Vedic Lifesciences
Competent Reliable Optimal

STUDY REPORT

STUDY No.

190909/ NNB/ PC

STUDY TITLE

ACUTE ORAL (GAVAGE) TOXICITY OF L-3-AMINOISOBUTYRIC ACID IN FEMALE SPRAGUE DAWLEY RATS

Sponsor's name and Address

NNB Nutrition China
Room 2009, Suning Huigu Building
#E8-2, No 268 Jiqingmen Street,
Nanjing, 210017, China

Facility Address

Vedic Lifesciences Pvt. Ltd.
118, Morya House, Andheri West,
Mumbai, Maharashtra
India

Vedic Lifesciences
Competent Reliable Optimal

Study No. 190909/ NNB/ PC

Study Report Approval

On Behalf of Vedic Lifesciences Pvt. Ltd.

Aswalkar

December 27, 2019
Date/Month/Year

Study Monitor- Dr Dipti Aswalkar

Yadav

December 27, 2019.
Date/Month/Year

QAU- Anil Yadav

On behalf of Sponsor

Date/Month/Year

Mina Wang

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in accordance with OECD Principles of Good Laboratory Practice (GLP), document no. ENV/MC/CHEM (98)17 (as revised in 1997) and adopted by the decision of the OECD Council [C(97)186/Final]. This study was performed in accordance with the Standard Operating Procedures and signed Study Plan.

There were no circumstances that might have affected the quality or integrity of the study.

No circumstances in the study have been left unreported which might have influence on the quality or integrity of the study.

This is also to certify that the results presented in this report are complete, true and accurate reflection of the raw data obtained during the conduct of the study and I, hereby accept the responsibility for the validity of the data.

A Yadav

December 27, 2019.
Date/Month/Year

Mr. Anil Yadav
Quality Assurance

TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS

°C	Degree Celsius
B. wt	Body weight
Conc.	Concentration
CoA	Certificate of Analysis
F	Female(s)
G	Gram (s)
GHS	Globally Harmonised System
H	Hour/s
Kg	Kilogram/s
LD_{50}	Lethal Dose 50%
Mg	Milligram
Min	Minute/s
mL	Milliliter
RT	Room Temperature
RH	Relative Humidity
SOP	Standard Operating Procedure
UDP	UP and Down Procedure

2. SUMMARY

Acute oral toxicity study was conducted to evaluate the toxicity of L-3-Aminoisobutyric Acid upon a single oral administration to Sprague Dawley Rats followed by observation for 14 days. The study was also intended to identify the LD_{50} of L-3-Aminoisobutyric Acid. The method followed was as per the OECD Guidelines for Testing of Chemicals, Number 425.

L-3-Aminoisobutyric Acid was initially tested at a dose of 175 mg/kg b.wt. in Step 1 with one female rat, slope 4 was followed for this study. Based on the survival pattern of the previously dosed animal after 48 hours, further doses were decided as per OECD TG 425 StatPgm.

The experiment consisted of seven steps with one female rat at each step treated with L-3-Aminoisobutyric Acid by oral gavage administration at a dose of 175 mg/kg b.wt. for Step 1, 310 mg/kg b.wt. for Step 2, 550 mg/kg b.wt. for Step 3, 980 mg/kg b.wt. for Step 4; and 2000 mg/kg b.wt. for Step 5, 6 & 7. The test item was formulated in vehicle (distilled water) at a concentration of 17.5, 31.0, 55.0, 98.0 mg/mL for step 1, 2, 3 & 4, respectively; and 200 mg/mL for step 5, 6 & 7 respectively. The test item was administered at a dose volume of 10 mL/kg body weight.

The animals were observed twice daily throughout the treatment period for mortality and moribundity. The animals were also observed for clinical signs approximately at 30 minutes, 1, 2, 3 and 4 hours on day 0 post dosing; and twice daily during the experimental i.e. day 1 to day 14 for each step. The body weight was recorded on day 0 (prior to dosing), day 7 and day 14. At the end of experimental period (day 14), the animals were euthanized and subjected to a detailed gross pathological examination.

No treatment related clinical signs, mortality and moribundity was observed throughout the experiment period.

All the animals gained body weight over the course of the study as compared to day 0. There were no gross pathological changes observed in any of the treated animals.

Conclusion

Based on the results of this study, the median lethal dose (LD_{50}) of L-3-Aminoisobutyric Acid after single oral administration to female Sprague Dawley Rats, observed over a period of 14 days is greater than 2000 mg/kg body weight, and the test item is classified as 'Category 5' based on The Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

3. OBJECTIVE

The objective of this study was to assess the toxicity of L-3-Aminoisobutyric Acid following single oral (gavage) administration to female Sprague Dawley rats.

4. TEST GUIDELINE

The study procedures described in the study report meet the requirements of the following guideline:

OECD Guidelines for Testing of Chemicals Section 4: Health Effects: No. 425, Acute Oral Toxicity – Up-and-Down-Procedure (UDP); Adopted: 3rd October 2008.

5. SAFETY PRECAUTIONS

Safety measures were adopted to ensure adequate personal health and safety. Aprons, gloves, cap and face mask were used in addition to protective laboratory wares and rubber slipper to avoid inhalation or skin contact with the test item.

6. AMENDMENT AND DEVIATION PROCEDURES

There was no amendment or deviation to the study plan generated.

7. STUDY INFORMATION

Study title	:	Acute Oral Toxicity Study (Up and Down Procedure) of L-3-Aminoisobutyric Acid in Sprague Dawley Rats.
Study number	:	190909/ NNB/ PC
Monitoring Scientist	:	Dr. Dipti Aswalkar

8. STUDY SCHEDULE

Study Initiation Date		:	17 October 2019
Experimental Start Date		:	17 October 2019
Acclimatization period	Step-1	:	17 October 2019 to 22 October 2019
	Step-2	:	17 October 2019 to 25 October 2019
	Step-3	:	17 October 2019 to 28 October 2019

	Step-4	:	17 October 2019 to 01 November 2019
	Step-5	:	17 October 2019 to 04 November 2019
	Step-6	:	17 October 2019 to 07 November 2019
	Step-7	:	17 October 2019 to 11 November 2019
Treatment Dates	Step-1	:	23 October 2019
	Step-2	:	26 October 2019
	Step-3	:	29 October 2019
	Step-4	:	02 November 2019
	Step-5	:	05 November 2019
	Step-6	:	08 November 2019
	Step-7	:	12 November 2019
Necropsy	Step-1	:	06 November 2019
	Step-2	:	09 November 2019
	Step-3	:	12 November 2019
	Step-4	:	16 November 2019
	Step-5	:	19 November 2019
	Step-6	:	22 November 2019
	Step-7	:	26 November 2019
Experimental Completion Date		:	26 November 2019
Draft Report Submission Date		:	11 December 2019
Study Completion date		:	27 December 2019

9. TEST ITEM INFORMATION

The details of the test item information given in the following section were as provided by the sponsor (Refer Annexure 3 -CoA)

Test Item name	: L-3-Aminoisobutyric Acid
Appearance	: White crystalline powder
Batch Number	: 20190801
Assay (Dry Basis)	: 99.64%
Manufacture date	: August 17, 2019

Assay (Dry Basis)	:	99.64%
Manufacture date	:	August 17, 2019
Expiry date	:	August 16, 2021
Storage conditions	:	Stored in tightly sealed container, away from moisture and direct sunlight (ambient temperature).

Note: The identity, stability and composition of the test item were the responsibility of the Sponsor. No analysis of the test item was conducted at Test facility to confirm it.

9.1. VEHICLE AND JUSTIFICATION

Based on the solubility and syringibility test distilled water was selected as a vehicle.

9.2. DOSE FORMULATIONS

The dose formulations were prepared shortly before each dosing for all the steps.

The required quantity of test item (175.01 mg for Step 1, 310.09 mg for Step 2, 550.05 mg for Step 3, 980.03 mg for Step 4, 2000.02 mg for Step 5, 2000.05 mg for Step 6 and 2000.03 mg for Step 7) was received from Test Item Control Office and was transferred to a mortar and triturated with pestle. 3-4 mL of the vehicle was added to the mortar and again triturated. This was then transferred to a calibrated volumetric flask. 1-2 mL of vehicle was used twice to rinse the mortar and pestle. Sufficient quantity of vehicle was then added to make up to the final volume of 10 mL. This was then transferred back to the beaker and mixed properly.

10. TEST SYSTEM DETAILS

Animal species	:	Rats
Strain	:	Sprague Dawley
Justification for selection of species	:	Recommended by the guideline
Sex	:	Female (nulliparous and non-pregnant)
Number of animals per step	:	1 animal per step.

Age of animals	:	9 - 12 weeks
Body weight range	:	166.45 to 195.61 g at the time of dosing (weight variation was within an interval of ± 20% of mean weight of previously dosed animals)
Total No. of Animals	:	15 animals were received for the study and 7 animals were used for the treatment. Unused animals were returned to the animal house.
Identification	:	The animals were marked (towards the tip of tail) with the temporary animal numbers at start of acclimatization. The animals were marked with permanent animal numbers (towards the base of tail) with different colour indelible marker pen before the start of test item administration. Each cage card was having details like Study No., Study Code, Species & Strain, Step, Dose, Sex, Animal No., Cage No.

11. ANIMAL HUSBANDRY

a). Acclimatization: Animals were acclimatized for minimum 5 days prior to the treatment. At the time of receipt and before selection for dosing, animals were subjected to health assessment and only animals without any visible sign of illness were used for the study.

b). Environmental Conditions: Animals were housed with adequate fresh air supply (12-15 air changes per hour), temperature of 19.8 to 23.8 °C, relative humidity range of 41 to 66 % and photo period of 12 h light and 12 h dark. The temperature and relative humidity was recorded once daily.

c). Housing: One animal per cage was housed in polycarbonate cages (size: 410 X 282 X 150 mm as length, width and height respectively) with stainless steel top grills having facilities for holding feed and water. Enrichment was provided in the individual cage.

d). Bedding: Sterilized Comfort (Corn Cob), Batch number: 015 was used as bedding material. Chemical and microbial analysis reports were archived along with study file.

e). Feed: Rat/Mice Pellet feed (Batch Number: 023) was provided *ad libitum* Proximate, contaminant and microbial analysis report were maintained along the study file.

f). Water: Reverse osmosis water was provided *ad libitum* throughout the study period. The results of analysis of water samples were maintained along the study file.

g). Animal Selection: The animals were manually selected in such a way that the weight variation was within ± 20 % of previously dosed animals. To enable this, the animals with highest body weight were selected at each step.

12. EXPERIMENTAL DESIGN

Step	Dose	No. of Animals	Animal Number
Step - 1	175 mg/Kg b.wt.	1	001
Step - 2	310 mg/Kg b.wt.	1	002
Step - 3	550 mg/Kg b.wt.	1	003
Step - 4	980 mg/Kg b.wt.	1	004
Step - 5	2000 mg/Kg b.wt.	1	005
Step - 6	2000 mg/Kg b.wt.	1	006
Step - 7	2000 mg/Kg b.wt.	1	007

12.1. DOSE SELECTION

Since there is no information about toxicity or LD_{50} of test item, the starting dose of 175 mg/kg/b.wt was selected as per guidelines' recommendation. As per the sponsor suggestion Slope 4 was followed for this study. Based on the mortality or survival pattern of the previously dosed animal after 48 hours, further doses were decided as per OECD TG 425 StatPgm.

12.2. 12.2. ROUTE OF ADMINISTRATION

Test item was administered by oral route and it was selected based on the guideline requirement.

12.3. JUSTIFICATION FOR CHOICE OF VEHICLE

Based on the solubility and syringibility test distilled water was selected as a vehicle.

12.4. DOSE FORMULATION PREPARATION

The test item was formulated in distilled water. The dose formulations were prepared shortly before each dosing for all the steps. The required quantity of test item (175.01 mg for Step 1, 310.09 mg for Step 2, 550.05 mg for Step 3, 980.03 mg for Step 4, 2000.02 mg for Step 5, 2000.05 mg for Step 6 and 2000.03 mg for Step 7) was received from Test Item Control Office and was transferred to a mortar and triturated with pestle. 3-4 mL of the vehicle was added to the mortar and again triturated. This was then transferred to a calibrated volumetric flask. 1-2 mL of vehicle was used twice to rinse the mortar and pestle. Sufficient quantity of vehicle was then added to make up to the final volume of 10 mL. This was then transferred back to the beaker and mixed properly.

12.5. TREATMENT

The animals received a single dose of the test item based on their body weight by oral gavage administration after being fasted overnight (approximately 15 hours), but with free access to water. Food was supplied approximately 3 hours post treatment. The dose administered to individual rat was adjusted according to its body weight recorded prior to dosing. The dose volume was 10 mL/kg body weight.

Note: Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer. The left-over formulations were disposed as per in house Standard Operating Procedure.

13. IN-LIFE OBSERVATIONS

The following observations were carried out during the study.

13.1. MORBIDITY/MORTALITY

All animals were observed for morbidity/mortality twice daily during the study period (once on holidays).

13.2. CLINICAL SIGNS OF TOXICITY

Animals were observed for clinical signs of toxicity, at least once daily during acclimatization, at 30 min and 1, 2, 3 and 4 hrs post-dosing on day 0; and thereafter twice daily for the 14-day observation period (once on holidays and day of sacrifice for each step). Animals were observed for changes in skin, fur, eyes and mucous membranes and respiratory, circulatory and nervous system.

13.3. BODY WEIGHT

Individual animal body weights were recorded on the day of animal receipt, selection and on the day of dosing before administration of test item (day 0) and on Day 7 and 14. Percent body weight changes were calculated.

13.4. NECROPSY

At the end of the observation period, all the surviving animals were sacrificed using carbon dioxide asphyxiation in euthanasia chamber and subjected to gross pathological evaluation.

13.5. STATISTICAL ANALYSIS

AOT425 StatPgm was used to select the doses, stopping criterion and estimation of LD_{50}.

14. RESULTS

14.1. CLINICAL SIGNS, MORBIDITY/MORTALITY

No clinical signs, mortality and moribundity was observed in any of the test item treated animals (Table 1).

14.2. BODY WEIGHT

All the animals gained body weight over the course of the study in all the steps as compared to day 0 (Refer Table 2).

14.3. GROSS PATHOLOGY

No gross pathological changes were observed in any of the treated animals. (Table 3)

15. CONCLUSION

Based on the results of this study, the median lethal dose (LD_{50}) of L-3-Aminoisobutyric Acid after single oral administration to female Sprague Dawley Rats, observed over a period of 14 days is **greater 2000 mg/kg body weight** and it is classified as **'Category 5'** based on The Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

16. ARCHIVING

The study plan, raw data, slides and study report will be maintained in the archives of Vedic Lifesciences Pvt Ltd. for 9 years from the date of completion of the study. The soft copies of the study plan, study report and compiled data will be copied to compact disc and will be archived for 9 years from the date of completion of the study. A sample of the test item will be maintained in the archives of Vedic Lifesciences Pvt Ltd. till the expiry date of the test item. At the end of the archiving period, the sponsor's instructions will be sought either to extend the archiving period or to return the archived material to the sponsor or for the disposal.

17. REFERENCES

a. Compendium of CPCSEA 2018, Ministry of Environment, Forests and Climate Change, Government of India.

b. Guide 2011 - Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources. Commission on Life Sciences. National Research Council. National Academy Press. Washington, D.C. 2011.

c. Guidance Document on the Recognition, Assessment and Use of Clinical signs as humane endpoints for Experimental Animals used in Safety Evaluation. ENV/JM/MONO (2000)7. OECD, December, 2000.

d. OECD Guidelines for Testing of Chemicals No. 425, "Acute Oral Toxicity – Up and Down Procedure", adopted 03rd October 2008.

e. United Nations Economics Commission for Europe: Globally Harmonized System of Classification and Labelling of Chemicals (GHS); ST/SG/AC.10/30/Rev.7, 2017.

TABLE 1 - SUMMARY OF CLINICAL SIGN OBSERVATION AND MORBIDITY/MORTALITY

Step - 1 **Dose: 175 mg/kg Body weight** **Sex: Female**

Animal No.	Day																															
	0						1		2		3		4		5		6		7		8		9		10		11		12		13		14
	hour(s)						I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I
	PD	0.5	1	2	3	4																											
001	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0

Step - 2 **Dose: 310 mg/kg Body weight** **Sex: Female**

Animal No.	Day																															
	0						1		2		3		4		5		6		7		8		9		10		11		12		13		14
	hour(s)						I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I
	PD	0.5	1	2	3	4																											
002	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0

Step - 3 **Dose: 550 mg/kg Body weight** **Sex: Female**

Animal No.	Day																															
	0						1		2		3		4		5		6		7		8		9		10		11		12		13		14
	hour(s)						I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I
	PD	0.5	1	2	3	4																											
003	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0

Step - 4 **Dose: 980 mg/kg Body weight** **Sex: Female**

Animal No.	Day																															
	0						1		2		3		4		5		6		7		8		9		10		11		12		13		14
	hour(s)						I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I
	PD	0.5	1	2	3	4																											
004	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0

PD: Pre dose; I: 1st observation; II: 2nd observation; 0: Normal; -: Not observed; 0.5 hours: 30 minutes

TABLE 1 Continued

Step - 5 **Dose: 2000 mg/kg Body weight** **Sex: Female**

Animal No.	Day																															
	0						1		2		3		4		5		6		7		8		9		10		11		12		13		14
	hour(s)						I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I
	PD	0.5	1	2	3	4																									
005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	0	0

Step - 6 **Dose: 2000 mg/kg Body weight** **Sex: Female**

Animal No.	Day																															
	0						1		2		3		4		5		6		7		8		9		10		11		12		13		14
	hour(s)						I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I
	PD	0.5	1	2	3	4																									
006	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	0	0	0	0

Step - 7 **Dose: 2000 mg/kg Body weight** **Sex: Female**

Animal No.	Day																															
	0						1		2		3		4		5		6		7		8		9		10		11		12		13		14
	hour(s)						I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I
	PD	0.5	1	2	3	4																									
007	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0

PD: Pre dose; I: 1st observation; II: 2nd observation; 0: Normal; -: Not observed; 0.5 hours: 30 minutes

TABLE 2 - INDIVIDUAL ANIMAL BODY WEIGHT (G) AND DOSE ADMINISTRATION DETAILS

Step & Dose (mg/kg b.wt.)	Animal No.	Body weight (g)	Dose Volume (mL)	Body weight (g)		% Change in Body weight	
		Day 0		Day 7	Day 14	Day 0-7	Day 0 -14
Step 1 & 175	001	195.61	2.0	206.61	212.49	5.62	8.63
Step 2 & 310	002	195.41	2.0	209.73	226.21	7.33	15.71
Step 3 & 550	003	193.23	1.9	209.80	225.94	8.58	16.93
Step 4 & 980	004	187.92	1.9	203.01	221.09	8.03	17.65
Step 5 & 2000	005	167.73	1.7	178.91	185.73	6.67	10.73
Step 6 & 2000	006	166.45	1.7	182.34	200.02	9.55	20.17
Step 7 & 2000	007	169.94	1.7	188.09	195.93	10.68	15.29

TABLE 3 - INDIVIDUAL ANIMAL GROSS PATHOLOGICAL FINDINGS

Step & Dose (mg/kg b.wt.)	Animal Number	Fate of animals	External	Internal
Step 1 & 175	**001**	TS	NAD	NAD
Step 2 & 310	**002**	TS	NAD	NAD
Step 3 & 550	**003**	TS	NAD	NAD
Step 4 & 980	**004**	TS	NAD	NAD
Step 5 & 2000	**005**	TS	NAD	NAD
Step 6 & 2000	**006**	TS	NAD	NAD
Step 7 & 2000	**007**	TS	NAD	NAD

TS: Terminal Sacrifice; NAD = No Abnormality Detected

ANNEXURE 1 - AOT425 STATPGM (VERSION: 1.0) TEST RESULTS AND RECOMMENDATIONS

Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program
Date/Time: 29 November 2019, 17:17:13
Data file name: work.dat
Last modified: 29/11/2019 17:17:13
Test type: Main Test
Limit dose (mg/kg): 2000
Assumed LD_{50} (mg/kg): Default
Assumed sigma (mg/kg): 0.249999
Recommended dose progression: 2000, 980, 550, 310, 175, 98, 55, 31, 17.5, 9.8, 5.5, 3.1, 1.75

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	001	175	O	O
2	002	310	O	O
3	003	550	O	O
4	004	980	O	O
5	005	2000	O	O
6	006	2000	O	O
7	007	2000	O	O

(X = Died, O = Survived)
Dose Recommendation: The main test is complete.
Stopping criteria met: 3 at Limit Dose.

SUMMARY OF LONG-TERM RESULTS:

Dose (mg/kg)	O	X	Total
175	1	0	1
310	1	0	1
550	1	0	1
980	1	0	1
2000	3	0	3
All Doses	7	0	7

Statistical Estimate based on long term outcomes: **The LD_{50} is greater than 2000 mg/kg.**

ANNEXURE 2- DOSE PROGRESSIONS - OECD TG 425

Slope = 1	2	3	4	5	6	7	8
		37.5			37.5		
				44			41
						47	
	55		55		55		55
						65	
				69			73
		81			82		
			99			91	97
				109	120		
						126	129
175	175	175	175	175	175	175	175
						240	230
				275	260		
			310			340	310
		375			375		
				440			410
						470	
	550		550		550		550
						650	
				690			730
		810			820		
			990			910	970
				1090	1200		
						1260	1290
1750	1750	1750	1750	1750	1750	1750	1750
						2400	2300
				2750	2600		
			3100				3100
					3750	3400	
							4100
5000	5000	5000	5000	5000	5000	5000	5000

ANNEXURE 3 - CERTIFICATE OF ANALYSIS L-3-AMINOISOBUTYRIC ACID

Nanjing Nutrabuilding Bio-tech Co., Ltd.

CERTIFICATE OF ANALYSIS
L-3-Aminoisobutyric Acid

Product Name	L-3-Aminoisobutyric Acid		
Batch No.	20190801	Quantity	Sample
Manufacturing Date	2019.08.17	Expiry Date	2021.08.16

Test Item	Specification	Result	Method
Physical and Chemical Examination			
Appearance	White crystalline powder	Conforms	Organoleptic
Loss on Drying	NMT 0.5%	0.12%	USP<731>
Assay (Dry Basis)	NLT 98.0%	99.64%	Titration
D-3-Aminoisobutyric Acid	NMT 1.0%	Not detected	HPLC
Residue on Ignition	NMT 0.2%	0.04%	USP<281>
Heavy Metals	NMT 20 ppm	< 20 ppm	ICP-MS
Arsenic (As)	NMT 2.0 ppm	< 2.0 ppm	ICP-MS
Lead (Pb)	NMT 2.0 ppm	< 2.0 ppm	ICP-MS
Mercury (Hg)	NMT 1.0 ppm	< 1.0 ppm	ICP-MS
Cadmium (Cd)	NMT 1.0 ppm	< 1.0 ppm	ICP-MS
Microbiological Examination			
Total Plate Count	NMT 10000 cfu/g	< 10000 cfu/g	USP<61>
Yeast & Mold	NMT 1000 cfu/g	< 1000 cfu/g	USP<61>
Coliform	NMT 10 mpn/g	< 10 mpn/g	MPN
E.Coli	Negative in 10 g	Negative	USP<62>
Salmonella	Negative in 10 g	Negative	USP<62>
S.Aureus	Negative in 10 g	Negative	USP<62>
Others			
Storage Condition	Stored in tightly sealed container, away from moisture and direct sunlight.		
Packing	Double PE bags in a net 25kg cardboard drum.		
Shelf Life	24 months from manufacturing date.		
Conclusion	Meet the Enterprise Standard.		

Jiangsu Life Science and Technology Innovation Park, Xixia District, Nanjing, China 210046

ANNEXURE 4 - CERTIFICATE OF ANALYSIS OF FEED

VRK Nutritional Solutions
VRK's "Scientist's Choice" Laboratory Animal Feed
202, Ganga Celidium (Dhan Ganga Business Centre),
Gangadham Phase - I, Bibwewadi-Kondhwa Road, Pune - 411 037. India.
Tel. : +91 20 2424 1169. E-mail : vrkgroup2009@gmail.com
www.vrknutritionalsolutions.com

CERTIFICATE OF ANALYSIS

Name of the Product: Rat/Mice Pellet Feed

Description: A whitish brown colored pellets
Pellet Size : 10-14 mm Solid Pellets
Batch No: 023

Date of Mfg.: 13.08.2019
Date of Sampling: 14.08.2019
Date of Reporting: 14.08.2019
Date of Expiry :12.08.2020

1. **Proximate analysis :**

No.	Test parameters	Detected values(in percentage)	Standard values(in percentage)
1	Moisture	8.48	12 MAX
2	Crude Protein	18.25	18 MIN
3	Crude Fat	3.54	3 MIN
4	Crude fiber	5.40	6 MAX
5	Calcium	1.20	1 MIN
6	Phosphorus	0.54	0.5 MIN
7	Total ash	6.00	6 MAX
8	Carbohydrates	64.00	60-65 MAX
9	Energy	3090 kcal/kg	3000 kcal/kg MIN

2. **Microbiological examination:**

No.	Test parameters	Detected values	Standard values
1	Total Bacterial count	36 Cfu * /gm	$1*10^4$
2	Escherichia coli	NIL**	NIL
3	Pseudomonas aeruginosa	NIL**	NIL
4	Staphylococcus aureus	NIL**	NIL
5	Salmonella spp	NIL**	NIL
6	Total mould count	00 Cfu * /gm	1-2
7	Aflatoxin B1	<10 ppb	<10 ppb
8	Aflatoxin B2	<10 ppb	<10 ppb
9	Aflatoxin G1	<10 ppb	<10 ppb
10	Aflatoxin G2	<10 ppb	<10 ppb

* cfu - colony forming unit.
**Nil : Not detected in first dilution (microorganisms below detectable limits)

Instructions : 1. Store the feed in cool, dry and well ventilated place off the floor.
2. Use within specified period.
3. Stop usage of feed if found defective.

For VRK Nutritional Solutions

Dr. [illegible] Kulkarni

VRK NUTRITIONAL SOLUTIONS PUNE MICRO 047

Authorized Photo copy 12/10/2019
(Signature Date)

ANNEXURE 5 - CERTIFICATE OF ANALYSIS OF WATER

BHAGAVATHI ANA LABS

TEST REPORT

Certificate No.: TC-5040
NABL HY-001
ULR : TC504019000008482P

	Test Report No.	BALPL/19-20/CL-1738	
	Lab Sample Code	27173807	
	Issue Date	10/08/2019	
	Customer Ref.	PO No. ERF/PO/19-20/044	
	Ref.Date	27/07/2019	
Sample Details: RO Water	Date of Registration		27/07/2019
	Date of commencement of testing		27/07/2019
	Date of completion of testing		03/08/2019
	Sample Condition of receipt		Found Ok
Sample Collected By: Mr. John Kennedy			Ambient
Qty. Received: 5ltr x 1No. and 1ltr x 1no.	SAMPLE TESTED AS RECEIVED		
Tests Required: As per Quote : IND.BH.41.19.0577/FOC, Dated : 26/07/2019			

Sample collected and submitted by the representative of BALPL

TEST RESULTS

S.No.	Test Parameters	UOM	Test Method	Requirement (Acceptable Limit) as per IS 10500:2012	Permissible Limit in the absence of alternate source as per IS 10500:2012	Result
	Table 1 Organoleptic and Physical Parameters					
1	Colour	Hazen Units	IS:3025 (P.4)	5 max	15 max	<1
2	Odour	-	IS:3025 (P.5)	Agreeable	Agreeable	Agreeable
3	pH	-	IS:3025 (P.11)	6.5 to 6.5	No Relaxation	6.8
4	Taste*	-	IS:3025 (P.7&8)	Agreeable	Agreeable	Agreeable
5	Turbidity	NTU	IS:3025 (P.10)	1 max	5 max	1
6	Total Dissolved Solids	mg/l	IS:3025 (P.16)	500 max	2000 max	49
	Table 2 General Parameters Concerning Substances Undesirable in Excessive amounts					
7	Aluminium as Al*	mg/l	IS 3025 (P.2)	0.03 max	0.2 max	<0.01
8	Ammonia (as total ammonia-N)	mg/l	APHA 4500 NH3-F	0.5 max	No relaxation	<0.05
9	Anionic Detergents as MBAS	mg/l	Annex K of IS 13428-2005	0.2 max	1.0 max	<0.1
10	Barium as Ba*	mg/l	IS 3025 (P.2)	0.7 max	No relaxation	<0.01
11	Boron as B*	mg/l	IS 3025 (P.2)	0.5 max	1.0 max	<0.01
12	Calcium as Ca	mg/l	IS:3025 (P.40)	75 max	200 max	4.4
13	Chloramines as Cl2*	mg/l	IS:3025 (P.26)	4.0 max	No relaxation	<0.1
14	Chlorides as Cl	mg/l	IS:3025 (P.32)	250 max	1000 max	10.2
15	Copper as Cu*	mg/l	IS 3025 (P.2)	0.05 max	1.5 max	<0.01
16	Fluoride as F	mg/l	APHA 4500 F-D	1.0 max	1.5 max	1.02

Hymavathi
Senior chemist
Authorized Signatory

Bhagavathi Ana Labs Pvt. Ltd.
(A Bureau Veritas Group Company)
Plot No. 7-2 C 7 & 8/4, Industrial Estate,
Sanathnagar, Hyderabad 500 018
TELANGANA, INDIA
Tel.: +91 40 68144100 / 68144101

Authorised Photo copy 17/10/2019

ANNEXURE 5 Continued

BHAGAVATHI ANA LABS

TEST REPORT

Certificate No.: TC-5040
NABL HY-001
ULR : TC504019000008482P

		Test Report No.	BALPL/19-20/CL-1738
		Lab Sample Code	27173807
		Issue Date	10/08/2019
		Customer Ref.	PO No. ERF/PO/19-20/044
		Ref.Date	27/07/2019
Sample Details	RO Water	Date of Registration	27/07/2019
		Date of commencement of testing	27/07/2019
		Date of completion of testing	03/08/2019
Sample Collected By	Mr. John Kennedy	Sample Condition of receipt	Found Ok
Qty. Received	5ltr x 1no. and 1ltr x 1no.		Ambient
Tests Required	As per Quote . IND.BH.41.18.0577/FOC, Dated : 26/07/2019	SAMPLE TESTED AS RECEIVED	

Sample collected and submitted by the representative of BALPL

TEST RESULTS

S.No.	Test Parameters	UOM	Test Method	Requirement (Acceptable Limit) as per IS 10500:2012	Permissible Limit in the absence of alternate source as per IS 10500:2012	Result
17	Residual free chlorine, if chlorinated	mg/l	IS:3025 (P 26)	0.2 min	1.0 min	<0.01
18	Iron as Fe*	mg/l	IS 3025 (P.2)	0.3 max	No Relaxation	<0.01
19	Magnesium as Mg	mg/l	IS:3025 (P.46)	30 max	100 max	<1
20	Manganese as Mn*	mg/l	IS 3025 (P.2)	0.1 max	0.3 max	<0.01
21	Mineral Oil	mg/l	IS:3025 (P .39)	0.5 max	No Relaxation	<0.1
22	Nitrate as NO3	mg/l	APHA 4500 NO3-B	45 max	No Relaxation	1.61
23	Phenolic Compounds as C6H5OH	mg/l	IS:3025 (P.43)	0.001 max	0.002 max	<0.001
24	Selenium as Se*	mg/l	IS 3025 (P.2)	0.01 max	No Relaxation	<0.01
25	Silver as Ag*	mg/l	IS 3025 (P.2)	0.1 max	No Relaxation	<0.01
26	Sulphate as SO4	mg/l	IS:3025 (P.24)	200 max	400 max	<1
27	Sulphide as H2S	mg/l	IS 3025 (P.29)	0.05 max	No Relaxation	<0.01
28	Total Alkalinity CaCO3	mg/l	IS:3025 (P.23)	200 max	600 max	18.27
29	Total Hardness as CaCO3	mg/l	IS:3025 (P.21)	200 max	600 max	13.2
30	Zinc as Zn*	mg/l	IS 3025 (P.2)	5 max	15 max	<0.01
	Table 3 Parameters Concerning Toxic Substances					
31	Cadmium as Cd*	mg/l	IS 3025 (P.2)	0.003 max	No relaxation	<0.003
32	Cyanide as CN	mg/l	IS:3025 (P.27)	0.05 max	No relaxation	<0.1
33	Lead as Pb*	mg/l	IS 3025 (P.2)	0.01 max	No relaxation	<0.01

Hymavathi
Senior chemist
Authorized Signatory

Bhagavathi Ana Labs Pvt. Ltd.
(A Bureau Veritas Group Company)
Plot No. 7-2-C 7 & 8/4, Industrial Estate,
Sanathnagar, Hyderabad - 500 018.
TELANGANA, INDIA
Tel.: +91 40 68144100 / 68144101

Authorized Photo copy 13/10/2019

ANNEXURE 5 Continued

BHAGAVATHI ANA LABS

TEST REPORT

Certificate No.: TC-5040
NABL HY-001
ULR : TC504019000008482P

		Test Report No.	BALPL/19-20/CL-1730
		Lab Sample Code	27173807
		Issue Date	10/08/2019
		Customer Ref.	PO No. ERF/PO/19-20/044
		Ref.Date	27/07/2019
Sample Details	RO Water	Date of Registration	27/07/2019
		Date of commencement of testing	27/07/2019
		Date of completion of testing	03/08/2019
Sample Collected By -	Mr. John Kennedy	Sample Condition of receipt	Found Ok
Qty. Received	5ltr x 1no. and 1ltr x 1no.		Ambient
Tests Required	As per Quote - IND.BH.41.18.0577/FOC, Dated : 26/07/2019	SAMPLE TESTED AS RECEIVED	

Sample collected and submitted by the representative of BALPL

TEST RESULTS

S.No.	Test Parameters	UOM	Test Method	Requirement (Acceptable Limit) as per IS 10500:2012	Permissible Limit in the absence of alternate source as per IS 10500:2012	Result
34	Mercury as Hg*	mg/l	IS 3025 (P.2)	0.001 max	No relaxation	<0.001
35	Molybdenum as Mo*	mg/l	IS 3025 (P.2)	0.07 max	No relaxation	<0.01
36	Nickel as Ni*	mg/l	IS 3025 (P.2)	0.02 max	No relaxation	<0.01
37	Polychlorinated biphenyls	mg/l	Annex M of IS 13428	0.0005 max	No relaxation	<0.00001
38	Polynuclear Aromatic hydrocarbons	mg/l	APHA 6440	0.0001 max	No relaxation	<0.00001
39	Total Arsenic as As*	mg/l	IS 3025 (P.2)	0.01 max	0.05 max	<0.01
40	Total Chromium as Cr*	mg/l	IS 3025 (P.2)	0.05 max	No relaxation	<0.01
	Trihalomethanes					
41	Bromoform	mg/l	EPA 524.3	0.1 max	No relaxation	<0.0005
42	Dibromochloromethane	mg/l	EPA 524.3	0.1 max	No relaxation	<0.0005
43	Bromodichloromethane	mg/l	EPA 524.3	0.06 max	No relaxation	<0.0005
44	Chloroform	mg/l	EPA 524.3	0.2 max	No relaxation	<0.0005
	Table 5. Pesticide Residues					
45	Aldrin & Dieldrin	µg/l	USEPA 508	0.03 max	-	<0.01
46	o,p- DDT	µg/l	USEPA 508	1 max	-	<0.01
47	p,p - DDT	µg/l	USEPA 508	1 max	-	<0.01
48	o,p- DDE	µg/l	USEPA 508	1 max	-	<0.01
49	p,p - DDE	µg/l	USEPA 508	1 max	-	<0.01

Hymavathi
Senior chemist
Authorized Signatory

Bhagavathi Ana Labs Pvt. Ltd.
(A Bureau Veritas Group Company)
Plot No 7-2-C 7 & 8/A, Industrial Estate,
Sanathnagar, Hyderabad - 500 018
TELANGANA, INDIA
Tel.: +91 40 68144100 / 68144101

Authorized Photo copy

ANNEXURE 5 Continued

BHAGAVATHI ANA LABS

TEST REPORT

Certificate No.: TC-5040
NABL HY-001
ULR : TC504019000006482P

		Test Report No.	BALPL/19-20/CL-1736
		Lab Sample Code	27173807
		Issue Date	10/08/2019
		Customer Ref.	PO No. ERF/PO/19-20/044
		Ref.Date	27/07/2019
Sample Details	RO Water	**Date of Registration**	27/07/2019
		Date of commencement of testing	27/07/2019
		Date of completion of testing	03/08/2019
		Sample Condition on receipt	Found Ok
Sample Collected By	Mr. John Kennedy.		Ambient
Qty. Received	5ltr x 1no. and 1ltr x 1no.	**SAMPLE TESTED AS RECEIVED**	
Tests Required	As per Quote : IND.BH.41.18.0577/POC, Dated : 25/07/2019		

Sample collected and submitted by the representative of BALPL

TEST RESULTS

S.No.	Test Parameters	UOM	Test Method	Requirement (Acceptable Limit) as per IS 10500:2012	Permissible Limit in the absence of alternate source as per IS 10500:2012	Result
50	o,p- DDD	µg/l	USEPA 508	1 max	-	<0.01
51	p,p - DDD	µg/l	USEPA 508	1 max	-	<0.01
52	Lindane	µg/l	USEPA 508	2 max	-	<0.01
53	Endosulfan (sum of Isomers & Endosulfah sulphate)	µg/l	USEPA 508	0.4 max	-	<0.01
54	Alpha-HCH	µg/l	USEPA 508	0.01 max	-	<0.01
55	Beta-HCH	µg/l	USEPA 508	0.04 max	-	<0.01
56	Delta-HCH	µg/l	USEPA 508	0.04 max	-	<0.01
57	Methyl Parathion	µg/l	USEPA 8141A	0.3 max	-	<0.01
58	Alachlor	µg/l	USEPA 525.2	20 max	-	<0.01
59	Atrazine	µg/l	USEPA 8141A	2 max	-	<0.01
60	Butachlor	µg/l	USEPA 8141A	125 max	-	<0.01
61	Chlorpyrifos	µg/l	USEPA 8141A	30 max	-	<0.01
62	Ethion	µg/l	USEPA 1657A	3 max	-	<0.01
63	Isoproturon	µg/l	USEPA 532	9 max	-	<0.01
64	Malathion	µg/l	USEPA 8141A	190 max	-	<0.01
65	Monocrotophos	µg/l	USEPA 8141A	1 max	-	<0.01
66	Phorate	µg/l	USEPA 8141A	2 max	-	<0.01
67	2,4-D	µg/l	USEPA 515.1	30 max	-	<0.01

Hymavathi
Senior chemist
Authorized Signatory

Bhagavathi Ana Labs Pvt. Ltd.
(A Bureau Veritas Group Company)
Plot No. 7-2-C 7 & 8/4, Industrial Estate,
Sanathnagar, Hyderabad - 500 018.
TELANGANA, INDIA
Tel.: +91 40 68144100 / 68144101

Authorized Photo copy (Sign & Date) 13/8/2019

ANNEXURE 5 Continued

BHAGAVATHI ANA LABS

TEST REPORT

Certificate No.: TC-5040
NABL HY-001
ULR : TC504019000008482P

		Test Report No.	BALPL/19-20/CL-1738
		Lab Sample Code	27173807
		Issue Date	10/08/2019
		Customer Ref.	PO No. ERF/PO/19-20/044
		Ref.Date	27/07/2019
Sample Details	RO Water	Date of Registration	27/07/2019
		Date of commencement of testing	27/07/2019
		Date of completion of testing	03/08/2019
Sample Collected By	Mr. John Kennedy	Sample Condition on receipt	Found Ok Ambient
Qty. Received	5ltr x 1no. and 1ltr x 1no.	SAMPLE TESTED AS RECEIVED	
Tests Required	As per Quote - IND.BH.41.18.0577/FOC, Dated : 26/07/2019		

Sample collected and submitted by the representative of BALPL

TEST RESULTS

S.No.	Test Parameters	UOM	Test Method	Requirement (Acceptable Limit) as per IS 10500:2012	Permissible Limit in the absence of alternate source as per IS 10500:2012	Result
	Table 6 Bacterial Quality of Drinking Water					
1	Total Coliform bacteria	per 100ml	IS 15185:2010	Shall not be detectable in any 100ml of sample	-	Absent
2	E.coli or thermotolerant coliform bacteria (Fecal Coliforms)	per 100ml	IS 15185:2010	Shall not be detectable in any 100ml of sample	-	Absent

Remarks: (1) The submitted sample complies to IS 10500:2012 water for Drinking purpose with respect to above tested parameters.
* marked parameters are not covered under NABL Scope.

B.Jyothi
Assistant Manager
Authorized Signatory

Bhagavathi Ana Labs Pvt. Ltd.
(A Bureau Veritas Group Company)
Plot No. 7-2-C 7 & 8/4, Industrial Estate,
Sanathnagar, Hyderabad - 500 018.
TELANGANA, INDIA
Tel.: +91 40 68144100 / 68144101

Authorised Signatory 17/10/2019

ANNEXURE 6 - CERTIFICATE OF ANALYSIS OF BEDDING MATERIAL

ISO 9001-2008 CERTIFIED

Central Govt. Approved for AGMARK

NABL

NIKHIL ANALYTICAL & RESEARCH PVT. LTD.

Opposite Sadhana Petrol Pump, Kolhapur Road, Sangli - 416 416 Maharashtra (Bharat)

• Email : nikhil_lab@yahoo.com • Phone : +919552574418

CERTIFICATE OF ANALYSIS

L1/5663/S			04/09/2019
Name/ Organization	VRK Nutritional Solutions, MIDC Mirej.		
Sample Description	Corn Cob		
Sample Collected by	Party	Sample Received on	20/08/2019
Sample Analysed by	Smt. Aishwarya	Analysis Completed on	04/09/2019
Reference	Batch No. 015, Mfd. 12/08/2019		

Sr.	Parameter	Unit	Value
A.	**Aflatoxins**		
1.	Aflatoxin G1	ppb	<10
2.	Aflatoxin G2	ppb	<10
3.	Aflatoxin B1	ppb	<10
4.	Aflatoxin B2	ppb	<10
B.	**Pesticide Residues**		
1.	2, 4 DDE	ppb	<0.01
2.	4, 4 DDE	ppb	<0.01
3.	4, 4 DDD	ppb	<0.01
4.	4, 4 DDT	ppb	<0.01
5.	Alpha HCH	ppb	<0.01
6.	Beta HCH	ppb	<0.01
7.	Delta HCH	ppb	<0.01
8.	Dieldrin	ppb	<0.01
9.	Lindane	ppb	<0.01
10.	Malathion	ppb	<0.10
	Microbiological Analysis		
1.	Total Bacterial Count	cfu/g	15×10^3
2.	Total Fungal Count	cfu/g	17×10^1
3.	*Escherichia coli*	cfu/g	Absent
4.	*Salmonella* Spp.	cfu/g	Absent
5.	*Staphylococcus aureus*	cfu/g	Absent
6.	*Pseudomonas aeruginosa*	cfu/g	Absent

Authorized Photo copy (Name & Date)

Analyst / Lab In-Charge

Managing Director
Nikhil Suhas Khambe
B Tech (Bio-tech)

Note : The report can not be used for court purpose. we are not responsible for any legal matter.

IAS-NRL

FOOD, FEED, WATER, SOIL, PLANT MATERIAL, ORGANIC MANURE, CHEMICAL, BIOLOGICAL FERTILIZER, PGR, AYURVEDIC & PHARMACEUTICALS, INDUSTRIAL MATERIAL, SOLID WASTE, WASTE WATER, AIR POLLUTION, ENVIRONMENTAL MONITORING & ETP CONSTRUCTION.

AGMARK Approval No. 0-1103/01/2015/Lab From Ministry of Agriculture, Department of Marketing & Inspection, Govt of India & State Govt. Approved for Soil & Water Analysis (ENG/STLR No. 1107/2021, Approved for Fertilizer Testing