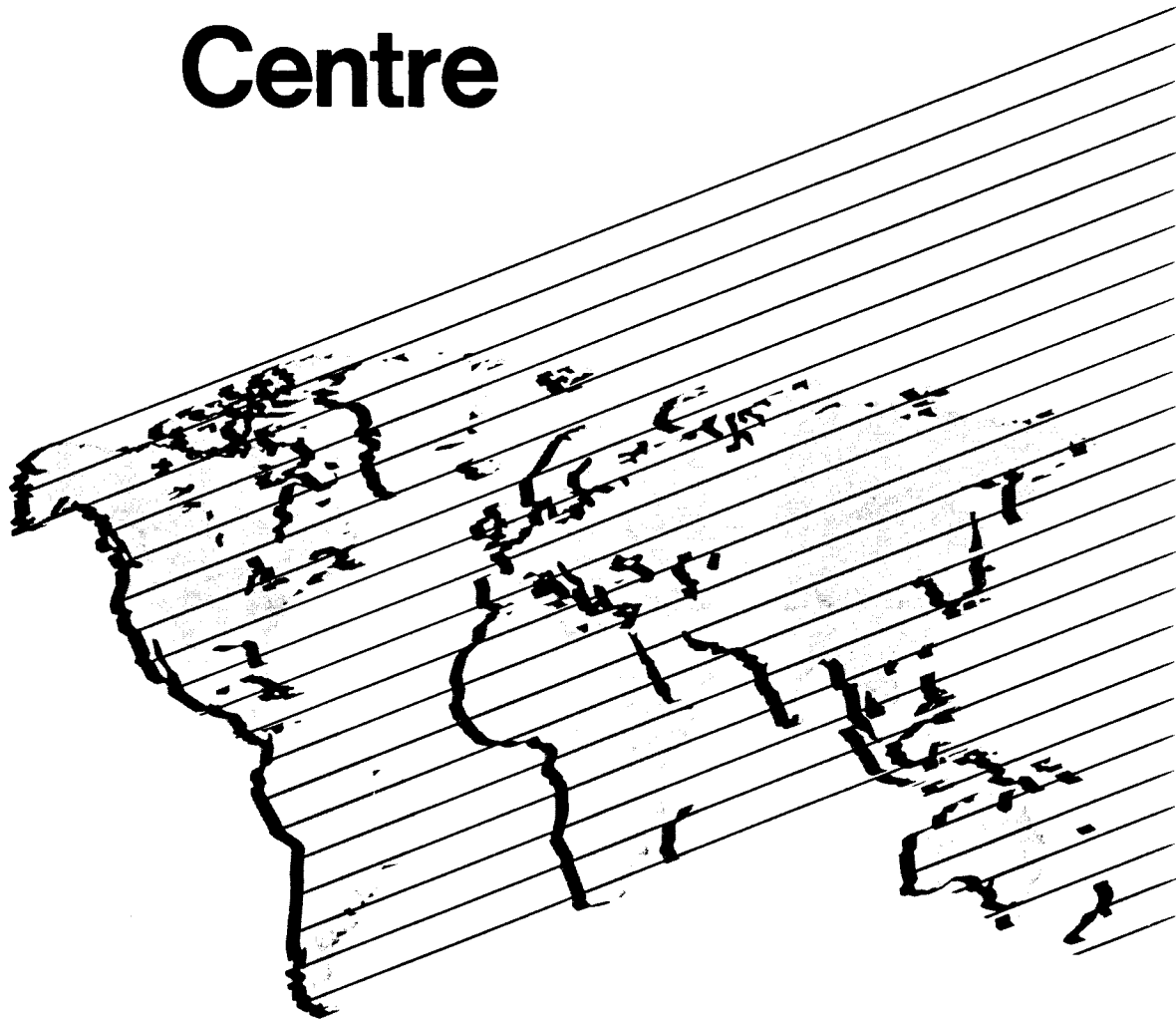


HRC Report

TREHALOSE CRYSTALS
ACUTE ORAL TOXICITY
TO THE RAT

**Huntingdon
Research
Centre**



TREHALOSE CRYSTALS
ACUTE ORAL TOXICITY TO THE RAT

Sponsor

Hayashibara Biochemical Laboratories Inc.,
2-3, Shimoishi 1-chome,
Okayama-shi,
Okayama-ken 700,
JAPAN.

Testing facility

Huntingdon Research Centre Ltd.,
P.O. Box 2,
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Report issued 17 February 1995

CONTENTS

	Page
COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS	3
QUALITY ASSURANCE STATEMENT	4
RESPONSIBLE PERSONNEL	5
SUMMARY	6
INTRODUCTION	7
TEST SUBSTANCE	8
EXPERIMENTAL PROCEDURE	9
RESULTS	12
CONCLUSION	12
TABLES	
1. Individual bodyweights	13
2. Individual bodyweight changes	13

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.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health and Social Security 1986 and subsequent revision, Department of Health, 1989.

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



Lewis A. McRae, M.I.A.T., M.I.Sc.T.,
Study Director,
Huntingdon Research Centre Ltd.

17 February 1995

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Certain studies such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

Date(s) of inspection


14-18 November 1994

Date(s) of reporting inspection findings
to the Study Director and HRC Management

18 November 1994

Date of reporting audit findings to the
Study Director and HRC Management

23 December 1994


.....
Jacqueline Cahill,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.


.....
Date

RESPONSIBLE PERSONNEL

Lewis A. McRae, M.I.A.T., M.I.Sc.T.,
Study Director,
Department of Industrial Toxicology.

A handwritten signature in cursive script, appearing to read 'L. McRae', is written over a horizontal dotted line.

SUMMARY

A study was performed to assess the acute oral toxicity of Trehalose Crystals to the rat. The method followed was that described in the OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

A group of ten fasted rats (five males and five females) was given a total dose by oral gavage of the test substance, formulated in distilled water, at a dose level of 16.0 g/kg bodyweight. All animals were killed and examined macroscopically on Day 15, the end of the observation period.

There were no deaths. Clinical signs of reaction to treatment were limited to piloerection and soft to liquid faeces. Recovery was complete in all instances by Day 4.

A slightly low bodyweight gain was recorded for one female on Day 8; this rat achieved the anticipated gain on Day 15. All other rats achieved satisfactory bodyweight gains throughout the study.

No abnormalities were recorded at the macroscopic examination on Day 15.

The acute lethal oral dose to rats of Trehalose Crystals was found to be greater than 16 g/kg bodyweight.

INTRODUCTION

The study was designed to assess the toxicity of Trehalose Crystals following a single oral dose to the rat. The rats were dosed by oral gavage as the test substance may be ingested accidentally.

The study was conducted in compliance with the OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

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

The dose level for the study was selected by the Study Sponsor.

The protocol was approved by the Study Director and HRC Management on 11 October 1994 and by the Sponsor on 19 October 1994.

The experimental phase of the study was undertaken between 26 October and 15 November 1994.

TEST SUBSTANCE

Identity: Trehalose Crystals

Chemical name: α,α -Trehalose (α -D-glucopyranosyl α -D-glucopyranoside)

Intended use: Food additive

Appearance: White crystalline powder

Storage conditions: Room temperature

Batch number: 931129

Expiry date: 28 May 1995

Purity: 100%

Date received: 3 October 1994

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Equal numbers of healthy male and female CD rats of Sprague-Dawley origin (Hsd/Ola:Sprague-Dawley(CD)) were obtained from Harlan Olac Ltd., Bicester, Oxon, England.

They were in the weight range of 95 to 114 g and approximately four to seven weeks of age prior to dosing (Day 1) in the main study. All the rats were acclimatised to the experimental environment for a period of five days prior to the start of the main study.

The rats were allocated without conscious bias to cages within the treatment group. They were housed in groups of up to five rats of the same sex in metal cages with wire mesh floors in Building R14 Room 6.

A standard laboratory rodent diet (Biosure LAD 1) and drinking water were provided *ad libitum*. Access to food only was prevented overnight prior to and approximately 3 to 4 hours after dosing.

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 at source, as conducted, usually weekly by the supplier, are made available to Huntingdon Research Centre Ltd. (as quarterly summaries).

Animal room temperature was set to achieve a temperature of $22 \pm 3^{\circ}\text{C}$. Relative humidity was not controlled but was anticipated to be in the range 30 - 70% R.H. Permanent daily recordings of these parameters was made and these are archived with other Department raw data. Any slight deviation in temperature and humidity that may have occurred had no impact on the study in the opinion of the Study Director. Air exchange was maintained at 10 to 15 air changes per hour and lighting 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

Trehalose Crystals was prepared at a maximum practical concentration of 80% w/v in distilled water and administered at a volume of 10 ml/kg bodyweight. To achieve a total dosage of 16 g/kg bodyweight the formulated test substance was administered as two equal dosage volumes over a one hour period giving a total dose volume of 20 ml/kg bodyweight.

The test substance was prepared on the day of dosing.

The absorption of the test substance was not determined.

The homogeneity, stability and purity of the test substance were the responsibility of the Sponsor.

TREATMENT PROCEDURE

Preliminary study

A preliminary study was carried to establish the feasibility of administration of a dosage of 16 g/kg bodyweight by dosing two male and two female rats at 16 g/kg bodyweight.

Main study

A group of ten rats (five males and five females) was treated at 16 g/kg bodyweight.

Control animals

No control animals were included in this study.

ADMINISTRATION OF TEST SUBSTANCE

The appropriate dose volume of the test substance was administered to each rat by oral gavage using a syringe and plastic catheter (8 choke).

The day of dosing was designated Day 1.

OBSERVATIONS

Mortality

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

Clinical signs

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1 (a period of six hours). On subsequent days animals were observed once in the morning and again at the end of the experimental day (with the exception of Day 15 - morning only). This latter observation was at approximately 16.30 hours on week days or 11.30 hours on Saturdays and Sundays. The nature and severity of the clinical signs and time were recorded at each observation.

The animals on the preliminary and main studies were observed for 5 and 14 days respectively after dosing.

Bodyweight

The bodyweight of each rat on the main study was recorded on Days 1 (prior to dosing), 8 and 15. Individual weekly bodyweight changes were calculated.

TERMINAL STUDIES

Termination

All animals on 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 abdominal and thoracic cavities. The macroscopic appearance of all tissues was recorded.

ARCHIVES

All raw data and study related documents generated during the course of the study at HRC, together with a copy of the final report will be lodged in the Huntingdon Research Centre Ltd., Archives.

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 client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's knowledge.

RESULTS

PRELIMINARY STUDY

The results of the preliminary study indicated that the acute lethal oral dose to male and female rats of Trehalose Crystals was greater than 16 g/kg bodyweight.

MAIN STUDY

There were no deaths following two oral doses of Trehalose Crystals at 8 g/kg bodyweight (a total dosage of 16 g/kg bodyweight).

CLINICAL SIGNS

Piloerection was observed in all rats within five minutes of dosing and throughout the remainder of Day 1 and also at later intervals during the study. There were no other signs with the exception of soft to liquid faeces which was evident in one male during the latter part of Day 1 only. Recovery, as judged by external appearance and behaviour, was complete by either Day 3 or day 4 (females) or Day 4 (males).

BODYWEIGHT (Tables 1 and 2)

A slightly low bodyweight gain was recorded for one female on Day 8; this rat achieved the anticipated gain on Day 15. All other rats achieved anticipated bodyweight gains throughout the study.

MACROSCOPIC EXAMINATION

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

CONCLUSION

The acute lethal oral dose to rats of Trehalose Crystals was found to be greater than 16 g/kg bodyweight.

TABLE 1

Individual bodyweights (g)

Sex	Dose (g/kg)	Animal number & ear mark	Bodyweight (g) at		
			Day 1	Day 8	Day 15
♂	16	1 RP	114	177	223
		2 LP	105	177	231
		3 RPLP	105	179	236
		4 RIRO	107	176	223
		5 LILO	107	195	261
♀	16	6 RP	104	143	168
		7 LP	95	137	163
		8 RPLP	103	137	154
		9 RIRO	101	146	169
		10 LILO	103	143	167

TABLE 2

Individual bodyweight changes (g)

Sex	Dose (g/kg)	Animal number & ear mark	Bodyweight gains (g) at	
			Week 1	Week 2
♂	16	1 RP	63	46
		2 LP	72	54
		3 RPLP	74	57
		4 RIRO	69	47
		5 LILO	88	66
♀	16	6 RP	39	25
		7 LP	42	26
		8 RPLP	34	17
		9 RIRO	45	23
		10 LILO	40	24