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Institute for Toxicological Studies

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Registration No 6 / 1999 / CPCSEA
Ministry of Social Justice and Empowerment, Government of India

PROTOCOL

Study Title

Mucous Membrane Irritation Test
of **Transgenic eggplant - Brinjal**
(Solanum melongena L.)
in Female Rabbit

Testing Facility

INTOX PVT. LTD.
375, Uravade, Tal. Mulshi,
Dist. Pune - 412 108
INDIA

Sponsor

Maharashtra Hybrid Seeds Co. Ltd.
Resham Bhavan, 4th floor
78, Veer Nariman Road
Mumbai - 400 020
INDIA

PROTOCOL NO. : P03.163

Date : 02 July, 2003

Total number of pages in this protocol : 11

CONTENTS

I. SUMMARY

Study Title	:	Mucous Membrane Irritation Test in Female Rabbit
Test Article	:	**Transgenic eggplant - Brinjal *(Solanum melongena L.)***
Study Number	:	Will be assigned upon authorization of the study.
Study Schedule	:	To be decided later
Monitoring Scientist	:	Dr. M. K. Sharma
Test Species	:	New Zealand White Rabbit Three females per group, 14 to 18 weeks old
Route of administration	:	Intravaginal
Duration of exposure	:	4 hours
Dose	:	0.1 g
Observations	:	All signs of ill health or reaction to treatment will be noted. The vaginal mucous membranes of each rabbit will be examined and the irritation will be assessed according to the numerical scoring system of Draize et. al. 1965.

II. INTRODUCTION

GENERAL

Sponsor	:	**Maharashtra Hybrid Seeds Co. Ltd.** Resham Bhavan, 4th floor 78, Veer Nariman Road Mumbai - 400 020 INDIA
Testing Facility	:	**INTOX PVT. LTD.** 375, Uravade, Tal. Mulshi, Dist. Pune - 412 108 INDIA

OBJECTIVE

The objective of Mucous Membrane Irritation Test in female rabbit will be to assess the possible irritation likely to arise from exposure of the mucous membranes to the test article. In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant effects on the mucous membrane of the rabbit is an important initial step. Information derived from this study serves to indicate the existance of possible hazards likely to arise from exposure of the mucous membrane to the test substance. This test provides a rational basis of risk assessment in man.

REGULATORY REFERENCES

A. Test Guidelines

The study will be conducted in compliance with the "Guidelines for Toxicity Evaluation of Transgenic Vegetables", Department of Biotechnology, Ministry of Science and Technology, Government of India, August 1998.

B. Good Laboratory Practices

The study will be conducted in compliance with the principles of Good Laboratory Practice as set forth in OECD Principles of Good Laboratory Practice (OECD, 1998).

PERSONNEL

Study Director	:	Dr. M. P. Pore $_{M.V.Sc.}$
Study Supervision and Animal Care	:	Dr. P. K. Pawar $_{M.V.Sc.}$

III. MATERIALS AND METHODS

TEST ARTICLE

The Sponsor is responsible for characterisation of the test article.The information supplied by the Sponsor is given below. Certificate of analysis provided by the Sponsor will be appended with the report.

Test Article	:	**Transgenic eggplant - Brinjal** ***(Solanum melongena L.)***
Characteristics	:	Solid
Supplied by	:	**Maharashtra Hybrid Seeds Co. Ltd.** Resham Bhavan, 4th floor 78, Veer Nariman Road Mumbai - 400 020 INDIA

TEST SYSTEM AND MANAGEMENT

Species	:	Rabbit
Strain	:	New Zealand White
Source	:	Breeding laboratories of INTOX PVT. LTD.
Age at start of treatment	:	14 to 18 weeks
Body weight range	:	1.5 to 2.5 kg
Number of animals per group	:	Three females
No. of groups	:	Four (Total Number of Animals - 12) Group 1 - Control Group 2 - Non transgenic vegetable Group 3 - Non transgenic vegetable (commercially available) Group 4 - Transgenic eggplant - Brinjal
Selection of Animals	:	The vaginal mucosa of each animal will be observed one day prior to the initiation of the study. Only healthy females free from vaginal discharge will be selected for study.
Acclimation	:	At least one week in experimental room after veterinary examination.

Husbandry

Environmental conditions	: Air conditioned rooms with 10 - 15 air changes per hour, temperature between 17^0- $23\ ^0$C, relative humidity 30 - 70% and illumination cycle set to 12 hours light and 12 hours dark.
Accomodation	: Singly, in stainless steel cages provided with wire mesh bottom and facilities for feeder and water bottle.
Diet	: Standard `Amrut' brand pelleted rabbit feed manufactured by M/s Nav Maharashtra Chakan Oil Mills Ltd., Pune, ad libitum
Water	: Water, supplied by Pune Municipal Corporation and passed through 'Aqua Guard On Line Water Filter', will be provided in glass bottles, ad libitum

EXPERIMENTAL PROCEDURE

Test Article Preparation

The test article will be finely ground and instilled in vagina.

Treatment

On test day, 0.1 g of the transgenic vegetables or non transgenic vegetables will be placed in the upper half of vaginal vault of each animal. The rabbits will be fixed in rabbit-holder during application. After 4 hours exposure residual test article will be flushed with lukewarm tap water. Control animals will be handled in the similar way except test article application.

IV. OBSERVATIONS

Body Weights

Body weights will be recorded on the day of application.

Clinical Signs

Rabbits will be observed daily for pharmaco-toxic signs throughout the study period.

Irritation Scoring

After exposure period of 4 hours, the vaginal mucosa of each animal will be examined at 30-60 minutes and 24, 48 and 72 hours for local irritation. If indicated, further observations will be made at on 7th day and 14th day to evaluate the reversibility of the effects observed. The irritation will be assessed according to the following numerical scoring system (Draize et. al., 1965).

SCALE FOR SCORING OF LOCAL IRRITATION

	Values
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 slight eschar formation (injuries in depth)	4
Maximum possible erythema score - 4	
Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight Oedema (slight swelling of Vulvar lips)	2
Moderate oedema (moderate swelling of Vulvar lips)	3
Severe oedema (severe swelling of Vulvar lips with protrusion)	4
Maximum possible oedema score - 4	

Evaluation of results

The individual scores for erythema and oedema at the designated observation times 30-60 minutes and at 24, 48 and 72 hours after application is used to calculate an irritancy index.

The irritancy index is obtained by totalling the scores for erythema and oedema after 30-60minutes and at 24, 48 and 72 hours. The resulting total is divided by the number of rabbits and the factor 4.

Classification is based on the following scale :

Irritance index	classification
0.0	non-irritant
0.1 - 2.0	slightly irritant
2.1 - 5.0	moderately irritant
5.1 - 8.0	severely irritant

The Irritancy Index on which the scores for erythema and oedema are based is not the only measure of mucous membrane irritation. The classification "Irritant" is applicable only if the erythema and oedema are reversible during the period of the study. A comprehensive evaluation also takes into accout any other dermal changes which may occur.

V. REPORTING

Two copies of final report will be submitted to the Sponsor.

The final report will include, but not be limited to the following :

The name and address of the Sponsor, the testing facility and the study schedule.

A description of test article, including concentration, purity, composition and other appropriate characteristics of the test article, as provided by the Sponsor.

A description of test animals including species, strain, source, number, sex, body weight range, age, housing conditions, diet etc.

A description of methods used.

Summary

Introduction

Materials and Methods

A description of all results including local irritation

Table of individual animal irritation score and degree of irritation

A summary of pharmaco-toxic signs, if any

Conclusion

References for experimental methodology

Principal personnel participating in the study

The Quality Assurance Statement, signed by the Quality Assurance Manager.

The GLP compliance Statement, signed by the Study Director.

The storage locations of all raw data, specimens and the report.

VI. AMENDMENTS TO PROTOCOL

Alterations to the experimental design will only be made following documented discussion between the Study Director and the Sponsor. If immediate action is necessary verbal agreement with the Sponsor will be confirmed as soon as possible by protocol amendment. Minor changes of the protocol which do not influence the procedures or the outcome of the study may be subject to the discretion of the Study Director, but will be mentioned in the study report.

VII. ARCHIVES

All specimens, raw data and other documents generated during the course of this study together with a copy of the final report, will be stored in the Archives of INTOX PVT. LTD., Pune, for a period of five years from the date of submission of final report.

VIII. QUALITY ASSURANCE UNIT REVIEW

The Quality Assurances Unit will conduct inspections of the various phases of the study and of certain repetitive operations to ensure the quality and integrity of the study. The final report will be reviewed by Quallity Assurance Unit comparing individual findings against raw data.

02 July, 2003

PROTOCOL APPROVAL

Mucous Membrane Irritation Test of

Transgenic eggplant - Brinjal *(Solanum melongena L.)* in Female Rabbit

This **Protocol No. P03.000,** (Mucous Membrane Irritation Test of **Transgenic eggplant - Brinjal *(Solanum melongena L.)*** in Female Rabbit) has been mutually agreed and signed.

Protocol prepared by :

..... Date : 02.07.2003

(Dr. M. P. Pore, Study Director)

Protocol approved on behalf of Testing Facility Management :

..... Date : 02-07-03

(Dr. P. Y. Naik, Technical Director)

Protocol approved on behalf of Sponsor :

..... Date : 02/07/03

(Dr. M. K. Sharma, General Manager)

Mucous membrane irritation test

SUMMARY results:

Mucous membrane irritation test was performed with **Transgenic Bt Brinjal containing *cry1A(c) gene (Solanum melongena L.)*** in female New Zealand White rabbits in full compliance with the "Guidelines for Toxicity Evaluation of Transgenic Vegetable", Department of Biotechnology, Ministry of Science and Technology, Government of India, August 1998.

Total nine female rabbits were used in this study. Three rabbits were treated with transgenic vegetable, three with non-transgenic vegetable, three with non-transgenic vegetable (commercially available) and three remained untreated and served as control. The test article, **Transgenic Bt Brinjal containing *cry1A(c) gene (Solanum melongena L.),*** non-transgenic vegetable or non-transgenic vegetable (commercially available) in the amount of 0.1 g was introduced into the vagina of each of three female animals. Local irritation to vaginal mucous membrane was evaluated at 1, 24, 48 and 72 hours after the treatment following the method of Draize J. H., 1965.

The test article, **Transgenic Bt Brinjal containing *cry1A(c) gene (Solanum melongena L.),*** non-transgenic vegetable and non-transgenic vegetable (commercially available), did not cause any erythema or edema to vaginal mucous membrane throughout the observation period of 72 hours after application. Throughout the study no clinical signs of intoxication were observed in any of the rabbits.

Based on the average irritation index (0.0), **Transgenic Bt Brinjal containing *cry1A(c) gene (Solanum melongena L.)*** is classified as non-irritant to mucous membrane in rabbit.

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Registration No 6 / 1999 / CPCSEA
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PROTOCOL

STUDY TITLE

Subchronic Oral (90 Day) Toxicity Study
of **Transgenic eggplant - Brinjal**
(Solanum melongena L.)
in Rat

Testing Facility
INTOX PVT. LTD.
375, Uravade, Tal. Mulshi,
Dist. Pune - 412 108
INDIA

Sponsor
Maharashtra Hybrid Seeds Co. Ltd.
Resham Bhavan, 4th floor
78, Veer Nariman Road
Mumbai - 400 020
INDIA

PROTOCOL NO. : P03.166

DATE : 02 July, 2003

Total number of pages in this protocol : 12

CONTENTS

I. INTRODUCTION

GENERAL

Sponsor	:	**Maharashtra Hybrid Seeds Co. Ltd.** Resham Bhavan, 4th floor 78, Veer Nariman Road Mumbai - 400 020 INDIA
Testing Facility	:	**INTOX PVT. LTD.** 375, Uravade, Tal. Mulshi, Dist. Pune - 412 108 INDIA

OBJECTIVE

The objective of Subchronic Oral (90-day) Toxicity Study in rat will be to assess the toxicological profile of **Transgenic eggplant - Brinjal *(Solanum melongena L.)*** when administered daily, for 90 consecutive days. This study will provide information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The results of this study should provide information on target organs, the possibilities of cumulation and can provide an estimate of a no-observed-adverse-effect-level of exposure which can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

REGULATORY REFERENCES

A. TEST GUIDELINES

The study will be conducted in compliance with the "Guidelines for Toxicity Evaluation of Transgenic Vegetables", Department of Biotechnology, Ministry of Science and Technology, Government of India, August 1998.

B. Good Laboratory Practices

The study will be conducted in compliance with the principles of Good Laboratory Practice as set forth in the OECD Principles of Good Laboratory Practices, OECD, 1998.

PERSONNEL

Study Director	:	Dr. M. P. Pore M.V.Sc.
Study Supervisor	:	Mr. S. D. Borate M.Sc.
Animal Care	:	Dr. P. K. Pawar M.V.Sc.
Pathology	:	Dr. B. V. Jalnapurkar M.V.Sc., Ph.D, F.R.V.C.S. (Sweden)

II. MATERIALS AND METHODS

TEST ARTICLE

The Sponsor is responsible for characterization of the test article. Information provided by the Sponsor is presented below as a summary. Certificate of analysis of the test article provided by the Sponsor will be appended to the final report.
@

Test Article	:	**Transgenic eggplant - Brinjal *(Solanum melongena L.)***
Characteristics	:	Solid
Manufactured by	:	**Maharashtra Hybrid Seeds Co. Ltd.** Resham Bhavan, 4th floor 78, Veer Nariman Road Mumbai - 400 020 INDIA

TEST SYSTEM AND MANAGEMENT

Species	:	Rat
Strain	:	Sprague Dawley
Source	:	Bred and reared at INTOX PVT. LTD.
Age at start of study	:	6 to 8 weeks
Weight range at start of the study	:	The weight variation of animals used shall not exceed ± 20 % of the mean weight for each sex.
Identification	:	By cage tag and corresponding picric acid colour body markings
Number of animals per dose group	:	Twenty - 10 males and 10 females
Acclimation	:	At least one week in experimental room after veterinary examination.
Randomization	:	After acclimation and veterinary examination, the rats will be randomly selected in groups of males and females

Husbandry

Environmental : Air conditioned rooms with 10-15 air changes per hour, temperature between 19-250C, relative humidity 30-70 %, and illumination cycle set to 12 hours light and 12 hours dark.

Accommodation : Groups of five animals of similar sex in polypropylene cages with stainless steel grill tops, facilities for food and water bottle, and bedding of clean paddy husk.

Diet : `Amrut' brand standard pelleted rat feed manufactured by M/s Nav Maharashtra Chakan Oil Mills Ltd., Pune, ad libitum.

Water : Water, supplied by Pune Municipal Corporation and passed through 'Aqua Guard On Line Water Filter', will be provided in glass bottles, ad libitum

STUDY DESIGN

Prior to final assignment to the study, the animals will be subjected to a veterinary examination to ensure that the selected rats are in a good state of health.

As tabulated below, groups of 10 rats of each sex will be administered **Transgenic eggplant - Brinjal *(Solanum melongena L.)*** by oral gavage, 5 days/week, for 90 days and then will be sacrificed and subjected to a complete necropsy.

Dose Group	Treatment	Treatment Level (mg/kg)
I	Vehicle control	10 ml/kg
II	Non-transgenic vegetable	1000
III	Non-transgenic vegetable (commercially available)	1000
IV	Transgenic eggplant	250*
V	Transgenic eggplant	500*
VI	Transgenic eggplant	1000

*If no mortality is observed at 1000 mg/kg in dose range finding study, the main study will include only high dose (1000 mg/kg) of transgenic eggplant.

FORMULATION OF TEST ARTICLE

The test article i.e. concentrated paste or cryogenic dehydrated powder of transgenic eggplant - Brinjal will be finely ground and will be suspended in peanut oil. Formulation of the test article will be prepared shortly before dosing on each day. Formulations for different doses will vary in concentrations to allow a constant dosage volume. The dose volume will not exceed 10 ml per kg body weight.

ADMINISTRATION OF TEST ARTICLE

The test article, will be administered by oral gavage to each rat daily, 5 days/week, for 90 days. The animals will be dosed at approximately the same time each day where possible using a stainless steel intubation needle fitted onto a suitably graduated glass syringe. The dosage volume administered to individual rat will be adjusted according to its most recently recorded body weights.

Treatment in this manner will be continued once a day, five days a week, for a period of 90 days. Vehicle control group animals will be treated with penut oil at the same dosage volume i. e. 10 ml/kg body weight.

OBSERVATIONS

Following observations will be made during the course of treatment.

Mortality

Throughout the study, all cages will be checked early on each working day and again in the afternoon to look for dead or moribund animals to allow necropsy examination to be carried out during the working hours of that day.

All rats that will be killed in extremis, or found dead in the cage will be subjected to detailed necropsy examination and a full spectrum of tissue samples will be preserved in 10 % neutral buffered formalin.

Clinical Signs

All signs of ill health, together with any behavioural changes or reaction to treatment will be recorded for individual animals. Dated and signed records of appearance, change and disappearance of clinical signs will be maintained on clinical history sheets for individual animals.

General Clinical Examinations

The rats will be daily subjected to general cageside clinical examinations, at the same time each day, and at suitable intervals after dosing, considering the peak period of anticipated effects after dosing.

Detailed Clinical Examinations

The rats will be subjected to detailed clinical examinations before initiation of the treatment (to allow for within-subject comparisons) and weekly thereafter during the treatment period. These observations will be made outside the home cage in a standard arena and preferably at the same time. Signs noted will include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity such as lacrimation, piloerection, pupil size, unusual respiratory pattern. Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, sterotypies or bizarre behaviour will also be recorded.

Body weight

The weight of each rat will be recorded at the time of allocation of animals to groups, on the day of commencement of treatment, weekly thereafter and at necropsy.

Food Consumption
The quantity of food consumed will be recorded daily.

Water Intake
Water intake will be recorded daily.

PATHOLOGY

Clinical Pathology
On completion of 90 days of treatment, samples of blood will be drawn, from the recurrent tarsal vein of male and female rats from each group and collected in tubes containing EDTA / Heparin as an anticoagulant.
Food will be removed overnight from animals to be sampled for laboratory investigations.
The estimations that will be performed on blood samples have been listed below, together with an abbreviated title (used in Appendices and Tables).

Haematology
The following estimations will be performed using 'Erba Hemolab-8 Hematology Analyser' (US Tech Inc., Fort Washington, MD, USA) :
Haemoglobin (Hb)
Packed cell volume (PCV)
Total red cell count (Total RBC)
Total white cell count (Total WBC)
Absolute erythrocyte indices :
- Mean corpuscular volume (MCV)
- Mean corpuscular haemoglobin (MCH)
- Mean corpuscular haemoglobin concentration (MCHC)

Clotting time, Prothrombin Time (PT) and Erythrocyte Sedimentation Rate (ESR) will be performed manually using standard techniques.

Clinical Chemistry
The following parameters will be analysed using the Erba Chem-5 plus Selective Multiparametric Clinical Chemistry Analyser (Transasia Bio-Medicals Ltd., India) using standard methodology :
- Total Protein
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Histamine
- Alkaline phosphatase (ALP)
- Glucose
- Urea Nitrogen (UN)
- Non-protein nitrogen (NPN)
- Bilirubin, total
- Acetylcholinesterase (AChE)
- Immunoglobulin profile (IgM, IgA, IgE)

Terminal Studies

Necropsy Examination

On completion of 90 days of treatment, all surviving rats will be sacrificed by carbondioxide anaesthesia. Complete necropsies will be carried out on all animals including those which will die during the study by the veterinary pathologist.

All the tissues listed in Appendix 1, from all animals, will be preserved in 10% neutral buffered formalin. In addition, samples of any macroscopically abnormal tissues will be preserved, along with samples of adjacent normal tissue.

Organ Weights

The following organs from all animals killed at the scheduled sacrifices will be trimmed of any adherent tissue, as appropriate, fat and weighed wet as soon as possible to avoid drying :

kidneys, liver, adrenals, testes, spleen, brain, ovaries,

Values of these organs as percent of necropsy body weights will be estimated (relative organ weights).

Histopathological Examination

Tissues preserved for microscopic examination in this study are listed in Appendix 1. These tissues will be embedded in paraffin wax, sectioned at five micrometres and stained with haematoxylin and eosin. Histopathological examinations of these organs will only be conducted if gross lesions are noted.

Disposal

The carcass will be mutilated by using Calcium Hydroxide and buried deep.

STATISTICAL ANALYSIS

Body Weights and organ weights

Bartlett's test (Bartlett,193) will be performed on each set of data to ensure that variance of the sets are homogenous. In case of homogenous set of data ANOVA will be performed to determine the treatment effects, and Dunnett's test (Dunnett, 1964) will be employed as appropriate.
In case of heterogenous data, F test will be carried out to determine which pairs of groups are heterogenous. This will be followed by Cochran's or Student's t tests, as appropriate.

Haematology and Clinical Chemistry

Bartlett's test will be performed on each set of data to ensure that variance of the sets were homogenous. In case of homogenous set of data ANOVA and / or t test will be carried out. In case of heterogenous data, F test will be carried out to determine which pairs of groups are heterogenous. This will be followed by Cochran's or Student's t tests, as appropriate

III. REPORTING

Two copies of final report will be submitted to the Sponsor.

The final report will include, but not be limited to the following :

The name and address of the Sponsor, the testing facility and the study schedule.

A description of test article, including concentration, purity, composition and other appropriate characteristics of the test article as provided by the Sponsor.

A description of test animals including species, strain, source, number, sex, body weight range, age, housing conditions, diet etc.

A description of methods used.

A description of the doses, dose regimen.

Individual and Summary of mortality data

individual and Summary of clinical signs

Tables of mean and individual body weights and food consumption

Tables of mean and individual haematology and clinical chemistry parameters

Tables of mean and individual organ weights and organ / body weight ratios

Tables of individual and summary of gross pathology findings

Tables of individual and summary of histopathology findings, if any.

Narrative discussion of parameters evaluated

Conclusion

References for experimental methodology

Principal personnel participating in the study

Quality assurance statement

Compliance statement

The storage location of raw data, specimens and reports.

IV. AMENDMENTS TO PROTOCOL

Alterations to the experimental design will only be made following documented discussion between the Study Director and the Sponsor. If immediate action is necessary verbal agreement with the Sponsor will be confirmed as soon as possible by protocol amendment. Minor changes of the protocol which do not influence the procedures or the outcome of the study may be subjected to the discretion of the Study Director, but will be mentioned in the study report.

V. ARCHIVES

All specimens, raw data and other documents generated during the course of this study together with a copy of the final report, will be stored in the Archives of INTOX PVT. LTD., for five years after submission of final report.

VI. QUALITY ASSURANCE UNIT REVIEW

The Quality Assurances Unit will conduct inspections of the various phases of the study and of certain repetitive operations, at the intervals specified by the Good Laboratory Practice Regulations. The dates on which the findings of these inspections are reported to the Study Director and to Management will be specified in the final report.

The final report will be reviewed by Quality Assurance Unit comparing individual findings against raw data and comparing the statements and results presented in the report with individual data presented in the Appendices of the report.

APPENDIX 1

List of Tissues Preserved for Histopathological Examination

All Gross lesions
Thyroid
Lungs
Heart
Brain
Stomach
Duodenum
Jejunum
Ileum
Colon
Liver
Spleen
Kidneys
Adrenals
Testes / Ovaries
Uterus
Prostrate

APPENDIX 2

PROTOCOL APPROVAL

Subchronic Oral (90 Day) Toxicity Study
of **Transgenic eggplant - Brinjal *(Solanum melongena L.)*** in Rat

This **Protocol No. P03.161,** Subchronic Oral - 90 Day
Toxicity Study of **Transgenic eggplant - Brinjal *(Solanum melongena L.)*** in Rat
has been mutually agreed and signed.

Protocol prepared by :

..... Date : 02.07.03

(Dr. M. P. Pore, Study Director)

Protocol approved :

..... Date : 02-07-03

(Dr. P. Y. Naik, Technical Director)

Protocol approved on behalf of Sponsor :

..... Date : 02/07/03.

(Dr. M. K. Sharma, General Manager)

Subchronic oral 90 days toxicity study

SUMMARY results

Subchronic Oral (90 day) Toxicity Study of **Transgenic Bt brinjal containing *cry 1 A(c) gene*** in Sprague Dawley rat was performed in compliance with the "Guidelines for Toxicity Evaluation of Transgenic Vegetables", Department of Biotechnology, Ministry of Science and Technology, Government of India, August 1998 and in accordance with the mutually agreed protocol.

Groups of ten male and ten female Sprague Dawley rats were administered **Transgenic Bt brinjal containing *cry 1 A(c) gene*** suspended in peanut oil by oral gavage daily at the dose of 1000 mg/kg body weight, five days a week, for 90 days and were sacrificed on day 91 to evaluate its toxicity. One concurrent group of ten male and ten female rats was similarly treated with non-transgenic brinjal in peanut oil, one group of ten male and ten female rats was treated with non-transgenic brinjal (commercially available) in peanut oil, while a fourth group of ten male and ten female rats was gavaged with peanut oil only, and served as vehicle control.

Dose range finding study on was performed prior to the main study. The results of this 14 day dose range finding study were considered as a basis for selection of doses for the main 90 day study.

The rats were examined daily for signs of toxicity, morbidity and mortality. They were subjected to detailed clinical examination before initiation of the study and weekly thereafter during the exposure period and at termination. Body weights were recorded weekly. Food and water consumption was recorded weekly. Laboratory investigations were performed on blood at termination of the study. All animals sacrificed terminally were subjected to a detailed necropsy and weights of certain organs were recorded. Histopathological evaluation was performed on tissues showing gross pathological changes during necropsy.

There was no incidence of treatment related mortality in rats exposed to **Transgenic Bt brinjal containing *cry 1 A(c) gene,*** non-transgenic brinjal or non-transgenic brinjal

(commercially available) at 1000 mg/kg body weight. The test articles did not induce any remarkable and treatment related clinical abnormalities in rats treated at the dose of 1000 mg/kg. No mortality or abnormal clinical signs were observed in animals treated with vehicle control article.

Transgenic Bt brinjal containing *cry 1 A(c) gene,* non-transgenic brinjal, non-transgenic brinjal (commercially available), did not have any adverse effect on the body weight gain and average daily food and water consumption by the male and female rats treated at the dose level of 1000 mg/kg.

The hematological parameters of hemoglobin, packed cell volume, total RBC counts, total and differential WBC counts, RBC indices, clotting time, prothrombin time and erythrocyte sedimentation rate of male and female rats, exposed to the test articles at the level of 1000 mg/kg were found to be comparable to those of the vehicle control animals at termination of the treatment.

The test article, **Transgenic Bt brinjal containing *cry 1 A(c) gene,*** non-transgenic brinjal, non-transgenic brinjal (commercially available), treated at the level of 1000 mg/kg, did not alter the plasma levels of total protein, alanine aminotransferase, asparate aminotransferase, histamine, alkaline phosphatase, glucose, urea nitrogen, non-protein nitrogen, total bilirubin, acetylcholinesterase and immunoglobulins (IgM, IgA, IgE) in male and female rats.

The values of absolute and relative weights of liver, kidneys, adrenals, spleen, brain, testes and ovaries of male and female rats treated with **Transgenic Bt brinjal containing *cry 1 A(c) gene*,** non-transgenic brinjal, non-transgenic brinjal (commercially available), at 1000 mg/kg were found to be comparable with those of the vehicle control rats at the end of treatment period.

Transgenic Bt brinjal containing *cry 1 A(c) gene,* non-transgenic brinjal and non-transgenic brinjal (commercially available), at the dose level of 1000 mg/kg, did not induce any remarkable and treatment related gross pathological alterations in any of the tissues of exposed rats, as evident at the detailed necropsy examination carried out at termination of the study.

There were isolated instances of necropsy findings such as reddening of lungs, dilated kidney pelvis, distended uterus and abscess in salivary gland. The gross pathological changes observed during necropsy were confirmed histologically. The abscess noted grossly in salivary gland was confirmed histologically. Lungs reddening noted at necropsy in four animals, was identified as acute congestion. The incidence of

pathological lesions being extremely small, and not dose dependent, was not considered to be of toxicological significance.

Based on the findings of this study, the no-observed-adverse-effect-level (NOAEL) of **Transgenic Bt brinjal containing *cry 1 A(c) gene*** in Sprague Dawley rats, following oral administration for 90 days was found to be more than 1000 mg/kg body weight.