



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

SUB CHRONIC ORAL TOXICITY STUDY

WITH

NHH 44 Bt-COTTON SEEDS

Report for:

**UNIVERSITY OF AGRICULTURAL SCIENCES
AGRICULTURAL RESEARCH STATION
DHARWAD-580007
KARNATAKA**

Guidelines:

**‘DBT, Guidelines for Toxicity and Allergenicity Evaluation of
Transgenic Seeds, Plants and Plant parts’**

Prepared by :

**DEPARTMENT OF TOXICOLOGY
SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH
(A Unit of Shriram Scientific & Industrial Research Foundation)**

19, University Road, Delhi – 110 007

Tel. 27667267, 27667860, 27667436

Fax No. 91+011-27667676, 27667207,

E. Mail : sridlhi@vsnl.com



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled ‘Sub chronic oral toxicity study’ with ‘NHH 44Bt-Cotton Seeds’ has been audited and examined with respect to the study protocol and the Standard Operating Procedures in accordance to ‘DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts’ for non clinical laboratory studies.

The report provides true and accurate record of results obtained.

The dates of inspections & dates on which findings were reported to the study director & SRI management are given below:

<u>Phases of study</u>	<u>Dates of Inspection</u>	<u>Dates of Reporting</u>
Protocol	15.12.2006	15.12.2006
Conduct	19.12.2006	19.12.2006
	08.01.2007	08.01.2007
	26.02.2007	26.02.2007
	19.03.2007	19.03.2007
Records/ Raw data	15.04.2007	15.04.2007
Report	05.05.2007	05.05.2007

**Sr. SCIENTIST
QUALITY ASSURANCE**

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



Confidential

PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE

We, the undersigned take overall responsibility to conduct the work described in the study entitled ‘Sub chronic oral toxicity study’ with ‘NHH 44 Bt-Cotton Seeds’ performed with respect to the study protocol and the Standard Operating Procedures in accordance to ‘DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts’ for non-clinical laboratory studies.

All the raw data, documentation, protocol and copy of final report are retained in the archives at Shriram Institute for Industrial Research, Delhi.

STUDY DIRECTOR SCIENTIST PATHOLOGY HEAD, DEPT. OF TOXICOLOGY

Approved for issue

**DEPUTY DIRECTOR
(MANAGEMENT)**

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



Confidential

PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

SCIENTIFIC PERSONNEL INVOLVED IN THE STUDY

Dr. RAJUL SAXENA, M.V.Sc.
(Scientist Pathology)

Dr. VIVEK SRIVASTAVA , M.V.Sc.
(Research Associate)

Ms. LITHA THOMAS, M.Sc.
(Analyst)

Mr. ALISHA N. SINGH , D.M.L.T.
(Lab Technician)

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



Confidential

PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

INDEX

S. No.	Contents	Page No.
1.	Quality Assurance Statement	2
2.	Statement of Compliance with Good Laboratory Practice	3
3.	Scientific personnel involved in the study	4
4.	Summary	7
5.	Introduction	8
6.	Objective	9
7.	Test Substance	10
8.	Experimental design	11
9.	Experimental procedure	12
10.	Animal groups and dosage level	13
11.	Clinical Laboratory studies	15
12.	Terminal Studies	17
13.	Observations	18-19
14.	Result and Conclusion	20



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

	Tables	
15.	Appendix-1 Body Weights and Survival Data	21-44
16.	Appendix 2 Feed Consumption data	45-55
17.	Appendix 3 Haematology- Mean & individual data	56-63
18.	Appendix 4 Bio-Chemistry- Mean & individual data	64-71
19.	Appendix 5 Mean & absolute organ weight data	72-81
20.	Appendix 6 Intensity of Lesions (Pathological findings)	82-87
21.	Appendix 7 Intensity of Lesions (Histopathological findings)	88-93



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

SUMMARY

This study was designed to investigate the toxicological effects of 90 days (5 days / week) repeated oral administration of NHH 44 Bt-Cotton seeds to determine, whether any types of effects could be induced, and to serve as a basis for selecting appropriate range of dosage for future studies and for establishing safety criteria for human consumption.

Three groups of 20 rats each (10 males and 10 females) were made. First group was kept as control, which was given vehicle only i.e. corn oil. Second and third group was administered with NHH 44 Bt-Cotton seeds in powdered form at the dose level of 1000 mg/kg B.wt. and powdered NHH 44 Non Bt-Cotton seeds at the dose level of 1000 mg/kg B. wt. respectively.

The 'NHH 44 Bt-Cotton seeds and NHH 44 Non Bt-Cotton seeds' samples were prepared daily in corn oil (vehicle) for oral administration.

Under the conditions of this study, the 90 days repeated oral administration of 'NHH 44 Bt-Cotton seeds and NHH 44 Non Bt-Cotton seeds' at the dose level of 1000 mg/kg B.wt to wistar rats did not induce any observable toxic effects, when compared to its control counterpart. Hence, **No Observable Adverse Effect Level (N.O.A.E.L) = 1000 mg/kg B.wt.**



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

INTRODUCTION

This study was aimed to determine the sub-acute oral toxicity in wistar rats.

The given study was carried out with the dose level of 1000 mg/kg B. wt. of NHH 44 Bt-Cotton seeds and NHH 44 Non Bt-Cotton seeds along with the vehicle control as per the 'DBT guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant Parts'.



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

OBJECTIVES

To assess the toxicological effects of 90 days oral administration of NHH 44 Bt-Cotton seeds in comparison to the NHH 44 Non Bt-Cotton seeds and control (only vehicle) in rats so as to provide information on the possible health hazards likely to arise from repeated exposure over a limited period of time, based on 'DBT guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant Parts'.



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

TEST SUBSTANCE

The sponsor is responsible for the necessary characterization and evaluations of the test substance. The details of the test substance provided by the Sponsor are as follows :

PRODUCT NAME : NHH 44 Bt-COTTON SEEDS &
NHH 44 NON Bt-COTTON SEEDS

SPONSOR : UNIVERSITY OF AGRICULTURAL
SCIENCES , DHARWAD

MATERIAL DESCRIPTION : DARK BROWN COLOURED
SEEDS

PACKED IN : WHITE COLOURED PLASTIC
BAGS

DATE OF COMMENCEMENT : 19.12.2006
OF STUDY

DATE OF COMPLETION : 18.03.2007
OF STUDY

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



Confidential

PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

EXPERIMENTAL DESIGN

<u>STUDY LABORATORY</u>	Dept. of Toxicology Shriram Institute for Industrial Research 19, University Road, Delhi-110007 (INDIA)
<u>STRAIN AND SPECIES</u>	Wistar (albino) rats
<u>ANIMAL SOURCE</u>	Lab animal facility, SRI, Delhi
<u>SEX</u>	Male & Female
<u>WEIGHT RANGE</u>	120 ± 20 gms
<u>HUSBANDRY</u>	All animals were caged in a group of 5 according to sex in plastic cages fitted with wire mesh tops and having sterilized paddy husk bedding. Water and standard rat diet were provided <i>ad libitum</i> .
<u>ENVIRONMENTAL CONDITIONS</u>	Room temperature : 22 ± 3°C Relative humidity : 30 – 70 % Air exchange : 15 air changes / hour Lighting condition : 12 hrs light and 12 hrs dark cycle
<u>AGE OF ANIMALS</u>	6-8 weeks
<u>ACCLIMATIZATION PERIOD</u>	7 days
<u>DATE OF COMMENCEMENT OF STUDY</u>	19.12.2006
<u>DURATION OF STUDY</u>	90 days
<u>DATE OF TERMINATION OF STUDY</u>	18.03.2007
<u>NECROPSY DATE</u>	Terminal Sacrifice : 19.03.2007 20.03.2007 21.03.2007



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

EXPERIMENTAL PROCEDURE

Sample preparation

Powdered samples of NHH 44Bt-Cotton seeds and NHH 44Non Bt-Cotton seeds were freshly prepared every day in corn oil (vehicle) for repeated oral administration for 90 days.

Total 60 healthy adult male and female (30+30) rats were acclimatized for 7 days and animals were caged in a group of 5 according to sex in plastic cages fitted with wire mesh tops and having sterilized paddy husk bedding. The rats were randomized and assigned to 3 groups of 10 male and 10 female rats each and identified by cage tag and individual marking.

The first group was kept as control and was given only the vehicle i.e. corn oil. Second and third group was administered with NHH 44Bt-Cotton seeds and NHH 44Non Bt-Cotton seeds at the dose level of 1000 mg/kg B.wt. respectively.

The rats were observed daily for behaviour, appearance and toxicological signs and symptoms, if any.



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

Animal Group and Dosage Level

Group	Dose mg/kg B.wt	No. of animals Male +Female
1. Control	0.0	10 +10
2. NHH 44Bt-Cotton seeds	1000.0	10 +10
3. NHH 44Non Bt-Cotton seeds	1000.0	10 +10

Body weights : Recorded individually before treatment and at weekly intervals, thereafter group mean body weights were calculated.

Feed Consumption : Recorded individually at weekly intervals, thereafter group mean feed consumption were recorded .

Signs / symptoms : Recorded daily in terms of clinical manifestation, if any.

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



Confidential

PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

Mortalities : If any, subjected to detailed macroscopic examination and tissue samples were preserved for histopathological examination.

The following clinical laboratory determinations were made in all the animals of each group after termination of the experiment. The organs were weighed and subjected to detailed macroscopic / microscopic examinations.



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

CLINICAL LABORATORY STUDIES

Blood Sampling

Food was withdrawn overnight prior to collection of samples. 5-8 ml of blood was withdrawn by cardiac puncture under CO₂ asphyxiation prior to sacrifice.

Haematology

Following haematological estimations were performed on control and treated group of animals :

Haematocrit (Hct)	Differential Leucocyte Count (DLC)
Haemoglobin (Hb)	Neutrophils (N)
Total Erythrocyte Count (TEC)	Lymphocytes (L)
Platelet count	Basophils (B)
Monocyte (M)	Eosinophils (E)
Total Leucocyte Count (TLC)	



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

Biochemistry

Following estimation were performed on control and treated rats using Boehringer Mannheim diagnostic kits:

- (a) Blood sugar
- (b) Blood urea nitrogen (BUN)
- (c) Total protein (TP)
- (d) Albumin
- (e) Serum glutamic oxalo acetate transaminase (SGOT)
- (f) Serum glutamic pyruvic transaminase (SGPT)
- (g) Serum alkaline phosphatase (SAP)
- (h) Cholestrol



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

TERMINAL STUDIES

Autopsy : After completion of treatment period all animals from each group were sacrificed and subjected to detailed macroscopic examination.

The following organs were weighed :

Heart, kidneys, liver, lungs, spleen, adrenals, testis, ovaries, uterus and brain.

Histopathology: Microscopic examination of the following tissues from all animals from each group were carried out :

Stomach	Brain
Intestine	Heart
Lungs	Kidneys
Liver	Adrenals
Spleen	Testis
Uterus	Ovaries

Any other macroscopically abnormal tissue.

BIOSTATISTICAL METHOD USED

All the data were analysed by using standard student's t- test of analysis



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

OBSERVATIONS

Mortality and toxic signs

No mortality was observed in any of the test groups including the control group of animals. No toxic signs and symptoms were noticed in any of the two dose groups and the control group of animals.

Mean body weights

No significant differences were observed in the body weight gain (Appendix -1) pattern of the test groups when compared to the control group of animals.

Feed Consumption

The feed consumed by the rats in all the test groups was similar to that consumed by the animals of control group (Appendix-2).

Haematological evaluations

No significant changes were noted among the test groups and control group of animals with respect to haematological findings (Appendix-3) as all the parameters fell within the accepted limits of normal variations for albino rats.



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

Clinical Biochemistry evaluations

Serum Biochemistry evaluations ([Appendix-4](#)) disclosed no significant differences in the tests as well as the control groups of animals, as all the parameters fell within the accepted limits of normal variations.

Organ Weight

Absolute organ weights and their ratios (relative organ weights) with their respective body weights are shown in [Appendix-5](#). As there were no significant changes in the organ weights of the test animals, when compared with organ weights of control group animals, the test substance (NHH 44 Bt-Cotton seeds and NHH 44 Non Bt-Cotton seeds) was failing to suggest any specific target organ.

Necropsy examinations

Necropsy examination of animals did not reveal any significant changes in both the test groups i.e. NHH 44 Bt-Cotton seeds and NHH 44 Non Bt-Cotton seeds at the level of 1000 mg/kg B.wt when compared to its control counterparts, as shown in [Appendix 6](#).

Histopathological examination

No significant histopathological changes ([Appendix-7](#)) were noticed in the animals of NHH 44 Bt-Cotton seeds and NHH 44 Non Bt-Cotton seeds dosed at 1000 mg/kg B.wt when compared to its control counterparts.



PROJECT NO. : TOX- 355 B
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SUB CHRONIC ORAL TOXICITY
STUDY IN RATS
REPORT NO. : 000046990
DATE : 18.5.2007

RESULT WITH CONCLUSION

Under the conditions of this study, the 90 days repeated oral administration of NHH 44 Bt-Cotton seeds at the dose level of 1000 mg/kg B.wt for five days/ week to wistar rats did not induce any treatment related observable toxic effects, when compared to its corresponding NHH 44 Non Bt-Cotton seeds and the control group of animals treated with corn oil (vehicle) only.

Hence, No Observable Adverse Effect Level (N.O.A.E.L.) = 1000 mg/kg.
B.wt.

The sample has been conducted as per DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts.