DRAFT REPORT

ACUTE DERMAL TOXICITY STUDY OF VIGORILLE CREAM IN WISTAR RATS AS PER OECD GUIDELINE NO. 402

STUDY NO: VLTO-100212

Study Completion Date: xx.xx.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 No. VLTO-100212 entitled "Acute Dermal Toxicity of Vigorille cream in Wistar Rats as per OECD Guideline No. 402, Acute Toxic Class Method" was performed under our supervision. 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 of Vigorille cream in Wistar Rats as per OECD Guideline No. 402, Acute Toxic Class Method”, 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.

TABLE OF CONTENTS

SUMMARY TABLES

APPENDICES

QUALITY ASSURANCE STATEMENT

The Study No.: VLTO-100212, entitled “Acute Dermal Toxicity Study of Vigorille cream in Wistar Rats” has been inspected in the spirit of OECD Principles of Good Laboratory Practice {ENV/MC/CHEM (98) 17:1997}.

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 Management.** 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 VIGORILLE CREAM IN WISTAR RATS

1.2 STUDY NUMBER : VLTO-100212

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 :

a. Study Initiation date : 14.04.2010

b. Date of procurement of animals: 14.04.2010

c. Acclimatization: Start : 14.04.2010 End: 26.04.2010

d. Treatment date : 19.04.2010

e. Necropsy date : 03.05.2010

f. Experiment end date : xx.xx.2010

2.0 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	

3.0 SUMMARY

This study was conducted to generate information concerning the effects of test item Vigorille Cream upon short-term exposure in rats by dermal route. This study also helps to classify and label the test substance. This study provide information to develop dosage regimen in subchronic and other studies and provide information on dermal absorption and the mode of toxic action by dermal route.

Initial testing (limit dose) with 5 male and 5 female rats was performed at a dose level of 2000 mg/kg. Mortality does not occur at this dose level, no further dose levels were tested.

The animals were observed for clinical signs of toxicity and mortality at 30 min, 1h, 2h, 4h and 6h following dosing and thereafter once daily during the 14 day observation period. Body weights were recorded weekly.

At the completion of study, all the animals were sacrificed by CO2 asphyxiation. The animals were subjected to detailed necropsy examination.

Treatment with Vigrolle Cream at 2000 mg/kg dose level was well tolerated.

There were no clinical signs and mortalities noticed at dose tested. There were no changes in body weights at the dose tested. There were no external and internal gross pathological changes in any of the animals.

4.0 Conclusion

The LD50 of Vigorille cream when administered by dermal route was found to be >2000 mg/kg.

5.0 STUDY COMPLIANCE

The study was performed in accordance with the following:

a. The OECD Guidelines for Testing of Chemicals (No. 402, Section 4: Health Effects) “Acute Dermal Toxicity” (Adopted: 24 February 1987).

b. In the spirit of principles of Good Laboratory Practice (1997)

c. The recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, December 15th 1998 and the protocol approved by Institutional Animal Ethics Committee (IAEC).

6.0 OBJECTIVE

The objective of this study was to generate information concerning the effects of test item Vigorille cream upon short-term exposure in rats by dermal route. This study may also help to classify and label the test substance. This study provide information to develop dosage regimen in subchronic and other studies and provide information on dermal absorption and the mode of toxic action by dermal route.

7.0 MATERIALS AND METHODS

7.1 TEST ITEM INFORMATION

The test item information as furnished by the sponsor is as follows

Test Item name	:	VLTO-100212
Physical appearance	:	Pale Yellow Colored Cream
Batch No.	:	N/A
Manufacture date	:	N/A
Expiry date	:	N/A
Storage Conditions	:	+18 to +36^{o}C
Name of the manufacturer & Supplier	:	VEDIC LIFESCIENCES PVT. LTD. 203, Morya Landmark-I, Off Link Road, Andheri (W), Mumbai – 400 053

The responsibility for the correct identity and stability of the test item rests with the sponsor. The certificate of analysis of test item as provided by the sponsor is presented in Annexure 1.

7.2 TEST SYSTEM

7.2.1	**Animal species**	**:**	Rats
7.2.2	**Strain**	**:**	Wistar
7.2.3	**Justification for selection of species**	**:**	Rat is one of the recommended species by regulatory agencies for conducting acute toxicological studies among rodents.
7.2.4	**Source**	**:**	In-house bred animals
7.2.5	**No. of animals/dose & sex**	**:**	Limit dose: 5 Male + 5 Female Females were nulliparous and non-pregnant. Total of 10 male and 15 female animals were received.
7.2.6	**Body weight range at receipt**	**:**	Males: 212.6-229.8 g Females: 203.3-216.6 g
7.2.7	**Age at treatment**	**:**	8 to 9 Weeks
7.2.8	**Identification**	**:**	Ear notching and cage cards.

7.3 PERFORMANCE OF TEST

7.3.1 Husbandry

a). Conditions: Animals were housed under standard laboratory conditions, air-conditioned with adequate fresh air supply (Air changes 12-16 per hour), room temperature 20.1– 24.1^{o}C, relative humidity 53-66 %, with 12 hours light and 12 hours dark cycle. The temperature and relative humidity was recorded daily.

b). Housing: Single animal was housed per cage in standard polypropylene cages (Size: L 430 x B 270 x H 150 mm) with stainless steel top grill mesh having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube.

c). Acclimatization: The animals were acclimatized for a minimum period of five days to laboratory conditions and were observed for clinical signs daily. Veterinary examination of all the animals was recorded on the day of receipt.

d). Diet: The animals were fed *ad libitum* throughout the acclimatization and study period. Nutrilab rodent feed manufactured by M/S Tetragon Chemie Private Ltd, (Vetcare), Bangalore, India was provided.

e). Water: Water was provided *ad libitum* throughout the acclimatization and study period. Deep bore-well water passed through activated charcoal filter and exposed to ultraviolet rays in Aqua guard water filter cum purifier manufactured by Eureka Forbes Ltd., Mumbai, India was provided in plastic water bottles with stainless steel sipper tubes.

7.4 STUDY DESIGN

The following study design was adopted for the study:

Dose (mg/kg)	Animal Number	
	Males	Females
2000	1-5	6-10

Initial testing (Limit dose) with 5 male and 5 female rats was performed at a dose level of 2000 mg/kg. Mortality does not occur at this dose level, no further dose levels were tested.

7.5 ANIMAL PREPARATION

'Before 24 hours of the test item application, the fur was carefully removed from the dorsolateral region of the trunk of test animals by clipping and shaving without abrading the skin. The area shaved was approximately 10% of the total body surface area.

7.6 TREATMENT

The weighed test item was applied as a thin and uniform film on approximately 10% of the total body surface area. The test substance was held on the applied surface by covering the applied region with porous cotton gauze dressing and non-irritating adhesive tape. The test item was held in contact with the skin throughout a 24 hour exposure period. At the end of the exposure period, residual test item was washed using tap water. The test item applied was calculated as per the individual body weight of the animal recorded on the day of application and the dose.

8.0 OBSERVATIONS

The following observations were undertaken during the study.

8.1 CLINICAL SIGNS AND MORTALITY

All the animals were observed for clinical signs and mortality at 30-40 min, 1h (± 10 min), 2h (± 10 min), 4h (± 10 min) and 6h (± 10 min) following dosing and thereafter once daily during the 14 day observation period. One hour after removal of the test patch, the test item applied site was also being observed. Duration of observations was determined by the toxic reactions, rate of onset and length of recovery period. The appearance, change and disappearance of these signs were recorded.

8.2 BODY WEIGHTS

Individual animal body weights were recorded on day 1 before test item application and on day 7 and 14 during the study period.

8.3 PATHOLOGY

At the completion of the study period, the animals were subjected to following pathological examinations:

8.3.1 Necropsy

At the end of observation period, all rats were sacrificed by CO_2 asphyxiation. All animals in the study were subjected to a complete necropsy.

8.3.2 Histopathology

Histopathological examination was not carried out as there were no gross pathological changes noted at necropsy.

9.0 DATA COMPILATION

The computer printout of the data (in the form of appendix) was verified with the original raw data by the study group. The data on body weight and weight change were subjected to computer statistical processing wherever possible. All individual animal data was summarized and presented as tables. All findings were presented in the report as per the standard reporting procedure.

10.0 AMMENDMENTS AND DEVIATIONS

There were no amendments and deviations were occurred during conduct of the study.

11.0 ARCHIVING

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.

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12.0 RESULTS AND DISCUSSION

12.1 CLINICAL SIGNS AND MORTALITY

There were no clinical signs of toxicity and mortalities noticed in dose tested.

Refer Table - 1 and Appendix – 1

12.2 BODY WEIGHTS

No statistically significant change in body weight and percent body weight gain was noted over the study period at the limit dose of 2000 mg/kg.

Refer Table - 2 and Appendix - 2

12.3 PATHOLOGY

There were no external and internal gross pathological lesions noticed in any of the animals sacrificed at the end of the study.

Refer Table - 3 and Appendix – 3

13.0 CONCLUSION

The LD50 of VLTO-100212 when administered by dermal route was found to be >2000 mg/kg.

TABLE - 1

SUMMARY OF CLINICAL SIGNS AND MORTALITY

Refer Appendix - 1

Dose (mg/kg)	No. of Animals	Sex	Clinical signs	Mortality
2000	5	Male	NAD	0/5
	5	Female	NAD	0/5

NAD: No Abnormality Detected

TABLE - 2

SUMMARY OF BODY WEIGHTS (g) AND BODY WEIGHT GAIN (%)

Refer Appendix - 1

Dose (mg/kg)	No. of Animals	Sex		Body weights on day				% Body weight gain	
				1	7	14		1-7	1-14
2000	5	Male		245.92	265.58	288.62		7.99	17.37
				±5.51	±9.28	±6.39		±2.63	±1.44
	5	Female		223.96	233.62	238.90		4.31	6.68
				±5.64	±7.05	±7.45		±1.76	±2.39

Values are in Mean ± SD

TABLE - 3

SUMMARY OF GROSS NECROPSY FINDINGS

Refer Appendix – 3

Dose (mg/kg)	No. of Animals	Sex	Necropsy findings	
			External	Internal
2000	5	Male	NAD	NAD
	5	Female	NAD	NAD

NAD: No Abnormalities Detected

APPENDICES

APPENDIX - 1

INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY RECORD

Dose (mg/kg)	Animal No.	Sex	Study Day 1					Study Days												
			30 m	1h	2h	4h	6h	2	3	4	5	6	7	8	9	10	11	12	13	14
2000	1	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	2	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	3	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	4	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	5	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2000	6	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	7	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	8	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	9	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	10	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: Normal, M: Male, F: Female

APPENDIX – 2

INDIVIDUAL ANIMAL BODY WEIGHTS (g) AND BODY WEIGHT GAIN (%)

Dose (mg/kg)	Animal No.	Sex	Body weights on day				% Body weight gain	
			1	7	14		1-7	1-14
2000	1	M	244.3	263.1	284.3		7.7	16.4
	2	M	253.6	280.3	299.6		10.5	18.1
	3	M	239.8	256.4	286.3		6.9	19.4
	4	M	242.6	268.1	284.3		10.5	17.2
	5	M	249.3	260.0	288.6		4.3	15.8
2000	6	F	218.1	229.7	238.6		5.3	9.4
	7	F	222.4	236.4	240.3		6.3	8.0
	8	F	220.4	225.6	229.7		2.4	4.2
	9	F	232.4	244.1	250.1		5.0	7.6
	10	F	226.5	232.3	235.8		2.6	4.1

M: Male, F: Female

APPENDIX - 3

INDIVIDUAL ANIMAL GROSS NECROPSY FINDINGS

Dose (mg/kg)	Animal No.	Sex	Type of Death	Necropsy findings	
				External	Internal
2000	1	M	TS	NAD	NAD
	2	M	TS	NAD	NAD
	3	M	TS	NAD	NAD
	4	M	TS	NAD	NAD
	5	M	TS	NAD	NAD
2000	6	F	TS	NAD	NAD
	7	F	TS	NAD	NAD
	8	F	TS	NAD	NAD
	9	F	TS	NAD	NAD
	10	F	TS	NAD	NAD

NAD: No Abnormalities Detected, TS: Terminal Sacrifice, M: Male, F: Female