

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

IRRITATION TO MUCOUS MEMBRANE 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
(A Unit of Shriram Scientific & Industrial Research Foundation)**

19, University Road, Delhi – 110 007

Tel. 27667267, 27667860, 27667432

Fax No. 91+11-27667676, 27667207

E. Mail: sridlhi@vsnl.com



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled ‘Irritation to Mucous Membrane in Rabbits’ with NHH 44Bt-Cotton Seeds& NHH 44Non Bt-Cotton Seeds on rabbits has been 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 | 28.11.2006 | 28.11.2006 |
| Conduct | 30.11.2006 | 30.11.2006 |
| Records | 17.12.2006 | 17.12.2006 |
| Report | 22.03.2007 | 22.03.2007 |

Sr. SCIENTIST
QUALITY ASSURANCE

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE

We, the undersigned take overall responsibility to conduct the work described in the study entitled ‘Irritation to Mucous Membrane in rabbits’ with NHH 44Bt-Cotton Seeds& NHH 44Non 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



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

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)

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

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. | Index | 5 |
| 5. | Summary | 6 |
| 6. | Introduction | 7 |
| 7. | Objective | 8 |
| 8. | Test substance | 9 |
| 9. | Experimental Design | 10 |
| 10. | Husbandry | 11 |
| 11. | Acclimatization | 12 |
| 12. | Identification of animals | 13 |
| 13. | Experimental Procedure | 14 |
| | Dose | |
| 14. | Observations | 15 |
| | Skin reaction | |
| 15. | Evaluation of Irritation Index | 18 |
| 16. | Evaluation of Reactions | 19 |
| 17. | Result | 22 |



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 5 of 22

SUMMARY

In the current study, 0.1gm of the test samples i.e. NHH 44 Bt-Cotton Seed sand NHH 44 Non Bt-Cotton seeds, supplied by University of Agricultural Sciences, Dharwad was applied in powdered form to the vaginal mucous membrane of the female albino rabbits. After 4 hours i.e. the exposure period, the degree of irritation was recorded and scored at 1, 24, 48, 72 hours, 7 and 14 days.

The irritation index was calculated and found as 0.00, after examining the mucous membrane at 1, 24, 48, 72 hours, 7 and 14 days. Hence, under these conditions it was concluded that the test sample i.e. NHH 44 Bt-Cotton Seeds was **'non irritant'** to the mucous membrane of rabbits when compared with the NHH 44 Non Bt-Cotton Seeds.



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

INTRODUCTION

In the present study localized inflammatory response, on the mucous membrane of the rabbits is evaluated by a single application of the test substance as it involves the interaction of test substance with the sensory receptors in the skin.

This study serves to indicate the existence of possible hazards likely to arise from exposure on the mucous membrane to the test substance.



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

OBJECTIVE

To determine the degree of irritation produced by the test substance on the mucous membrane following a single dose application.

CHARACTERIZATION OF TEST AND CONTROL COTTONSEEDS

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



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

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 OF STUDY : 30.11.2006

DATE OF COMPLETION OF STUDY : 13.12.2006



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

EXPERIMENTAL DESIGN

ANIMALS

In the present study three female, young adult (nulliparous, non-pregnant) rabbits of the New Zealand White strain per group were taken.



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

HUSBANDRY

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

The room temperature was maintained at 22 ± 3 °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.



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

ACCLIMATIZATION

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

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 12 of 22

IDENTIFICATION OF ANIMALS

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



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

EXPERIMENTAL PROCEDURE

The exact quantity of the given test samples were applied carefully to the vaginal mucous membrane of the rabbits.

DOSE : 0.1 gm / animal



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 14 of 22

OBSERVATIONS

The mucous membrane of the rabbits were examined for any discharge and inflammatory reaction at 1, 24, 48, 72 hours, 7 and 14 days after dose application.

Page 15 of 22

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

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

| SKIN REACTION | SCORE |
|--------------------------------|-------|
| (A) ERYTHEMA | |
| No erythema | 0 |
| Very slight erythema | 1 |
| Well defined erythema | 2 |
| Moderate to severe erythema | 3 |
| Severe erythema (Beet redness) | 4 |

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 16 of 22

| 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 (moderate swelling of vulvar lips) | 3 |
| Severe edema (excessive swelling of vulvar lips with eversion of vaginal mucosa) | 4 |

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 17 of 22

EVALUATION OF IRRITATION INDEX

| EVALUATION | SCORE |
|---------------------|--------------|
| Non Irritant | 0.00 |
| Negligible Irritant | 0.00-0.40 |
| Slight Irritant | 0.41-1.99 |
| Moderate Irritant | 2.00-4.99 |
| Severe Irritant | 5.00-8.00 |



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

EVALUATION OF REACTIONS
TABLE-I
CONTROL

| | RABBIT NUMBERS | | | AVERAGE | COMBINED INDEX |
|----------------|-----------------------|-----------|-----------|----------------|-----------------------|
| | 1F | 2F | 3F | | |
| 1HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 24HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 48HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 72HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 7 DAYS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 14 DAYS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 19 of 22

EVALUATION OF REACTIONS TABLE-II NHH 44 Bt-COTTON SEEDS

| | RABBIT NUMBERS | | | AVERAGE | COMBINED INDEX |
|----------------|----------------|----|----|---------|----------------|
| | 1F | 2F | 3F | | |
| 1HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 24HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 48HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 72HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 7 DAYS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 14 DAYS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 20 of 22

EVALUATION OF REACTIONS TABLE-III NHH 44 NON Bt-COTTON SEEDS

| | RABBIT NUMBERS | | | AVERAGE | COMBINED INDEX |
|----------------|----------------|----|----|---------|----------------|
| | 1F | 2F | 3F | | |
| 1HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 24HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 48HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 72HOURS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 7 DAYS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |
| 14 DAYS | | | | | |
| Erythema Score | 0 | 0 | 0 | 0 | 0.00 |
| Edema Score | 0 | 0 | 0 | 0 | |



PROJECT NO. : TOX-355D
PRODUCT : NHH 44 Bt-COTTON SEEDS
STUDY : IRRITATION TO MUCOUS MEMBRANE
REPORT NO. : 000041399
DATE : 23.03.2007

Confidential

Page 21 of 22

RESULT

The given sample of NHH 44 Bt-Cotton Seeds was found to be '**non-irritant**' to the vaginal mucous membrane of the rabbits when compared to the NHH 44 Non Bt-cotton seeds.

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

Page 22 of 22