SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH

Confidential

PROJECT No.	**:**	**TOX/444**
PRODUCT	**:**	**Cry 1 C a 1 Protein**
STUDY	**:**	**ACUTE ORAL TOXICITY STUDY IN RATS**
REPORT No.	**:**	**000123466**
DATE	**:**	**07.04.2009**

ACUTE ORAL TOXICITY STUDY IN RATS

WITH

Cry 1 C a 1 PROTEIN

REPORT FOR

METAHELIX LIFE SCIENCES PVT. LTD.,
PLOT NO.-3, KIADB 4TH PHASE,
BOMMASANDRA,
BANGALORE-500 099, INDIA.

GUIDELINES
'DBT Guidelines '

TEST FACILITY

SHRIRAM TOXICOLOGY CENTRE

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH
(A Unit of Shriram Scientific & Industrial Research Foundation)
19, University Road, Delhi – 110 007
Tel. 27667267, 27667860, 27667432
Fax No. 91+11-27667676, 27667207
E. Mail: sridlhi@vsnl.com

GLP COMPLIANCE STATEMENT

Study No. : TOX/444
Test Item : Cry 1 C a 1 Protein
Study Title : Acute oral toxicity study in rats

I hereby attest to the authenticity of the study and guarantee that the data is correct and accurate to the best of my knowledge and that the study was performed by the procedure described in the Shriram Institute for Industrial Research Standard Operating Procedures. I hereby attest that this study was conducted in compliance with the principles of GLP as well as the Study protocol submitted to and approved by the sponsor. This report shall not be reproduced except in full, without the written approval of the Sponsor.

The study was performed to meet the requirements of the following guidelines: DBT

Dr.A.K.Tiwari		7/4/09
Study Director	Signature	Date

STATEMENT OF QUALITY ASSURANCE UNIT

Study No. : TOX/444
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Quality Assurance Unit of the testing facility inspected the conduct of study on the following dates:

Phases of study	Dates of Inspection	Dates of Reporting
Protocol	17.03.2009	18.03.2009
Study conduct	19.03.2009	21.03.2009
Records (Raw data)	25.03.2009	26.03.2009
Report	06.04.2009	06.04.2009

No findings were noticed during inspection, which would have impaired this study in any way. Report reflects the raw data

Dr. Binu Bhat
Quality Assurance Unit

Binu Bhat
Signature

07.04.2009
Date

STATEMENT OF COMPLIANCE

Study No. : TOX/444
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Study Title : Acute oral toxicity study in rats

We, the undersigned, take overall responsibility for the reliability of the work described in the study entitled ' Acute oral toxicity study in rats ' with 'Cry 1 C a 1 Protein' performed with respect to the study plan and the Standard Operating Procedures in compliance with Good Laboratory Practices (G.L.P) for non-clinical laboratory studies.

Dr. M. L. Aggarwal
Head Toxicology — Signature — Date 07/4/09

Dr. K. M. Chacko
Management — Signature — Date 07/4/09

PERSONNEL INVOLVED IN THE STUDY

Study Director : Dr. A. K. Tiwari

Pathology : Dr. A. K. Tiwari

Quality Assurance Unit : Dr. Binu Bhat

Study Personnel : Ms. Pooja Singh

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SUMMARY

In the assessment and evaluation of the toxic characteristics of a substance, determination of 'Acute oral toxicity study in rats' is usually an initial step.

This study was hence, designed to conduct acute oral toxicity of 'Cry 1 C a 1 Protein' in wistar rats sponsored by, 'Metahelix Life Sciences Private Limited, Bangalore'.

A batch consisting of 5 male & 5 female rats was administered with the single dose of the test protein orally at the dose level of 0.35 mg/rat (a limit test using a single dose equivalent to 10X of the estimated dietary exposure of the test protein) with the help of metallic cannula attached with tuberculin syringe. Similarly control groupI was administered with aqueous buffer and control group II was administered with vehicle i.e. Distilled water only. As no toxic sign and symptoms or mortality was observed in any of the treatment group.

At the end of the study, all the animals were sacrificed and their necropsy examination conducted.

Under the given conditions, no toxic signs and symptoms / mortality was observed at the dose of 0.35 mg of purified Cry 1 C a 1 protein in aqueous buffer to each rat.

INTRODUCTION

This study was carried out to determine the acute toxicity of 'Cry 1 C a 1 Protein' in Wistar rats when the test substance was administered orally to the animals.

OBJECTIVES

(a) To determine the acute oral toxicity of novel protein using a single dose toxicity method on minimum number of animals.

(b) This study provides information on possible health hazards likely to arise from dietary exposure to a novel protein.

TEST SUBSTANCE DETAILS

The sponsor is responsible for necessary evaluations of the test substance concerning the chemical purity, identity, stability and other required data. The details of the test substance provided by the Sponsor are:

Product name	:	Protein
Manufacturer and Supplier	:	Metahelix Life Sciences (P) Limited, Bangalore
Physical State	:	Liquid
Odour and appearance	:	No odour, Colorless
Solubility	:	Water

EXPERIMENTAL DESIGN

Name of species	:	***Rattus rattus albanicus***
Strain of the animals	:	Wistar
No. of animals used per group	:	5 male & 5 female
Weight range	:	160-180 gm
Age at start of treatment	:	8 to 9 weeks
Room No.	:	103
Route of administration	:	Oral
Vehicle used	:	Aqueous Buffer
Date of initiation of study	:	17.03.2009
Date of initiation of experiment	:	17.03.2009
Date of dosing	:	19.03.2009
Date of completion of experiment	:	02.04.2009
Date of completion of study	:	15.04.2009

IDENTIFICATION OF ANIMALS

Each cage was tagged having the description of study number, test substance code, dose, animal number, cage number, date of initiation and date of completion of the experiment.

The animals were also marked with the help of picric acid.

HUSBANDRY

All animals were randomly selected and caged in a group of 5 according to sex in Polypropylene cages fitted with wire mesh tops and having sterilized paddy husk bedding. The room temperature was maintained at 22 ± 3° C with 30 - 70 % relative humidity.

The room was ventilated at the rate of approximately 15 air changes per hour.

Lighting was controlled to give 12 hours artificial light (8 a.m. - 8 p.m.) each day.

DIET

Water and standard pelleted feed (Amrut Feeds Ltd.) was freely available to the experimental animals. There was no known contaminants in the feed & water at levels that would have interfered with the experiment results, obtained.

ACCLIMATIZATION

A minimum of 5 days acclimatization was allowed before the commencement of the study.

EXPERIMENTAL PROCEDURE

METHOD OF ADMINISTRATION

The animals were fasted overnight prior to dosing and for 3-4 hours after dosing. A batch consisting of 5 male & 5 female rats was administered with the single dose of the test protein orally at the dose level of 0.35 mg/rat (a limit test using a single dose equivalent to 10X of the estimated dietary exposure of the test protein) with the help of metallic cannula attached with tuberculin syringe. Similarly control groupI was administered with aqueous buffer and control group II was administered with vehicle i.e. Distilled water only.

Frequency of administration

The test protein was administered once only following overnight fasting.

STATISTICAL ANALYSIS

Parameters such as body weight changes were tabulated and calculated by student's 'T'- test

SACRIFICE AND NECROPSY

All the experimental animals were subjected to necropsy. All findings were recorded.

OBSERVATIONS

All experimental (treated and control) animals were observed for 14 days (Table-2). Observations were made at least once during first 30 minutes with special attention during first 4 hours on the day of dosing and daily thereafter for the total of 14 days or until reversible toxic signs subsided.

All gross visible toxic signs and symptoms were recorded (Table-3-5).

Body weights were recorded prior to dosing and weekly thereafter (Tables-6-8).

Feed consumption were recorded weekly (Table-9).

INSTRUMENTS USED

1. Sartorious Balance no. SRI / TOX / ANI / 039 for animal weighing.

TABLE - 1
TOXIC SIGN & SYMPTOMS

Dose Groups	Mortality		Toxic Signs/ Symptoms
	Male	Female	
Treatment I	0/5	0/5	No treatment related toxic sign & Symptoms/ Mortality was observed
Treatment II	0/5	0/5	No toxic sign & Symptoms/ Mortality was observed
Treatment III	0/5	0/5	No toxic sign & Symptoms/ Mortality was observed

RESULT

Under the conditions of the study, no toxic sign and symptoms / mortality was observed in any of the animals at the dose of 0.35 mg of purified Cry 1 C a 1 protein in aqueous buffer to each rat.

The compound has been tested as per 'DBT Guidelines ' for non-clinical laboratory studies.

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TABLE - 2
MORTALITY DATA

Dose	Time of Death (Days)														Cumulative Mortality
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
TREATMENT I															
MALE															
0.35 mg protein/rat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
FEMALE															
0.35 mg protein/rat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
TREATMENT II															
MALE															
Aqueous Buffer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
FEMALE															
Aqueous Buffer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
TREATMENT III															
MALE															
Distilled water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
FEMALE															
Distilled water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

TABLE - 3

SUMMARY OF OBSERVATIONS OF TREATMENT I GROUP (Test Protein)

Animal No.	Sex	Dose mg/Rat	Clinical Observations	Necropsy Observations
1	M	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
2	M	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
3	M	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
4	M	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
5	M	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
6	F	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
7	F	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
8	F	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
9	F	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
10	F	0.35	No treatment related toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.

TABLE - 4

SUMMARY OF OBSERVATIONS OF TREATMENT II GROUP (Aqueous Buffer)

Animal No.	Sex	Dose mg/Rat	Clinical Observations	Necropsy Observations
11	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
12	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
13	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
14	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
15	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
16	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
17	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
18	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
19	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
20	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.

TABLE - 5

SUMMARY OF OBSERVATIONS OF TREATMENT III GROUP (Distilled water)

Animal No.	Sex	Dose mg/Rat	Clinical Observations	Necropsy Observations
21	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
22	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
23	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
24	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
25	M	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
26	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
27	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
28	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
29	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.
30	F	0	No toxic sign & symptoms / mortality was noticed	No noteworthy finding was observed in the animal, which was sacrificed on day 15th after dosing.

TABLE – 6

MEAN BODY WEIGHT DATA OF TREATMENT I GROUP (Test protein)

Animal No.	Sex	Dose mg/Rat	Weight in gram		
			Day 1st	Day 7th	Day 14th
1	M	0.35	165	169	175
2	M	0.35	161	166	171
3	M	0.35	169	173	178
4	M	0.35	162	167	172
5	M	0.35	170	175	179
Mean ± S.D.			**165.40 ± 4.04**	**170.00 ± 3.87**	**175.00 ± 3.54**
6	F	0.35	170	176	180
7	F	0.35	164	168	174
8	F	0.35	160	166	170
9	F	0.35	168	172	179
10	F	0.35	165	170	174
Mean ± S.D.			**165.50 ± 3.85**	**170.40 ± 3.84**	**175.40 ± 4.09**

TABLE – 7

MEAN BODY WEIGHT DATA OF TREATMENT II GROUP (Aqueous Buffer)

Animal No.	Sex	Dose mg/Rat	Weight in gram		
			Day 1st	Day 7th	Day 14th
11	M	0	165	169	177
12	M	0	170	177	182
13	M	0	169	174	180
14	M	0	162	167	172
15	M	0	167	175	180
Mean ± S.D.			**166.60 ± 3.21**	**172.40 ± 4.22**	**178.20 ± 3.90**
16	F	0	171	176	182
17	F	0	164	170	175
18	F	0	168	173	178
19	F	0	170	176	181
20	F	0	165	169	175
Mean ± S.D.			**167.60 ± 3.05**	**172.80 ± 3.27**	**178.26 ± 3.27**

TABLE – 8

MEAN BODY WEIGHT DATA OF TREATMENT III GROUP (Distilled Water)

Animal No.	Sex	Dose mg/Rat	Weight in gram		
			Day 1st	Day 7th	Day 14th
21	M	0	171	175	180
22	M	0	165	169	175
23	M	0	169	175	180
24	M	0	164	169	175
25	M	0	160	165	172
Mean ± S.D.			**165.80 ± 4.32**	**170.60 ± 4.34**	**176.40 ± 3.51**
26	F	0	166	170	175
27	F	0	169	172	178
28	F	0	162	167	172
29	F	0	168	173	180
30	F	0	170	175	179
Mean ± S.D.			**167.00 ± 3.16**	**171.40 ± 3.05**	**176.80 ± 3.27**

TABLE - 9.1

WEEKLY FEED CONSUMPTION DATA OF MALE AND FEMALE RATS

Dose Levels	MALES (Mean ± SD)		FEMALES (Mean ± SD)	
	DAY 7	DAY 14	DAY 7	DAY 14
Treatment I	76.87 ± 0.89	77.66 ± 1.16	73.79 ± 1.73	71.96 ± 1.20
Treatment II	78.01 ± 1.11	77.84 ± 1.01	77.79 ± 1.03	76.83 ± 1.31
Treatment III	76.94 ± 1.50	76.86 ± 1.31	71.70 ± 0.84	71.76 ± 1.59

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TABLE - 9.2
FEED CONSUMPTION DATA OF MALE RATS

DAYS	TOTAL FEED WT. (In gms.)	Treatment I		Treatment II		Treatment III	
		Remai-ning feed (gms)	Feed consu-med (gms)	Remai-ning feed (gms)	Feed consu-med (gms)	Remai-ning feed (gms)	Feed consu-med (gms)
1	100	21.5	78.5	21.2	78.8	20.1	79.9
2	100	22.6	77.4	22.4	77.6	22.6	77.4
3	100	22.8	77.2	20.6	79.4	23.5	76.5
4	100	23.5	76.5	20.8	79.2	23.6	76.4
5	100	24.1	75.9	22.6	77.4	23.0	77.0
6	100	23.6	76.4	23.5	76.5	23.6	76.4
7	100	23.8	76.2	22.8	77.2	25.0	75.0
8	100	23.7	76.3	22.0	78.0	24.8	75.2
9	100	23.5	76.5	22.8	77.2	24.2	75.8
10	100	21.6	78.4	22.0	78.0	23.3	76.7
11	100	21.5	78.5	23.0	77.0	23.6	76.4
12	100	21.0	79.0	23.5	76.5	23.2	76.8
13	100	21.6	78.4	21.0	79.0	21.0	79.0
14	100	23.5	76.5	20.8	79.2	21.9	78.1

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TABLE - 9.3
FEED CONSUMPTION DATA OF FEMALE RATS

DAYS	TOTAL FEED WT. (In gms.)	Treatment I		Treatment II		Treatment III	
		Remai-ning feed (gms)	Feed consu-med (gms)	Remai-ning feed (gms)	Feed consu-med (gms)	Remai-ning feed (gms)	Feed consu-med (gms)
1	100	23.7	76.3	21.5	78.5	27.4	72.6
2	100	24.6	75.4	22.6	77.4	28.2	71.3
3	100	28.8	71.2	23.2	76.8	29.3	70.7
4	100	27.7	72.3	21.2	78.9	29.4	70.6
5	100	26.5	73.5	21.0	79.0	28.2	71.8
6	100	26.2	73.8	22.5	77.5	28.4	71.6
7	100	26.0	74.0	23.6	76.4	27.2	72.8
8	100	27.7	72.3	24.3	75.5	26.9	73.1
9	100	29.0	71.0	25.2	74.8	25.4	74.6
10	100	30.2	69.8	22.6	77.4	28.6	71.4
11	100	27.8	72.2	21.4	78.6	28.6	71.4
12	100	26.9	73.1	22.5	77.5	29.2	70.8
13	100	26.8	73.2	23.5	76.5	28.8	71.2
14	100	27.9	72.1	22.5	77.5	30.2	69.8