Oxy-Powder®

28-Day Safety Study

Sub-Chronic Toxicity Report

SUMMARY AND CONCLUSION

The Sub-Chronic oral toxicity study was designed and conducted to determine the toxicity profile of **Oxy Powder** when administered daily for 28 days in Sprague Dawley rats. In the acute toxicity test the compound was found to be non toxic at the dose level of 5000 mg/kg body weight. The dose has been selected on this basis and the justification provided on page 14 of this report.

Oxy Powder suspended in distilled water was administered to animals at the dose levels 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight. Two additional dose levels were added to the study as 0 mg/kg (Rev.) and 1000 mg/kg (Rev.), in order to study the reversibility or delayed occurrence of symptoms, if any. The control animals were administered with vehicle only.

Salient features of the study were as follows:

1) All the male and female animals from control and all the treated dose groups up to 1000 mg/kg survived throughout the dosing period of 28 days and the recovery period of 14 days.
2) No signs of intoxication were observed in male and female animals from different dose groups during the dosing period of 28 days and during the recovery period of 14 days.
3) Male and female animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days and the recovery period of 14 days.
4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days and the recovery period of 14 days.

5) Ophthalmoscopic examination, conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality.

6) Hematological analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment.

7) Biochemical analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment.

8) Functional battery observation tests conducted at termination revealed no abnormalities.

9) Urine analysis, conducted at the end of the dosing period in week 4 and at the end of recovery period in week 6, revealed no abnormality attributable to the treatment.

10) Organ weight data of male and female sacrificed at the end of the dosing period and at the end of the recovery period was found to be comparable with that of respective controls.

11) Gross pathological examination did not reveal any abnormality.

12) Histopathological examination did not reveal any abnormality.

Based on these findings the no observed effect level (NOEL) of **Oxy Powder** supplied by **Mayfair Clinical Education and Research Centre, Mumbai**, in Sprague Dawley rat via oral route, over a period of 28 days was found to be 1000 mg/kg body weight for male and female animals.

DR. R.M.BHIDE

STUDY DIRECTOR

PROJECT NO.12204

SUBCHRONIC ORAL TOXICITY STUDY (28 DAY)

OF OXY POWDER

IN THE SPRAGUE DAWLEY RAT

STUDY DIRECTOR
DR. R.M.BHIDE Ph.D.

TESTING FACILITY
INDIAN INSTITUTE OF TOXICOLOGY,
32/A/1, HADAPSAR INDUSTRIAL ESTATE,
PUNE - 411 013, INDIA.

DATA REQUIREMENTS
OECD GUIDELINE,
SECTION 4, TEST NO.408,
21 SEPTEMBER, 1998.

SPONSOR

MAYFAIR CLINICAL EDUCATION AND RESEARCH CENTRE,

MUMBAI

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STATEMENT OF COMPLIANCE

Project No. : 12204

Test Substance : **Oxy Powder**

Study Title : Subchronic Oral Toxicity Study (28 day) of Oxy Powder in the Sprague Dawley Rat

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. We hereby attest that this study was conducted in compliance with Protocol submitted to and approved by the sponsor.

The study also complies with the Schedule Y in Drugs and Cosmetic Act (IInd Amendment) Rules, 2005, Ministry of Health and Family Welfare, Government of

India, OECD Guideline for the testing of Chemicals No.408, "Repeated Dose 28 -day Oral Toxicity Study in Rodents" adopted on September 21st, 1998 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.**	_____	_____
Study Director	Signature	Date

Dr. P.R.Tikhe **Ph.D.**	_____	_____
Quality Assurance Unit	Signature	Date

Mr. V.M.Bhide **M.B.A.**	_____	_____
Director, Administration	Signature	Date

STATEMENT OF QUALITY ASSURANCE UNIT

Project No. : 12204

Test Substance : **Oxy Powder**

Study Title : Subchronic Oral Toxicity Study (28 day) of Oxy Powder in the Sprague Dawley Rat

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

10-05-2006,	Body weight data collection
11-05-2006,	Test substance administration
25-05-2006,	Food consumption data collection
08-06-2006,	Necropsy
22-06-2006,	Haematology technique

Raw data audit : 08-07-2006

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	Date

ARCHIVING

Project No.	: 12204
Test Substance	: **Oxy Powder**
Study Title	: Subchronic Oral Toxicity Study (28 day) of Oxy Powder in the Sprague Dawley Rat

Indian Institute of Toxicology takes the responsibility of archiving the following items for a period of two years. Protocol, raw data and a copy of final report, Wet tissue samples, histology slides and blocks.

Mr. V.M.Bhide **M.B.A.**

Director, Administration	Signature	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. & A.H.
Histopathology	: Dr. S.K.Bokan M.V.Sc.
Technician	: Dr. V.S.Jagadale M.V.Sc.

Biochemistry	: Mr. N.G.Bidkar B.Sc.
Histology	: Miss S.G.Tikhe B.Sc., D.M.L.T. Mr. S.N.Gaikwad B.Sc., D.M.L.T. Mrs. S.C.Gaikwad B.Sc., D.M.L.T.
Quality Assurance Audit	: Dr. P.R.Tikhe Ph.D.

PREFACE

General

Title	: Subchronic Oral Toxicity Study (28 day) of **Oxy Powder** in the Sprague Dawley Rat
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.	: 12204
Test Substance	: **Oxy Powder**

Schedule

Date of receipt of animals for treatment (day 0)	: 10/05/2006
Date of treatment (day 1)	: 11/05/2006
Date of termination of dosing (day 28)	: 07/06/2006
Date of sacrifice (day 29)	: 08/06/2006
Date of completion of recovery period 42 days	: 21/06/2006
Date of sacrifice 43 Day	: 22/06/2006
Date of reporting	: 12/07/2006

Objective

The objective of this study is to assess the toxicological profile of **Oxy Powder** after administering at three dose levels by oral route for 28 consecutive days to Sprague Dawley rats to determine the target organ of toxicity and no observed effect level

(NOEL). The study objective also includes the detection of delayed occurrence or reversibility of any signs / toxicity at the end of recovery period of 14 days.

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.

TEST SYSTEM

Species	: Rat
Strain	: Sprague Dawley
Source	: Indian Institute of Toxicology, Pune
Sex	: Male and female
Age	: 6 to 8 weeks
No. of animals per dose level	: 5 per sex (dose groups sacrificed on day 29) 5 per sex (reversal groups sacrificed on day 43)
Acclimation	: Seven days prior to dosing.
Veterinary examination	: Prior to and at the end of the acclimation period.
Identification of animals	: By cage number, animal number and individual marking on fur.
Diet	: Pelleted feed supplied by Nav Maharashtra Chakan Oil Mills Ltd., Pune
Water	: Aquaguard pure water in glass bottles *ad libitum*
Housing & Environment	: The animals were housed 5 each, of the same sex in 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.

Dose : Male
0 mg/kg, 0 mg/kg (Rev.), 250 mg/kg, 500 mg/kg, 1000 mg/kg and 1000 mg/kg (Rev.) body weight

: Female
0 mg/kg, 0 mg/kg (Rev.), 250 mg/kg, 500 mg/kg, 1000 mg/kg and 1000 mg/kg (Rev.) body weight

Body weight at start of Study

Male	Mean	: 96.66 g (= 100 %)
	Minimum	: 91.5 g (- 5.34 %)
	Maximum	: 100.5 g (+ 3.97 %)
	Total No. of animals	: 30
Female	Mean	: 87.96 g (= 100 %)
	Minimum	: 81.7 g (- 7.12 %)
	Maximum	: 97.3 g (+ 10.62 %)
	Total No. of animals	: 30

METHODS

Randomization, Numbering and Grouping of Animals

Sixty rats i.e. 30 male and 30 female healthy animals were randomly divided into four groups of 5 animals per sex for dosing up to 28 days and 5 animals per sex as reversal groups for control and high dose i.e. 0 mg/kg, 0 mg/kg (Rev.) 250 mg/kg, 500 mg/kg, 1000 mg/kg and 1000 mg/kg (Rev.) body weight. Animals were allowed acclimation period of 7 days to laboratory conditions prior to the initiation of treatment. Rats were assigned five per sex per cage wise and the individual animal was fur marked with picric acid. The females were nulliparous and nonpregnant. Randomization was conducted as per the Indian Institute of Toxicology Standard Operating Procedure on Randomization of Study Animals (SOP No.IIT/S-PSC/13).

Route of Administration

Oral (gavage), once daily for 28 consecutive days.

Justification for Selection of Sprague Dawley rats for the Study

1) One of the rodent species recommended as test system for the use in toxicity studies,

2) This test system has been demonstrated to be sensitive to toxins,

3) Widely used throughout industry for the evaluation of toxicity of various products,

4) Historical data and evidence at the facility.

Justification for Selection of Route

The oral route was selected for use because,

Oral route is considered to be a proposed therapeutic route.

Dose Preparation

Oxy Powder suspended in distilled water was administered to animals at the dose levels of 250 mg/kg, 500 mg/kg and 1000 mg/kg in the dose volume of 10 ml/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only.

Justification for Dose Selection

In acute toxicity test the compound was found to be non toxic at the dose level of 5000 mg/kg body weight. The doses selected for the study were 0 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight.

OBSERVATIONS

Clinical Signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during the period of the study.

Body Weight

The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study and at termination to calculate relative organ weights. The group mean body weights and percent body weight gain were calculated.

Food Consumption

The quantity of food consumed by groups consisting of five animals of each sex (or ten animals, 0 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg) and five animals of each sex (or ten animals, 0 mg/kg Rev. and 1000 mg/kg Rev.) was recorded weekly and the food consumption per animal was calculated for control and all the treated dose groups.

Ophthalmoscopy

The eyes of control and all the treated dose group animals were examined prior to the initiation of the dosing and in week 4 and in week 6 (for reversal group animals) of the study. Eye examination was carried out using a hand slit lamp after induction of mydriasis with 0.5% solution of tropicamide.

Functional Observations

Towards the end of the exposure period in week 4, sensory reactivity to stimuli of different types (auditory, visual and proprioceptive stimuli) and by grading different stimuli according to the Standard Operating Procedure for 'Conduct of functional observational battery' No.IIT/S-PSC/36, assessment of grip strength (Digital Grip Strength Meter, using Columbus Instrument) and motor activity assessment was conducted.

TERMINAL STUDIES

Laboratory Investigations

Following laboratory investigations were carried out on day 29 and on day 43, in animals fasted over-night. Blood samples were collected from orbital sinus following morning using sodium heparin (200 IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Prothrombin time analysis was conducted using citrate bulb (100 µl of 3.8% solution of sodium citrate for 1 ml of blood). Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Hematological Investigations

Following hematological parameters were studied using Beckman Coulter haematology analyzer.

Hb : Hemoglobin (g%)
RBC : Red Blood Corpuscles (x 10^6 /cmm)
HCT : Hematocrit (%)
MCV : Mean Corpuscular Volume (μm^3)
MCH : Mean Corpuscular Hemoglobin (pg)
MCHC : Mean Corpuscular Hemoglobin Concentration (%)
Platelets (x 10^3 /uL)
WBC : White Blood Corpuscles (x 10^3 /uL)

Analysis of the following parameters were performed manually:

Rt. : Reticulocyte (%)
N : Neutrophils (%)
L : Lymphocytes (%)
E : Eosinophils (%)
M : Monocytes(%)
B : Basophil (%)
Pt. : Prothrombin time (Sec.)

Biochemical Investigations

Following biochemical parameters were studied using VeTEX (Veterinary Chemistry Expert) Clinical Chemistry autoanalyser system.

Total Protein (g%)
BUN : Blood Urea Nitrogen (mg%)
ALT : Alanine Aminotransferase (IU/L)
AST : Aspartate Aminotransferase (IU/L)
AP : Alkaline Phosphatase (IU/L)
Blood Sugar (mg%)
Calcium (mg %)
Phosphorous (mg %)
γ GT : Gamma Glutamyl Transferase (U/L)
Bilirubin (mg %)
Albumin (g %)
Creatinine (mg %)
CPK : Creatine Phospho Kinase (IU/L)
Sodium (mmol/l)
Potassium (mmol/l)
Chloride (mmol/l)
Cholesterol (mg %)
Triglycerides (mg %)
LDH : Lactate De-Hydrogenase (IU/L)

Urine Analysis

Urine samples were collected in week 4 and in week 6 and the following parameters were studied.

The following estimations were performed using appropriate methodology as discussed below :

Volume (urine volume was collected from all male and female animals from each dose group using metabolic cages and collection duration was fixed at 16 hours.)
Appearance
Colour
pH = Multistix
Specific Gravity = Multistix
Proteins = Multistix
Glucose = Multistix
Ketones = Multistix
Bilirubin = Multistix
Urobilinogen = Multistix
Occult Blood = Multistix
Nitrite = Multistix

Results are reported according to the following convention :

Absent	= 0
Trace	= +
Small amount of analyte	= ++
Moderate amount of analyte	= +++
Large amount of analyte	= ++++

Microscopy

Urine samples were centrifuged at 1000 r.p.m. for 10 minutes.

P : Pus cells
E : Epithelial cells
C : Casts
R : RBC
Cr : Crystals

The presence and approximate frequency of these constituents are reported according to the following convention :

Grade	Description
0	None found in any field
1	Few found in some field
2	Few found in many field
3	Many found in many field

Necropsy

All the animals were sacrificed on day 29 except for reversal group animals which were sacrificed on day 43 i.e. post-dosing period of 14 days, using CO_2 asphyxiation technique (body weights mentioned in the table section are fasting body weights, Appendix No.IX and X). Necropsy of all animals was carried out and the weights of the following organs were recorded: Liver, kidneys, adrenals, epididymis, thymus, spleen, brain, heart, uterus and testes/ovaries. The organ weights were recorded as absolute values and their relative values (i.e. per cent of the body weight) were calculated.

Histopathology

Following tissue samples of organs from control and animals treated at the highest dose level of 1000 mg/kg, were preserved in 10% formalin and were subjected to histopathological examination.Adrenals, Aorta, Brain, Caecum, Colon, Duodenum, Epididymis, Gonads, Heart, Ileum, Jejunum, Kidneys, Liver, Lungs, Lymphnodes, Oesophagus, Prostate, Rectum, Sciatic nerve, Spleen, Sternum with bone marrow, Stomach, Seminal Vesicles, Spinal cord, Thymus, Thyroid / parathyroid, Trachea, Urinary Bladder and Uterus. Following tissue samples of organs from low and intermediate dose groups animals were preserved for histopathology examination.
Liver, kidneys, adrenals, epididymis, thymus, spleen, brain, heart, uterus and testes/ovaries.

Statistical Analysis

All findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were tabulated. Data on all parameters were evaluated by analysis (co-)variance followed by Student 't' test and Cochran 't' test. Histopathological observations were tabulated; evaluated and individual animal score is calculated according to degree and area by using software, LABCAT Module for Histopathology, Innovative Programming Associates, INC., Princeton, New Jersey.

RESULTS

Clinical Signs (Table B)

Male -

Group I (0 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.1 to 5).

Group II (0 mg/kg, Reversal) : Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.11 to 15).

Group III (250 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.21 to 25).

Group IV (500 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.31 to 35).

Group V (1000 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.41 to 45).

Group VI (1000 mg/kg, Reversal) : Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.51 to 55).

Female -

Group I (0 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.6 to 10).

Group II (0 mg/kg, Reversal) : Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.16 to 20).

Group III (250 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.26 to 30).

Group IV (500 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.36 to 40).

Group V (1000 mg/kg) : Animals were free of intoxicating signs throughout the dosing period of 28 days (animal nos.46 to 50).

Group VI (1000 mg/kg, Reversal) : Animals were free of intoxicating signs throughout the dosing period of 28 days and during the recovery period of 14 days (animal nos.56 to 60).

Mortality (Table C)

Male and Female -

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days and the post-dosing recovery period of 14 days.

Body Weight (Table D; App.I; Fig.I & II)

Male and Female -

Animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

During the post-dosing recovery period animals from 1000 mg/kg reversal group exhibited normal body weight gain when compared with that of respective controls.

Food Consumption (Table E; Fig.III & IV)

Male and Female -

During the dosing period and the post-dosing recovery period the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Ophthalmoscopy (Table F)

The eyes of control and all the treated dose group animals were examined prior to the initiation of the dosing and in week 4 and in week 6 (for reversal group animals) of the study. Ophthalmoscopic examination did not reveal any abnormality.

Functional Observations (Tables No.G; App. II, III, IV and V)

Functional observation tests conducted at termination revealed no abnormalities.

Hematological Investigations (Table H; App.VI)

Male and Female -

Hematological investigation's conducted on day 29 and on day 43 (for reversal group animals), revealed following significant changes in the values of different parameters studied when compared with that of respective controls, however the increase/decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Male: MCHC : Decreased values were obtained for animals from 500 mg/kg ($P<0.05$) and 1000 mg/kg ($P<0.01$) dose groups, sacrificed on day 29 and Total WBC: Increased values were obtained for animals from 500 mg/kg dose group, sacrificed on day 29 ($P<0.05$).

Female: MCV : Increased values were obtained for animals from 500 mg/kg dose group, sacrificed on day 29 ($P<0.05$) and MCV and MCH : Increased values were obtained for animals from 1000 mg/kg reversal group, sacrificed on day 43 ($P<0.05$).

Parameters	Laboratory range
MCV	44.5 to 69.0 (μm^3)
MCH	12.0 to 24.5 (pg)
MCHC	21.6 to 42.0 (%)
Total WBC	3.00 to 19.00 (x 10^3 /uL)

Biochemical Investigations (Table I; App.VII)

Male and Female -

Biochemical investigation's conducted on day 29 and on day 43 (for reversal group animals), revealed following significant changes in the values of different parameters studied when compared with that of respective controls, however, the values obtained were within normal biological and laboratory limits or the effect was not dose dependent.

Male: Blood Urea Nitrogen: Elevated levels were observed in animals from 500 mg/kg dose group, sacrificed on day 29 ($P<0.05$), LDH : Elevated levels were observed in animals from 1000 mg/kg dose group, sacrificed on day 29 ($P<0.05$) and Aspartate Aminotransferase: Decreased levels were observed in animals from 1000 mg/kg reversal group, sacrificed on day 43 ($P<0.01$).

Female: Alkaline Phosphatase: Elevated levels were observed in animals from 1000 mg/kg reversal group, sacrificed on day 43 ($P<0.05$).

Parameters	Laboratory range
Alkaline Phosphatase	50 to 80 (IU/L)
BUN	20 to 50 (mg%)
AST	30 to 70 (IU/L)
LDH	300 to 400 (IU/L)

Urine Analysis (Table J; App.VIII)

Male and Female -

Urine analysis of control and treated animals in week 4 and reversal group animals in week 6, revealed no abnormality.

Organ Weights (Table L, M; App.IX, X)

Male and Female –

In comparison with respective controls on day 29, organ weight data of animals from different dose groups was found to be comparable.

In comparison with respective controls on day 43, organ weight data of animals from 1000 mg/kg reversal group was found to be comparable.

Necropsy (Table M; App.XI)

Gross pathological examination did not reveal any abnormality.

Histopathology (Table N; App.XI)

Histopathological examination revealed focal lymphocytic infiltration and/or necrosis in liver, lymphocytic infiltration in the kidneys, focal lymphocytic infiltration in the heart, gliosis in the brain, interstitial pneumonitis in the lungs, eosinophillic infiltration in uterus were observed in few male and female animals from control and 1000 mg/kg dose group animals with similar quality and quantity and are considered incidental, gender and physiology related and are covered in the background data of the pathology.