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:
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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:

<table>
<thead>
<tr>
<th>Date</th>
<th>Audit Description</th>
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<tr>
<td>21 January 2002</td>
<td>Standard Test Method Compliance Audit</td>
</tr>
<tr>
<td>03 April 2002</td>
<td>Test Material Preparation</td>
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<td>24 April 2002</td>
<td>Animal Preparation</td>
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<td>10 April 2002</td>
<td>Dosing</td>
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<td>23 April 2002</td>
<td>Assessment of Response</td>
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<td>24 April 2002</td>
<td>Necropsy</td>
</tr>
<tr>
<td>§ 31 May 2002</td>
<td>Draft Report Audit</td>
</tr>
<tr>
<td>§ Date of QA Signature</td>
<td>Final Report Audit</td>
</tr>
</tbody>
</table>

§ 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 MScT 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.

J D Highton
Study Director

DATE: 03 JUL 2002
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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.
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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 (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:

<table>
<thead>
<tr>
<th>Dose Level (mg/kg)</th>
<th>Specific Gravity</th>
<th>Dose Volume (ml/kg)</th>
<th>Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>1.690</td>
<td>1.19</td>
<td>3</td>
</tr>
<tr>
<td>2000</td>
<td>1.690</td>
<td>1.19</td>
<td>3</td>
</tr>
</tbody>
</table>

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 (LD₅₀) 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

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

* = Animal killed *in extremis*
## Table 2

**Individual Clinical Observations**

<table>
<thead>
<tr>
<th>Dose Level mg/kg</th>
<th>Animal Number and Sex</th>
<th>Effects Noted After Dosing (Hours)</th>
<th>Effects Noted During Period After Dosing (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>½</td>
<td>1-0 Female</td>
<td>0 H</td>
<td>HLPARdRI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-1 Female</td>
<td>0 H</td>
<td>HLPARdRI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-2 Female</td>
<td>0 H</td>
<td>HLPARdRI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-0 Female</td>
<td>0 0 H</td>
<td>HLPARd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-1 Female</td>
<td>0 0 H</td>
<td>HLPARd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-2 Female</td>
<td>0 0 H</td>
<td>HLPARd</td>
</tr>
</tbody>
</table>

0 = No signs of systemic toxicity  
Dh = Dehydration  
Rd = Decreased respiratory rate  
Se = Red/brown staining around the eyes  
H = Hunched posture  
HL = HLPA RdRI  
HP = HP  
Rd = HLPA RdRI  
Ss = SeADhWtX  
P = Pilo-erection  
Pt = Ptosis  
A = Ataxia  
L = Lethargy  
X* = Animal killed in extremis
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Table 3  Individual Bodyweights and Weekly Bodyweight Changes

<table>
<thead>
<tr>
<th>Dose Level mg/kg</th>
<th>Animal Number and Sex</th>
<th>Bodyweight (g) at Day</th>
<th>Bodyweight Gain (g) During Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>2000</td>
<td>1-0 Female</td>
<td>226</td>
<td>264</td>
</tr>
<tr>
<td></td>
<td>1-1 Female</td>
<td>222</td>
<td>264</td>
</tr>
<tr>
<td></td>
<td>1-2 Female</td>
<td>202</td>
<td>245</td>
</tr>
<tr>
<td></td>
<td>2-0 Female</td>
<td>220</td>
<td>245</td>
</tr>
<tr>
<td></td>
<td>2-1 Female</td>
<td>228</td>
<td>251</td>
</tr>
<tr>
<td></td>
<td>2-2 Female</td>
<td>210</td>
<td>215</td>
</tr>
</tbody>
</table>

- = Animal dead
### Table 4: Individual Necropsy Findings

<table>
<thead>
<tr>
<th>Dose Level mg/kg</th>
<th>Animal Number and Sex</th>
<th>Time of Death</th>
<th>Macroscopic Observations</th>
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</thead>
<tbody>
<tr>
<td>2000</td>
<td>1-0 Female</td>
<td>Killed Day 14</td>
<td>No abnormalities detected</td>
</tr>
<tr>
<td></td>
<td>1-1 Female</td>
<td>Killed Day 3</td>
<td>No abnormalities detected</td>
</tr>
<tr>
<td></td>
<td>1-2 Female</td>
<td>Killed Day 14</td>
<td>No abnormalities detected</td>
</tr>
<tr>
<td></td>
<td>2-0 Female</td>
<td>Killed Day 4</td>
<td>No abnormalities detected</td>
</tr>
<tr>
<td></td>
<td>2-1 Female</td>
<td>Killed Day 14</td>
<td>No abnormalities detected</td>
</tr>
<tr>
<td></td>
<td>2-2 Female</td>
<td>Killed Day 14</td>
<td>No abnormalities detected</td>
</tr>
</tbody>
</table>
Appendix 1  Test Procedure with a Starting Dose of 2000 mg/kg Bodyweight

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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

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<td>Analytical Chemistry</td>
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<tr>
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<td>Environmental Fate</td>
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<tr>
<td>London Road</td>
<td>Environmental Toxicity</td>
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<td>Shardlow</td>
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<tr>
<td>Derbyshire</td>
<td>Phys/Chem Tests</td>
</tr>
<tr>
<td>DE72 2GD</td>
<td>Toxicology</td>
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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.

[Signature]

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority
SAFE PHARM LABORATORIES LTD

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- ACUTE TOXIC CLASS METHOD

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

DATE: 05 JUL 2002

J D Highton
Study Director