

Study Title

**Acute Oral Toxicity of *Bacillus thuringiensis* var. *kurstaki*
[Cry IA(c)] HD-73 protein in Albino Mice**

Author

Mark W. Naylor

Study Completed on

April 16, 1993

Performing Laboratories

**Monsanto Company
Agricultural Group / Environmental Health Laboratory
645 S. Newstead
St. Louis, MO 63110**

Laboratory Project ID

**Lab Study Number: 92197
Monsanto Study Number: ML-92-493**

Volume 15 of 17

0001 OF 0187

The Agricultural Group of Monsanto Company
New Products Division
Regulatory Sciences

Lab Study #: 92197
Monsanto Study #: ML-92-493
MSL #: 12708

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1) (A), (B), or (C).

Company Monsanto Company

Company Agent FRANK SERDY Date 2/15/94

Title REGULATORY AFFAIRS DIRECTOR Signature Frank Serdy

The Agricultural Group of Monsanto Company
New Products Division
Regulatory Sciences

Lab Study #: 92197
Monsanto Study #: ML-92-493
MSL #: 12708

STATEMENT OF COMPLIANCE

This study meets the requirements for 40 CFR Part 160, see performing laboratory Quality Assurance Audit Statement and Statement of Compliance.

Submitter

Frank Sedy

2/15/94

Sponsor

Ray J. H. H.

1/20/94

Study Director

(see page 23 of 187)

<u>TABLE OF CONTENTS</u>	<u>PAGE</u>
Confidentiality Statement	2
Statement of Compliance	3
Table of Contents	4
Notes to the Reviewer	5
Monsanto Toxicology Study Summary	6
Introduction	6
Purpose	7
Methods	7
Results	8
Conclusion	9
Performing Laboratory Report	10
Summary	11
Table of Contents	12
Trademarks	13
Notes to Reader	14
Introduction	15
Materials and Methods	15
Results	19
Discussion	20
References	21
Supplementary Study Information	22
Statement of Compliance	23
ESH Quality Assurance Audit Statement	24
EHL Decision-tree Statistical Analysis	25
Appendix 1: Summary Tables	27
Appendix 2: Inlife Data	39
Appendix 3: Gross Pathology Data	80
Appendices	
1 Study Protocol	181

The Agricultural Group of Monsanto Company
New Products Division
Regulatory Sciences

Lab Study #: 92197
Monsanto Study #: ML-92-493
MSL #: 12708

NOTES TO THE REVIEWER

The following is the Monsanto Product Toxicologist's summary of the attached Monsanto Company - Environmental Health Laboratory toxicology report. It is not considered part of the study and is not included in the Study director's or submitter's GLP compliance statements. It has been subjected to Monsanto Quality Assurance review. It is included solely as an aid to the reviewer.

All comments and questions related to this summary and the report should be addressed to:

Bruce Hammond, PhD., DABT
Manger of Toxicology
The Agricultural Group
Monsanto Company
(314) 694-2179

00050F0187

MONSANTO TOXICOLOGY STUDY SUMMARY

TEST SUBSTANCE: *Bacillus thuringiensis* var. *kurstaki*
[Cry IA(c)] HD-73 protein

STUDY TITLE: Acute Oral Toxicity of *Bacillus*
Thuringiensis Var. *Kurstaki* [Cry IA(c)]
HD-73 Protein in Albino Mice

TESTING LABORATORY: Monsanto Environmental Health
Laboratory, St. Louis, Missouri

LAB STUDY NO: 92197

MONSANTO STUDY NO: ML-92-493

FINAL REPORT ISSUED: April 12, 1993

SUMMARY PREPARED: January 10, 1994

INTRODUCTION

Cotton, *Gossypium hirsutum*, has been genetically engineered to be resistant to selected insect pests (Lepidoptera). Resistance was accomplished by the insertion of a gene from *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) which encodes for the production of a protein insecticidal to Lepidoptera larvae but safe to mammals, birds, fish and beneficial insects. A second gene encoding neomycin phosphotransferase II (NPTII) was also introduced as a marker to enable selection of cells containing the *B.t.k.* gene. Larvae of Lepidopteran pests are the most important insect pests impacting successful cotton production. Cotton typically requires numerous chemical insecticide treatments for Lepidoptera control. The cotton varieties containing the *B.t.k.* gene will significantly reduce chemical insecticide use in cotton and, therefore, provide a major benefit to cotton growers and the environment.

Prior to the commercialization of this insect resistant cotton variety, data and information were produced to demonstrate that this cotton variety is equivalent to current cotton varieties in composition and agronomic performance and that the proteins expressed by the inserted genes cause no adverse effect when consumed by domestic or wild animals and beneficial insects. Exposure to humans is not expected since protein is not present in the processed cotton fiber or oil at detectable levels, and cottonseed meal is not used in human foods. Areas of investigation included: molecular characterization of the introduced genes; biochemical characterization of the expressed *B.t.k.* protein; estimation of the levels of the *B.t.k.* protein in cotton products; safety of the *B.t.k.* protein to mammals and beneficial insects; and the environmental fate of the *B.t.k.* protein.

PURPOSE

The *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) HD-73 protein has been expressed in cotton plants to confer resistance to Lepidopteran insect damage. An acute oral toxicity test was undertaken to assess the potential mammalian toxicity of *B.t.k.* HD-73 protein. The experimental design for this study was adapted from Toxicology Guidelines for Biochemical Pest Control Agents, Subdivision M, Pesticide Assessment Guidelines, EPA, 1982.

METHODS

B.t.k. HD-73 protein (Lot no. 5025385) was administered by gavage to 3 groups of 10 CD-1 male and female mice. The test material was produced in *E. coli*. and purified to 68% of total protein (Volume 5). Characterization studies demonstrated the test material to be fully active and of suitable strength, purity and stability for use in studies assessing the safety of the *B.t.k.* protein expressed in insect resistant cotton plants (Volume 5). The *B.t.k.* protein produced in *E. coli*. bacteria was also shown to be equivalent to the *B.t.k.* protein produced in insect resistant cotton plants (Volume 4). Mice were selected for dosing instead of rats since there was a limited amount of test material available from bacterial production. Mice are considered an acceptable species for assessing mammalian safety since there is a considerable body of data regarding the toxicity of chemicals in mice.

The doses of *B.t.k.* HD-73 protein administered to mice were 0, 500, 1000 and 4200 mg/kg.¹ The highest dose given to mice was selected based on the maximum hazard dose concept as outlined in Subdivision M, Guidelines for biochemical pesticides. Another group of mice was gavaged with 6340 mg/kg bovine serum albumin (BSA) (Lot # 5192751, > 93% purity) and served as a protein control group. Since the *B.t.k.* HD-73 test material was 68% pure, it was considered necessary to dose mice with a higher dose of BSA to make the total mass of material given to mice equivalent for the BSA (protein) control and test group. The concentration of total mass in the dosing vehicle for the *B.t.k.* HD-73 test group was 94.6 mg/ml and for the BSA control group, 94.9 mg/ml (the value 94.9 mg/ml was corrected for % purity of protein in BSA, eg. 102 mg/ml X 93% (total protein) = 94.9 mg/ml). Thus, the total mass of material given to both groups of mice were similar. The dosing vehicle for *B.t.k.* HD-73 protein and BSA was 1 mM ammonium bicarbonate/10 mM cysteine pH 9.0 buffer. Controls were administered the same volume (66.66 ml/kg body weight or 2 ml/30 gram mouse) of dosing vehicle given to high dosage mice. The *B.t.k.* HD-73 protein dosing solution

¹ The final report states that the highest dosage tested was 4300 mg/kg. Examination of the dose confirmation data found a small calculation error after the final report (ML-92-493) was issued. The corrected high dosage administered was 4200 mg/kg.

was made up to the maximum concentration which could be achieved (94.6 mg/ml) based on solubility and remain a free flowing solution which could be injected through a gavage needle. Due to the solubility limit of *B.t.k* HD-73 protein in the vehicle, it was not possible to solubilize enough test material to administer 4200 mg/kg in one dose (Volume 14). Therefore, the total dose administered was subdivided into two equal doses which were given within approximately 3 hrs apart on day 1 of the study. Dosing was subdivided for both control groups in the same manner.

The dosing solutions were prepared on the same day of dosing to mice. Samples of the *B.t.k* HD-73 dosing solutions were collected shortly before and after dosing and frozen for subsequent dose confirmation analysis (Volume 14).

Mice were observed twice daily for signs of toxicity and food consumption was recorded daily. Food and water were provided *ad libitum* during the study. Body weights were recorded pre-test and on post-dosing day 7. All animals were sacrificed on post-dosing day 7 and 8 and subjected to a gross necropsy. Approximately 40 tissues were collected and saved for each animal on test.

RESULTS

No treatment related adverse effects were observed in animals dosed with *B.t.k* HD-73 protein or the control materials. There were no observations of abnormal clinical signs in any of the groups during the study.

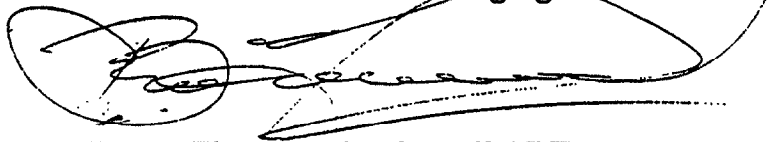
There were no statistically significant differences in body weight, cumulative body weight or food consumption between the vehicle or protein control groups and *B.t.k* HD-73 protein treated groups.

Several incidental pathologic changes were observed at necropsy which were randomly distributed among all groups and are commonly seen for the strain of mice used by the testing laboratory. None of these findings were considered related to treatment.

Results of the dose confirmation analysis (Volume 14) confirmed that the test material was stable in dosing solutions based on measurement of biological activity (insect bioassay) of dosing solutions after they were given to mice. The concentrations of the test material in dosing solutions were in good agreement with nominal concentrations based on insect bioassays and ELISA determinations. The appearance of the test material on SDS-PAGE gels was identical based on side-by-side comparisons of samples of dosing solutions collected before and after dosing of mice.

CONCLUSION

There were no treatment related adverse findings in any of the groups administered *B.t.k.* HD-73 protein by oral gavage at dosages up to 4200 mg/kg. The oral LD50 for *B.t.k.* HD-73 protein in mice is greater than 4200 mg/kg and the no-observed effect level is 4200 mg/kg.

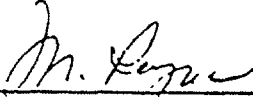


Bruce Hammond, PhD., DABT.,
Manager of Toxicology
Monsanto Agricultural Group

MONSANTO AGRICULTURAL GROUP ,
ENVIRONMENTAL HEALTH LABORATORY
645 S. NEWSTEAD
ST. LOUIS, MISSOURI 63110

Acute Oral Toxicity Study of
Bacillus Thuringiensis Var. *Kurstaki*
[Cry IA(c)] HD-73 Protein
in Albino Mice

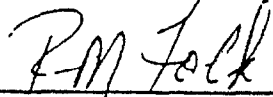
Study Number: 92197
Project Number: ML-92-493



M. S. Reyna, B.S.
Study Director

4/12/93

Date



R. M. Folk, Ph.D.
Director, Environmental Health Laboratory

3/30/93

Date

SUMMARY

Bacillus thuringiensis var. krustaki HD-73 [Cry IA(c)] designated as *Btk* HD-73 was administered orally by gavage in 2 equal doses given within 3 hours of each other on Day 1. Groups of ten CD-1 mice received total doses of 500, 1000 and 4300 mg/kg *Btk* HD-73. A vehicle control group was administered the vehicle, ammonium bicarbonate and cysteine solution, as a single dose of 66.66 ml/kg. Another control group (also termed a vehicle control) was administered bovine serum albumin (BSA) as a single target dose of 6340 mg/kg. In all the above cases, the target dose was divided into two equal amounts and given approximately three hours apart on Day 1 of the study. Clinical observations were performed, and body weights and food consumption were determined. All surviving animals were necropsied at study termination (Days 8-9).

There were no treatment-related effects on body weight, food consumption, survival, clinical observations or gross pathology.

Based on the above results, there were no adverse effects when *Btk* HD-73 was administered by gavage to mice at doses of up to 4300 mg/kg.

TABLE OF CONTENTS

TITLE PAGE	PAGE	1
SUMMARY	PAGE	2
TABLE OF CONTENTS	PAGE	3
TRADEMARKS	PAGE	4
NOTES TO READER	PAGE	5
INTRODUCTION	PAGE	6
MATERIALS AND METHODS	PAGE	6-9
RESULTS	PAGE	10
DISCUSSION	PAGE	11
REFERENCES	PAGE	12
SUPPLEMENTARY STUDY INFORMATION	PAGE	13
COMPLIANCE STATEMENT	PAGE	14
QUALITY ASSURANCE STATEMENT	PAGE	15*
EHL DECISION-TREE STATISTICAL ANALYSIS	PAGE	16-17
APPENDICES	FIRST <u>PAGE</u>	LAST <u>PAGE</u>
APPENDIX 1: SUMMARY TABLES	18	29
Table 1: Summary of Body Weight Data	19	20
Table 2: Summary of Cumulative Body Weight Changes	21	22
Table 3: Summary of Food Consumption Data	23	24
Table 4: Summary of Clinical Signs	25	25
Table 5: Summary of Terminal Body Weights	26	27
Table 6: Summary Incidence of Individual Gross Necropsy Alterations	28	29
APPENDIX 2: INLIFE OBSERVATIONS	30	70
Table 1: Individual Body Weight Data	31	40
Table 2: Individual Food Consumption Data	41	50
Table 3: Individual Clinical Signs	51	60
Table 4: Animal Termination History	61	70
APPENDIX 3: GROSS PATHOLOGY	71	171
Table 1: Individual Gross and Histopathology Data	72	171

TRADEMARKS

The following registered trademarks were used in this report:

<u>ITEM</u>	<u>REGISTERED TRADEMARK OF:</u>
RODENT CHOW	Purina Mills, Inc., St. Louis, MO
CD-1	Charles River Laboratories, Inc. Wilmington, MA

NOTES TO READER

TERMS

The following terms are used as column headings in some data tables:

- GEN** Generation (F0, F1, etc.) used in reproduction studies.
- PERIOD (PER)** A number corresponding to a specified interval within the study; used to facilitate data reporting.
- WINDOW** A series of days within a study when data could have been collected for the corresponding period. Actual data collection occurred on one or more of the days within the window.

ANIMAL IDENTIFICATION SYSTEM

The current EHL animal number format is YYXXXSGAANN where YYXXX is the study number, Y is the study year, and X is the sequence number within the year. The S represents the animal's sex, G is the primary group code, A's may represent the subgroup code (in most cases these characters are blank), and N is the animal's sequence number within the group (or subgroup), e.g., 99999M2 001. The animal designation may be further reduced by exclusion of the study number and blank subgroup codes, e.g., M2001. These abbreviated animal identifications will include five to seven characters, depending on the number of characters in the subgroup code.

INTRODUCTION

Purpose: To determine the toxicity of *Btk* HD-73 protein when administered as a single dose (divided in two equal amounts given several hours apart) by gavage to mice.

Date Protocol Signed by Study Director: November 12, 1992

Date Inlife Portion Started: November 17, 1992

Date Last Animal Sacrificed: November 25, 1992

Applicable Testing Guidelines: Performed in general accordance with provisions specified by EPA subdivision M testing guidelines for microbial and biochemical pest control agents.

Experimental Design: *Btk* HD-73 protein was administered by gavage to groups of ten CD-1 mice/sex as single target doses of 500, 1000 and 4300 mg/kg (active protein). A vehicle control group was administered the vehicle, ammonium bicarbonate and cysteine solution, as a single dose of 66.66 ml/kg. Another control group was administered bovine serum albumin (BSA) as a single target dose of 6340 mg/kg. In all the above cases, the target dose was divided into two equal amounts and given approximately three hours apart on Day 1 of the study. Clinical observations were performed, and body weights and food consumption were determined. All animals were necropsied at study termination (Days 8-9). Numerous tissues were retained but were not examined microscopically.

MATERIALS AND METHODS

Test Material and Vehicles

Source (Test Material and Vehicles): Agricultural Group, Monsanto Co.,
St. Louis, MO

Date Received: November 17, 1992

Identification: *Btk* HD-73 protein, prepared at three different
concentrations

EHL Substance Identification Code: T920142

Lot Number: 5025385

Identification: Vehicle - 1mM ammonium bicarbonate and 10mM cysteine,
at pH 9

EHL Substance Identification Code: T920143

Lot Number: 5192751

Identification: BSA

EHL Substance Identification Code: T920144

Lot Number: 5192746

Test Material Formulation

Group Designations and Dosages:

Treatment Level	Group Designations (Male, Female)	Target Dosage	Concentration	pH
Vehicle	MV1, FV1	66.66 ml/kg*	N/A	9.0
BSA	MV2, FV2	6340 mg/kg*	102 mg/ml	N/A
<i>Btk</i> HD-73	M1, F1	500 mg/kg*	11 mg/ml	N/A
<i>Btk</i> HD-73	M2, F2	1000 mg/kg*	22.4 mg/ml	N/A
<i>Btk</i> HD-73	M3, F3	4300 mg/kg*	94.6 mg/ml	N/A

The ammonium bicarbonate/cysteine solution was used as the vehicle, and all groups received a volume of 66.66 ml/kg divided into two equal amounts. The *Btk* HD-73 protein was 68% (by weight) active material and dose concentrations reflect a weight adjustment for 100% activity. The BSA dosage reflects an adjustment for 93% purity.

NOTE: Dosing sample preparation was not conducted at EHL (for further detail see reference section no. 6).

Animals

Species: Albino mouse

Strain: CD-1

Source: Charles River Breeding Laboratory, Portage MI

Date of Arrival at EHL: November 3, 1992

Quarantine and Acclimation Period: 14 days

Number Used in Study: 100 (50 males, 50 females). Any unhealthy animals were excluded from assignment to the study.

EHL 92197

Page 7

0016 OF 0187

Tissues Retained: Aorta, adrenals, brain, cecum, colon, duodenum, esophagus, eyes, femur with joint, gall bladder, gross lesions, heart, ileum, jejunum, kidneys, lungs (with mainstem bronchi), liver, lymph node (mesenteric and submaxillary), muscle (quadriceps femoris), ovaries, pancreas, pituitary, prostate, rectum, salivary gland (submaxillary), sciatic nerve, seminal vesicles, skin (with mammary tissue), spinal cord (cervical, thorax, lumbar), spleen, sternum with marrow, stomach, testes with epididymides, thymus, thyroid/parathyroid, trachea, uterus (corpus and cervix), urinary bladder

Fixatives: Eyes, 5% buffered neutral formalin/0.5% glutaraldehyde
Remaining tissues, 10% buffered neutral formalin

Statistics

The following procedures were used to detect statistically significant differences between treated animals and their respective controls:

Dunnett's Multiple Comparison Test (two-tailed) (1): Body weights, body weight changes and food consumption

Terminal body weights were evaluated by decision-tree statistical analysis procedures which, depending on the results of tests for normality (2) and homogeneity of variances (Bartlett's Test), utilized either parametric (Dunnett's Test and Linear Regression) or nonparametric (Kruskal-Wallis, Jonckheere's and/or Mann-Whitney Tests) routines to detect group differences and analyze for trend.

Other statistical routines used for some data were: Bartlett's Test (3) to evaluate homogeneity of variances, and Grubbs Test (4, 5) to detect outliers.

RESULTS

Inlife

Mortality

Refer to Appendix 1, Table 4; Appendix 2, Table 4. No unscheduled deaths occurred during the study.

Body Weight

Refer to Appendix 1, Tables 1 and 2; Appendix 2, Table 1. There were no statistically significant differences in mean body weight or in cumulative body weight gain, among the groups treated with either BSA or the *Btk* HD-73 protein, when compared to the vehicle control.

Food Consumption

Refer to Appendix 1, Table 3; Appendix 2, Table 2. There were no statistically significant differences in mean food consumption among the groups treated with either BSA or the *Btk* HD-73 protein, when compared to the vehicle control.

Clinical Signs

Refer to Appendix 1, Table 4; Appendix 2, Table 3. There were no abnormal clinical signs observed.

Gross Pathology

Refer to Appendix 1, Tables 5 and 6; Appendix 3, Table 1. There were no statistically significant differences in terminal body weight and there were no gross lesions considered related to treatment.

DISCUSSION

There were no adverse findings considered related to treatment among any of the treated groups.

Based on the above results, there were no adverse effects when *Btk* HD-73 was administered by gavage to mice at doses of up to 4300 mg/kg.

REFERENCES

1. Dunnett, C.W. A multiple comparison procedure for comparing several treatments with a control. Jour. Am. Stat. Assoc. 50: 1096-1121 (1955).
2. BMDP Biomedical Computer Programs P Series Manual, Health Sciences Computing Facility, UCLA, University of California Press (1977).
3. Dixon, W.J. and Massey, F.J. Jr. Introduction to Statistical Analysis, 3rd Edition. McGraw-Hill Company, NY (1969).
4. Grubbs, F.E. Procedure for detecting outlying observations in samples. Technometrics XI: 1-21 (1969).
5. Grubbs, F. E., Beck, G. Extension of sample sizes and percentage points for significance tests of outlying observations. Technometrics, XIV: 847-854 (1972).
6. Sammons, Doug. Monsanto's Plant Sciences, Chesterfield Village. Study No. 92-01-36-13, Experiment No. 92-427-716.

SUPPLEMENTARY STUDY INFORMATION

Study Performed at: Environmental Health Laboratory (EHL),
645 S. Newstead, St. Louis, MO 63110

Location of Study Protocol, Original Data, Retained Tissues, Final Report
and Facilities Records: Environmental Health Laboratory
Archives

Supervisory Personnel:

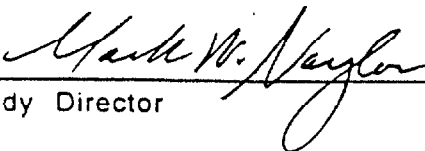
F. A. Ruecker, D.V.M., Dipl. A.C.V.P.
Manager, Pathology

L. D. Stout, D.V.M., M.S.
Manager, Toxicology Studies

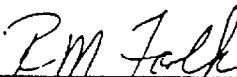
R. M. Folk, Ph.D.
Director, Environmental Health Laboratory

STATEMENT OF COMPLIANCE

The study EHL 92197 (ML-92-493) was conducted in general conformance with the appropriate provisions of the USEPA, FIFRA (40 CFR, Part 160) Good Laboratory Practice Standards. Test material characterization was the responsibility of the sponsor and was not performed by the EHL.



Study Director 6/3/93
Date



R. M. Folk 6/3/93
Date
Director, Environmental Health Laboratory

ESH QUALITY ASSURANCE AUDIT STATEMENT

Study Number: 92197
ML-92-493

Protocol Amendments: None

Study Title: Acute Oral Toxicity Study of Bacillus
Thuringiensis Var. Kurstaki [Cry IA(c)]
HD-73 Protein in Albino Mice

Dates of Inspections
and Communication
of Findings: November 24, 1992
December 14, 1992
January 07, 11, 26, 29, 1993
March 11, 1993

Quality Assurance
Review Conducted by: S.G. Arri
C.C. Deatherage
C.K. Russell

Results: The Quality Assurance review indicates the
final report accurately presents the raw
data as developed during the study. There
appears to be no significant deviation from
applicable GLP regulations that adversely
affected study quality or integrity.



John L. Henshaw
Director Quality & Compliance Assurance

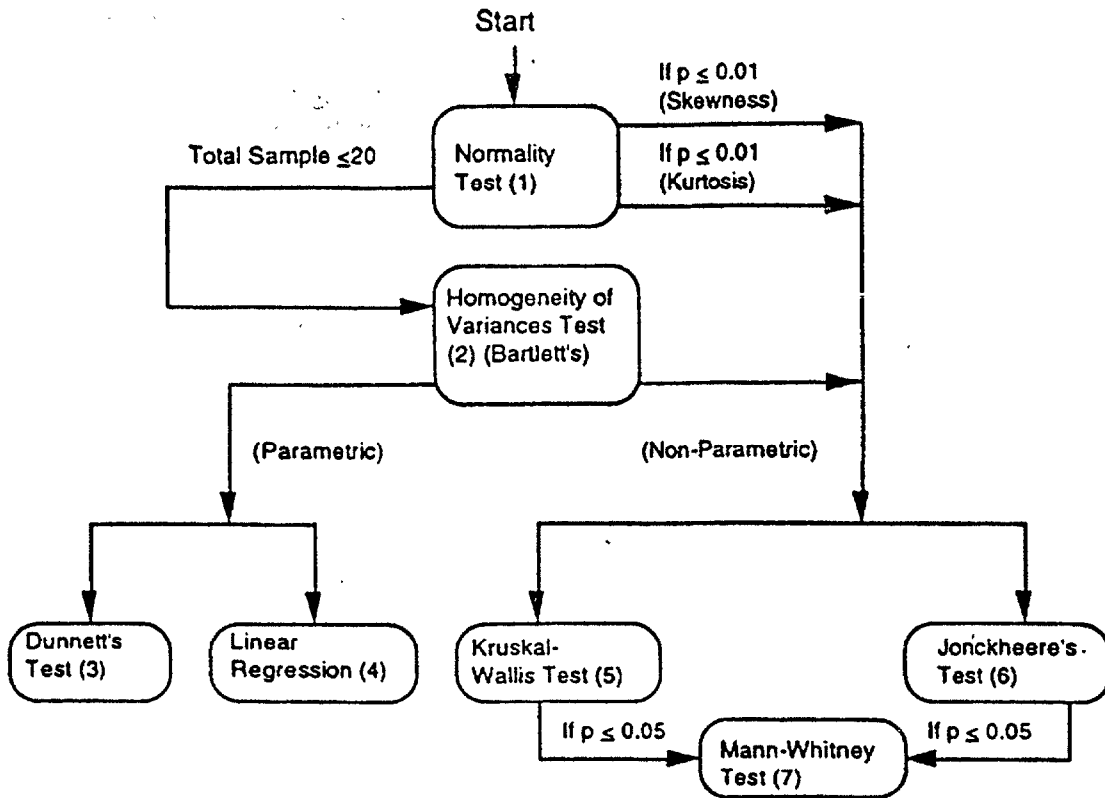
Date

3/23/93

EHL 92197

00240F0187

EHL DECISION-TREE STATISTICAL ANALYSIS



Note 1: Categorical data were analyzed with an Uncorrected Chi-square Test (8)

Note 2: Dunnett's and Mann-Whitney tests to detect group differences were performed two-tailed.

EHL DECISION-TREE STATISTICAL ANALYSIS

REFERENCES

1. BMDP Biomedical Computer Programs P Series Manual, Health Sciences Computing Facility, UCLA, University of California Press (1977).
2. Dixon, W.J. and Massey, F.J. Jr., Introduction to Statistical Analysis, 3rd Edition, McGraw-Hill Company, NY (1969).
3. Dunnett, C.W. A multiple comparison procedure for comparing several treatments with a control. Jour. Am. Stat. Assoc. 50: 1096-1121 (1955).
4. Draper, N.R. and Smith, H. Applied Regression Analysis. Wiley, NY (1966).
5. Breslow, N. A generalized Kruskal-Wallis test for comparing K-Samples subject to unequal patterns of censorship. Biometrika. 57: 579-594 (1970).
6. Hollander, M. and Wolfe, D.A. Nonparametric Statistical Methods. Wiley, NY (1973).
7. Mann, H.B. and Whitney, D.R. On a test of whether one of two random variables is stochastically larger than the other. Ann. Math. Stat. 18: 50-60 (1947).
8. Snedecor, G.W. and Cochran, W.G. Statistical Methods. Iowa State University Press, Ames, IA (1967).

Appendix 1

PAGE 18
EHL 92197

APPENDIX 1

Summary Tables

0027 OF 0187

THIS REPORT WAS GENERATED BY DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
BMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

SUMMARY OF BODY WEIGHT DATA (GM)

SEX: MALE

GROUP	TARGET DOSE	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
MV1	66.70 ML/KG	MEAN	28.4	29.8
VEHICLE CONTROL		STD. DEV.	1.97	2.07
VEHICLE		SAMPLE SIZE	10	10
MV2	6340. MG/KG	MEAN	28.4	30.1
VEHICLE CONTROL		STD. DEV.	2.09	2.52
BSA		SAMPLE SIZE	10	10
M1	500.0 MG/KG	MEAN	28.4	29.9
TEST GROUP		STD. DEV.	2.09	1.97
Btk Full Length HD-73		SAMPLE SIZE	10	10
M2	1080. MG/KG	MEAN	28.5	29.7
TEST GROUP		STD. DEV.	1.82	2.20
Btk Full Length HD-73		SAMPLE SIZE	10	10
M3	4380. MG/KG	MEAN	28.6	30.1
TEST GROUP		STD. DEV.	1.73	1.25
Btk Full Length HD-73		SAMPLE SIZE	10	10

--- LEGEND ---

* -- BONNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.05)
** -- BONNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.01)
BT -- BARTLETT'S TEST INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE AMONG VARIANCES OF THE DIFFERENT GROUPS (P<.01)
NA -- BONNETT'S TEST NOT APPROPRIATE FOR THIS GROUP/SEX/DATE

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 1

00280187

Table 1 Appendix 1

PAGE 19
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

SUMMARY OF BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

SEX: FEMALE

GROUP	TARGET DOSE	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV ?
FV1	66.70 ML/KG			
VEHICLE CONTROL		MEAN	24.2	24.8
VEHICLE		STD. DEV.	1.43	1.27
		SAMPLE SIZE	10	10
FV2	6340. MG/KG			
VEHICLE CONTROL		MEAN	24.3	24.9
BSA		STD. DEV.	1.54	2.14
		SAMPLE SIZE	10	10
F1	500.0 MG/KG			
TEST GROUP		MEAN	24.3	24.4
Btk Full Length HD-73		STD. DEV.	1.45	1.36
		SAMPLE SIZE	10	10
F2	1000. MG/KG			
TEST GROUP		MEAN	24.3	25.2
Btk Full Length HD-73		STD. DEV.	1.44	1.37
		SAMPLE SIZE	10	10
F3	4300. MG/KG			
TEST GROUP		MEAN	24.3	24.5
Btk Full Length HD-73		STD. DEV.	1.36	1.64
		SAMPLE SIZE	10	10

--- L E G E N D ---

- * -- DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.05)
- ** -- DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.01)
- BT -- BARTLETT'S TEST INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE AMONG VARIANCES OF THE DIFFERENT GROUPS (P<.01)
- NA -- DUNNETT'S TEST NOT APPROPRIATE FOR THIS GROUP/SEX/DATE

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 2

0029010187

Table 1 Appendix 1
PAGE 20
EHL 92197

STUDY NUMBER: 92197
DHEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

SUMMARY OF CUMULATIVE BODY WEIGHT CHANGES (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

REPORT PRINT DATE: 2-MAR-93
STUDY START DATE: 17-NOV-92

SEX: MALE

FROM DATE: 16-NOV-92
TO DATE : 23-NOV-92
DAY OF STUDY (FROM-TO): 0- 7

MV1 VEHICLE CONTROL 67 ML/KG VEHICLE	MEAN GM STD. DEV. SAMPLE SIZE	1.42 1.398 10
MV2 VEHICLE CONTROL 6340 MG/KG BSA	MEAN GM STD. DEV. SAMPLE SIZE	1.62 1.160 10
M1 TEST GROUP 500 MG/KG Btk Full Length HD-73	MEAN GM STD. DEV. SAMPLE SIZE	1.46 0.883 10
M2 TEST GROUP 1000 MG/KG Btk Full Length HD-73	MEAN GM STD. DEV. SAMPLE SIZE	1.20 0.884 10
M3 TEST GROUP 4300 MG/KG Btk Full Length HD-73	MEAN GM STD. DEV. SAMPLE SIZE	1.46 0.707 10

--- LEGEND ---

* - DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P < 0.05)
** - DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P < 0.01)
NA - DUNNETT'S TEST NOT APPROPRIATE FOR THIS PERIOD

STUDY NUMBER:92197

MOHSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 1

Table 2
Appendix 1
PAGE 21
EHL 92197

003010187

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

SUMMARY OF CUMULATIVE BODY WEIGHT CHANGES (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

REPORT PRINT DATE: 2-MAR-93
STUDY START DATE: 17-NOV-92

SEX: FEMALE

FROM DATE: 16-NOV-92
TO DATE : 23-NOV-92
DAY OF STUDY (FROM-TO): 0- 7

FV1 VEHICLE CONTROL 67 MG/KG VEHICLE	MEAN GM STD. DEV. SAMPLE SIZE	0.60 0.863 10
FV2 VEHICLE CONTROL 6340 MG/KG BSA	MEAN GM STD. DEV. SAMPLE SIZE	0.59 0.897 10
F1 TEST GROUP 500 MG/KG Btk Full Length HD-73	MEAN GM STD. DEV. SAMPLE SIZE	0.14 1.135 10
F2 TEST GROUP 1000 MG/KG Btk Full Length HD-73	MEAN GM STD. DEV. SAMPLE SIZE	0.88 0.721 10
F3 TEST GROUP 4300 MG/KG Btk Full Length HD-73	MEAN GM STD. DEV. SAMPLE SIZE	0.24 1.118 10

--- LEGEND ---

* - DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P < 0.05)
** - DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P < 0.01)
NA - DUNNETT'S TEST NOT APPROPRIATE FOR THIS PERIOD

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 2

Table 2 Appendix 1

PAGE 22
EHL 92197

00310F0187

STUDY NUMBER: 92197
 DMEH NUMBER: ML92493
 RTE OF ADMIN: ORAL (GAVAGE)

SUMMARY OF FOOD CONSUMPTION DATA

SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

SEX: MALE

FROM DATE : 17-NOV-92
 TO DATE : 23-NOV-92
 DAY OF STUDY (FROM-TO) : 1-7

GROUP	VEHICLE CONTROL	MEAN GM/DAY	STD. DEV.	SAMPLE SIZE
MV1	VEHICLE CONTROL 66.70 ML/KG VEHICLE	5.1BT	0.57	10
MV2	VEHICLE CONTROL 6340. MG/KG BSA	5.4	0.48	10
M1	TEST GROUP 500.0 MG/KG Btk Full Length HD-73	5.6	1.83	10
M2	TEST GROUP 1000. MG/KG Btk Full Length HD-73	5.0	0.63	10
M3	TEST GROUP 4300. MG/KG Btk Full Length HD-73	5.3	0.25	10

--- L E G E N D ---

- * -- DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.05)
- ** -- DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.01)
- BT -- BARTLETT'S TEST INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE AMONG VARIANCES OF THE DIFFERENT GROUPS (P<.01)
- NA -- DUNNETT'S TEST NOT APPROPRIATE FOR THIS GROUP/SEX/DATE

00320187

Table 3 Appendix 1 PAGE 23 EHL 92197

THIS REPORT WAS GENERATED F. DATA LOCKED THROUGH 15-DFC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

SUMMARY OF FOOD CONSUMPTION DATA
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

SEX: FEMALE

FROM DATE : 17-NOV-92
TO DATE : 23-NOV-92
DAY OF STUDY (FROM-TO) : 1-7

FV1	VEHICLE CONTROL	MEAN GM/DAY	5.2BT
	66.70 ML/KG	STD. DEV.	0.53
	VEHICLE	SAMPLE SIZE	10
FV2	VEHICLE CONTROL	MEAN GM/DAY	5.0
	6340. MG/KG	STD. DEV.	0.49
	BSA	SAMPLE SIZE	9
F1	TEST GROUP	MEAN GM/DAY	4.5
	500.0 MG/KG	STD. DEV.	1.22
	Btk Full Length HD-73	SAMPLE SIZE	10
F2	TEST GROUP	MEAN GM/DAY	4.7
	1000. MG/KG	STD. DEV.	0.55
	Btk Full Length HD-73	SAMPLE SIZE	10
F3	TEST GROUP	MEAN GM/DAY	5.2
	4300. MG/KG	STD. DEV.	0.48
	Btk Full Length HD-73	SAMPLE SIZE	10

--- LEGEND ---

- * -- DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.05)
- ** -- DUNNETT'S TEST (TWO-TAILED) INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE (P<.01)
- BT -- BARTLETT'S TEST INDICATES STATISTICALLY SIGNIFICANT DIFFERENCE AMONG VARIANCES OF THE DIFFERENT GROUPS (P<.01)
- NA -- DUNNETT'S TEST NOT APPROPRIATE FOR THIS GROUP/SEX/DATE

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 2

00330F0187

Table 3 Appendix 1

PAGE 24
EHL 92197

THIS REPORT WAS GENERATED ON DATA LOCKED THROUGH 15-DEC-1992

STUDY NUMBER: 92197
RTE OF ADMIN: ORAL (GAVAGE)
STUDY START DATE: 17-NOV-1992

SUMMARY OF CLINICAL SIGNS

REPORT PRINT DATE: 30-DEC-1992
SPECIES: MOUSE
STRAIN/BREED: CD-1

CATEGORY	OBSERVATION	GEN.	SEX	WINDOW	GROUP	NO. ANIMALS AFFECTED	NO. OF OCCURRENCES
DEATH	SCHEDULED SACRIFICE		M	D7-9	MV1	10	10
					MV2	10	10
					M1	10	10
					M2	10	10
					M3	10	10
			F	D7-9	FV1	10	10
					FV2	10	10
					F1	10	10
					F2	10	10
					F3	10	10

00340F0187

Table 4 Appendix 1

PAGE 25
EHL 92197

THIS REPORT WAS GENERATED: JM DATA LOCKED THROUGH 17-DEC-1992

STUDY NUMBER: 92197 SUMMARY OF TERMINAL BODY AND ORGAN WEIGHT DATA (GM) REPORT PRINT DATE: 28-DEC-1992
 RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
 STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ITEM OF INTEREST	GEN.	SEX	PERIOD	WINDOW	GROUP	MEAN	%CONTROL	STD.DEV.	N	STAT FLAGS
TERM. BODY WGT.		M	1	06-10	MV1	25.2800		1.6376	10	(PA)
					MV2	25.4200	(101)	2.2861	10	
					M1	25.2400	(100)	1.7219	10	
					M2	25.0100	(99)	1.9941	10	
					M3	25.3800	(100)	1.3440	10	
					FV1	21.4600		0.9868	10	(PA)
					FV2	21.3200	(99)	1.7612	10	
		F	1	06-10	F1	20.9200	(97)	1.5091	10	
					F2	21.7200	(101)	1.4482	10	
					F3	21.3000	(99)	1.5370	10	

Table 5
 Appendix 1
 PAGE 26
 EHL 92197

00350187

STUDY NUMBER: 92197 SUMMARY OF TERMINAL BODY AND ORGAN WEIGHT DATA (GM) REPORT PRINT DATE: 28-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1.

EXPLANATION OF STATISTICAL FLAGS

GENERAL	
Flag	Statistical Statement
NA	Statistics not done, insufficient or inappropriate data
NONCATEGORICAL DATA	
Flag	Statistical Statement
Parametric (PA)	
L-	The response is linearly related to dose (p<=0.05) with a negative slope
L--	The response is linearly related to dose (p<=0.01) with a negative slope
L+	The response is linearly related to dose (p<=0.05) with a positive slope
L++	The response is linearly related to dose (p<=0.01) with a positive slope
*	Significantly different from control (p<=0.05; Dunnett's)
**	Significantly different from control (p<=0.01; Dunnett's)
Nonparametric (NP)	
L-	The response is linearly related to dose (p<=0.05) with a negative slope
L--	The response is linearly related to dose (p<=0.01) with a negative slope
L+	The response is linearly related to dose (p<=0.05) with a positive slope
L++	The response is linearly related to dose (p<=0.01) with a positive slope
*	Significantly different from control (p<=0.05; Mann-Whitney)
**	Significantly different from control (p<=0.01; Mann-Whitney)
CATEGORICAL DATA	
Flag	Statistical Statement
C+	Significantly different from control (p<=0.05; uncorrected Chi square)
C++	Significantly different from control (p<=0.01; uncorrected Chi square)

00360187

Table 5 Appendix 1 PAGE 27
EHL 92197

STUDY NO: 92197

***** MONSANTO ENVIRONMENTAL HEALTH LAB *****

PAGE: 1

STUDY TYPE: SA SPECIES: MOUSE

PATHOLOGY SECTION
SUBSTANCE: Btk Full Length HD-73

PRINTED: 15-DEC-92

** SUMMARY INCIDENCE OF INDIVIDUAL GROSS NECROPSY ALTERATIONS **
SELECTION CRITERIA: SCHEDULED SACRIFICES PERIODS: 22-NOV-92:26-NOV-92

NO. IN GROUP AT RISK:	M A L E				
	MV1	MV2	M1	M2	M3
	10	10	10	10	10
LIVER					
-FOCUS, RED/PURPLE/BLACK	0	0	0	1	1
-FOCUS, BROWN/YELLOW/TAN	0	0	0	1	0
LUNG					
-FOCUS, RED/PURPLE/BLACK	0	0	0	0	0
LY. NODE, MESENT.					
-ABNORMAL COLOR; RED/PURPLE/BLACK	0	0	0	0	0
OVARY (IES)					
-CYST	0	0	0	0	0
LY. NODE, SUBMAX.					
-ENLARGED/PROMINENT	1	0	2	0	0
SPLEEN					
-ENLARGED	0	0	0	0	0
STOMACH					
-FOCUS, RED/PURPLE/BLACK	0	0	0	0	0
SEM. VESICLE(S)					
-ATROPHY/SMALL	0	1	0	0	0
UTERUS					
-ENLARGED/DILATED/DISTENDED	0	0	0	0	0

00370F0187

Table 6

Appendix 1

PAGE 28
EHL 92197

STUDY NO: 92197

***** MONSANTO ENVIRONMENTAL HEALTH LAB *****

PAGE: 1

STUDY TYPE: SA SPECIES: MOUSE

PATHOLOGY SECTION
SUBSTANCE: Btk Full Length HD-7J

PRINTED: 15-DEC-92

** SUMMARY INCIDENCE OF INDIVIDUAL GROSS NECROPSY ALTERATIONS **
SELECTION CRITERIA: SCHEDULED SACRIFICES PERIODS: 22-NOV-92:26-NOV-92

NO. IN GROUP AT RISK:	F E M A L E				
	FV1	FV2	F1	F2	F3
LIVER					
-FOCUS, RED/PURPLE/BLACK	0	0	0	0	0
-FOCUS, BROWN/YELLOW/TAN	0	0	0	0	0
LUNG					
-FOCUS, RED/PURPLE/BLACK	1	0	1	0	0
LY. NODE, MESENT.					
-ABNORMAL COLOR, RED/PURPLE/BLACK	1	0	0	0	0
OVARY (IES)					
-CYST	0	2	1	0	0
LY. NODE, SUBMAX.					
-ENLARGED/PROMINENT	0	0	1	0	0
SPLEEN					
-ENLARGED	2	1	0	0	0
STOMACH					
-FOCUS, RED/PURPLE/BLACK	0	0	1	0	0
SEM. VESICLE(S)					
-ATROPHY/SMALL	0	0	0	0	0
UTERUS					
-ENLARGED/DILATED/DISTENDED	3	1	3	0	6

003810187

Table 6 Appendix 1 PAGE 29
EHL 92197

APPENDIX 2

Inlife Observations

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-OEC-92

STUDY NUMBER: 92197
DHEM NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: MALE
SUBSTANCE : VEHICLE
TARGET DOSE : 66.70 ML/KG

ANIMAL	DATE (1992): DAY OF STUDY:	PRE-	23-NOV
		TEST	7
92197MV1 001		25.4	26.3
92197MV1 002		26.9	29.0
92197MV1 003		26.1	28.2
92197MV1 004		30.6	32.8
92197MV1 005		29.0	30.0
92197MV1 006		27.5	31.7
92197MV1 007		28.4	29.8
92197MV1 008		28.7	27.6
92197MV1 009		31.5	31.7
92197MV1 010		30.0	31.2

0040010187

Table 1
Appendix 2
PAGE 31
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 1

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEN NUMBER: ML92493
RTE OF ADMIN: ORAL (CAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: MALE
SUBSTANCE : BSA
TARGET DOSE : 6340. MG/KG

ANIMAL	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
92197MV2 001		30.8	30.5
92197MV2 002		27.2	29.3
92197MV2 003		28.0	28.7
92197MV2 004		26.1	26.9
92197MV2 005		24.7	26.4
92197MV2 006		27.7	29.8
92197MV2 007		29.0	31.3
92197MV2 008		30.3	33.3
92197MV2 009		31.1	34.4
92197MV2 010		29.5	30.0

004100187

Table 1
Appendix 2
PAGE 32
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEN NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP SEX: MALE
SUBSTANCE : 8tk Full Length HD-73
TARGET DOSE : 500.0 MG/KG

ANIMAL	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
92197M1 001		25.0	26.5
92197M1 002		27.1	29.0
92197M1 003		26.5	28.0
92197M1 004		30.5	32.8
92197M1 005		27.7	29.0
92197M1 006		27.4	28.9
92197M1 007		30.8	32.5
92197M1 008		28.5	30.6
92197M1 009		29.2	30.9
92197M1 010		31.6	30.7

00420F0187

Table 1
Appendix 2
PAGE 33
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 3

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 1000. MG/KG

SEX: MALE

ANIMAL	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
92197M2 001		27.3	28.8
92197M2 002		29.0	31.0
92197M2 003		26.0	27.1
92197M2 004		26.2	27.6
92197M2 005		27.4	26.4
92197M2 006		28.9	30.3
92197M2 007		28.1	29.3
92197M2 008		31.0	32.3
92197M2 009		30.2	32.5
92197M2 010		30.9	31.7

Table 1
Appendix 2
PAGE 34
EHL 92197

004310187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DHEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE
STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 4300. MG/KG

SEX: MALE

ANIMAL	DATE (1992):		PRE-TEST	23-NOV 7
	DAY OF STUDY:	TEST		
92197M3 001			28.1	30.2
92197M3 002			26.1	28.4
92197M3 003			27.7	29.4
92197M3 004			26.9	28.3
92197M3 005			27.3	29.7
92197M3 006			29.6	30.5
92197M3 007			28.7	29.6
92197M3 008			30.8	31.2
92197M3 009			29.4	31.2
92197M3 010			31.5	32.2

004400187

Table 1
Appendix 2

PAGE 35
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: HL92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: FEMALE
SUBSTANCE : VEHICLE
TARGET DOSE : 66.70 ML/KG

ANIMAL	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
92197FV1 001		22.8	23.0
92197FV1 002		26.5	25.2
92197FV1 003		22.6	24.3
92197FV1 004		24.9	26.5
92197FV1 005		25.7	26.6
92197FV1 006		24.1	24.1
92197FV1 007		22.7	23.8
92197FV1 008		23.1	23.7
92197FV1 009		25.7	26.2
92197FV1 010		24.1	24.8

00450187

Table 1
Appendix 2

PAGE 36
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: FEMALE
SUBSTANCE : BSA
TARGET DOSE : 6340. MG/KG

ANIMAL	DATE (1992):	
	PRE- DAY OF STUDY:	23-NOV 7
92197FV2 001	22.5	23.4
92197FV2 002	26.1	25.6
92197FV2 003	26.8	29.1
92197FV2 004	25.5	26.9
92197FV2 005	24.9	26.3
92197FV2 006	23.2	23.0
92197FV2 007	22.7	22.8
92197FV2 008	22.9	22.7
92197FV2 009	23.7	23.8
92197FV2 010	25.1	25.7

00460187

Table 1 Appendix 2

PAGE 37
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
OMEN NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CO-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 500.0 MG/KG

SEX: FEMALE

ANIMAL	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
92197F1 001		23.0	23.4
92197F1 002		23.6	22.4
92197F1 003		25.8	26.5
92197F1 004		25.2	23.1
92197F1 005		25.7	25.8
92197F1 006		23.7	24.9
92197F1 007		24.6	24.6
92197F1 008		22.7	23.9
92197F1 009		22.2	23.7
92197F1 010		26.4	26.0

00470187

Table 1
Appendix 2

PAGE 38
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL BODY WEIGHT DATA (GM)
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 1000. MG/KG

SEX: FEMALE

ANIMAL	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
92197F2 001		25.4	26.1
92197F2 002		23.7	25.3
92197F2 003		22.7	23.5
92197F2 004		25.8	27.2
92197F2 005		24.4	24.6
92197F2 006		25.2	26.5
92197F2 007		22.2	24.4
92197F2 008		23.9	24.1
92197F2 009		26.6	26.6
92197F2 010		23.0	23.4

004810187

Table 1 Appendix 2

PAGE 39
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP SEX: FEMALE
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 4300. MC/KG

ANIMAL	DATE (1992): DAY OF STUDY:	PRE- TEST	23-NOV 7
92197F3 001		25.7	27.5
92197F3 002		26.3	26.2
92197F3 003		22.9	23.6
92197F3 004		22.5	22.5
92197F3 005		25.5	25.4
92197F3 006		22.8	24.6
92197F3 007		23.9	22.8
92197F3 008		23.3	24.1
92197F3 009		24.5	22.8
92197F3 010		25.1	25.4

00490F0187

Table 1
Appendix 2
PAGE 40
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEW NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL
SUBSTANCE : VEHICLE
TARGET DOSE : 66.70 ML/KG

SEX: MALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7
ANIMAL GM GM/DAY

92197MV1 001	26	4.3
92197MV1 002	31	5.2
92197MV1 003	31	5.1
92197MV1 004	35	5.9
92197MV1 005	33	5.5
92197MV1 006	34	5.6
92197MV1 007	30	4.9
92197MV1 008	35	5.9
92197MV1 009	28	4.7
92197MV1 010	26	4.4

005010187

Table 2 Appendix 2 PAGE 41
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 1

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMZH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA

SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL
SUBSTANCE : BSA
TARGET DOSE : 6340. MG/KG

SEX: MALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7
ANIMAL GM GM/DAY

92197HV2 001	36	6.0
92197HV2 002	31	5.2
92197HV2 003	31	5.1
92197HV2 004	30	5.0
92197HV2 005	29	4.8
92197HV2 006	34	5.6
92197HV2 007	33	5.6
92197HV2 008	35	5.9
92197HV2 009	37	6.2
92197HV2 010	30	5.0

00510F0187

Table 2
Appendix 2

PAGE 42
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DHEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA

SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 500.0 MG/KG

SEX: MALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7
ANIMAL CM CM/DAY

92197M1	001	65	10.8
92197M1	002	30	5.0
92197M1	003	31	5.1
92197M1	004	32	5.4
92197M1	005	30	5.0
92197M1	006	30	4.9
92197M1	007	31	5.1
92197M1	008	31	5.2
92197M1	009	32	5.3
92197M1	010	27	4.5

0052010187

Table 2
Appendix 2

PAGE 43
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP SEX: MALE
SUBSTANCE : Btk Full Length HD-71
TARGET DOSE : 1000. MG/RC

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7

ANIMAL	GM	GM/DAY
92197H2 001	30	5.0
92197H2 002	32	5.4
92197H2 003	27	4.5
92197H2 004	30	5.0
92197H2 005	23	3.8
92197H2 006	36	6.0
92197H2 007	27	4.4
92197H2 008	34	5.6
92197H2 009	32	5.4
92197H2 010	30	4.9

005310187

Table 2
Appendix 2
PAGE 44
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
OMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA
SPECIES: MOUSE STRAIN/BREED: CO-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 4300. MG/KG

SEX: MALE

DATES FROM-TO: 17NOV-23NOV

DAY FROM-TO: 1-7

ANIMAL GM GM/DAY

92197M3	001	33	5.5
92197M3	002	33	5.4
92197M3	003	32	5.3
92197M3	004	30	5.0
92197M3	005	34	5.7
92197M3	006	29	4.8
92197M3	007	32	5.3
92197M3	008	33	5.4
92197M3	009	34	5.6
92197M3	010	32	5.3

0054M0187

Table 2
Appendix 2

PAGE 45
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 2

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DHEH NUMBER: HL92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA

SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 11-NOV-92

GROUP : VEHICLE CONTROL
SUBSTANCE : VEHICLE
TARGET DOSE : 66.70 ML/KG

SEX: FEMALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7

ANIMAL	GM	GM/DAY
92197FV1 001	30	5.0
92197FV1 002	32	5.3
92197FV1 003	37	6.2
92197FV1 004	31	5.1
92197FV1 005	29	4.9
92197FV1 006	30	5.0
92197FV1 007	30	5.0
92197FV1 008	26	4.3
92197FV1 009	32	5.3
92197FV1 010	35	5.8

00550F0187

Table 2 Appendix 2

PAGE 46
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: HL92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA

SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL
SUBSTANCE : BSA
TARGET DOSE : 6340. MG/KG

SEX: FEMALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7
ANIMAL

ANIMAL	GM	GM/DAY
92197FV2 001		
92197FV2 002	29	4.8
92197FV2 003	33	5.5
92197FV2 004	31	5.2
92197FV2 005	31	5.2
92197FV2 006	25	4.2
92197FV2 007	26	4.3
92197FV2 008	34	5.7
92197FV2 009	30	5.1
92197FV2 010	31	5.1

00560187

Table 2
Appendix 2
PAGE 47
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 3

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA

SPECIES: HOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 500.0 MG/KG

SEX: FEMALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7
ANIMAL GM GM/DAY

ANIMAL	GM	GM/DAY
92197F1 001	26	4.3
92197F1 002	19	3.1
92197F1 003	26	4.4
92197F1 004	20	3.3
92197F1 005	27	4.5
92197F1 006	27	4.5
92197F1 007	42	7.0
92197F1 008	24	4.0
92197F1 009	23	3.8
92197F1 010	38	6.3

005700187

Table 2
Appendix 2
PAGE 48
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA

SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 1000. MG/KG

SEX: FEMALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7
ANIMAL GM GM/DAY

92197F2	001	25	4.1
92197F2	002	33	5.5
92197F2	003	24	4.0
92197F2	004	30	4.9
92197F2	005	33	5.5
92197F2	006	29	4.9
92197F2	007	24	4.0
92197F2	008	30	4.9
92197F2	009	28	4.6
92197F2	010	30	5.0

00580187

Table 2 Appendix 2

PAGE 49
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL FOOD CONSUMPTION DATA
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-7J
TARGET DOSE : 4300. MG/KG

SEX: FEMALE

DATES FROM-TO: 17NOV-23NOV
DAY FROM-TO: 1-7
ANIMAL

ANIMAL	GM	GM/DAY
92197F3 001	32	5.3
92197F3 002	29	4.8
92197F3 003	30	4.9
92197F3 004	34	5.7
92197F3 005	30	5.0
92197F3 006	36	6.1
92197F3 007	27	4.4
92197F3 008	29	4.9
92197F3 009	32	5.3
92197F3 010	33	5.5

Table 2
Appendix 2

PAGE 50
EHL 92197

00590F0187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL CLINICAL SIGNS
SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: MALE
SUBSTANCE : BSA
TARGET DOSE : 6340. MG/KG

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
--------	------------------------	-----------------	----------	-------------

** NO ABNORMAL CLINICAL SIGNS RECORDED **

00610F0187

Table 3
Appendix 2
PAGE 52
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (CAVAGE)

INDIVIDUAL CLINICAL SIGNS
SPECIES: MOUSE STRAIN/BREED: CD-1
STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 500.0 MG/KG

SEX: MALE

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION

** NO ABNORMAL CLINICAL SIGNS RECORDED **				

006200187

Table 3
Appendix 2
PAGE 58
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL CLINICAL SIGNS
SPECIES: MOUSE STRAIN/BREED: CD-1
STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 1000. MG/KG

SEX: MALE

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
--------	------------------------	-----------------	----------	-------------

** NO ABNORMAL CLINICAL SIGNS RECORDED **

Table 3
Appendix 2
PAGE 54
EHT 92197

00630187

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEN NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)
INDIVIDUAL CLINICAL SIGNS
SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92
GROUP : TEST GROUP SEX: MALE
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 4300. MG/KG

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
--------	---------------------	--------------	----------	-------------

** NO ABNORMAL CLINICAL SIGNS RECORDED **

00640F0187

Table 3
Appendix 2
PAGE 55
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMZH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL CLINICAL SIGNS

SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL
SUBSTANCE : VEHICLE
TARGET DOSE : 66.70 ML/KG

SEX: FEMALE

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
--------	------------------------	-----------------	----------	-------------

.. NO ABNORMAL CLINICAL SIGNS RECORDED ..

00650F0187

Table 3
Appendix 2
PAGE 56
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEN NUMBER: HL92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL CLINICAL SIGNS
SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: FEMALE
SUBSTANCE : BSA
TARGET DOSE : 6340. MC/KG

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
--------	------------------------	-----------------	----------	-------------

** NO ABNORMAL CLINICAL SIGNS RECORDED **

Table 3
Appendix 2
PAGE 57
EHL 92197

00660187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
SITE OF ADMIN: ORAL (CAVAGE)
INDIVIDUAL CLINICAL SIGNS
SPECIES: MOUSE
STRAIN/BREED: CD-1
SEX: FEMALE
STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 500.0 MG/KC

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
** NO ABNORMAL CLINICAL SIGNS RECORDED **				

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEN NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)
SPECIES: MOUSE
STRAIN/BREED: CD-1
STUDY START DATE: 17-NOV-92
GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 1000. MG/KG
SEX: FEMALE

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
--------	------------------------	-----------------	----------	-------------

** NO ABNORMAL CLINICAL SIGNS RECORDED **

006810187

Table 3
Appendix 2
PAGE 59
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

INDIVIDUAL CLINICAL SIGNS
SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 4300. MG/KG

SEX: FEMALE

ANIMAL	DATE OF OBSERVATION	DAY OF STUDY	CATEGORY	OBSERVATION
--------	------------------------	-----------------	----------	-------------

** NO ABNORMAL CLINICAL SIGNS RECORDED **

006910187

Table 3
Appendix 2
PAGE 60
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 4

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
 DMR NUMBER: ML92493
 RTE OF ADMIN: ORAL (GAVAGE)

ANIMAL TERMINATION HISTORY
 SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: MALE
 SUBSTANCE : VEHICLE
 TARGET DOSE : 66.70 ML/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197MV1 001	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 002	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 003	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 004	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 005	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 006	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 007	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 008	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 009	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV1 010	24-NOV-92	8	SCHEDULED SACRIFICE

Table 4
 Appendix 2
 PAGE 61
 ENL 92197

007010187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

ANIMAL TERMINATION HISTORY

SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL SEX: MALE
SUBSTANCE : BSA
TARGET DOSE : 6340. MG/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197MV2 001	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 002	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 003	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 004	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 005	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 006	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 007	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 008	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 009	24-NOV-92	8	SCHEDULED SACRIFICE
92197MV2 010	24-NOV-92	8	SCHEDULED SACRIFICE

Table 4
Appendix 2
PAGE 62
EHL 92197

0071010187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197 ANIMAL TERMINATION HISTORY
DNEH NUMBER: ML92493
RTZ OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP SEX: MALE
SUBSTANCE : Bck Full Length HD-73
TARGET DOSE : 500.0 MG/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197M1 001	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 002	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 003	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 004	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 005	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 006	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 007	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 008	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 009	24-NOV-92	0	SCHEDULED SACRIFICE
92197M1 010	24-NOV-92	0	SCHEDULED SACRIFICE

0072010187

Table 4
Appendix 2
PAGE 63
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DREH NUMBER: HL92493
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP SEX: MALE
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 1000. MG/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197M2 001	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 002	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 003	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 004	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 005	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 006	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 007	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 008	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 009	24-NOV-92	8	SCHEDULED SACRIFICE
92197M2 010	24-NOV-92	8	SCHEDULED SACRIFICE

00730F0187

Table 4
Appendix 2
PAGE 64
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197 ANIMAL TERMINATION HISTORY
 DMEH NUMBER: ML92493
 RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92
 GROUP : TEST GROUP SEX: MALE
 SUBSTANCE : Btk Full Length HD-73
 TARGET DOSE : 4300. MG/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197M3 001	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 002	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 003	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 004	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 005	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 006	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 007	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 008	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 009	24-NOV-92	8	SCHEDULED SACRIFICE
92197M3 010	24-NOV-92	8	SCHEDULED SACRIFICE

00740187

Table 4 Appendix 2 PAGE 65 EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMZH NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE)

ANIMAL TERMINATION HISTORY

SPECIES: MOUSE STRAIN/BREED: CD-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL
SUBSTANCE : VEHICLE
TARGET DOSE : 66.70 ML/KG

SEX: FEMALE

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197FV1 001	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 002	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 003	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 004	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 005	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 006	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 007	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 008	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 009	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV1 010	25-NOV-92	9	SCHEDULED SACRIFICE

00750F0187

Table 4
Appendix 2

PAGE 66
EHL 92197

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
DMEH NUMBER: HL92493
RTE OF ADMIN: ORAL (GAVAGE)

ANIMAL TERMINATION HISTORY

SPECIES: MOUSE STRAIN/BREED: CO-1

STUDY START DATE: 17-NOV-92

GROUP : VEHICLE CONTROL
SUBSTANCE : BSA
TARGET DOSE : 6340. MG/KG

SEX: FEMALE

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197FV2 001	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 002	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 003	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 004	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 005	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 006	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 007	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 008	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 009	25-NOV-92	9	SCHEDULED SACRIFICE
92197FV2 010	25-NOV-92	9	SCHEDULED SACRIFICE

Table 4
Appendix 2

PAGE 67
EHL 92197

007610187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197 ANIMAL TERMINATION HISTORY
DMEH NUMBER: ML92493 SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92
RTE OF ADMIN: ORAL (GAVAGE)
GROUP : TEST GROUP SEX: FEMALE
SUBSTANCE : Blk Full Length HD-73
TARGET DOSE : 500.0 MG/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197F1 001	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 002	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 003	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 004	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 005	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 006	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 007	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 008	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 009	25-NOV-92	9	SCHEDULED SACRIFICE
92197F1 010	25-NOV-92	9	SCHEDULED SACRIFICE

Table 4 Appendix 2

PAGE 66
EHL 92197

00770187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197
 DNEH NUMBER: ML92493
 RTE OF ADMIN: ORAL (GAVAGE)
 ANIMAL TERMINATION HISTORY
 SPECIES: MOUSE STRAIN/BREED: CD-1
 STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP SEX: FEMALE
 SUBSTANCE : Btk Full Length HD-73
 TARGET DOSE : 1000. MG/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197F2 001	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 002	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 003	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 004	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 005	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 006	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 007	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 008	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 009	25-NOV-92	9	SCHEDULED SACRIFICE
92197F2 010	25-NOV-92	9	SCHEDULED SACRIFICE

Table 4 Appendix 2

PAGE 69
 EHL 92197

007810187

THIS REPORT WAS GENERATED FROM DATA LOCKED THROUGH 15-DEC-92

STUDY NUMBER: 92197 ANIMAL TERMINATION HISTORY
DMEN NUMBER: ML92493
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE STRAIN/BREED: CD-1 STUDY START DATE: 17-NOV-92

GROUP : TEST GROUP SEX: FEMALE
SUBSTANCE : Btk Full Length HD-73
TARGET DOSE : 4300. MC/KG

ANIMAL	DATE OF DEATH	DAY OF STUDY	TYPE OF DEATH
92197F3 001	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 002	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 003	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 004	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 005	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 006	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 007	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 008	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 009	25-NOV-92	9	SCHEDULED SACRIFICE
92197F3 010	25-NOV-92	9	SCHEDULED SACRIFICE

00790F0187

Table 4
Appendix 2
PAGE 70
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 4

Appendix 3

PAGE 71
EHL 92197

APPENDIX 3

Gross Pathology

00800F0187

Necropsy data locked on 17-DEC-92

(06)

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197MV1 001	SEX: MALE	PROSECTOR : (ESGOLI)
ANIMAL STATUS CODE: S	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: ()
		PATHOLOGIST : (MMCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 22.4

--- GROSS OBSERVATIONS ---

ILEUM
NOTE: IN CASSETTE 1L.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
-------------------------------------	-------------------------

NO TISSUES EXAMINED.

Table 1
Appendix 3
PAGE 72
EHL 92197

00810F0187

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (CAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197MVI 002	SEX: MALE	PROSECTOR : (BASCHW)
ANIMAL STATUS CODE: 5	HOURS POST MORTEM = 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: ()
		PATHOLOGIST : (MMDCO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.3

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
-------------------------------------	-------------------------

NO TISSUES EXAMINED.

Table 1

Appendix 3

PAGE 73
EHL 92197

00821F0187

Necropsy data locked on 17-DEC-92

(88)

STUDY NUMBER: 92197 . INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197MV1 003 SEX: MALE PROSECTOR : (RPCRUE)
ANIMAL STATUS CODE: S HOURS POST MORTEM = 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WIS. BY: ()
PATHOLOGIST : (***KDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.4

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

00830F0187

Table 1
Appendix 3

PAGE 74
EHL 92197

Necropsy data locked on 17-DEC-92

(00)

STUDY NUMBER: 92197
SITE OF ADMIN: ORAL (CAVAGE)
STUDY START DATE: 17-NOV-1992

INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA

REPORT PRINT DATE: 17-DEC-1992
SPECIES: MOUSE
STRAIN/BREED: CD-1

ANIMAL NO: 92197MV1 004 SEX: MALE PROSECTOR : (HJBOYD)
ANIMAL STATUS CODE: S HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: []
PATHOLOGIST : (MMCD0)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 27.5

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1
Appendix 3

PAGE 75
EHL 92197

00840F0187

Necropsy data locked on 17-DEC-92

(00)

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (CAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197MVI 005	SEX: MALE	PROSECTOR : (ESCOLI)
ANIMAL STATUS CODE: S	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: []
		PATHOLOGIST : (MMCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.9

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
-------------------------------------	-------------------------

NO TISSUES EXAMINED.

Table 1
Appendix 3
PAGE 76
EHL 92197

00850187

[00]

Necropsy data locked on 17-DEC-92

STUDY NUMBER: 92197
 RTE OF ADMIN: ORAL (CAVAGE)
 STUDY START DATE: 17-NOV-1992
 ANIMAL NO: 92197M1 007 SEX: MALE
 ANIMAL STATUS CODE: S HOURS POST MORTEM - 0

INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA

REPORT PRINT DATE: 17-DEC-1992
 SPECIES: MOUSE
 STRAIN/BREED: CD-1

PROSECTOR : (BASCHW)
 ORGAN WTS BY: (
 PATHOLOGIST : (MPCDO)

DATE OF NECROPSY: 24-NOV-92

--- ORGAN WEIGHTS (GM) and 1 BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 25.2

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

NO TISSUES EXAMINED.

DISTRIBUTION (SEVERITY)

00870F0187

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197HV1 008 SEX: MALE PROSECTOR : (ESGOLI)
ANIMAL STATUS CODE: S HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: { }
PATHOLOGIST : (HMCDO)

--- ORGAN WEIGHTS (GM) and 1 BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 23.8

--- GROSS OBSERVATIONS ---

LY. NODE, SUBMAX.
ENLARGED/PROMINENT -- ENLARGED - ONE, 0.2 X 0.3 CM.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

00880187

Table 1
Appendix 3
PAGE 79
EHL 92197

Necropsy data locked on 17-DEC-92

{00}

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197MVI 010 SEX: MALE PROSECTOR : (MJB0YD)
ANIMAL STATUS CODE: S HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: ()
PATHOLOGIST : (MHCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 26.4

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1 Appendix 3

PAGE 81
EHL 92197

00900187

Necropsy data locked on 17-DEC-92

(00)

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197MV2 001 SEX: MALE PROSECTOR : (BASCHM)
ANIMAL STATUS CODE: S HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: ()
PATHOLOGIST : (MDCO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 25.7

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1 Appendix 3

PAGE 82
EHL 92197

00910187

Necropsy data locked on 17-DEC-92

(88)

STUDY NUMBER: 92197
RTE OF ADMIN: ORAL (GAVAGE)
STUDY START DATE: 17-NOV-1992

INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA

REPORT PRINT DATE: 17-DEC-1992
SPECIES: MOUSE
STRAIN/BREED: CD-1

ANIMAL NO: 92197MV2 003 SEX: MALE
ANIMAL STATUS CODE: 5 HOURS POST MORTEM - 0
DATE OF NECROPSY: 24-NOV-92
PROSECTOR : (BASCHW)
ORGAN WIS. BY: []
PATHOLOGIST : (MPCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.8

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1
Appendix 3

PAGE 84
EHL 92197

00930F0187

STUDY NUMBER:92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 11

Necropsy data locked on 17-DEC-92

(00)

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197MV2 004	SEX: MALE	PROSECTOR : (MJBOYD)
ANIMAL STATUS CODE: 5	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: ()
		PATHOLOGIST : (***CDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 22.7

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1
Appendix 3
PAGE 85
EHL 92197

00940F0187

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197MV2 006 SEX: MALE PROSECTOR : (BASCHW)
ANIMAL STATUS CODE: 5 HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: ()
PATHOLOGIST : (MPCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 25.2

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1 Appendix 3

PAGE 87
EHL 92197

00960F0187

Necropsy data locked on 17-DEC-92

[00]

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197MV2 007	SEX: MALE	PROSECTOR : (MJBOYD)
ANIMAL STATUS CODE: S	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: []
		PATHOLOGIST : (MHCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 26.5

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1
Appendix 3
PAGE 88
EHL 92197

00970187

Necropsy data locked on 17-DEC-92

[00]

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197MV2 009	SEX: MALE	PROSECTOR : {RPGROE}
ANIMAL STATUS CODE: S	HOURS POST MORTEM = 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: { }
		PATHOLOGIST : {MMCD01}

--- ORGAN WEIGHTS (%M) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 29.

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED

0099010187

Table 1
Appendix 3
PAGE 90
EHL 92197

(90)

Necropsy data locked on 17-DEC-92

STUDY NUMBER: 92197
 RTZ OF ADMIN: ORAL (GAVAGE)
 STUDY START DATE: 17-NOV-1992
 REPORT PRINT DATE: 17-DEC-1992
 SPECIES: MOUSE
 STRAIN/BREED: CD-1

INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA

ANIMAL NO: 92197V2 010 SEX: MALE
 ANIMAL STATUS CODE: 5 HOURS POST MORTEM = 0 DATE OF NECROPSY: 24-NOV-92
 PROSECTOR : (BASCHW)
 ORGAN WTS. BY: (
 PATHOLOGIST : (HMCDO)

--- ORGAN WEIGHTS (GM) and BODY WEIGHT ---

TERMINAL BODY WEIGHT --- 25.0

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Necropsy data locked on 17-DEC-92

(00)

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M1 005	SEX: MALE	PROSECTOR : (BASCHW)
ANIMAL STATUS CODE: S	HOURS POST MORTEM = 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WIS. BY: ()
		PATHOLOGIST : (MMMCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 23.9

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1

Appendix 3

PAGE 96
EHL 92197

010500187

Necropsy data locked on 17-DEC-92

{00}

STUDY NUMBER: 92197
RTE OF ADMIN: ORAL (CAVAGE)
STUDY START DATE: 17-NOV-1992
INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA
REPORT PRINT DATE: 17-DEC-1992
SPECIES: MOUSE
STRAIN/BREED: CD-1

ANIMAL NO: 92197M1 008 SEX: MALE
ANIMAL STATUS CODE: S HOURS POST MORTEM = 0 DATE OF NECROPSY: 24-NOV-92
PROSECTOR : (BASCHW)
ORGAN WTS. BY: ()
PATHOLOGIST : (MMKDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.9

--- GROSS OBSERVATIONS ---

LY.NODE, SUBMAX.
ENLARGED/PROMINENT -- ENLARGED - ONE, 0.2 X 0.4 CM.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS
NO TISSUES EXAMINED.

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
NO TISSUES EXAMINED.	

010810187

Table 1
Appendix 3
PAGE 99
EHL 92197

Necropsy data locked on 17-DEC-92

[00]

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M1 009	SEX: MALE	PROSECTOR : (ESGOLI)
ANIMAL STATUS CODE: S	HOURS POST MORTEM = 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: []
		PATHOLOGIST : (HMCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 26.5

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
-------------------------------------	-------------------------

NO TISSUES EXAMINED.

0109010187

Table 1 Appendix 3 PAGE 100 EHL 92197

```

STUDY NUMBER: 92197                INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA                REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)        SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992     STRAIN/BREED: CD-1
-----
ANIMAL NO: 92197M1 010    SEX: MALE    PROSECTOR : (RPGRUE)
ANIMAL STATUS CODE: S    HOURS POST MORTEM = 0    DATE OF NECROPSY: 24-NOV-92    ORGAN WIS. BY: ( )
                                     PATHOLOGIST : (MHCDO)

```

--- ORGAN WEIGHTS (GM) and V BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 25.9

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1 Appendix 3 PAGE 101
EHL 92197

01100F0187

Necropsy data locked on 17-DEC-92

{00}

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA .	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M2 002	SEX: MALE	PROSECTOR : (MJBOYD)
ANIMAL STATUS CODE: S	HOURS POST MORTEM = 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WIS. BY: ()
		PATHOLOGIST : (MMCDO)

--- ORGAN WEIGHTS (GM) and A BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 26.3

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

01120F0187

Table 1
Appendix 3
PAGE 103
EHL 92197

Necropsy data locked on 17-DEC-92

(00)

STUDY NUMBER: 92197
RTE OF ADMIN: ORAL (GAVAGE)
STUDY START DATE: 17-NOV-1992

INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA

REPORT PRINT DATE: 17-DEC-1992
SPECIES: MOUSE
STRAIN/BREED: CD-1

ANIMAL NO: 92197M2 003 SEX: MALE
ANIMAL STATUS CODE: S HOURS POST MORTEM = 0 DATE OF NECROPSY: 24-NOV-92
PROSECTOR : (ESGOLI)
ORGAN WTS. BY: ()
PATHOLOGIST : (MPCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 22.9

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

01130187

Table 1
Appendix 3
PAGE 104
EHL 92197

Necropsy data locked on 17-DEC-92

106,

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197M2 004 SEX: MALE PROSECTOR : (BASCHW)
ANIMAL STATUS CODE: 5 HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: ()
PATHOLOGIST : (MHCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 22.4

--- GROSS OBSERVATIONS ---

LIVER
FOCUS, BROWN/YELLOW/TAN -- LEFT LATERAL LOBE - SEROSA, < 0.1
CM, ROUND, POORLY-DEMARCATED, YELLOW, FLAT FOCUS.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

0114010187

Table 1
Appendix 3
PAGE 105
EHL 92197

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
 RTE OF ADMIN: ORAL (CAVAGE) SPECIES: MOUSE
 STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197M2 007 SEX: MALE PROSECTOR : (ESGOLI)
 ANIMAL STATUS CODE: S HOURS POST MORTEM = 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: ()
 PATHOLOGIST : (MMCD0)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.1

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
NO TISSUES EXAMINED.	

Table 1
Appendix 3

PAGE 108
EHL 92197

01170F0187

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (CAVAGE) SPECIES: HOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197M2 008 SEX: MALE PROSECTOR : (RPGRUE)
ANIMAL STATUS CODE: S HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: ()
PATHOLOGIST : (MPCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 27.1

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1
Appendix 3
PAGE 109
EHL 92197

01180187

Necropsy data locked on 17-DEC-92

{00}

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M2 009	SEX: MALE	PROSECTOR : (BASCHW)
ANIMAL STATUS CODE: S	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: ()
		PATHOLOGIST : (MMCD0)

--- ORGAN WEIGHTS (GM) and 1 BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 27.4

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
NO TISSUES EXAMINED.	

Table 1
Appendix 3
PAGE 110
EHL 92197

011900187

Necropsy data locked on 17-DEC-92

106,

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M2 010	SEX: MALE	PROSECTOR : (MJBOYD)
ANIMAL STATUS CODE: 5	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: ()
		PATHOLOGIST : (MCMCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 27.3

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
NO TISSUES EXAMINED.	

012000187

Table 1 Appendix 3 PAGE 111 EHL 92197

Necropsy data locked on 17-DEC-92

(66)

STUDY NUMBER: 92197
 RATE OF ADMIN: ORAL (GAVAGE)
 STUDY START DATE: 17-NOV-1992
 REPORT PRINT DATE: 17-DEC-1992

INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA

 ANIMAL NO: 92197M3 001 SEX: MALE PROSECTOR : (BASCHM)
 ANIMAL STATUS CODE: S HOURS POST MORTEM - 0 DATE OF NECROPSY: 24-NOV-92 ORGAN HTS. BY: (
 PATHOLOGIST : (MARCDO)

--- ORGAN WEIGHTS (GM) and 1 BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.8

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1

Appendix 3

PAGE 112
EHL 92197

STUDY NUMBER: 92197

MONSANTO ENVIRONMENTAL HEALTH LABORATORY

PAGE 41

0121 OF 0187

Necropsy data locked on 17-DEC-92

{00}

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M3 002	SEX: MALE	PROSECTOR : (ESGOLI)
ANIMAL STATUS CODE: S	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: []
		PATHOLOGIST : (MMDCO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 23.6

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
NO TISSUES EXAMINED.	

0122010187

Table 1 Appendix 3

PAGE 113
EHL 92197

Necropsy data locked on 17-DEC-92

100:

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M3 003	SEX: MALE	PROSECTOR : (BASCHW)
ANIMAL STATUS CODE: S	HOURS POST MORTEM = 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: ()
		PATHOLOGIST : (HMCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 24.9

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

Table 1
Appendix 3

PAGE 114
EHL 92197

01230F0187

Necropsy data locked on 17-DEC-92

{00}

STUDY NUMBER: 92197
RTE OF ADMIN: ORAL [GAVAGE]
STUDY START DATE: 17-NOV-1992

INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA

REPORT PRINT DATE: 17-DEC-1992
SPECIES: MOUSE
STRAIN/BREED: CD-1

ANIMAL NO: 92197M3 004 SEX: MALE
ANIMAL STATUS CODE: S HOURS POST MORTEM = 0 DATE OF NECROPSY: 24-NOV-92

PROSECTOR : [MJBOYD]
ORGAN WTS. BY: []
PATHOLOGIST : [***MCD0]

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 23.3

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

012400187

Table 1
Appendix 3
PAGE 115
EHL 92197

Necropsy data locked on 17-DEC-92

{00;

STUDY NUMBER: 92197	INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA	REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE)		SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992		STRAIN/BREED: CD-1

ANIMAL NO: 92197M3 005	SEX: MALE	PROSECTOR : (RPGRUE)
ANIMAL STATUS CODE: S	HOURS POST MORTEM - 0	DATE OF NECROPSY: 24-NOV-92
		ORGAN WTS. BY: ()
		PATHOLOGIST : (MPCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 25.2

--- GROSS OBSERVATIONS ---

LIVER
FOCUS, RED/PURPLE/BLACK -- MOST LOBES - SEROSA, MULTIPLE,
< 0.1 - 0.1 CM, IRREGULAR, CONFLUENT, BRIGHT RED, FLAT FOCI.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS	DISTRIBUTION (SEVERITY)
-------------------------------------	-------------------------

NO TISSUES EXAMINED.

012500187

Table 1 Appendix 3 PAGE 116 EHL 92197

Necropsy data locked on 17-DEC-92

{00,

```
STUDY NUMBER: 92197              INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA          REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN:  ORAL (GAVAGE)                                           SPECIES:  MOUSE
STUDY START DATE:  17-NOV-1992                                         STRAIN/BREED:  CD-1
-----
ANIMAL NO: 92197M3  006  SEX: MALE                                       PROSECTOR   : (ESGOLJ)
ANIMAL STATUS CODE: S  HOURS POST MORTEM  =    0   DATE OF NECROPSY: 24-NOV-92   ORGAN WTS. BY: (      )
                                                             PATHOLOGIST  : (MMCD0)
```

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 25.8

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

012600187

Table 1 Appendix 3

PAGE 117
EHL 92197

Necropsy data locked on 17-DEC-92

1081

STUDY NUMBER: 92197 INDIVIDUAL GROSS AND HISTOPATHOLOGY DATA REPORT PRINT DATE: 17-DEC-1992
RTE OF ADMIN: ORAL (GAVAGE) SPECIES: MOUSE
STUDY START DATE: 17-NOV-1992 STRAIN/BREED: CD-1

ANIMAL NO: 92197M3 008 SEX: MALE PROSECTOR : (MJBOYD)
ANIMAL STATUS CODE: S HOURS POST MORTEM = 0 DATE OF NECROPSY: 24-NOV-92 ORGAN WTS. BY: { }
PATHOLOGIST : (HMCDO)

--- ORGAN WEIGHTS (GM) and % BODY WEIGHT ---

TERMINAL BODY WEIGHT -- 26.8

--- GROSS OBSERVATIONS ---

NO GROSS ABNORMALITIES.

--- MICROSCOPIC TISSUE OBSERVATIONS ---

TISSUE / HISTOPATHOLOGICAL FINDINGS

DISTRIBUTION (SEVERITY)

NO TISSUES EXAMINED.

0128010187

Table 1 Appendix 3

PAGE 119
EHL 92197

ATTACHMENT 7

A 90 Day Feeding Study with Cottonseed in Goats

FINAL REPORT

**A 90 Day Feeding Study with
Cotton seed in Goat**

Study sponsor

**MAHARASHTRA HYBRID SEEDS
COMPANY LIMITED**

**RESHAM BHAVAN, 4TH FLOOR,
78, VEER NARIMAN ROAD,
MUMBAI-400 020 (INDIA)**

Performing Laboratory

INDUSTRIAL TOXICOLOGY RESEARCH CENTRE

(Council of Scientific & Industrial Research)

**POST BOX 80, M.G. MARG,
LUCKNOW-226 001, INDIA**

Final Report
Laboratory Project Identification
Study Number: CNP 062-22
November, 1998

STUDY TITLE
**A 90 DAY FEEDING STUDY WITH COTTONSEED
IN GOAT**

Principal Investigator
Co-Principal Investigator

Dr. K.K. Dutta
Dr. R.K.S. Dogra

Study Monitor
Dr. M. Krishnan
Monsanto Enterprises Limited, Mumbai

Contributors

Dr. Ravi Shanker
Dr. V. Misra
Dr. Kr. P. Singh
Dr. D.C. Purohit

Dr. A.K. Saxena
Dr. S. Khanna
Mr. N. Mathur
Mr. K.K. Gupta

Dr. S. Khandelwal
Dr. A.K. Prasad
Mr. N. Garg
Mr. B.D. Bhattacharji

Technical Assistants

Mr.. Kewal Lal
Mr. L.J. Shukla
Mr. S.D. Pandey
Mr. V.K. Jain

Mr. S.N. Srivastava
Mr. Pradeep Kumar
Mr. U. Mani

Mr. B.K. Majhi
Mr. S.P. Dhruv
Mr. Dheer Kumar

Coordinator and Head, BusinessDevelopment Group
Dr.P.N Viswanathan

Head of the Institution

Dr. P.K. SETH
DIRECTOR

Performing laboratory

Industrial Toxicology Research Centre
Post Box 80, Mahatma Gandhi Marg
Lucknow-226 001
Fax: 0522-228227
E-mail:intox@itrc.sirnetd.ernet.in

CONTENTS

	Page
▪ Statement of No Data Confidentiality Claim	3
▪ Quality Assessment Statement	4
▪ Statement of Particulars of Study	5
▪ Abbreviations	6
▪ Abstract	7
▪ Introduction	7
▪ Materials & Methods	9
▪ Results	16
▪ Discussion	20
▪ Acknowledgements	21
▪ References	21
▪ Tables	23
▪ Appendix 1 (Data Supplied by MAHYCO)	44

Report on the Analysis of 'Bt-cotton' Seeds

Statement of No Data Confidentiality claims

This is to certify that the Performing Laboratory is not making any claim of confidentiality for any information contained in this study.

This performing Laboratory also states that the issues related to raw data and materials will be stored in the laboratory's archives and will be accessible to the authorized scientists of the laboratory and/or persons authorized by the sponsors.

Laboratory : Industrial Toxicology Research, Centre, Lucknow
Principal Investigator : Dr. K.K. Dutta
Title : Scientist E-2

Signature : K.K. Dutta

Date : 06/11/98

Quality Assurance Statement

This is to certify that Quality Assurance Unit of the Performing Laboratory (Industrial Toxicology Research Centre) has monitored this study and reviewed the study data and report.

-----06 11 1998

Quality Assurance Representative Date

Performing Laboratory

Study Number : CNP ó 062 ó 22

Title : A 90 DAY FEEDING STUDY WITH COTTONS
IN GOAT

Facility : Industrial Toxicology Research Centre Post Box
No. 80, Mahatma Gandhi Marg Lucknow 226001
Uttar Pradesh
Fax : 0522 ó 228227
E-mail: intox@itrc.sirnetd.ernet.in

Study Director : Dr. K.K. Dutta

Co-Study Director : Dr. R.K.S.Dogra

Investigators : Dr Ravi Shanker, Dr S Khandelwal Dr. S Khanna,
Dr Kr P Singh, Dr A.K Saxena, Dr D C Purohit, Dr
V Misra, Dr A K Prasad, Mr N Mathur, Mr N Garg

Study Initiation Date : 13.01.1998

Experimentation
Termination Date : 18.05.1998

Record Retention : All study specific raw data, protocol, final reports
and facility records will be retained in the archives
at the Industrial Toxicology Research Centre,
Lucknow. These will be made available to the
authorized personnel from the sponsors.

Signature of Approval

K.K Dutta 06/11/98
Study Director Date

Co-Study Director 06/11/98
Date

Study Sponsor 11/11/98
Date

Abbreviations

B.t.	:	Bacillus thuringiensis
B.t.k	:	Bacillus thuringiensis Kurstaki
C	:	Degree Celsius
DLC	:	Cubic millimeter
EEC	:	European Economic Commission
F	:	Degree Fahrenheit
GOT	:	Glutamate Oxaloacetate Transaminase
G	:	Gram
ITRC	:	Industrial Toxicology Research Centre
IRC	:	Insect Resistant Cotton
L	:	Litres
Mg	:	Milligram
OECD	:	Organisation for Economic Co-operation and Development
R	:	Replicates
RBC	:	Red Blood Corpuscles
SOP	:	Standard Operating Procedure
TR	:	Treatment
U/L	:	Enzyme Unit of Activity per Litre
WBC	:	White Blood Corpuscles
WHO	:	World Health Organisation

ABSTRACT

A 90 day goat feeding study was conducted with hybrid cottonseed. Treatment groups included Goats fed cottonseed that was genetically modified to contain a protein from *Bacillus thuringiensis* subsp. *Kurstaki* (B.t.k), and control groups were fed cottonseed that did not contain the B.t. protein. All cotton seed was provided by M/s Maharashtra hybrid Seeds Company Ltd. Mumbai, India. Twenty four male and twenty four female goats of the Barberi breed were divided by the random block method into four treatment groups. Each contained 6 male and 6 female goats. Each treatment group was fed cottonseed mixed with concentrate ration for 90 day period. The treatment groups were as follows: Tr.1, Hybrid MCU-5 with Bt; Tr.2, Hybrid MCU-5 without Bt; Tr.3, Hybrid LRA with Bt; and Tr.4 Hybrid LRA without Bt. The ration (feed concentrate only) fed to the animals was analysed for chemical composition (crude protein, fat, acid detergent fiber, vitamin A& E, and minerals). The drinking water was also analysed for chemical composition.

Feed intake, weight gain, haematology and serum enzymes were measured for each goat during the feeding period of study. At the end of the study, the animals were assessed for gross pathology and histopathology. It is concluded from results of the above analyses, that it is safe for animal feed as control cottonseed without the Bt protein. Although some gross pathology and histopathological differences were observed across the 48 goats in the entire study, none of the differences were attributable to any of the cottonseed feeding treatments, and were typical for goats feeding on cottonseed.

INTRODUCTION

The objective of this study was to compare the wholesomeness of cottonseeds with Cry IA(c) protein (referred to as Bt. Protein) with control seeds that do not contain the Cry IA (c) protein. Cottonseeds of cottonline with Bt and control cotton lines were administered to the goats through their diet for 90 days.

Data was generated using two cotton lines-with and without Bt proteins (Treatments shown in the table below). The comparative assessment was to help achieve an insight into whether there are any differences in the wholesomeness and feed safety of Bt and non-Bt cotton.

MCU5 (with Bt)	MCU5 (without Bt Control)	LRA (with Bt)	LRA(without Bt Control)
Tr.1	Tr.2	Tr.3	Tr.4

Justification for Using goat as a model: The toxicity testing protocol was selected on the basis of the eventual use of the product. There are standard procedures available for pre-marketing safety evaluation of consumer products and recommendations have been enacted by WHO and EEC scientific committee for food and feed ingredient (Bartosek et al., 1982).

It is generally agreed that both rodent and non-rodent species be included in the subchronic and chronic studies, recognizing that a number of factors might dictate the number and choice of species (OECD 1993). Since the present study involves safety evaluation of cottonseed which is widely used as a feed ingredient for domestic animals (cattle, buffaloes, sheep, goat and poultry) in India, a goat as an appropriate model was used. Subchronic 90-day feeding schedule as recommended for rodents was followed in the present study of crushed cotton seed. Moreover, recommendations also exist that in the case of pesticides, tests be carried out on farm animals that might accidentally be exposed (Sperlinger, 1981; Bartosek et al., 1982)

The goat (*Capra hircus*) forms an excellent experimental animal for physiological and biomedical research (Dougherty, 1976; Pathak and Bhowmik, 1996). The factors which make it an especially suitable experimental animal include its size, easy in handling, its preference for clean and dry surroundings, relatively dry faeces, docile

and intelligent nature and resistance to many diseases like trypanosomiasis, tuberculosis, brucellosis etc. The goat is widely used in nutrition and metabolic studies (Jindal, 1984). Goat lung is suitable for understanding the high altitude pulmonary adjustment and response to polluted environment (Atwal and Sweeny, 1971). It is suitable for haematological studies as its blood coagulation values simulate those of human beings. The maturation of goat spermatozoa during transit through epididymis also resembles that of human beings (Jindal and Panda, 1980). In immunological research and vaccine production goat has few equals. Heamalin (1980) has also advocate their use in mineral and nutrition research as a cheap experimental model for cattle. The results of goat studies can also be applied to human beings as many of physiological parameters of goat are similar to human beings (Jindal, 1984). Since this species comes under the food chain for humans in this country, safety evaluation of transgenic products using goats is desirable.

The safety data via feeding generated on the animal found in local environment would help in the resolution of local concerns about the risk from long-term use of transgenic products, entering directly or indirectly into the human food chain. Safety information generated on animals abundantly available in local environment and closely linked with human habitat is more ensuring to human than the extrapolation of data from distantly related species (Ghosh, 1977).

The present report is an outcome of a 90 day cottonseed (crushed) feeding study in male and female goats.

MATERIALS AND METHODS

Test Material: The two lines of cotton seeds were studied for safety in the present study. Both lines had their Bt and non Bt counter parts as follows:

Tr.1 ó with Bt protein; Cotton line MCU-5 (Test Substance)

Tr.2 ó without Bt protein; Cotton line MCU-5 (Control Substance)

Tr.3 ó with Bt protein; Cotton line LRA (Test Substance)

Tr.4 ó without Bt protein; Cotton line LRA (Control Substance)

Animals and feeding schedule: The 90 day feeding study with crushed cottonseeds was conducted on 48 goats (*Capra hircus*) obtained from the State Animal Husbandry Department of Uttar Pradesh. The Goats were of the Indian Barberi breed in the age group 11.5 to 12.5 months and having body weight range of 14.0 to 19.2 kg. All the goats were acclimated for 15 days and received antihelminthic drugs for removal of endoparasites and drugs for removal of ectoparasites. For the 90 day study, the goats were distributed by the random block design method into four treatment groups consisting of 12 goats (6 male and 6 female), based on sex and body weight. As per the approved protocol, the 90 day feeding study was designed to evaluate any subchronic effects of cottonseed ingested with a standard diet during the active growth phase of the goat. The four treatment groups were designated as Tr.1, Tr.2, Tr.3 and Tr.4 Group. They were fed with one of the cottonseed lines as detailed below:

Tr.1 Group consisted of 6 male and 6 female goats and termed as Tr.1 M & Tr.1 F respectively. These goats were fed with crushed cottonseeds of cotton line MCU-5 having Bt protein, as test substance along with other feed ingredients.

Tr.2 Group consisted of 6 male and 6 female goats and termed as Tr.2 M and Tr.2 F respectively. These goats were fed with crushed cottonseeds of cotton line MCU-5 having Bt protein, as control substance along with other feed ingredients.

Tr.3 Group consisted of 6 male and 6 female goats and termed as Tr.3 M and Tr.3 F respectively. These goats were fed with crushed cottonseeds of cotton line LRA having Bt protein, as test substance, along with other feed ingredients.

Tr.4 Group consisted of 6 male and 6 female goats and termed as Tr.4 M and Tr.4 F respectively. These goats were fed with crushed cottonseeds of cotton line LRA having no Bt protein, as control substance along with other feed ingredients.

Goats were identified with an identification number (I.D.No.) around their neck and with numbered tag fixed into the ear, individually. Another token bearing group colour

viz (Yellow for Tr.1, Blue for Tr.2, Green for Tr.3 and Red for Tr.4) was also tied around the neck of goats according to their grouping (Treatment groups).

Goats belonging to each group were housed separately, each goat had a separate manager in the respective pens, bearing the same I.D. No. on the wall of the pen and feed containers as around their neck and on their ear. Goat pens were constructed with concrete and cement. No bedding material was used inside the goat pens as per protocol. Goats were maintained under strict hygienic veterinary care in their pens at the Gheru Campus of ITRC, Lucknow. The ambient temperature of the goat pens was maintained between 25-30⁰C with the help of hanging electric heaters (during winter) and by desert coolers and ceiling fans during the summer. The relative humidity of goat pens ranged between 50-55% (as per SOP No. 9 for goat husbandry¹). All the goats were provided with 13 hours dark and 11 hours light photo period in summer (March to May 1998) which is essential for normal physiology of goats. The light and dark periods were maintained as described on SOP No. 9. The ambient room temperature, relative humidity and photoperiod duration were recorded daily.

Feed and Water: Goats were offered feed concentrate (having 37 parts cornmeal, 20 parts wheat bran, 12.5 parts cottonseeds, 12.5 parts groundnut cake, 15 parts crushed gram, 0.5 parts salt (sodium chloride) and 2.5 part mineral mixture) and green grass. The feed concentrate itself was 10% of the total feed viz. concentrate and green grass. All goats received 12.5% non-Bt cottonseeds in their feed concentrate right from the day of their arrival at the Gheru Campus till initiation of the pilot Study. Goats were offered feed concentrate as well as green grass (*Cynodon dactylon*) as per their individual body weights. The amount of feed offered to each goat was calculated considering that 17kg adult goat (mean value of recommended 15 to 18 kg body weight of goats in the protocol) consumes 2.7 kg green grass and 300 g feed concentrate. The amount of feed offered to each goat every day was calculated according to their individual body weight. Daily feed uptake was recorded for each goat. The body temperature was also recorded for each goat weekly. The body weight of goats was also recorded weekly by weighing them in an Avery platform balance. All the goats were provided with boiled, cooled and filtered drinking water ad libitum.

Feed analysis: Feed ingredients for one week were prepared by crushing whole cereal grains using a MILCENTØ grinding machine (as per SOP No. 10 for mixing, grinding and blending of feed). Crushed cottonseed samples were daily blended to the feed mix to avoid any rancidity problems. Crushed feed ingredients viz. gram, groundnut cake, corn meal and wheat bran were separately stored in 20 litre plastic drums with lids and marked individually with the name of the ingredients. Feed mix, containing concentrate mixture and crushed cottonseed was prepared daily to avoid rancidity problems. The ingredients were mixed thoroughly with plastic scoops 10 times manually and offered to goats as per their individual body weight daily.

Feed concentrate components and cotton varieties were weekly sent for analysis of crude protein, fat, acid detergent fibre, calcium, sodium, potassium, phosphorous, magnesium, copper, zinc, manganses, iron and vitamins A and E.

Random sample analysis of raw feed mixture was carried out once in a month to test the homogeneity of mixing of feed ingredients and crushed cottonseeds. The average analytical composition of concentrate ration was as under:

Consultants	Tr.1 n=14	Tr.2 n=14	Tr.3 n=14	Tr.4 n=14
Crude Protein (g/100g)	22.980 (8.75-41.86)	22.220 (7.70-39.42)	26.100 (11.29-44.57)	24.190 (12.11-37.62)
Fat(g/100g)	13.500 (8.96-17.59)	16.150 (9.96-20.10)	10.630 (8.28-16.76)	12.290 (8.42-18.76)
Acid Detergent Fibre(%)	54.100 (33.78-66.78)	54.220 (34.99-70.15)	55.070 (29.77-67.73)	53.370 (36.79-67.29)
Vitamin A(mg/g)	BDL	BDL	BDL	BDL
Vitamin E(mg/100g)	51.990 (35.81-75.38)	39.730 (28.09-68.14)	38.950 (28.07-66.18)	40.570 (21.43-76.95)
Phosphorus(mg/100g)	126.420 (82.00-165)	129.510 (88.9-155.9)	129.560 (71.1-186.6)	97.150 (63.7-149.8)

contd í í

Consultants	Tr.1 n=14	Tr.2 n=14	Tr.3 n=14	Tr.4 n=14
Sodium (mg/100g)	93.390 (63.62-295.90)	79.720 (59.6-114.6)	92.680 (59.68-272.14)	76.800 (48.62-124.1)
Potassium(mg/100g)	991.840 (723-1338)	920.040 (676.58-1089.90)	953.050 (693.9-1143.5)	815.360 (354-1105)
Zinc (mg/100g)	0.115 (0-0.249)	0.075 (0.012-0.161)	0.130 (0-0.311)	0.079 (0.012-0.137)
Magnesium (mg/100g)	96.320	87.345	88.610	82.180
Copper(mg/100g)	1.666 (0.025-11.37)	0.589 (0-6.19)	0.220 (0-1.246)	0.389 (0.012-2.435)
Manganese(mg/100g)	0.117 (0.024-0.537)	0.078 (0-0.308)	0.102 (0-1.654)	0.073 (0-0.199)

BDL = Below detection limits

N = 14 weekly samples.

The values are based on one determination only.

The range is given in parenthesis.

Cottonseed used in the feed concentrate of each treatment group was analysed for proximate components and gossypol at several intervals during the 90 day feeding period. Results of these analyses are presented in Apendix 1. There were no meaningful differences in nutrient and toxicant composition between Bt and Non-Bt (Controls) cottonseed for each of the two cultivars.

Water Analysis: Drinking water provided to goats was analysed once a month for drinking water parameters, as specified in the ITRC drinking water analysis protocol. This also included the testing for various pathogens.

Exercise: Goats were provided with 30 minutes exercise daily. During the exercise, goats wore Muffe Masks covering their mouth so that they may not eat any vegetation or foliage material during the course of exercise. Care was taken that male and female groups of goat underwent exercise separately.

Health status of Goats: A veterinary officer of the state animal husbandry department checked the health status of all 48 goats at the time of initiation of the feeding experiment (0 day). In between the course of the feeding study, the same state veterinary officer twice checked the health status of all the 48 experimental goats.

Autopsy of experimental goats: The cottonseed feeding in 24 male goats was initiated on Feb. 12, 1998 and continued till May 12, 1998. Animals were sacrificed on May 13, 1998, however one male goat (I.D.No.27, Tr.2) died on 9.5.98 (86th day of experiment). Similarly, cottonseed feeding in 24 female goats was initiated on Feb. 18, 1998 and continued till May 18, 1998. Animals were sacrificed on May 19, 1998, however one animal (I.D. No.2, Tr.2) died on 6.5.98 (77th day of experiment). At the termination of the 90 day study, male goats were sacrificed humanely under anaesthesia on May 13, 1998 and female goats on May 19, 1998 respectively. Before sacrifice, goats were weighed and anaesthetized by intravenous injection of Penta barbitone (Douglas et al., 1971; UFAW Handbook, 1987; Guide for the Care and Use of Laboratory Animals, 1996). Animals were dissected on an operation table using shadow less lamp. The following organs viz. adrenals, kidneys, testes/ovaries, liver, spleen, stomachs, heart and brain were taken out for observing gross lesions and histopathology and were preserved in buffered formalin (Bancroft and Stevens, 1996). The organ weights of adrenals, heart, liver, gonads (testes or ovaries), brain, kidneys and spleen were also recorded.

Disposal of carcasses: The carcasses of 48 experimental goats were buried in 4 pits, 6 feet deep and 6 feet wide along with solid crystals of sodium chloride and quick lime. The burial site was overlaid with coal tar and fenced with barbed wires (Linton, 1934).

Hematology Studies: The following hematological parameters were carried out at zero, forty five and ninety days, for all the goats in the experiment: (a) total RBC count, (b) Total WBC count, (c) Differential leucocyte count (d) Haemoglobin concentration and (e) Clotting time, assayed (Schalm and Jain, 1975; Maile, 1977).

Clinical biochemistry: The following parameters viz. total serum protein, GOT, GPT, alkaline phosphatase and acid phosphatase were carried out in serum of goats at day zero, forty five and ninety, by \pm Auto Analyser (Boehringer Mannheim) \emptyset . The detailed methodology for the assay of clinical biochemistry was as per standard procedures (Karmen, 1955; Hillman, 1971; Weichoelbaum, 1946).

Histopathological Studies: Processing of tissues (Liver, Lung, Kidney, Heart, all the four Stomachs, Small intestine, Colon, Testes/Ovaries, Adrenals, Spleen, Thymus) of male and female goats was carried out as per standard procedures. Five micron thick sections were stained with haematoxylin and eosin; and with special stains if necessary (Bancroft and Stevens, 1996).

Chemical and Reagents: All chemicals used in this study viz. formaldehyde, dyes, sodium chloride, hydrochloric acid, glacial acetic acid, methanol etc. were of AnalaR Grade. Double distilled water or deionised water was used for making solutions and reagents.

Statistical Analysis: Analysis of variance technique was used to analyse the significant differences in mean values of different haematological and biochemical parameters between different days. The analysis was carried out for each parameter separately. Homogeneity of variance between treatment groups and normality assumption were verified before using analysis of variance. Where the variations were significant, data was log transformed and analysed. Inter group comparisons were done by calculating least significant differences at 5 per cent level of significance, using residual degrees of freedom and residual mean squares. The data is free from sampling and non sampling errors. Raw data were carefully transferred to a computer for statistical calculation.

Data were thrice compared with original raw data on Data sheet/GLP data books for avoidance of any transcription error. The statistical analysis has been carried out in 46 goats excluding the two goats which died 5 days and 14 days before the termination of experiment. These 46 goats were sufficient for statistical calculations and the death of 2 goats did not affect significantly the statistical analysis of data. The statistical analysis was made between Tr.1 and Tr.2 and between Tr.3 and Tr.4 at different time intervals and the days effect within the group.

RESULTS

The male and female goats of all the treatment groups were fed with feed concentrate mixture and green grass as per protocol agreed up on by the sponsor. Daily in life observations of the experimental goats, animal husbandry, feed intake, body weight changes, and body temperature are given in Tables 1-8.

Feed concentrate intake: All the goats consumed almost total feed concentrate offered to them and no concentrate was left. The male goats of Tr.1 group consumed an average of 275.0 g per day as compared to Tr.2 group which consumed 273.6g. The animals of Tr.3 group consumed an average of 284.1 g per day as compared to 285.2 g consumed by Tr.4 group. Similarly, the female goats of Tr. 1 group consumed an average of 257.8 g per day as compared to Tr.2 group which consumed 275.7 g. The animals of Tr.3 group consumed an average of 255.7 g per day as compared to Tr.4 group which consumed 265.1 g (Table 1,5). There were no statistically significant differences in feed concentrate intake between the treatment group comparison (Tr.1 to Tr.2 male; Tr.3 to Tr.4 male; Tr.1 to Tr.2 females; Tr.3 to Tr.4 females).

Roughage (green grass) intake: The weekly average of per day roughage (green grass) intake in male and female goats of all the four treatment groups did not show any significant change (Tables 2,6).

Average weekly body weight gain: The average weekly weight gain for male and female goats showed non-significant increase in four treatment groups of both the sexes (Tables 3,7).

Body temperature measurements: There was no statistically significant change in body temperature of goats in four treatment groups of both the sexes (Tables 4,8).

Other daily observations: Three male goats (I.D. No.9, 26, 27) out of 24 male goats developed diarrhoea; I.D. No. 9 and 26 on 7.4.98; I.D. No. 27 on 5.5.98. They recovered on 14.4.98 and 13.4.98 respectively, but I.D.No. 27 died on 9.5.98. five days before the sacrifice schedule on 13.5.1998. Two female goats (I.D. No. 45, 44) developed diarrhoea on 8.4.98 and 15.4.98 respectively and recovered on 18.4.98 and 25.5.98 respectively. Two goats (I.D. No. 33, 44) were lethargic and sluggish throughout the experimentation. One goat (I.D. No.2) died on 6.5.1998 two weeks before the sacrifice schedule on 19.5.98, as summarized below:

Tr. Group	Sex	I.D. No.	Lethargy		Diarrhoea		Died on
			Affected	Recovered	Affected	Recovered	
Tr.2	M	27	---	---	7.4.98	13.4.98	---
Tr.2	M	27	---	---	5.5.98	---	9.5.98
Tr.3	M	09	---	---	7.4.98	14.4.98	---
Tr.4	M	26	---	---	7.4.98	12.4.98	---
Tr.2	F	33	11.2.98	12.2.98	---	---	---
Tr.2	F	02	11.2.98	12.2.98	---	---	6.5.98
Tr.3	F	45	---	---	8.4.98	18.4.98	---
Tr.4	F	44	11.2.98	12.2.98	---	---	---

The death of two goats was recorded under note to file for feed consumption, growth etc. In-house veterinarian's report on necropsy of two goats has been mentioned in Autopsy finding of goat (Tables 10, 14).

Gross Pathology Observations: At the termination of the study all goats were sacrificed and dissected for gross pathology observations. These observations are

summarized in Table 9 to 16 for all male and female goats in all of the treatment groups. There were no gross pathology observations that were correlated to any of the treatments. The most common observations were excessive fat and watery fluid in the abdominal cavity, congested lungs, and enlarged gallbladder. These observations were made on some of the goats in all of the male and female treatment groups. These gross pathology observations are typical for ruminant animals consuming cottonseed in their diet and are primarily due to gossypol toxicity (Hudson et al., 1988; East et al., 1994). Typical gross pathology observations in ruminants that are associated with gossypol toxicity are increased fluid in the abdominal and thoracic cavities and edema of various tissues that include the gall bladder and ventral subcutaneous tissues.

Hematological Observations : Hematological observations at 0, 45 and 90 days of study are as under: All the treatment groups in male goats showed a higher value of total red blood cell count ($p < 0.05$) at 90 days as compared to 0 day (Table 17). In female goats, Tr2 and Tr.4 displayed a significantly higher value ($p < 0.05$) at 90 days as compared to 9 day (Table 18).

The total white blood cell count showed higher values ($p < 0.01$) in male goats of Tr.1 group as compared to R.2, and 90 days and all the treatment groups showed a significantly lowered value ($p < 0.001$) at 45 days as compared to 0 day (Table 19). In female goats, there was no statistical change (Table 20).

In male goats, the mean values of neutrophil count of Tr.2 group showed an increasing trend as compared to Tr.1 group at 45 and 90 days (Table 21). In female goats, no statistical change was observed (Table 22).

The lymphocyte count in male goats of Tr.1 group showed higher values ($p < 0.05$) at 45 and 90 days as compared to Tr.2 group (Table 23). The female goats showed no change of statistical significance (Table 24). The eosinophil counts and monocyte counts showed no variations of statistical significance in all the treatments groups of both the sexes (Tables 25, 26, 27, 28).

The female goats receiving cottonseed with Bt protein and those receiving cottonseed without Bt protein for 90 days, showed no treatment related changes in gastrointestinal tract (rumen, reticulum omasum, abomasum, small intestine, colon), lungs, heart, adrenals, spleen and thymus. The hepatocytes of animals receiving cottonseed with Bt protein or those receiving cottonseed without Bt protein developed hydropic changes as well as degenerative changes of nuclei (pyknosis, karyorrhexis, karyolysis) or necrosis. The kidneys developed shrinkage of glomerular tuft and desquamative changes of epithelium in some tubules along with presence of hyaline casts in their lumen. The ovaries showed well formed follicles in various stages of development.

Discussion

Cotton lines with or without Bt protein, when fed to four groups of male and female goats (Tr.1 Tr.2, Tr.3 and Tr.4) through their regular diet for 90 days produced similar effects. The increased quantity of fluid in the abdominal and thoracic cavities, enlargement of liver and diarrhoea, observed in the present study are consistent with the reported literature on cottonseed and gossypol toxicity, and are not attributed to the Bt protein as they were observed in both the Bt and non-Bt treatment groups. Moreover, under natural habitat, goats do not deliberately feed on high amounts of cottonseed but also match their feed intake with equal amount of exercise that they are able to do during the day. In the present study, as the goats were fed with appreciable amounts of cottonseed diet and also were confined to their pens for most part of the 24 hour cycle, possibly these animals could not utilize their calories, which may have resulted in deposition of fat in their abdominal cavity. Although there were some gross pathology and histopathological findings among the 48 goats in the feeding study, all the observations were typical for goats exposed to cottonseed containing gossypol in their diet. None of the observations were treatment related, and were observed among goats fed control cotton seed without the Bt protein and among goats fed cottonseed containing the Bt protein. It was shown in a companion study that gossypol levels are equivalent in the Bt and non-Bt cottonseed used in the 90 day feeding study (appendix

1), therefore, the gross pathology and histopathology findings were expected to be equally distributed among the different treatment groups.

Therefore the analysis of data of the 90 days feeding study, as per the protocol, did not show any differences in the response of goats of both sexes exposed to either Bt or non-Bt crushed cottonseed in their diet. It is concluded from safe for animal feed as control cottonseed without the Bt protein. The cottonseeds used in this study were produced by M/S Maharashtra Hybrid Seeds Company Ltd., Mumbai, India.

Acknowledgement: Assistance of Mr. Surendra Singh, Ms. Rashmi Saxena and Ms. Anvia Srivastava for histopathological preparations; of Mr. A.K. Pandey for feed analysis; of Mr. B.M. Pandey, Mr. Hari Ram, Mr. R.B. Singh, Mr. Ram Kumar, Mr. Mohan for maintenance of goats is duly acknowledged. The timely services of scientific and technical personnel of Instrumentation Section and Water Analysis Section is greatly acknowledged. Thanks are also due to Mr. Ram Lal, Mr. Kishan Lal and Mr. M.C. Sharma for photography work.

References

- Atwal, O.S & Sweeny, R.R. (1971) American Journal of Veterinary Research 32: 1990. Bancroft, J.D, and Stevens, A. Theory and Practice of Histological Techniques, Churchill, Livingstone (1996).
- Bartosek, I., Gaitani, A. & Pacei, E. (Eds). Animals in toxicological Research , Raven Press, New York, 181-208 (1982).
- Dougherty, R.S. Journal of American Veterinary Medical Association. Vol. 169, 1121 (1976).
- Douglas, J.S. and Woodnott, D.P. (Eds.) The I.A.T. Manual of Laboratory Animal Practice and Techniques, Charles C. Thomas Publishers, Illinois, pp 231 (1971).
- East, N.E., Anderson, M., Lowenstine, L.J. (1994) Apparent gossypol-included toxicosis in adult dairy goats. J. Am. Vet. Med. Assoc. 204: 642-643.
- Gosh, P.K. (1997). Transgenic Plants and Biosafety Concerns in India. Current Science Vol. 72(3), 172-179.

Guide for the Care and use of Laboratory Animals. (1996).Published by Institute of Laboratory Animal Resources, Commission of Life Sciences. National Research Council U.S.A. pp.66.

Heamalin 1980. Cited from Jindal 1984.

Hillman, G.(1971). Z. Clin. Chem. U. Clin. Biochem., 9,273.

Hudson, L.M., Kerr, L.A., Maslin, W.R. (1988). Gossypol toxicosis in a herd of beef calves. Am. Ver. Med. Assoc. 192: 1303.

Jindal, S.K. Goat Production. Cosmos Publication, New Delhi, 9-10 (1984).

Jindal, S.K. & Panda, J.N. (1980). Andrologia Vol. 12, 328.

Karmen, A. (1955) J. Clin. Invest. 34: 131.

Linton, R.G. Veterinary Hygiene. W. Green and Sons Ltd., Edinbrough, pp 324 (1934). Miale, J.B. Laboratory Medicine Haematology. C.W. Mosby Co. Louis, USA (1977). Pathak, S.K. & Bhowmik, M.K. 1996. Indian Journal of Veterinary Pathology. Vol. 20, 55.

OECD Guidelines for Testing of Chemicals, Section IV Health effects (No.407-409). Organisation for Economic Co-operation and Development, Paris (1993).

Schalm, O.W., Jain, N.C., Carrol, E.J. Veterniary Haematology, Lea and Febiger, Philadelphia, (1975).

Sperlinger, D. (Ed.), Animals in Research: New Peprspective in Animal Experimentation. John Wiley & Sons, (1981).

The UFAW Handbook of the Care of Management of Laboratory Animals. Pool,e T.B. (Ed.), Long man Group U.K Ltd. Pp. 522 (1987).

Weicholbaum, T.E. (1946). Amer. J. Clin. Path., 16, 40.

Table 1: Weekly average of per day concentrate intake (gms) in each male goat of different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	264.2	19.4	264.6	23.7	269.3	23.6	268.3	20.5
2	271.8	19.3	266.4	27.5	273.1	19.6	272.7	24.7
3	265.3	21.7	270.2	25.2	267.7	23.2	270.3	29.4
4	264.8	17.3	267.2	23.4	276.2	33.3	271.7	31.3
5	272.1	24.0	265.8	25.8	283.7	38.2	279.2	31.5
6	276.6	22.7	267.5	29.0	278.3	24.7	283.8	29.4
7	274.8	25.2	264.4	31.3	291.6	35.1	288.5	30.6
8	280.8	25.8	280.6	27.7	292.1	30.2	292.8	29.5
9	280.8	26.8	280.0	30.1	291.3	30.5	288.1	31.4
10	281.2	26.9	281.5	34.5	288.7	29.3	292.8	34.2
11	282.0	25.5	280.3	34.9	289.4	31.0	295.1	35.0
12	280.0	25.3	284.2	37.1	293.0	30.4	299.6	36.0
13	281.1	27.5	284.6*	37.8	299.3	29.3	304.5	37.2
Average	275.0		273.6		284.1		285.2	

* Based on 5 animals as I.D. No. 27 died on 5.5.98

Table 2: Weekly average of per day roughage intake (Kgs) in each male goat of different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	1.423	0.10	1.543	0.14	1.622	0.21	1.468	0.11
2	1.391	0.21	1.359	0.16	1.458	0.24	1.442	0.22
3	1.369	0.26	1.431	0.14	1.407	0.24	1.365	0.32
4	1.403	0.18	1.350	0.06	1.399	0.16	1.337	0.18
5	1.402	0.14	1.417	0.13	1.446	0.11	1.406	0.17
6	1.387	0.13	1.398	0.22	1.483	0.07	1.503	0.14
7	1.476	0.10	1.479	0.11	1.572	0.09	1.459	0.19
8	1.491	0.12	1.534	0.05	1.533	0.03	1.497	0.08
9	1.519	0.07	1.493	0.19	1.559	0.12	1.663	0.17
10	1.542	0.08	1.607	0.11	1.502	0.16	1.648	0.14
11	1.588	0.10	1.697	0.10	1.623	0.09	1.695	0.16
12	1.635	0.13	1.642	0.06	1.676	0.07	1.673	0.04
13	1.600	0.02	1.581*	0.0	1.590	0.02	1.595	0.03
Average	1.479		1.502		1.528		1.519	

* Based on 5 animals as I.D. No. 27 died on 5.5.98

Table 3: Weekly body weight (Kgs) in male goats in different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	15.83	1.26	15.88	1.57	16.03	1.55	15.98	1.49
2	15.78	1.29	15.93	1.54	16.02	1.94	15.90	1.96
3	15.63	1.29	16.03	1.55	16.25	2.14	15.98	1.74
4	15.78	1.31	16.10	1.61	16.46	2.15	16.16	1.73
5	15.95	1.31	16.20	1.62	16.62	2.11	16.41	1.75
6	16.10	1.40	16.28	1.65	16.85	2.01	16.72	1.75
7	16.32	1.44	16.46	1.76	17.07	1.94	17.01	1.80
8	16.37	1.44	16.46	1.81	17.08	1.79	17.11	1.93
9	16.38	1.40	16.55	1.86	17.05	1.74	17.25	1.96
10	16.48	1.38	16.62	1.86	17.11	16.96	17.40	1.97
11	16.53	1.46	16.71	1.90	17.18	1.70	17.52	2.02
12	16.67	1.46	16.85	1.94	17.41	1.63	17.68	2.18
13	16.83	.50	17.78*	1.28	17.65	1.63	18.00	2.27
Average	1.00	0.88	1.38	1.07	1.61	0.67	2.02	1.18

* Based on 5 animals as I.D. No. 27 died on 5.5.98

** Average weight gain over 13 weeks

Table 4: Weekly body temperature ($^{\circ}$ F) in male goats in different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	101.7	0.48	101.3	0.50	102.3	0.45	101.9	0.50
2	101.6	1.29	101.0	0.48	101.9	0.48	101.6	0.48
3	101.5	0.57	101.0	0.61	101.8	0.45	101.7	0.34
4	101.4	0.40	101.2	0.24	100.9	0.18	101.0	0.24
5	101.3	0.30	101.5	0.68	101.0	0.40	101.3	0.64
6	101.0	0.20	101.2	0.30	101.1	0.41	101.1	0.22
7	100.1	0.22	101.1	0.25	101.2	0.51	101.2	0.20
8	100.3	0.22	100.8	0.38	100.8	0.25	100.9	0.51
9	100.8	0.37	100.9	0.34	100.9	0.18	101.0	0.13
10	100.8	0.42	100.9	0.47	100.9	0.25	101.1	0.09
11	101.0	0.24	101.0	0.30	100.9	0.29	100.6	0.24
12	101.1	0.15	100.8	0.00	100.9	0.27	100.8	0.23
13	100.7	0.43	101.0*	0.39	100.8	0.31	100.9	0.19
Average	101.1		101.0		101.2		101.2	

* Based on 5 animals as I.D. No. 27 died on 5.5.98

Table 5: Weekly average of per day concentrate intake (gms) in each female goat of different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	242.1	25.2	261.4	17.9	235.6	19.3	247.0	16.6
2	239.9	31.3	258.5	21.9	231.4	17.6	245.5	14.2
3	243.9	35.7	254.2	31.5	245.0	16.0	248.6	16.4
4	245.8	40.6	275.8	23.6	253.8	13.6	259.3	16.8
5	251.3	35.1	277.6	25.1	258.8	19.2	261.7	19.7
6	257.4	30.2	279.7	26.2	259.6	18.5	265.6	18.7
7	267.7	23.2	279.7	27.9	262.5	17.8	273.2	21.1
8	264.8	23.6	280.0	25.7	259.1	18.5	270.0	21.6
9	264.3	24.0	281.2	24.7	258.3	22.1	271.5	24.6
10	263.8	27.3	286.5	26.2	260.5	20.1	273.7	25.3
11	266.1	25.3	289.2*	25.2	264.0	20.7	276.1	27.6
12	271.3	22.1	280.1*	16.0	266.7	18.2	277.1	27.7
13	273.2	21.6	282.3*	16.7	269.3	21.9	378.4	29.6
Average	257.8		275.7		255.7		265.1	

* Based on 5 animals as I.D. No. 2 died on 6.5.98

Table 6: Weekly average of per day roughage intake (Kgs) in each female goat of different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	1.328	0.19	1.258	0.12	1.223	0.06	1.229	0.07
2	1.273	0.11	1.242	0.08	1.250	0.16	1.187	0.02
3	1.297	0.07	1.331	0.14	1.289	0.07	1.246	0.07
4	1.569	0.14	1.596	0.16	1.335	0.12	1.343	0.10
5	1.565	0.13	1.663	0.17	1.356	0.09	1.404	0.13
6	1.580	0.16	1.664	0.12	1.467	0.14	1.512	0.16
7	1.448	0.10	1.588	0.07	1.411	0.10	1.497	0.13
8	1.452	0.15	1.498	0.12	1.305	0.16	1.464	0.25
9	1.404	0.22	1.571	0.13	1.371	0.25	1.381	0.26
10	1.488	0.18	1.574	0.11	1.524	0.17	1.474	0.23
11	1.578	0.06	1.628*	0.08	1.621	0.07	1.609	0.04
12	1.609	0.05	1.588*	0.02	1.588	0.04	1.588	0.04
13	1.563	0.08	1.623*	0.04	1.605	0.07	1.591	0.03
Average	1.474		1.527		1.411		1.425	

* Based on 5 animals as I.D. No. 2 died on 6.5.98

Table 7: Weekly body weight (Kgs) in female goats in different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	15.01	1.33	15.72	1.51	14.75	0.81	15.01	1.19
2	15.26	1.42	15.72	1.51	14.96	0.81	15.05	0.91
3	15.35	1.37	15.92	1.50	15.01	0.85	15.23	0.96
4	15.41	1.35	16.05	1.53	15.06	0.87	15.38	1.00
5	15.51	1.32	16.20	1.59	15.17	0.91	15.58	1.08
6	15.65	1.26	16.46	1.59	15.21	0.96	15.80	1.17
7	15.71	1.26	16.61	1.51	15.23	0.98	15.88	1.25
8	15.78	1.25	16.78	1.47	15.27	1.00	15.95	1.37
9	15.83	1.24	16.90	1.48	15.37	1.04	16.05	1.44
10	15.87	1.22	16.93	1.46	15.43	1.09	16.13	1.49
11	15.87	1.20	16.42*	0.85	15.56	1.17	16.21	1.63
12	15.95	1.17	16.56*	0.87	15.71	1.26	16.30	1.67
13	16.15	1.15	16.76*	0.87	15.91	1.23	16.47	1.67
Average	1.13	0.91	1.60	0.68	1.16	0.84	1.45	0.95

* Based on 5 animals as I.D. No. 2 died on 6.5.98

** Weogjt gain over 13 weeks

Table 8: Weekly body temperature (°F) in female goats in different treatment groups

WEEKS	Tr1		Tr2		Tr3		Tr4	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
1	101.7	0.49	101.6	0.66	101.4	0.44	101.3	0.15
2	101.3	0.29	101.5	0.60	101.3	0.25	101.0	0.31
3	101.3	0.57	101.2	0.61	100.7	0.31	101.1	0.73
4	101.6	0.58	101.5	0.67	100.7	0.36	100.9	0.46
5	101.3	0.22	101.3	0.58	100.7	0.10	100.9	0.36
6	101.1	0.15	101.6	0.61	101.6	0.30	100.9	0.48
7	100.7	0.39	101.0	0.20	101.0	0.20	101.1	0.30
8	101.0	0.33	100.9	0.39	100.8	0.31	100.6	0.43
9	101.0	0.20	100.8	0.23	100.5	0.43	100.9	0.38
10	100.9	0.14	101.0	0.13	101.0	0.41	101.1	0.15
11	101.0	0.20	100.6*	0.08	101.0	0.20	100.6	0.16
12	100.9	0.22	101.0*	0.19	100.8	0.22	100.8	0.15
13	100.9	0.36	100.9*	0.27	101.0	0.37	100.9	0.40
Average	101.1		101.2		100.9		101.0	

* Based on 5 animals as I.D. No. 2 died on 6.5.98

Table 9 : Autopsy findings in male goats (Tr.3) fed with cottonseed containing Bt protein

I.D.No.		Observations
7	--	Excessive fluid in abdominal cavity.
10	--	Presence of watery fluid in the abdominal cavity.
13	--	Presence of watery fluid in the abdominal cavity and thoracic cavity
15	--	Nothing abnormal Detected (NAD).
22	--	Watery fluid in the thoracic cavity
25	--	NAD.

Table 10 : Autopsy findings in male goats (Tr.4) fed with cottonseed without containing Bt protein

I.D.No.		Observations
8	--	Excessive fat in abdominal cavity enlarged gall bladder
12	--	Excessive fat in abdominal cavity, enlarged gall bladder
18	--	Liver showed nutmeg appearance on cut surface. Presence of fat in abdominal
24	--	Excessive fat in abdominal cavity.
27	--	Diarrhoea, intestine congested and filled with watery fluid. Died 9.5.98, 5 days before completion of feeding study.
28	--	NAD

Table 11 : Autopsy findings in male goats (Tr.3) fed with cottonseed containing Bt protein

I.D.No.		Observations
9	--	Excessive fat in abdomen, one adrenal hypertrophied, another hypotrophied.
13	--	Rumen contained semisolid food material.
17	--	NAD
19	--	Rumen filled with semisolid food material.
20	--	Watery fluid in abdominal cavity. Mesentric lymph nodes prominent Only one testis present.
29	--	Watery fluid in abdominal cavity.

Table 12 : Autopsy findings in male goats (Tr.4) fed with cottonseed without containing Bt protein

I.D.No.		Observations
14	--	Excessive fat in abdominal cavity.
16	--	NAD
21	--	Excessive fat in abdominal cavity.
26	--	NAD
30	--	Excessive fat in abdominal cavity.

Table 13 : Autopsy findings in female goats (Tr.1) fed with cottonseed containing Bt protein

I.D.No.		Observations
1	--	Excessive fat in abdominal cavity.
6	--	NAD
39	--	Watery fluid in abdominal cavity.
41	--	NAD
46	--	NAD
48	--	Excessive fat in abdominal cavity.

Table 14 : Autopsy findings in female goats (Tr.2) fed with cottonseed without containing Bt protein : Control

I.D.No.		Observations
2	--	Lung congested, gall bladder congested, Rumen failed with pungent fluid material, No food left over, omasum impacted with food abomasums empty mucous layer congested, small intestine failed with food Smelling fluid material. Died on 6.5.98, two weeks before completion of feeding study.
3	--	NAD
4	--	Small intestine and colon contained semisolid food material.
33	--	Excessive fat in abdominal cavity.
35	--	Watery fluid in abdominal cavity Mesentric lymph node slightly enlarged.
36	--	NAD

Table 15 : Autopsy findings in female goats (Tr.3) fed with cottonseed containing Bt protein

I.D.No.		Observations
32	--	Excessive fat in abdominal cavity.
34	--	Semisolid food material in reumen
38	--	NAD
42	--	Excessive fat in abdominal cavity.
43	--	Fluid in abdominal cavity.
45	--	NAD

Table 16 : Autopsy findings in female goats (Tr.4) fed with cottonseed without containing Bt protein : Control

I.D.No.		Observations
5	--	Fat in abdominal cavity.
31	--	Fat in abdominal cavity. Gall blader enlarged
37	--	NAD
40	--	NAD
44	--	NAD
47	--	NAD

Table 17 : RBC counts in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	5.26 \pm 1.77	6	6.94 \pm 1.02	6	8.32 \pm 1.22
II	6	5.70 \pm 1.53	6	6.18 \pm 1.28	5*	7.77 \pm 1.60
III	6	5.85 \pm 1.27	6	6.74 \pm 0.68	6	7.74 \pm 1.32
IV	3 ⁺	6.53 \pm 0.75	6	7.23 \pm 0.72	6	7.95 \pm 1.15

RBC counts expressed as million/cu mm

*goat died; ⁺sample spoilt

Table 18 : RBC counts in female goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	7.82 \pm 1.82	6	8.40 \pm 0.90	6	9.42 \pm 1.80
II	6	7.01 \pm 1.55	6	8.17 \pm 0.69	5*	8.76 \pm 2.06
III	6	8.24 \pm 1.31	6	8.87 \pm 1.19	6	9.65 \pm 0.98
IV	6	6.53 \pm 0.75	6	7.48 \pm 0.83	6	9.15 \pm 1.38

RBC counts expressed as million/cu mm

*goat died; + sample spoilt

Table 19 : WBC counts in female goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	13.23 \pm 1.40	6	10.85 \pm 1.99	6	11.32 \pm 1.90
II	6	11.75 \pm 2.02	6	9.37 \pm 1.30	5*	9.82 \pm 1.07
III	6	11.27 \pm 1.23	6	8.87 \pm 1.19	6	9.65 \pm 0.98
IV	3 ⁺	11.95 \pm 0.51	6	9.58 \pm 1.27	6	9.51 \pm 1.70

WBC counts expressed as thousand/cu mm

*goat died; + sample spoilt

Table 20 : WBC counts in female goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	13.58 \pm 2.97	6	11.89 \pm 2.63	6	11.24 \pm 1.48
II	6	11.42 \pm 2.56	6	9.79 \pm 1.58	5*	8.84 \pm 1.96
III	6	12.11 \pm 2.21	6	10.09 \pm 1.70	6	8.97 \pm 1.28
IV	6	15.19 \pm 2.21	6	11.83 \pm 1.93	6	9.53 \pm 1.45

WBC counts expressed as thousand/cu mm

*goat died; + sample spoilt

Table 21 : Neutrophil counts in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean ± SD	N	45 Mean ± SD	N	Mean± SD
I	6	28.2±2.1	6	31.3±3.8	6	29.8±5.1
II	6	29.2±2.5	6	35.5±2.4	5*	37.8±2.6
III	6	32.3±1.7	6	35.6±3.5	6	34.8±2.9
IV	6	33.2±2.4	6	36.6±5.5	6	34.8±2.2

DLC values expressed as percentage of total leucocyte count

*goat died

Table 22 : Neutrophil counts in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean ± SD	N	45 Mean ± SD	N	Mean± SD
I	6	28.3±3.4	6	32.8±4.7	6	32.3±4.4
II	6	33.3±3.8	6	36.8±4.2	5*	35.4±3.9
III	6	32.8±3.1	6	32.2±2.5	6	34.5±2.2
IV	6	28.0±2.5	6	29.6±3.1	6	31.0±3.1

DLC values expressed as percentage of total leucocyte count

*goat died

Table 23 : Lymphocyte counts in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	65.5±2.9	6	63.8±3.2	6	65.3±5.0
II	6	65.0±2.3	6	59.5±2.2	5*	58.0±2.2
III	6	62.5±2.3	6	59.5±2.6	6	60.7±2.5
IV	6	61.5±1.5	6	59.5±3.6	6	60.8±1.8

DLC values expressed as percentage of total leucocyte count

*goat died

Table 24 : Lymphocyte counts in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	65.8±4.3	6	62.5±5.5	6	62.8±4.1
II	6	65.0±3.7	6	58.2±4.2	5*	60.6±3.3
III	6	62.7±3.6	6	63.0±2.5	6	61.7±2.5
IV	6	66.5±3.1	6	65.5±2.9	6	64.8±3.5

DLC values expressed as percentage of total leucocyte count

*goat died

Table 25 : Eosinophil counts in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	4.3±1.1	6	3.3±0.9	6	3.3±0.7
II	6	4.2±0.7	6	3.8±0.9	5*	2.8±0.7
III	6	3.5±0.7	6	3.5±1.3	6	2.8±0.9
IV	6	3.8±0.7	6	3.2±1.8	6	2.8±0.9

DLC values expressed as percentage of total leucocyte count

*goat died

Table 26 : Eosinophil counts in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	3.7±0.9	6	3.3±0.4	6	3.3±0.7
II	6	3.8±0.9	6	3.5±1.2	5*	3.2±0.7
III	6	3.3±0.7	6	3.5±0.8	6	2.5±0.5
IV	6	4.2±0.7	6	3.0±1.5	6	3.0±1.1

DLC values expressed as percentage of total leucocyte count

*goat died

Table 27 : Monocyte counts in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	2.0 \pm 0.6	6	1.5 \pm 0.5	6	1.5 \pm 0.5
II	6	1.5 \pm 0.8	6	1.2 \pm 0.7	5*	1.4 \pm 0.8
III	6	1.7 \pm 0.7	6	1.3 \pm 0.5	6	1.7 \pm 0.5
IV	6	1.5 \pm 0.8	6	1.3 \pm 0.7	6	1.5 \pm 0.9

DLC values expressed as percentage of total leucocyte count

*goat died

Table 28 : Monocyte counts in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	1.7 \pm 0.5	6	1.3 \pm 0.5	6	1.5 \pm 0.5
II	6	1.5 \pm 0.5	6	1.2 \pm 0.7	5*	1.4 \pm 0.8
III	6	1.7 \pm 0.7	6	1.3 \pm 0.5	6	1.7 \pm 0.5
IV	6	1.5 \pm 0.8	6	1.3 \pm 0.7	6	1.5 \pm 0.8

DLC values expressed as percentage of total leucocyte count

*goat died

Table 29 : Haemoglobin (Hb) values in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	6.45 \pm 2.03	6	6.43 \pm 1.48	6	5.75 \pm 1.08
II	6	6.65 \pm 1.88	6	5.96 \pm 2.07	5*	6.66 \pm 2.03
III	6	7.32 \pm 1.50	6	8.20 \pm 0.87	6	6.93 \pm 0.91
IV	3 ⁺	7.67 \pm 1.10	6	8.33 \pm 0.91	6	7.25 \pm 1.64

Hb values expressed as g/dl
*goat died; + sample spoilt

Table 29 : Haemoglobin (Hb) values in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean \pm SD	N	45 Mean \pm SD	N	Mean \pm SD
I	6	8.50 \pm 1.22	6	7.71 \pm 1.27	6	7.31 \pm 2.17
II	6	8.55 \pm 1.44	6	6.95 \pm 1.75	5*	6.84 \pm 3.09
III	6	8.76 \pm 1.30	6	8.30 \pm 1.51	6	8.78 \pm 1.51
IV	6	8.07 \pm 0.55	6	7.38 \pm 0.80	6	7.40 \pm 0.71

Hb values expressed as g/dl
*goat died; + sample spoilt

Table 31 : Clothing time in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean ± SD	N	45 Mean ± SD	N	Mean± SD
I	6	4.09±0.14	6	4.92±0.86	6	4.08±0.41
II	6	4.09±0.19	6	3.59±1.03	5*	3.56±0.29
III	6	4.07±0.46	6	3.94±0.92	6	3.32±0.14
IV	6	3.86±0.76	6	4.17±0.44	6	3.48±0.29

Clothing time expressed in minutes.

*goat died

Table 32 : Clothing time in male goats at different days of treatment

Treatment Group	0		Days			90
	N	Mean ± SD	N	45 Mean ± SD	N	Mean± SD
I	6	4.48±0.53	6	4.59±0.21	6	3.21±0.20
II	6	4.27±0.15	6	3.94±0.66	5*	3.32±0.05
III	6	4.45±0.26	6	4.29±0.13	6	3.39±0.14
IV	6	4.47±0.30	6	4.24±0.50	6	3.41±0.38

Clothing time expressed in minutes.

*goat died

Table 33 : Serum Aspartate Aminotransferase (GOT) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	119.0±14.3	6	134.7±45.2	6	82.1±6.3
II	5 ⁺	100.0±16.6	6	104.2±34.7	5*	89.7±13.5
III	5 ⁺	139.0±42.3	6	177.8±45.8	6	94.3±8.6
IV	6	120.0±20.5	6	152.0±46.3	4 ⁺	92.5±10.4

GOT values expressed as U/L

*goat died ; + sample spoilt

Table 34 : Serum Aspartate Aminotransferase (GOT) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	117.7±8.2	6	125.8±14.9	6	140.4±27.9
II	6	117.3±29.7	6	176.0±79.7	5*	159.2±67.5
III	6	102.8±12.0	6	160.3±43.7	6	142.0±46.9
IV	5*	132.2±63.7	6	80.4±21.4	6	112.0± 9.4

GOT values expressed as U/L

*goat died ; + sample spoilt

Table 35 : Serum Alanine Aminotransferase (GPT) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	20.9±6.1	6	19.4±5.9	6	17.9±4.3
II	6	23.9±10.2	5 ⁺	18.3±8.8	5*	19.1±6.8
III	5 ⁺	28.6±12.3	6	17.5±5.6	6	22.0±4.4
IV	6	23.7±5.3	6	20.5±5.5	4 ⁺	18.7±4.8

GPT values expressed as U/L

*goat died ; + sample spoilt

Table 36 : Serum Alanine Aminotransferase (GPT) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean± SD
I	6	22.5±6.3	6	26.3±8.3	6	21.1±2.4
II	6	22.3±4.1	6	22.4±2.2	5*	24.1±1.8
III	6	21.7±11.1	6	24.3±2.6	6	27.4±3.0
IV	5 ⁺	26.1±6.1	3 ⁺	24.1±3.7	6	22.2±1.9

GPT values expressed as U/L

*goat died ; + sample spoilt

Table 37 : Serum Alkaline Phosphatase (ALP) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
I	6	182.0±90.7	6	240.2±117.6	6	221.2±102.9
II	6	317.3±229.7	5 ⁺	473.6±291.7	5*	406.2±205.0
III	5 ⁺	279.4±153.2	6	265.5±222.7	6	300.8±108.3
IV	6	268.8±230.9	6	340.0±263.6	4 ⁺	370.2±305.0

ALP values expressed as U/L

*goat died ; + sample spoilt

Table 38 : Serum Alkaline Phosphatase (ALP) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
I	6	569.3±427.5	6	680.0±663.7	6	501.5±879.2
II	6	351.7±112.3	5 ⁺	218.0±43.9	5*	170.0±53.7
III	5 ⁺	783.8±794.0	6	331.2±323.3	6	676.3±582.3
IV	5 ⁺	221.8±56.9	6	270.7±230.1	6	256.8±218.4

ALP values expressed as U/L

*goat died ; + sample spoilt

Table 39 : Serum total protein (TP) levels in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
I	6	6.4 ± 0.9	6	6.7 ± 0.9	6	6.6 ± 0.8
II	6	5.6 ± 0.7	5 ⁺	6.3 ± 0.9	5*	6.1 ± 0.8
III	5 ⁺	6.2 ± 1.0	6	6.8 ± 0.2	6	6.5 ± 0.2
IV	6	5.5 ± 0.5	6	6.7 ± 0.6	4 ⁺	6.5 ± 0.9

TP values expressed as g/dl
 *goat died ; + sample spoilt

Table 40 : Serum total protein (TP) values in female goats at different days of treatment

Treatment Group	Days					
	0		45		90	
N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
I	6	7.9 ± 0.8	6	8.0 ± 0.5	6	6.8 ± 0.5
II	6	7.6 ± 0.9	5 ⁺	8.0 ± 0.7	5*	6.7 ± 1.1
III	5 ⁺	7.8 ± 0.4	6	5.9 ± 0.6	6	6.9 ± 0.3
IV	6	7.0 ± 1.0	6	7.1 ± 0.8	6	6.9 ± 0.7

TP values expressed as g/dl
 *goat died ; + sample spoilt

Table 41 : Serum Acid Phosphatase (ACP) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
I	6	4.9 ± 1.3	6	2.7 ± 0.2	6	3.8 ± 1.1
II	6	5.1 ± 1.4	5 ⁺	3.1 ± 0.5	5*	3.6 ± 1.2
III	5 ⁺	5.6 ± 1.0	6	3.3 ± 0.3	6	3.9 ± 1.1
IV	6	4.7 ± 0.6	6	0.4 ± 0.6	4 ⁺	3.4 ± 0.6

 ACP values expressed as U/L

*goat died ; + sample spoilt

Table 42 : Serum Acid Phosphatase (ACP) values in male goats at different days of treatment

Treatment Group	Days					
	0		45		90	
N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
I	6	6.9 ± 2.6	6	4.0 ± 0.8	6	3.2 ± 0.8
II	6	6.0 ± 2.6	6	3.9 ± 1.3	5*	2.9 ± 0.5
III	6	9.9 ± 1.2	6	3.1 ± 1.1	6	3.6 ± 0.6
IV	5 ⁺	7.8 ± 2.7	6	4.0 ± 1.3	6	3.7 ± 0.6

 ACP values expressed as U/L

*goat died ; + sample spoilt

REPORT ON THE ANALYSIS OF “Bt-cotton” SEEDS

V.SUBRAMAIAN & Associates
MAHYCO, R and D, KALLAKAL

Cottonseed is a rich source of oil for human consumption and processed cottonseed meal is used for animal feed. The cotton crop is often infested with insect pests such as cotton bollworm and tobacco budworm. One of the ways to control these insect pests is to introduce the *Bacillus thuringiensis* (Bt gene), which produces insecticidal proteins that are toxic to the insect pests. It has been reported that these insecticidal proteins cause no deleterious effect to organisms such as beneficial insects, birds, fish and mammals including humans. Earlier reports indicated that the Bt (insect protected) cotton cultivars are comparatively similar in chemical composition to commercial varieties. However, speculation still exists about the safe use of Bt plant products such as grains. Therefore the chemical composition of Bt cottonseed of two cultivars were evaluated in comparison with that of the parental controls. The levels of protein, oil, ash, carbohydrate, calories and gossypol contents were determined.

MATERIALS AND METHODS

Cottonseed samples of two cultivars, MCU-5 and LRA that are Bt and their parental controls without Bt were selected for the study. The seeds were supplied from MAHYCO Research Farm at Jalna. Seeds were harvested from the field and stored. Seed samples at seven different intervals were used for chemical analysis. Ginned and delinted seeds were used for proximate analysis. Ginned, delinted and dehusked seeds were used for total gossypol analysis. The husk was removed manually and the endosperm (meat) was separated. The whole seed/meat was ground in a coffee grinder to obtain uniform flour particles and used for chemical analysis.

Proximate Analysis.

Proximate analysis (protein, oil, ash, carbohydrate and moisture) of cottonseed was performed using standard analytical methods. Ash content was measured according AOAC method (1990 a). Protein content was estimated by determining the total

Nitrogen according to the Kjeldahl method and the values were multiplied by 6.25 to calculate the total protein (AOAC 1990 b). Oil content was determined by Soxhlet extraction method (AOA 1990 c). Moisture content was determined by loss on drying at 100°C to constant weight as described in AOAC method (1990 d). Carbohydrate was estimated by difference using the fresh weight derived data and the following equation (USDA 1975 a):

$$\% \text{ Carbohydrate} = 100\% (\% \text{ protein} + \% \text{ oil} + \% \text{ ash} + \% \text{ moisture})$$

Calories were calculated using the factors with the fresh weight-derived data and the following equation (USDA 1975 b):

$$\text{Calories (kcal/100g)} = (4 \times \% \text{ protein}) + (9 \times \% \text{ oil}) + (4 \times \% \text{ Carbohydrate})$$

Measurement of Total Gossypol:

Cotton meal samples were extracted with acidified aminopropanol in dimethyl formamide solution. Total gossypol content was determined using the aniline reaction procedure (AOCS 1989). The gossypol levels were corrected for moisture content in the sample. The reference standard, gossypol was obtained from Sigma Chemical Company, U.S.A.

RESULTS AND OBSERVATIONS

Cottonseed is used for animal feed, primarily for cattle, chicken and fish. The oil extracted from the seed is used for human consumption and also for industrial uses. The composition of cottonseed indirectly gives its feed, and food value. Previous studies by other workers indicated that the Bt-protected cotton cultivars were compositionally equivalent to untreated cotton cultivars, and also consistent with the commercial cotton varieties (Berberich et al., 1996).

A study on chemical composition of cottonseed including gossypol content, using MCU-5 and LRA cultivars was made. Statistical analysis of data was not done. The comparative data on Bt and control seeds for total gossypol levels are shown in Table 1. The total gossypol levels in cotton meals from the Bt cotton cultivars were lower than the control in both the cultivars, particularly in the first three stages of seed

sampling (Table 1). A negligible increase was observed in the later sampling stages. These levels were well within the range of 0.39 to 1.70 % total gossypolin cottonseed reported for cotton varieties by earlier workers. From the safety point of view, total gossypol levels in the Bt. Cotton cultivars were comparable to or lower than those of non-Bt controls and conform to earlier reported values (Berberich et al., 1996).

Proximate analysis of major components such as protein, oil, ash, carbohydrate and moisture were determined in Bt and control cottonseed samples. Calorie values were derived by calculation. The results obtained for the two cultivars are shown in Table 2. There were no appreciable difference in values for various constituents except oil content, between Bt and control cottonseeds. Oil content was slightly lower in Bt cottonseed than that of controls in the cultivar MCU-5, but the values were within the normal literature range for commercial cotton varieties. This difference may not represent a meaningful difference in nutritional value of the seed. However, it has reflected in minor a difference in calorie values (Table2). The values for the above constituents conform to the earlier reported values in the literature (Berberich et al., 1996).

The cottonseed lots used to prepare feed for the 90 day goat feeding study were analysed for proximate components and gossypol. Result of these analyses are shown in Table 3. There were no meaningful differences in nutrient and toxicant composition between Bt and non-Bt (control) cottonseed within a cultivar.

CONCLUSIONS

On the basis of our analytical data with two cotton cultivars, it is concluded that the composition of various nutrients in the seeds of the Bt-protected cotton, is equivalent and/or similar to that of parental control plants. Further, no variation was observed in levels of total gossypol, which is an anti-nutritional component, between Bt and control cottonseed. There were no meaningful differences in nutrient and toxicant content of between Bt and non-Bt cottonseed used in the 90 day goat feeding study.

REFERENCES

- AOAC. Ash. Method 923.03 (modified). In Official Methods of Analysis, 15th ed.; Helrich, K., Ed.; Association of Official Analytical Chemists: Arlington, Va, 1990a
- AOAc, Protein (Nx6.25). Methods 955.04C, 979.09 (modified). In Official Methods of Analysis, 15th ed.; Helrich, K., Ed.; Association of official Analytical Chemists, Arlington, VA, 1990b.
- AOAC. Total fat. Method 960.39 (modified). In official Methods of Analysis, 15th ed.; Helrich, K.; Ed.; Association of Official Analytical Chemist; Arlington, Va., 1990c.
- AOAC. Moisture. Methods 926.08, 925.09 (modified). In Official Methods of Analysis, 15th ed.; Helrich, K. Ed.; Association of Official Analytical Chemist; Arlington, Va, 1990d.
- USDA. Composition of Foods (carbohydrates). In Agricultural Handbook 8; U.S. Department of Agriculture; Washington, D.C., 1975a.
- USDA. Composition of Foods (calories). In Agricultural Hand Book 8; U.S. Department of Agriculture; Washington, D.C., 1975b.
- AOCS. Total Gossypol. Method Ba 8-78, In Official Methods and Recommended Practices of the American Oil Chemists Society, 4th Ed; firestone, D. Ed.; American Oil Chemists Society; Champaign, IL, 1989.
- Berberich, S.A.; Ream, J.E.; Jackson, T.L.; Wood, R.; Stipanovic, R.; Harvey, P.; Patzer, S.; Fuchs, R.L. The composition of insect-protected cottonseed is equivalent to that of conventional cottonseed. J. Agric. Food Chem. 1996, 44, 365-371.

Table 1

Total Gossypol Content (%) in Cottonseed Samples at Different Samples at Different Dates of Sampling

Dates of Sampling	Total Gossypol Content (%)*			
	MCU-5		LRA	
	Bt	Control	Bt	Control
07.2.98	1.14	1.52	1.09	1.57
14.2.98	1.31	1.66	1.21	1.46
21.2.98	1.11	1.27	1.39	1.46
28.2.98	1.36	1.19	1.46	1.50
25.4.98	1.20	1.39	1.46	1.44
09.5.98	1.44	1.27	1.38	1.50
16.5.98	1.54	1.21	1.50	1.27

*Moisture-free basis.

Bt.: *Bacillus thuringiensis*.

Table 2
Range and Mean Values of Proximate Analysis in Cottonseed Samples*

Characteristics	MCU-5				LRA			
	Range Mean	Bt Mean	Control Range Mean		Bt Range mean		Coontrol Range	
Protein %	25.2-29.3	26.7	24.9-28.2	26.6	26.9-28.8	27.9	26.8-28.9	27.8
Oil %	19.324.2	21.0	23.3-25.4	24.2	19.3-22.5	20.6	20.8-21.5	21.2
Ash%	2.8-4.3	4.0	3.8-4.2	4.0	3.8-4.7	4.1	3.7-4.1	3.9
Carbohydrates	46.8-50.9	48.3	43.9-47.7	45.2	45.0-49.1	47.4	45.8-48.3	47.2
Calories/100g	479.1-506.9	488.9	500.0-510.8	504.5	477.8-496.7	486.4	489.0-491	490.5
Moisture %	4.7-5.7	5.2	4.6-5.9	5.2	4.6-6.3	5.4	4.5-6.1	5.2

*The values were divided across 7 dates of sampling for the respective treatments. Protein, oil, ash, carbohydrate and calories reported on moisture-free basis. the values are mean of at least two determinations.
Bi-treated: insect-protected plants with *Bacillus thuringiensis*.

Table 3
Proximate Composition and Gossypol Content of Cottonseeds a from insect Protected with Bt and Control Lines

Date of Collection	Treatment No.	Cultivar	Bt/C	Protein%	Oil%	Ash%	Carbohy drates	Calorie/s 100 g	Gossypol%
07.02.98	TR-1	MCU-5	BT	29.3	19.3	4.3	47.1	479.1	1.14
	TR-2	MCU-5	C	25.9	24.7	4.0	45.4	507.2	1.52
	TR-3	LRA	Bt	28.3	19.9	4.1	47.7	483.1	1.09
	TR-4	LRA	C	28.9	21.1	3.9	46.1	489.4	1.57
07.03.98*	TR-1	MCU-5	Bt	25.9	21.1	4.0	49.2	489.4	1.24
	TR-2	MCU-5	C	25.6	23.7	3.9	46.8	502.6	1.23
	TR-3	LRA	Bt	29.9	20.4	4.1	46.5	485.3	1.43
	TR-4	LRA	C	26.2	20.7	3.8	49.3	488.0	1.48
07.03.98*	TR-1	MCU-5	Bt	26.7	19.5	4.0	49.8	481.6	1.54
	TR-2	MCU-5	C	24.9	23.3	4.1	47.7	500.0	1.21
	TR-3	LRA	Bt	28.2	19.6	4.1	48.1	481.7	1.50
	TR-4	LRA	C	27.6	21.0	.8	47.6	490.1	1.27

The values are mean of duplicate determination.

Bt: *Bacillus thuringiensis*

C: Untreated control

*The gossypol values given in 07.03.98 are mean obtained from values from two dates of sampling that were closed to 7.03.98