

**SafePharm
Laboratories**

**N-ACETYL-L-HYDROXYPROLINE:
ACUTE ORAL TOXICITY IN THE RAT
- ACUTE TOXIC CLASS METHOD**

SPL PROJECT NUMBER: 732/093

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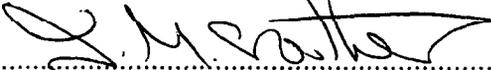
QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("General Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

01 March 2001	Standard Test Method Compliance Audit
01, 17 May 2001	Test Material Preparation
23 May 2001	Animal Preparation
14 May 2001	Dosing
01 May 2001	Assessment of Response
15 May 2001	Necropsy
§ 25 June 2001	Draft Report Audit
§ Date of QA Signature	Final Report Audit
§	Evaluation specific to this study



 For Safepharm Quality Assurance Unit*

DATE: 26 NOV 2001

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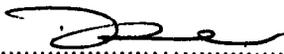
JV Johnson BSc; G Wren ONC; R Hurst

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.


..... DATE: **26 NOV 2001**

D M Dreher BSc (Hons)
Study Director

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**N-ACETYL-L-HYDROXYPROLINE:
ACUTE ORAL TOXICITY IN THE RAT
- ACUTE TOXIC CLASS METHOD**

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD (CrI: CD[®] (SD) IGS BR) strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method" (adopted 22 March 1996)
- Commission Directive 96/54/EC Method B1 tris Acute Toxicity (Oral) – Acute Toxic Class Method

Method. A group of three fasted females was treated with the test material at a dose level of 2000 mg/kg bodyweight. This was followed by a group of three fasted animals of the other sex at the same dose level.

The test material was administered orally as a solution in distilled water. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. There were no deaths.

Clinical Observations. There were no signs of systemic toxicity noted in female animals. Hunched posture and lethargy were noted in male animals during the day of dosing. All males appeared normal one day after dosing.

Bodyweight. All animals showed expected gains in bodyweight over the study period.

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD₅₀) of the test material in the Sprague-Dawley CD (CrI: CD[®] (SD) IGS BR) strain rat was estimated from the flow chart in Appendix 1 as being greater than 2500 mg/kg bodyweight.

The test material does not meet the criteria for classification according to EU labelling regulations Commission Directive 93/21/EEC.

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1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material following a single oral administration in the Sprague-Dawley CD (CrI: CD[®] (SD) IGS BR) strain rat. The method was designed to meet the requirements of the following:

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The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 24 May 2001 and 13 June 2001.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	N-ACETYL-L-HYDROXYPROLINE
Description	:	white solid
Lot number	:	000703
Date received	:	23 April 2001
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Preparation of Test Material

For the purpose of the study the test material was freshly prepared, as required, as a solution at the appropriate concentration in distilled water.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Male and female Sprague-Dawley CD (CrI: CD[®] (SD) IGS BR) strain rats were supplied by Charles River (UK) Ltd, Margate, Kent, UK. On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by indelible ink-marking on the tail and a number written on a cage card. At the start of the study the animals weighed at least 200g, and were approximately eight weeks of age.

The animals were housed in groups of three by sex in solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (Rat and Mouse Expanded Diet No.1, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminants that would reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

3.2 Procedure

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Rats	
			Male	Female
2000	200	10	-	3
2000	200	10	3	-

All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to the fasted bodyweight at the time of dosing. Treatment of animals was sequential. Sufficient time was allowed between each sex to confirm the survival of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity $\frac{1}{2}$, 1, 2 and 4 hours after dosing and subsequently once daily for fourteen days.

Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment.

At the end of the observation period the animals were killed by cervical dislocation. All animals were subjected to gross pathological examination. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

3.3 Evaluation of Data

Data evaluations included the relationship, if any, between the exposure of the animal to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight changes, mortality and any other toxicological effects.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test material was made as shown in the schematic diagram in Appendix 1.

The results were evaluated according to Commission Directive 93/21/EEC for classification and labelling of dangerous substances and preparations.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

5. RESULTS

5.1 Mortality Data

Individual mortality data are given in Table 1.

There were no deaths.

5.2 Clinical Observations

Individual clinical observations are given in Table 2.

There were no signs of systemic toxicity noted in female animals. Hunched posture was noted in all male animals during the day of dosing with lethargy noted in two animals during the day of dosing. All male animals appeared normal one day after dosing.

5.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 3.

All animals showed expected gains in bodyweight over the study period.

5.4 Necropsy

Individual necropsy findings are given in Table 4.

No abnormalities were noted at necropsy.

6. CONCLUSION

The acute oral median lethal dose (LD₅₀) of the test material in the Sprague-Dawley CD (CrI: CD[®] (SD) IGS BR) strain rat was estimated from the flow chart in Appendix 1 as being greater than 2500 mg/kg bodyweight.

The test material does not meet the criteria for classification according to EU labelling regulations Commission Directive 93/21/EEC.

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Table 1 Mortality Data

Dose Level mg/kg	Sex	Number of Animals Treated	Deaths During Day of Dosing (Hour)				Deaths During Period After Dosing (Days)								Deaths	
			½	1	2	3-4	1	2	3	4	5	6	7	8-14		
2000	Female	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	Male	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3

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Table 2 Individual Clinical Observations

Dose Level mg/kg	Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
		½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2000	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-0 Male	HL	HL	HL	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Male	H	H	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 Male	HL	HL	HL	H	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity
H = Hunched posture
L = Lethargy

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Table 3 Individual Bodyweights and Weekly Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	1-0 Female	213	245	275	32	30
	1-1 Female	215	231	261	16	30
	1-2 Female	230	251	280	21	29
	2-0 Male	241	289	351	48	62
	2-1 Male	224	261	344	37	83
	2-2 Male	219	266	339	47	73

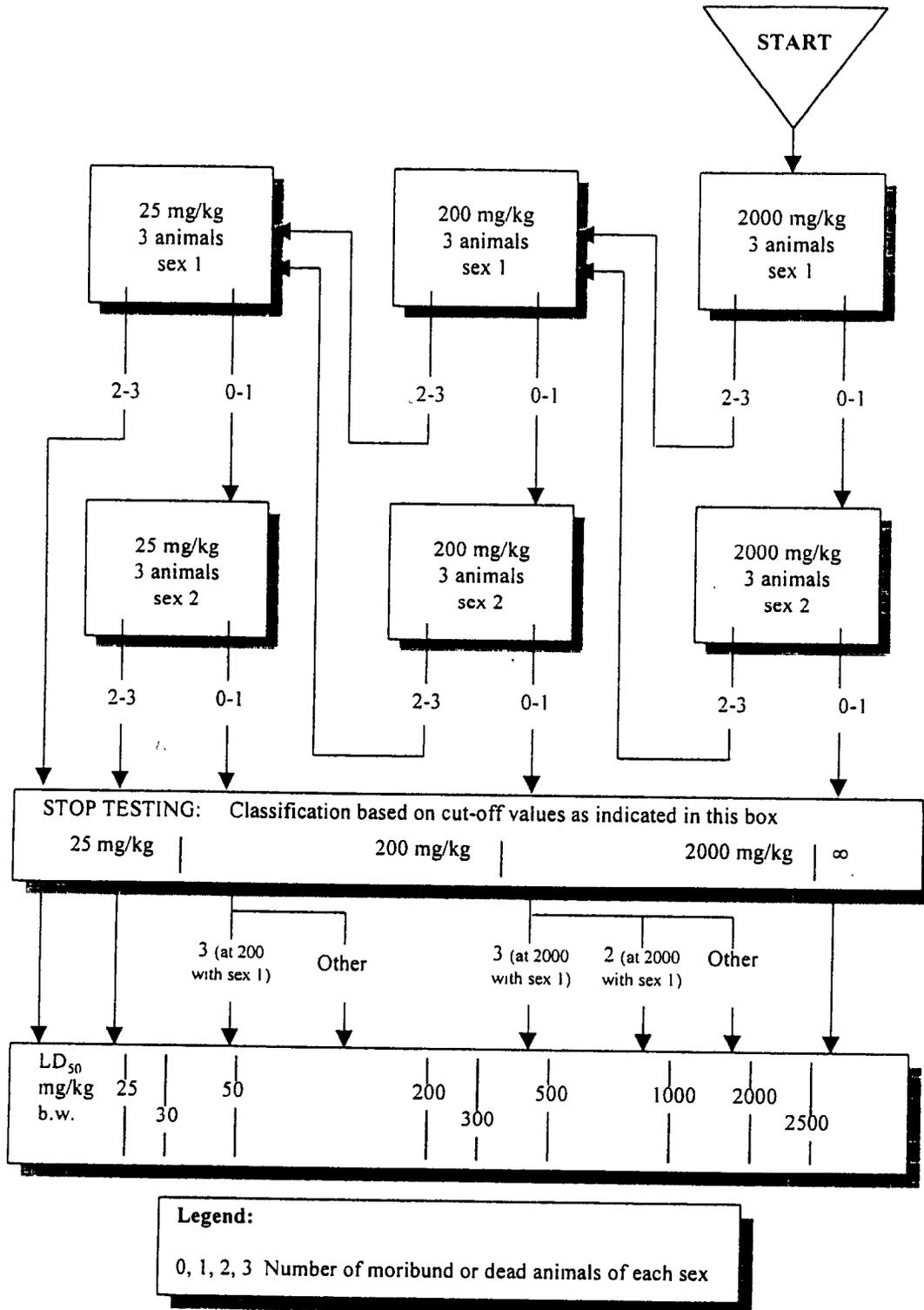
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Table 4 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Macroscopic Observations
2000	1-0 Female	No abnormalities detected
	1-1 Female	No abnormalities detected
	1-2 Female	No abnormalities detected
	2-0 Male	No abnormalities detected
	2-1 Male	No abnormalities detected
	2-2 Male	No abnormalities detected

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Appendix 1 Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight



Appendix 2 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM****GOOD LABORATORY PRACTICE****STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC****LABORATORY**

**SafePharm Laboratories Ltd
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD**

TEST TYPE

**Analytical Chemistry
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Tests
Toxicology**

DATE OF INSPECTION

28 February 2000

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
26/4/00

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority