FLAIR LABS

FACILITY FOR LABORATORY ANIMAL AND INVIVO RESEARCH

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FINAL REPORT

ACUTE ORAL TOXICITY STUDY OF RELYON (CLO_2) IN SWISS ALBINO MICE

GUIDELINE: SCHEDULE – Y

STUDY No.: FLAIR/ PC / 202

DATE : August 13, 2013

STUDY SPONSOR

PROPHYLAXIS,
14,Nilkanth Ind. Society,
1 B/H,Hariom Dyeing,Ved Road.
SURAT,GUJRAT

TESTING FACILITY

FLAIR LABS

(FACILITY FOR LABORATORY ANIMAL AND INVIVO RESEARCH)
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PALSANA, SURAT (GUJARAT), 394 315

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ABBREVIATIONS

PC – Preclinical
w/w – Weight by weight
M.Sc. – Master in Science
mg – miligram
NOAEL – No Observed Adverse Effect Level
ml – mililitre
°C – Degree Celsius
pg – picogram
NAD – No Abnormalities Detected
SD – Standard Deviation
% - Percentage
M.V.Sc – Master in Veterinary Science
µg or mcg -Microgram
kg - kilogram
gm – Grams
No. - Number
Ltd. – Limited
N – Normal
NP – Not Performed
n – Number of Observations
CPCSEA – Committee for the Purpose of Control and Supervision of Experiments on Animals

STATEMENT OF COMPLIANCE

This study has been performed in accordance with the described procedures as mentioned in the study plan. Experimental work with the animals confirms strictly with the guidelines of The Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA) and the Principles of Good Laboratory Practices involving experimental animals. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have potential bearing on the validity and reproducibility of this experimental study.

It is assured that the reported results represent the raw data obtained during the course of the study. The information contained in this report is authentic and accurate.

Dr.Rajendra Patil
(Study Director)

Signature & Date 13/8/2013

INSTITUTIONAL ANIMAL ETHICS COMMITTEE APPROVAL

The Institutional Animal Ethics Committee (IAEC) of Flair Labs has reviewed the submitted protocol entitled "Acute Oral Toxicity Study of Relyon (clo_2)in Swiss Albino Mice" to conduct the experiments with the laboratory animals and their utilization according to the guideline of CPCSEA and the procedures described in the study plan. It has been found that the proposed study meets the CPCSEA guidelines involving experimental animals.

13.08.2013

Mrs. Y Anuradha, M.Sc. **(Signature & Date)**
Chairman, IAEC,
Flair Labs, Palsana, Dist : Surat – 394 315

STATEMENT OF QUALITY ASSURANCE

Study Number	:	FLAIR/PC/202
Study Title	:	Acute Oral Toxicity Study of Relyon (clo_2)in Swiss Albino Mice
Test Item	:	Relyon (clo_2)

The present study was audited in phases to assess the adherence with the procedures mentioned in the Standard Operating Procedures and Study Plan by the Quality Assurance Unit of FLAIR LABS. The details of the findings were recorded and reported to the management. Audit reports are maintained as part of the raw data. It is assured that the reported results completely represent the raw data obtained during the course of the study. The information contained in this report is authentic and accurate.

Mr.V.G.S.Sharma
(Quality Assurance)

13/08/2013
Signature & Date

STUDY DETAIL

Study Title	:	Acute Oral Toxicity Study of Relyon (clo_2) in Swiss Albino Mice
Test Item	:	Relyon (clo_2)
Study Number	:	FLAIR/PC/202
Route	:	Oral
Species/Strain	:	Swiss Albino / Mice

Test Item Details (as provided by the Sponsor)

Test Item	:	Relyon (clo_2)
Batch No.	:	2012032701
Physical Appearance	:	Saffron yellow liquid solution
Manufacturing Date	:	March. 27,2012

Sponsor	:	PROPHYLAXIS, SURAT,GUJRAT
Test Facility	:	FLAIR LABS (Facility for Laboratory Animals & In-Vivo Research) Plot No.B/510, Opp. Gujarat Eco-Textile Park, N.H. 8, Palsana, Dist : Surat (Gujarat), 394 315
Study Director	:	Dr.Rajendra Patil FLAIR LABS (Facility for Laboratory Animals & In-Vivo Research) Plot No.B/510, Opp. Gujarat Eco-Textile Park,N.H. 8, Palsana, Dist : Surat (Gujarat), 394 315

STUDY SCHEDULE

Study Initiation Date	:	15/06/2013
Acclimation Start Date	:	27/06/2013
Experimental Start Date	:	04/07/2013
Experimental Completion Date	:	18/07/2013
Study Completion Date	:	13/08/2013

PERSONNEL INVOLVED IN THE STUDY

Study Director	:	Dr. Rajendra M Patil (M.V.Sc. - Pathology)
Study Personnel	:	Mr. Nilesh Sonawane (B.Sc.D.M.L.T. – Biochemistry)
Pathologist	:	Dr. Rajendra M Patil (M.V.Sc. - Pathology)
Quality Assurance	:	Mr.V.G.S.Sharma (M.Sc.– Bio science)
Animal House Veterinarian	:	Dr. Amol Murkar (M.V.Sc. – Pathology)

SUMMARY AND CONCLUSION

The present study was conducted to evaluate the safety and toxicity profile of Relyon (clo_2) developed by PROPHYLAXIS, U-12,BOMBAY MARKETUMERWADA,SURAT,GUJRAT, after single dose administration in Swiss Albino Mice.

The Test Item Relyon (clo_2) formulation was administered as single dose by oral gavage to mice at 3 graded dose levels viz. 10, 20 and 40 ml/kg body weight/day (76 ,152 and 304 mg/kg body weight respectively)and concurrently the control group animals were administered with vehicle..

Following are the observations made during conduct of the study :

1) Single dose administration of Relyon (clo_2) in mice showed mortality at the dose level of 40ml/kg body weight and showed clinical signs at 20 and 40 ml/kg body weight.
2) Gross and External observations did not reveal any changes indicative of treatment related effects up to the dose level of 40 ml/kg.

The result confirms that there were treatment attributed behavioral alterations, preterminal deaths or adverse effect on body weight data during 14 days of observation period. Therefore, it is concluded that Maximum Tolerated Dose (MTD) for Relyon (clo_2) is 20 ml/kg body weight after single administration in Swiss Albino Mice through oral route.

1.0 INTRODUCTION

Relyon is recognised as a superior water disinfectant alternative to chlorine and has become increasingly popular as a water purification treatment. Chlorine is being phased out due to growing concerns over its carcinogenic by-products, principally trihalomethanes (THMs).

The Chlorine Dioxide obtained by using RELYON components having shelf life as per kinetic half time >30 days.RELYON Chlorine Dioxide deactivates micro organisms by attacking and penetrating their cell wall, disrupting the transport of nutrients across the cell wall and inhibiting protein synthesis. Since this action occurs regardless of the metabolic state of the organism, RELYON is highly effective against dormant organisms and spores.

2.0 OBJECTIVE

The objective of this study was to evaluate the effects of Relyon (clo_2) in Swiss Albino Mice after single dose exposure by oral route. This study may provide a rationale for risk assessment in human.

3.0 TEST METHOD PRINCIPLE

The Test Item Relyon (clo_2) was orally administered once at 3 graded dose levels to swiss albino mice. During the study period animals were observed closely, each day for signs of toxicity.

4.0 SAFETY PRECAUTIONS

Gloves, cap, face mask and goggles (where ever required) were used in addition to protective body garments and foot cover to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test item.

5.0 REGULATORY GUIDELINES

The study was conducted in accordance with the general principles of Schedule "Y", 2005. Drug and Cosmetics (II Amendment) Rules, Repeated Dose Toxicity Studies, Ministry of Health and Family Welfare, Government of India. The study was conducted in accordance with the Standard Operating Procedures of Flair Labs, Palsana, Dist : Surat.

6.0 ANIMAL WELFARE

All scientists working with laboratory animals had deep ethical consideration for the animals during various experimental activities in this study and the study was performed as per the recommendations set forth in:

Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility, The Gazette of India, 1998.

7.0 HUSBANDARY

7.1 Institutional Animal Ethics Committee Approval

Protocol for general procedures and use of animals for conducting Toxicity study has been reviewed and approved by the Institutional Animal Ethics Committee (Protocol No. FLAIR/IAEC/004/004/2013).

7.2 Animal Room Conditions

Experimental animals were housed in animal facility with adequate environmental conditions of temperature 22 $\pm$ 3°C and relative humidity 30-70 %. The 12-hour light and 12 hour dark cycle was maintained manually throughout the study.

7.3 Housing

Animals (Mice) were housed in groups of five of the same sex in sterilized standard polypropylene cages, each with stainless steel top grill having facilities for pelleted food and drinking water in autoclavable polypropylene bottles. Steam sterilized clean paddy husk was used as bedding material and it was changed at least twice a week or as frequently as required.

7.4 Feed and Water

Rodent feed in pellet form, manufactured by VRK, Nutritional Solutions, Sangli, Maharashtra, India was provided *ad libitum.* Potable water filtered by Reverse Osmosis System provided *ad libitum* to animals in sterilized polypropylene bottles with steel tube having smooth edges.

7.5 Room Sanitation

Each day floor of the experimental room was cleaned and mopped twice with disinfectant solution.

8.0 TEST SYSTEM DETAILS

The study was designed to use minimum possible number of animals to fulfil the objectives of the experiment, good scientific justification & requirements of regulatory guidelines.

Species	:	Mice
Strain	:	Swiss Albino
Sex	:	Male & Female
Age at the time of Treatment	:	Between 8 to 10 Weeks
No. of animals per group	:	10 (5 Males + 5 Females)
No. of groups	:	4 Groups (Control + 3 Treated)

9.0 JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM

Mice are one of the recommended species, as test system, for the conduct of toxicological studies. Further, Swiss albino mice have been used widely for the evaluation of product safety. Extensive data is available for comparative analysis with the mice as the test species.

10.0 CHEMICALS AND REAGENTS

Normal Saline, 10%, Buffered Formalin, Carbon Dioxide Gas

11.0 PROCEDURES

11.1 Criteria for the Selection of Animals

After veterinary health examination animals of uniform body weight were selected for the experiment. Selected females were nulliparous and non-pregnant. The individual body weight of animals was within $\pm$ 20 % of group mean body weight range. The groups mean body weights of all groups were approximately within the stipulated range.

11.2 Acclimation

Animals were acclimatised for a minimum period of seven days and observed for general health before commencement of the experiment.

11.3 Randomization and Grouping of Animals

Before start of the experiment animals were randomly assigned to different groups (G 1 to G 4) using validated in house developed randomization procedure. Each group consisted of ten mice (5 male and 5 females).

11.4 Animal and Cage Identification

All animals within the cage were identified by tail marking. A label with details of cage number, project number, species, strain, and sex, dose and route of treatment along with identification numbers of animals within it was attached to each cage.

12.0 EXPERIMENTAL PROCEDURE

12.1 Experimental Design

Group	Dose (ml/kg)	Animal/Group	
		Male	Female
I	0	5	5
II	10	5	5
III	20	5	5
IV	40	5	5

12.2 Duration of Experiment

Animals were treated once by oral route. All the animals were observed throughout the treatment period till the date of termination. At termination all surviving animals were sacrificed humanely and gross pathology data was collected.

13.0 PREPARATION AND ADMINISTRATION OF DOSES

Test Item details and Certificate of Analysis (COA) are provided as Annexure I. The doses selected for the study by the oral route were 10, 20 and 40 ml/kg. To attain the required dose in mg/kg was 76,152 and 304 respectively.

13.1 Preparation of the Doses

1. Take a dark container of 500ml.
2. Fill the container with 450 ml of potable water.
3. Add component A (25 ml) into the water.
4. Then add component B (25 ml) into the water.
5. Close the container and keep 7 hour in 40 °c in dark place for the reaction to generate relyon (clo_2)of 7600 ppm

13.2 Dose Administration

After preparation of the dose formulations, animals were treated at the dose volume of 10 20 and 40 ml/kg body weight. Actual dose volumes were calculated on the basis of recent body weight for all individuals and the Test Item was administered through oral route using gavage. Animals of the control group were treated with distilled water at the volume of 40 ml/kg body weight.

13.3 Route of Administration and Justification for Selection

Test formulation and vehicle were administered through oral route to fulfill the regulatory requirement and this may provide a rationale for risk assessment in human.

14.0 OBSERVATIONS

14.1 Mortality and Clinical Signs

All the animals were observed daily throughout the experimental period of 14 days for mortality, morbidity or clinical signs of toxicity, if any.

14.2 Body Weights

Body weights were recorded on the day of randomization (prior to day of dosing), Day 0 (first day of dosing), 7, 14, of the experimental period.

14.3 Necropsy

All surviving animals on the day of termination were sacrificed humanely with an over dose of carbon dioxide and subjected to gross necropsy. The findings were recorded in relevant format and are part of the raw data file.

15.0 STATISTICAL ANALYSIS

Body weights were subjected to Bartlett's test to assess the homogeneity of variance followed by t test Analysis of Variance (ANOVA) and Dunnett's t-test. Where the data do not meet the homogeneity of variance, Student's t-test was performed to calculate significance. Significance and non significance at 1% and 5% level both were considered for the analysis of the data.

16.0 RESULTS

16.1 Mortality and Clinical Signs

(Reference: Table No. 1 - 2 / Appendix No.1 - 2)

On the day of dosing clinical signs were recorded immediately (0 hrs), thereafter at 10 min, 30 min, 2 hrs, 4 hrs, 6 hrs and 8 hrs. Further clinical signs were recorded every day throughout the observation period for all the animals from control and treatment groups. Clinical sign at the dose level of 20 ml/kg treated dose group showed dullness and at 40 ml/kg treated animal showed dullness and. Mortality of 2 male and 4 female recorded at the dose level of 40 ml /kg treated dose only.

16.2 Body Weights

(Reference: Table No. 3 – 4 / Appendix No. 3 - 4)

The mean of body weight data recorded on Day prior to dosing, 0(Day of dosing), 7, 14, for treatment groups did not show any statistically significant changes in comparison to the control group. Body weight of animals was observed to show a normal increasing pattern throughout the study period.

16.3 External and Gross

(Reference: Table No. 5 – 6 / Appendix No. 5 - 6)

No external observations or gross lesions were recorded in either the treatment or control group animals. No spontaneous or incidental findings were observed in any of the animals.

17.0 CONCLUSION

Present study was conducted to assess the single dose toxicological profile of Relyon (clo_2) in Swiss Albino Mice at 10, 20 and 40 ml/kg body weight/day (76, 152 and 304 mg/kg body weight respectively)

The result confirms that there were treatment attributed behavioral alterations, preterminal deaths or adverse effect on body weight data during 14 days of observation period. Therefore, it is concluded that Maximum Tolerated Dose (MTD) for Relyon (clo_2) is 20 ml/kg body weight (or 152 mg/kg body weight) after single administration in Swiss Albino Mice through oral route.

18.0 DATA COMPILATION

All the findings such as mortality, clinical signs, body weight and body weight changes, and gross observations have been tabulated.

19.0 ARCHIVING

Raw data and study related documents generated during the course of the study at Flair Labs, along with a copy of the final report will be archived at the premises for period of 2 year.

20.0 REFERENCES

1. Handbook of Toxicology, 2nd Edition, Edited by Michael J. Derelanko and Mannfred A. Hollinger, ISBN 0-8493-0370-2, CRC Press LLC.

2. Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatment groups with a control.*J.Am.Stat.Assoc.*50 pp.1096-1121.

3. Annexure E IS: 1453, 2004, New Delhi.

4. Committee for Medicinal Products for Human Use (CHMP) Draft Guideline on "Repeat Dose Toxicity"; 21 February 2008.

21.0 SUMMARY TABLES

TABLE 1

SUMMARY OF CLINICAL SIGNS AND MORTALITY – MALE

Group	Dose (ml/kg)	Clinical Sign	Mortality
I	0	N	0
II	10	N	0
III	20	66	0
IV	40	66,50	2/5

Abbreviation : N - Normal, 0 - No Mortality,66-Dullnes,50-Piloerection

TABLE 2

SUMMARY OF CLINICAL SIGNS AND MORTALITY – FEMALE

Group	Dose (ml/kg)	Clinical Sign	Mortality
I	0	N	0
II	10	N	0
III	20	66	0
IV	40	66,50	4/5

Abbreviation : N - Normal, 0 - No Mortality,66-Dullnes,50-Piloerection

TABLE 3

SUMMARY OF GROUP MEAN BODY WEIGHT & CHANGE– MALE

SUMMARY TABLE - MALES BODY WEIGHT (g)					
Study Day	**Group**	**I**	**II**	**III**	**IV**
	Dose	**0 ml/kg**	**10 ml/kg**	**20 ml/kg**	**40 ml/kg**
0	**Mean**	**24.5**	**24.4**	**24.3**	**24.3**
	± SD	0.8	0.7	0.8	0.3
	n	5	5	5	5
7	**Mean**	**27.9**	**27.6**	**27.4**	**26.7**
	± SD	0.7	0.6	0.7	0.6
	n	5	5	5	3
14	**Mean**	**29.1**	**29.0**	**28.7**	**27.8**
	± SD	0.9	1.0	0.8	0.6
	n	5	5	5	3
% BODY WEIGHT CHANGE					
Study Day	**Group**	**I**	**II**	**III**	**IV**
	Dose	**0 ml/kg**	**10 ml/kg**	**20 ml/kg**	**40 ml/kg**
0 - 7	**Mean**	**8.1**	**6.7**	**6.2**	**3.4**
	± SD	0.7	2.0	0.8	0.3
	n	5	5	5	3
0 - 14	**Mean**	**12.9**	**12.0**	**11.1**	**7.8**
	± SD	2.7	1.2	1.1	0.2
	n	5	5	5	3

TABLE 4

SUMMARY OF GROUP MEAN BODY WEIGHT – FEMALE

SUMMARY TABLE - FEMALES BODY WEIGHT (g)					
Study Day	**Group**	**I**	**II**	**III**	**IV**
	Dose	**0 ml/kg**	**10 ml/kg**	**20 ml/kg**	**40 ml/kg**
0	**Mean**	**24.4**	**24.2**	**24.4**	**24.2**
	± SD	0.8	0.7	0.8	1.5
	n	5	5	5	5
7	**Mean**	**27.3**	**27.1**	**26.7**	**25.6**
	± SD	0.8	0.9	0.8	0.0
	n	5	5	5	1
14	**Mean**	**28.8**	**28.8**	**28.0**	**26.5**
	± SD	0.5	0.5	0.6	0.0
	n	5	5	5	1
% BODY WEIGHT CHANGE					
Study Day	**Group**	**I**	**II**	**III**	**IV**
	Dose	**0 ml/kg**	**10 ml/kg**	**20 ml/kg**	**40 ml/kg**
0 - 7	**Mean**	**6.1**	**5.1**	**3.7**	**2.8**
	± SD	1.2	2.4	1.3	0.0
	n	5	5	5	1
0 - 14	**Mean**	**12.0**	**11.8**	**8.7**	**6.4**
	± SD	2.5	2.3	3.2	0.0
	n	5	5	5	1

TABLE 5

SUMMARY OF EXTERNAL AND GROSS FINDINGS – MALE

Group	Dose (ml/kg)	External observation	Gross Pathology
I	0	N	NAD
II	10	N	NAD
III	20	N	NAD
IV	40	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

TABLE 6

SUMMARY OF EXTERNAL AND GROSS FINDINGS – FEMALE

Group	Dose (ml/kg)	External observation	Gross Pathology
I	0	N	NAD
II	10	N	NAD
III	20	N	NAD
IV	40	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

22.0 APPENDICES

APPENDIX 1

INDIVIDUAL ANIMAL CLINICAL SIGNS – MALE

Group :	I (Control)	Dose :	0	ml/kg

Expt. Day	Animals Number				
	1	2	3	4	5
0 hrs	N	N	N	N	N
10 min	N	N	N	N	N
30 min	N	N	N	N	N
2 hrs	N	N	N	N	N
4 hrs	N	N	N	N	N
6 hrs	N	N	N	N	N
8 hrs	N	N	N	N	N
1	N	N	N	N	N
2	N	N	N	N	N
3	N	N	N	N	N
4	N	N	N	N	N
5	N	N	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N
9	N	N	N	N	N
10	N	N	N	N	N
11	N	N	N	N	N
12	N	N	N	N	N
13	N	N	N	N	N
14	N	N	N	N	N

Abbreviations : N = Normal

APPENDIX 1 *Cont...*

INDIVIDUAL ANIMAL CLINICAL SIGNS – MALE

Group :	II	Dose :	10	ml/kg

Expt. Day	Animals Number				
	11	12	13	14	15
0 hrs	N	N	N	N	N
10 min	N	N	N	N	N
30 min	N	N	N	N	N
2 hrs	N	N	N	N	N
4 hrs	N	N	N	N	N
6 hrs	N	N	N	N	N
8 hrs	N	N	N	N	N
1	N	N	N	N	N
2	N	N	N	N	N
3	N	N	N	N	N
4	N	N	N	N	N
5	N	N	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N
9	N	N	N	N	N
10	N	N	N	N	N
11	N	N	N	N	N
12	N	N	N	N	N
13	N	N	N	N	N
14	N	N	N	N	N

Abbreviations : N = Normal

APPENDIX 1 *Cont...*

INDIVIDUAL ANIMAL CLINICAL SIGNS – MALE

Group :	III	Dose :	20	ml/kg

Expt. Day	Animals Number				
	21	22	23	24	25
0 hrs	66	66	66	66	66
10 min	66	66	66	66	66
30 min	66	66	66	66	66
2 hrs	66	66	66	66	66
4 hrs	66	66	66	66	66
6 hrs	66	66	66	66	66
8 hrs	66	66	66	66	66
1	66	66	66	66	66
2	66	66	66	66	66
3	66	66	66	66	66
4	N	66	N	66	N
5	N	66	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N
9	N	N	N	N	N
10	N	N	N	N	N
11	N	N	N	N	N
12	N	N	N	N	N
13	N	N	N	N	N
14	N	N	N	N	N

Abbreviation : N - Normal, ,66-Dullnes

APPENDIX 1 *Cont...*

INDIVIDUAL ANIMAL CLINICAL SIGNS – MALE

Group :	IV	Dose :	40	ml/kg

Expt. Day	Animals Number				
	31	32	33	34	35
0 hrs	66,50	66,50	66,50	66,50	66,50
10 min	66,50	66,50	66,50	66,50	66,50
30 min	66,50	66,50	66,50	66,50	66,50
2 hrs	66,50	66,50	66,50	66,50	66,50
4 hrs	66,50	66,50	66,50	66,50	66,50
6 hrs	66,50	66,50	66,50	66,50	66,50
8 hrs	66,50	66,50	66,50	66,50	66,50
1	66,50	66,50	N	N	66,50
2	66,50	66,50	1	N	66,50
3	66,50	66,50		1	66,50
4	66,50	N			N
5	66,50	N			N
6	66,50	N			N
7	N	N			N
8	N	N			N
9	N	N			N
10	N	N			N
11	N	N			N
12	N	N			N
13	N	N			N
14	N	N			N

Abbreviation : N - Normal, 1 – Found Dead,66-Dullnes,50-Piloerection

APPENDIX 2

INDIVIDUAL ANIMAL CLINICAL SIGNS – FEMALE

Group :	I (Control)	Dose :	0	ml/kg

Expt. Day	Animals Number				
	6	7	8	9	10
0 hrs	N	N	N	N	N
10 min	N	N	N	N	N
30 min	N	N	N	N	N
2 hrs	N	N	N	N	N
4 hrs	N	N	N	N	N
6 hrs	N	N	N	N	N
8 hrs	N	N	N	N	N
1	N	N	N	N	N
2	N	N	N	N	N
3	N	N	N	N	N
4	N	N	N	N	N
5	N	N	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N
9	N	N	N	N	N
10	N	N	N	N	N
11	N	N	N	N	N
12	N	N	N	N	N
13	N	N	N	N	N
14	N	N	N	N	N

Abbreviations : N = Normal

APPENDIX 2 *Cont...*

INDIVIDUAL ANIMAL CLINICAL SIGNS – FEMALE

Group :	II	Dose :	10	ml/kg

Expt. Day	Animals Number				
	16	17	18	19	20
0 hrs	N	N	N	N	N
10 min	N	N	N	N	N
30 min	N	N	N	N	N
2 hrs	N	N	N	N	N
4 hrs	N	N	N	N	N
6 hrs	N	N	N	N	N
8 hrs	N	N	N	N	N
1	N	N	N	N	N
2	N	N	N	N	N
3	N	N	N	N	N
4	N	N	N	N	N
5	N	N	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N
9	N	N	N	N	N
10	N	N	N	N	N
11	N	N	N	N	N
12	N	N	N	N	N
13	N	N	N	N	N
14	N	N	N	N	N

Abbreviations : N = Normal

APPENDIX 2 *Cont...*

INDIVIDUAL ANIMAL CLINICAL SIGNS – FEMALE

Group :	III	Dose :	20	ml/kg

Expt. Day	Animals Number				
	26	27	28	29	30
0 hrs	66	66	66	66	66
10 min	66	66	66	66	66
30 min	66	66	66	66	66
2 hrs	66	66	66	66	66
4 hrs	66	66	66	66	66
6 hrs	66	66	66	66	66
8 hrs	66	66	66	66	66
1	66	66	66	66	66
2	66	66	66	66	66
3	66	N	66	66	66
4	66	N	N	66	N
5	N	N	N	N	N
6	N	N	N	N	N
7	N	N	N	N	N
8	N	N	N	N	N
9	N	N	N	N	N
10	N	N	N	N	N
11	N	N	N	N	N
12	N	N	N	N	N
13	N	N	N	N	N
14	N	N	N	N	N

Abbreviation : N – Normal,66-Dullnes

APPENDIX 2 *Cont...*

INDIVIDUAL ANIMAL CLINICAL SIGNS – FEMALE

Group :	IV	Dose :	40	ml/kg

Expt. Day	Animals Number				
	36	37	38	39	40
0 hrs	66,50	66,50	66,50	66,50	66,50
10 min	66,50	66,50	66,50	66,50	66,50
30 min	66,50	66,50	66,50	66,50	66,50
2 hrs	66,50	66,50	66,50	66,50	66,50
4 hrs	66,50	66,50	66,50	66,50	66,50
6 hrs	66,50	66,50	66,50	66,50	66,50
8 hrs	66,50	66,50	66,50	66,50	66,50
1	66,50	N	1	N	66,50
2	66,50	N		1	66,50
3	66,50	1			66,50
4	66,50				1
5	66,50				
6	N				
7	N				
8	N				
9	N				
10	N				
11	N				
12	N				
13	N				
14	N				

Abbreviation : N - Normal, 1 – Found Dead,66-Dullnes,50-Piloerection

APPENDIX 3

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – MALE

Group :	I (Control)	Dose : (ml/kg)	0

Expt. Day	Animals No. & Body Weight (g)				
	1	2	3	4	5
0	23.3	24.3	24.6	25.3	25.1
7	26.8	27.6	28.0	28.5	28.6
14	28.5	28.3	28.8	29.5	30.6
Expt.Day	% Body Weight Change				
0 - 7	8.9	7.0	8.5	8.0	7.9
0 - 14	15.9	9.7	11.6	11.7	15.5

APPENDIX 3 *Cont...*

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – MALE

Group :	II	Dose : (ml/kg)	10

Expt. Day	Animals No. & Body Weight (g)				
	11	12	13	14	15
0	23.2	24.2	24.7	25	24.8
7	27.2	27.1	27.3	27.8	28.5
14	27.6	28.5	29.2	30.1	29.4
Expt.Day	% Body Weight Change				
0 - 7	9.7	6.3	4.6	5.3	7.5
0 - 14	11.3	11.8	11.9	14.0	10.9

APPENDIX 3 *Cont...*

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – MALE

Group :	III	Dose : (ml/kg)	20

Expt. Day	Animals No. & Body Weight (g)				
	21	22	23	24	25
0	23.2	24.1	24.6	24.3	25.3
7	26.5	26.9	27.5	28.0	28.3
14	27.8	28.0	28.8	29.6	29.3
Expt.Day	% Body Weight Change				
0 - 7	6.9	5.1	6.6	5.7	6.8
0 - 14	12.1	9.4	11.6	11.7	10.6

APPENDIX 3 *Cont...*

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – MALE

Group :	IV	Dose : (ml/kg)	40

Expt. Day	Animals No. & Body Weight (g)				
	31	32	33	34	35
0	24.1	24	24.2	24.8	24.5
7	26.2	26.5	-	-	27.3
14	27.3	27.6	-	-	28.5
Expt.Day	% Body Weight Change				
0 - 7	3.6	3.5	-	-	3.0
0 - 14	7.9	7.8	-	-	7.5

APPENDIX 4

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – FEMALE

Group :	I (Control)	Dose : (ml/kg)	0

Expt. Day	Animals No. & Body Weight (g)				
	6	7	8	9	10
0	23.1	24.2	24.3	25.1	25.2
7	26.5	26.8	27.0	27.6	28.5
14	28.5	28.9	28.1	29.0	29.5
Expt.Day	% Body Weight Change				
0 - 7	6.9	5.9	4.7	5.3	7.5
0 - 14	14.9	14.2	8.9	10.7	11.3

APPENDIX 4 *Cont...*

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – FEMALE

Group :	II	Dose : (ml/kg)	10

Expt. Day	Animals No. & Body Weight (g)				
	16	17	18	19	20
0	23.2	23.8	24.6	24.7	24.8
7	25.8	26.8	27.9	26.8	28.0
14	28.6	28.1	29.0	28.8	29.3
Expt.Day	% Body Weight Change				
0 - 7	4.0	7.2	7.3	1.5	5.7
0 - 14	15.3	12.4	11.5	9.1	10.6

APPENDIX 4 *Cont...*

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – FEMALE

Group :	III	Dose : (ml/kg)	20

Expt. Day	Animals No. & Body Weight (g)				
	26	27	28	29	30
0	23.3	24.1	24.4	24.8	25.3
7	25.4	26.5	26.8	27.6	27.2
14	27.9	27.0	28.6	28.3	28.0
Expt.Day	% Body Weight Change				
0 - 7	2.4	5.2	4.7	4.2	2.3
0 - 14	12.5	7.1	11.7	6.8	5.3

APPENDIX 4 *Cont...*

INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT CHANGE (%) – FEMALE

Group :	IV	Dose : (ml/kg)	40

Expt. Day	Animals No. & Body Weight (g)				
	36	37	38	39	40
0	23.1	23.2	23.5	24.3	26.8
7	25.6	-	-	-	-
14	26.5	-	-	-	-
Expt.Day	% Body Weight Change				
0 - 7	2.8	-	-	-	-
0 - 14	6.4	-	-	-	-

APPENDIX 5

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – MALE

Group	I (Control)	Dose : (ml/kg)	0

Animal No.	External Observations	Gross Pathology Observations
1	N	NAD
2	N	NAD
3	N	NAD
4	N	NAD
5	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

APPENDIX 5 *Cont...*

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – MALE

Group	II	Dose : (ml/kg)	10

Animal No.	External Observations	Gross Pathology Observations
11	N	NAD
12	N	NAD
13	N	NAD
14	N	NAD
15	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

APPENDIX 5 *Cont...*

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – MALE

Group	III	Dose : (ml/kg)	20

Animal No.	External Observations	Gross Pathology Observations
21	N	NAD
22	N	NAD
23	N	NAD
24	N	NAD
25	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

APPENDIX 5 *Cont...*

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – MALE

Group	IV	Dose : (ml/kg)	40

Animal No.	External Observations	Gross Pathology Observations
31	N	NAD
32	N	NAD
33	N	NAD
34	N	Autolysis
35	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

APPENDIX 6

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – FEMALE

Group	I (Control)	Dose : (ml/kg)	0

Animal No.	External Observations	Gross Pathology Observations
6	N	NAD
7	N	NAD
8	N	NAD
9	N	NAD
10	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

APPENDIX 6 *Cont...*

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – FEMALE

Group	II	Dose : (ml/kg)	10

Animal No.	External Observations	Gross Pathology Observations
16	N	NAD
17	N	NAD
18	N	NAD
19	N	NAD
20	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

APPENDIX 6 *Cont...*

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – FEMALE

Group	III	Dose : (ml/kg)	20

Animal No.	External Observations	Gross Pathology Observations
26	N	NAD
27	N	NAD
28	N	NAD
29	N	NAD
30	N	NAD

Abbreviations : N = Normal, NAD = No Abnormalities Detected

APPENDIX 6 *Cont...*

INDIVIDUAL ANIMAL EXTERNAL, GROSS EXAMINATION – FEMALE

Group	IV	Dose : (ml/kg)	40

Animal No.	External Observations	Gross Pathology Observations
36	N	NAD
37	N	NAD
38	N	Autolysis
39	N	NAD
40	N	Autolysis

Abbreviations : N = Normal, NAD = No Abnormalities Detected

ANNEXURE I

CERTIFICATE OF ANALYSIS

PROPHYLAXIS

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CERTIFICATE OF ANALYSIS
QUALITY CONTROL DEPARTMENT

Ref. No.(MOA)	**PRO/03/1 dtd. 27 March 2012**	**AR No.**	**20120327017660**
Name of the Sample	**RELYON**	**Dt. of Receipt of Sample**	**27 March, 2012**
Generic Name	**Water disinfectant**		
Manufactured by	**Self**		

Batch No.	D/M	D/E	Batch Size	Packaging Type	Sample Quantity
2012032701	March. 27	30 days as per kinetic half time	1000 Kg.	HDPE bottle 100 ml	1L

Name of Tests	Specifications	Result of Analysis
Description	Saffron yellow liquid solution	Saffron yellow liquid solution
Specific Gravity	--------	**1.1**
pH	--------	**1.45**

Assay:
Molecular formula : ClO2

Active ingredient	Unit	Result
Chlorine Dioxide	**0.76%**	**0.766 %**

Opinion: In the opinion of the under signed the sample was found to comply as per the specification above.

For PROPHYLAXIS

A. Dhanani
Authorised Signatory

REPORT APPROVAL

ACUTE ORAL TOXICITY STUDY OF RELYON (CLO_2) IN SWISS ALBINO MICE

Prepared by:

Signature.....**Date**..13/8/2013.....
Dr.Rajendra Patil
(Study Director)

Approved by:

Signature.....**Date**..13.08.2013.....
Dr.Balaji Madrewar
(Test Facility Management)