SafePharm Laboratories

13F-SFA-MONOMER:

ACUTE ORAL TOXICITY IN THE RAT - FIXED DOSE METHOD

SPL PROJECT NUMBER: 1458/0061

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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:

	16 November 2006	Standard Test Method Compliance Audit
	06 February 2007	Test Material Preparation
	14 February 2007	Animal Preparation
	15 February 2007	Dosing
	12, 14 February 2007	Assessment of Response
	14 February 2007	Necropsy
§	13 April 2007	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 10 MAY 2007

For Safepharm Quality Assurance Unit*

***Authorised QA Signatures:**
Head of Department:
Deputy Head of Department:
Senior Audit Staff:

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). 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 2004/9/EC and 2004/10/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, South Africa, 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: 10/5/07

Study Director

CONTENTS

QUALITY ASSURANCE REPORT		**2**
GLP COMPLIANCE STATEMENT		**3**
CONTENTS		**4**
SUMMARY		**5**
1.	**INTRODUCTION**	**6**
2.	**TEST MATERIAL AND EXPERIMENTAL PREPARATION**	**6**
2.1	Description, Identification and Storage Conditions	6
2.2	Experimental Preparation	6
3.	**METHODS**	**7**
3.1	Animals and Animal Husbandry	7
3.2	Procedure	7
3.3	Evaluation of Data	8
4.	**ARCHIVES**	**9**
5.	**RESULTS**	**10**
5.1	Mortality	10
5.2	Clinical Observations	10
5.3	Bodyweight	10
5.4	Necropsy	10
6.	**CONCLUSION**	**10**
Table 1	Individual Clinical Observations and Mortality Data	11
Table 2	Individual Bodyweights and Bodyweight Changes	12
Table 3	Individual Necropsy Findings	13
Appendix 1	Flow Chart for the Sighting Test	14
Appendix 2	Flow Chart for the Main Test	15
Appendix 3	Statement of GLP Compliance in Accordance with Directive 2004/9/EC	16

13F-SFA-MONOMER:

ACUTE ORAL TOXICITY IN THE RAT - FIXED DOSE METHOD

SUMMARY

Introduction. The study was performed to assess the acute oral toxicity of the test material in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for Testing of Chemicals No 420 "Acute Oral Toxicity - Fixed Dose Method" (adopted 17 December 2001)
- Method B1 *bis* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

Method. Following a sighting test in which there was no mortality at a dose level of 2000 mg/kg, an additional four fasted female animals were given a single oral dose of undiluted test material, at a dose level of 2000 mg/kg bodyweight. 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.

Bodyweight. All animals showed expected gains in bodyweight.

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 strain rat was estimated to be greater than 2000 mg/kg bodyweight (Globally Harmonised Classification System Category – Unclassified).

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1. INTRODUCTION

The study was performed to assess the acute oral toxicity of the test material in the Sprague-Dawley CD strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for Testing of Chemicals No 420 "Acute Oral Toxicity - Fixed Dose Method" (adopted 17 December 2001)
- Method B1 *bis* Acute Toxicity (Oral) of Commission Directive 2004/73/EC

The test system was chosen because the rat has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 06 February 2007 and 26 February 2007.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	13F-SFA-MONOMER
Description	:	clear colourless liquid
Batch number	:	061115
Date received	:	24 November 2006
Storage conditions	:	room temperature in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

2.2 Experimental Preparation

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 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 were eight to twelve weeks of age. The bodyweight variation did not exceed ±20% of the mean bodyweight of any previously dosed animal.

The animals were housed in groups of up to four in suspended 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) 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

Using available information on the toxicity of the test material, 2000 mg/kg was chosen as the starting dose.

Dose Level (mg/kg)	Specific Gravity	Dose Volume (ml/kg)	Number of Rats
			Female
2000	1.549	1.30	1

In the absence of toxicity at a dose level of 2000 mg/kg, an additional group of animals was treated as follows:

Dose Level (mg/kg)	Specific Gravity	Dose Volume (ml/kg)	Number of Rats
			Female
2000	1.549	1.30	4

A total of five animals were therefore treated at a dose level of 2000 mg/kg in the study.

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 its fasted bodyweight at the time of dosing.

Clinical observations were made ½, 1, 2, and 4 hours after dosing and subsequently once daily for fourteen days. Morbidity and mortality checks were made twice daily.

Individual bodyweights were recorded on Day 0 (the day of dosing) and on Days 7 and 14.

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

3.3 Evaluation of Data

The test material will be classified according to Annex 3 of the OECD Guidelines for Testing of Chemicals No. 420 "Acute Oral Toxicity - Fixed Dose Method" (adopted 17 December 2001) as shown in the Flow Chart in Appendix 2.

Evaluation of data included identification of the number of animals that died during the study (or that were killed for humane reasons), and determination of the nature, severity, onset and duration of the toxic effects. If possible, the signs of evident toxicity were described. Evident toxicity refers to the toxic effects of sufficient severity that administration of the next higher dose level could result in development of severe signs of toxicity and probable mortality. Effects on bodyweights and abnormalities noted at necropsy were also identified.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test material was made.

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

Individual clinical observations and mortality data are given in Table 1.

5.1 Mortality

There were no deaths.

5.2 Clinical Observations

No signs of systemic toxicity were noted.

5.3 Bodyweight

Individual bodyweights and bodyweight changes are given in Table 2.

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

5.4 Necropsy

Individual necropsy findings are given in Table 3.

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 strain rat was estimated to be greater than 2000 mg/kg bodyweight (Globally Harmonised Classification System Category – Unclassified).

Table 1 Individual Clinical Observations and Mortality Data

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
	2-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-1 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-2 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-3 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity

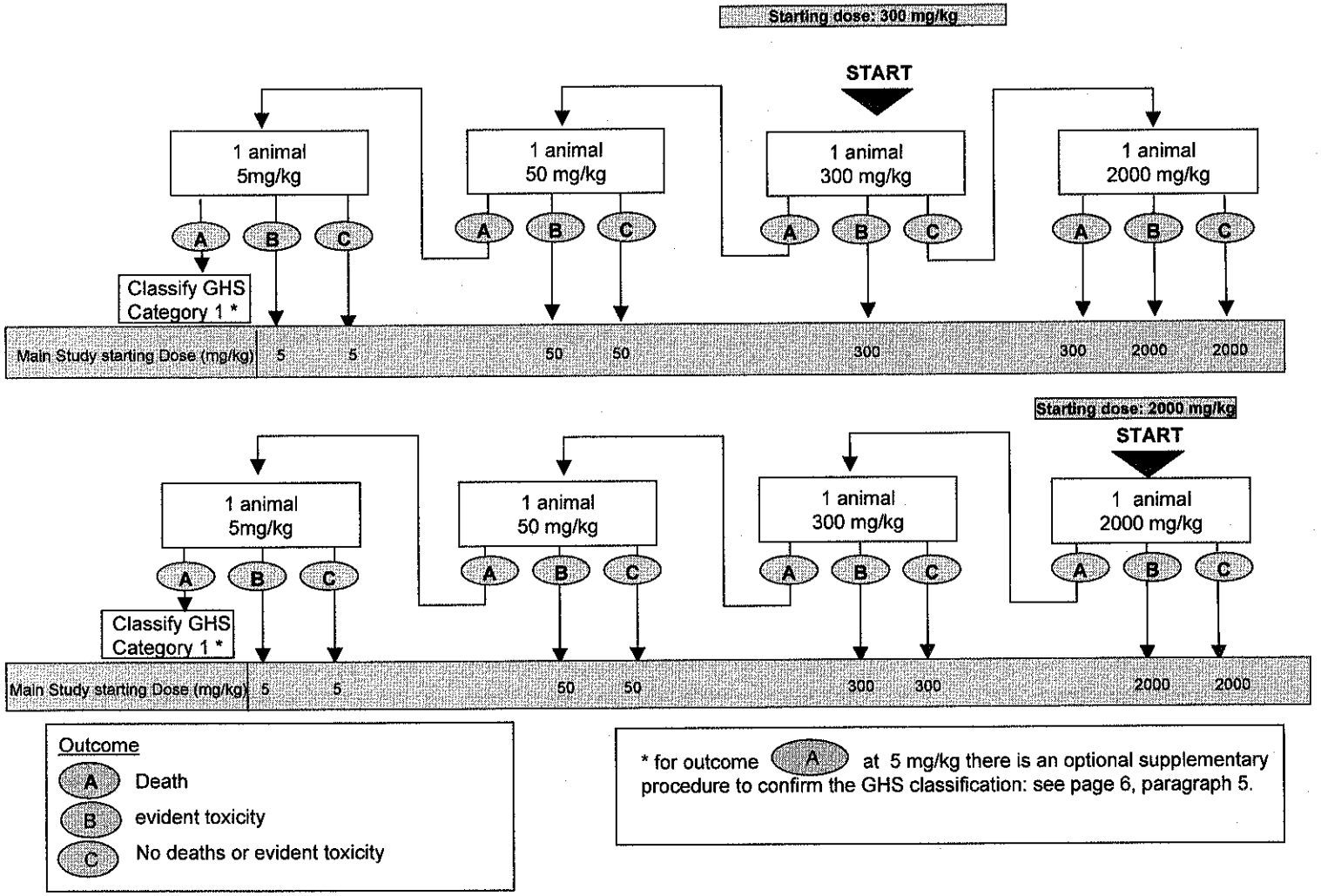
Table 2 Individual Bodyweights and Bodyweight Changes

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	203	213	243	10	30
	2-0 Female	230	261	283	31	22
	2-1 Female	221	251	277	30	26
	2-2 Female	222	263	290	41	27
	2-3 Female	237	273	298	36	25

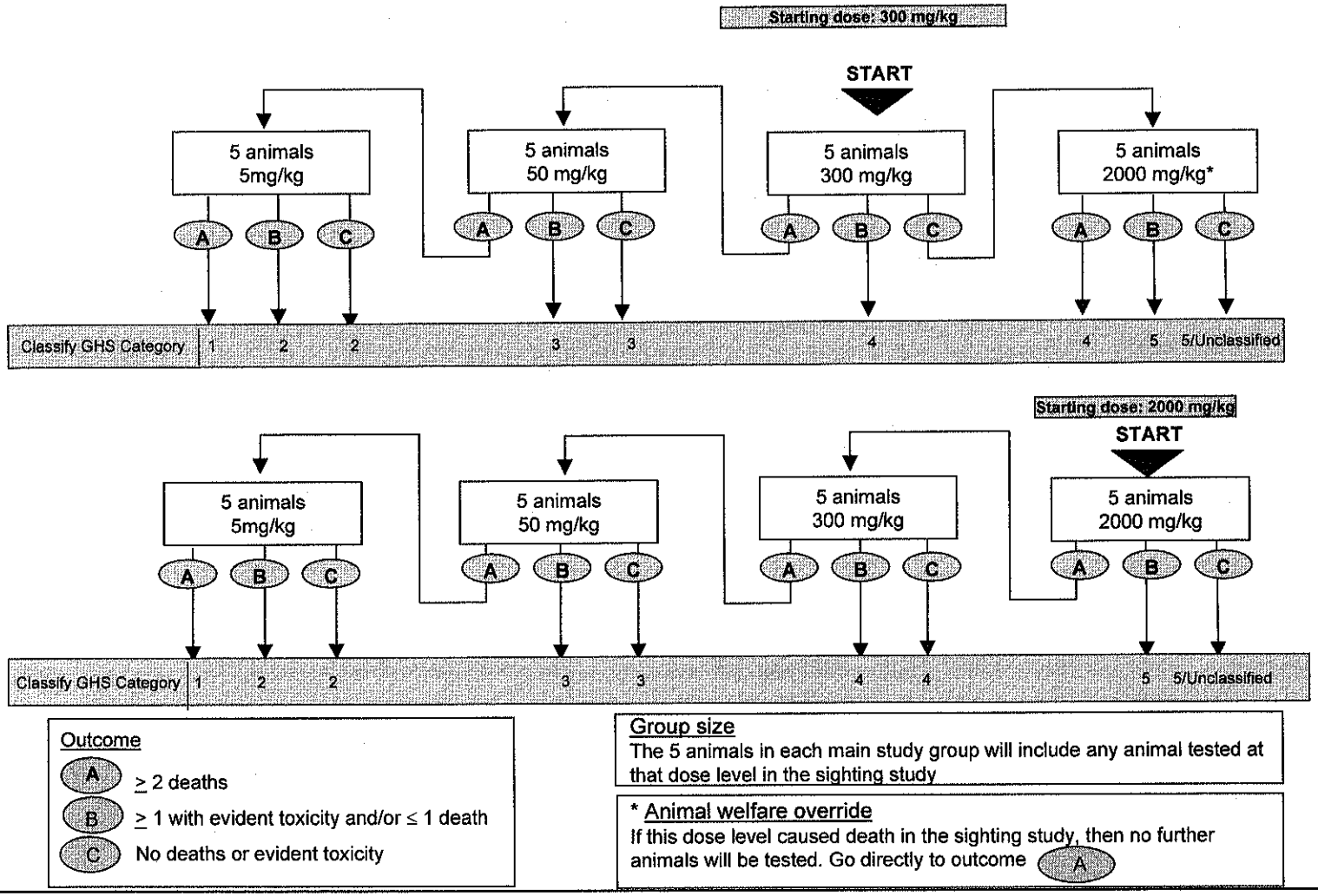
Table 3 Individual Necropsy Findings

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

Appendix 1 Flow Chart for the Sighting Test



Appendix 2 Flow Chart for the Main Test



Appendix 3 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

DH Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

LABORATORY

SafePharm Laboratories Ltd.
Shardlow Business Park
London Road
Shardlow
Derby
DE72 2GD

TEST TYPE

Analytical Chemistry
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

30th August 2005

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

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

10/05.

Head, UK GLP Monitoring Authority