DRAFT REPORT

ACUTE DERMAL TOXICITY OF PRSOLUTION GEL AS PER OECD GUIDELINE NO. 402

STUDY NO: VLTO-100213

Study Completion Date: 26.04.2010

SPONSOR

DM CONTACT MANAGMENT
100-645 TYEE ROAD, VICTORIA
BC V9A6X5, CANADA

TEST FACILITY

VEDIC LIFESCIENCES PVT. LTD.
203, MORYA LANDMARK-I,
OFF LINK ROAD, ANDHERI (W),
MUMBAI – 400 053
INDIA

STATEMENT OF COMPLIANCE

To the best of our knowledge and belief, this Study entitled "Acute Dermal toxicity study of Prosolution Gel in Rat was performed under my supervision in compliance with the test guideline laid down in OECD – 402" The objectives laid down in the study protocol were achieved.

No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Jayesh Chaudhary
CEO, Vedic Lifesciences Pvt. Ltd.

Deepali jadhav
Executive

CERTIFICATE

We certify that the work reported here is a true and authentic report of the study entitled, ‘‘Acute Dermal toxicity study of Prosolution gel in Rat as per OECD Guideline 402 “ based on the experiment conducted in one of the partnered Toxicology Laboratory Services of VEDIC LIFESCIENCES PVT LTD (B-203 Morya Landmark I, Off New Link Road, Andheri (W), Mumbai - 400 053,) India. The results presented here are faithful reflection of data collected during the study.

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QUALITY ASSURANCE STATEMENT

The Study, entitled “Acute Dermal toxicity study of Prosolution Gel in Rat ” has been inspected in the spirit of OECD Guideline 402.

This study was inspected and findings reported to Management and to the Study Director.

Inspections were performed according to the Standard Operating Procedures of the Quality Assurance Unit. The report was audited against the approved study plan and pertinent raw data and accurately reflects the raw data.

STATEMENT OF CONFIDENTIALITY

This report which contains **CONFIDENTIAL** and **PROPRIETARY** information of **DM contact managment.** will not be disclosed to anyone except the employees of this company wherever necessary or to persons authorized by law or judicial judgment without the expressed or written approval of Sponsor.

DECLARATION

The Study Director hereby declares that the work was performed under his supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

1. STUDY DETAILS

1.1 TITLE : Acute Dermal toxicity study of Prosolution Gel in Rat.

1.2 STUDY NUMBER : VLTO-100213

1.3 TESTING FACILITY : VEDIC LIFESCIENCES PVT. LTD,
203, Morya Landmark-I,
Off Link Road, Andheri (W),
Mumbai – 400 053, INDIA

1.4 SPONSOR : DM CONTACT MANAGMENT,
100-645, Tyee Road
Victoria, Bc V9a 6x5,
Canada

1.5 STUDY SCHEDULE :

Study initiation date : 29/01/10

Experimental starting date : 10/03/10

Experimental completion date : 01/04/10

Study completion date : 26/04/10

2. MONITORING PERSONNEL

Sl. No.	Responsibility	Personnel	Signature with date
1.	MONITORING SCIENTIST	DEEPALI JADHAV VEDIC LIFESCIENCES PVT.LTD MUMBAI	
2.	SPONSOR'S NOMINEE	JAYESH CHAUDHARY VEDIC LIFESCIENCES PVT.LTD MUMBAI	

3. SUMMARY

Acute Dermal toxicity study of Prosolution Gel in Sprague Dawley Rats was performed as per the OECD Guidelines No. 402, "Acute Dermal Toxicity" Adopted: 24 Feb 1987, OECD Guideline for Testing of Chemicals Section 4,

Single Dermal dose of Prosolution Gel, was applied to shaven skin of male and female rats to assess its acute dermal toxicity at a dose of 2000 mg/kg, did not cause death and no evident toxic clinical signs were observed.

DOSE RANGE FINDING

The Prosolution Gel was applied to shaven skin of one male and one female rat at the dose 2000 mg/kg body weight. The application site was covered with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. The test site was further covered in bandage cloths that secured gauze dressing and test substance. Neck collars were put to each animal to avoid ingestion.

Prosolution Gel did not cause any mortality and also no evident signs of toxicity were observed during the 7 days observation period of post dosing. Hence, 2000 mg/kg dose was selected for the main study.

MAIN STUDY

In the main study, the Prosolution Gel was applied to shaven skin as a single dose to group of five male and five female rats at the dose of 2000 mg/kg body weight. The animals were observed for mortality and signs of intoxication for a period of 14 days post-dosing and their body weights were recorded at weekly intervals. Necropsy was performed on all rats at termination of the study.

Prosolution Gel did not cause any mortality and no evident signs of toxicity were observed in male and female rats treated at 2000 mg/kg during the observation period of 14 days post-dosing. No adverse effects on the body weight gain by rats treated at 2000 mg/kg were observed. No gross pathological alterations were detected in the treated rats at terminal necropsy.

The median lethal dose (LD50) of the Prosolution Gel in Sprague Dawley rats by dermal route was estimated to be more than 2000 mg/kg body weight.

4 OBJECTIVE

Objective of this Acute Dermal toxicity study in rat was to assess the toxic characteristics of Prosolution Gel, when applied to skin as single dermal route.

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. It provides information on health hazards likely to arise from a short-term exposure by the dermal route. Data from an acute dermal toxicity study may serve as a basis for classification and labeling. It is an initial step in establishing a dosage regimen in subchronic and other studies and may provide information on dermal absorption and the mode of toxic action of a substance by this route.

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5. MATERIALS & METHODS

5.1 TEST ARTICLE

The following information was provided about the test article.

Test article	: Prosolution Gel
Characteristics	: Colorless viscous jelly like.
Batch No.	: NA
Date of Manufacture	: NA
Date of Expiry	: NA
Purity	: NA
Sponsor	: DM CONTACT MANAGMENT, 100-645, Tyee Road Victoria, Bc V9a 6x5, Canada

5.2 TEST SYSTEM

Test system	: Rat
Strain	: Sprague Dawley
Source	: Bred and reared at, India.
Age	: 9 to 10 weeks.
Body weight range at Initiation	: 203 g to 230 g
Identification	: By cage tag and corresponding colour body marking
Number of dose groups	: Three G 1 : 2000mg/kg (DRF) G 2 : 2000mg/kg
Number of animals per group	: 1 M and 1F for DRF. 5 M and 5 F Main study.
Acclimation	: One week in experimental room after veterinary examination
Randomization	: After acclimation and veterinary examination animals were randomly selected.

Husbandry

Environmental conditions	: Air conditioned rooms with 10 –15 air changes per hour, temperature between 19-250C, relative humidity 30-70% and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark.
Accommodation	: Individually housed in polypropylene cages with stainless Steel grill top, facilities for food and water bottle, and Bedding of clean paddy husk.
Acclimatization	: The animal will be acclimatized for a minimum period of five days to laboratory conditions and will be observed for clinical signs daily. Veterinary examination of all the animals will be recorded on the day of receipt.

Diet : 'Amrut' brand pelleted standard Rats and mice feed manufactured by Pranav Agro Industries ltd. Sangli, was provided ad libitum.

Water : Potable water passed through reverse osmosis filtration system and exposed to u.v. ray was provided ad libitum in glass bottles with stainless steel sipper tubes.

Principle of the test

The test substance is applied to the skin in graduated doses to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects and deaths are made. Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied. Animals showing severe and enduring signs of distress and pain may need to be humanely killed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out.

Dose Levels and Justification

Limit test

As described in Guideline a limit dose of 2000 mg/kg body weight in a group of 5 male and 5 female animals and if compound related mortality is not produced, a full study may not be needed.

2000 mg/kg body weight was selected as a starting dose level based on the recommendation of OECD 402 Guide line.

The toxicity of the test article following single dermal exposure was assessed using male and female rats. The rats were observed for incidence of mortality and signs of intoxication for 14 days after the application of test article.

Preparation of Animals

Preparation of animal skin : Approximately 24 hrs. before application, hair around the trunk between flank and shoulders was closely clipped with a small electric clipper exposing 10 % of the total body surface. Care must be taken to avoid abrading the skin, which could alter its permeability.

5.3 STUDY DESIGN

After an acclimation period the rats were weighed and the required numbers of animals were randomly allocated to the treatment groups. Selection of dose levels for the study was made on the basis of the results of dose range finding study.

Dose Range Finding Study

Dose range finding study was conducted using two (1M + 1F) rats at the dose of 2000 mg/kg. This group of rats were administered Prosolution gel as a single dose and was observed for the incidence of mortality and clinical sign for 7 days.

Main Study

On the basis of results of dose range finding study, the main study was conducted using 5 male and 5 female rats at dose of 2000 mg/kg. As described below, this group of rats were administered topically Prosolution gel as a single dose and was observed for the incidence of mortality and clinical sign for 14 days.

Group No.	Dose (mg/kg)	Male Rats		Female Rats	
		No. of Rats	ID	No. of Rats	ID
2	2000	5	R091- R095	5	R096 – R100

5.4 DOSE FORMULATION

The test article was weighed on cotton gauze and applied on the pre shaven skin. The test article dose was calculated based on the recent body weight record before application.

5.5 ADMINISTRATION OF TEST ARTICLE

One day prior to treatment, hair was removed with electric clippers from the dorsolumbar region exposing an area equivalent to approximately 10% of the total body surface. The dose applied to individual rat was adjusted according to its body weight that was recorded just before treatment. The Prosolution gel was applied to the shaved skin to each rat as a single dose using a suitable applicator. A porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. The test site was further covered in bandage cloths that secured gauze dressing and test substance. Neck collars were put to each animal to avoid ingestion.

The animals were housed individually with plastic collar around their necks during the exposure period of 24 hours to prevent ingestion of the test material. After 24 hours thorough wiping cleaned the site of application and the animals were transferred to individual cages.

5.6 OBSERVATIONS

Mortality

On the day of dosing, all animals were observed for mortality at 30 min, 1, 2, 4 and 6 hours following topical application and thereafter they were observed once a day for 14 days.

Clinical signs

The treated animals were observed for signs of intoxication, at 30 min, 1, 2, 4 and 6 hours after topical application and thereafter once a day for 14 days. The appearance, progress and disappearance of the signs were recorded.

The animals were examined particularly for changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity such as lacrimation, piloerection, pupil size and unusual respiratory pattern. Changes, if any, in gait, posture and responses to handling as well as the presence of clonic or tonic movements, stereotypies or bizarre behaviour were also recorded.

Body weights

The body weights of rats were individually recorded before dosing and at weekly intervals thereafter. Group mean body weights were calculated.

Necropsy

All animals were sacrifices at the end of the observation period and subjected to a complete necropsy. As no gross pathological findings were encountered in any of the organs, histopathological examination was not conducted.

6. RESULTS

6.1 Mortality

Prosolution Gel tested at the dose level of 2000 mg/kg, did not cause any mortality in treated rats, during the observation period of 14 days post dosing (Table 1).

6.2 Clinical Signs

No evident signs of toxicity and skin reaction were observed in treated rats throughout the observation period of 14 days at 2000 mg/kg (Table 2).

6.3 Body Weights

The body weight gain by treated rats was not affected during the 14 days observation period (Table 3).

6.4 Necropsy

No gross pathological alterations were encountered in any of the female rats sacrificed at termination of the study (Table 4).

6.5 LD50

The median lethal dose (LD50) Prosolution Gel after dermal route as a single dose in Sprague Dawley rats, both male and female rats was found to be more than 2000 mg/kg body weight.

7. CONCLUSION

Prosolution Gel to Sprague Dawley rat as a single dose by dermal route did not cause any mortality in any of the treated male and female rats at 2000 mg/kg body weight. No signs of evident toxicity and no skin reactions were observed at 2000 mg/kg in this study.

No adverse effect on body weight gain by treated rats was recorded during the 14 days post-treatment observation period. It also did not induce any gross pathological alterations in any of the rats, as evident at necropsy.

Based on these results, the acute dermal toxicity study of Prosolution Gel in Sprague Dawley rats performed as per the OECD Guideline 402, was found to be more than 2000 mg/kg body weight.

8. ARCHIVES

All test article, raw data and other documents generated during the course of this study together with a copy of final report will be stored in the archives of Vidic Lifesciences, Mumbai, India for a period of one year from the date of submission of final report.

TABLE 1

Survival Data

Male Rats

Group & Dose mg/kg	G 2 2000
Days	No. of surviving Rats / initial no. of Rats
0 - 7	5/5
8 - 14	5/5

Female Rats

Group & Dose mg/kg	G 2 2000
Days	No. of surviving Rats / initial no. of Rats
0 - 7	5/5
8 - 14	5/5

TABLE 2

Summary of Clinical Signs

Male Rats

Group & Dose mg/kg	**G 2 2000**
Clinical Signs	Incidence (No. of animals with findings / Initial no. animals)
No abnormality detected	5/5

Female Rats

Group & Dose mg/kg	**G 2 2000**
Clinical Signs	Incidence (No. of animals with findings / Initial no. animals)
No abnormality detected	5/5

TABLE 3

SUMMARY OF BODY WEIGHTS (g)

Male Rats

Group & Dose mg/kg		Days		
		0	7	15
G 2 2000	Mean	222.6	229.4	234.8
	± S. D.	7.9	9.0	8.3
	N	5	5	5

Female Rats

Group & Dose mg/kg		Days		
		0	7	15
G 2 2000	Mean	211.8	213.8	220.8
	± S. D.	9.3	12.4	9.8
	N	5	5	5

TABLE 4

SUMMARY OF NECROPSY FINDINGS

Male Rats

Group & Dose mg/kg	G 2 2000
Necropsy findings	Incidence (No. of rats with findings / Initial no. of rats)
No abnormality detected	5/5

Female Rats

Group & Dose mg/kg	G 2 2000
Necropsy findings	Incidence (No. of rats with findings / Initial no. of rats)
No abnormality detected	5/5

APPENDIX 1

CLINICAL SIGNS AND MORTALITY DATA

Group : G 2 Dose : 2000 mg/kg

Sr. No.	Animal ID	Animal Mark	Observations at: hrs.					Days													
			½	1	2	4	6	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Male																					
1	R091	H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	R092	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	R093	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	R094	HB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	R095	HT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Female																					
6	R096	BT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	R097	HBT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	R098	RHL	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	R099	LHL	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	R100	RFL	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mortality		Total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		%	0																		

*Number of animals died / Number of animals treated

APPENDIX 2

INDIVIDUAL ANIMAL BODY WEIGHTS (g)

Group : G 2 Dose : 2000 mg/kg

Animal ID	Day 0	Day 7	Day 14
Male Rats			
R091	210	215	221
R092	224	230	237
R093	230	238	241
R094	221	228	234
R095	228	236	241
Female Rats			
R096	203	203	213
R097	206	209	215
R098	225	233	235
R099	207	205	214
R100	218	219	227

APPENDIX 3

INDIVIDUAL ANIMAL FATE & NECROPSY FINDINGS

Group: G 2 Dose : 2000 mg/kg

Animal ID	Fate	Necropsy Findings
Male Rats		
R091	TS	NAD
R092	TS	NAD
R093	TS	NAD
R094	TS	NAD
R095	TS	NAD
Female Rats		
R096	TS	NAD
R097	TS	NAD
R098	TS	NAD
R099	TS	NAD
R100	TS	NAD

TS – Terminal Sacrifice NAD – No Abnormalities Detected