



**PROJECT : TOX-355a**  
**PRODUCT NAME : NHH 44 Bt-COTTON SEEDS**  
**STUDY : ACUTE ORAL TOXICITY STUDY IN RATS**  
**REPORT NO. : 000041398**  
**DATE : 23.03.2007**

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**ACUTE ORAL TOXICITY STUDY IN RATS**

**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](mailto:sridlhi@vsnl.com)*



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### **QUALITY ASSURANCE STATEMENT**

This is to certify that the work described in the study report entitled ‘Acute Oral Toxicity Study in rats’ with ‘NHH 44 Bt-Cotton Seeds & NHH 44 Non Bt-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’ in compliance with Good laboratory Practices (G.L.P) 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	27.11.2006	27.11.2006
Conduct	30.11.2006	30.11.2006
Records/ Raw data	14.12.2006	14.12.2006
	15.01.2006	15.01.2006
Report	20.03.2007	20.03.2007

**Sr. SCIENTIST**  
**QUALITY ASSURANCE**



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**STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE**

We, the undersigned take overall responsibility to conduct the work described in the study entitled ‘Acute Oral Toxicity Study in rats’ with NHH 44 Bt-Cotton Seeds & NHH 44 Non 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)**



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**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.**  
**(Project Trainee)**



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## **SUMMARY**

In the assessment and evaluation of the toxic characteristics of a substance, determination of ‘Acute Oral Toxicity’ is usually an initial step.

This study was hence, designed to conduct acute oral toxicity of ‘NHH 44 Bt-Cotton Seeds & NHH 44 Non Bt-Cotton Seeds’ provided by University of Agricultural Sciences, Dharwad, in rats.

Three groups consisting of 5 male and 5 female rats were selected and two groups were administered with the test substance orally at the dose level of 5000 mg/kg body weight with the help of metallic cannula attached with tuberculin syringe, while the first group was kept as control and was dosed with vehicle (corn oil) only, the second group of animals was fed on NHH 44 Bt-Cotton Seeds and the third group on NHH 44 Non Bt-Cotton Seeds.

No mortality was observed in any sex of any group at 5000 mg/kg b.wt. dose level. Hence, no further testing was required.

Under the conditions of this study, the single oral administration of ‘NHH 44 Bt-Cotton Seeds’ and ‘NHH 44 Non Bt-Cotton Seeds’ at the dose level of 5000 mg/kg b.wt to wistar rats did not induce any treatment related observable toxic effects, when compared to its control group of animal treated with corn oil (vehicle) only.



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## **INTRODUCTION**

This study was carried out to determine the acute oral toxicity of ‘NHH 44 Bt-Cotton seeds’ and ‘NHH 44 Non Bt-Cotton Seeds ’ in wistar rats.

This study is an initial step in establishing a dosage regimen in sub-chronic and other toxicity studies and may provide information on the mode of toxic action of the test substance after single dose administration.



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## **OBJECTIVE**

- (a) To determine the acute oral toxicity using a minimum number of animals at each step to enable the classification of the compound according to any of the commonly used test system.
  
- (b) This acute toxic class method provides information both for hazard assessment and for hazard classification purposes.



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### **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 : 30.11.2006  
OF STUDY**

**DATE OF COMPLETION : 14.12.2006  
OF STUDY**



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## EXPERIMENTAL DESIGN

Name of species : *Rattus rattus albanicus*  
Strain of the animals : Wistar  
\*No. of animals used per group : 5 Male, 5 Female  
Age of the animals used : 6 to 7 weeks  
Weight range : 160-180 gm  
Acclimatization period : 7 Days  
Route of administration : Oral  
Vehicle used : Corn oil

\* A limit test at one dose level of 5000 mg/kg body weight was carried out with 5 male and 5 female rats.



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## **HUSBANDRY**

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. The room temperature was maintained at  $22 \pm 3^{\circ} \text{C}$  with 30 - 70 % relative humidity.

The room was ventilated at the rate of approximately 15 air changes per hour.

Lighting was controlled to give 12 hours artificial light (8 a.m. - 8 p.m.) each day.

## **DIET**

Water and standard rat pelleted feed (Amrut feeds Ltd.) was freely available to the experimental rats.



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### **IDENTIFICATION OF ANIMALS**

Each cage was tagged having the details of animal group number, product name, dosage level, date of initiation and date of completion.

The animals were also individually marked with the help of marking ink.



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### **METHOD OF ADMINISTRATION**

The animals were normally fasted for 18 hours before and 4 hours after dosing. Three groups of 5 male and 5 female rats each were designated for the study. First group of animals were administered with vehicle (corn oil ) only. Second group of animals were administered with NHH 44 Bt-Cotton Seeds orally at the highest recommended dose level of 5000 mg/kg body weight with the help of metallic cannula attached with tuberculin syringe. Third group was similarly administered orally with NHH 44 Non Bt-Cotton Seeds. No mortality was observed in any sex at this dose level. So, no further testing was required.

### **Frequency of administration**

The test article was administered once only following an overnight fast.



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## CLINICAL LABORATORY STUDIES

The following clinical laboratory determinations were made in the animals of the test as well control groups.

### Blood sampling

3-5 ml of blood was withdrawn by cardiac puncture under light Carbon dioxide anesthesia prior to sacrifice.

### Haematology

Following haematological estimations were performed on control and treated group of animals using Beckman Coulter Haematology Analyser A<sup>c</sup>.T-diff.

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



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### **Serum Biochemistry**

Following estimations were performed on control and treated rats using Hitachi Biochemistry Analyser 902 (Roche).

- (a) Blood Sugar
- (b) Blood Urea
- (c) Total Protein (TP)
- (d) Albumin
- (e) Serum Glutamic Oxalo Acetate Transaminase (SGOT)
- (f) Serum Glutamic Pyruvic Transaminase (SGPT)
- (g) Serum Alkaline Phosphatase (SAP)

### **SACRIFICE AND NECROPSY**

All the experimental animals were subjected to necropsy at the end of the study. All findings not considered normal were recorded.



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### **Histopathology**

Microscopic examination of the following tissues from all animals of the control and treated group were carried out:

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

Any other macroscopically abnormal tissue.

### **BIO-STATISTICAL ANALYSIS**

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



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## **RESULTS**

### **Mortality and toxic signs**

No mortality was recorded (Table- 1.01 &1.02) in the control as well as test groups (NHH 44 Bt-Cotton Seeds and in NHH 44 Non Bt-Cotton Seeds) of animals. No toxic sign or symptoms (Table-1.03) were noticed in any test groups ( NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds) of animals as compared to control group of animals .

### **Mean body weights**

No significant differences were observed in the percentile body weight (Table 1.04-1.07) in control and test groups (NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds) of animals.



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### **Haematological evaluations**

There were no significant changes in haematological parameters (Tables 2.01-2.05) in control and test groups (NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds) of animals. These parameters fell within the accepted limits of normal variations for rats.

### **Clinical biochemistry evaluations**

Serum Biochemistry evaluations (Tables 3.01-3.05) disclosed no significant differences in control and test groups (NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds) 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 Table No. (4.01-4.10). No significant changes could be noticed in the organ weights of all the test groups (NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds) of animals, when compared with the organ weights of control group animals.



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### **Necropsy Finding**

Necropsy examination (Tables 5.01-5.03) did not reveal any significant gross pathological changes in any of the test groups (NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds) when compared with the control group of animals.

### **Histopathological Finding**

No remarkable histopathological changes (Tables 6.01-6.03) were noticed in the animals treated with ( NHH 44 Bt-Cotton Seeds along with NHH 44 Non Bt-Cotton Seeds) when compared with the control group (vehicle only) of animals.

### **Conclusion**

Under the conditions of this study, the single oral administration of (NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds) at the dose level of 5000 mg/kg b.wt to wistar rats did not induce any treatment related observable toxic effects, when compared to its control group of animal treated with vehicle (corn oil) only.



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**TABLE –1.01**  
**LD<sub>50</sub> ASSAY - MORTALITY DATA OF MALE RATS**

Dosage level mg/kg	Time of Death ( Days )														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
<b>CONTROL- VEHICLE ONLY</b>																
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
<b>NHH 44 Bt-COTTON SEEDS</b>																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
<b>NHH 44 NON Bt-COTTON SEEDS</b>																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5



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**TABLE -1.02**  
**LD<sub>50</sub> ASSAY - MORTALITY DATA OF FEMALE RATS**

Dosage level mg/kg	Time of Death (Days)														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
<b>CONTROL- VEHICLE ONLY</b>																
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
<b>NHH 44 Bt-COTTON SEEDS</b>																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
<b>NHH 44 NON Bt-COTTON SEEDS</b>																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

No toxic signs & symptoms / mortality was observed in control group of animals.