



SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH

Confidential

PROJECT NO. : TOX-355C
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : PRIMARY SKIN IRRITATION TEST IN RABBITS
REPORT NO. : 000041400
DATE : 23.03.2007

PRIMARY SKIN IRRITATION TEST IN RABBITS

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 ‘Primary Skin Irritation Test in Rabbits’ with NHH 44Bt-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.

<u>Phases of study</u>	<u>Dates of Inspection</u>	<u>Dates of Reporting</u>
Protocol	27.11.2006	28.11.2006
Conduct	30.11.2006	30.11.2006
Records	16.12.2006	16.12.2006
Report	22.03.2007	22.03.2007

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 ‘Primary Skin Irritation Test in Rabbits’ 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

In the present study, 0.5 gm of the test samples i.e .NHH 44 Bt-Cotton Seeds & NHH 44 Non Bt-Cotton Seeds supplied by University of Agricultural Sciences, Dharwad was applied in powdered form to the skin of albino rabbits. After 4 hours i.e. the exposure period, the degree of irritation was read and scored at 1, 24, 48, 72 hours, 7 and 14 days after the patch removal.

The primary skin irritation index was calculated and came as 0.00 after 4 hours of the patch removal. Hence, it was concluded that the test substance NHH 44Bt-Cotton Seeds was '**non-irritant**' to the skin of rabbits when compared with the NHH 44 Non Bt-Cotton Seeds .



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INTRODUCTION

Primary skin irritation is the production of reversible inflammatory changes in the skin following the application of a test substance as it involves the interaction of chemicals with the sensory receptors in the skin at the site of application.

On the basis, of this study, the possible hazard likely to arise from exposure of the skin to the test substance can be assessed.



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OBJECTIVE

To evaluate local irritant effects to rabbit skin following a single dose application of the test samples.

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 : 30.11.2006
OF STUDY

DATE OF COMPLETION : 13.12.2006
OF STUDY



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ANIMALS

In the present study three young adult rabbits of the New Zealand White Strain were taken per sample / per group.



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HUSBANDRY

All animals were housed in metal cages fitted with perforated floors. Water and standard rabbit feed were given *ad libitum*.

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

The light conditions were controlled to give 12 hours artificial light (8 a.m. - 8 p.m.) each day.



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ACCLIMATIZATION

A minimum of 7 days acclimatization was allowed before the commencement of the study.



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IDENTIFICATION OF ANIMALS

Each rabbit cage was attached with a tag marked with the animal number, the test and the product name.



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EXPERIMENTAL PROCEDURE

SKIN PREPARATION

Twenty-four hours before the test (dose application), hair on the back and flanks of each rabbit were closely clipped exposing (approximately 6cm² area) of skin.

DOSE : 0.5 gm / animal



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APPLICATION PROCEDURE

The test samples (NHH 44Bt-Cotton seeds and NHH 44Non Bt-Cotton seeds) were evenly applied to a small area (approximately 6 cm square) of the closely clipped skin of each rabbit. The site of application was covered with a cotton gauze patch, which was kept in contact with skin by means of semi-occlusive dressing.

At the termination of 4 hours exposure period, the bandages / gauze were removed and treatment sites were cleaned with wet gauze to remove any residual test substance.



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OBSERVATIONS

Skin reaction at the site of application was subjectively assessed and scored once daily at 1, 24, 48, 72 hours, 7 and 14 days after patch removal (post test observation period) accordingly.

The reaction at the site of application was assessed and scored according to the following numerical system:

SKIN REACTION	SCORE
(A) ERYTHEMA AND ESCHAR FORMATION	
- No erythema	0
- Very slight erythema (barely perceptible)	1
- Well defined erythema	2
- Moderate to severe erythema	3
- Severe erythema (beet redness to eschar formation)	4



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SKIN REACTION	SCORE
---------------	-------

(B) EDEMA FORMATION

- | | |
|--|---|
| - No edema | 0 |
| - Very slight edema (barely perceptible) | 1 |
| - Slight edema (edges of area well raised) | 2 |
| - Moderate edema (raised approx.1mm) | 3 |
| - Severe edema (raised more than 1mm and extending beyond area of exposure) | 4 |
-



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EVALUATION OF PRIMARY SKIN IRRITATION INDEX

EVALUATIONS	SCORE
Non Irritant	0.0
Negligible Irritant	0.1- 0.4
Slight Irritant	0.41-1.9
Moderate Irritant	2.0 - 4.9
Severe Irritant	5.0 - 8.0



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EVALUATION OF REACTIONS (Draize's Method)
TABLE-I
CONTROL GROUP

	Rabbit Numbers			Average	Combined Index
	1M	2M	3M		
1 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
24 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
48 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
72 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
7 Days					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
14 Days					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	



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EVALUATION OF REACTIONS (Draize's Method)
TABLE-II
NHH 44 Bt-COTTON SEEDS

	Rabbit Numbers			Average	Combined Index
	1M	2M	3M		
1 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
24 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
48 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
72 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
7 Days					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
14 Days					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	



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EVALUATION OF REACTIONS (Draize's Method)
TABLE-III
NHH 44 NON Bt-COTTON SEEDS

	Rabbit Numbers			Average	Combined Index
	1M	2M	3M		
1 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
24 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
48 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
72 Hrs.					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
7 Days					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	
14 Days					
Erythema Score	0	0	0	0.00	0.00
Edema Score	0	0	0	0.00	



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RESULT

The given sample of NHH 44Bt-Cotton Seeds was found to be '**non-irritant**' to the skin of rabbits when compared with the sample of NHH 44Non Bt-Cotton Seeds .

The sample has been tested as per 'DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts' for non clinical laboratory studies.