SafePharm Laboratories

C6-2AL:

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 408/329

AUTHOR: J D Highton

STUDY SPONSOR:

Asahi Glass Co., Ltd.
1-12-1, Yurakucho
Chiyoda-ku
TOKYO 100-8405
JAPAN

TEST FACILITY:

Safepharm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
U.K.

Telephone: (01332) 792896

Facsimile: (01332) 799018

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:

	21 January 2002	Standard Test Method Compliance Audit
	03 April 2002	Test Material Preparation
	24 April 2002	Animal Preparation
	10 April 2002	Dosing
	23 April 2002	Assessment of Response
	24 April 2002	Necropsy
§	31 May 2002	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: - 4 JUL 2002

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**

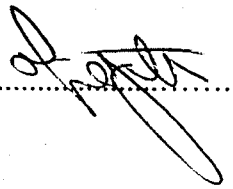
Head of Department:	JR Pateman CBiol MIBiol DipRQA FRQA
Deputy Head of Department:	JM Crowther MIScT MRQA
Senior Audit Staff:	JV Johnson BSc MRQA; G Wren ONC MRQA; R Hurst MRQA

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: 03 JUL 2002

J D Highton
Study Director

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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 (Crl: 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 17 December 2001)

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 further group of three fasted females at the same dose level.

The test material was administered orally undiluted. Clinical signs and bodyweight development were monitored during the study. All animals were subjected to gross necropsy.

Mortality. Two animals were killed *in extremis* three or four days after dosing.

Clinical Observations. Signs of systemic toxicity commonly noted were hunched posture, lethargy, pilo-erection, decreased respiratory rate, laboured respiration and ataxia with additional signs of ptosis, emaciation, dehydration, tiptoe gait and red/brown staining around the eyes and/or snout. Surviving animals recovered five or six days after dosing.

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

Necropsy. No abnormalities were noted at necropsy.

Conclusion. The acute oral median lethal dose (LD_{50}) of the test material in the female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rat was estimated from the flow chart in Appendix 1 as being greater than 2000 mg/kg bodyweight.

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

C6-2AL:

ACUTE ORAL TOXICITY IN THE RAT - ACUTE TOXIC CLASS METHOD

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 (Crl: 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 17 December 2001)

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 08 May 2002 and 29 May 2002.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	C6-2AL
Description	:	clear colourless liquid
Batch number	:	001002
Date received	:	25 March 2002
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 used as supplied. The specific gravity was determined and used to calculate the appropriate dose volume for the required dose level.

3. METHODS

3.1 Animals and Animal Husbandry

Female Sprague-Dawley CD (Crl: 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 animals 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 were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean initial bodyweight of the first treated group.

The animals were housed in groups of three 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 (Certified Rat and Mouse Diet (Code 5LF2) supplied by PMI Nutrition International, Nottingham, 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.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2 Procedure

In the absence of data regarding the toxicity of the test material, 2000 mg/kg was chosen as the starting dose.

Groups of fasted animals were treated as follows:

Dose Level (mg/kg)	Specific Gravity	Dose Volume (ml/kg)	Number of Rats
			Female
2000	1.690	1.19	3
2000	1.690	1.19	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 group to confirm the survival of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently once daily for up to fourteen days.

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

At the end of the observation period the surviving animals were killed by cervical dislocation. All animals, including those killed *in extremis* during the study, 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.

Two animals were killed *in extremis* three or four days after dosing.

5.2 Clinical Observations

Individual clinical observations are given in Table 2.

Signs of systemic toxicity commonly noted were hunched posture, lethargy, pilo-erection, decreased respiratory rate, laboured respiration and ataxia with additional signs of ptosis, emaciation, dehydration, tiptoe gait and red/brown staining around the eyes and/or snout. Surviving animals recovered five or six days after dosing.

5.3 Bodyweight

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

The surviving 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 (LD_{50}) of the test material in the female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rat was estimated from the flow chart in Appendix 1 as being greater than 2000 mg/kg bodyweight.

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

Table 1 Mortality Data

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

* = Animal killed *in extremis*

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	H	HLPA RdRl	HLPA RdRl	HP	HP	HP	HP	0	0	0	0	0	0	0	0	0	0
	1-1 Female	0	H	HLPA RdRl	HPA RdRl	HPSs	HPSs	HLPPtRdRlSsEm SeADhWtX*											
	1-2 Female	0	H	HLPA RdRl	HPA RdRl	HPSs	HP	HP	HP	H	0	0	0	0	0	0	0	0	0
	2-0 Female	0	0	HL	HLPA Rd	HLPRd	HRd	HRd	HLPPtARdRlSs EmSeDhWtX*										
	2-1 Female	0	0	HL	HLPA RdRl	HLPSs	H	H	H	0	0	0	0	0	0	0	0	0	0
	2-2 Female	0	0	HLPRd	HLPA Rd	HLPRd Ss	H	H	H	0	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity
Dh = Dehydration
Rd = Decreased respiratory rate
Se = Red/brown staining around the eyes
H = Hunched posture
Em = Emaciation
Rl = Laboured respiration
Wt = Tiptoe gait
L = Lethargy
P = Pilo-erection
Ss = Red/brown staining around the snout
X* = Animal killed *in extremis*
A = Ataxia
Pt = Ptosis

Table 3 Individual Bodyweights and Weekly Bodyweight Changes

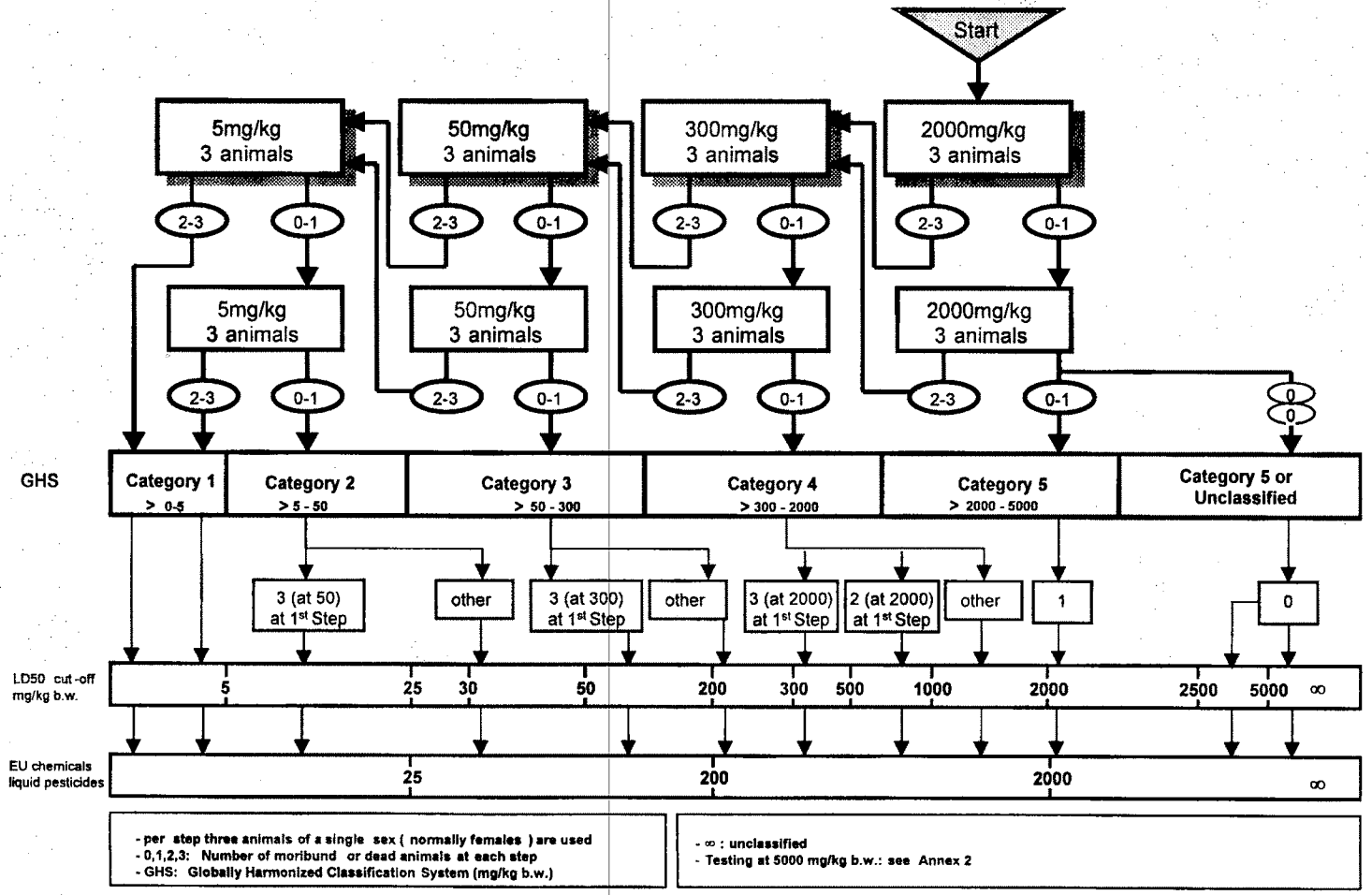
Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight (g) at Death	Bodyweight Gain (g) During Week	
		0	7	14		1	2
2000	1-0 Female	226	264	294		38	30
	1-1 Female	222	-	-	170	-	-
	1-2 Female	202	245	252		43	7
	2-0 Female	220	-	-	165	-	-
	2-1 Female	228	251	265		23	14
	2-2 Female	210	215	248		5	33

- = Animal dead

Table 4 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
2000	1-0 Female	Killed Day 14	No abnormalities detected
	1-1 Female	Killed Day 3	No abnormalities detected
	1-2 Female	Killed Day 14	No abnormalities detected
	2-0 Female	Killed Day 4	No abnormalities detected
	2-1 Female	Killed Day 14	No abnormalities detected
	2-2 Female	Killed Day 14	No abnormalities detected

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

SAFEPHARM LABORATORIES LTD

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I verify that this is an exact copy of the original report which is located in the Archives of Safepharm Laboratories Ltd., Derby, UK.

..... DATE: 05 JUL 2002

J D Highton
Study Director