12-JUL-2006
PROJECT NO.12203

STATEMENT OF COMPLIANCE

Project No. : 12203

Test Substance : **Oxy Powder**

Study Title : Acute Oral Toxicity of **Oxy Powder** in Sprague Dawley Rats

We hereby attest to the authenticity of the study and guarantee that the data is correct and accurate to the best of our knowledge and that the study was performed by the procedure described in the Indian Institute of Toxicology Standard Operating Procedures for the testing of chemicals. We hereby attest that this study was conducted in compliance with Protocol submitted to and approved by the sponsor.

This study was performed in full compliance with the OECD Guideline for the Testing of Chemicals (No. 420, Section 4: Health Effects) "Acute Oral Toxicity - Fixed Dose Method" Adopted on 17th December 2001, Schedule "Y" in drug and cosmetics (Eighth Amendment) Rules 1988, Ministry of Health and Family Welfare, Government of India and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Indian Institute of Toxicology Registration No.15/1999/CPCSEA).

Dr. R.M.Bhide Ph.D.		12/7/2006
Study Director	Signature	Date
Dr. P.R.Tikhe Ph.D.	Tikhe	12-7-06
Quality Assurance Unit	Signature	Date
Mr. V.M.Bhide M.B.A.	VBhide	12.07.2006
Director, Administration	Signature	Date

Data Requirements

OECD Guidelines,
Section 4, Test No.420,
17th December, 2001.

Dr.R.M.Bhide Ph.D.
Study Director

Testing Facility
Indian Institute of Toxicology
32/A/1, Hadapsar Industrial Estate,
Pune - 411 013.
India.

Sponsor

Mayfair Clinical Education and Research Centre, Mumbai

12-JUL-2006
PROJECT NO.12203

STATEMENT OF QUALITY ASSURANCE UNIT

Project No. : 12203

Test Substance : **Oxy Powder**

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Quality Assurance Unit of the testing facility inspected the conduct of study on the following dates :

22/04/2006, Test substance preparation
27/04/2006, Clinical observation

Raw data audit - 15/05/2006

Final report audit - 12/07/2006

No inspection led to findings which had to be reported to the management or would have impaired this study in any way.

Dr. P.R.Tikhe Ph.D.

Quality Assurance Unit | Signature | 12-7-06 Date

12-JUL-2006
PROJECT NO.12203

ARCHIVING

Project No. : 12203

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Indian Institute of Toxicology takes the responsibility of archiving Protocol, Raw data and all the relevant material generated during the study along with a copy of final report for a period of two years.

Mr. V.M.Bhide M.B.A. — Director, Administration

Signature

12.07.2006
Date

PERSONNEL INVOLVED IN THE STUDY

Study Director	: Dr. R.M.Bhide Ph.D.
Head Administration	: Mr. V.M.Bhide M.B.A.
Animal Care	: Dr. S.D.Bhande B.V.Sc.
Technician	: Mr. D.G.Shirsath B.Sc., Applied Toxicology
Quality Assurance Audit	: Dr. P.R.Tikhe Ph.D.

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SUMMARY AND CONCLUSION

The study now reported was designed to determine the acute oral toxicity profile of **Oxy Powder** in Sprague Dawley rats.

The sighting study did not result in any signs of intoxication at the dose level of 2000 mg/kg body weight and the animal survived, therefore, one animal was treated with the higher dose of 5000 mg/kg body weight. No signs of intoxication were observed in animals treated at the dose level of 5000 mg/kg body weight. Therefore the main study was continued at the dose level of 5000 mg/kg body weight.

The main study did not result in any signs of intoxication at the dose level of 5000 mg/kg body weight. All animals survived through the study period of 14 days.

Gross pathological examination did not reveal any abnormalities.

It was concluded that the acute toxicity study of **Oxy Powder** supplied by **Mayfair Clinical Education and Research Centre, Mumbai,** when administered via oral route in Sprague Dawley rats falls into the category 5 criteria of Globally Harmonised System (GHS).

DR.R.M.BHIDE
STUDY DIRECTOR

STUDY DIRECTOR

PREFACE

General

Study Title	: Acute Oral Toxicity of **Oxy Powder** in Sprague Dawley Rats
Sponsor	: **Mayfair Clinical Education and Research Centre, Mumbai**
Monitoring Scientist	: **Dr. J. K. Lalla**
Testing Facility	: Indian Institute of Toxicology, 32/A/1, Hadapsar Industrial Estate, Pune - 411 013.
Project No.	: 12203

Schedule

Sighting study :

Date of treatment	: 20/04/2006 and 22/04/2006
Date of termination	: 04/05/2006 and 06/05/2006

Main study :

Date of treatment	: 24/04/2006
Date of termination	: 08/05/2006
Date of reporting	: 12/07/2006

Guidelines

This study was performed in full compliance with the OECD Guideline for the Testing of Chemicals (No. 420, Section 4: Health Effects) "Acute Oral Toxicity - Fixed Dose Method" Adopted on 17th December 2001.

Schedule "Y" in drug and cosmetics (Eighth Amendment) Rules 1988, Ministry of Health and Family Welfare, Government of India.

OBJECTIVES

The purpose of this study is to assess the Toxicological profile of **Oxy Powder** to a single administration via oral route to Sprague Dawley rats. The animals were observed for 14 days or more, depending on the occurrence of toxic symptoms. The results of acute toxicity study were useful for selection of doses for repeated dose toxicity study and may also provide preliminary information on the target organ of toxicity.

MATERIALS AND METHODS

TEST SUBSTANCE

Sponsor	: **Mayfair Clinical Education and Research Centre, Mumbai**
Label on Sample	: **Oxy Powder**
Characteristics of Sample	: Consistency - Solid (Capsule) Colour - White

Disclaimer :

The above physiochemical data of test substance is supplied by the Sponsor. All responsibility with regards to the accuracy and authenticity of this information remains with the Sponsor. The test lab is not responsible for any variations with the batch number supplied.

Preparation of Dose

Sighting study :

Dose : Treatment - Female - 2000 mg/kg body weight
- 5000 mg/kg body weight

Main study :

Dose : Treatment - Female - 5000 mg/kg body weight

Dose volume : 10 ml/kg.

Vehicle : Distilled water

Procedure : The test substance was suspended in distilled water to obtain 200.0 mg/ml and 500.0 mg/ml strength of suspensions. The test substance was administered in the dose volume of 10 ml/kg body weight.

The formulation was prepared fresh on the day of dosing.

TEST SYSTEM

Species	: Rat
Strain	: Sprague Dawley
Source	: I.I.T. Animal house
Sex	: Female
Age	: 5 to 8 weeks
No. of animals per dose	: Sighting study : One and Main study : Four
Acclimation	: Five days prior to dosing.
Veterinary examination	: Before allocation of animals to different doses after the completion of acclimation period.
Identification of animals	: By cage number and individual marking on fur.
Diet	: Pelleted feed *ad libitum* supplied by Nav Maharashtra Chakan Oil Mills Ltd., Pune
Water	: Aquaguard pure water in glass bottles *ad libitum*
Housing & Environment	: Sighting study : One animal per polypropylene cages provided with bedding of husk. Main study : Maximum 5 animals per polypropylene cages provided with bedding of husk. The temperature was maintained between 20 & 24 °C and relative humidity between 30 and 70%; 10-15 air changes per hour and 12 hours each of dark and light cycle was maintained.

Rationale for Selection of Sprague Dawley Rat as Test System

1) One of the recommended rodent species by the regulatory authorities for conducting preclinical toxicity studies among rodents, as it is a sensitive species for expression of toxic responses.

2) Rat is recommended rodent species for conducting acute toxicity studies as per OECD guidelines.
3) Availability of the historical control data at the facility.

Route of Administration and Reason for Choice

Oral route of administration is the proposed therapeutic route of administration in human being.

Justification for Selection of Doses

Sighting study :

Dose (mg/kg body weight)	No. of animals	Mortality
2000	1	0/1
5000	1	0/1

Results : Based on the sighting study following dose was selected for the main study.

Main study :

Dose (mg/kg body weight)	No. of animals
5000	4

Randomization and Numbering of Animals

Eleven healthy female rats, acclimatized to laboratory conditions for 5 days prior to dosing, were used in this study. Animals were randomly assigned to the cages and the individual animal was fur marked with picric acid. The females were nulliparous and nonpregnant.

Preparation of Animals

The rats were deprived of feed for 16 hours before and 3 hours after the administration of the test substance. Water was not withheld during this period.

EXPERIMENTAL PROCEDURE

The test substance, suspended in distilled water was administered by gavage to rats using a ball-tipped intubation needle (18 G) fitted on to a syringe as per SOP on Test

Article/Substance (TA/S) administration - Gavage/Intubation, (Sop No.IIT/S-PSC/16.2).

Allocation of animals.

Sighting study :

Species/ strain	Group No.	Animal Nos. Female	Dose (mg/kg)	Concentration (mg/ml)	Route
Rats/Sprague Dawley	I	1	2000	200	Oral
	II	1	5000	500	

Main study :

Species/ strain	Group No.	Animal Nos. Female	Dose (mg/kg)	Concentration (mg/ml)	Route
Rats/Sprague Dawley	I	4	5000	500	Oral

OBSERVATIONS

Clinical Signs of Intoxication

Observations of clinical signs were made at 10 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours and 6 hours after dosing on day 1 and once daily thereafter for 14 days at approximately same time.

Cageside observations included changes in the skin, fur, eyes and mucous membrane. It also included respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavioral pattern. Particular attention was directed to the observation of tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma.

Mortality

Twice daily.

Body Weight

Individual animal body weight were recorded following the period of fasting on day 0, weekly thereafter and at termination on day 15. Changes in body weights were calculated and recorded.

Gross Pathology

Macroscopic examination was performed on animals found dead and animals sacrificed at the end of the observation period of 14 days.

Histopathology

Necropsy examination did not reveal any gross abnormality hence histopathological examination was not carried out.

RESULTS

Clinical Signs of Intoxication and Mortality (Table No.A, B & App. No.I, II)

Sighting study :

Group I - Animal treated at the dose level of 2000 mg/kg body weight did not result in any signs of intoxication during the study period of 14 days. Animal survived through the study period of 14 days.

Group II - Animal treated at the dose level of 5000 mg/kg body weight did not result in any signs of intoxication during the study period of 14 days. Animal survived through the study period of 14 days.

Main Study:

Group I -Animals treated at the dose level of 5000 mg/kg body weight did not result in any signs of intoxication during the study period of 14 days. All animals survived through the study period of 14 days.

Body Weight (Table No.C & App.No.III)

Sighting study :

Group I (2000 mg/kg) - Percent body weight gain after 7 days and 14 days was found to be 14.46% and 32.93% respectively.

Group II (5000 mg/kg) – Percent body weight gain after 7 days and 14 days was found to be 18.77% and 36.19% respectively.

Main study :

Group I (5000 mg/kg) - Percent body weight gain after 7 days and 14 days was found to be 15.41% and 30.88% respectively.

Gross Pathology Findings (Table No.D & App.IV)

Macroscopic examination of animals sacrificed at termination revealed no abnormalities.

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Table No. A

Summary of Mortality Record

Under the conditions of the present study, the following mortality rates were recorded:

Sighting study :

Group No.	Dose (mg/kg body weight)	Mortality	
		Absolute	Relative %
I	2000	0/1	0
II	5000	0/1	0

Main study :

Group No.	Dose (mg/kg body weight)	Mortality	
		Absolute	Relative %
I	5000	0/4	0

Table No.B

Summary of Clinical Signs of Intoxication

Sighting study :

Group No.	Dose mg/kg	Observed Signs	Total Number of Animals	Animal Nos.	Period of signs in days From - to
I	2000	Nil	1	1	1 - 14
II	5000	Nil	1	1	1 - 14

Main study :

Group	Dose		Total		Period of signs in

No.	mg/kg	Observed Signs	Number of Animals	Animal Nos.	days from - to
I	5000	Nil	4	2 - 5	1 - 14

Table No.C

Mean Body Weight and Percent Body Weight Gain

Sighting study :

Group No.	Dose (mg/kg body weight)	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
I	2000	124.50	142.50	14.46	165.50	16.14	32.93
II	5000	133.20	158.20	18.77	181.40	14.66	36.19

Main study :

Group No.	Dose (mg/kg body weight)	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
I	5000	130.08	150.00	15.41	170.05	13.39	30.88

Table No.D

Summary of Gross Pathology Findings

Sighting study :

Site and lesion observed	Group	I	II
NAD		1	1

Main study :

Site and lesion observed	Group	I
NAD		2 – 5

NAD = No Abnormality Detected

Appendix No.I

Individual Animal - Mortality Record

Sighting study :

Group No.	Dose mg/kg	Animal No.	Mortality	
			Absolute	Relative %
I	2000	1	0	0
II	5000	1	0	0

Main study :

Group No.	Dose mg/kg	Animal No.	Mortality	
			Absolute	Relative %
I	5000	2	0	0
		3	0	0
		4	0	0
		5	0	0

Appendix No.II

Individual Animal - Clinical Signs of Intoxication

Sighting study:

Group No.	Dose mg/kg	Animal No.	Observed Signs	Period of signs in days from - to
I	2000	1	Nil	1 - 14
II	5000	1	Nil	1 - 14

Main study:

Group No.	Dose mg/kg	Animal No.	Observed Signs	Period of signs in days from - to
I	5000	2	Nil	1 - 14
		3	Nil	1 - 14
		4	Nil	1 - 14
		5	Nil	1 - 14

Appendix No.III

Individual Animal - Body Weight and Percent Body Weight Gain

Sighting study :

Group : I

Dose : 2000 mg/kg body weight

Animal No.	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
1	124.5	142.5	14.46	165.5	16.14	32.93

Sighting study :

Group : II

Dose : 5000 mg/kg body weight

Animal No.	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
1	133.2	158.2	18.77	181.4	14.66	36.19

Appendix No. III (Contd.)

Individual Animal - Body Weight and Percent Body Weight Gain

Main study :

Group : I

Dose : 5000 mg/kg body weight

Animal No.	Body weight Day 1	Body weight Day 7	% body weight gain day 1-7	Body weight Day 14	% body weight gain day 7- 14	% body weight gain day 1- 14
2	137.8	155.3	12.70	174.8	12.56	26.85
3	125.4	146.9	17.15	168.0	14.36	33.97
4	133.0	151.2	13.68	170.0	12.43	27.82
5	124.1	146.6	18.13	167.4	14.19	34.89

Appendix No. IV

Individual Animal - Gross Pathology Findings

Sighting study :

Group : I

Dose : 2000 mg/kg body weight

Animal No.	Fate	Gross Pathology Findings
1	TS	NAD

Group : II

Dose : 5000 mg/kg body weight

Animal No.	Fate	Gross Pathology Findings
1	TS	NAD

TS = Terminal sacrifice
NAD = No abnormality detected

Appendix No.IV (Contd.)

Individual Animal - Gross Pathology Findings

Main study :

Group : I

Dose : 5000 mg/kg body weight

Animal No.	Fate	Gross Pathology Findings
2	TS	NAD
3	TS	NAD
4	TS	NAD
5	TS	NAD

TS = Terminal sacrifice
NAD = No abnormality detected