



SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH

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PROJECT NO. : TOX-355E
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : SKIN SENSITIZATION STUDY ON GUINEA PIGS
REPORT NO. : 000041401
DATE : 23.03.2007

SKIN SENSITIZATION STUDY ON GUINEA PIGS

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
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QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled ‘Skin Sensitization Study on Guinea Pigs ’ 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	25.12.2006	25.12.2006
Conduct	28.12.2006	28.12.2006
Records	30.01.2007	30.01.2007
Report	21.03.2007	21.03.2007

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 ‘Skin Sensitization Study on Guinea pigs’ 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

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SUMMARY

NHH 44 Bt-Cotton Seeds (powdered) was moistened with water and applied to the shaved skin of 10 young adult guinea pigs for 6 hours a day, three times a week for three weeks until 9 applications were made in the induction (sensitization) phase. Similarly, NHH 44 Non Bt-Cotton Seeds was also applied to the guinea pigs and a control group of 5 guinea pigs was maintained which was not treated in the induction phase. After the last induction exposure the animals were left untreated for two weeks before conducting the challenge phase.

The day before the challenge, the hair from each guinea pig including 5 additional untreated animals (controls) was removed with a clipper from the mid back area and test substance was moistened and applied to the shaved area of the test and control guinea pigs. The test material was removed after 6 hours and the skin reaction at the site of application was assessed and scored immediately after challenge patch removal, and again after 24 hours and 48 hours.

Under the conditions of this study, the repeated application of “NHH 44 Bt-Cotton Seeds” did not induce dermal sensitization (allergies) to the skin of any of the guinea pigs when compared to its corresponding “NHH 44 Non Bt-Cotton Seeds” and the control group of animals.



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INTRODUCTION

This study was designed to determine the potential of the 'NHH 44 Bt-Cotton Seeds and NHH 44 Non Bt-Cotton Seeds' samples to elicit an immunological response through contact with the skin for human allergic contact dermatitis.



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OBJECTIVE

1. This test method is used to determine whether the test substance will elicit dermal sensitization in guinea pigs.
2. The rationale for this practice is based on the fact that the guinea pig has been shown to be the best animal model for human allergic contact dermatitis.

CHARACTERIZATION OF TEST AND CONTROL COTTONSEEDS

The test and control cotton seeds were characterized by the sponsor prior to their use in the study.



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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 : 28.12.2006
OF STUDY

DATE OF COMPLETION : 01.02.2007
OF STUDY



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EXPERIMENTAL DESIGN

Name of species : *Cavia porcellus*

No. of animals used per dose : 10 young adult healthy guinea pigs for the NHH 44 Bt Cotton Seeds , 10 for Non NHH 44 Bt Cotton Seeds and 5 for negative control were used.

Weight range : 300-500 gm

Acclimatization period : 7 Days

Route of administration : Dermal

ANIMAL GROUPS AND TEST APPLICATIONS

Group	No. of animals	No. of applications	
		Induction	Challenge
Control	5	0	1
(NHH 44Bt-Cotton Seeds)	10	9	1
(NHH 44 Non Bt-Cotton Seeds)	10	9	1



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HUSBANDRY

The animals were kept in group of 10 for test group and 5 for control group animals per pan with proper identification.

The room temperature was maintained at $22 \pm 3^{\circ}$ 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 pelleted feed (Amrut feeds Ltd.) was freely available to all the animals.



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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 marked individually with the help of marking ink.



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EXPERIMENTAL PROCEDURE (BUEHLER METHOD)

The dermal sensitization study comprised of two test phases -

1. Induction Phase
2. Challenge Phase

Sample preparation

The NHH 44 Bt-Cotton Seeds as well as NHH 44 Non Bt-Cotton Seeds were powdered and applied after moistening it with water.



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1. Induction Phase

A day before the test, the hair was clipped from the mid back area of 20 guinea pigs designated as test animals. The sample was applied by moistening the powdered cotton seeds with water and 1 inch x 1 inch gauze patch was applied to the test area. The gauze patch was covered with non-reactive tape and wrapped with bandage. The test patches were removed after 6 hours and the test area was cleaned with water and observations for the presence of erythema and oedema were recorded. The test article application procedure was repeated 3 times each week for 3 weeks; total 9 applications were made to the test area. After the last application of the induction phase these animals were left untreated for 2 weeks before conducting the challenge phase. A control group of 5 guinea pigs was also maintained which was not treated in the induction phase.



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2. Challenge Phase

The day before the challenge phase test, the hair of each guinea pig of test group and 5 additional untreated animals (Control) was removed with a clipper from the mid back area.

The test substance (prepared by moistening the crushed cotton seeds with water) of NHH 44 Bt-Cotton Seeds and Non NHH 44 Bt-Cotton Seeds was applied respectively to the shaved area of the control and the test guinea pigs and the test area was covered with non reactive gauze bandage. The test patches were removed after 6 hours.

Three observations for the presence of erythema and oedema were made-

1. Immediately after challenge patch removal.
2. After 24 hours.
3. After 48 hours.



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The following scoring criteria was used for evaluating the skin reaction:

SCORING CRITERIA FOR TEST

Reaction	Description	Score
Erythema	Erythema and eschar formation	
	- No erythema	0
	- Very slight erythema (Barely perceptible)	1
	- Well defined erythema (Pale red in colour)	2
	- Moderate to severe erythema (Red and area well defined)	3
	- Severe erythema (Beet redness to slight eschar formation)	4

(contd on page 17)



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Oedema

Oedema formation

- No oedema 0
 - Very slight oedema 1
(Barely perceptible)
 - Slight oedema 2
(Edges of area well defined
by definite raising)
 - Moderate to severe oedema 3
(Edges raised approx. 1mm)
 - Severe oedema 4
(Raised more than 1mm and
extending beyond area of exposure)
-



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RATING OF SENSITIZATION RESPONSE

Sensitized	Grades	Classification
0 to 8	I	No different than control
9 to 28	II	Mild
29 to 28	III	Moderate
65 to 80	IV	Strong
81 to 100	V	Extreme



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OBSERVATION

No noticeable dermal irritancy (erythema & oedema, Table - 1) was observed in any test and control guinea pigs during the induction phase and after the challenge phase.



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RESULT

Under the conditions of this study, the repeated application of “NHH 44 Bt-Cotton Seeds ” did not induce dermal sensitization (allergies) to the skin of any of the guinea pigs when compared to its corresponding “NHH 44 Non Bt-Cotton Seeds” and the control group of animals.

The samples have been tested in accordance with Guidelines for Toxicity and Allergenicity, Evaluation of Transgenic seeds, plants and plant parts, Department of Biotechnology, Ministry of Science and Technology, Government of India for non-clinical laboratory studies.



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TABLE NO. 1
SUMMARY OF ERYTHEMA AND OEDEMA SCORE

Group	Induction Phase		Challenge Phase	
	Erythema	Oedema	Erythema	Oedema
Control	-	-	0	0
NHH 44Bt- Cotton Seeds	0	0	0	0
NHH 44Non Bt- Cotton Seeds	0	0	0	0



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TABLE NO. 2
INDUCTION PHASE
EVALUATION OF REACTION
NHH 44 Bt-COTTON SEEDS

Application No.	Skin Reaction	Guinea Pig Number										Average	Combined Index
		1	2	3	4	5	6	7	8	9	10		
1.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
2.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
3.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
4.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
5.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
6.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
7.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
8.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
9.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	



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TABLE NO. 3
INDUCTION PHASE
EVALUATION OF REACTION
NHH 44 NON Bt-COTTON SEEDS

Application No.	Skin Reaction	Guinea Pig Number										Average	Combined Index
		1	2	3	4	5	6	7	8	9	10		
1.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
2.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
3.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
4.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
5.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
6.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
7.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
8.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	
9.	Erythema	0	0	0	0	0	0	0	0	0	0	0	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	



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TABLE NO. 4
CHALLENGE PHASE
EVALUATION OF SKIN REACTION
NHH 44 Bt-COTTON SEEDS

Skin Reaction	Time (hours)	Treated Animals (NHH 44 Bt-Cotton Seeds)										Average Index	Combined Index	Untreated Animals (control)					Average Index	Combined Index
		1	2	3	4	5	6	7	8	9	10			1	2	3	4	5		
Erythema	After	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	
Oedema	6 hrs.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	After	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	
Oedema	24 hrs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	After	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	
Oedema	48 hrs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



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TABLE NO. 5
CHALLENGE PHASE
EVALUATION OF SKIN REACTION
NHH 44 NON Bt-COTTON SEEDS

Skin Reaction	Time (hours)	Treated Animals (NHH 44 Non Bt-Cotton Seeds)										Average	Combined Index	Untreated Animals (control)					Average	Combined Index	
		1	2	3	4	5	6	7	8	9	10			1	2	3	4	5			
Erythema	After	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	
Oedema	6 hrs.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	After	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	
Oedema	24 hrs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythema	After	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	
Oedema	48 hrs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0