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

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

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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)

(MANAGEMENT)

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 OF STUDY	:	16.08.2007
DATE OF COMPLETION OF STUDY	:	31.08.2007

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

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 ± 3° 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.

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.

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.

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

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

TABLE –1.01
LD_{50} ASSAY - MORTALITY DATA OF MALE RATS

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

TABLE –1.02

LD_{50} ASSAY - MORTALITY DATA OF FEMALE RATS

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

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.

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

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

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