ACUTE ORAL TOXICITY STUDY OF GEN F -20 PLUS SPRAY IN WISTAR RATS

STUDY No.: 110702/DM/PC

SPONSOR
LEADING EDGE MARKETING
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NASSAU BAHAMAS

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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 GEN F-20 PLUS SPRAY 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 GEN F-20 PLUS SPRAY 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 No.: 110702/DM/PC, entitled "Acute Oral Toxicity Study of Gen F-20 Plus Spray 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 the Management and Study Director on the dates given below.

Dates	Inspection Phases	Reporting Dates
	Initiation Phase:	
05.08.2011	Final study plan review	05.08.2011
	In-Life Phase:	
11.08.2011	Formulation preparation, Body weight, dosing and clinical signs observation sighting study Step-1	11.08.2011
13.08.2011	Formulation preparation, Body weight, dosing and clinical signs observation Main study	13.08.2011
25.08.2011	Necropsy of Sighting study – Step-I	25.08.2011
27.08.2011	Necropsy of Main study	27.08.2011

Inspections were performed according to the Standard Operating Procedures of the VEDIC LIFESCIENCES PVT. LTD. 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 which 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.110702/DM/PC "Acute Oral Toxicity Study of Gen F-20 Plus Spray in Wistar Rats" was performed in the spirit of OECD Principles of Good Laboratory Practices {ENV/MC/CHEM (98) 17: 1997}.

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
mL	-	Milliliter
n	-	Number of animals
NAD	-	No Abnormality Detected
N	-	Normal
SD	-	Standard Deviation
mL/kg	-	Milliliter/ Kilogram
%	-	Percent
mg/kg	-	Milligrams per kg
F	-	females
TS	-	Terminal sacrifice

1. STUDY DETAILS

1.1 Study Title : Acute Oral Toxicity Study of Gen F-20 Plus Spray in Wistar Rats

1.2 Study Number : 110702/DM/PC

1.3 Sponsor Details

Sponsor : LEADING EDGE MARKETING
PO Box CR-56766, Suite 1210
NASSAU BAHAMAS

1.5 Test Facility : VEDIC LIFE SCIENCES PVT. LTD.
203, Morya Landmark-1,
Off new link road, Andheri (West),
Mumbai-400 053
INDIA

1.6 Study Schedule :

a. Study Initiation date : 05.08.2011

b. Date of procurement of animals : 06.08.2011

c. Acclimatization: Start : 06.08.2011 End : 12.08.2011

d. Treatment date : Sighting Study - Step I : 11.08.2011
Sighting Study - Step II : 12.08.2011
Main Study : 13.08.2011

e. Necropsy date : Sighting Study - Step I : 25.08.2011
Sighting Study - Step II : 26.08.2011
Main Study : 27.08.2011

f. Experiment end date : 27.08.2011

2. MONITORING PERSONNEL

The following personnel involved in the conduct of the study.

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 Gen F-20 Plus 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.

The Sighting study was conducted in one female rat by administering 10 mL/kg body weight of Gen F-20 Plus Spray by oral gavage. There were no clinical signs noticed in sighting study step-I at 10 mL/kg. As per the guideline, sighting study step-II was conducted in one female rat by administering 20 mL/kg body weight. The animal did not reveal any clinical signs of toxicity and mortality for 24 hours. Hence as per the guideline, main study was conducted in another 4 female rats by administering a dose of 20 mL/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 20 mL/kg body weight did not reveal any clinical signs of toxicity and mortality. There were no treatment related changes in body weight and body weight gain up to 20 mL/kg. 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 Gen F-20 Plus Spray is nontoxic up to 20 mL/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 compliance 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. 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) guidelines for laboratory animals facility published in the gazette of India, December 15th 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 Gen F-20 Plus Spray 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 test item information furnished by the sponsor is presented below:

Common name	: Gen F-20 Plus Spray
Physical Appearance	: Clear liquid with berry flavor
Test Item code by Test facility	: D109 TOX 093
Manufactured Date	: Sept 2010
Expiry Date	: May 2013
Batch No.	: 111037
Storage conditions	: Ambient (+18 to + 36°C)
Name of the Supplier	: D. M. Contact

The responsibility for the correct identity and stability of the test item rests with the sponsor.

7.2 Test System

7.3.1	**Animal species**	**:**	Rats
7.3.2	**Strain**	**:**	Wistar
7.3.3	**Justification for selection of species**	**:**	Rat is one of the recommended species by regulatory agencies for conducting acute toxicological studies among rodents.
7.3.4	**Source**	**:**	In-house bred animals
7.3.5	**No. of animals**	**:**	6 Female Rats. Females were nulliparous and non-pregnant.
7.3.6	**Body weight range at receipt**	**:**	142.13 to 158.63g
7.3.7	**Age at treatment**	**:**	8-9 Weeks
7.3.8	**Identification**	**:**	Acclimatization period: Tail marking by marker pen and cage cards. Treatment period: Body marking by turmeric solution and cage cards.

7.2.1 Performance of the Test

7.2.1.1 Husbandry

a. Conditions **:** Animals were housed under standard laboratory conditions, air-conditioned with adequate fresh air supply (Air changes 12-15 per hour), room temperature 20.4°C to 23.8°C, relative humidity 57-66 %, with 12 hours light and 12 hours dark cycle. The temperature and relative humidity was recorded daily.

b. Housing **:** Minimum of two animals were housed in a standard polypropylene cage (Size: L 430 x B 285 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.

7.2.1.2 Study Design

Sighting Study

The sighting study was conducted to select appropriate dose for the main study. A starting dose of 10 mL/kg (Step-I) was selected because of unavailability of sufficient toxicological data on the test item.

The test item was administered by oral gavage in a single dose of 10 mL/kg 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 20 mL/kg body weight, no clinical signs of toxicity or mortalities noticed at 20 mL/kg.

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

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 20 mL/kg body weight.

There were no clinical sign of toxicity or mortalities noticed in any of the animals in the tested dose.

7.2.1.3 Administration of test item

The animals were fasted overnight prior to dosing. 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 sighting study step-I animals and 20 mL/kg body weight for sighting study step-II and main study animals. Food was offered 3-4 hours followed by dosing.

7.3 Observations

The following observations will be undertaken during the study.

7.3.1 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.

7.3.2 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.

7.3.3 Pathology

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

7.3.3.1 Necropsy and gross pathology

All the animals were sacrificed by using CO_2 asphyxiation and subjected to necropsy and detailed gross pathological examination 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. The data on body weight and body weight gain were subjected to computer statistical processing wherever possible. 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 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. 1/2– Sponsor's copy
b). Copy No. 2/2 – Archives

11. ARCHIVING

All materials and data generated from the experiment will be stored at archives of the test facility. The study plan, raw data and final report will be maintained in the archives of Vedic Lifesciences Pvt. Ltd. for 9 years from the date of completion of the study. A sample of the test item will be archived and maintained for a period of 5 years from the date of completion of the study or till the expiry date whichever is earlier. At the end of archiving 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.

12. RESULTS AND DISCUSSSION

12.1 Clinical signs and mortality

There were no toxicologically significant clinical signs of toxicity/mortality noticed in the doses tested.

Refer Table -1 and Appendix -1

12.2 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

12.3 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

13. CONCLUSION

From the present study, it was concluded that the test item Gen F-20 Plus Spray is nontoxic up to 20 mL/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.

14. TABLES

TABLE 1.SUMMARY OF CLINICAL SIGNS AND MORTALITY

Refer Appendix – 1

Study Type	Dose (mL/kg)	No. of Animals	Sex	Clinical signs	Mortality
Sighting Study - Step-I	**10**	1	Female	N	0/1
Sighting Study - Step-II	**20**	1	Female	N	0/1
Main Study	**20**	4	Female	N	0/4

N: Normal

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

Refer Appendix - 2

Study Type	Dose (mL/kg)	No. of Animals	Sex		Body weight on days			% Body weight gain	
					1	7	14	1-7	1-14
Sighting Study - Step-I	**10**	1	Female		160.50	178.55	182.55	11.25	13.74
Sighting Study - Step-II	**20**	1	Female		170.99	187.28	195.64	9.53	14.42
Main Study	**20**	4	Female	Mean ± SD	**162.13 ±22.48**	**179.56 ±17.33**	**192.21 ±14.17**	**11.23 ±4.66**	**19.35 ±7.80**

SD: Standard Deviation

TABLE 3. SUMMARY OF GROSS PATHOLOGICAL FINDINGS

Refer Appendix - 3

Study Type	Dose (mL/kg)	No. of Animals	Sex	Necropsy findings	
				External	Internal
Sighting Study - Step-I	**10**	1	Female	NAD	NAD
Sighting Study - Step-II	**20**	1	Female	NAD	NAD
Main Study	**20**	4	Female	NAD	NAD

NAD: No Abnormalities Detected

15. APPENDICES

APPENDIX 1. INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY RECORD

Study Type	Dose (mL/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	10	Ra1507	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sighting Study - Step-II	20	Ra1508	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Main Study	20	Ra1509	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Ra1510	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Ra1511	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		Ra1512	F	N	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) BODY WEIGHT GAIN (%)

Study Type	Dose (mL/kg)	Animal No.	Sex	Body weight on days			% Body weight gain	
				1	7	14	1-7	1-14
Sighting Study - Step-I	**10**	Ra1507	F	160.50	178.55	182.55	11.25	13.74
Sighting Study - Step-II	**20**	Ra1508	F	170.99	187.28	195.64	9.53	14.42
Main Study	**20**	Ra1509	F	145.67	166.36	184.28	14.20	26.51
		Ra1510	F	141.15	163.57	176.73	15.88	25.21
		Ra1511	F	188.06	198.89	207.51	5.76	10.34
		Ra1512	F	173.64	189.41	200.31	9.08	15.36

F: Female

APPENDIX 3. INDIVIDUAL ANIMAL GROSS PATHOLOGICAL FINDINGS

Study Type	Dose (mL/kg)	Animal No.	Sex	Fate	Gross Pathological findings	
					External	Internal
NAD	**10**	Ra1507	F	TS	NAD	
NAD	**20**	Ra1508	F	TS	NAD	
Main Study	**20**	Ra1509	F	TS	NAD	NAD
		Ra1510	F	TS	NAD	NAD
		Ra1511	F	TS	NAD	NAD
		Ra1512	F	TS	NAD	NAD

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