ACUTE ORAL TOXICITY STUDY OF PROSOLUTION PILLS IN WISTAR RATS

STUDY NO: 110501/DM/PC

Study Completion Date: 21.07. 2011

SPONSOR

LEADING EDGE MARKETING
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NASSAU BAHAMAS

SPONSOR'S CONTACT PERSON

DM CONTACT MANAGEMENT
100-645 TYEE ROAD, VICTORIA
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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 ORAL TOXICITY STUDY OF PROSOLUTION PILLS IN WISTAR RATS” was performed under my supervision in compliance with the test guidelines laid down in OECD-420”. 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	Vijay Gokarn
MD, Vedic Lifesciences Pvt. Ltd.	Assistant project manager- Technical

CERTIFICATE

We certify that the work reported here is a true and authentic report of the study entitled, "Acute Oral Toxicity Study of Prosolution Pills in Wistar Rats, as per the OECD guidelines-420 ", based on the experiment conducted in one of the partnered Toxicology Laboratory Services of VEDIC LIFESCIENCES PVT LTD (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 Oral Toxicity Study of Prosolution Pills in Wistar Rats" has been inspected in the spirit of OECD Guidelines.

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

Dates	Inspection Phases	Reporting Dates
	Initiation Phase:	
14.06.2011	Final study plan review	14.06.2011
	In-Life Phase:	
21.06.2011	Formulation preparation, body weight and dosing of sighting study step-I	21.06.2011
23.06.2011	Formulation preparation, body weight and dosing of main study	23.06.2011
05.07.2011	Necropsy of sighting study step-I	05.07.2011
07.07.2011	Necropsy of main study	08.07.2011
	Reporting Phase:	
11.07.2011	Draft report review	11.07.2011
21.07.2011	Final report review	21.07.2011

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 **LEADING EDGE MARKETING** 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.

STATEMENT OF GLP COMPLIANCE

The Study No. 110501/DM/PC "Acute Oral Toxicity Study of Prosolution Pills in Wistar Rats" was performed in the spirit of OECD Principles of Good Laboratory Practices.

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.

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOLS

CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals.

GHS: Globally Harmonized System

OECD: Organization for Economic Co-operation and Development

IAEC: Institutional Animal Ethics Committee

Bwt: Body Weight

g: Gram

h/hr: Hour

kg: Kilogram

min: Minute

mg: milligram

ml :milliliter

n: Number of animals

NAD: No abnormality detected

N: Normal

SD: Standard Deviation

1. STUDY DETAILS

1.1 TITLE : **Acute Oral Toxicity Study of Prosolution Pills in Wistar Rats**

1.2 STUDY NUMBER : **110501/DM/PC**

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

1.4 SPONSOR'S CONTACT PERSON : DM CONTACT MANAGEMENT
100-645 Tyee Road
Victoria, Bc V9a6x5
Canada

1.5 STUDY SCHEDULE :

a. Study Initiation : 14.06.2011

b. Date of procurement of animals: 16.06.2011

c. Acclimatization: Start : 16.06.2011
End : 22.06.2011

d. Treatment date : Sighting Study - Step I: 21.06.2011
Step II: 22.06.2011
Main Study: 23.06.2011

e. Necropsy date : Sighting Study - Step I:
05.07.2011
Step II: 06.07.2011
Main Study: 07.07.2011

f. Experiment end date : 07.07.2011

Study completion date : 21.07.2011

2. MONITORING PERSONNEL

Sr. No.	Designation	Personnel	Signature with date
1.	Assistant project manager-Technical	VIJAY GOKARN VEDIC LIFESCIENCES PVT.LTD MUMBAI	
2.	Managing Director	JAYESH CHAUDHARY VEDIC LIFESCIENCES PVT.LTD MUMBAI	

3. SUMMARY

The test item Prosolution Pills was evaluated for Acute Oral Toxicity in Wistar rats as per the OECD guideline for the testing of chemicals, "Acute Oral Toxicity - Fixed dose procedure", Test No. 420, adopted by the council on 17th December 2001.

A starting dose of 300 mg/kg was selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg body weight as per the sequential dose selection flow chart provided in the Annexure-2 of the OECD Test No. 420 guideline.

The Sighting study was conducted in one female rat by administering 300 mg/kg body weight of Prosolution Pills by oral gavage. There were no clinical signs noticed in sighting study step-I at 300 mg/kg body weight. As per the guideline, sighting study step-II was conducted in one female rat by administering 2000 mg/kg body weight. The animal did not reveal any clinical signs of toxicity and mortality for 24 hours. Hence as per the Annexure-3 of the guideline, main study was conducted in another 4 female rats by administering a dose of 2000 mg/kg body weight of test item by oral gavage as a single dose. All the animals in the study were observed for 14 days. Body weight of all the animals were recorded on day 1(pre-Dose), 7 and 14 of the sighting and main study. On day 15, the animals were subjected to gross necropsy.

The animals at 2000 mg/kg body weight did not reveal any clinical signs of toxicity and mortality. There were no treatment related changes in body weights and body weight gain up to 2000 mg/kg body weight. There were no external and internal gross pathological changes noticed during the necropsy of animals.

Based on the results of the study, the test item Prosolution Pills is non toxic up to 2000 mg/kg body weight when administered as a single dose by oral gavage to Wistar Rats and can be classified GHS category 5/Unclassified according to the Globally Harmonized System (GHS) for classification of chemicals.

4. STUDY COMPLIANCE

The study was performed in accordance with the following:

a. The study was conducted following the OECD Guidelines for Testing of Chemicals (No. 420, Section 4: Health Effects) on conduct of "Acute Oral Toxicity - Fixed Dose Method" (Adopted: 17th December 2001).

b. In the spirit of OECD Principles of Good Laboratory Practices (1997).

c. The standard operating procedures at VEDIC LIFESCIENCES PVT. LTD. and as per the mutually agreed study plan with the sponsor.

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

5. SAFETY PRECAUTIONS

Gloves, cap, face mask was used in addition to protective body garments and rubber slipper to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test item.

6. OBJECTIVE

The objective of this study was to assess the toxic potential of Prosolution Pills when administered by oral gavage in a single dose to female rats at one or more defined doses.

7. MATERIALS AND METHODS

7.1 Test Item Information

The following information was provided about the test article.

Test article : Prosolution pills

Batch No. : T/122

Date of Manufacture : Dec. 2010

Date of Expiry : Nov. 2012

Storage condition : Room Temperature

Sponsor : DM CONTACT MANAGEMENT
100-645 Tyee Road
Victoria, Bc V9a6x5
Canada

7.2 Vehicle

Carboxy methyl cellulose (0. 5% w/v) was used as a vehicle for formulation preparation.

7.3 Justification for selection of vehicle

The test item was not soluble in water, hence Carboxy methyl cellulose (0. 5% w/v) was used as a suspending agent.

Carboxy methyl cellulose is commonly used as vehicle in oral toxicity studies.

7.4 Test System

7.4.1 Animal species : Rats

7.4.2 Strain : Wistar

7.4.3 Justification for selection of species : Rat is one of the recommended species by regulatory agencies for conducting acute toxicological studies among rodents.

7.4.4 Source : In-house bred animals

7.4.5 No. of animals and sex : 6 Female Rats
Female were nulliparous and non-pregnant

7.4.6 Body weight range at receipt: 143.15-158.38g

7.4.7 Age at treatment : 8-9 weeks

7.4.8 Identification : Acclimatization period: Tail marking by marker pen and cage cards
Treatment period: Body marking by turmeric solution and cage cards.

7.5 Methods

7.5.1 Performance of the Test

7.5.1.1 Husbandry

a. Conditions : Animals were housed under standard laboratory conditions, air-conditioned with adequate froesh air supply (Air changes 12-15 per hour), room temperature 20.2 C to 23.8oC, relative humidity 57-62 %, with 12 hours light and 12 hours dark cycle. The temperature and relative humidity was recorded daily.

b. Housing : Maximum of two animals were housed in a standard polypropylene cage (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. Sterilized paddy husk was provided as a bedding material.

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 and on 5th day of acclimatization.

d. Diet :The animals were fed ad libitum throughout the acclimatization and study period. Nutrilab rodent feed (Manufactured by Provimi Animal Nutrition Pvt 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.

7.5.1.2 Study Design

7.5.1.3 Sighting Study

The sighting study was conducted to select appropriate dose for the main study. A starting dose of 300 mg/kg body weight (Step-I) was selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg body weight as per the sequential dose selection flow chart provided in the guideline because of unavailability of sufficient toxicological data on the test item. The flow chart for the sighting study is presented as an Annexure -I.

The test item was administered by oral gavage in a single dose of 300 mg/kg body weight to single female rat for sighting study step-I. There were no clinical signs of toxicity or mortality noticed. And another single female rat was selected randomly for sighting study step-II at 2000 mg/kg body weight, no clinical signs of toxicity or mortality noticed at 2000 mg/kg body weight.

For each sighting study steps a period of 24 hours observation was allowed for any clinical signs and mortality to conduct the main study.

7.5.1.4 Main Study

In the absence of clinical signs of toxicity or mortality in the sighting study step-II, the main study was conducted by using four female rats which was administered by oral gavage in a single dose of 2000 mg/kg body weight.

There were no clinical sign of toxicity or mortality noticed in any of the animals in the tested dose. The flow chart for main study is presented as an Annexure - II.

7.5.1.5 Dose formulation

The weighed test item was finely grinded in a mortar with the help of pestle. The grinded test item was suspended in 0.5% w/v Carboxy methyl cellulose to get desired concentration as per the dose. Formulation of the test item was prepared shortly before

dosing. The homogeneity of the test formulation was maintained by continuous stirring on the magnetic stirrer during dosing.

7.5.1.6 Administration of test item

The animals were fasted overnight prior to dosing (about 16-18 hours). Water was provided during fasting period. The test item was administered by oral gavage to each rat as a single dose, using gavaging needle. The dosage volume administered to individual rat was adjusted according to its body weight recorded on the day of dosing. The dose volume was 10 ml/kg body weight for all animals. Food was offered 3-4 hours followed by dosing.

7.6 Observations

The following observations were undertaken during the study.

7.6.1 Clinical signs and mortality

All the animals were observed for clinical signs and mortality at 30-40min, 1hr (±10min), 2hr (±10min), 3hr (±10min) and 4hr (±10min) on day 1 followed by dosing and thereafter once daily for clinical signs and twice daily for mortality/morbidity during the 14 day observation period.

7.6.2 Body weight

Individual animal body weight was recorded on day 1 before test item administration and on day 7 and 14 during the study period.

7.6.3 Pathology

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

7.6.3.1 Necropsy and gross pathology

All the animals were sacrificed by using CO2 asphyxiation and subjected to necropsy and detailed gross pathological examination was done and the observations were recorded.

8. DATA COMPILATION

The computer printouts of the data (in the form of appendix) were verified with the original raw data. All individual animal data were summarized and presented as tables. All findings were presented in the report as per the standard reporting procedure.

9. AMENDMENTS AND DEVIATIONS

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

10. REPORT DISTRIBUTION

Three copies of the Study Report prepared will be distributed as mentioned below

a). Copy No. 1/3 & 2/3 - Sponsor's copy

b). Copy No. 3/3 - Archives, VEDIC LIFESCIENCES PVT. LTD.

11. AUDITING OF THE REPORT

After completion of the report writing, draft report along with the raw data was sent to the QAU for auditing. After complying with the QAU findings draft report was sent to the Sponsor and based on the feedback, final report will be prepared and again sent to QAU for final audit.

12. ARCHIVING

All materials and data generated from the experiment will be stored at archives of the test facility. The study plan, raw data, specimens (If any), and final report will be maintained for 5 years from the date of completion of the study. A sample of the test item was archived at Vedic Lifesciences Pvt. Ltd. facility for a period of 2 years from the date of completion of the study or till the expiry date whichever is earlier. At the end of this period, the Sponsor's instructions will be sought to either extend the archiving period or return the archived material to the Sponsor or for the material to be disposed off.

13. RESULTS AND DISCUSSSION

13.1 Clinical signs and mortality

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

Refer Table - 1 and Appendix - 1

13.2 Body weight

There were no treatment related changes in body weight and percent body weight gain noticed over the study period at all the doses tested.

Refer Table - 2 and Appendix - 2

13.3 Pathology

There were no gross pathological changes noticed in any of the animals sacrificed at the end of the study.

Refer Table - 3 and Appendix - 3

14. CONCLUSION

From the present study, it was concluded that the test item Prosolution Pills is non toxic up to 2000 mg/kg body weight when administered as a single dose by oral gavage to Wistar Rats and can be classified GHS category 5/Unclassified according to the Globally Harmonized System (GHS) for classification of chemicals.

TABLES

TABLE 1: SUMMARY OF CLINICAL SIGNS AND MORTALITY

Refer Appendix-1

STUDY TYPE	DOSE (mg/kg)	No. OF ANIMALS	SEX	CLINICAL SIGNS	MORTALITY
Sighting Study-Step-I	300	1	Female	N	0/1
Sighting Study-Step-II	2000	1	Female	N	0/1
Main Study	2000	4	Female	N	0/4

N: Normal

TABLE 2: SUMMARY OF BODY WEIGHT (g) AND BODY WEIGHT GAIN (%)

Refer Appendix- 2

STUDY TYPE	DOSE (mg/kg)	No. OF ANIMALS	SEX		BODY WEIGHT ON DAYS			% BODY WEIGHT GAIN	
					1	7	14	1-7	7-14
Sighting Study-Step-I	300	1	Female		147.32	165.13	181.67	12.09	23.32
Sighting Study-Step-II	2000	1	Female		151.70	171.95	180.81	13.35	19.19
Main Study	2000	4	Female	Mean±SD	150.46 ±8.33	174.16 ±11.83	187.35 ±7.31	15.72 ±3.28	24.61 ±2.70

SD: Standard deviation

TABLE 3: SUMMARY OF GROSS PATHOLOGICAL FINDINGS

Refer Appendix -3

STUDY TYPE	DOSE (mg/kg)	No. OF ANIMALS	SEX	NECROPSY FINDINGS	
				EXTERNAL	INTERNAL
Sighting Study-Step-I	300	1	Female	NAD	NAD
Sighting Study-Step-II	2000	1	Female	NAD	NAD
Main Study	2000	4	Female	NAD	NAD

NAD: No abnormalities detected

APPENDICES

APPENDIX 1: INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY RECORD

Study Type	Dose (mg/kg)	Animal No.	Sex	Study Day 1					Study Days												
				30-40 min	1hr (±10 min)	2hr (±10 min)	3hr (±10 min)	4hr (±10 min)	2	3	4	5	6	7	8	9	10	11	12	13	14
Sighting Study - Step-I	300	Ra1311	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sighting Study - Step-II	2000	Ra1312	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Main Study	2000	Ra1313	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Ra1314	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Ra1315	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Ra1316	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N: Normal, F: Female, min: minutes, hr: hours

APPENDIX 2: INDIVIDUAL ANIMAL BODY WEIGHT (g) AND BODY WEIGHT GAIN (%)

Study Type	Dose (mg/kg)	Animal No.	Sex	Body weight on days			% Body weight gain	
				1	7	14	1-7	1-14
Sighting Study - Step-I	300	Ra1311	F	147.32	165.13	181.67	12.09	23.32
Sighting Study - Step-II	2000	Ra1312	F	151.70	171.95	180.81	13.35	19.19
Main Study	2000	Ra1313	F	162.64	188.61	197.80	15.97	21.62
		Ra1314	F	148.41	178.09	182.66	20.00	23.08
		Ra1315	F	146.88	168.62	186.95	14.80	27.28
		Ra1316	F	143.90	161.31	181.98	12.10	26.46

F: Female

APPENDIX 3: INDIVIDUAL ANIMAL GROSS PATHOLOGICAL FINDINGS

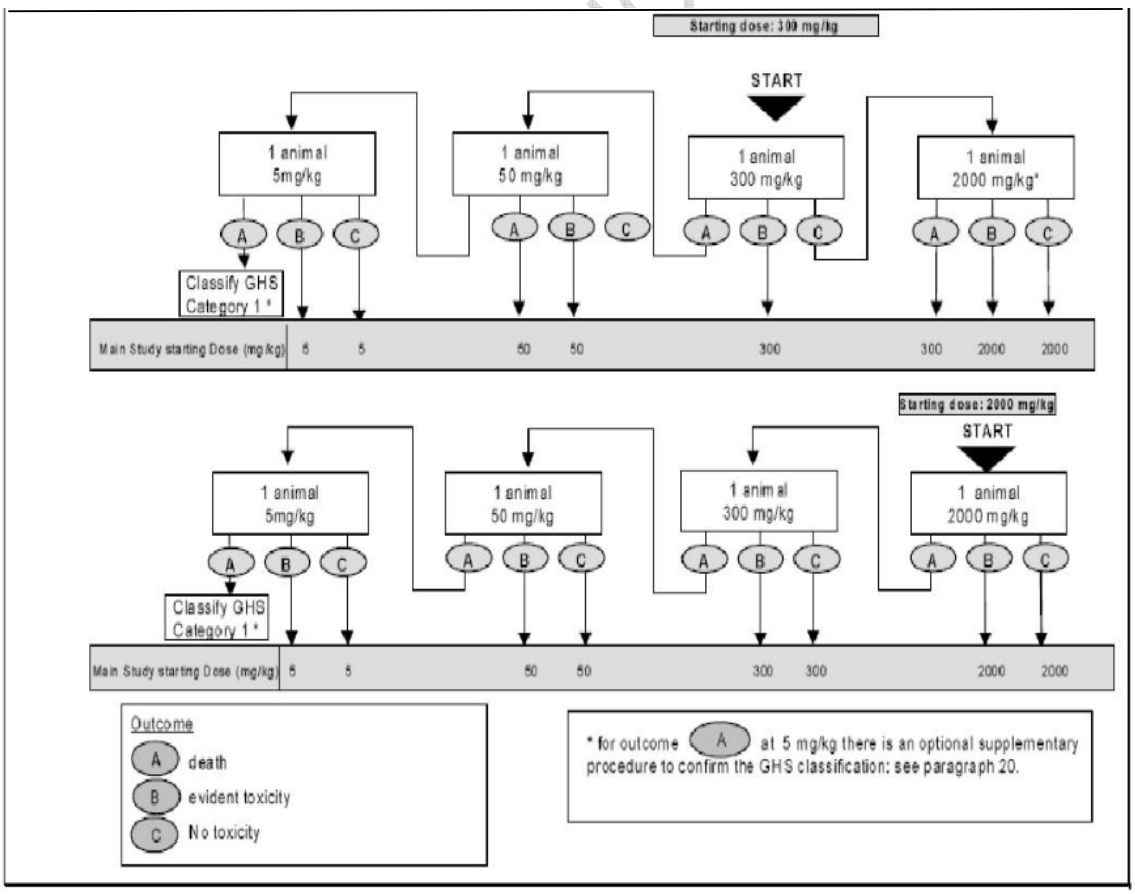
Study Type	Dose (mg/kg)	Animal No.	Sex	Fate	Gross Pathological findings	
					External	Internal
Sighting Study - Step-I	300	Ra1311	F	TS	NAD	NAD
Sighting Study - Step-II	2000	Ra1312	F	TS	NAD	NAD
Main Study	2000	Ra1313	F	TS	NAD	NAD
		Ra1314	F	TS	NAD	NAD
		Ra1315	F	TS	NAD	NAD
		Ra1316	F	TS	NAD	NAD

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

ANNEXURES

ANNEXURE 1: FLOW CHART FOR THE SIGHTING STUDY

OECD/OCDE



ANNEXURE 2. FLOW CHART FOR THE MAIN STUDY

OECD/OCDE