



PROJECT : TOX-346
PRODUCT : Bt COTTONSEEDS
STUDY : ACUTE ORAL TOXICITY STUDY IN RATS
REPORT NO. : 000041134
DATE : 22.03.2007

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ACUTE ORAL TOXICITY STUDY IN RATS

WITH

Bt COTTONSEEDS

Report for :

METAHELIX LIFE SCIENCES PRIVATE LIMITED
PLOT NO.3, KIADB 4th PHASE, BOMMASANDRA,
BANGALORE-560 099, INDIA

Guidelines:

**‘DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic
Seeds, Plants and Plant Parts’**

Prepared by :

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QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled 'Acute oral toxicity study in wistar rats' with 'Bt Cottonseeds' has been audited and examined with respect to the study protocol and the Standard Operating Procedures in accordance to 'DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts' in compliance with Good laboratory Practices (G.L.P) for non clinical laboratory studies.

The report provides true and accurate record of results obtained.

The dates of inspections & dates on which findings were reported to the study director & SRI management are given below:

<u>Phases of study</u>	<u>Dates of Inspection</u>	<u>Dates of Reporting</u>
Protocol	10.11.2006	10.11.2006
Conduct	13.11.2006	13.11.2006
Records/ Raw data	30.11.2006	30.11.2006
Report	22.03.2007	22.03.2007

Binnu Bhat
Sr. SCIENTIST
QUALITY ASSURANCE

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STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE

We, the undersigned take overall responsibility to conduct the work described in the study entitled 'Acute oral toxicity study in Wistar rats' with Bt Cottonseeds 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.

Ashish
STUDY DIRECTOR *P. S. Chauhan*
SCIENTIST PATHOLOGY *M. G. Gaurwal*
HEAD, DEPT. OF TOXICOLOGY

Approved for issue

[Signature]
DEPUTY DIRECTOR
(MANAGEMENT)

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SCIENTIFIC PERSONNEL INVOLVED IN THE STUDY

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SUMMARY

In the assessment and evaluation of the toxic characteristics of a substance, determination of ‘acute oral toxicity’ is usually an initial step.

This study was hence, designed to conduct acute oral toxicity of ‘Bt Cottonseeds’ provided by M/s Metahelix Life Sciences Private Limited, in wistar rats.

Three groups consisting of 5 male and 5 female rats each were designated for the study. The first group was kept as control that was dosed with vehicle only, the second group of animals was administered with the Non-Bt cotton seeds (Sample-I) in powdered form orally at the dose level of 5000 mg/kg body weight with the help of metallic cannula attached with tuberculin syringe and the third group of animals was treated similarly with powdered Bt cotton seeds (Sample-II).

No toxic signs and symptoms / mortality was observed in any test group as well as the control group of animals. Hence no further testing was required.

Under the conditions of this study, the single oral administration of ‘Bt Cottonseeds (Sample-II)’ at the dose level of 5000 mg/kg b.wt to wistar rats did not induce any treatment related observable toxic effects, when compared to its corresponding “Non-Bt cotton seeds (Sample-I)” and the control group of animals treated with corn oil (vehicle) only.



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INTRODUCTION

This study was carried out to determine the acute oral toxicity of 'Bt Cottonseeds' in wistar rats.

A limit test was conducted at the dose level 5000 mg/kg body weight taking 5 male and 5 female each and as no mortality was observed at this dose, no further testing was required.



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OBJECTIVE

- (a) To determine the acute oral toxicity using a minimum number of animals at each step to enable the classification of the compound according to any of the commonly used system.

- (b) This acute toxic class method provides information both for hazard assessment and for hazard classification purposes.

CHARACTERIZATION OF TEST AND CONTROL COTTONSEEDS

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

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TEST SUBSTANCE

PRODUCT NAME : Bt COTTONSEEDS

SPONSOR : METAHELIX LIFE SCIENCES
PRIVATE LIMITED

MATERIAL DESCRIPTION : YELLOWISH BROWN COLOURED
POWDER

PACKED IN : BROWN COLOURED PAPER
CARTONS

DATE OF COMMENCEMENT : 13.11.2006
OF STUDY

DATE OF COMPLETION : 27.11.2006
OF STUDY



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EXPERIMENTAL DESIGN

Name of species : *Rattus rattus albanicus*
Strain of the animals : Wistar
*No. of animals used per dose : 5 Male, 5 Female
Age of the animals used : 6 to 7 weeks
Weight range : 160-180 gm
Acclimatization period : 7 Days
Route of administration : Oral
Vehicle used : Corn oil

* A limit test at one dose level of 5000 mg/kg body weight was carried out with 5 male and 5 female rats.

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HUSBANDRY

All animals were caged in a group of 5 according to sex in plastic cages fitted with wire mesh tops and having sterilized paddy husk bedding. The room temperature was maintained at $22 \pm 3^{\circ}$ C with 40 - 70 % relative humidity.

The room was ventilated at the rate of approximately 15 air changes per hour.

Lighting was controlled to give 12 hours artificial light (8 a.m. - 8 p.m.) each day.

DIET

Water and standard pelleted feed (Amrut feeds Ltd.) was freely available to the experimental rats.



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IDENTIFICATION OF ANIMALS

Each cage was tagged having the details of animal group number, product name and the dosage level.

The animals were also marked with the help of marking ink.



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METHOD OF ADMINISTRATION

The animals were normally fasted for 18 hours before and 4 hours after dosing. A batch of 5 male and 5 female rats was administered the test substance orally at the highest dose level of 5000 mg/kg body weight with the help of metallic cannula attached with tuberculin syringe. No mortality was observed in any sex at this dose level. So, no further testing was required.

The control group of animals was similarly treated with corn oil only.

Frequency of administration

The test article was administered once only following an overnight fast.



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CLINICAL LABORATORY STUDIES

The following clinical laboratory determinations were made in the animals of the test as well control groups.

Blood sampling

3-5 ml of blood was withdrawn by cardiac puncture under light Carbon dioxide anesthesia prior to sacrifice.

Haematology

Following haematological estimations were performed on control and treated group of animals using Beckman Coulter Haematology Analyser A^c.T-diff.

Haematocrit (Hct)	Differential Leucocyte counts (DLC)
Haemoglobin (Hb)	Neutrophils (N)
Total Erythrocyte count (TEC)	Lymphocytes (L)
Platelet count	Basophils (B)
Total Leucocyte count (TLC)	Monocyte (M)
	Eosinophils (E)



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Serum Biochemistry

Following estimations were performed on control and treated rats using Hitachi Biochemistry Analyser 902 (Roche).

- (a) Blood sugar
- (b) Blood urea
- (c) Total protein (TP)
- (d) Albumin
- (e) Serum glutamic oxalo acetate transaminase (SGOT)
- (f) Serum glutamic pyruvic transaminase (SGPT)
- (g) Serum alkaline phosphatase (SAP)

SACRIFICE AND NECROPSY

All the experimental animals were subjected to necropsy, whether they were sacrificed or died during study. All findings not considered normal were recorded.



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Histopathology

Microscopic examination of the following tissues from all animals of the control and treated group were carried out :

Stomach	Brain
Ileum	Heart
Liver	Lungs
Spleen	Kidneys
Testis	Adrenals
Ovaries	Uterus

Any other macroscopically abnormal tissue.

BIO-STATISTICAL ANALYSIS

Student's t-test.



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RESULTS

Mortality and toxic signs

No mortality was recorded (Table- 1.01 &1.02) in the control and test groups Non-Bt Cottonseeds (Sample-I) and in Bt Cottonseeds (Sample -II) of animals. No toxic signs or symptoms (Table-1.03) were noticed in the control and in any test groups Non-bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample - II) of animals.

Mean body weights

No significant differences were observed in the percentile body weight (Table 1.04-1.07) is noticed in control and test groups Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample -II) of animals.



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Haematological evaluations

There was no significant changes in haematological parameters (Tables 2.01-2.05) in control and test groups Non-bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) of animals. These parameters fell within the accepted limits of normal variations for albino rats.

Clinical biochemistry evaluations

Serum Biochemistry evaluations (Tables 3.01-3.05) disclosed no significant differences in control and test groups Non-bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) of animals, as all the parameters fall with in the accepted limits of normal variations.

Organ Weight

Absolute organ weights and their ratios (relative organ weights) with their respective body weights are shown in Table No. (4.01-4.10). No significant changes could be noticed in the organ weights of all the test groups Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) of animals of animals, when compared with the organ weights of control group animals.



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Necropsy Finding

Necropsy examination (Tables 5.01-5.03) did not reveal any significant gross pathological changes in any of the test groups Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) when compared with the control group of animals.

Histopathological Finding

No remarkable histopathological changes (Tables 6.01-6.03) were noticed in the animals treated with Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) when compared with the control group (vehicle only) of animals.

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TABLE –1.01
LD₅₀ ASSAY - MORTALITY DATA OF MALE RATS

Dosage level mg/kg	Time of Death (Days)														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
CONTROL- DOSED WITH VEHICLE ONLY																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
NON –BT COTTONSEEDS (SAMPLE-I)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
BT COTTONSEEDS (SAMPLE-II)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

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TABLE –1.02

LD₅₀ ASSAY - MORTALITY DATA OF FEMALE RATS

Dosage level mg/kg	Time of Death (Days)														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
CONTROL- DOSED WITH VEHICLE ONLY																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
NON –BT COTTONSEEDS (SAMPLE-I)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
BT COTTONSEEDS (SAMPLE-II)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

No toxic signs & symptoms / mortality was observed in control group of animals.

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TABLE - 1.03

SUMMARY OF OBSERVATIONS

(MALES & FEMALES)

Group & Dosage level (mg / kg B.wt)	Clinical Observations	Necropsy Observations
Control group (Vehicle only) was noticed.	No toxic signs or symptoms	No noteworthy findings 5000
Non-Bt Cottonseeds (Sample-I) 5000	No treatment related toxic signs or symptoms was noticed.	No noteworthy findings
Bt Cottonseeds (Sample-II) 5000	No treatment related toxic signs or symptoms was noticed.	No noteworthy findings

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TABLE - 1.04

MEAN PERCENTILE BODY WEIGHT DATA OF RATS

Group No.	Sex	Day 0	Day 7	Day 14	Sex	Day 0	Day 7	Day 14
CONTROL	Male	100.00 ± 0.00	103.83 ± 0.45	107.33 ± 0.78	Female	100.00 ± 0.00	104.40 ± 0.46	108.07 ± 0.76
	NON-Bt COTTONSEEDS SAMPLE-I	Male	100.00 ± 0.00	103.90 ± 0.56		107.46 ± 0.87	Female	100.00 ± 0.00
Bt COTTONSEEDS SAMPLE-II	Male	100.00 ± 0.00	104.17 ± 0.52	107.64 ± 0.85	Female	100.00 ± 0.00	104.39 ± 0.29	108.07 ± 0.31

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TABLE – 1.05

WEEKLY PERCENTILE BODY WEIGHT DATA OF RATS

GROUP : CONTROL - Vehicle only

S.No.	Sex	Day 0	Day 7	Day14	S.No.	Sex	Day 0	Day 7	Day14
1	M	100.00	103.93	107.87	1	F	100.00	104.94	108.64
2	M	100.00	103.39	106.78	2	F	100.00	104.09	107.60
3	M	100.00	103.91	106.70	3	F	100.00	104.85	109.09
4	M	100.00	103.43	106.86	4	F	100.00	104.17	107.74
5	M	100.00	104.49	108.43	5	F	100.00	103.93	107.30
Mean		100.00	103.83	107.33	Mean		100.00	104.40	108.07
± S.D.		± 0.00	± 0.45	± 0.78	± S.D.		± 0.00	± 0.46	± 0.76

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TABLE – 1.0

WEEKLY PERCENTILE BODY WEIGHT DATA OF RATS

GROUP : NON-Bt COTTONSEEDS (SAMPLE-I) DOSE : 5000 mg/kg b.wt.

S.No.	Sex	Day 0	Day 7	Day14	S.No.	Sex	Day 0	Day 7	Day14
1	M	100.00	103.91	107.26	1	F	100.00	104.94	108.02
2	M	100.00	103.33	107.22	2	F	100.00	104.14	107.69
3	M	100.00	103.95	106.78	3	F	100.00	104.79	108.98
4	M	100.00	104.79	108.98	4	F	100.00	104.24	107.88
5	M	100.00	103.53	107.06	5	F	100.00	103.73	108.07
Mean ± S.D.		100.00 ± 0.00	103.90 ± 0.56	107.46 ± 0.87	Mean ± S.D.		100.00 ± 0.00	104.37 ± 0.50	108.13 ± 0.50

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TABLE – 1.07
WEEKLY PERCENTILE BODY WEIGHT DATA OF RATS
 GROUP : Bt COTTONSEEDS (SAMPLE-II)

DOSE : 5000 mg/kg b.wt.

S.No.	Sex	Day 0	Day 7	Day14	S.No.	Sex	Day 0	Day 7	Day14
1	M	100.00	104.62	108.09	1	F	100.00	104.29	108.59
2	M	100.00	104.07	108.14	2	F	100.00	104.44	107.78
3	M	100.00	103.35	106.15	3	F	100.00	104.05	108.09
4	M	100.00	104.60	108.05	4	F	100.00	104.85	107.88
5	M	100.00	104.19	107.78	5	F	100.00	104.32	108.02
Mean ± S.D.		100.00 ± 0.00	104.17 ± 0.52	107.64 ± 0.85	Mean ± S.D.		100.00 ± 0.00	104.39 ± 0.29	108.07 ± 0.31

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TABLE – 1.08

AVERAGE WEEKLY BODY WEIGHT(in grams) DATA OF RATS

Group No.	Sex	Day 0	Day 7	Day 14	Sex	Day 0	Day 7	Day 14
CONTROL	Male	177.40 ± 1.52	184.20 ± 2.17	190.40 ± 2.41	Female	168.80 ± 6.14	176.20 ± 5.72	182.50 ± 5.59
	NON-Bt COTTONSEEDS (SAMPLE-I)	Male	174.60 ± 5.77	181.40 ± 5.46		187.60 ± 5.32	Female	164.80 ± 3.35
Bt COTTONSEEDS (SAMPLE-II)	Male	173.00 ± 4.30	180.20 ± 4.09	186.20 ± 3.77	Female	168.20 ± 7.70	176.00 ± 7.97	182.20 ± 8.04

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TABLE - 1.09

WEEKLY BODY WEIGHT DATA (in gms) OF RATS

GROUP : CONTROL - Vehicle only

S.No.	Sex	Day 0	Day 7	Day14	S.No.	Sex	Day 0	Day 7	Day14
1	M	178	185	192	1	F	162	170	176
2	M	177	183	189	2	F	171	178	184
3	M	179	186	191	3	F	165	173	180
4	M	175	181	187	4	F	168	175	181
5	M	178	186	193	5	F	178	185	191
Mean		177.40	184.20	190.40			168.80	176.20	182.50
± S.D.		± 1.52	± 2.17	± 2.41			± 6.14	± 5.72	± 5.59

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TABLE - 1.10

WEEKLY BODY WEIGHT DATA (in gms) OF RATS

GROUP : NON-Bt COTTONSEEDS (SAMPLE I)

DOSE : 5000 mg /kg b.wt.

S.No.	Sex	Day 0	Day 7	Day14	S.No.	Sex	Day 0	Day 7	Day14
1	M	179	186	192	1	F	162	170	175
2	M	180	186	193	2	F	169	176	182
3	M	177	184	189	3	F	167	175	182
4	M	167	175	182	4	F	165	172	178
5	M	170	176	182	5	F	161	167	174
Mean ± S.D.		174.60 ± 5.77	181.40 ± 5.46	187.60 ± 5.32	Mean ± S.D.		164.80 ± 3.35	172.00 ± 3.67	178.20 ± 3.77

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TABLE - 1.11

WEEKLY BODY WEIGHT DATA (in gms) OF RATS

GROUP : Bt COTTONSEEDS (SAMPLE II)

DOSE : 5000 mg /kg b.wt.

S.No.	Sex	Day 0	Day 7	Day14	S.No.	Sex	Day 0	Day 7	Day14
1	M	173	181	187	1	F	163	170	177
2	M	172	179	186	2	F	180	188	194
3	M	179	185	190	3	F	173	180	187
4	M	174	182	188	4	F	165	173	178
5	M	167	174	180	5	F	168	169	175
Mean ± S.D.		173.00 ± 4.30	180.20 ± 4.09	186.20 ± 3.77	Mean ± S.D.		168.20 ± 7.70	176.00 ± 7.97	182.20 ± 8.04

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PRODUCT : Bt COTTONSEEDS
STUDY : ACUTE ORAL TOXICITY STUDY IN RATS
REPORT NO. : 000041134
DATE : 22.03.2007

TABLE - 2.01

MEAN HAEMATOLOGY DATA OF MALE RATS

Parameters	Control (Vehicle only)	Non-Bt Cottonseeds(Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds(Sample II) 5000 mg /kg b.wt.
WBC (X10⁵)	7.80 ± 1.67	7.48 ± 1.17	7.78 ± 1.23
<u>Differential</u> Lymphocytes %	84.60 ± 1.14	82.60 ± 2.88	83.00 ± 2.74
Neutrophils %	14.20 ± 0.84	15.80 ± 2.86	15.20 ± 2.59
Eosinophils %	0.60 ± 0.55	1.00 ± 0.00	0.80 ± 0.45
Monocytes %	0.60 ± 0.55	0.60 ± 0.55	1.00 ± 0.00
Basophils %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
R.B.C. (x10 ⁶) %	8.14 ± 0.48	8.24 ± 0.42	8.58 ± 0.54
Hb (gm %)	15.78 ± 1.12	15.30 ± 0.54	15.86 ± 1.12
Hct %	46.70 ± 3.98	45.60 ± 2.25	47.18 ± 3.08
Platelets (x10 ³)	8.78 ± 0.73	9.64 ± 0.84	9.52 ± 0.53

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TABLE – 2.02

MEAN HAEMATOLOGY DATA OF FEMALE RATS

Parameters	Control (Vehicle only)	Non-Bt Cottonseeds(Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds(Sample II) 5000 mg /kg b.wt.
WBC (X10³)	7.37 ± 1.52	6.94 ± 1.38	7.19 ± 1.14
<u>Differential</u> Lymphocytes %	77.81 ± 1.58	76.04 ± 1.92	76.73 ± 2.74
Neutrophils %	12.92 ± 1.79	14.64 ± 2.70	13.98 ± 3.11
Eosinophils %	0.60 ± 0.55	0.92 ± 0.71	0.69 ± 0.55
Monocytes %	0.60 ± 0.55	0.60 ± 0.55	0.75 ± 0.55
Basophils %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
R.B.C. (x10 ⁶) %	7.48 ± 0.30	7.41 ± 0.50	7.83 ± 0.36
Hb (gm %)	14.66 ± 0.58	13.92 ± 0.68	14.61 ± 0.66
Hct %	43.74 ± 2.19	41.21 ± 2.50	43.37 ± 1.63
Platelets (x10 ³)	8.52 ± 1.27	8.92 ± 1.66	9.00 ± 1.56

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TABLE - 2.03

HAEMATOLOGY DATA OF RATS

GROUP: Control

DIFFERENTIAL											
Animal No.	Sex	WBC Count (x 10 ³)	L%	N%	E%	M%	B%	RBC Count (x 10 ⁶)	Hb gm %	Hct %	Platelet Count (x 10 ⁵)
1	M	6.5	84.0	15.0	0.0	1.0	0.0	8.5	16.6	49.8	8.4
2	M	8.9	85.0	14.0	1.0	0.0	0.0	8.5	16.5	49.5	9.4
3	M	10.2	86.0	13.0	0.0	1.0	0.0	8.4	16.3	48.5	7.7
4	M	6.9	83.0	15.0	1.0	1.0	0.0	7.4	13.9	40.3	9.0
5	M	6.5	85.0	14.0	1.0	0.0	0.0	7.9	15.6	45.4	9.4
Mean		7.80	84.60	14.20	0.60	0.60	0.00	8.14	15.78	46.70	8.78
±		±	±	±	±	±	±	±	±	±	±
S.D		1.67	1.14	0.84	0.55	0.55	0.00	0.48	0.12	3.98	0.73
1	F	7.6	85.0	14.0	1.0	0.0	0.0	7.6	15.0	44.5	11.0
2	F	6.8	86.0	12.0	1.0	1.0	0.0	8.2	16.1	49.2	11.2
3	F	8.5	84.0	16.0	0.0	0.0	0.0	8.4	16.3	48.9	9.3
4	F	6.7	87.0	12.0	0.0	1.0	0.0	8.2	16.3	47.9	8.9
5	F	10.4	83.0	15.0	1.0	1.0	0.0	8.0	16.4	50.2	8.4
Mean		7.37	77.81	12.92	0.60	0.60	0.00	7.48	14.66	43.74	8.52
±		±	±	±	±	±	±	±	±	±	±
S.D		1.52	1.58	1.79	0.55	0.55	0.00	0.30	0.58	2.19	1.27

L = Lymphocytes

N = Neutrophils

E = Eosinophils

M = Monocytes

B = Basophils

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TABLE - 2.04

HAEMATOLOGY DATA OF RATS

GROUP: NON-Bt COTTONSEEDS (SAMPLE I) DOSE : 5000 mg /kg b.wt

DIFFERENTIAL											
Animal No.	Sex	WBC Count (x 10 ³)	L%	N%	E%	M%	B%	RBC Count (x 10 ⁶)	Hb gm %	Hct %	Platelet Count (x 10 ⁵)
1	M	6.2	85.0	14.0	1.0	0.0	0.0	7.6	14.5	42.0	9.5
2	M	8.7	82.0	16.0	1.0	1.0	0.0	8.4	15.6	46.8	9.1
3	M	8.4	81.0	18.0	1.0	0.0	0.0	8.7	15.6	47.8	9.1
4	M	6.3	79.0	19.0	1.0	1.0	0.0	8.1	15	45.0	11.1
5	M	7.8	86.0	12.0	1.0	1.0	0.0	8.4	15.8	46.4	9.4
Mean		7.48	82.60	15.80	1.00	0.60	0.00	8.24	15.30	45.60	9.64
±		±	±	±	±	±	±	±	±	±	±
S.D		1.17	2.88	2.86	0.00	0.55	0.00	0.42	0.54	2.25	0.84
1	F	6.1	80.0	19.0	1.0	0.0	0.0	7.1	14	40.1	10.7
2	F	8.2	84.0	14.0	1.0	1.0	0.0	7.6	14.6	42.8	8.9
3	F	6.3	82.0	17.0	1.0	0.0	0.0	7.9	15.1	43.8	8.3
4	F	7.2	83.0	16.0	0.0	1.0	0.0	8.4	15.8	45.4	8.4
5	F	9.4	85.0	12.0	2.0	1.0	0.0	8.1	15.2	46.6	12.1
Mean		6.94	76.04	14.64	0.92	0.60	0.00	7.41	13.92	41.21	8.92
±		±	±	±	±	±	±	±	±	±	±
S.D		1.38	1.92	2.70	0.71	0.55	0.00	0.50	0.68	2.50	1.66

L = Lymphocytes

N = Neutrophils

E = Eosinophils

M = Monocytes

B = Basophils

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TABLE - 2.05

HAEMATOLOGY DATA OF RATS

GROUP: Bt COTTONSEEDS (SAMPLE II) DOSE : 5000 mg /kg b.wt

DIFFERENTIAL											
Animal No.	Sex	WBC Count (x 10 ³)	L%	N%	E%	M%	B%	RBC Count (x 10 ⁶)	Hb gm %	Hct %	Platelet Count (x 10 ⁵)
1	M	9.4	79	19	1	1	00	8	14.2	42.6	9.3
2	M	7.6	82	16	1	1	00	9.1	16.6	49.8	8.8
3	M	6	86	12	1	1	00	8.8	16.7	48.1	9.5
4	M	7.7	83	15	1	1	00	8	15.2	45.6	9.8
5	M	8.2	85	14	0	1	00	9	16.6	49.8	10.2
Mean		7.78	83.00	15.20	0.80	1.00	0.00	8.58	15.86	47.18	9.52
±		±	±	±	±	±	±	±	±	±	±
S.D		1.23	2.74	2.59	0.45	0.00	0.00	0.54	1.12	3.08	0.53
1	F	6.7	88	10	1	1	01	8.9	15.9	46.7	10.5
2	F	6.5	82	18	0	0	05	8	15.4	46.2	12.5
3	F	7.8	81	17	1	1	00	8.5	16.9	49.7	9.6
4	F	8.1	84	15	1	0	01	8.1	15.2	45.6	9.3
5	F	9.3	85	14	0	1	02	8.4	15.7	46.1	8.4
Mean		7.19	76.73	13.98	0.69	0.75	0.00	7.83	14.61	43.37	9.00
±		±	±	±	±	±	±	±	±	±	±
S.D		1.14	2.74	3.11	0.55	0.55	0.00	0.36	0.66	1.63	1.56

L = Lymphocytes

N = Neutrophils

E = Eosinophils

M = Monocytes

B = Basophils

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TABLE - 3.01

MEAN BIOCHEMISTRY DATA ON MALE RATS

Parameters	Control (Vehicle only)	Non-Bt Cottonseeds(Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds(Sample II) 5000 mg /kg b.wt.
Urea (mg %)	39.58 ± 5.09	44.30± 7.05	33.04 ± 4.14
Glucose (mg %)	134.80 ± 4.35	121.20 ± 29.50	147.20 ± 32.46
Total Protein (gm %)	6.78 ± 0.26	7.30 ± 0.49	7.12 ± 0.50
S.G.P.T (I.U)	61.90 ± 4.06	57.22 ± 11.01	58.30 ± 9.30
S.G.O.T (I.U)	64.90 ± 13.66	53.84 ± 5.85	65.02 ± 9.24
Albumin (gm %)	3.84 ± 0.29	4.22 ± 0.33	4.18 ± 0.37
SAP (I.U)	91.80 ± 17.63	66.00 ± 14.63	66.80 ± 19.37
Cholestrol (mg %)	49.00 ± 5.48	57.00 ± 11.81	52.60 ± 7.54

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TABLE - 3.02

MEAN BIOCHEMISTRY DATA ON FEMALE RATS

Parameters	Control (Vehicle only)	Non-Bt Cottonseeds (Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds (Sample II) 5000 mg /kg b.wt.
Urea (mg %)	30.70 ± 2.10	34.06 ± 3.02	36.68 ± 4.89
Glucose (mg %)	173.60 ± 27.13	128.20 ± 26.01	163.20 ± 22.38
Total Protein (gm %)	7.16 ± 0.63	7.76 ± 0.34	7.16 ± 0.75
S.G.P.T (I.U)	41.92 ± 12.03	52.18 ± 7.38	53.68 ± 11.37
<i>S.G.O.T (I.U)</i>	51.38 ± 4.40	64.82 ± 12.36	61.50 ± 2.93
Albumin (gm %)	4.32 ± 0.31	4.22 ± 0.26	4.24 ± 0.42
SAP (I.U)	67.40 ± 10.88	79.60 ± 10.45	73.60 ± 8.96
Cholestrol (mg %)	68.80 ± 9.81	64.00 ± 7.62	61.80 ± 9.26

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TABLE - 3.03
BIOCHEMISTRY DATA OF RATS (VEHICLE ONLY)
GROUP : CONTROL

Animal No.	Sex	Urea (mg %)	Glucose (mg %)	Total Protein (gm%)	S.G.P.T I.U.	S.G.O.T I.U.	Cholestrol (mg%)	Albumin (gm%)	SAP I.U.
1	M	40.9	172	7.1	6.8	68.8	45	4.2	115
2	M	46.3	192	6.4	7.5	47.4	57	3.7	102
3	M	35	97	6.7	8.1	79.4	49	3.6	90
4	M	34	116	6.8	6.6	54.1	51	3.6	69
5	M	41.7	97	6.9	6.8	74.8	43	4.1	83
Mean		39.58	134.80	6.78	61.90	64.90	49.00	3.84	91.80
±		±	±	±	±	±	±	±	±
S.D		5.09	44.35	0.26	4.06	13.66	5.48	0.29	17.63
1	F	32.7	184	6.8	41.7	43.7	67	4.3	56
2	F	32.2	203	7.5	56.6	55	64	3.8	79
3	F	28.8	141	8.1	48.8	52.8	59	4.6	79
4	F	31.7	149	6.6	24.6	52.7	69	4.4	63
5	F	28.1	191	6.8	37.9	52.7	85	4.5	60
Mean		30.70	173.60	7.16	41.92	51.38	68.80	4.32	67.40
±		±	±	±	±	±	±	±	±
S.D		2.10	27.13	0.63	12.03	4.40	9.81	0.31	10.88

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TABLE - 3.04

BIOCHEMISTRY DATA OF RATS

GROUP: NON-Bt COTTONSEEDS (SAMPLE I) DOSE : 5000 mg /kg b.wt

Animal No.	Sex	Urea (mg %)	Glucose (mg %)	Total Protein (gm%)	S.G.P.T I.U.	S.G.O.T I.U.	Cholestrol (mg%)	Albumin (gm%)	SAP I.U.
1	M	34.2	87	7.3	49.2	48.4	41	3.7	61
2	M	44.3	141	6.9	66.1	61.3	54	4.5	83
3	M	45.1	141	8.1	41.9	53.5	66	4.1	59
4	M	54.1	91	7.3	63.3	58	71	4.3	79
5	M	43.8	146	6.9	65.6	48	53	4.5	48
Mean		44.30	121.20	7.30	57.22	53.84	57.00	4.22	66.00
±		±	±	±	±	±	±	±	±
S.D		7.05	29.50	0.49	11.01	5.85	11.81	4.22	14.63
1	F	30.7	149	7.8	56.8	54.6	51	4.6	89
2	F	33.5	132	7.8	52.3	62.5	66	4.2	84
3	F	36.2	100	8.1	46.5	85.6	71	3.9	86
4	F	38	103	7.9	61.7	56.5	67	4.1	76
5	F	31.9	157	7.2	43.6	64.9	65	4.3	63
Mean		34.06	128.20	7.76	52.18	64.82	64.00	4.22	79.60
±		±	±	±	±	±	±	±	±
S.D		3.02	26.01	0.34	7.38	12.36	7.62	0.26	10.45

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TABLE - 3.05

BIOCHEMISTRY DATA OF RATS

GROUP: Bt COTTONSEEDS (SAMPLE II) DOSE : 5000 mg /kg b.wt

Animal No.	Sex	Urea (mg %)	Glucose (mg %)	Total Protein (gm%)	S.G.P.T I.U.	S.G.O.T I.U.	Cholestrol (mg%)	Albumin (gm%)	SAP I.U.
1	M	32.4	147	7.3	43.2	58.6	65	4.4	100
2	M	32.3	144	7.9	59.3	70.7	53	4.7	68
3	M	38.9	106	6.7	66	57	49	3.9	56
4	M	34.2	142	6.7	65.8	60.3	45	3.8	56
5	M	27.4	197	7	57.2	78.5	51	4.1	54
Mean		33.04	147.20	7.12	58.30	65.02	52.60	4.18	66.80
±		±	±	±	±	±	±	±	±
S.D		4.14	32.46	0.50	9.30	9.24	7.54	0.37	19.37
1	F	34.3	142	6.8	65.4	63.6	48	3.9	76
2	F	45.3	192	6.4	62.1	57.6	57	3.7	64
3	F	33.9	181	6.8	38.4	60.3	67	4.4	78
4	F	34	157	7.5	45.6	60.9	71	4.5	85
5	F	35.9	144	8.3	56.9	65.1	66	4.7	65
Mean		36.68	163.20	7.16	53.68	61.50	61.80	4.24	73.60
±		±	±	±	±	±	±	±	±
S.D		4.89	22.38	0.75	11.37	2.93	9.26	0.42	8.96

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TABLE - 4.01

MEAN PERCENTILE ORGAN WEIGHT DATA OF MALE RATS

Dosage Group	Mean body wt. (gm)	Lung (gm)	Liver (gm)	Kidney (gm)	Testis (gm)	Adrenal (gm)	Heart (gm)	Spleen (gm)	Brains (gm)
Control (Vehicle only)	190.4 ± 2.41	0.80 ± 0.02	4.15 ± 0.33	0.95 ± 0.01	1.48 ± 0.01	0.03 ± 0.00	0.43 ± 0.02	0.29 ± 0.02	1.02 ± 0.01
Non-Bt Cotton seeds SAMPLE-I 5000 mg/kg b.wt	187.6 ± 5.32	0.78 ± 0.04	4.12 ± 0.25	0.90 ± 0.09	1.43 ± 0.03	0.04 ± 0.01	0.42 ± 0.02	0.29 ± 0.03	1.02 ± 0.01
Bt Cotton seeds SAMPLE-II 5000 mg/kg b.wt	86.2 ± 3.77	0.77 ± 0.03	4.23 ± 0.30	0.91 ± 0.06	1.46 ± 0.02	0.04 ± 0.00	0.42 ± 0.01	0.27 ± 0.01	1.02 ± 0.02

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TABLE - 4.02

MEAN PERCENTILE ORGAN WEIGHT DATA OF FEMALE RATS

Dosage Group	Mean body wt. (gm)	Lung (gm)	Liver (gm)	Kidney (gm)	Ovary (gm)	Utreus (gm)	Adrenal (gm)	Heart (gm)	Spleen (gm)	Brains (gm)
Control (Vehicle only)	182.5 ± 5.59	0.74 ± 0.02	4.17 ± 0.06	0.80 ± 0.03	0.08 ± 0.01	0.13 ± 0.02	0.04 ± 0.00	0.43 ± 0.02	0.26 ± 0.02	1.02 ± 0.02
Non-Bt Cotton seeds SAMPLE-I 5000 mg/kg b.wt	178.2 ± 3.77	0.73 ± 0.02	4.21 ± 0.06	0.79 ± 0.01	0.07 ± 0.01	0.12 ± 0.02	0.04 ± 0.01	0.41 ± 0.01	0.25 ± 0.02	1.02 ± 0.01
Bt Cotton seeds SAMPLE-II 5000 mg/kg b.wt	182.2 ± 8.04	0.74 ± 0.01	4.15 ± 0.10	0.79 ± 0.04	0.08 ± 0.01	0.12 ± 0.01	0.04 ± 0.00	0.42 ± 0.02	0.26 ± 0.03	1.01 ± 0.02

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TABLE - 4.03
AVERAGE ORGAN WEIGHT DATA OF MALE RATS

Dosage Group	Mean body wt. (gm)	Lung (gm)	Liver (gm)	Kidney (gm)	Testis (gm)	Adrenal (gm)	Heart (gm)	Spleen (gm)	Brains (gm)
Control (Vehicle only)	190.4 ± 2.41	1.51 ± 0.05	7.90 ± 0.54	1.81 ± 0.04	2.82 ± 0.05	0.06 ± 0.001	0.82 ± 0.04	0.55 ± 0.04	1.93 ± 0.04
Non-Bt Cotton seeds SAMPLE-I 5000 mg/kg b.wt	187.6 ± 5.32	1.47 ± 0.11	7.72 ± 0.44	1.69 ± 0.21	2.72 ± 0.13	0.07 ± 0.01	0.79 ± 0.05	0.54 ± 0.07	1.91 ± 0.06
Bt Cotton seeds SAMPLE-II 5000 mg/kg b.wt	186.2 ± 3.77	1.44 ± 0.08	7.87 ± 0.54	1.70 ± 0.14	2.73 ± 0.08	0.07 ± 0.01	0.77 ± 0.03	0.51 ± 0.02	1.89 ± 0.04

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TABLE - 4.04
AVERAGE ORGAN WEIGHT DATA OF FEMALE RATS

Dosage Group	Mean body wt. (gm)	Lung (gm)	Liver (gm)	Kidney (gm)	Ovary (gm)	Uterus (gm)	Adrenal (gm)	Heart (gm)	Spleen (gm)	Brains (gm)
Control (Vehicle only)	182.50 ± 5.59	1.36 ± 0.08	7.60 ± 0.14	1.47 ± 0.09	0.14 ± 0.03	0.24 ± 0.04	0.07 ± 0.01	0.78 ± 0.06	0.48 ± 0.05	1.87 ± 0.02
Non-Bt Cotton seeds SAMPLE-I 5000 mg/kg b.wt	178.2 ± 3.77	1.30 ± 0.05	7.50 ± 0.06	1.41 ± 0.05	0.12 ± 0.02	0.21 ± 0.03	0.07 ± 0.01	0.73 ± 0.03	0.44 ± 0.05	1.82 ± 0.06
Bt Cotton seeds SAMPLE-II 5000 mg/kg b.wt	182.2 ± 8.04	1.35 ± 0.09	7.56 ± 0.14	1.45 ± 0.12	0.14 ± 0.02	0.22 ± 0.04	0.07 ± 0.01	0.78 ± 0.07	0.47 ± 0.09	1.85 ± 0.07

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PROJECT : TOX-346
PRODUCT : Bt COTTONSEEDS
STUDY : ACUTE ORAL TOXICITY STUDY IN RATS
REPORT NO. : 000041134
DATE : 22.03.2007

TABLE - 4.05
 ABSOLUTE & RELATIVE ORGAN WEIGHT DATA OF MALE RATS
 GROUP: CONTROL (VEHICLE ONLY)

Animal No	Sex	Body Weight in gms	LUNG		LIVER		KIDNEY		GONADS		ADRENAL		HEART		SPLEEN		BRAIN	
			Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt
1	M	192	1.55	0.81	7.51	3.91	1.83	0.95	2.85	1.48	0.06	0.03	0.84	0.44	0.57	0.3	1.96	1.02
2	M	189	1.47	0.78	8.50	4.5	1.79	0.95	2.79	1.48	0.06	0.03	0.80	0.42	0.52	0.28	1.90	1.01
3	M	191	1.53	0.8	7.48	3.92	1.80	0.94	2.83	1.48	0.05	0.03	0.84	0.44	0.55	0.29	1.94	1.02
4	M	187	1.45	0.78	8.47	4.50	1.75	0.94	2.75	1.47	0.05	0.03	0.77	0.41	0.50	0.27	1.88	1.01
5	M	193	1.57	0.81	7.52	3.90	1.86	0.96	2.87	1.49	0.07	0.04	0.87	0.45	0.59	0.31	1.98	1.03
Mean		190.40	1.51	0.80	7.90	4.15	1.81	0.95	2.82	1.48	0.06	0.03	0.82	0.43	0.55	0.29	1.93	1.02
± S.D.		± 2.41	± 0.05	± 0.02	± 0.54	± 0.32	± 0.04	± 0.01	± 0.05	± 0.01	± 0.001	± 0.001	± 0.04	± 0.02	± 0.04	± 0.02	± 0.04	± 0.01

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TABLE - 4.06
 ABSOLUTE & RELATIVE ORGAN WEIGHT DATA OF MALE RATS
 NON-Bt COTTONSEEDS (SAMPLE I) DOSE : 5000 mg /kg b.wt.

Animal No	Sex	Body Weight in gms	LUNG		LIVER		KIDNEY		GONADS		ADRENAL		HEART		SPLEEN		BRAIN	
			Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt
1	M	192	1.56	0.81	7.50	3.91	1.85	0.96	2.83	1.47	0.07	0.04	0.83	0.43	0.61	0.32	1.95	1.02
2	M	193	1.58	0.82	7.51	3.89	1.87	0.97	2.85	1.4	0.08	0.04	0.85	0.44	0.60	0.31	1.99	1.03
3	M	189	1.47	0.78	8.51	4.5	1.80	0.95	2.78	1.47	0.06	0.03	0.81	0.43	0.53	0.28	1.92	1.02
4	M	182	1.37	0.75	7.52	4.13	1.46	0.80	2.57	1.41	0.07	0.04	0.73	0.4	0.48	0.26	1.84	1.01
5	M	182	1.35	0.74	7.55	4.15	1.45	0.80	2.59	1.42	0.06	0.03	0.74	0.41	0.47	0.26	1.85	1.02
Mean ± S.D.		187.60 ± 5.32	1.47 ± 0.11	0.78 ± 0.04	7.72 ± 0.27	4.12 ± 0.25	1.69 ± 0.21	0.90 ± 0.09	2.72 ± 0.13	1.43 ± 0.03	0.07 ± 0.008	0.04 ± 0.01	0.79 ± 0.05	0.42 ± 0.02	0.54 ± 0.07	0.29 ± 0.03	1.91 ± 0.06	1.02 ± 0.01

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TABLE- 4.07
ABSOLUTE & RELATIVE ORGAN WEIGHT DATA OF MALE RATS

Bt COTTONSEEDS (SAMPLE II) DOSE : 5000 mg /kg b.wt.

Animal No	Sex	Body Weight in gms	LUNG		LIVER		KIDNEY		GONADS		ADRENAL		HEART		SPLEEN		BRAIN	
			Wt.in gm	% Bod y wt	Wt.in gm	% Body wt	Wt.i n gm	% Body wt	Wt.in gm	% Bod y wt	Wt.i n gm	% Body wt	Wt.i n gm	% Body wt	Wt.i n gm	% Bod y wt	Wt.i n gm	% Bod y wt
1	M	187	1.46	0.78	8.46	4.52	1.74	0.93	2.76	1.48	0.08	0.04	0.76	0.41	0.51	0.27	1.87	1.00
2	M	186	1.45	0.78	8.44	4.54	1.72	0.92	2.73	1.40	0.07	0.04	0.74	0.40	0.50	0.27	1.84	0.99
3	M	190	1.51	0.79	7.46	3.93	1.80	0.95	2.80	1.47	0.08	0.04	0.81	0.43	0.54	0.28	1.95	1.03
4	M	188	1.49	0.79	7.41	3.94	1.79	0.95	2.75	1.46	0.07	0.04	0.79	0.42	0.51	0.27	1.90	1.01
5	M	180	1.31	0.73	7.56	4.20	1.45	0.81	2.59	1.44	0.07	0.04	0.76	0.42	0.50	0.28	1.89	1.05
Mean ± S.D.		186.2 ± 3.77	1.49 ± 0.04	0.77 ± 0.03	7.87 ± 0.54	4.23 ± 0.30	1.70 ± 0.14	0.91 ± 0.06	2.73 ± 0.08	1.46 ± 0.02	0.07 ± 0.01	0.04 ± 0.00	0.77 ± 0.03	0.42 ± 0.01	0.51 ± 0.02	0.27 ± 0.01	1.89 ± 0.04	1.02 ± 0.02

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TABLE - 4.08

ABSOLUTE & RELATIVE ORGAN WEIGHT DATA OF FEMALE RATS
GROUP : CONTROL (VEHICLE ONLY)

Anim al No	Sex	Body Weig ht in gms	LUNG		LIVER		KIDNEY		OVARY		UTREUS		ADRENAL		HEART		SPLEEN		BRAIN	
			Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt	Wt.in gm	% Body wt
1	F	176	1.27	0.72	7.46	4.24	1.38	0.78	0.10	0.06	0.19	0.11	0.06	0.03	0.72	0.41	0.41	0.23	1.79	1.02
2	F	184	1.38	0.75	7.61	4.14	1.48	0.80	0.19	0.10	0.30	0.16	0.08	0.04	0.80	0.43	0.52	0.28	1.92	1.04
3	F	180	1.32	0.73	7.53	4.18	1.42	0.79	0.12	0.07	0.23	0.13	0.08	0.04	0.76	0.42	0.47	0.26	1.85	1.03
4	F	181	1.34	0.74	7.56	4.18	1.44	0.80	0.14	0.08	0.26	0.14	0.07	0.04	0.77	0.43	0.49	0.27	1.88	1.04
5	F	191	1.48	0.77	7.82	4.09	1.62	0.85	0.15	0.08	0.22	0.12	0.08	0.04	0.87	0.46	0.53	0.28	1.89	0.99
Mean ± S.D.		182.5 0 ± 6.90	1.36 ± 0.08	0.74 ± 0.02	7.60 ± 0.14	4.17 ± 0.06	1.47 ± 0.09	0.80 ± 0.03	0.14 ± 0.03	0.08 ± 0.01	0.24 ± 0.04	0.13 ± 0.02	0.07 ± 0.01	0.04 ± 0.00	0.78 ± 0.06	0.43 ± 0.02	0.48 ± 0.05	0.26 ± 0.02	0.87 ± 0.05	1.02 ± 0.02

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TABLE - 4.09
ABSOLUTE & RELATIVE ORGAN WEIGHT DATA OF FEMALE RATS
NON-Bt COTTONSEEDS (SAMPLE I) DOSE : 5000 mg /kg b.wt.

Animal No	Sex	Body Weight in gms	LUNG		LIVER		KIDNEY		OVARY		UTREUS		ADRENAL		HEART		SPLEEN		BRAIN	
			Wt. in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt .in gm	% Body wt	Wt .in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt. in gm	% Body wt	Wt. in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt. in gm	% Body wt
1	F	175	1.26	0.72	7.45	4.26	1.38	0.79	0.11	0.06	0.19	0.11	0.06	0.03	0.71	0.41	0.41	0.23	1.77	1.01
2	F	182	1.36	0.75	7.56	4.15	1.48	0.81	0.13	0.07	0.25	0.14	0.08	0.04	0.76	0.42	0.50	0.27	1.8	1.03
3	F	182	1.35	0.74	7.57	4.16	1.46	0.80	0.15	0.08	0.24	0.13	0.07	0.04	0.77	0.42	0.49	0.27	1.89	1.04
4	F	178	1.30	0.73	7.49	4.21	1.39	0.78	0.12	0.07	0.20	0.11	0.07	0.04	0.73	0.41	0.43	0.24	1.79	1.01
5	F	174	1.24	0.71	7.44	4.28	1.36	0.78	0.11	0.06	0.17	0.10	0.06	0.03	0.69	0.40	0.38	0.22	1.75	1.01
Mean ± S.D.		178.2 ± 3.77	1.30 ± 0.05	0.73 ± 0.02	7.50 ± 0.06	4.21 ± 0.06	1.41 ± 0.05	0.79 ± 0.01	0.12 ± 0.02	0.07 ± 0.01	0.21 ± 0.03	0.12 ± 0.02	0.07 ± 0.01	0.04 ± 0.01	0.73 ± 0.03	0.41 ± 0.01	0.44 ± 0.05	0.25 ± 0.02	1.82 ± 0.06	1.02 ± 0.01

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TABLE - 4.10
ABSOLUTE & RELATIVE ORGAN WEIGHT DATA OF FEMALE RATS
Bt COTTONSEEDS (SAMPLE II) DOSE : 5000 mg /kg b.wt..

Animal No	Sex	Body Weight in gms	LUNG		LIVER		KIDNEY		OVARY		UTREUS		ADRENAL		HEART		SPLEEN		BRAIN	
			Wt. in gm	% Bod y wt	Wt .in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt .in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt. in gm	% Bod y wt	Wt. in gm	% Bod y wt
1	F	177	1.30	0.73	7.47	4.22	1.35	0.76	0.12	0.07	0.19	0.11	0.07	0.04	0.72	0.41	0.40	0.23	1.81	1.02
2	F	194	1.48	0.76	7.79	4.02	1.64	0.85	0.17	0.09	0.28	0.14	0.08	0.04	0.89	0.46	0.59	0.30	1.93	0.99
3	F	187	1.39	0.74	7.59	4.06	1.50	0.80	0.16	0.09	0.25	0.13	0.08	0.04	0.0	0.43	0.53	0.28	1.92	1.03
4	F	178	1.31	0.74	7.50	4.21	1.37	0.77	0.13	0.07	0.21	0.12	0.07	0.04	0.75	0.42	0.43	0.24	1.80	1.01
5	F	175	1.26	0.72	7.44	4.25	1.39	0.79	0.12	0.07	0.19	0.11	0.06	0.03	0.73	0.40	0.40	0.23	1.78	1.02
Mean ± S.D.		182.2 ± 8.04	1.35 ± 0.09	0.74 ± 0.01	7.56 ± 0.14	4.15 ± 0.10	1.45 ± 0.12	0.79 ± 0.04	0.14 ± 0.02	0.08 ± 0.01	0.22 ± 0.04	0.12 ± 0.01	0.07 ± 0.01	0.04 ± 0.00	0.78 ± 0.07	0.42 ± 0.02	0.47 ± 0.09	0.26 ± 0.03	1.85 ± 0.07	1.01 ± 0.02

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TABLE - 5.01
INTENSITY OF GROSS PATHOLOGICAL LESIONS
GROUP-CONTROL (VEHICLE ONLY) IN RATS

ANIMAL NO.										
ORGAN	MALE RATS					FEMALE RATS				
	1	2	3	4	5	1	2	3	4	5
Lungs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Liver	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gonads	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	Mild dis- colouration	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Adrenal	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gastrointesti- nal Tract	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD- No Abnormal Development

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TABLE -5.02
INTENSITY OF GROSS PATHOLOGICAL LESIONS
NON-Bt COTTONSEEDS (SAMPLE I) DOSE : 5000 mg /kg b.wt.

ANIMAL NO.										
ORGAN	MALE RATS					FEMALE RATS				
	1	2	3	4	5	1	2	3	4	5
Lungs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Liver	NAD	NAD	Mild dis-colouration	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gonads	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Adrenal	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gastrointestinal Tracts	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD- No Abnormal Development

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TABLE -5.03

INTENSITY OF GROSS PATHOLOGICAL LESIONS

Bt COTTONSEEDS (SAMPLE II) DOSE : 5000 mg /kg b.wt.

ANIMAL NO.										
ORGAN	MALE RATS					FEMALE RATS				
	1	2	3	4	5	1	2	3	4	5
Lungs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Liver	NAD	NAD	NAD	NAD	NAD	NAD	Mild dis-colouration	NAD	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gonads	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Adrenal	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gastrointestinal Tracts	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD- No Abnormal Development

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TABLE - 6.01
INTENSITY OF HISTOPATHOLOGICAL LESIONS
GROUP-CONTROL (VEHICLE ONLY) IN RATS

ANIMAL NO.										
ORGAN	MALE RATS					FEMALE RATS				
	1	2	3	4	5	1	2	3	4	5
Lungs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Liver	NAD	Focal fatty changes	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gonads	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Adrenal	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gastrointestinal Tract	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD- No Abnormal Development

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TABLE -6.02

INTENSITY OF HISTOPATHOLOGICAL LESIONS
NON-Bt COTTONSEEDS (SAMPLE I) DOSE : 5000 mg /kg b.wt.

ANIMAL NO.										
ORGAN	MALE RATS					FEMALE RATS				
	1	2	3	4	5	1	2	3	4	5
Lungs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Liver	NAD	NAD	NAD	NAD	NAD	NAD	NAD	Mild inflammatory changes	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gonads	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Adrenal	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gastrointesti -nal Tracts	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD- No Abnormal Development

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TABLE -6.03
INTENSITY OF HISTOPATHOLOGICAL LESIONS
Bt COTTONSEEDS (SAMPLE II) DOSE : 5000 mg /kg b.wt.

ANIMAL NO.										
ORGAN	MALE RATS					FEMALE RATS				
	1	2	3	4	5	1	2	3	4	5
Lungs	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Liver	NAD	NAD	NAD	NAD	Focal fatty changes	NAD	NAD	NAD	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gonads	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Adrenal	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Gastrointestinal Tracts	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

NAD- No Abnormal Development



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(A unit of Shriram Scientific and Industrial Research Foundation)

An ISO - 9001:2000 Certified Institute

TEST CERTIFICATE

000041134

Issued to :

METAHELIX LIFE SCIENCES PVT. LTD.
PLOT NO. 3, KIADB 4TH PHASE,
BOMMASANDRA
BANGALORE - 560099KARNATAKA

J.O.No.

TOX/346 A

Reg.No.

4612570

Date

22-03-2007

GC-01 (REV-04)

Your Ref.No.

--

Kind Attn: DR. M.J. VASUDEVA RAO , PRESIDENT

Sample Particulars :

Date

One sample of "Bt Cottonseeds" was received for acute oral toxicity study in wistar rats.

Material Description

: Non-Bt Cottonseeds (Sample-I)- Yellowish brown coloured powder
Bt Cottonseeds (Sample-II)- Yellowish brown coloured powder.

Sponsor

: Metahelix Life Sciences Private Limited
Plot no.3, KIADB 4th Phase, Bommasandra,
Bangalore-560 099, India.

TEST RESULTS

Acute Oral toxicity study in wistar rats

Under the conditions of this study, the single oral administration of 'Bt Cottonseeds (Sample-II)' at the dose level of 5000 mg/kg B.wt. did not induce any treatment related observable toxic effects, when compared to its corresponding Non-Bt Cottonseeds (Sample-I) and the control group of animals treated with corn oil (vehicle) only.

The sample has been conducted as per DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts.

(Annexure enclosed)

DOR : 06-11-2006

DOC : 22-03-2007


22/3/07
AUTHORISED SIGNATORY
(EMPLOYEE CODE: 6006)

19, University Road, Delhi - 110007.

E-Mail: qad@shriraminstitute.org Website: <http://www.shriraminstitute.org>

Ph: 91-11-27667267, 27667983, 27667860

Fax: 91-11-27667676, 27667207

PCR & ELISA CONFIRMATION OF BIOSAFETY COTTONSEED MATERIAL

Objective: Quality Control of the cottonseed material from cry1C-9124 based intrahirsutum hybrids to be used for the biosafety studies; despatched on 11th September 2006.

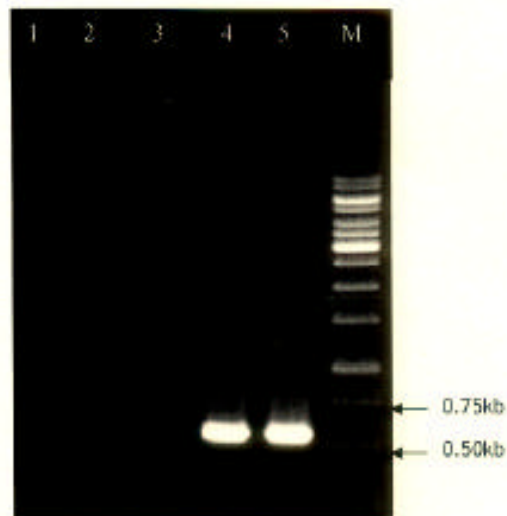
1. Confirmation of transgenic nature by PCR specific to the transgene
2. Confirmation of presence of Cry1C protein and its quantitation by ELISA

PCR confirmation

PCR was performed on Eppendorf Mastercycler Gradient machine with the following primers:

Upper: 5'-CCT CGC CAT TCT TCG TGA TTC C
 Lower: 5'-GGT TGG CCT CCC TTC CGT AGA TA

1. H₂O CONTROL
2. -VE CONTROL (LEAF)
3. NON TRANSGENIC SEED DNA
4. TRANSGENIC SEED DNA
5. +VE CONTROL



EXPECTATION- 0.58 KB

Results and conclusion

As expected amplification from cry1C was observed in case of transgenic and positive control proving the presence of the gene. Water and negative controls were clear indicating the absence of gene.



ELISA confirmation

Quantitative ELISA for Cry1C protein was performed using the Quantiplate kit for Cry1C (Envirologix, USA; Catalog No. AP 007) according to the manufacturer's protocol

Sl no	Entry ID	A ₄₅₀	Cry1C concentration (µg/g on fresh wt)
1	Blank	0.09	NA
2	1 ppb standard	0.3	0.92
3	5 ppb standard	1.44	5.2
4	10 ppb standard	2.21	9.93
5	Nontransgenic	0.092	NA
6	Transgenic	2.9	13.08

Results

The absorbance value observed at 450nm for nontransgenic sample was nearly the same as blank and no colour development was observed in case of nontransgenic. Blue colour development was observed in case of transgenic samples indicating the presence of Cry1C protein.

Declaration

I hereby declare that the certificate of quality presented above is true to the best of my knowledge and is made on the basis of experiments carried out in our premises.

Val. Ramanathan

Head- Genomics

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT : TOX-346A
PRODUCT : Bt COTTONSEEDS
STUDY : ADDENDUM TO REPORT NO. 000041134 FOR ACUTE
ORAL TOXICITY STUDY IN RATS
DATE : 29.09.2007

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ADDENDUM TO REPORT NO. 000041134 FOR

ACUTE ORAL TOXICITY STUDY IN RATS

WITH

Bt COTTONSEEDS

Report for:

**METAHELIX LIFE SCIENCES PRIVATE LIMITED
PLOT NO.3, KIADB 4th PHASE, BOMMASANDRA,
BANGALORE-560 099, INDIA**

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

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E. Mail: sridlhi@vsnl.com

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



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QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled 'Addendum to Report No. 000041134 for Acute Oral Toxicity Study in Rats' with 'Bt Cottonseeds' has been examined in accordance to the '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.

Binu Bhat
Sr. SCIENTIST
QUALITY ASSURANCE

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT : TOX-346A
PRODUCT : Bt COTTONSEEDS
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STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE

We, the undersigned take overall responsibility to conduct the work described in the study entitled 'Addendum to Report No. 000041134 for Acute Oral Toxicity Study in Rats' with Bt Cottonseeds performed with respect to 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

JOINT DIRECTOR
(MANAGEMENT)

SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH



PROJECT : TOX-346A
PRODUCT : Bt COTTONSEEDS
STUDY : ADDENDUM TO REPORT NO. 000041134 FOR ACUTE
ORAL TOXICITY STUDY IN RATS
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TEST SUBSTANCE

The sponsor is responsible for necessary characterization and evaluation of the test substance. The details of the test substance provided by the sponsor are as follows:

PRODUCT NAME : NON-Bt COTTONSEEDS (SAMPLE I)
& Bt COTTONSEEDS (SAMPLE II)

SPONSOR : METAHELIX LIFE SCIENCES
PRIVATE LIMITED

MATERIAL DESCRIPTION : YELLOWISH BROWN COLOURED
POWDER

PACKED IN : BROWN COLOURED PAPER
CARTONS

DATE OF COMMENCEMENT : 16.08.2007
OF STUDY

DATE OF COMPLETION : 31.08.2007
OF STUDY

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PROJECT : TOX-346A
PRODUCT : Bt COTTONSEEDS
STUDY : ADDENDUM TO REPORT NO. 000041134 FOR ACUTE
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DATE : 29.09.2007

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EXPERIMENTAL DESIGN

Name of species : *Rattus rattus albanicus*
Strain of the animals : Wistar
No. of animals used per dose : 5 Male, 5 Female
Age of the animals used : 6 to 7 weeks
Weight range : 160-180 gm
Acclimatization period : 7 Days
Route of administration : Oral
Vehicle used : Corn oil

GROUP AND DOSAGE LEVEL

Group	Dosage Level (mg/kg B.wt.)	No. of animals	
		Male	Female
Control group (Vehicle only)	0.00	5	5
Non-Bt Cottonseeds (Sample-I)	5000	5	5
Bt Cottonseeds (Sample-II)	5000	5	5

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HUSBANDRY

All animals were caged in a group of 5 according to sex in plastic cages fitted with wire mesh tops and having sterilized paddy husk bedding. The room temperature was maintained at $22 \pm 3^{\circ}$ C with 30 - 70 % relative humidity. The room was ventilated at the rate of approximately 15 air changes per hour.

Lighting was controlled to give 12 hours artificial light (8 a.m. - 8 p.m.) each day.

Water and standard pelleted feed (Amrut feeds Ltd.) was freely available to the experimental rats.

IDENTIFICATION OF ANIMALS

Each cage was tagged having the details of animal group number, product name, dosage level, date of initiation and date of completion.

The animals were also marked with the help of picric acid.

DOSE ADMINISTRATION

The animals were normally fasted for 18 hours before and 4 hours after dosing. Three groups of 5 male and 5 female rats each were designated for the study. First group of animals was administered with Non-Bt Cottonseeds (Sample-I) in powdered form orally at the highest dose level of 5000 mg/kg body weight with

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the help of metallic cannula attached with tuberculin syringe. Second group was similarly administered orally with powdered Bt Cottonseeds (Sample-II).

The control group of animals was also treated with corn oil only.

Frequency of administration

The test article was administered once only following an overnight fast.

OBSERVATION

Mortality and toxic signs

Mortality and clinical sign and symptoms were recorded during the observation period of 14 days after dose administration.

CLINICAL LABORATORY STUDIES

The following clinical laboratory determinations were made in the animals of the test as well control groups as supplement to the main report (Report No. 000041134) entitled 'Acute oral toxicity study in rats with Bt Cottonseeds'.

Blood sampling

3-5 ml of blood was withdrawn by cardiac puncture under light Carbon dioxide anesthesia prior to sacrifice.

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Serum Biochemistry

Following estimations were performed on control and treated rats using Hitachi Biochemistry Analyser 902 (Roche).

- a. Bilirubin
- b. Acetylcholinesterase (AchE)
- c. Serum histamine level

BIO-STATISTICAL ANALYSIS

Student's t-test.

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RESULTS

Mortality and toxic signs

Mortality and clinical sign and symptoms were recorded during the observation period of 14 days after dose administration.

No mortality was recorded (Table- 1.01 &1.02) in the animals of control and test groups i.e. Non-Bt Cottonseeds (Sample-I) and Bt Cottonseeds (Sample-II) of animals. No toxic signs or symptoms (Table-1.03) were noticed in the control and in any test groups i.e. Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) animals.

Clinical biochemistry evaluations

Serum Biochemistry evaluations for Bilirubin and Acetylcholinesterase (AchE) (Tables 2.01-2.05) disclosed no significant differences in control and test groups Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) of animals, as all the parameters fell within the accepted limits of normal variations. Serum histamine level was found negligibly in all the test groups and was comparable to the the control group of animals.

Conclusion

Under the conditions of this study, the single oral administration of 'Non-Bt Cottonseeds (Sample –I)' and 'Bt Cottonseeds (Sample –II)' at the dose level of 5000 mg/kg b.wt to wistar rats did not induce any treatment related observable toxic effects with regards to bilirubin and acetylcholinesterase (AchE) and

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serum histamine, when compared to its control group of animal treated with corn oil (vehicle) only.

TABLE –1.01
LD₅₀ ASSAY - MORTALITY DATA OF MALE RATS

Dosage level mg/kg	Time of Death (Days)														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
CONTROL- DOSED WITH VEHICLE ONLY																
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
NON-BT COTTONSEEDS (SAMPLE-I)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
BT COTTONSEEDS (SAMPLE-II)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

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TABLE –1.02
LD₅₀ ASSAY - MORTALITY DATA OF FEMALE RATS

Dosage level mg/kg	Time of Death (Days)														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
CONTROL- DOSED WITH VEHICLE ONLY																
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
NON –BT COTTONSEEDS (SAMPLE-I)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
BT COTTONSEEDS (SAMPLE-II)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

No toxic signs & symptoms / mortality was observed in control group of animals.

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TABLE - 1.03
SUMMARY OF OBSERVATIONS (MALES & FEMALES)

Group & Dosage level (mg / kg B.wt)	Clinical Observations	Necropsy Observations
Control group (Vehicle only) was noticed.	No toxic signs or symptoms	No noteworthy findings
Non-Bt Cottonseeds (Sample-I) 5000	No treatment related toxic signs or symptoms was noticed.	No noteworthy findings
Bt Cottonseeds (Sample-II) 5000	No treatment related toxic signs or symptoms was noticed.	No noteworthy findings

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TABLE - 2.01
MEAN BIOCHEMISTRY DATA ON MALE RATS

Parameters	Control (Vehicle only)	Non-Bt Cottonseeds (Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds (Sample II) 5000 mg /kg b.wt.
Bilirubin	0.09 ± 0.01	0.07±0.02	0.06±0.01
Acetylcholinesterase (AChE)	562.2±104.25	592.4±76.11	629.4±87.08

* Serum histamine was present negligibly.

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TABLE -2.02
MEAN BIOCHEMISTRY DATA ON FEMALE RATS

Parameters	Control (Vehicle only)	Non-Bt Cottonseeds (Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds (Sample II) 5000 mg /kg b.wt.
Bilirubin	0.08±0.01	0.07±0.01	0.07±0.01
Acetylcholinesterase (AchE)	1435.8±292.47	1706.6±344.21	1346.8±332.19

* Serum histamine was present negligibly.

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TABLE -2.03
BIOCHEMISTRY DATA OF Rats (VEHICLE only)
GROUP: CONTROL

Animal No.	Sex	Bilirubin	Acetyl cholinesterase (AchE)
1	M	0.10	476
2	M	0.08	684
3	M	0.09	494
4	M	0.10	489
5	M	0.10	668
Mean ± S.D		0.09 ± 0.01	562.2±104.25
1	F	0.07	1575
2	F	0.06	932
3	F	0.11	1685
4	F	0.09	1483
5	F	0.09	1504
Mean ± S.D		0.08±0.01	1435.8±292.47

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TABLE -2.04
BIOCHEMISTRY DATA OF RATS
GROUP: NON-Bt COTTONSEEDS (SAMPLE I) DOSE : 5000 mg /kg b.wt.

Animal No.	Sex	Bilirubin	Acetyl cholinesterase (AchE)
1	M	0.09	673
2	M	0.05	509
3	M	0.08	530
4	M	0.07	668
5	M	0.06	582
Mean ± S.D		0.07±0.02	592.4±76.11
1	F	0.07	1713
2	F	0.08	1893
3	F	0.07	2063
4	F	0.08	1716
5	F	0.08	1148
Mean ± S.D		0.07±0.01	1346.8±332.19

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TABLE -2.05
BIOCHEMISTRY DATA OF RATS
GROUP: Bt COTTONSEEDS (SAMPLE II) DOSE: 5000 mg /kg b.wt

Animal No.	Sex	Bilirubin	Acetyl cholinesterase (AchE)
1	M	0.07	526
2	M	0.07	637
3	M	0.05	566
4	M	0.06	671
5	M	0.08	747
Mean ± S.D		0.06±0.01	629.4±87.08
1	F	0.08	1833
2	F	0.07	1134
3	F	0.06	1520
4	F	0.09	1246
5	F	0.06	1001
Mean ± S.D		0.07±0.01	1346.8±332.19



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ADDENDUM TO REPORT NO. 000041134 FOR

ACUTE ORAL TOXICITY STUDY IN RATS

WITH

Bt COTTONSEEDS

Report for:

METAHELIX LIFE SCIENCES PRIVATE LIMITED
PLOT NO.3, KIADB 4th PHASE, BOMMASANDRA,
BANGALORE-560 099, INDIA

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)

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QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled ‘Addendum to Report No. 000041134 for Acute Oral Toxicity Study in Rats’ with ‘Bt Cottonseeds’ has been examined in accordance to the ‘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 the results obtained


Dinu Bhat
Sr. SCIENTIST
QUALITY ASSURANCE




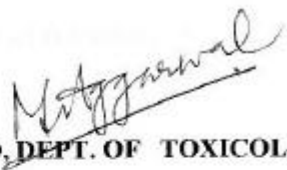
STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE

We, the undersigned take overall responsibility to conduct the work described in the study entitled ‘Addendum to Report No. 000041134 for Acute Oral Toxicity Study in Rats’ with Bt Cottonseeds performed with respect to 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.


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


JOINT DIRECTOR (MANAGEMENT)



SHRIRAM INSTITUTE FOR INDUSTRIAL RESEARCH
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PRODUCT : Bt COTTONSEEDS
STUDY : ADDENDUM TO REPORT NO. 000041134 FOR
ACUTE ORAL TOXICITY STUDY IN RATS

TEST SUBSTANCE

The sponsor is responsible for necessary characterization and evaluation of the test substance. The details of the test substance provided by the sponsor are as follows:

PRODUCT NAME : NON-Bt COTTONSEEDS (SAMPLE I)
& Bt COTTONSEEDS (SAMPLE II)

SPONSOR : METAHELIX LIFE SCIENCES
PRIVATE LIMITED

MATERIAL DESCRIPTION : YELLOWISH BROWN COLOURED
POWDER

PACKED IN : BROWN COLOURED PAPER
CARTONS



EXPERIMENTAL DESIGN

Name of species : *Rattus rattus albanicus*
Strain of the animals : Wistar
No. of animals used per dose : 5 Male, 5 Female
Age of the animals used : 6 to 7 weeks
Weight range : 160-180 gm
Acclimatization period : 7 Days
Route of administration : Oral
Vehicle used : Corn oil
Date of commencement of study : 14.12.2007
Date of completion of study : 28.12.2007

GROUP AND DOSAGE LEVEL

Group	Dosage Level (mg/kg B.wt.)	No. of animals	
		Male	Female
Control group (Vehicle only)	0.00	5	5
Non-Bt Cottonseeds (Sample-I)	5000	5	5
Bt Cottonseeds (Sample-II)	5000	5	5



HUSBANDRY

All animals were caged in a group of 5 according to sex in plastic cages fitted with wire mesh tops and having sterilized paddy husk bedding. The room temperature was maintained at $22 \pm 3^{\circ}$ C with 30 - 70 % relative humidity.

The room was ventilated at the rate of approximately 15 air changes per hour.

Lighting was controlled to give 12 hours artificial light (8 a.m. - 8 p.m.) each day.

Water and standard pelleted feed (Amrut feeds Ltd.) was freely available to the experimental rats.

IDENTIFICATION OF ANIMALS

Each cage was tagged having the details of animal group number, product name, dosage level, date of initiation and date of completion.

The animals were also marked with the help of picric acid.



DOSE ADMINISTRATION

The animals were normally fasted for 18 hours before and 4 hours after dosing. Three groups of 5 male and 5 female rats each were designated for the study. First group of animals was administered with Non-Bt Cottonseeds (Sample-I) in powdered form orally at the highest dose level of 5000 mg/kg body weight with the help of metallic cannula attached with tuberculin syringe. Second group was similarly administered orally with powdered Bt Cottonseeds (Sample-II).

The control group of animals was also treated with corn oil only.

Frequency of administration

The test article was administered once only following an overnight fast.

Mortality and toxic signs

Mortality and clinical sign and symptoms were recorded during the observation period of 14 days after dose administration.



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PROJECT : TOX-346A
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STUDY : ADDENDUM TO REPORT NO. 000041134 FOR
ACUTE ORAL TOXICITY STUDY IN RATS

CLINICAL LABORATORY STUDIES

The following clinical laboratory determinations were made in the animals of the test as well control groups as supplement to the main report (Report No. 000041134) entitled 'Acute oral toxicity study in rats with Bt Cottonseeds'.

Blood sampling

3-5 ml of blood was withdrawn by cardiac puncture under light Carbon dioxide anesthesia prior to sacrifice.

Haematology

The Prothrombin time and ESR of the blood samples taken from the control and the treated rats by 'Fully Automatic Cell Counter (Inst.no. SRI/TOX/INST/19)'.

Biochemistry

Non-protein nitrogen estimation was performed on control and treated rats using 'Fully Automatic Biochemistry Analyser (Inst.no. SRI/TOX/INST/18)'.

BIO-STATISTICAL METHOD USED

Student's t-test.



OBSERVATIONS

Mortality and toxic signs

No mortality was recorded (Table- 1.01 &1.02) in the animals of control and test groups i.e. Non-Bt Cottonseeds (Sample-I) and Bt Cottonseeds (Sample-II) of animals. No toxic signs or symptoms (Table-1.03) were noticed in the control and in any test groups i.e. Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II) animals.

Haematology evaluations

Haematological evaluation for the parameters 'Prothrombin time' and 'ESR' (Tables 2.01-2.04) did not show significant differences in the control and the test groups 'Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II)' of animals, as all the parameters fell within the accepted limits of normal variations.

Clinical biochemistry evaluations

Serum Biochemistry evaluations for Non-protein nitrogen (Tables 3.01-3.04) disclosed no significant differences in control and test groups 'Non-Bt Cottonseeds (Sample-I) along with Bt Cottonseeds (Sample-II)' of animals, as all the parameters fell within the accepted limits of normal variations.



RESULTS WITH CONCLUSION

Under the conditions of this study, the single oral administration of ‘Non-Bt Cottonseeds (Sample-I)’ and ‘Bt Cottonseeds (Sample-II)’ at the dose level of 5000 mg/kg b.wt to rats did not induce any treatment related observable toxic effects with regards to the haematological parameters i.e. Prothrombin Time and ESR as well as the biochemical parameter i.e. Non-protein nitrogen, when compared to its control group of animal treated with corn oil (vehicle) only.

The sample has been conducted as per DBT, Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts.



TABLE –1.01
LD₅₀ ASSAY - MORTALITY DATA OF MALE RATS

Dosage level mg/kg	Time of Death (Days)														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
CONTROL- DOSED WITH VEHICLE ONLY																
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
NON-BT COTTONSEEDS (SAMPLE-I)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
BT COTTONSEEDS (SAMPLE-II)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5



TABLE –1.02
LD₅₀ ASSAY - MORTALITY DATA OF FEMALE RATS

Dosage level mg/kg	Time of Death (Days)														Cumulative Mortality	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
CONTROL - DOSED WITH VEHICLE ONLY																
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
NON-BT COTTONSEEDS (SAMPLE-I)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
BT COTTONSEEDS (SAMPLE-II)																
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

No toxic signs & symptoms / mortality was observed in control group of animals.



TABLE - 1.03
SUMMARY OF OBSERVATIONS (MALES & FEMALES)

Group & Dosage level (mg / kg B.wt)	Clinical Observations	Necropsy Observations
Control group (Vehicle only)	No toxic signs or symptoms was noticed.	No noteworthy findings
Non-Bt Cottonseeds (Sample-I) 5000	No treatment related toxic signs or symptoms was noticed.	No noteworthy findings
Bt Cottonseeds (Sample-II) 5000	No treatment related toxic signs or symptoms was noticed.	No noteworthy findings



TABLE - 2.01
MEAN HAEMATOLOGY DATA ON RATS

Parameters	Sex	Control (Vehicle only)	Non-Bt Cottonseeds (Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds (Sample II) 5000 mg /kg b.wt.
Prothrombin Time (sec.)	Male	19.60 ± 1.14	18.40 ± 1.14	17.20 ± 1.30
	Female	19.20 ± 0.84	18.20 ± 1.30	18.20 ± 1.30
ESR (mm/hr.)	Male	18.40 ± 1.14	17.40 ± 1.14	18.00 ± 1.58
	Female	19.20 ± 0.84	18.20 ± 0.84	18.40 ± 1.14



TABLE -2.02
HAEMATOLOGY DATA OF RATS (VEHICLE ONLY)
GROUP: CONTROL

Animal No.	Sex	Prothrombin Time (sec.)	ES R (mm/hr.)
1	M	21	18
2	M	19	20
3	M	20	17
4	M	18	19
5	M	20	18
Mean		19.60	18.40
±		±	±
S.D		1.14	1.14
1	F	20	19
2	F	19	20
3	F	20	18
4	F	19	19
5	F	18	20
Mean		19.20	19.20
±		±	±
S.D		0.84	0.84



TABLE -2.03
HAEMATOLOGY DATA OF RATS
GROUP: NON-Bt COTTONSEEDS (SAMPLE I) DOSE: 5000 mg /kg b.wt.

Animal No.	Sex	Prothrombin Time (sec.)	ES R (mm/hr.)
1	M	18	19
2	M	20	18
3	M	19	16
4	M	18	17
5	M	17	17
Mean		18.40	17.40
±		±	±
S.D		1.14	1.14
1	F	20	19
2	F	19	18
3	F	17	17
4	F	18	18
5	F	17	19
Mean		18.20	18.20
±		±	±
S.D		1.30	0.84



TABLE -2.04
HAEMATOLOGY DATA OF RATS
GROUP: Bt COTTONSEEDS (SAMPLE II) DOSE: 5000 mg /kg b.wt

Animal No.	Sex	Prothrombin Time (sec.)	ESR (mm/hr.)
1	M	16	17
2	M	17	19
3	M	19	18
4	M	18	16
5	M	16	20
Mean		17.20	18.00
±		±	±
S.D		1.30	1.58
1	F	17	19
2	F	19	17
3	F	20	20
4	F	18	18
5	F	17	18
Mean		18.20	18.40
±		±	±
S.D		1.30	1.14



TABLE - 3.01
MEAN BIOCHEMISTRY DATA ON RATS

Parameter	Sex	Control (Vehicle only)	Non-Bt Cottonseeds (Sample I) 5000 mg /kg b.wt.	Bt Cottonseeds (Sample II) 5000 mg /kg b.wt.
Non-protein nitrogen (mg/dl)	Male	45.88 ± 0.88	45.86 ± 1.14	46.16 ± 0.99
	Female	44.60 ± 0.86	45.04 ± 0.96	45.00 ± 0.99



TABLE -3.02
BIOCHEMISTRY DATA OF Rats (VEHICLE only)
GROUP: CONTROL

Animal No.	Sex	Non-protein nitrogen (mg/dl)
1	M	45.2
2	M	45.1
3	M	47.2
4	M	45.6
5	M	46.3
Mean		45.88
±		±
S.D		0.88
1	F	44.6
2	F	45.2
3	F	44.2
4	F	45.6
5	F	43.4
Mean		44.60
±		±
S.D		0.86



TABLE -3.03
BIOCHEMISTRY DATA OF RATS
GROUP: NON-Bt COTTONSEEDS (SAMPLE I) DOSE: 5000 mg /kg b.wt.

Animal No.	Sex	Non-protein nitrogen (mg/dl)
1	M	46.2
2	M	45.0
3	M	47.6
4	M	45.8
5	M	44.7
Mean		45.86
±		±
S.D		1.14
1	F	44.2
2	F	44.0
3	F	46.3
4	F	45.1
5	F	45.6
Mean		45.04
±		±
S.D		0.96



TABLE -3.04
BIOCHEMISTRY DATA OF RATS
GROUP: Bt COTTONSEEDS (SAMPLE II) DOSE: 5000 mg /kg b.wt

Animal No.	Sex	Non-protein nitrogen (mg/dl)
1	M	46.8
2	M	44.2
3	M	45.6
4	M	47.4
5	M	46.8
Mean		46.16
±		±
S.D		1.28
1	F	45.8
2	F	44.2
3	F	45.4
4	F	44.6
5	F	46.7
Mean		45.00
±		±
S.D		0.99