

Annexure to Report No. : 000073685

Dt : 28.02.2004

TOXICOLOGY STUDY REPORT

PROJECT NO. : TOX / 290

SPONSOR : NATH SEEDS (NEW DELHI)
309- MEGHDOOT,
94 NEHRU PLACE,
NEW DELHI

SUBJECT : IRRITATION TO MUCOUS MEMBRANE
IN RABBITS

PRODUCT : Bt- COTTON SEEDS (NATH SEEDS) ALONG
WITH NON-TRANSGENIC COTTON SEEDS

MATERIAL DESCRIPTION : BROWN COLORED COTTON SEEDS

RESULT : NON-IRRITANT
The given sample of Bt- cotton seeds (Nath seeds) was found to be **non-irritant** to the vaginal mucous membrane of the rabbits, when compared to its corresponding non-transgenic cotton seeds.

TOTAL NO. OF : 7
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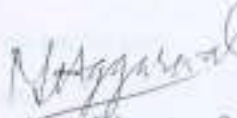
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BT-COTTON SEEDS (NATH SEEDS)
BIOSAFETY STUDY (IRRITATION TO MUCOUS MEMBRANE IN RABBITS)

QUALITY ASSURANCE STATEMENT

The work described in this report was performed under my supervision as study director in accordance with guidelines for Toxicity and Allergenicity, Evaluation of Transgenic seeds, Plants and Plant parts (Adoption O.E.C.D guideline No. 405), Department of Biotechnology, Ministry of Science and Technology, Govt. of India for non-clinical laboratory studies

The report provides true and accurate record of results obtained.


**Asst. Director &
Chief, Toxicology**

The following scientific and supervisory personnel were involved in the study :

Dr. Binu Bhat

Dr. Dharendra Singh

Manoj Kumar

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (IRRITATION TO MUCOUS MEMBRANE IN RABBITS)

OBJECTIVE

To determine the potential of irritation produced by sample of Bt cotton seeds (Nath seeds) along with non-transgenic cotton seeds on the vaginal mucous membrane following a single application.

EXPERIMENTAL DESIGN

ANIMALS

Six female young adult (nulliparous, non- pregnant) rabbits of the New Zealand White strain per sample were used.

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 24 ± 2 °C with 40-70 % relative humidity.

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

Acclimatization

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

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BT-COTTON SEEDS (NATH SEEDS)
BIOSAFETY STUDY (IRRITATION TO MUCOUS MEMBRANE IN RABBITS)

EXPERIMENTAL PROCEDURE

Sample Preparation

The Bt-cotton seeds (Nath seeds) as well as the Non-transgenic cotton seeds samples were crushed and was weighed and applied as such carefully to the vaginal mucous membrane of the rabbit.

Dose : 0.1 gm

OBSERVATIONS

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

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (IRRITATION TO MUCOUS MEMBRANE IN RABBITS)

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
(B) OEDEMA FORMATION	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well raised)	2
Moderate oedema (moderate swelling of vulvar lips)	3
Severe oedema (excessive swelling of vulvar lips with eversion of vaginal mucosa)	4

EVALUATION OF IRRITATION INDEX

EVALUATION	SCORE
Non Irritant	0
Negligible Irritant	0-0.40
Slight Irritant	0.41-1.99
Moderate Irritant	2.00-4.99
Severe Irritant	5.00-8.00

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 BT-COTTON SEEDS (NATH SEEDS)
 BIOSAFETY STUDY (IRRITATION TO MUCOUS MEMBRANE IN RABBITS)

EVALUATION OF REACTIONS (Draize Test)**Bt-cotton seeds (Nath seeds)**

	RABBIT NUMBERS						AVERAGE	COMBINED INDEX
	1F	2F	3F	4F	5F	6F		
24HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
48HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
72HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 7th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 14th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0

Non-transgenic cotton seeds

	RABBIT NUMBERS						AVERAGE	COMBINED INDEX
	1F	2F	3F	4F	5F	6F		
24HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
48HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
72HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 7th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 14th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0

Note : F – Female

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (IRRITATION TO MUCOUS MEMBRANE IN RABBITS)

Result :

The given sample of Bt- cotton seeds (Nath Seeds) was found to be **non-irritant** to the vaginal mucous membrane of the rabbits, when compared to its corresponding non-transgenic cotton seed.

The sample has been tested as per the guidelines for Toxicity and Allergenicity, Evaluation of Transgenic seeds, Plants and Plant parts parts (Adoption O.E.C.D guideline No. 405), Department of Biotechnology, Ministry of Science and Technology, Govt. of India for non-clinical laboratory studies.

TOXICOLOGY STUDY REPORT

PROJECT NO. : TOX/ 290

SPONSOR : NATH SEEDS (NEW DELHI)
309- MEGHDOOT,
94- NEHRU PLACE,
NEW DELHI-110 019.

SUBJECT : PRIMARY SKIN IRRITATION TEST ON
RABBITS

PRODUCT : Bt- COTTON SEEDS (NATH SEEDS) ALONG
WITH NON-TRANSGENIC COTTON SEEDS

MATERIAL DESCRIPTION : BROWN COLORED COTTON SEEDS

RESULT : NON - IRRITANT
The given sample of Bt- cotton seeds (Nath seeds)
was found to be **non-irritant** to the skin of the
rabbits, when compared to its corresponding non-
transgenic cotton seed.

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SHIRIRAM INSTITUTE**BT-COTTON SEEDS (NATH SEED)
BIOSAFETY STUDY (PRIMARY SKIN IRRITATION TEST ON RABBITS)****QUALITY ASSURANCE STATEMENT**

The work described in this report was performed under my supervision as study director in accordance to the guidelines for Toxicity and Allergenicity Evaluation of Transgenic seeds, Plants and Plant parts parts (Adoptive O.E.C.D guideline No. 404), Department of Biotechnology, Ministry of Science and Technology, Govt. of India for non-clinical laboratory studies.

The report provides true and accurate record of results obtained.


**Asst. Director &
Chief, Toxicology**

The following scientific and supervisory personnel were involved in the study :

Dr. Binu Bhat

Dr. Dharendra Singh

Manoj Kumar

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (PRIMARY SKIN IRRITATION TEST ON RABBITS)

OBJECTIVE

To evaluate the potential for local primary irritant effects to rabbit skin following a single application of Bt cotton seeds (Nath Seeds) and non-transgenic cotton seeds.

EXPERIMENTAL DESIGN

Animals

Six (3 Male and 3 Female) adult rabbits of the New Zealand White Strain per sample were used.

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 $24 \pm 2^\circ \text{C}$ with 40 - 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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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (PRIMARY SKIN IRRITATION TEST ON RABBITS)

Acclimatization

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

EXPERIMENTAL PROCEDURE

Skin Preparation :

Twenty four hours before the test (dose application) the hair on the back and flanks of each rabbit was closely clipped.

Dose : 0.5 gm

Sample preparation :

The Bt-cotton seed (Nath seeds) as well as the Non-transgenic cotton seed samples were crushed and was weighed and applied as such carefully to the skin of the rabbits.

Application Procedure

The given Bt-cotton seed (Nath seeds) and non-transgenic cotton seed samples were crushed and evenly applied to a small moistened area of the closely clipped skin of each rabbit. The site of application was covered with cotton gauze patch, which was held in contact with skin by means of semi-occlusive dressing.

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BT-COTTON SEEDS (NATH SEEDS) PRIMARY SKIN IRRITATION TEST ON RABBITS

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

OBSERVATIONS

Skin reactions at the site of applications were assessed and scored at 24, 48, 72 hours, day 7th and day 14th after the dose application (post test observation period) 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

(B) OEDEMA FORMATION

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

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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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (PRIMARY SKIN IRRITATION TEST ON RABBITS)

EVALUATION OF REACTIONS (Draize's Method)

Bt-cotton seeds (Nath seeds)

	RABBIT NUMBERS						AVERAGE	COMBINED INDEX
	1M	2M	3M	4F	5F	6F		
24HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
48HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
72HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 7th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 14th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0

Non-transgenic cotton seeds

	RABBIT NUMBERS						AVERAGE	COMBINED INDEX
	1M	2M	3M	4F	5F	6F		
24HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
48HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
72HOURS								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 7th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0
DAY 14th								
Erythema Score	0	0	0	0	0	0	0	0
Edema Score	0	0	0	0	0	0	0	0

Note : F – Female

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (PRIMARY SKIN IRRITATION TEST ON RABBITS)

Result

The given sample of Bt-cotton seeds (Nath Seeds) was found to be **non-irritant** to the skin of the rabbits, when compared to its corresponding non-transgenic cotton seed.

The sample has been tested as per the guidelines for toxicity and allergenicity, evaluation of transgenic seeds, plants and plant parts (Adoption O.E.C.D guideline No. 404), Department of Biotechnology, Ministry of Science and Technology, Government of India, for non-clinical laboratory studies.

Annexure to Report No. : 000073685

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TOXICOLOGY STUDY REPORT

PROJECT NO. : TOX/ 290

SPONSOR : NATH SEEDS (NEW DELHI)
309, MEGHDOOT,
94, NEHRU PLACE,
NEW DELHI-110 019.

SUBJECT : DERMAL SENSITIZATION STUDY IN
GUINEA PIGS (BUEHLER METHOD)

PRODUCT : BT- COTTON SEEDS (NATH SEEDS) ALONG
WITH NON TRANSGENIC COTTON SEEDS

MATERIAL DESCRIPTION : BROWN COLORED COTTON SEEDS

RESULTS : Under the conditions of this study the repeated
application of Bt-cotton seeds (Nath seeds) did not
induce dermal sensitization in guinea pigs when
compared to its corresponding non-transgenic
cotton seeds.

NO. OF PAGES : 15

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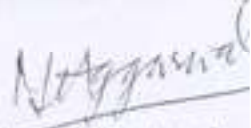
BT-COTTON SEEDS (NATH SEEDS)
BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

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SHRIRAM INSTITUTE**BT-COTTON SEEDS (NATH SEEDS)
BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)****QUALITY ASSURANCE STATEMENT**

The work described in this report was performed under my supervision as study director in accordance with Guidelines for toxicity and allergenicity, Evaluation of Transgenic seeds, plants and plant parts (Adoption O.E.C.D guideline No. 406), Department of Biotechnology, Ministry of Science and Technology, Government of India for non-clinical laboratory studies.


**Asstt. Director &
Chief of Toxicology**

The following scientific and supervisory personnel were involved in the study :

Dr. Binu Bhat

Dr. Dharendra Singh

Mr. Manoj Kumar

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

SCOPE

This study was designed to demonstrate the potential of the Bt-cotton seeds (Nath seeds) and non-transgenic cotton seed samples to elicit an immunological response through contact with the skin.

SIGNIFICANCE AND USE

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.

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

OBJECTIVE

To compare the dermal sensitization potential of “ Bt-cotton seeds (Nath Seeds) and non-transgenic seeds” in guinea pigs as per Buehler Method.

SUMMARY WITH CONCLUSION

Bt-cottonseeds (Nath Seeds) as well as non-transgenic cotton seeds were crushed and moistened with water were applied to the shaved skin of 10 young adult guinea pigs per group for 6 hours a day, three times a week for three weeks until 9 applications were made in the induction (sensitization) phase. A control group of 5 guinea pigs was also 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 (negative controls) was removed with a clipper from the mid back area and a paste of the test article was 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.

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BT-COTTON SEEDS (NATH SEED) BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

Under the conditions of this study, the repeated application of Bt- cotton seeds (Nath Seeds) did not induce dermal sensitization (allergies) in any of the test guinea pigs, when compared to its corresponding non-transgenic cotton seed.

Procedure

The animals were divided into two groups. One group was given the extract of Bt-cotton seeds and the other group was given the extract of non-transgenic cotton seeds.

Results

The results showed that there was no allergic reaction in any of the guinea pigs in either group.

Environmental conditions

Room Temperature

24 ± 2°C

Relative Humidity (%)

50 ± 20

Air Change

12 air changes per hour

Lighting

12 hours light and 12 hours dark

Air Humidity

20-80% relative humidity

These conditions were maintained throughout the study.

SHRIRAM INSTITUTE**BT-COTTON SEEDS (NATH SEED
BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)****EXPERIMENTAL DESIGN**

Animals 10 young adult healthy guinea pigs for the Bt-cottonseed group, 10 for non-transgenic and 5 for negative control group were used.

Husbandry The animals were caged individually with proper identification in plastic cages fitted with wire mesh tops and floors.

The standard guinea pig diet, water and green vegetables were freely available.

Environmental conditions

Room Temperature : $24 \pm 2^{\circ}\text{C}$

Relative Humidity (%) : 50 ± 20

Air Changes : 15 Air changes / hour

Lighting : 12 hours light & dark alternatively

Acclimatization : A minimum 7 days period of acclimatization was allowed before commencing the study.

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BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)
BT-COTTON SEEDS (NATH SEEDS)

ANIMAL GROUPS AND TEST APPLICATIONS

Group	No. of animals	No. of applications	
		Induction	Challenge
Negative Control	5	0	1
Control (Non-Transgenic)	10	9	1
Test (Bt Cotton seeds)	10	9	1

EXPERIMENTAL PROCEDURE (BUEHLER METHOD)

The dermal sensitization study comprised of two test phases -

1. Induction Phase
2. Challenge Phase

Sample preparation :

The Bt-cotton seeds (Nath seeds) as well as the Non-transgenic cotton seed samples were crushed and a paste (prepared by moistening the crushed sample with water) was prepared daily before application.

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. A paste (prepared by moistening the crushed cotton seed sample with water) of the sample of Bt cotton seeds as well as non-transgenic cotton seeds backed by 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.

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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

The test patches were removed after 6 hours 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.

2. Challenge Phase

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

The paste (prepared by moistening the crushed cotton seeds with water) of the samples of Bt cotton seeds (Nath Seeds) and non-transgenic cotton seed was applied respectively to the shaved area of the test and the control guinea pigs and tapped in place. The trunk of each animal was wrapped with bandage to maintain the test site. 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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BT-COTTON SEEDS (NATH SEEDS)
BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

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

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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BT-COTTON SEEDS (NATH SEEDS) BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

OBSERVATION

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

RESULT

Under the conditions of this study, the repeated application of "Bt-cotton seeds (Nath Seeds)" did not induce dermal sensitization (allergies) to the skin of any of the guinea pigs when compared to its corresponding non-transgenic cotton seeds.

The samples have been tested in accordance with Guidelines for toxicity and allergenicity, Evaluation of Transgenic seeds, plants and plant parts (Adoption O.E.C.D guideline No. 406), Department of Biotechnology, Ministry of Science and Technology, Government of India for non-clinical laboratory studies.

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BT-COTTON SEEDS (NATH SEEDS)
 BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

TABLE NO. 1
 SUMMARY OF ERYTHEMA AND OEDEMA SCORE

Group	Induction Phase		Challenge Phase	
	Erythema	Oedema	Erythema	Oedema
Negative control (Untreated)	-	-	0	0
Control (Non-Transgenic- cotton seeds)	0	0	0	0
Test (Transgenic- cotton seeds)	0	0	0	0

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BT-COTTON SEEDS (NATH SEEDS)
 BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

TABLE NO. 2
 INDUCTION PHASE
 EVALUATION OF REACTION
 NON-TRANSGENIC 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	0.0
	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	0.0
	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	0.0
	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	0.0
	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	0.0
	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	0.0
	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	0.0
	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	0.0
	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	0.0
	Oedema	0	0	0	0	0	0	0	0	0	0	0		

SHRIRAM INSTITUTE

BT-COTTON SEEDS (NATH SEEDS)
 BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

TABLE NO. 3
 INDUCTION PHASE
 EVALUATION OF REACTION
 Bl. COTTON SEEDS (NATH 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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BT-COTTON SEEDS (NATH SEEDS)
 BIOSAFETY STUDY (DERMAL SENSITIZATION STUDY IN GUINEA PIGS)

TABLE NO. 4
CHALLENGE PHASE
EVALUATION OF SKIN REACTION

Skin Reaction	Time (hours)	Treated Animals (BT.Cottonseeds)										Average Combined Index	Untreated Animals (Negative control)					Average Combined Index
		1	2	3	4	5	6	7	8	9	10		1	2	3	4	5	
Erythema After	6 hrs.	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
Erythema After	24 hrs	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
Erythema After	48 hrs	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

Skin Reaction	Time (hours)	Treated Animals (Non-Transgenic)										Average Combined Index	Untreated Animals (Negative control)					Average Combined Index
		1	2	3	4	5	6	7	8	9	10		1	2	3	4	5	
Erythema After	6 hrs.	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
Erythema After	24 hrs	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
Erythema After	48 hrs	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