

Annexure to Report No. : 000066702

Dt.: 14.11.03

TOXICOLOGY STUDY REPORT

PROJECT NO. : TOX/ 289

SPONSOR : J. K. AGRI – GENETICS,
(A DIVISION OF J. K. INDUSTRIES LTD.)
1-10-177, 4TH FLOOR, VARUN TOWERS,
BEGUMPET, HYDERABAD - 500016

SUBJECT : PRIMARY SKIN IRRITATION TEST ON
RABBITS

PRODUCT : CRUSHED WHOLE SEED OF TRANSGENIC
COTTON LINE JKC738 (Lot No. F3815) ALONG
WITH NON TRANSGENIC COTTON SEED
LINE JKC738 (Lot No. F 3815)

MATERIAL : BROWN COLORED CRUSHED WHOLE SEED
DESCRIPTION

RESULT : NON - IRRITANT
The given sample of Crushed whole seed of
transgenic cotton line JKC738 were found to be
non-irritant to the skin of the rabbits, when
compared to its corresponding non-transgenic
crushed cotton seed line JKC738.

TOTAL NO. OF : 7
PAGES


SCIENTIST
PATHOLOGY


DY. DIRECTOR

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(A Unit of Shriram Scientific & Industrial Research Foundation, Delhi)
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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
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, 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED PRIMARY SKIN IRRITATION TEST ON RABBITS

OBJECTIVE

To evaluate the potential for local primary irritant effects to rabbit skin following a single application of crushed transgenic cotton seed line & crushed non-transgenic cotton seed.

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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED 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 exact quantity of crushed transgenic cotton seed was weighed and applied as such carefully to the of the closely clipped skin of each rabbit.

Application Procedure

The given crushed transgenic and non-transgenic cotton seed sample were 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
PRIMARY SKIN IRRITATION TEST ON RABBITS

EVALUATION OF REACTIONS

Crushed whole seed transgenic cotton line JKC738

| | 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 |

Crushed whole seed Non-transgenic cotton line JKC 738

| | 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
PRIMARY SKIN IRRITATION TEST ON RABBITS

EVALUATION OF REACTIONS (Draize's Method)

Result

The given sample of crushed whole seed transgenic cotton line JKC 738 was found to be **non-irritant** to the skin of the rabbits, when compared to its corresponding crushed whole seed non-transgenic cotton line JKC 738.

The sample has been tested as per the 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.

Annexure to Report No. : 000066702

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TOXICOLOGY STUDY REPORT

PROJECT NO. : TOX / 286

SPONSOR : J. K. AGRI – GENETICS,
(A DIVISION OF J. K. INDUSTRIES LTD.)
1-10-177, 4TH FLOOR, VARUN TOWERS,
BEGUMPET, HYDERABAD - 500016SUBJECT : IRRITATION TO MUCOUS MEMBRANE
IN RABBITSPRODUCT : CRUSHED WHOLE SEED OF TRANSGENIC
COTTON LINE JKC738 (Lot. No. F 3815) ALONG
WITH NON TRANSGENIC COTTON SEED LINE
JKC738 (Lot. No. F 3815)MATERIAL
DESCRIPTION : BROWN COLORED CRUSHED WHOLE SEEDRESULT : NON-IRRITANT
The given sample of Crushed whole seed of
Transgenic cotton line JKC738 was found to be
non-irritant to the vaginal mucous membrane of
the rabbits, when compared to its corresponding
non-transgenic crushed cotton seed line JKC738TOTAL NO. OF : 7
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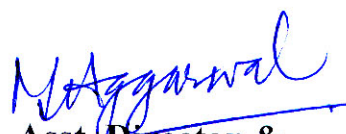
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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
IRRITATION TO MUCOUS MEMBRANE IN RABBITS

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, 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED IRRITATION TO MUCOUS MEMBRANE IN RABBITS

OBJECTIVE

To determine the potential of irritation produced by sample of crushed transgenic cotton seed along with crushed non-transgenic cotton seed 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
IRRITATION TO MUCOUS MEMBRANE IN RABBITS

EXPERIMENTAL PROCEDURE

Sample Preparation

The crushed Transgenic as well as the Non-transgenic cotton seed samples were 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
IRRITATION TO MUCOUS MEMBRANE IN RABBITS

EVALUATION OF REACTIONS

Crushed transgenic cotton seed

| | 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 crushed cotton seed

| | 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED IRRITATION TO MUCOUS MEMBRANE IN RABBITS

Result :

The given sample of crushed whole transgenic cotton line JKC738 was found to be **non-irritant** to the vaginal mucous membrane of the rabbits, when compared to its corresponding non-transgenic crushed cotton seed line JKC738.

The sample has been tested as per the guidelines for Toxicity and Allergenicity, Evaluation of Transgenic seeds, Plants and Plant parts, Department of Biotechnology, Ministry of Science and Technology, Govt. of India for non-clinical laboratory studies.

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SPONSOR : J.K. AGRI - GENETICS
(A DIVISION OF J.K.INDUSTRIES LTD)
1-10-177, 4th FLOOR, VARUN TOWERS,
BEGUMPET, HYDERABAD - 500016

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

PRODUCT : CRUSHED WHOLE SEED OF TRANSGENIC
COTTON LINE JKC738 (Lot No. F3815) ALONG
WITH NON- TRANSGENIC COTTON SEED
JKC738 (Lot No. F 3815)

MATERIAL DESCRIPTION : BROWN COLORED CRUSHED WHOLE SEED

RESULTS : Under the conditions of this study the repeated
application of crushed whole transgenic cotton seed
line JKC738 did not induce dermal sensitization in
guinea pigs when compared to its corresponding
crushed non-transgenic cotton seed line JKC738.

Total No. of Pages : 15

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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED DERMAL SENSITIZATION STUDY IN GUINEA PIGS

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**CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
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 guidelines No. 401), 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. Dhirendra Singh

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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED DERMAL SENSITIZATION STUDY IN GUINEA PIGS

SCOPE

This study was designed to demonstrate the potential of the crushed whole seed transgenic and non-transgenic cotton 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED DERMAL SENSITIZATION STUDY IN GUINEA PIGS

OBJECTIVE

To compare the dermal sensitization potential of “crushed transgenic and non-transgenic cotton seeds” in guinea pigs as per Buehler Method.

SUMMARY WITH CONCLUSION

Transgenic as well as non-transgenic crushed cotton seeds 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.

Under the conditions of this study, the repeated application of crushed whole transgenic cotton seed line JKC738 did not induce dermal sensitization (allergies) in any of the test guinea pigs, when compared to its corresponding crushed non-transgenic cotton seed line JKC738.

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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED DERMAL SENSITIZATION STUDY IN GUINEA PIGS

EXPERIMENTAL DESIGN

| | | | |
|---------------------------------|---|---|--|
| Animals | 10 young adult healthy guinea pigs for the transgenic 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 ± 2°C | |
| Relative Humidity (%) | : | 50 ± 20 | |
| Air Changes | : | 15 Air changes / hour | |
| Lighting | : | 12 hours light & dark alternatively | |
| Acclimatisation | : | A minimum 7 days period of acclimatisation was allowed before commencing the study. | |

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 (Transgenic) | 10 | 9 | 1 |

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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
DERMAL SENSITIZATION STUDY IN GUINEA PIGS

EXPERIMENTAL PROCEDURE (BUEHLER METHOD)

The dermal sensitization study comprised of two test phases -

1. Induction Phase
2. Challenge Phase

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 whole cotton seed sample with water) of the sample of Transgenic 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.

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.

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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
DERMAL SENSITIZATION STUDY IN GUINEA PIGS

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 sample of transgenic cotton 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.

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

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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
DERMAL SENSITIZATION STUDY IN GUINEA PIGS

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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE ALONG WITH NON TRANSGENIC COTTON SEED 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 crushed "transgenic cotton seeds" did not induce dermal sensitization (allergies) to the skin of any of the guinea pigs when compared to its corresponding crushed 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 guidelines No. 401), Department of Biotechnology, Ministry of Science and Technology, Government of India for non-clinical laboratory studies.

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**CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
DERMAL SENSITIZATION STUDY IN GUINEA PIGS

TABLE NO. 2
INDUCTION PHASE
EVALUATION OF REACTION
NEGATIVE CONTROL

| 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
DERMAL SENSITIZATION STUDY IN GUINEA PIGS

TABLE NO. 3
INDUCTION PHASE
EVALUATION OF REACTION
CRUSHED 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 |
| | 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
DERMAL SENSITIZATION STUDY IN GUINEA PIGS

TABLE NO. 4
INDUCTION PHASE
EVALUATION OF REACTION
CRUSHED TRANSGENIC COTTON SEED

| 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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CRUSHED WHOLE SEED OF TRANSGENIC COTTON LINE
ALONG WITH NON TRANSGENIC COTTON SEED
DERMAL SENSITIZATION STUDY IN GUINEA PIGS

TABLE NO. 5
CHALLENGE PHASE
EVALUATION OF SKIN REACTION

| Skin Reaction | Time (hours) | Treated Animals (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 | 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 |

| 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 | 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 |