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Repeated dose (14-day) oral toxicity study by gavage with enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity in Wistar rats Advinus G6596

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STUDY TITLE

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *BACILLUS AMYLOLIQUEFACIENS* CONTAINING AMYLOMALTASE ACTIVITY IN WISTAR RATS

STUDY No: G6596

AUTHOR: Mr. P. M. SATHISH

STUDY COMPLETED ON: 22 December 2009

SPONSOR

DSM FOOD SPECIALTIES
PO BOX 1, 2600 MA DELFT
THE NETHERLANDS

TEST FACILITY

TOXICOLOGY
DEPARTMENT OF SAFETY ASSESSMENT
ADVINUS THERAPEUTICS PRIVATE LIMITED
POST BOX No. 5813, PLOT Nos. 21 & 22
PEENYA II PHASE, BANGALORE - 560 058
INDIA

STATEMENT OF CONFIDENTIALITY

The report contains **confidential** and **proprietary** information of DSM Food Specialties, PO Box 1, 2600 MA Delft, The Netherlands, which will not be disclosed to anyone except the employees of this company or to persons authorised by law or judicial judgement, without an expressed or a written approval of DSM Food Specialties, PO Box 1, 2600 MA Delft, The Netherlands.

STATEMENT OF GLP COMPLIANCE

The Study No.: G6596 was performed according to OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation. This study was conducted in accordance with the standard operating procedures of Advinus Therapeutics Private Limited and the mutually agreed study plan signed by Study Director on 29 June 2009 and Monitoring Scientist on 06 July 2009.

DECLARATION

The Study Director hereby declares that the work was performed under his supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

Mr. P. M. Sathish
Study Director

22 December 2009
Date

QUALITY ASSURANCE STATEMENT

Study No.: G6596, entitled " Repeated dose (14-Day) oral toxicity study by gavage with enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity in Wistar Rats " has been inspected in accordance with the OECD Principles of Good Laboratory Practice for the testing of chemicals as specified by International [C (97) 186/Final] Legislation.

This study was inspected and findings reported to Management and Study Director on the dates shown below:

Inspection Date	Study Phase	Reporting date
	INITIATION PHASE	
24.06.2009	Study plan review	24.06.2009
	IN-LIFE PHASE	
06.07.2009	Acclimatization	13.07.2009
07.07.2009	Body weights, test item preparation and administration as oral gavage, cage change, feed input and sampling for active ingredient analysis.	
21.07.2009	Necropsy and Blood Collection	27.07.2009
	REPORTING PHASE	
09.09.2009 to 16.09.2009	Draft report review	16.09.2009
25.11.2009	Final report review	25.11.2009

Inspections were performed according to the Standard Operating Procedures of the test facility's Quality Assurance Unit. The report was inspected against the approved study plan and pertinent raw data and accurately reflects the raw data.

Date: 22 Dec, 2009.

(Mr. SATISH MURTHY V.)
Head, GLP Section,
Quality Assurance Unit
Advinus Therapeutics Private Limited, Bangalore

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOLS

Alb	Albumin
A.I.	Active Ingredient
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
App	Appendix / Appendices
Approx.	Approximately
APTT	Activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
A/G	Albumin/Globulin ratio
Baso	Basophils
BUN	Blood Urea Nitrogen
Bwt	Body weight
Ca	Calcium
Cl	Chloride
Creat	Creatinine
DLC	Differential Leukocyte Count
Eosi	Eosinophils
EDTA	Ethylene Diamine Tetra Acetic Acid
F	Female
fl	Femtolitre
g	gram
G	Giga
G.	Group
GGT	Gamma Glutamyl Transpeptidase
Glu	Fasting Glucose
Glob	Globulin
H	Height
Hgb	Haemoglobin
Hct	Haematocrit
h	Hours
K	Potassium
Kg	kilogram
L	litre
Lymp	Lymphocytes
M	Male
m	metre
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
mEq	milli Equivalent
mg	milligram
mL	millilitre
micro	microcytes
min	minute
mm	millimetre
mmol	millimole
Mono	Monocytes
NA	Not Applicable
Na	Sodium
NAD	No Abnormality Detected
Neut	Neutrophils
No.	Number
pg	picogram
Pi	Inorganic phosphorus
Plt	Platelets
PT	Prothrombin Time
RBC	Red Blood Corpuscles
rpm	revolutions per minute
Ref.App.	Reference Appendix
s	seconds
SD	Standard Deviation
T	Tera
T.Bil	Total Bilirubin
T.Chol	Total Cholesterol
T.Pro	Total plasma protein
TOS	Total organic solids
U	Units
W	Width
WBC	White Blood Corpuscles
%	Percent
µmol	micromole
°C	Degree Celsius
°F	Degree Fahrenheit
µ	microns

TABLE OF CONTENTS

STUDY TITLE 1

STATEMENT OF CONFIDENTIALITY 2

STATEMENT OF GLP COMPLIANCE 2

DECLARATION 2

QUALITY ASSURANCE STATEMENT **ERROR! BOOKMARK NOT DEFINED.**

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOLS 4

TABLE OF CONTENTS 5

1. STUDY DETAILS 9

2. STUDY PERSONNEL 10

3. SUMMARY 11

4. OBJECTIVE 12

5. STUDY GUIDELINE 12

6. MATERIALS AND METHODS 12

6.1 Materials 12

6.1.1 Test Item Information 12

6.1.2 Test System 13

6.2 Methods 14

6.2.1 Performance of the Test 14

6.2.1.1 Husbandry 14

6.2.1.2 Dose Selection 15

6.2.1.3 Grouping 15

6.2.1.4 Group Allocation and Number of Animals 16

6.2.1.5 Route of Administration and Justification 16

6.2.1.6 Identity of the Test Item 16

6.2.1.7 Justification for the Selection of Vehicle 16

6.2.1.8 Stability of the Test Item 16

6.2.1.9 Test item preparation and administration 17

6.2.1.10 Dose Formulation analysis 17

6.2.1.11 Treatment 18

6.3 Observations 18

6.3.1 Physical Examination, General Clinical Signs and Pre-terminal Deaths 18
6.3.2 Body Weights and Food Intake.....18
6.3.3 Clinical Laboratory Investigations.....18
6.3.4 Pathology 20
6.4 STATISTICAL ANALYSES.....22
7. RESULTS.....23
7.1 Analyses of the Test Item in Dose Solutions 23
7.2 In-Life Data.....23
7.2.1 Physical Examination, General Clinical Signs and Mortality 23
7.2.2 Body Weights and Net Weight Gains.....23
7.2.3 Food Intake 23
7.2.4 Laboratory Investigations 23
7.2.5 Terminal Fasting Body Weights, Organ Weights and Organ Weight Ratios...24
7.2.6 Gross Pathology.....24
7.2.7 Histopathology.....24
8. DISCUSSION.....25
9. CONCLUSION.....25
10. REFERENCES 26
11. ARCHIVING.....28
12. REPORT DISTRIBUTION.....28
13. TABLES 29
TABLE 1. Details of Experimental Layout, Treatment, Clinical Pathology investigations and Sacrifice Schedule 30
TABLE 2. Summary of Physical Examination, General Clinical Signs and Mortality.....31
TABLE 3. Summary of Body Weights (g) 32
TABLE 4. Summary of Cumulative Net Body Weight Gains (g) 33
TABLE 5. Summary of Cagewise Average Food Intake (g/rat/day).....34
TABLE 6. Summary of Haematological Values at Termination – Males 35
TABLE 7. Summary of Haematological Values at Termination – Females.....37

TABLE 8. Summary of Clinical Chemistry Values at Termination – Males 39

TABLE 9. Summary of Clinical Chemistry Values at Termination – Females.....40

TABLE 10. Summary of Terminal Fasting Body Weights and Organ Weights – Males 41

TABLE 11. Summary of Terminal Fasting Body Weights and Organ Weight Ratios – Males 42

TABLE 12. Summary of Terminal Fasting Body Weights and Organ Weights – Females.....43

TABLE 13. Summary of Terminal Fasting Body Weights and Organ Weight Ratios – Females.....44

TABLE 14. Summary of Necropsy Findings.....45

TABLE 15. Summary of Histopathological Findings.....46

14. FIGURES.....47

FIGURE 1. Body Weight and Growth Curves – Males 48

FIGURE 2. Body Weight and Growth Curves – Females.....48

FIGURE 3. Food Consumption Curves – Males 49

FIGURE 4. Food Consumption Curves – Females 49

15. APPENDICES 50

APPENDIX 1. Individual Physical Examination, Clinical Signs and Mortality – Males.....51

APPENDIX 2. Individual Physical Examination, Clinical Signs and Mortality – Females 52

APPENDIX 3. Individual Body Weights (g) - Males.....53

APPENDIX 4. Individual Cumulative Net Body Weight Gains (g) - Males 54

APPENDIX 5. Individual Body Weights (g) - Females.....55

APPENDIX 6. Individual Cumulative Net Body Weight Gains (g) - Females.....56

APPENDIX 7. Cagewise Average Food Intake (g/rat/day).....57

APPENDIX 8. Individual Haematological Values at Termination – Males 58

APPENDIX 9. Individual Haematological Values at Termination – Females.....60

APPENDIX 10. Individual Clinical Chemistry Values at Termination – Males 62

APPENDIX 11. Individual Clinical Chemistry Values at Termination – Females....63

APPENDIX 12. Individual Terminal Fasting Body Weights and Organ Weights – Males.....64

APPENDIX 13. Individual Terminal Fasting Body Weights and Organ Weight Ratios – Males.....65

APPENDIX 14. Individual Terminal Fasting Body Weights and Organ Weights – Females 66

APPENDIX 15. Individual Terminal Fasting Body Weights and Organ Weight Ratios – Females 67

APPENDIX 16. Individual Gross Pathological and Histopathological Findings – Males.....68

APPENDIX 17. Individual Gross Pathological and Histopathological Findings – Females 71

APPENDIX 18. Gavage Preparation Data.....74

APPENDIX 19. Batch Analysis Data.....75

APPENDIX 20. Certificate of Analysis 76

APPENDIX 21. Deviation from the Approved Study Plan.....77

16. ANNEXURES 78

ANNEXURE 1. Contaminant Analysis Report for Bedding Material (Paddy Husk) 79

ANNEXURE 2. Analysis Report - Animal Diet Sample.....81

ANNEXURE 3. Feed Contaminant Analysis Report for Ssniff Rats/Mice Diet - Maintenance Meal.....82

ANNEXURE 4. Analysis Report - Water Sample.....84

ANNEXURE 5. Contaminant Analysis Report for Water Sample.....85

ANNEXURE 6. GLP Certificate – Germany 87

ANNEXURE 7. GLP Certificate – The Netherlands.....88

ANNEXURE 8. GLP Certificate – India 89

1. STUDY DETAILS

Study Title	:	Repeated dose (14-Day) oral toxicity study by gavage with enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity in Wistar Rats
Test Item	:	Enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity
Study Number	:	G6596
Sponsor	:	DSM Food Specialties PO box 1, 2600 MA Delft The Netherlands
Test Facility	:	Toxicology Department of Safety Assessment Advinus Therapeutics Private Limited Post Box No. 5813, Plot Nos. 21 & 22 Peenya II Phase, Bangalore – 560 058 India

Study schedule

• Study initiation date	:	29 June 2009	
• Experimental start date	:	02 July 2009	
• Acclimatisation	:	Start: 02 July 2009	End: 06 July 2009
• Treatment	:	Start: 07 July 2009	End: 20 July 2009
• Date of sacrifice	:	21 July 2009	
• Experiment end date	:	30 July 2009	
• Study completion date	:	22 December 2009	

2. STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name, Responsibility, Section / Department	Function	Sign and Date
Mr. P. M. SATHISH M.Sc. Study Director, Toxicology Department of Safety Assessment	Overall in-charge for the conduct of the study and report preparation	22 December 2009
Dr. RAVI.K. B. M.V.Sc. Technical co-ordinator and Study Veterinarian, Toxicology Department of Safety Assessment	Assistance in conduct of the study, report preparation and Veterinary services	20 DEC 2009
Mr. M.V.SUMAN M.Sc. Assistant, Toxicology Department of Safety Assessment	Assistance in conduct of the study	21 DEC 2009
Mr. M. RAMAKRISHNA BHAT M.Sc. Laboratory Investigations, Laboratory Services Department of Safety Assessment	Analyst – Haematology & Clinical chemistry	21 DEC 2009
Ms. SUSHMA RANGANATH M.Sc. (Agri.) Analytical Chemist Residue/Analytical Section (Resigned on 16.10.2009 - Signed by Study Director on her behalf)	Analytical service	22 December 2009
Dr. NATARAJU G.J. M.V.Sc. Pathologist, Pathology Department of Safety Assessment	Study Pathologist	21 Dec. 2009
Mr. NAVEEN KUMAR.R. M.Sc. Necropsy and Histotechniques, Department of Safety Assessment	Necropsy	21 dec 2009
Mr. N. SHIVANAND B.Com. Data Entry, Data Analysis and Report Compilation, EDP Department of Safety Assessment	Documentation & Report preparation	21 Dec 2009
Mr. JAYARAM.P. M.Sc. Data Analysis, EDP Department of Safety Assessment	Data Analysis	21/12/09

3. SUMMARY

The test item, enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity was tested for its toxicity potential when administered orally for 14 consecutive days to Wistar rats. The test item was dissolved in "MilliQ" water and administered by oral gavage at doses of 100, 300 and 1000 mg TOS /kg bwt/day to low (G2), mid (G3) and high dose (G4) groups of rats, respectively at a volume of 20 mL/kg bwt/day. The rats in the concurrent vehicle control group (G1) received "MilliQ" water (the vehicle) without the test item at a volume of 20 mL/kg bwt/day.

All the groups consisted of 6 male and 6 female rats. The identity of the test item was provided by the Sponsor by a Certificate of Analysis. As per the information provided in the Certificate of Analysis by the Sponsor, the test item preparations at concentrations of 91, 273 and 909 mg/mL and undiluted test item were stable at room temperature for 4 hours and also for 7 days when stored at 4°C.

The dose formulations were analysed for protein content twice i.e., on Days 1 and 8 of the treatment period. Results of analysis showed that the mean concentrations were within ±10 % variation against the nominal concentrations of 5.0, 15.0 and 50.0 mg/mL.

Rats were observed for mortality, clinical signs, physical abnormalities, changes in body weights and food consumption. Clinical pathology investigations (haematology and clinical chemistry) were performed at sacrifice. The rats were subjected to detailed necropsy at terminal sacrifice. The stomachs from the control and high dose groups were subjected to histopathological examination.

The results of the study indicated that the oral administration of test item Enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity to Wistar rats at dose levels of 100, 300 and 1000 mg TOS/kg bwt/day did not cause any effect on general health and growth, body weight and food consumption. No treatment-related changes were observed in the haematology and clinical chemistry parameters, organ weights and organ weight ratios. Gross pathology and histopathology examination did not reveal any treatment related changes.

In view of the results described above, no changes of toxicological significance were noted among the animals that received doses up to 1000 mg TOS/kg bwt/day or 18181.81 mg enzyme preparation /kg bwt/day, this dose level is considered to be the No Observed Effect Level (NOEL) in Wistar rats, under the test conditions employed.

4. OBJECTIVE

The purpose of this repeated dose (14-day) oral toxicity study was to assess the systemic toxicity potential of the test item when administered orally by gavage to Wistar rats and also to determine the doses to be used for subsequent toxicity study.

5. STUDY GUIDELINE

The study was conducted as per OECD 407 for testing of chemicals, "Repeated Dose 28-day Oral Toxicity Study in Rodents" adopted on 03 October 2008 (OECD, 2008).

However, this study was a pre-study which did not include recovery groups for the control and high dose groups. Further, clinical examination and functional observations were not done. Blood collection, haematology, clinical chemistry, gross pathology, organ collection, organ weights and histopathology were done as per the study plan.

6. MATERIALS AND METHODS

6.1 Materials

6.1.1 Test Item Information

(as furnished by the Sponsor)

Test item	:	Enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity
Common Name (active ingredient)	:	Meltamase
Name to be used in the report	:	Enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity
Chemical name (IUPAC)	:	Amylomaltase 2.4.1.25
Batch No.	:	MEG.GRZ.0905
Batch Manufactured by (Name and Address)	:	DSM Nutritional Products Emil-Barell-Str 3 79639 Grenzach-Wyhlen
Supplier (Name and Address)	:	DSM Food Specialties PO box 1, 2600MA Delft The Netherlands

Manufactured Date	:	February 2009
Expiry Date	:	January 2010
Purity to be stated in the report	:	5.50%
Physical appearance	:	Brown liquid
Storage conditions	:	Deep Frozen (-10 to -20ºC)

Note: 1. The test item was received in good condition at the test facility on 17.06.2009
2. Test Item code by Test Facility: D03-08.

6.1.2 Test System

Species	:	Wistar rats - HsdCpb: WU rats conventionally bred (In-house random bred)
Source	:	Toxicology Department of Safety Assessment Advinus Therapeutics Private Limited Bangalore 560 058, India
Justification for the selection of species	:	Rat is the standard rodent species used for toxicity assessment and also recommended by various regulatory authorities.
No. of Groups	:	4 groups: Vehicle control (G1) Low dose (G2) Mid dose (G3) High dose (G4)
No. of rats / group	:	12 rats (6 males + 6 females) Total number of rats - 24 males + 24 females
Date of birth	:	03.06.2009
Age at treatment	:	5 weeks

Mean body weights (g)
Mean ± SD at the start of treatment :

	Males	Females
G1:	100.40 ± 9.43	92.09 ± 7.42
G2:	100.02 ± 8.81	92.00 ± 5.41
G3:	101.18 ± 8.92	92.12 ± 4.48
G4:	100.83 ± 9.55	91.42 ± 4.75

Identification	:	The animals were individually identified by accession numbers, cage cards and turmeric solution as permanent body marking.
Acclimatization	:	After physical examination, for ascertaining good health and suitability for the study, the rats were acclimatized for five days before start of the treatment. Females used in the study were nulliparous and non-pregnant. During acclimatization, animals were temporarily identified using crystal violet solution as body marking.

6.2 Methods

6.2.1 Performance of the Test

6.2.1.1 Husbandry

Room Number

Laboratory Room No. SC-27

Conditions:

Rats were housed under standard laboratory conditions, air conditioned with adequate fresh air supply (12-15 air changes/hour). Environment: with temperature 20-24°C, relative humidity 66 -68%, with 12 hours light and 12 hours dark cycle.

The maximum and minimum temperature and relative humidity in the experimental room was recorded once daily. The relative humidity in the experimental room was calculated daily from dry and wet bulb temperature recordings.

Housing:

Two rats per sex per cage were housed in solid floor sterilized standard polysulfone cages (size: L 425 x B 266 x H 175 mm) with stainless steel top grill having facilities for holding pelletted food and drinking water in polycarbonate bottle with stainless steel sipper tubes.

Bedding:

Steam sterilized clean paddy husk was used as bedding and changed along with the cage at least twice a week.

Contaminant analysis report of the bedding material (paddy husk) is presented in Annexure 1

Diet: *ad libitum*

Ssniff rats/mice pellet food - maintenance meal - manufactured by Ssniff Spezialdiäten GmbH., Ferdinand-Gabriel-Weg 16, D-59494 Söest, Germany.

Analysis and contaminant analysis reports of Ssniff rats/mice diet – maintenance meal are given in Annexures 2 and 3, respectively.

Water: *ad libitum*

Deep bore-well water passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier manufactured by Eureka Forbes Ltd., Mumbai - 400 001, India.

Analysis and contaminant analysis reports of water sample are given in Annexures 4 and 5, respectively.

6.2.1.2 Dose Selection

Three dose levels of 100 mg (G2), 300 mg (G3) and 1000 mg (G4) TOS /kg bwt/day, Since Total Organic Solids (TOS) = 5.50 % (Certificate of Analysis: TOS = Dry Matter-Ash) these levels corresponding to 1818.18, 5454.54 and 18181.81 mg enzyme preparation /kg bwt/day have been selected for the study in consultation with the Sponsor.

In addition to the test doses, a concurrent vehicle control group was included.

6.2.1.3 Grouping

Grouping was done a day prior to initiation of treatment by body weight stratification and distribution as follows: the rats procured for the study were weighed and segregated depending upon body weight ranges. Males in the range of 71-100 g and females in the range of 71-110 g were selected for the study. Rats were randomly distributed to all groups to attain group mean body weights not varying ± 20% for each sex. The rats which were not selected or with extreme body weights were discarded under deep Isoflurane Anesthesia.

6.2.1.4 Group Allocation and Number of Animals

The selected male and female rats were assigned to vehicle control and different treatment groups as shown below:

Group No.	Group	Colour of cage card	Doses in TOS (mg/kg bwt/day)	Concentration (mg TOS /mL)	Dose volume (mL/kg)	No. of rats	Sex	Rat Numbers	
								From	To
G1	Vehicle control	White	0	0	20	6 6	M F	Ri1571 Ri1595	Ri1576 Ri1600
G2	Low dose	Yellow	100	5	20	6 6	M F	Ri1577 Ri1601	Ri1582 Ri1606
G3	Mid dose	Green	300	15	20	6 6	M F	Ri1583 Ri1607	Ri1588 Ri1612
G4	High dose	Pink	1000	50	20	6 6	M F	Ri1589 Ri1613	Ri1594 Ri1618

6.2.1.5 Route of Administration and Justification

Oral by gavage, dose is expressed as mg/kg bwt/day. Oral route was chosen as this is a potential route of human exposure.

6.2.1.6 Identity of the Test Item

The identity of the test item was provided by the study Sponsor by a Certificate of Analysis. The responsibility for the correct identity, stability test results and the purity of the test item rests with the Sponsor.

6.2.1.7 Justification for the Selection of Vehicle

Since the test item is an enzyme in liquid form and based on the experience of previous studies conducted with similar test item at this test facility, “MilliQ" water was chosen as vehicle to prepare the dose solution.

6.2.1.8 Stability of the Test Item

As per the information provided in the Certificate of Analysis by the Sponsor, the test item preparations at concentrations of 91, 273 and 909 mg/mL and undiluted test item were stable at room temperature for 4 hours and also for 7 days when stored at 4°C.

6.2.1.9 Test item preparation and administration

The dose formulations were prepared on the first day of the treatment and at 3 – 4 day intervals thereafter (within the stability period). Refer Appendix 18.

The test item was thawed to room temperature and pH of the test item solution was recorded using pre-calibrated pH meter. The pH of the unfrozen test item solution was adjusted to 6.91 to 6.99 using sodium bicarbonate solution.

The test material enzyme preparation of *Bacillus amyloliquefaciens* containing amylomaltase activity has a Total Organic Solids of 5.50 % and was adjusted to 100%. All the doses were based on the TOS.

To prepare the test item solutions, 22.73 (G2), 68.18 (G3) and 227.27 (G4) mL test item was separately measured and volume was made up to 250.0 mL with "MilliQ" water to get the desired test item concentrations of 5 (90.9), 15 (272.7) and 50 (909.1) mg/mL for low, mid and high dose groups respectively. The dose volume administered to each rat was at an equivolume of 20.0 mL/kg bwt throughout the study.

The prepared dose solutions were made into required number of aliquots, depending on daily requirement. The remaining aliquots were stored at 2 - 8 °C and were used daily. The rats in the vehicle control group were administered "MilliQ" water at a volume of 20.0 mL/kg bwt/day.

The dose volume was calculated for individual animal on the first day of the treatment and was adjusted according to the body weights recorded during subsequent intervals of the treatment period.

The test item volume, volume of the test item prepared and administered varied depending on the body weights of the rats recorded during different intervals of treatment period and was recorded in the raw data.

6.2.1.10 Dose Formulation analysis

Dose solutions prepared on 07 July 2009 (Day 1) and 14 July 2009 (Day 8) were analysed for protein content by Micro-Kjeldahl method. The determination of protein content in enzyme samples was as follows: the total nitrogen content in the sample was determined by digesting the sample with concentrated sulphuric acid and digestion mixture. A known volume of digested sample was distilled by using Micro-Kjeldahl distillation unit and the total nitrogen content was calculated. The protein content was calculated from the total nitrogen content in the sample by multiplying the total nitrogen content with a factor of 6.25.

6.2.1.11 Treatment

The dose solutions were administered orally by gavage to the rats of the specific groups daily at approximately same time each day for 14 consecutive days. Similarly, the vehicle was administered to rats in the vehicle control group for 14 consecutive days.

6.3 Observations

6.3.1 Physical Examination, General Clinical Signs and Pre-terminal Deaths

a. Physical Examination

Physical examination was carried out prior to test item administration on Days 1, 8 and 14 of the treatment period.

b. General Clinical Signs and Mortality

Clinical signs were observed once daily. All animals were observed for morbidity and mortality twice daily or once on holidays.

6.3.2 Body Weights and Food Intake

a. Body weights:

Individual body weights were recorded prior to test item administration (Day 1) and on Days 5, 8, 12 and 14 of the treatment period.

b. Food intake:

Food consumption was monitored on Days 8 and 14. The food output was subtracted from the food input and the resultant value was divided by the number of rats per cage to determine the food intake/rat/week and was divided by the number of days to obtain food consumption g/rat/day.

6.3.3 Clinical Laboratory Investigations

a. Blood collection:

At the end of the treatment period, all the surviving rats were fasted overnight (water allowed) and blood was collected on Day 15 from abdominal aorta under isoflurane anaesthesia. An aliquot of blood was collected in tubes containing 3.2% sodium citrate solution for determination of coagulation parameters and the remaining blood was collected into EDTA and heparinized tubes for haematology and clinical chemistry respectively.

After haematology analysis, whole blood samples were discarded. Following clinical chemistry analysis, the remaining plasma samples were frozen till the data was reviewed by the Study Director.

b. Haematology:

The following haematological parameters were determined using ADVIA 2120 haematology system (Bayer Health Care LLC, USA).

Sl. No.	Parameter	Abbreviations	Units
1	Haematocrit	HCT	L/L
2	Haemoglobin	HGB	g/L
3	Mean Corpuscular Haemoglobin	MCH	pg
4	Mean Corpuscular Haemoglobin Concentration	MCHC	g/L
5	Mean Corpuscular Volume	MCV	fL
6	Mean Platelet Volume	MPV	fL
7	Platelets	Plat	G/L
8	Red Blood Corpuscles	RBC	T/L
9	White Blood Corpuscles	WBC	G/L
10	Absolute and percentage differential leukocyte count #	DLC@	T/L and %

#: Additionally blood smears were prepared from the haematology (K_2EDTA tube) sample. The blood smears were stained by Wright's stain (solution). However these blood smears were not subjected to differential leukocyte count by conventional microscopy and were discarded.

@: Differential Leukocyte parameters and their respective abbreviations are: Neutrophils (Neut), Lymphocytes (Lymph), Monocytes (Mono), Eosinophils (Eos) and Basophils (Baso).

Coagulation:

Blood samples collected for coagulation analysis were centrifuged at 5000 rpm for 5 minutes for separation of plasma. Prothrombin time (PT) and Activated partial thromboplastin time (APTT) analysis were carried out using STart-4 coagulation analyser (Diagnostica stago, 92600 Asnieres, France).

c. Clinical chemistry:

Plasma was separated in a refrigerated centrifuge at 5000 rpm for 10 minutes and analysed using Roche/Hitachi 902 Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) Automatic Analyser for the following parameters:

Sl. No.	Parameter (Abbreviation)	Abbreviations	Units
1	Glucose	Glu	mmol/L
2	Total Bilirubin	T.Bil	µmol/L
3	Creatinine	Creat	µmol/L
4	Inorganic phosphorous	Pi	mmol/L
5	Total plasma protein	T.Pro	g/L
6	Albumin	Alb	g/L
7	Globulin [calculated values]	Glob	g/L
8	Albumin/Globulin ratio	A/G Ratio	-
9	Gamma Glutamyl Transpeptidase	GGT	U/L
10	Blood Urea Nitrogen	BUN	mmol/L
11	Alanine Amino Transferase	ALT	U/L
12	Aspartate Amino Transferase	AST	U/L
13	Alkaline phosphatase	ALP	U/L
14	Total Cholesterol	T.Chol	mmol/L
15	Calcium	Ca	mmol/L
16	Chloride	Cl	mEq/L
17	Sodium	Na	mEq/L
18	Potassium	K	mEq/L

6.3.4 Pathology

a. Necropsy:

All rats in the study were subjected to gross necropsy and the findings were recorded. The rats were fasted overnight (water allowed), anaesthetized with isoflurane, weighed and exsanguinated and were subjected to detailed necropsy.

b. Organ weights:

The following organs were weighed from all animals: thymus, epididymides, brain, heart, adrenals, gonads, spleen, liver and kidneys. The organ weight ratios were determined as percentages of body weights.

c. Tissue collection

On completion of the gross pathology examination the tissues and organs noted below were collected and weighed from all animals. Organ to the body weight ratio (%) was calculated by using the fasting body weight.

The tissues from all rats were preserved in 10% buffered neutral formalin:

Tissue	Organ weight	Collection and preservation	Microscopic examination
All gross lesions		X	X
Brain (Cerebrum, Cerebellum and medulla)	X	X	
Spinal cord (cervical, thoracic and lumbar)		X	
Stomach		X	X
Duodenum		X	
Jejunum		X	
Ileum (with Peyer's patches)		X	
Cecum		X	
Colon		X	
Rectum		X	
Liver	X	X	
Kidneys	X	X	
Adrenals	X	X	
Spleen	X	X	
Heart	X	X	
Thymus	X	X	
Thyroid		X	
Trachea		X	
Lungs #		X	
Testes@	X	X	
Epididymides	X	X	
Ovaries	X	X	
Uterus with cervix	X	X	
Seminal vesicles		X	
Coagulating glands		X	
Prostate		X	
Urinary bladder		X	
Mesenteric lymph nodes		X	
Sciatic nerves		X	
Bone with marrow (Femur)		X	
Mandibular lymph nodes		X	
Bone marrow smear (from Femur)		X	

#: Inflated with fixative and then immersed in formalin.
@: Collected in modified Davidson's fluid
X: Activity carried out.

d. Histopathology

The stomachs from the vehicle control and the high dose groups were subjected to histopathological examination. Since there were no test item related microscopic changes in high dose group, the stomach in remaining groups were not processed for microscopic examination.

The stomach was processed for routine paraffin embedding and 5 micron sections were stained with Mayer's Haematoxylin Eosin stain. Unused tissues were archived.

6.4 STATISTICAL ANALYSES

Results of statistical analysis have been reported in the form of Mean ± SD and sample size.

The statistical analysis of the experimental data were carried out using the in-house developed and validated package in Excel and also using licensed copies of SYSTAT Statistical package ver.12.0. All quantitative variables like body weight, net weight gain, food consumption, clinical pathology investigation (Haematology and Clinical Chemistry) and organ weight data were compared by Bartlett's test for homogeneity of variances within the group before performing a One-factor ANOVA modelling by treatment groups. When the data were found to be non-optimal (non-normal or heteroschedastic), ANOVA were done using suitable transformation. Comparison of means between treatment groups and control group was done using Dunnett's 't' test when the overall treatment, 'F' test is found to be significant.

When a significant difference in values over the control group was observed in a minimum of two treatment groups with linear increase or decrease, the dose correlation co-efficient was estimated and subjected to t-test.

All analyses and comparisons were evaluated at the 5% ($P \leq 0.05$) level. Statistically significant differences ($P \leq 0.05$), indicated by the aforementioned tests were designated by the superscripts throughout the report as stated below:

+/-: Significantly higher (+)/lower (-) than the control group
d: Signifiant dose correlation

7. RESULTS

Details of experimental layout, treatment, clinical laboratory investigations and sacrifice schedule are furnished in Table 1.

7.1 Analyses of the Test Item in Dose Solutions

Refer Appendix 19.

The concentrations of test item in the gavage solutions were determined on Days 1 and 8 of the treatment period based on the protein value of test item. The mean analyzed test item concentrations were with in the acceptance criteria of ± 10 % as against the nominal concentrations of 5.0, 15.0 and 50.0 mg/mL for low, mid and high dose groups respectively.

7.2 In-Life Data

7.2.1 Physical Examination, General Clinical Signs and Mortality

Refer Table 2, Appendices 1 and 2

No clinical signs or mortality were observed at any of the tested dose levels.

7.2.2 Body Weights and Net Weight Gains

Refer Tables 3 and 4, Appendices 3 to 6, Figures 1and 2

No significant changes were observed in the mean body weights and net weight gains at any of the tested doses in either sex.

7.2.3 Food Intake

Refer Table 5, Appendix 7, Figures 3 and 4

No significant changes were observed in the food consumption at any of the tested doses in either sex.

7.2.4 Laboratory Investigations

Haematology: Refer Tables 6 and 7, Appendices 8 and 9

Males and Females:

Haematological investigation did not reveal any significant changes at any of the tested doses except for significantly higher APTT values (males and females) and lower MCHC (females) in the 300 mg/kg group as compared to control group

The increased APTT values in the 300 mg/kg group was considered incidental as there were no significant changes in the other related parameters like prothrombin time, platelets count and liver function parameters (coagulation factors synthesis). The decrease observed in the MCHC at 300 mg/kg group females is also considered incidental as there were no changes in the RBC and Haemoglobin concentration. Moreover, similar changes were not observed at the 1000 mg/kg dose group.

Clinical Chemistry: Refer Tables 8 and 9, Appendices 10 and 11

Males and females:

Biochemical investigation revealed statistically significant higher creatinine and total cholesterol level in the 300 mg/kg group males and lower albumin and A/G ratio in the 1000 mg/kg group females, compared to control group.

The decrease in the albumin and A/G ratio is marginal (about 8 to 12 %) and as there were no changes in the total protein and globulin levels, the observed significance is considered as incidental finding. The increase in creatinine and total cholesterol in the 300 mg/kg group males is considered not treatment related as similar changes were not observed at 1000 mg/kg dose group.

7.2.5 Terminal Fasting Body Weights, Organ Weights and Organ Weight Ratios

Refer Tables 10 to 13, Appendices 12 to 15

There were no significant differences in the terminal fasting body weights, organ weights and organ weight ratios in both males and females.

7.2.6 Gross Pathology

Refer Table 14, Appendices 16 and 17

A single incidence of bilateral dilated pelvis in kidney observed in the 300 mg/kg group female (Ri1609) was microscopically associated with dilatation of pelvis and is considered as incidental finding.

Thus, there were no test item related gross pathological findings in both males and females

7.2.7 Histopathology

Refer Table 15, Appendices 16 & 17

There were no test item related microscopic findings in the stomach of all the males and females.

8. DISCUSSION

The purpose of this Repeated dose (14-day) oral toxicity study was to assess the systemic toxicity potential of the test item when administered orally by gavage to rats and also to determine the doses to be used for subsequent toxicity study.

This study consisted of one control (G1 – 0 mg/kg) and three treatment groups i.e., low (G2 – 100 mg TOS /kg), mid (G3 – 300 mg TOS /kg) and high dose (G4 – 1000 mg TOS /kg). Each group consisted of 6 male and 6 female rats. The control and treated groups were administered “MilliQ" water and test item mixed solutions respectively by oral gavage once daily for 14 consecutive days.

Animals from all the groups were observed for clinical signs, physical abnormalities, changes in body weights and food consumption. Laboratory investigations on haematology and clinical chemistry were performed at sacrifice. The rats were subjected to detailed necropsy at terminal sacrifice. Stomachs from the control and high dose groups were subjected to histopathological examination.

There were no treatment-related findings in daily observation, body weights, food consumption, haematology, clinical chemistry, terminal fasting body weights, organ weights and their ratios and gross and histopathology.

9. CONCLUSION

In view of the results described above, no changes of toxicological significance were noted among the animals that received doses up to 1000 mg TOS/kg bwt/day or 18181.81 mg enzyme preparation /kg bwt/day, this dose level is considered to be the No Observed Effect Level (NOEL) in Wistar rats, under the test conditions employed.

10. REFERENCES

1. OECD Principles of Good Laboratory Practice (as revised in 1997), Environmental Directorate, Organisation for Economic Co-operation and Development, Paris 1998.

2. OECD Guideline No. 407, Repeated Dose 28-day Oral Toxicity Study in Rodents" adopted on 3 October 2008 (OECD, 2008).

3. Levene H. 1960. Robust tests for equality of variances. In: Olkin I, Ghurye SG, Hoeffding W, Madow WG, Mann HB, editors. Contributions to probability and statistics. Stanford (CA): Stanford University Press. P 278-292.

4. Non Parametric Statistics in Behavioral Science Sidney Seigel and N.J.Castallan, McGraw Hill Publishing.

5. Shapiro SS, Wilk MB. 1965. An analysis of variance test for normality (complete samples). Biometrika 52(3-4): 591-611.

6. Glucose (Glu) mmol/l:

GOD-POD method: Trinder. P., Ann Clin Biochem., 6:24, 1969.

2. Blood Urea Nitrogen (BUN) mmol/l:

Urease-GLDH method: Tiffany et al., Clin Chem., 18:829, 1972.

3. Total Plasma Protein (Tot.Pro.) g/l:

Biuret method: Doumas et al., Clin Chem., 27:1642, 1981.

4. Aspartate Amino transferase (AST) U/l:

Infinity AST reagent based on recommendations of IFCC : IFCC method for AST J Clin Chem Clin Biochem., 24:497-510, 1986.

5. Alanine Amino transferase (ALT) U/l:

Infinity ALT reagent based on recommendations of IFCC : IFCC Expert panel on enzymes part 3 J Clin Chem Clin Biochem., 24:481-495, 1986.

6. Gamma Glutamyl Transpeptidase (GGT) U/l:

Szasz G., Persijn JP., et al.,: New substrates for measuring γ-glutamyl-transpeptidase activity J Clin Chem Clin Biochem., 12:228, 1973.

7. Total Bilirubin (Tot. Bil) µmol/l:

Walters MI., Gerade HW: An ultramicro method for the determination of conjugated and total bilirubin in serum or plasma; Microchem J., 15:231, 1970.

8. Creatinine (Creat) µmol/l:

Jaffe's kinetic method: Fabing DL, Ertingshausen G: Automated reaction rate method for determination of serum Creatinine with the "Centrifichem"; Clin Chem., 17:696, 1971.

9.Chloride (Cl) mmol/L:

Tietz NW. Clinical guide to Laboratory Tests. Philadelpian Pa: WB Saunders Co; 1983 : 110, 398, 446. Kaplan L, Pesce A.Clinical Chemistry theory, analysis and correlation. St. Louis, Mo:CV Mosby Co; 1984:1061, 1077.

10. Albumin (Alb) g/l:

Bromocresol-green method: Doumas B.T., et al.; In standard methods of Clin Chem Vol 7,(175-189), 1972 Academic Press, Chicago, USA

11. Inorganic Phosphorus (Pi) mmol/l:

Molybdate Method: Daly JA, Ertingshausen G; Direct method for determining inorganic phosphorus in serum with the "Centrifichem"; Clin Chem., 18:263, 1972.

12. Total Cholesterol (Chol) mmol/l:

CHOD-POD method: Allain CC, PoonLS, Chan CSG, Richmond W and Fu PC; Clin Chem., 20:470-475, 1974; Roeschlau P, Bernt E and Gruber WA., J Clin Chem Clin Biochem., 12:226, 1974.

13. Sodium (Na): mEq/l:

Using ion sensitive electrode principle (indirect potentiometry).

14. Potassium (K): mEq/l:

Using ion sensitive electrode principle (indirect potentiometry).

11. ARCHIVING

Advinus will archive at the archives of the test facility the following for 15 years after completion of the study: study plan, raw data, draft and final reports. A sample of the test item was sent to the archives from the test item stores before the first dispensing. This sample shall be stored for a period of 2 years from the date of this final report or till next GLP inspection, whichever is later, however not beyond 15 years. All tissue specimens will be archived for 5 years, blocks and slides will be archived for 12 years after which these will be handed over to the Sponsor or preserved longer at the cost of the sponsor.

12. REPORT DISTRIBUTION

Sponsor:	One signed final report in original (Copy No. 1/2) and a soft copy in PDF format.
Archives:	One signed final report in original (Copy No. 2/2).

13. TABLES

TABLE 1. Details of Experimental Layout, Treatment, Clinical Pathology investigations and Sacrifice Schedule

Group No.	Dose (mg TOS/kg bwt/day)	No. of rats per group		Treatment period (days)	Laboratory investigations		Pathology		Sacrifice (15^{th} day)
		Males	Females		Haematology	Clinical Chemistry	Gross pathology and Organ weights	Histopathology of stomachs	
G1	0	6	6	14	+	+	+	+	+
G2	100	6	6	14	+	+	+	-	+
G3	300	6	6	14	+	+	+	-	+
G4	1000	6	6	14	+	+	+	+	+

+: Yes -: Not done

TABLE 2. Summary of Physical Examination, General Clinical Signs and Mortality

Refer Appendix: 1 and 2

PARAMETERS	Sex	Males				Females			
	Group No.	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (mgTOS/kg bwt/day)	0	100	300	1000	0	100	300	1000
	No. of rats	6	6	6	6	6	6	6	6
1. GENERAL AFFECTIONS		0	0	0	0	0	0	0	0
2. NEUROLOGICAL AFFECTIONS		0	0	0	0	0	0	0	0
3. RESPIRATORY AFFECTIONS		0	0	0	0	0	0	0	0
4. EYE AFFECTIONS		0	0	0	0	0	0	0	0
5. GASTRO INTESTINAL AFFECTIONS		0	0	0	0	0	0	0	0
6. SKIN AFFECTIONS		0	0	0	0	0	0	0	0
7. UROGENITAL AFFECTIONS		0	0	0	0	0	0	0	0
8. MORTALITY		0	0	0	0	0	0	0	0

TABLE 3. Summary of Body Weights (g)

Refer Appendix: 3 and 5

Group Dose (mg TOS/kg bwt/day)		Days 1	5	8	12	14
		Males				
G1 0	Mean	100.40	123.88	139.93	161.98	173.61
	SD	9.43	10.75	12.17	14.69	17.06
	N	6	6	6	6	6
G2 100	Mean	100.02	124.32	141.06	159.59	170.92
	SD	8.81	10.43	12.94	16.70	17.62
	N	6	6	6	6	6
G3 300	Mean	101.18	125.28	141.07	162.54	175.38
	SD	8.92	11.81	12.00	15.06	15.35
	N	6	6	6	6	6
G4 1000	Mean	100.83	123.06	140.24	160.26	164.45
	SD	9.55	13.48	15.91	20.65	20.51
	N	6	6	6	6	6
		Females				
G1 0	Mean	92.09	106.97	118.79	129.01	134.66
	SD	7.42	7.66	9.79	11.87	12.12
	N	6	6	6	6	6
G2 100	Mean	92.00	106.71	116.84	127.13	131.62
	SD	5.41	6.03	6.65	6.87	8.11
	N	6	6	6	6	6
G3 300	Mean	92.12	105.87	116.41	128.39	131.48
	SD	4.48	3.96	3.17	3.65	4.85
	N	6	6	6	6	6
G4 1000	Mean	91.42	107.22	115.99	126.10	132.01
	SD	4.75	7.03	6.28	6.77	7.36
	N	6	6	6	6	6

TABLE 4. Summary of Cumulative Net Body Weight Gains (g)

Refer Appendix: 4 and 6

Group Dose (mg TOS/kg bwt/day)		Days			
		5	8	12	14
		Males			
G1	Mean	23.48	39.53	61.58	73.21
0	SD	4.08	5.25	7.90	9.62
	N	6	6	6	6
G2	Mean	24.30	41.03	59.57	70.90
100	SD	2.62	5.18	9.91	10.64
	N	6	6	6	6
G3	Mean	24.09	39.88	61.36	74.20
300	SD	4.18	5.22	9.13	8.84
	N	6	6	6	6
G4	Mean	22.24	39.42	59.44	63.62
1000	SD	5.10	8.76	13.32	13.22
	N	6	6	6	6
		Females			
G1	Mean	14.88	26.71	36.92	42.58
0	SD	2.25	6.18	9.06	9.44
	N	6	6	6	6
G2	Mean	14.71	24.84	35.13	39.62
100	SD	1.74	2.46	2.61	4.03
	N	6	6	6	6
G3	Mean	13.75	24.30	36.27	39.36
300	SD	2.22	2.63	5.22	5.55
	N	6	6	6	6
G4	Mean	15.80	24.57	34.68	40.59
1000	SD	4.71	4.88	5.33	6.72
	N	6	6	6	6

TABLE 5. Summary of Cagewise Average Food Intake (g/rat/day)

Refer Appendix: 7

Group Dose (mg TOS/kg bwt/day)		Weeks 1	Weeks 2
		Males	
G1 0	Mean	16.98	18.82
	SD	0.40	0.59
	N	3	3
G2 100	Mean	17.45	19.27
	SD	0.59	1.08
	N	3	3
G3 300	Mean	16.81	19.07
	SD	0.31	0.56
	N	3	3
G4 1000	Mean	16.85	18.50
	SD	1.57	2.38
	N	3	3
		Females	
G1 0	Mean	14.25	14.45
	SD	0.72	1.34
	N	3	3
G2 100	Mean	14.32	14.42
	SD	0.21	0.18
	N	3	3
G3 300	Mean	14.18	14.49
	SD	0.23	0.23
	N	3	3
G4 1000	Mean	13.81	15.15
	SD	0.52	1.13
	N	3	3

TABLE 6. Summary of Haematological Values at Termination – Males

Refer Appendix: 8

Group No. Dose (mg TOS/kg bwt/day)		WBC G/L	RBC T/L	HGB g/L	HCT L/L	MCV fL	MCH pg	MCHC g/L	MPV fL	Plat G/L	PT s	APTT s
G1	Mean	6.37	7.39	141.17	0.444	60.23	19.13	317.50	12.08	1441.50	17.02	7.27
0	SD	1.19	0.23	5.56	0.011	1.95	0.55	7.87	0.30	81.07	2.19	1.96
	N	6	6	6	6	6	6	6	6	6	6	6
G2	Mean	5.90	7.31	143.17	0.439	60.00	19.62	327.00	10.82	1605.83	16.30	6.62
100	SD	1.91	0.26	3.92	0.018	1.47	0.22	9.01	1.29	85.39	0.28	1.88
	N	6	6	6	6	6	6	6	6	6	6	6
G3	Mean	5.98	7.33	144.17	0.454	62.00	19.72	318.00	11.15	1597.00	16.97	9.85 +
300	SD	1.73	0.30	4.62	0.019	2.48	0.64	6.81	0.90	68.85	0.23	1.65
	N	6	6	6	6	6	6	6	6	6	6	6
G4	Mean	7.77	7.44	144.83	0.449	60.40	19.48	322.33	11.68	1558.50	16.38	8.17
1000	SD	2.09	0.27	2.48	0.013	0.74	0.47	5.24	0.79	166.14	0.76	1.14
	N	6	6	6	6	6	6	6	6	6	6	6

+: Significantly higher than the control group

TABLE 6 contd. Summary of Haematological Values at Termination – Males

Refer Appendix: 8

Group No. Dose (mg TOS/kg bwt/day)		Neut %	Lymp %	Mono %	Eosi %	Baso %	Abs Neut G/L	Abs Lymp G/L	Abs Mono G/L	Abs Eosi G/L	Abs Baso G/L
G1	Mean	14.80	79.93	2.78	1.07	0.40	0.93	5.10	0.18	0.07	0.03
0	SD	2.47	2.71	0.72	1.01	0.13	0.13	1.09	0.06	0.06	0.01
	N	6	6	6	6	6	6	6	6	6	6
G2	Mean	11.48	83.80	2.70	0.73	0.37	0.67	4.96	0.16	0.04	0.02
100	SD	3.96	3.98	0.72	0.39	0.16	0.29	1.67	0.07	0.02	0.01
	N	6	6	6	6	6	6	6	6	6	6
G3	Mean	12.55	81.92	3.08	1.03	0.37	0.74	4.90	0.19	0.06	0.02
300	SD	1.61	1.00	0.78	0.64	0.08	0.20	1.46	0.09	0.02	0.01
	N	6	6	6	6	6	6	6	6	6	6
G4	Mean	13.48	81.13	3.00	0.82	0.40	1.00	6.34	0.24	0.06	0.03
1000	SD	6.99	6.92	0.86	0.28	0.11	0.52	1.99	0.13	0.01	0.02
	N	6	6	6	6	6	6	6	6	6	6

TABLE 7. Summary of Haematological Values at Termination – Females

Refer Appendix: 9

Group No. Dose (mg TOS/kg bwt/day)		WBC G/L	RBC T/L	HGB g/L	HCT L/L	MCV fL	MCH pg	MCHC g/L	MPV fL	Plat G/L	PT s	APTT s
G1	Mean	5.07	7.77	149.83	0.459	59.17	19.33	326.50	11.70	1592.00	15.68	6.23
0	SD	1.92	0.39	5.19	0.009	2.13	0.46	6.28	0.47	229.53	0.60	0.64
	N	6	6	6	6	6	6	6	6	6	6	6
G2	Mean	4.13	7.86	147.00	0.464	59.05	18.72	316.67	11.62	1510.17	15.10	5.88
100	SD	0.83	0.24	2.97	0.005	1.88	0.63	5.85	0.26	133.17	0.36	0.77
	N	6	6	6	6	6	6	6	6	6	6	6
G3	Mean	4.68	7.73	144.17	0.460	59.52	18.65	313.50 -	11.67	1480.17	15.10	11.32 +
300	SD	1.83	0.40	6.59	0.030	1.79	0.52	7.71	0.53	198.69	2.32	2.65
	N	6	6	6	6	6	6	6	6	6	6	6
G4	Mean	4.47	7.79	149.33	0.471	60.50	19.20	317.33	11.72	1543.67	16.20	7.28
1000	SD	1.13	0.41	6.98	0.030	2.18	0.53	8.59	0.60	210.05	1.55	1.79
	N	6	6	6	6	6	6	6	6	6	6	6

+/-:Significantly higher (+)/lower (-) than the control group

TABLE 7 contd. Summary of Haematological Values at Termination – Females

Refer Appendix: 9

Group No. Dose (mg TOS/kg bwt/day)		Neut %	Lymp %	Mono %	Eosi %	Baso %	Abs Neut G/L	Abs Lymp G/L	Abs Mono G/L	Abs Eosi G/L	Abs Baso G/L
G1	Mean	13.80	81.35	2.63	0.85	0.35	0.76	4.07	0.14	0.04	0.02
0	SD	6.64	6.58	0.88	0.24	0.10	0.66	1.32	0.06	0.01	0.01
	N	6	6	6	6	6	6	6	6	6	6
G2	Mean	12.53	82.93	2.13	1.13	0.35	0.50	3.44	0.09	0.04	0.02
100	SD	3.50	3.45	0.36	0.38	0.19	0.12	0.77	0.03	0.01	0.01
	N	6	6	6	6	6	6	6	6	6	6
G3	Mean	17.10	77.33	3.12	1.17	0.43	0.92	3.49	0.16	0.06	0.02
300	SD	8.50	9.37	0.91	0.56	0.21	0.91	0.82	0.10	0.04	0.01
	N	6	6	6	6	6	6	6	6	6	6
G4	Mean	13.65	81.42	2.68	1.00	0.35	0.60	3.66	0.11	0.04	0.02
1000	SD	4.49	4.77	1.20	0.55	0.05	0.23	1.06	0.04	0.02	0.01
	N	6	6	6	6	6	6	6	6	6	6

TABLE 8. Summary of Clinical Chemistry Values at Termination – Males

Refer Appendix: 10

Group No. Dose (mg TOS/kg bwt/day)		Glu mmol/L	BUN mmol/L	T.Pro g/L	AST U/L	ALT U/L	ALP U/L	GGT* U/L	T.Bil µmol/L	Creat µmol/L	Alb g/L	Pi mmol/L	Ca mmol/L	T.Chol mmol/L	Glob g/L	A/G	Na mEq/L	K mEq/L	Cl mEq/L
G1	Mean	8.04	5.73	60.17	76.17	39.17	209.33	1.50	3.63	13.50	39.15	2.99	2.70	1.81	21.02	1.87	138.27	4.21	89.82
0	SD	1.63	0.64	2.07	8.91	10.28	21.68	1.05	0.41	2.59	1.15	0.18	0.04	0.17	1.54	0.13	2.07	0.63	1.53
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
G2	Mean	8.54	6.19	60.55	69.67	36.00	202.83	1.17	3.67	15.50	39.35	2.98	2.68	1.84	21.20	1.86	139.25	4.07	90.08
100	SD	0.57	0.69	1.84	6.56	7.82	16.87	1.17	0.52	3.39	0.94	0.30	0.10	0.31	1.20	0.09	1.85	0.40	1.15
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
G3	Mean	8.09	5.75	62.07	77.50	38.17	229.00	1.50	3.59	20.17 +	39.35	2.95	2.76	2.33 +	22.72	1.75	140.38	4.03	91.42
300	SD	0.71	0.88	1.54	9.52	5.78	36.13	1.22	0.61	4.62	2.19	0.22	0.07	0.20	1.78	0.22	1.77	0.25	1.78
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
G4	Mean	7.55	5.62	59.85	66.50	35.67	196.17	1.83	3.53	13.83	37.50	3.00	2.65	1.89	22.35	1.70	138.92	4.28	91.30
1000	SD	0.61	1.34	1.86	5.86	7.37	23.81	0.98	0.56	2.64	1.46	0.23	0.05	0.38	2.33	0.22	1.18	0.19	1.09
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

+: Significantly higher than the control group

*: GGT: Lower Limit of Quantification (LLOQ): 3U/l. Not considered for Statistical Analysis.

TABLE 9. Summary of Clinical Chemistry Values at Termination – Females

Refer Appendix: 11

Group No. Dose (mg TOS/kg bwt/day)		Glu mmol/L	BUN mmol/L	T.Pro g/L	AST U/L	ALT U/L	ALP U/L	GGT* U/L	T.Bil µmol/L	Creat µmol/L	Alb g/L	Pi mmol/L	Ca mmol/L	T.Chol mmol/L	Glob g/L	A/G	Na mEq/L	K mEq/L	Cl mEq/L
G1	Mean	8.18	5.50	63.47	68.83	33.67	144.33	0.67	3.41	16.50	42.32	2.47	2.65	1.85	21.15	2.01	140.57	4.11	94.02
0	SD	0.82	1.06	1.81	6.05	6.77	34.35	0.82	0.46	1.64	1.52	0.28	0.06	0.27	1.12	0.14	0.94	0.30	1.19
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
G2	Mean	7.64	6.20	61.35	69.50	37.50	138.33	1.50	3.23	16.33	40.80	2.52	2.66	2.10	20.55	1.99	139.60	3.60	91.92
100	SD	0.90	0.78	1.38	3.27	5.39	15.44	0.84	0.28	3.50	1.63	0.07	0.03	0.23	1.07	0.17	0.80	0.31	1.70
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
G3	Mean	8.19	5.38	62.02	67.83	36.33	139.50	1.50	3.27	17.00	40.37	2.58	2.60	1.78	21.65	1.87	140.22	4.07	92.78
300	SD	0.95	1.16	1.72	5.23	4.03	11.62	0.84	0.30	2.19	1.61	0.25	0.06	0.26	0.81	0.10	1.36	0.56	1.88
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
G4	Mean	8.59	5.85	61.12	67.50	30.67	149.67	1.83	3.07	16.50	38.97 -	2.63	2.60	2.13	22.15	1.76 -	140.22	3.98	93.53
1000	SD	0.80	1.47	2.47	2.88	4.89	14.49	0.75	0.43	3.39	1.94	0.16	0.04	0.39	1.43	0.14	0.88	0.34	1.38
	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6

-:Significantly lower than the control group

*: GGT: Lower Limit of Quantification (LLOQ): 3U/l. Not considered for Statistical Analysis.

TABLE 10. Summary of Terminal Fasting Body Weights and Organ Weights – Males

Refer Appendix: 12

Group No. Dose (mg TOS/kg bwt/day)		Fasting Bwt (g)	Organ weights (g)								
			Adrenals	Testes	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Epididym
G1	Mean	166.81	0.059	2.102	1.482	6.332	0.646	0.542	0.662	1.886	0.339
0	SD	13.53	0.006	0.342	0.191	0.778	0.088	0.091	0.052	0.086	0.064
	N	6	6	6	6	6	6	6	6	6	6
G2	Mean	163.31	0.060	2.164	1.533	6.344	0.622	0.545	0.650	1.857	0.304
100	SD	16.56	0.008	0.216	0.204	0.794	0.058	0.122	0.065	0.098	0.060
	N	6	6	6	6	6	6	6	6	6	6
G3	Mean	168.05	0.062	2.234	1.570	6.770	0.624	0.600	0.695	1.839	0.373
300	SD	15.37	0.010	0.377	0.217	1.309	0.126	0.137	0.056	0.041	0.092
	N	6	6	6	6	6	6	6	6	6	6
G4	Mean	161.62	0.058	2.108	1.415	5.909	0.597	0.557	0.631	1.802	0.320
1000	SD	19.97	0.003	0.451	0.189	1.063	0.088	0.175	0.072	0.030	0.048
	N	6	6	6	6	6	6	6	6	6	6

TABLE 11. Summary of Terminal Fasting Body Weights and Organ Weight Ratios – Males

Refer Appendix: 13

Group No. Dose (mg TOS/kg bwt/day)		Fasting Bwt (g)	Organ weight ratios (%)								
			Adrenals	Testes	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Epididym
G1	Mean	166.81	0.035	1.254	0.887	3.802	0.387	0.324	0.397	1.136	0.203
0	SD	13.53	0.001	0.112	0.056	0.443	0.044	0.033	0.017	0.100	0.031
	N	6	6	6	6	6	6	6	6	6	6
G2	Mean	163.31	0.037	1.327	0.937	3.884	0.382	0.332	0.399	1.145	0.185
100	SD	16.56	0.005	0.077	0.046	0.275	0.029	0.052	0.025	0.109	0.028
	N	6	6	6	6	6	6	6	6	6	6
G3	Mean	168.05	0.037	1.322	0.932	4.005	0.371	0.354	0.414	1.103	0.220
300	SD	15.37	0.004	0.118	0.065	0.472	0.059	0.057	0.019	0.120	0.038
	N	6	6	6	6	6	6	6	6	6	6
G4	Mean	161.62	0.037	1.293	0.875	3.639	0.370	0.339	0.391	1.129	0.198
1000	SD	19.97	0.005	0.127	0.024	0.222	0.032	0.063	0.015	0.134	0.016
	N	6	6	6	6	6	6	6	6	6	6

TABLE 12. Summary of Terminal Fasting Body Weights and Organ Weights – Females

Refer Appendix: 14

Group No. Dose (mg TOS/kg bwt/day)		Fasting Bwt (g)	Organ weights (g)								
			Adrenals	Ovaries	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Uterus*
G1	Mean	127.82	0.071	0.073	1.265	4.815	0.468	0.357	0.564	1.758	0.419
0	SD	11.24	0.009	0.013	0.056	0.513	0.059	0.043	0.039	0.115	0.053
	N	6	6	6	6	6	6	6	6	6	6
G2	Mean	126.12	0.067	0.078	1.163	4.646	0.416	0.328	0.545	1.737	0.333
100	SD	7.88	0.009	0.016	0.106	0.375	0.065	0.035	0.035	0.072	0.061
	N	6	6	6	6	6	6	6	6	6	6
G3	Mean	126.8	0.059	0.073	1.214	4.827	0.411	0.354	0.563	1.735	0.363
300	SD	2.31	0.004	0.012	0.104	0.203	0.049	0.021	0.029	0.101	0.080
	N	6	6	6	6	6	6	6	6	6	6
G4	Mean	125.07	0.066	0.080	1.224	4.649	0.419	0.357	0.513	1.753	0.437
1000	SD	5.54	0.007	0.018	0.104	0.228	0.071	0.029	0.036	0.083	0.136
	N	6	6	6	6	6	6	6	6	6	6

*: With cervix

TABLE 13. Summary of Terminal Fasting Body Weights and Organ Weight Ratios – Females

Refer Appendix: 15

Group No. Dose (mg TOS/kg bwt/day)		Fasting Bwt (g)	Organ weight ratios (%)								
			Adrenals	Ovaries	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Uterus*
G1	Mean	127.82	0.056	0.058	0.994	3.765	0.366	0.279	0.443	1.379	0.333
0	SD	11.24	0.006	0.012	0.054	0.191	0.031	0.015	0.035	0.079	0.068
	N	6	6	6	6	6	6	6	6	6	6
G2	Mean	126.12	0.053	0.062	0.924	3.683	0.330	0.260	0.432	1.381	0.264
100	SD	7.88	0.005	0.011	0.084	0.183	0.045	0.016	0.019	0.090	0.044
	N	6	6	6	6	6	6	6	6	6	6
G3	Mean	126.8	0.047	0.057	0.957	3.808	0.324	0.279	0.444	1.368	0.286
300	SD	2.31	0.004	0.009	0.078	0.167	0.038	0.014	0.024	0.074	0.060
	N	6	6	6	6	6	6	6	6	6	6
G4	Mean	125.07	0.053	0.064	0.978	3.717	0.334	0.286	0.410	1.403	0.349
1000	SD	5.54	0.008	0.016	0.062	0.087	0.045	0.019	0.027	0.055	0.108
	N	6	6	6	6	6	6	6	6	6	6

*: With cervix

TABLE 14. Summary of Necropsy Findings

Refer Appendix: 16 and 17

PARAMETERS	Sex	Males				Females			
	Group. No.	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (mg TOS/kg bwt/day)	0	100	300	1000	0	100	300	1000
	No. of rats	6	6	6	6	6	6	6	6
1. No. dead during treatment		0	0	0	0	0	0	0	0
2. No. moribund sacrificed		0	0	0	0	0	0	0	0
3. No. terminally sacrificed		6	6	6	6	6	6	6	6
4. No. examined for gross pathology		6	6	6	6	6	6	6	6
5. No. showing gross pathology		0	0	0	0	0	0	1	0
A. No. showing external pathology		0	0	0	0	0	0	0	0
B. No. showing visceral organ pathology		0	0	0	0	0	0	1	0
1. Kidney bilateral – pelvis dilated		0	0	0	0	0	0	1	0

TABLE 15. Summary of Histopathological Findings

Number in ( ): No. of tissues evaluated/group

Refer Appendix: 16 and 17

TISSUE AND OBSERVATION	Sex	MALES				FEMALES			
	Group No.	G1	G2	G3	G4	G1	G2	G3	G4
	Doses in TOS (mg TOS/kg bwt/day)	0	100	300	1000	0	100	300	1000
	No. of rats	6	6	6	6	6	6	6	6
	No. of rats examined	6	0	0	6	6	0	1	6
1. STOMACH		(6)	(-)	(-)	(6)	(6)	(-)	(-)	(6)
Tissue present no change		6	-	-	6	6	-	-	6
2. KIDNEYS		(-)	(-)	(-)	(-)	(-)	(-)	(1)	(-)
Dilated pelvis		-	-	-	-	-	-	1	-

14. FIGURES

FIGURE 1. Body Weight and Growth Curves – Males

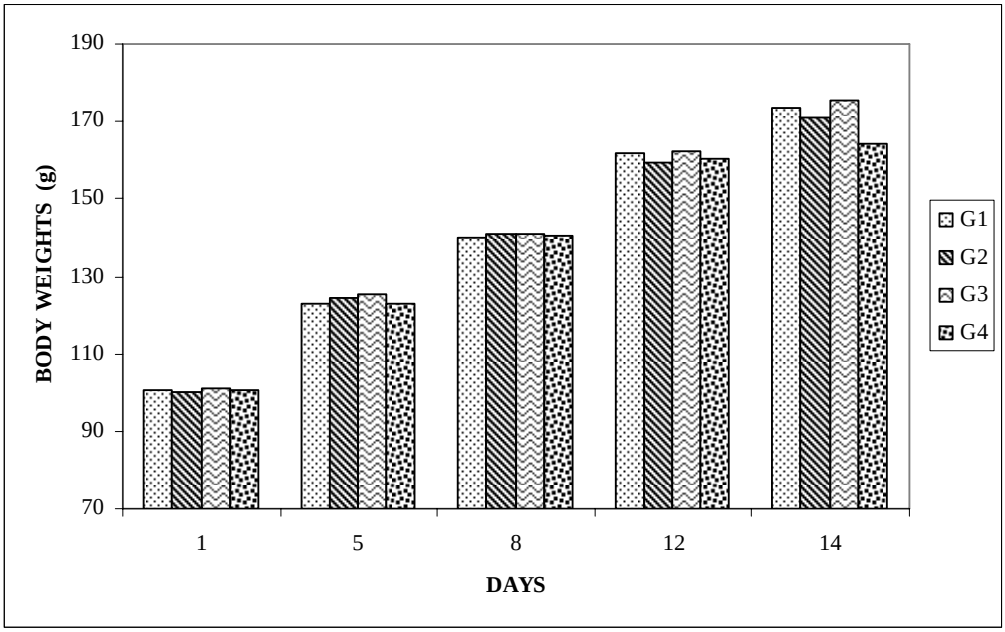


FIGURE 2. Body Weight and Growth Curves – Females

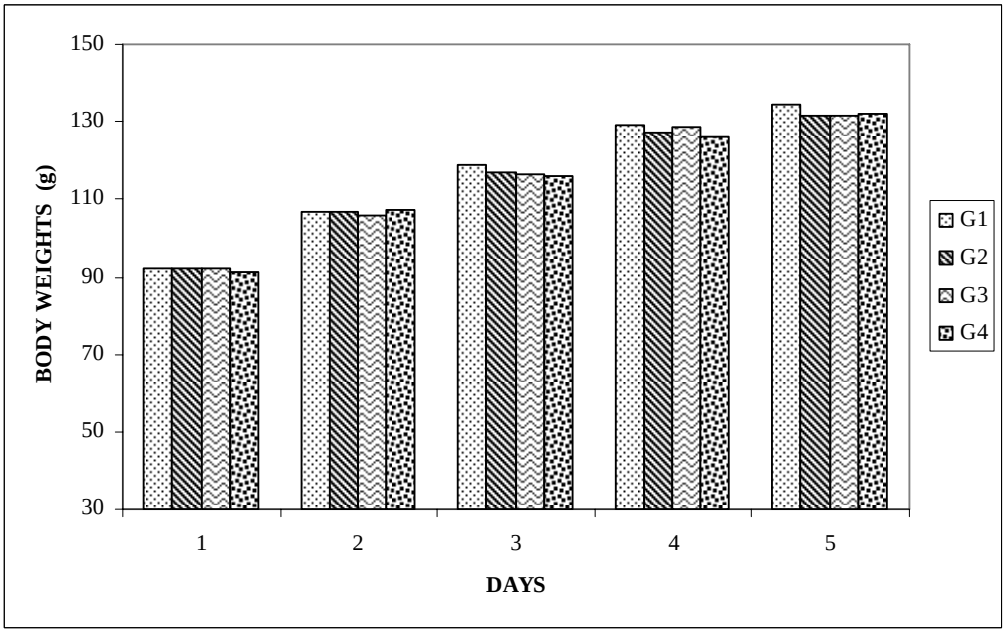


FIGURE 3. Food Consumption Curves – Males

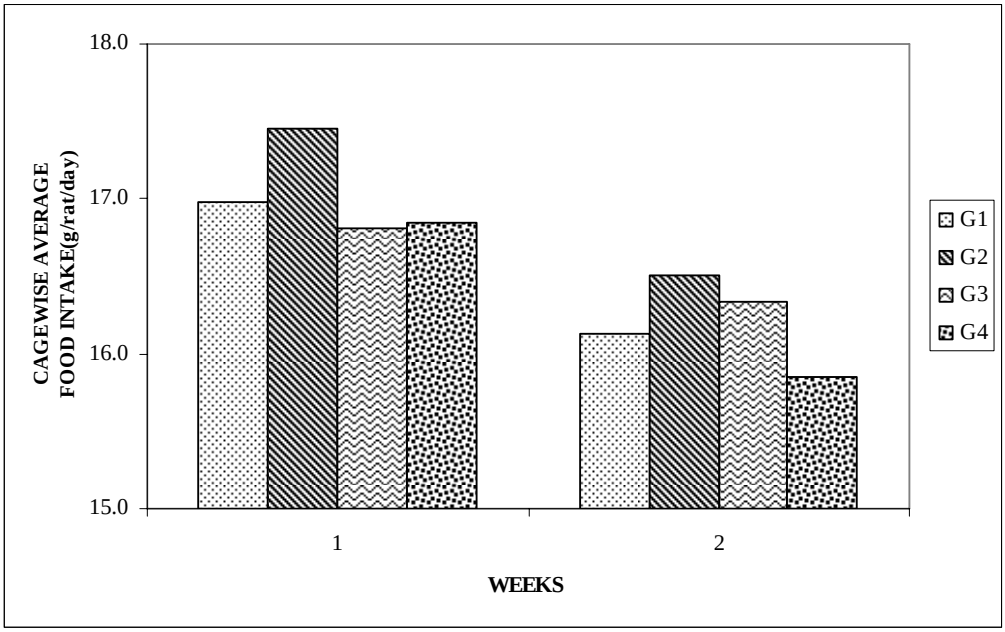
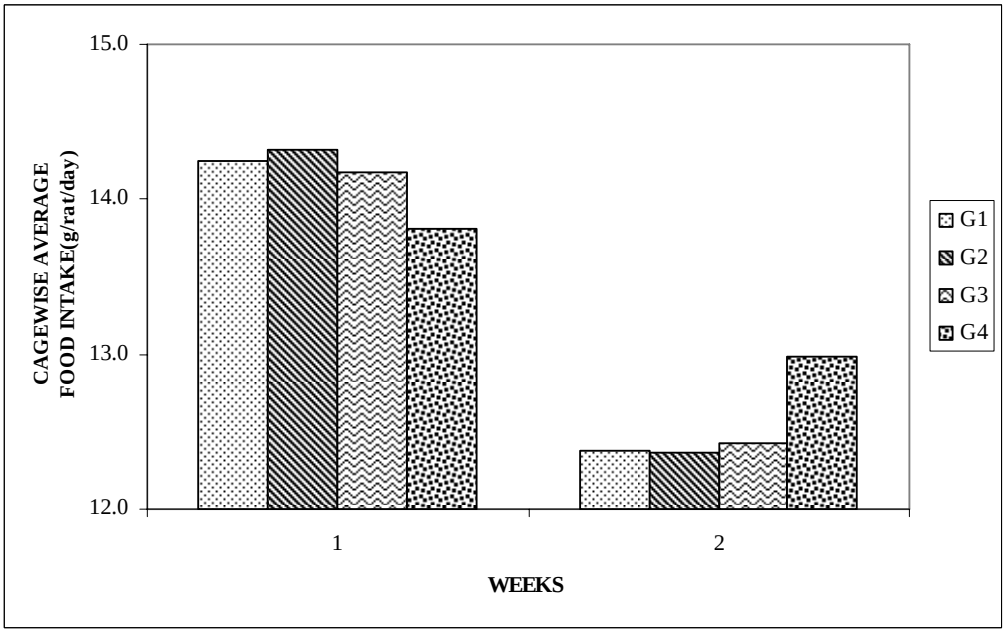


FIGURE 4. Food Consumption Curves – Females



15. APPENDICES

APPENDIX 1. Individual Physical Examination, Clinical Signs and Mortality – Males

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Physical Examination and Clinical Signs
G1	Ri1571	NAD
0	Ri1572	NAD
	Ri1573	NAD
	Ri1574	NAD
	Ri1575	NAD
	Ri1576	NAD
G2	Ri1577	NAD
100	Ri1578	NAD
	Ri1579	NAD
	Ri1580	NAD
	Ri1581	NAD
	Ri1582	NAD
G3	Ri1583	NAD
300	Ri1584	NAD
	Ri1585	NAD
	Ri1586	NAD
	Ri1587	NAD
	Ri1588	NAD
G4	Ri1589	NAD
1000	Ri1590	NAD
	Ri1591	NAD
	Ri1592	NAD
	Ri1593	NAD
	Ri1594	NAD

NAD: No Abnormality Detected

Note: Physical examination carried out during acclimatization period for all animals did not reveal any abnormalities.

APPENDIX 2. Individual Physical Examination, Clinical Signs and Mortality – Females

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Physical Examination and Clinical Signs
G1	Ri1595	NAD
0	Ri1596	NAD
	Ri1597	NAD
	Ri1598	NAD
	Ri1599	NAD
	Ri1600	NAD
G2	Ri1601	NAD
100	Ri1602	NAD
	Ri1603	NAD
	Ri1604	NAD
	Ri1605	NAD
	Ri1606	NAD
G3	Ri1607	NAD
300	Ri1608	NAD
	Ri1609	NAD
	Ri1610	NAD
	Ri1611	NAD
	Ri1612	NAD
G4	Ri1613	NAD
1000	Ri1614	NAD
	Ri1615	NAD
	Ri1616	NAD
	Ri1617	NAD
	Ri1618	NAD

NAD: No Abnormality Detected

Note: Physical examination carried out during acclimatization period for all animals did not reveal any abnormalities.

APPENDIX 3. Individual Body Weights (g) - Males

Group Dose (mg TOS/kg bwt/day)	Rat No.	Days				
		1	5	8	12	14
G1	Ri1571	105.21	130.51	149.81	177.81	189.42
0	Ri1572	82.45	106.31	120.25	139.59	147.55
	Ri1573	98.90	123.48	137.27	160.26	169.45
	Ri1574	109.14	136.70	150.31	175.91	190.69
	Ri1575	104.68	128.60	149.30	166.68	182.61
	Ri1576	102.03	117.66	132.66	151.61	161.94
G2	Ri1577	107.81	131.43	150.01	167.28	180.43
100	Ri1578	92.02	117.24	132.56	147.79	159.54
	Ri1579	86.60	107.84	121.85	139.50	148.91
	Ri1580	106.12	131.52	151.53	173.93	187.85
	Ri1581	106.75	135.15	154.46	180.93	190.77
	Ri1582	100.83	122.75	135.92	148.12	158.01
G3	Ri1583	87.02	104.61	120.54	135.35	146.91
300	Ri1584	108.80	137.06	154.98	177.51	191.12
	Ri1585	101.52	129.34	144.91	172.82	180.81
	Ri1586	94.32	118.47	134.85	158.39	172.76
	Ri1587	105.93	131.82	147.96	169.38	184.51
	Ri1588	109.51	130.35	143.15	161.81	176.19
G4	Ri1589	84.52	103.38	120.11	134.58	143.48
1000	Ri1590	103.39	130.74	154.14	178.93	160.32
	Ri1591	111.16	140.70	161.34	188.30	200.33
	Ri1592	108.22	129.46	143.93	163.47	174.04
	Ri1593	96.19	112.70	126.73	144.09	148.82
	Ri1594	101.47	121.40	135.21	152.19	159.70

APPENDIX 4. Individual Cumulative Net Body Weight Gains (g) - Males

Group Dose (mg TOS/kg bwt/day)	Rat No.	Days			
		5	8	12	14
G1	Ri1571	25.30	44.60	72.60	84.21
0	Ri1572	23.86	37.80	57.14	65.10
	Ri1573	24.58	38.37	61.36	70.55
	Ri1574	27.56	41.17	66.77	81.55
	Ri1575	23.92	44.62	62.00	77.93
	Ri1576	15.63	30.63	49.58	59.91
G2	Ri1577	23.62	42.20	59.47	72.62
100	Ri1578	25.22	40.54	55.77	67.52
	Ri1579	21.24	35.25	52.90	62.31
	Ri1580	25.40	45.41	67.81	81.73
	Ri1581	28.40	47.71	74.18	84.02
	Ri1582	21.92	35.09	47.29	57.18
G3	Ri1583	17.59	33.52	48.33	59.89
300	Ri1584	28.26	46.18	68.71	82.32
	Ri1585	27.82	43.39	71.30	79.29
	Ri1586	24.15	40.53	64.07	78.44
	Ri1587	25.89	42.03	63.45	78.58
	Ri1588	20.84	33.64	52.30	66.68
G4	Ri1589	18.86	35.59	50.06	58.96
1000	Ri1590	27.35	50.75	75.54	56.93
	Ri1591	29.54	50.18	77.14	89.17
	Ri1592	21.24	35.71	55.25	65.82
	Ri1593	16.51	30.54	47.90	52.63
	Ri1594	19.93	33.74	50.72	58.23

APPENDIX 5. Individual Body Weights (g) - Females

Group Dose (mg TOS/kg bwt/day)	Rat No.	Days				
		1	5	8	12	14
G1	Ri1595	96.94	111.38	123.82	134.07	138.60
0	Ri1596	91.25	104.89	110.80	117.79	123.90
	Ri1597	93.66	105.03	114.50	121.05	125.97
	Ri1598	80.03	95.49	106.36	117.26	122.90
	Ri1599	88.91	106.48	125.21	138.95	145.34
	Ri1600	101.73	118.54	132.07	144.91	151.26
G2	Ri1601	87.11	100.57	112.64	122.82	126.11
100	Ri1602	99.34	114.48	127.87	137.15	145.31
	Ri1603	84.49	98.64	108.08	117.40	122.58
	Ri1604	95.00	107.30	116.04	126.63	129.69
	Ri1605	92.35	108.83	117.70	126.93	129.73
	Ri1606	93.70	110.44	118.70	131.83	136.28
G3	Ri1607	91.44	102.62	114.08	122.45	123.95
300	Ri1608	91.88	107.13	119.22	133.29	137.40
	Ri1609	95.26	106.05	116.63	127.46	129.78
	Ri1610	84.31	100.39	111.33	128.13	129.03
	Ri1611	97.48	111.72	119.48	130.89	134.01
	Ri1612	92.33	107.28	117.74	128.11	134.70
G4	Ri1613	89.37	106.77	115.87	127.11	133.49
1000	Ri1614	89.78	97.06	107.36	116.82	119.99
	Ri1615	84.99	100.80	109.58	119.30	126.94
	Ri1616	91.05	111.07	122.09	134.02	140.17
	Ri1617	98.85	113.29	119.27	131.63	134.27
	Ri1618	94.49	114.33	121.78	127.73	137.22

APPENDIX 6. Individual Cumulative Net Body Weight Gains (g) - Females

Group Dose (mg TOS/kg bwt/day)	Rat No.	Days			
		5	8	12	14
G1	Ri1595	14.44	26.88	37.13	41.66
0	Ri1596	13.64	19.55	26.54	32.65
	Ri1597	11.37	20.84	27.39	32.31
	Ri1598	15.46	26.33	37.23	42.87
	Ri1599	17.57	36.30	50.04	56.43
	Ri1600	16.81	30.34	43.18	49.53
G2	Ri1601	13.46	25.53	35.71	39.00
100	Ri1602	15.14	28.53	37.81	45.97
	Ri1603	14.15	23.59	32.91	38.09
	Ri1604	12.30	21.04	31.63	34.69
	Ri1605	16.48	25.35	34.58	37.38
	Ri1606	16.74	25.00	38.13	42.58
G3	Ri1607	11.18	22.64	31.01	32.51
300	Ri1608	15.25	27.34	41.41	45.52
	Ri1609	10.79	21.37	32.20	34.52
	Ri1610	16.08	27.02	43.82	44.72
	Ri1611	14.24	22.00	33.41	36.53
	Ri1612	14.95	25.41	35.78	42.37
G4	Ri1613	17.40	26.50	37.74	44.12
1000	Ri1614	7.28	17.58	27.04	30.21
	Ri1615	15.81	24.59	34.31	41.95
	Ri1616	20.02	31.04	42.97	49.12
	Ri1617	14.44	20.42	32.78	35.42
	Ri1618	19.84	27.29	33.24	42.73

APPENDIX 7. Cagewise Average Food Intake (g/rat/day)

Group Dose (mg TOS/kg bwt/day)	Rat No. From	Rat No. To	Weeks 1	Weeks 2
	Males			
G1	Ri1571	Ri1572	16.59	18.16
0	Ri1573	Ri1574	17.38	19.01
	Ri1575	Ri1576	16.97	19.30
G2	Ri1577	Ri1578	17.54	19.25
100	Ri1579	Ri1580	16.82	18.20
	Ri1581	Ri1582	17.99	20.35
G3	Ri1583	Ri1584	16.54	18.45
300	Ri1585	Ri1586	17.15	19.54
	Ri1587	Ri1588	16.75	19.21
G4	Ri1589	Ri1590	16.62	17.81
1000	Ri1591	Ri1592	18.53	21.14
	Ri1593	Ri1594	15.41	16.54
	Females			
G1	Ri1595	Ri1596	13.81	13.29
0	Ri1597	Ri1598	13.85	14.14
	Ri1599	Ri1600	15.08	15.92
G2	Ri1601	Ri1602	14.43	14.48
100	Ri1603	Ri1604	14.44	14.21
	Ri1605	Ri1606	14.08	14.56
G3	Ri1607	Ri1608	14.05	14.62
300	Ri1609	Ri1610	14.45	14.23
	Ri1611	Ri1612	14.04	14.62
G4	Ri1613	Ri1614	13.21	16.45
1000	Ri1615	Ri1616	14.15	14.51
	Ri1617	Ri1618	14.08	14.49

APPENDIX 8. Individual Haematological Values at Termination – Males

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	WBC G/L	RBC T/L	HGB g/L	HCT L/L	MCV fL	MCH pg	MCHC g/L	MPV fL	Plat G/L	PT s	APTT s
G1	Ri1571	8.74	6.98	137	0.446	64.0	19.7	307	12.6	1348	16.8	6.8
0	Ri1572	6.15	7.32	134	0.431	59.0	18.3	310	11.8	1411	15.5	5.9
	Ri1573	5.86	7.46	141	0.441	59.2	18.9	319	12.1	1414	15.6	5.0
	Ri1574	5.80	7.64	146	0.458	59.9	19.1	318	12.0	1548	15.9	9.9
	Ri1575	5.51	7.53	149	0.456	60.5	19.8	328	12.2	1535	17.0	9.4
	Ri1576	6.13	7.39	140	0.434	58.8	19.0	323	11.8	1393	21.3	6.6
G2	Ri1577	3.55	7.62	147	0.463	60.8	19.3	317	12.1	1650	16.1	10.3
100	Ri1578	8.48	7.15	142	0.440	61.6	19.9	324	12.5	1494	16.8	6.3
	Ri1579	5.53	7.13	141	0.412	57.8	19.8	343	10.3	1517	16.1	6.3
	Ri1580	4.04	7.66	149	0.454	59.2	19.5	328	9.4	1713	16.3	6.1
	Ri1581	7.41	7.05	139	0.432	61.3	19.7	321	11.0	1604	16.4	4.9
	Ri1582	6.39	7.25	141	0.430	59.3	19.5	329	9.6	1657	16.1	5.8
G3	Ri1583	5.97	7.23	139	0.436	60.3	19.3	320	11.0	1483	16.7	12.3
300	Ri1584	8.68	6.87	143	0.452	65.7	20.8	317	12.7	1667	16.8	9.4
	Ri1585	4.81	7.44	150	0.470	63.2	20.2	319	11.6	1594	17.2	11.1
	Ri1586	4.59	7.67	148	0.483	63.0	19.3	306	10.9	1558	17.1	10.0
	Ri1587	7.38	7.15	139	0.437	61.1	19.5	319	10.2	1623	17.2	8.0
	Ri1588	4.43	7.59	146	0.445	58.7	19.2	327	10.5	1657	16.8	8.3
G4	Ri1589	6.00	7.34	141	0.442	60.2	19.2	319	11.6	1435	17.2	6.8
1000	Ri1590	6.99	7.79	148	0.469	60.2	19.0	316	10.2	1605	17.1	6.7
	Ri1591	11.83	7.26	145	0.445	61.3	20.0	326	12.0	1665	15.9	9.0
	Ri1592	7.96	7.78	147	0.462	59.5	19.0	319	12.5	1375	16.9	8.5
	Ri1593	7.27	7.26	144	0.435	59.9	19.8	330	12.1	1457	15.6	9.4
	Ri1594	6.59	7.23	144	0.443	61.3	19.9	324	11.7	1814	15.6	8.6

APPENDIX 8 contd. Individual Haematological Values at Termination – Males

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Neut %	Lymp %	Mono %	Eosi %	Baso %	Abs Neut G/L	Abs Lymp G/L	Abs Mono G/L	Abs Eosi G/L	Abs Baso G/L
G1	Ri1571	11.7	83.5	2.8	0.8	0.3	1.02	7.30	0.24	0.07	0.03
0	Ri1572	16.8	77.4	3.3	0.5	0.5	1.03	4.76	0.20	0.03	0.03
	Ri1573	13.7	79.2	2.5	3.1	0.5	0.80	4.64	0.14	0.18	0.03
	Ri1574	12.6	83.2	2.3	0.6	0.4	0.73	4.82	0.13	0.03	0.02
	Ri1575	17.8	78.3	1.9	0.9	0.5	0.98	4.31	0.10	0.05	0.03
	Ri1576	16.2	78.0	3.9	0.5	0.2	0.99	4.78	0.24	0.03	0.01
G2	Ri1577	11.8	83.4	2.2	1.4	0.3	0.42	2.96	0.08	0.05	0.01
100	Ri1578	12.0	83.8	2.0	0.6	0.4	1.02	7.11	0.17	0.05	0.03
	Ri1579	18.5	76.7	3.0	1.0	0.2	1.02	4.24	0.17	0.06	0.01
	Ri1580	9.5	86.5	2.2	0.6	0.6	0.39	3.50	0.09	0.02	0.02
	Ri1581	6.6	88.4	2.9	0.4	0.5	0.49	6.55	0.21	0.03	0.03
	Ri1582	10.5	84.0	3.9	0.4	0.2	0.67	5.37	0.25	0.03	0.01
G3	Ri1583	15.1	80.3	2.8	0.7	0.3	0.90	4.79	0.17	0.04	0.02
300	Ri1584	12.1	83.1	2.9	0.4	0.5	1.05	7.21	0.25	0.04	0.04
	Ri1585	13.1	82.4	2.7	0.8	0.3	0.63	3.96	0.13	0.04	0.01
	Ri1586	12.0	81.4	3.1	2.2	0.4	0.55	3.74	0.14	0.10	0.02
	Ri1587	10.2	82.6	4.6	0.8	0.3	0.76	6.09	0.34	0.06	0.02
	Ri1588	12.8	81.7	2.4	1.3	0.4	0.56	3.62	0.11	0.06	0.02
G4	Ri1589	14.8	79.4	3.5	1.1	0.3	0.89	4.76	0.21	0.07	0.02
1000	Ri1590	7.0	88.0	2.9	0.5	0.3	0.49	6.15	0.20	0.04	0.02
	Ri1591	6.4	86.6	4.2	0.6	0.5	0.75	10.24	0.50	0.07	0.06
	Ri1592	25.0	69.8	2.8	0.6	0.5	1.99	5.56	0.22	0.05	0.04
	Ri1593	10.9	85.4	1.6	1.0	0.3	0.79	6.21	0.11	0.07	0.02
	Ri1594	16.8	77.6	3.0	1.1	0.5	1.10	5.11	0.20	0.07	0.04

APPENDIX 9. Individual Haematological Values at Termination – Females

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	WBC G/L	RBC T/L	HGB g/L	HCT L/L	MCV fL	MCH pg	MCHC g/L	MPV fL	Plat G/L	PT s	APTT s
G1	Ri1595	4.46	7.74	150	0.451	58.3	19.4	332	11.3	1589	15.2	6.1
0	Ri1596	2.40	8.44	160	0.475	56.2	18.9	336	11.4	1502	14.9	6.9
	Ri1597	6.15	7.93	149	0.457	57.7	18.8	325	11.9	1276	15.6	6.8
	Ri1598	8.12	7.68	147	0.460	59.9	19.2	320	12.4	1489	16.6	5.3
	Ri1599	4.91	7.37	147	0.453	61.5	20.0	325	11.2	1931	15.9	5.7
	Ri1600	4.39	7.43	146	0.456	61.4	19.7	321	12.0	1765	15.9	6.6
G2	Ri1601	5.08	7.70	144	0.459	59.6	18.7	313	12.0	1461	15.7	4.9
100	Ri1602	4.14	7.85	146	0.458	58.3	18.7	320	11.4	1564	15.0	5.2
	Ri1603	4.78	8.01	146	0.462	57.7	18.2	316	11.8	1451	14.8	5.9
	Ri1604	3.68	8.28	149	0.468	56.5	18.0	318	11.3	1305	15.2	5.8
	Ri1605	4.31	7.65	152	0.467	61.0	19.8	325	11.5	1685	15.2	6.6
	Ri1606	2.77	7.69	145	0.471	61.2	18.9	308	11.7	1595	14.7	6.9
G3	Ri1607	3.77	7.58	142	0.451	59.5	18.7	315	10.9	1763	14.3	10.3
300	Ri1608	3.89	7.57	144	0.449	59.4	19.1	321	11.4	1693	14.2	7.6
	Ri1609	4.26	8.11	146	0.463	57.1	18.0	315	11.6	1408	14.4	15.0
	Ri1610	8.19	7.27	140	0.438	60.3	19.3	320	12.1	1355	14.3	13.7
	Ri1611	3.05	8.32	156	0.519	62.4	18.7	300	12.4	1273	13.6	10.9
	Ri1612	4.90	7.55	137	0.441	58.4	18.1	310	11.6	1389	19.8	10.4
G4	Ri1613	4.43	7.74	147	0.443	57.3	19.0	331	11.1	1682	19.2	9.8
1000	Ri1614	3.24	8.38	162	0.522	62.3	19.3	309	12.1	1386	15.2	9.1
	Ri1615	6.36	7.20	142	0.440	61.2	19.8	324	11.7	1623	14.9	7.3
	Ri1616	3.71	7.52	147	0.473	63.0	19.6	311	11.2	1850	15.7	5.6
	Ri1617	3.96	8.00	146	0.469	58.6	18.3	312	11.5	1429	16.2	5.9
	Ri1618	5.11	7.88	152	0.478	60.6	19.2	317	12.7	1292	16.0	6.0

APPENDIX 9 contd. Individual Haematological Values at Termination – Females

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Neut %	Lymp %	Mono %	Eosi %	Baso %	Abs Neut G/L	Abs Lymp G/L	Abs Mono G/L	Abs Eosi G/L	Abs Baso G/L
G1	Ri1595	13.8	79.9	3.7	0.9	0.5	0.62	3.56	0.16	0.04	0.02
0	Ri1596	15.7	80.7	1.3	1.3	0.2	0.38	1.94	0.03	0.03	0.00
	Ri1597	10.7	84.7	2.2	0.8	0.4	0.66	5.21	0.14	0.05	0.03
	Ri1598	25.7	69.7	2.5	0.7	0.3	2.08	5.66	0.20	0.06	0.03
	Ri1599	10.5	84.0	3.5	0.8	0.3	0.52	4.12	0.17	0.04	0.01
	Ri1600	6.4	89.1	2.6	0.6	0.4	0.28	3.91	0.12	0.03	0.02
G2	Ri1601	8.7	86.8	2.3	0.6	0.3	0.44	4.41	0.12	0.03	0.02
100	Ri1602	7.9	87.5	2.0	1.5	0.3	0.33	3.62	0.08	0.06	0.01
	Ri1603	12.6	82.0	2.6	1.2	0.7	0.60	3.92	0.13	0.06	0.03
	Ri1604	14.8	81.5	1.7	0.9	0.2	0.55	3.00	0.06	0.03	0.01
	Ri1605	14.8	81.0	1.8	1.0	0.4	0.64	3.49	0.08	0.04	0.02
	Ri1606	16.4	78.8	2.4	1.6	0.2	0.46	2.18	0.07	0.04	0.01
G3	Ri1607	11.3	85.2	1.8	0.9	0.4	0.43	3.21	0.07	0.03	0.02
300	Ri1608	10.9	82.9	3.9	0.8	0.4	0.43	3.23	0.15	0.03	0.02
	Ri1609	13.0	81.9	2.6	0.9	0.8	0.55	3.49	0.11	0.04	0.03
	Ri1610	33.5	59.3	4.3	1.6	0.3	2.74	4.86	0.36	0.13	0.03
	Ri1611	15.6	77.2	3.3	2.1	0.5	0.48	2.36	0.10	0.06	0.02
	Ri1612	18.3	77.5	2.8	0.7	0.2	0.90	3.80	0.14	0.04	0.01
G4	Ri1613	8.1	86.1	3.9	0.4	0.4	0.36	3.81	0.17	0.02	0.02
1000	Ri1614	15.5	77.0	4.3	1.9	0.3	0.50	2.49	0.14	0.06	0.01
	Ri1615	9.1	87.4	1.4	0.8	0.4	0.58	5.55	0.09	0.05	0.02
	Ri1616	15.7	80.9	1.9	0.8	0.3	0.58	3.00	0.07	0.03	0.01
	Ri1617	13.4	81.7	2.8	0.7	0.3	0.53	3.24	0.11	0.03	0.01
	Ri1618	20.1	75.4	1.8	1.4	0.4	1.03	3.85	0.09	0.07	0.02

APPENDIX 10. Individual Clinical Chemistry Values at Termination – Males

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Glu mmol/L	BUN mmol/L	T.Pro g/L	AST U/L	ALT U/L	ALP U/L	GGT* U/L	T.Bil µmol/L	Creat µmol/L	Alb g/L	Pi mmol/L	Ca mmol/L	T.Chol mmol/L	Glob g/L	A/G	Na mEq/L	K mEq/L	Cl mEq/L
G1	Ri1571	10.87	5.64	61.0	76	35	210	2	3.28	12	37.9	2.75	2.70	2.03	23.1	1.64	137.4	4.51	89.0
0	Ri1572	7.88	5.91	58.6	74	59	228	1	3.28	17	39.1	2.98	2.75	1.88	19.5	2.01	136.9	4.16	87.9
	Ri1573	6.61	4.85	61.7	79	39	210	2	3.69	11	40.6	3.05	2.73	1.80	21.1	1.92	140.2	3.34	89.6
	Ri1574	7.33	6.81	61.9	68	37	236	1	4.34	16	39.9	3.26	2.70	1.67	22.0	1.81	139.9	3.92	90.5
	Ri1575	6.63	5.42	61.1	68	36	196	3	3.39	11	39.7	2.83	2.65	1.55	21.4	1.86	140.0	4.10	92.4
	Ri1576	8.93	5.73	56.7	92	29	176	0	3.77	14	37.7	3.05	2.66	1.90	19.0	1.98	135.2	5.23	89.5
G2	Ri1577	8.52	6.18	62.7	65	32	185	0	3.13	18	40.4	3.06	2.71	1.99	22.3	1.81	138.1	4.44	90.1
100	Ri1578	8.64	5.59	59.0	72	29	219	2	4.01	14	39.2	2.82	2.55	1.82	19.8	1.98	140.2	3.96	90.8
	Ri1579	8.32	6.17	61.0	74	50	204	0	3.78	13	39.5	2.58	2.70	1.75	21.5	1.84	137.7	3.78	89.2
	Ri1580	8.91	7.39	61.5	79	40	182	1	4.46	12	40.3	3.47	2.81	1.78	21.2	1.90	142.6	4.60	89.0
	Ri1581	9.25	6.36	61.4	61	34	223	3	3.47	21	38.8	3.03	2.74	1.38	22.6	1.72	138.6	3.55	89.4
	Ri1582	7.59	5.43	57.7	67	31	204	1	3.14	15	37.9	2.89	2.59	2.31	19.8	1.91	138.3	4.11	92.0
G3	Ri1583	7.40	5.14	61.5	75	40	190	3	4.27	18	40.5	2.98	2.70	2.19	21.0	1.93	138.9	4.49	91.5
300	Ri1584	9.08	5.36	61.6	71	41	253	0	2.67	15	37.4	2.86	2.72	2.47	24.2	1.55	139.5	3.97	90.7
	Ri1585	7.20	6.88	63.2	74	29	255	2	3.33	19	38.1	2.68	2.80	2.54	25.1	1.52	138.7	3.74	90.1
	Ri1586	8.22	4.91	62.5	92	35	222	2	3.90	23	39.1	2.92	2.77	2.45	23.4	1.67	141.2	3.93	90.3
	Ri1587	8.57	5.35	59.6	86	38	270	0	4.13	28	37.8	3.34	2.87	2.01	21.8	1.73	140.6	3.96	91.0
	Ri1588	8.06	6.85	64.0	67	46	184	2	3.26	18	43.2	2.91	2.70	2.30	20.8	2.08	143.4	4.07	94.9
G4	Ri1589	7.14	3.73	58.9	67	37	194	1	3.42	13	39.5	2.95	2.67	2.22	19.4	2.04	138.5	3.97	90.7
1000	Ri1590	6.93	4.71	62.6	62	40	225	1	3.16	11	36.4	2.69	2.70	1.38	26.2	1.39	140.1	4.50	92.3
	Ri1591	8.59	7.59	61.7	64	38	193	3	3.21	16	38.7	3.14	2.68	1.45	23.0	1.68	139.0	4.24	91.2
	Ri1592	7.75	5.48	58.4	76	45	223	1	3.86	12	35.5	2.79	2.62	2.24	22.9	1.55	137.1	4.43	89.7
	Ri1593	7.71	6.45	59.4	60	25	170	3	4.49	18	37.5	3.10	2.57	1.96	21.9	1.71	140.3	4.19	92.7
	Ri1594	7.15	5.78	58.1	70	29	172	2	3.01	13	37.4	3.31	2.66	2.09	20.7	1.81	138.5	4.34	91.2

*: GGT: Lower Limit of Quantification (LLOQ): 3U/l. Not considered for Statistical Analysis.

APPENDIX 11. Individual Clinical Chemistry Values at Termination – Females

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Glu mmol/L	BUN mmol/L	T.Pro g/L	AST U/L	ALT U/L	ALP U/L	GGT* U/L	T.Bil µmol/L	Creat µmol/L	Alb g/L	Pi mmol/L	Ca mmol/L	T.Chol mmol/L	Glob g/L	A/G	Na mEq/L	K mEq/L	Cl mEq/L
G1	Ri1595	7.38	6.73	64.0	68	39	150	1	3.33	17	41.8	2.61	2.71	2.03	22.2	1.88	140.2	3.85	93.8
0	Ri1596	9.00	5.47	64.7	69	30	106	2	3.72	17	44.8	2.15	2.54	1.62	19.9	2.25	140.6	4.58	96.4
	Ri1597	6.95	5.61	64.2	78	34	112	0	3.61	16	42.2	2.79	2.69	1.79	22.0	1.92	141.8	4.12	93.7
	Ri1598	8.58	4.05	60.2	70	44	148	0	3.33	16	40.6	2.13	2.65	1.70	19.6	2.07	140.7	4.21	93.7
	Ri1599	8.39	6.54	65.1	69	29	202	1	3.87	19	43.3	2.67	2.65	2.31	21.8	1.99	141.1	3.73	93.3
	Ri1600	8.78	4.57	62.6	59	26	148	0	2.58	14	41.2	2.49	2.67	1.62	21.4	1.93	139.0	4.19	93.2
G2	Ri1601	8.73	6.23	60.7	75	36	153	1	2.78	16	40.2	2.58	2.62	1.99	20.5	1.96	139.8	3.33	90.6
100	Ri1602	6.69	5.48	62.8	70	36	118	1	3.52	15	41.4	2.44	2.68	2.17	21.4	1.93	138.6	3.80	90.2
	Ri1603	6.42	5.22	61.8	71	29	136	1	3.04	12	40.0	2.50	2.68	1.79	21.8	1.83	139.3	3.47	90.4
	Ri1604	8.13	7.14	61.8	67	45	137	2	3.22	20	43.1	2.62	2.62	1.98	18.7	2.30	140.1	3.72	93.7
	Ri1605	8.14	7.01	62.1	68	38	127	3	3.48	14	41.7	2.45	2.69	2.46	20.4	2.04	139.0	4.05	92.8
	Ri1606	7.75	6.12	58.9	66	41	159	1	3.33	21	38.4	2.51	2.67	2.23	20.5	1.87	140.8	3.22	93.8
G3	Ri1607	9.13	4.65	61.6	60	38	135	2	3.11	17	40.2	2.35	2.64	1.54	21.4	1.88	141.6	4.28	93.8
300	Ri1608	6.90	7.08	64.7	68	36	137	2	3.13	18	42.2	2.44	2.64	2.08	22.5	1.88	139.7	3.65	92.3
	Ri1609	9.24	4.74	60.7	70	34	125	0	3.10	20	39.7	2.30	2.53	1.66	21.0	1.89	141.4	4.18	96.0
	Ri1610	8.63	5.55	60.8	75	43	160	2	3.28	15	38.0	2.89	2.66	2.09	22.8	1.67	138.1	3.92	91.0
	Ri1611	7.49	6.31	63.6	70	36	137	2	3.86	18	42.2	2.81	2.53	1.79	21.4	1.97	139.5	4.99	91.1
	Ri1612	7.73	3.97	60.7	64	31	143	1	3.11	14	39.9	2.69	2.60	1.52	20.8	1.92	141.0	3.39	92.5
G4	Ri1613	9.00	5.63	59.4	67	34	140	1	3.32	11	38.6	2.55	2.65	1.95	20.8	1.86	140.9	3.73	92.7
1000	Ri1614	8.58	5.91	59.4	69	32	136	3	3.36	16	38.1	2.63	2.60	1.70	21.3	1.79	140.2	4.17	95.5
	Ri1615	8.26	3.54	58.2	64	31	156	2	3.00	15	37.0	2.62	2.56	2.54	21.2	1.75	140.9	3.46	92.4
	Ri1616	7.22	8.15	63.7	72	21	163	2	2.99	21	39.9	2.73	2.63	2.67	23.8	1.68	139.2	4.22	92.0
	Ri1617	9.59	5.78	61.9	65	32	135	2	3.45	18	37.8	2.85	2.57	1.94	24.1	1.57	141.0	3.94	94.6
	Ri1618	8.87	6.08	64.1	68	34	168	1	2.27	18	42.4	2.39	2.58	1.95	21.7	1.95	139.1	4.38	94.0

*: GGT: Lower Limit of Quantification (LLOQ): 3U/l. Not considered for Statistical Analysis.

APPENDIX 12. Individual Terminal Fasting Body Weights and Organ Weights – Males

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Fasting Bwt (g)	Organ weights (g)								
			Adrenals	Testes	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Epididym
G1	Ri1571	184.39	0.067	2.476	1.762	7.460	0.613	0.618	0.714	1.979	0.410
0	Ri1572	149.77	0.051	1.736	1.330	6.875	0.552	0.433	0.580	1.877	0.282
	Ri1573	162.34	0.059	2.012	1.428	5.883	0.715	0.459	0.652	2.002	0.263
	Ri1574	178.87	0.065	2.404	1.551	6.470	0.714	0.638	0.672	1.796	0.324
	Ri1575	170.44	0.056	2.300	1.588	6.044	0.740	0.615	0.719	1.829	0.419
	Ri1576	155.07	0.056	1.686	1.234	5.257	0.543	0.490	0.634	1.830	0.337
G2	Ri1577	172.9	0.050	2.307	1.717	6.453	0.637	0.465	0.659	1.841	0.295
100	Ri1578	151.37	0.062	1.849	1.331	6.154	0.533	0.553	0.568	1.731	0.238
	Ri1579	143.89	0.058	2.080	1.355	5.912	0.593	0.475	0.573	1.895	0.307
	Ri1580	173.79	0.075	2.205	1.708	7.036	0.706	0.577	0.705	2.021	0.388
	Ri1581	186.07	0.060	2.473	1.731	7.355	0.647	0.767	0.721	1.856	0.354
	Ri1582	151.84	0.056	2.068	1.357	5.154	0.617	0.433	0.674	1.797	0.239
G3	Ri1583	142.26	0.051	1.576	1.300	5.360	0.567	0.404	0.611	1.874	0.250
300	Ri1584	186.47	0.080	2.631	1.925	8.577	0.804	0.704	0.765	1.845	0.536
	Ri1585	172.63	0.058	2.342	1.668	6.686	0.613	0.640	0.740	1.811	0.353
	Ri1586	161.4	0.065	2.204	1.530	6.229	0.602	0.584	0.699	1.885	0.375
	Ri1587	178.57	0.062	2.537	1.589	8.108	0.718	0.776	0.700	1.848	0.358
	Ri1588	166.98	0.055	2.114	1.409	5.662	0.439	0.491	0.655	1.773	0.365
G4	Ri1589	140.19	0.057	1.673	1.167	5.165	0.594	0.448	0.575	1.769	0.278
1000	Ri1590	171.62	0.063	2.531	1.542	6.294	0.588	0.616	0.685	1.821	0.391
	Ri1591	193.33	0.057	2.739	1.680	7.788	0.760	0.884	0.749	1.778	0.359
	Ri1592	166.39	0.056	2.165	1.482	5.971	0.578	0.488	0.613	1.792	0.317
	Ri1593	142.29	0.058	1.673	1.262	4.831	0.494	0.407	0.565	1.798	0.266
	Ri1594	155.87	0.057	1.868	1.357	5.405	0.569	0.499	0.598	1.851	0.311

APPENDIX 13. Individual Terminal Fasting Body Weights and Organ Weight Ratios – Males

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Fasting Bwt (g)	Organ weight ratios (%)								
			Adrenals	Testes	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Epididym
G1	Ri1571	184.39	0.036	1.343	0.956	4.046	0.332	0.335	0.387	1.073	0.222
0	Ri1572	149.77	0.034	1.159	0.888	4.590	0.369	0.289	0.387	1.253	0.188
	Ri1573	162.34	0.036	1.239	0.880	3.624	0.440	0.283	0.402	1.233	0.162
	Ri1574	178.87	0.036	1.344	0.867	3.617	0.399	0.357	0.376	1.004	0.181
	Ri1575	170.44	0.033	1.349	0.932	3.546	0.434	0.361	0.422	1.073	0.246
	Ri1576	155.07	0.036	1.087	0.796	3.390	0.350	0.316	0.409	1.180	0.217
G2	Ri1577	172.90	0.029	1.334	0.993	3.732	0.368	0.269	0.381	1.065	0.171
100	Ri1578	151.37	0.041	1.222	0.879	4.066	0.352	0.365	0.375	1.144	0.157
	Ri1579	143.89	0.040	1.446	0.942	4.109	0.412	0.330	0.398	1.317	0.213
	Ri1580	173.79	0.043	1.269	0.983	4.049	0.406	0.332	0.406	1.163	0.223
	Ri1581	186.07	0.032	1.329	0.930	3.953	0.348	0.412	0.387	0.997	0.190
	Ri1582	151.84	0.037	1.362	0.894	3.394	0.406	0.285	0.444	1.183	0.157
G3	Ri1583	142.26	0.036	1.108	0.914	3.768	0.399	0.284	0.429	1.317	0.176
300	Ri1584	186.47	0.043	1.411	1.032	4.600	0.431	0.378	0.410	0.989	0.287
	Ri1585	172.63	0.034	1.357	0.966	3.873	0.355	0.371	0.429	1.049	0.204
	Ri1586	161.40	0.040	1.366	0.948	3.859	0.373	0.362	0.433	1.168	0.232
	Ri1587	178.57	0.035	1.421	0.890	4.541	0.402	0.435	0.392	1.035	0.200
	Ri1588	166.98	0.033	1.266	0.844	3.391	0.263	0.294	0.392	1.062	0.219
G4	Ri1589	140.19	0.041	1.193	0.832	3.684	0.424	0.320	0.410	1.262	0.198
1000	Ri1590	171.62	0.037	1.475	0.898	3.667	0.343	0.359	0.399	1.061	0.228
	Ri1591	193.33	0.029	1.417	0.869	4.028	0.393	0.457	0.387	0.920	0.186
	Ri1592	166.39	0.034	1.301	0.891	3.589	0.347	0.293	0.368	1.077	0.191
	Ri1593	142.29	0.041	1.176	0.887	3.395	0.347	0.286	0.397	1.264	0.187
	Ri1594	155.87	0.037	1.198	0.871	3.468	0.365	0.320	0.384	1.188	0.200

APPENDIX 14. Individual Terminal Fasting Body Weights and Organ Weights – Females

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Fasting Bwt (g)	Organ weights (g)								
			Adrenals	Ovaries	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Uterus*
G1	Ri1595	131.48	0.066	0.073	1.313	5.104	0.414	0.388	0.552	1.829	0.398
0	Ri1596	116.27	0.066	0.084	1.233	4.167	0.416	0.330	0.546	1.629	0.439
	Ri1597	120.59	0.081	0.088	1.212	4.654	0.433	0.316	0.560	1.829	0.469
	Ri1598	118.04	0.059	0.052	1.205	4.350	0.476	0.308	0.533	1.594	0.479
	Ri1599	136.59	0.079	0.072	1.335	5.512	0.503	0.401	0.642	1.820	0.385
	Ri1600	143.93	0.076	0.068	1.294	5.103	0.563	0.397	0.550	1.849	0.343
G2	Ri1601	120.05	0.064	0.072	1.072	4.552	0.391	0.330	0.528	1.829	0.319
100	Ri1602	137.47	0.073	0.079	1.307	4.916	0.515	0.392	0.570	1.764	0.357
	Ri1603	115.70	0.056	0.072	1.221	3.999	0.384	0.289	0.513	1.671	0.241
	Ri1604	125.77	0.070	0.063	1.205	4.996	0.443	0.304	0.550	1.727	0.427
	Ri1605	125.62	0.060	0.076	1.016	4.516	0.436	0.321	0.508	1.639	0.340
	Ri1606	132.11	0.079	0.108	1.158	4.896	0.324	0.334	0.599	1.792	0.314
G3	Ri1607	122.99	0.058	0.066	1.096	4.726	0.424	0.339	0.579	1.618	0.353
300	Ri1608	127.36	0.060	0.077	1.201	5.156	0.393	0.329	0.586	1.910	0.366
	Ri1609	125.56	0.066	0.073	1.217	4.681	0.351	0.354	0.532	1.713	0.355
	Ri1610	127.88	0.054	0.092	1.150	4.921	0.498	0.384	0.586	1.687	0.304
	Ri1611	127.20	0.060	0.071	1.404	4.886	0.389	0.346	0.520	1.781	0.287
	Ri1612	129.80	0.057	0.056	1.216	4.593	0.410	0.373	0.576	1.699	0.514
G4	Ri1613	127.04	0.066	0.062	1.206	4.737	0.426	0.339	0.523	1.820	0.651
1000	Ri1614	116.38	0.079	0.101	1.112	4.245	0.334	0.342	0.520	1.735	0.326
	Ri1615	120.90	0.064	0.057	1.251	4.682	0.411	0.340	0.484	1.618	0.537
	Ri1616	129.85	0.067	0.073	1.267	4.833	0.435	0.415	0.530	1.820	0.451
	Ri1617	125.38	0.060	0.090	1.118	4.543	0.366	0.349	0.459	1.704	0.312
	Ri1618	130.89	0.061	0.094	1.391	4.854	0.541	0.357	0.560	1.822	0.342

*: With cervix

APPENDIX 15. Individual Terminal Fasting Body Weights and Organ Weight Ratios – Females

Group No. Dose (mg TOS/kg bwt/day)	Rat No.	Fasting Bwt (g)	Organ weight ratios (%)								
			Adrenals	Ovaries	Kidneys	Liver	Thymus	Spleen	Heart	Brain	Uterus*
G1	Ri1595	131.48	0.050	0.056	0.999	3.882	0.315	0.295	0.420	1.391	0.303
0	Ri1596	116.27	0.057	0.072	1.060	3.584	0.358	0.284	0.470	1.401	0.378
	Ri1597	120.59	0.067	0.073	1.005	3.859	0.359	0.262	0.464	1.517	0.389
	Ri1598	118.04	0.050	0.044	1.021	3.685	0.403	0.261	0.452	1.350	0.406
	Ri1599	136.59	0.058	0.053	0.977	4.035	0.368	0.294	0.470	1.332	0.282
	Ri1600	143.93	0.053	0.047	0.899	3.545	0.391	0.276	0.382	1.285	0.238
G2	Ri1601	120.05	0.053	0.060	0.893	3.792	0.326	0.275	0.440	1.524	0.266
100	Ri1602	137.47	0.053	0.057	0.951	3.576	0.375	0.285	0.415	1.283	0.260
	Ri1603	115.70	0.048	0.062	1.055	3.456	0.332	0.250	0.443	1.444	0.208
	Ri1604	125.77	0.056	0.050	0.958	3.972	0.352	0.242	0.437	1.373	0.340
	Ri1605	125.62	0.048	0.060	0.809	3.595	0.347	0.256	0.404	1.305	0.271
	Ri1606	132.11	0.060	0.082	0.877	3.706	0.245	0.253	0.453	1.356	0.238
G3	Ri1607	122.99	0.047	0.054	0.891	3.843	0.345	0.276	0.471	1.316	0.287
300	Ri1608	127.36	0.047	0.060	0.943	4.048	0.309	0.258	0.460	1.500	0.287
	Ri1609	125.56	0.053	0.058	0.969	3.728	0.280	0.282	0.424	1.364	0.283
	Ri1610	127.88	0.042	0.072	0.899	3.848	0.389	0.300	0.458	1.319	0.238
	Ri1611	127.20	0.047	0.056	1.104	3.841	0.306	0.272	0.409	1.400	0.226
	Ri1612	129.80	0.044	0.043	0.937	3.539	0.316	0.287	0.444	1.309	0.396
G4	Ri1613	127.04	0.052	0.049	0.949	3.729	0.335	0.267	0.412	1.433	0.512
1000	Ri1614	116.38	0.068	0.087	0.955	3.648	0.287	0.294	0.447	1.491	0.280
	Ri1615	120.9	0.053	0.047	1.035	3.873	0.34	0.281	0.4	1.338	0.444
	Ri1616	129.85	0.052	0.056	0.976	3.722	0.335	0.32	0.408	1.402	0.347
	Ri1617	125.38	0.048	0.072	0.892	3.623	0.292	0.278	0.366	1.359	0.249
	Ri1618	130.89	0.047	0.072	1.063	3.708	0.413	0.273	0.428	1.392	0.261

*: With cervix

APPENDIX 16. Individual Gross Pathological and Histopathological Findings – Males

Group No.	Rat No.	Doses (mg TOS/kg bwt/day)	Gross	Microscopic
G1	Ri1571	0	NAD	STOMACH: Tissue present no change
G1	Ri1572	0	NAD	STOMACH: Tissue present no change
G1	Ri1573	0	NAD	STOMACH: Tissue present no change
G1	Ri1574	0	NAD	STOMACH: Tissue present no change
G1	Ri1575	0	NAD	STOMACH: Tissue present no change
G1	Ri1576	0	NAD	STOMACH: Tissue present no change
G2	Ri1577	100	NAD	Tissues not examined
G2	Ri1578	100	NAD	Tissues not examined
G2	Ri1579	100	NAD	Tissues not examined
G2	Ri1580	100	NAD	Tissues not examined

1: Minimal, 2: Mild, 3: Moderate, 4: Severe

contd.

APPENDIX 16 contd. Individual Gross Pathological and Histopathological Findings – Males

Group No.	Rat No.	Doses (mg TOS/kg bwt/day)	Gross	Microscopic
G2	Ri1581	100	NAD	Tissues not examined
G2	Ri1582	100	NAD	Tissues not examined
G3	Ri1583	300	NAD	Tissues not examined
G3	Ri1584	300	NAD	Tissues not examined
G3	Ri1585	300	NAD	Tissues not examined
G3	Ri1586	300	NAD	Tissues not examined
G3	Ri1587	300	NAD	Tissues not examined
G3	Ri1588	300	NAD	Tissues not examined
G4	Ri1589	1000	NAD	STOMACH: Tissue present no change

1: Minimal, 2: Mild, 3: Moderate, 4: Severe

contd.

APPENDIX 16 contd. Individual Gross Pathological and Histopathological Findings – Males

Group No.	Rat No.	Doses (mg TOS/kg bwt/day)	Gross	Microscopic
G4	Ri1590	1000	NAD	STOMACH: Tissue present no change
G4	Ri1591	1000	NAD	STOMACH: Tissue present no change
G4	Ri1592	1000	NAD	STOMACH: Tissue present no change
G4	Ri1593	1000	NAD	STOMACH: Tissue present no change
G4	Ri1594	1000	NAD	STOMACH: Tissue present no change

1: Minimal, 2: Mild, 3: Moderate, 4: Severe

APPENDIX 17. Individual Gross Pathological and Histopathological Findings – Females

Group No.	Rat No.	Doses (mg TOS/kg bwt/day)	Gross	Microscopic
G1	Ri1595	0	NAD	STOMACH: Tissue present no change
G1	Ri1596	0	NAD	STOMACH: Tissue present no change
G1	Ri1597	0	NAD	STOMACH: Tissue present no change
G1	Ri1598	0	NAD	STOMACH: Tissue present no change
G1	Ri1599	0	NAD	STOMACH: Tissue present no change
G1	Ri1600	0	NAD	STOMACH: Tissue present no change
G2	Ri1601	100	NAD	Tissues not examined
G2	Ri1602	100	NAD	Tissues not examined
G2	Ri1603	100	NAD	Tissues not examined
G2	Ri1604	100	NAD	Tissues not examined

1: Minimal, 2: Mild, 3: Moderate, 4: Severe

contd.

APPENDIX 17 contd. Individual Gross Pathological and Histopathological Findings – Females

Group No.	Rat No.	Doses (mg TOS/kg bwt/day)	Gross	Microscopic
G2	Ri1605	100	NAD	Tissues not examined
G2	Ri1606	100	NAD	Tissues not examined
G3	Ri1607	300	NAD	Tissues not examined
G3	Ri1608	300	NAD	Tissues not examined
G3	Ri1609	300	KIDNEYS(Bilateral): Pelvis dilated	KIDNEYS: Dilated pelvis-bilateral
G3	Ri1610	300	NAD	Tissues not examined
G3	Ri1611	300	NAD	Tissues not examined
G3	Ri1612	300	NAD	Tissues not examined
G4	Ri1613	1000	NAD	STOMACH: Tissue present no change

1: Minimal, 2: Mild, 3: Moderate, 4: Severe

contd.

APPENDIX 17 contd. Individual Gross Pathological and Histopathological Findings – Females

Group No.	Rat No.	Doses (mg TOS/kg bwt/day)	Gross	Microscopic
G4	Ri1614	1000	NAD	STOMACH: Tissue present no change
G4	Ri1615	1000	NAD	STOMACH: Tissue present no change
G4	Ri1616	1000	NAD	STOMACH: Tissue present no change
G4	Ri1617	1000	NAD	STOMACH: Tissue present no change
G4	Ri1618	1000	NAD	STOMACH: Tissue present no change

1: Minimal, 2: Mild, 3: Moderate, 4: Severe

APPENDIX 18. Gavage Preparation Data

Date of mixing	Group No.	Dose (mg TOS/kg bwt/day)	Test Item (mL)	Total volume made up with "MilliQ" water (mL)
07.07.2009,	G1	0	0	250.0
	G2	100	22.73	250.0
	G3	300	68.18	250.0
	G4	1000	227.27	250.0
11.07.2009	G1	0	0	150.0
	G2	100	13.64	150.0
	G3	300	40.91	150.0
	G4	1000	136.36	150.0
14.07.2009	G1	0	0	250.0
	G2	100	22.73	250.0
	G3	300	68.18	250.0
	G4	1000	227.27	250.0
18.07.2009	G1	0	0	150.0
	G2	100	13.64	150.0
	G3	300	40.91	150.0
	G4	1000	136.36	150.0

APPENDIX 19. Batch Analysis Data

Date	Batch No.	Group	Nominal concentration (mg/mL)	Mean protein content in the sample (mg/mL)	Test item concentration in samples (mg/mL)	Analysed concentration of test item as TOS
07.07.2009	I	G1	0.0	0.0	0.0	0.0
		G2	5.0	1.96	89.09	4.90
		G3	15.0	5.94	270.00	14.85
		G4	50.0	20.02	910.00	50.05
14.07.2009	II	G1	0.0	0.0	0.0	0.0
		G2	5.0	1.98	90.00	4.95
		G3	15.0	5.98	271.82	14.95
		G4	50.0	20.06	911.82	50.15

$$\text{Test item concentration in the sample (mg/mL)} = \frac{\text{Mean protein content in the sample (mg/mL)} \times 100}{\text{Per cent protein content in the test item}}$$

$$\text{Analysed concentration of test item as TOS (mg/mL)} = \text{Test item concentration in the sample (mg/mL)} \times \text{\% TOS}/100$$

NOTE: % protein content in the test item is 2.20 %(w/w) and % TOS is 5.50 as furnished by the Sponsor in the Certificate of Analysis.

APPENDIX 20. Certificate of Analysis

DSM Food Specialties B.V.
R&D/REG

DSM

Page 1 of 1

CERTIFICATE OF ANALYSIS

Name of the product	**Meltamase**
Batch no.	MEG.GRZ.0905
Status	Batch for toxicity study
Date of manufacture	February 2009
Date of expiration	12 months (provisionally)
Active component	Meltamase
Date of issue	16 June 2009

Parameter	Method	Unit	Result
	Characterization data		
Meltamase activity	B1903	ATU / g	2280
Dry Matter	60485	% (w/w)	6.65
Ash	60328	% (w/w)	1.15
TOS	Calculation	% (w/w)	5.50
Proteins by Kjeldahl Nitrogen x 6.25	61804	% (w/w)	2.20
	Stability data; > 90% residual activity		
Stability at 4°C, undiluted	B1903	Days	7
Stability at 4°C, 91 mg / ml	B1903	Days	7
Stability at 4°C, 273 mg / ml	B1903	Days	7
Stability at 4°C, 909 mg / ml	B1903	Days	7
Stability at RT, undiluted	B1903	Hours	4
Stability at RT, 91 mg / ml	B1903	Hours	4
Stability at RT, 273 mg / ml	B1903	Hours	4
Stability at RT, 909 mg / ml	B1903	Hours	4

Signature R&D QESH: 	Remarks (if any): CoA drafted for toxicity study

#55271

APPENDIX 21. Deviation from the Approved Study Plan

Sl. No.	AS IN STUDY PLAN	DESCRIPTION OF DEVIATION
1.	**1. STUDY DETAILS (Page 4/19)** **Study Schedule:** Study completion: Latest by September/October 2009	 **Study Schedule:** Study completion: Latest by December 2009
Impact of Deviation: None. This did not affect the outcome of the study or the interpretation of the results.		

Date: 22 December 2009

(Mr. P. M. Sathish)
STUDY DIRECTOR

16. ANNEXURES

ANNEXURE 1. Contaminant Analysis Report for Bedding Material (Paddy Husk)

LUFA-ITL GmbH

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AGROLAB Laborgruppe
www.agrolab.de

LUFA - ITL Dr.-Hell-Str. 6, 24107 Kiel

ADVINUS THERAPEITICS PRIVATE LIMITED
PEENYA INDUSTRIAL AREA
0 BANGALORE-560 058
INDIEN

Date 26.02.2009
Customer no. 1209576
Page 1 of 2

TEST REPORT

Sample No. 651329

Order No. **582713**
Sample Arrival **11.02.2009**
Sample code **Sample 6 Bedding material - Paddy Husk**
data of sampling: 23.01.2009
batch-no.: PH-19
Sample packing **plastic bag**

	Unit	Result	Declaration		Substance	Method
Mycotoxins						
Aflatoxine B1	µg/kg	**2,2**			OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine B2	µg/kg	**<1,0**			OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G1	µg/kg	**<1,0**			OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G2	µg/kg	**<1,0**			OM	HPLC-VDLUFA Bd. III, 16.1.4
PCB						
PCB 28	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
PCB 52	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
PCB 101	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
PCB 118	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
PCB 138	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
PCB 153	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
PCB 180	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Organochlorous-Pesticides GC-Multiresidueanalysis						
Aldrin	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Dieldrin	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Endrin	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Chlorodane alpha	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Chlorodane gamma	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Chlorodane-oxy	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Endosulfan alpha	mg/kg	**0,011**			OM	acc. to §64 LFGB L00.00-34
Endosulfan beta	mg/kg	**0,038**			OM	acc. to §64 LFGB L00.00-34
Endosulfansulfat	mg/kg	**0,037**			OM	acc. to §64 LFGB L00.00-34
HCB (Hexachlorobenzene)	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
epsilon-HCH	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
HCH-alpha	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
HCH-beta	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
HCH-delta	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
HCH-gamma (gammexane)	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Heptachlor	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-cis	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-trans	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
o,p-DDD	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34

LUFA-ITL GmbH

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AGROLAB Laborgruppe
www.agrolab.de

Date 26.02.2009
Customer no. 1209576
Page 2 of 2

Sample No. 651329

	Unit	Result	Declaration		Substance	Method
o,p-DDE	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
o,p-DDT	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
p,p-DDD	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
p,p-DDE	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
p,p-DDT	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Methoxychlor	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Quintozene	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Tecnazene	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Tetradifon	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Nitrofen	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Organo-Phosphorous Pesticides GC-Multiresidueanalysis						
Bromophos (-methyl)	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Bromophos-ethyl	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorfenvinphos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorpyrifos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorpyrifos-methyl	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorthion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Diazinone	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Dichlorvos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Dimethoate	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Ethione	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Fenitrothione	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Fenthion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Malathion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Mecarbame	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Methidathion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Parathion-ethyl	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Parathion-methyle	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Pirimiphos-ethyle	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Pirimiphos-methyle	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Profenofos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Sulfotep	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34

Explanation: "<", n.d.: not detected, below limit of detection .
The actual limit of detection can be different to the standard value for a particular analysis due to matrix effects or insufficient sample volume.
Remark: OM=original matter, DM=dry matter

LUFA - ITL Dr. Reutter, Tel. 0431/1228-230

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ADVINUS THERAPEITICS PRIVATE LIMITED

The analytical results are valid for the delivered sample material only. The testing period is the time between the receipt of the sample and the reporting date. Validation of results is not possible for samples of unknown origin .

ANNEXURE 2. Analysis Report - Animal Diet Sample

ADVINUS THERAPEUTICS PRIVATE LIMITED
21 & 22, PEENYA INDUSTRIAL AREA, II PHASE
BANGALORE - 560 058

ANALYSIS REPORT - ANIMAL DIET SAMPLE

From: Analytical R & D Department
Advinus, Bangalore-560 058

To: Department of Safety Assessment
Advinus, Bangalore-560 058

Our Ref. No.: WC/TFS/1957 Date: 29/06/2009

Sample Details: Name : Complete diet for Rat/Mice (pellet) maintenance Sampling Date: 21/04/2009

Batch No. : 107 2008

Supplier : Ssniff Spezialdiaten, GmbH, Germany.

Manufacturer: Ssniff Spezialdiaten, GmbH, Germany

ANALYSIS RESULTS
(Analysis on "as is basis")

Sl. No.	Parameter	(%)
1.	Moisture	8.5
2.	Crude protein (N x 6.25)	18.6
3.	Crude fat (Ether extract)	3.5
4.	Crude fibre	8.0
5.	Total ash	6.4
6.	Acid insoluble ash	0.8
7.	Nitrogen free extract	55.0
8.	Calcium (Ca)	1.0
9.	Phosphorus (P)	0.8

29/06/2009
Analytical R&D Department.

ANNEXURE 3. Feed Contaminant Analysis Report for Ssniff Rats/Mice Diet - Maintenance Meal

LUFA-ITL GmbH

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AGROLAB Laborgruppe
www.agrolab.de

LUFA - ITL Dr.-Hell-Str. 6, 24107 Kiel

ADVINUS THERAPEITICS PRIVATE LIMITED
PEENYA INDUSTRIAL AREA
0 BANGALORE-560 058
INDIEN

Date 26.02.2009
Customer no. 1209576

TEST REPORT

Sample No. 651324

Order No. **582713**
Sample Arrival **11.02.2009**
Sample code **Sample 1 Complete diet for rats/mice-Maintenance pellet**
data of sampling: 21.01.2009
batch-no.:8291274
Sample packing **plastic bag**

	Unit	Result	Declaration		Substance	Method
Mycotoxins						
Aflatoxine B1	µg/kg	<1,0			OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine B2	µg/kg	<1,0			OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G1	µg/kg	<1,0			OM	HPLC-VDLUFA Bd. III, 16.1.4
Aflatoxine G2	µg/kg	<1,0			OM	HPLC-VDLUFA Bd. III, 16.1.4
PCB						
PCB 28	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
PCB 52	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
PCB 101	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
PCB 118	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
PCB 138	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
PCB 153	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
PCB 180	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Organochlorous-Pesticides GC-Multiresidueanalysis						
Aldrin	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Dieldrin	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Endrin	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Chlorodane alpha	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Chlorodane gamma	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Chlorodane-oxy	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Endosulfan alpha	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Endosulfan beta	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Endosulfansulfat	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
HCB (Hexachlorobenzene)	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
epsilon-HCH	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
HCH-alpha	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
HCH-beta	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
HCH-delta	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
HCH-gamma (gammexane)	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Heptachlor	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-cis	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
Heptachlorepoxide-trans	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34
o,p-DDD	mg/kg	<0,005			OM	acc. to §64 LFGB L00.00-34

LUFA-ITL GmbH

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AGROLAB Laborgruppe
www.agrolab.de

Date 26.02.2009
Customer no. 1209576
Page 2 of 2

Sample No. 651324

	Unit	Result	Declaration		Substance	Method
o,p-DDE	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
o,p-DDT	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
p,p-DDD	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
p,p-DDE	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
p,p-DDT	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Methoxychlor	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Quintozene	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Tecnazene	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Tetradifon	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Nitrofen	mg/kg	**<0,005**			OM	acc. to §64 LFGB L00.00-34
Organo-Phosphorous Pesticides GC-Multiresidueanalysis						
Bromophos (-methyl)	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Bromophos-ethyl	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorfenvinphos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorpyrifos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorpyrifos-methyl	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Chlorthion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Diazinone	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Dichlorvos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Dimethoate	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Ethione	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Fenitrothione	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Fenthion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Malathion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Mecarbame	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Methidathion	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Parathion-ethyl	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Parathion-methyle	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Pirimiphos-ethyle	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Pirimiphos-methyle	mg/kg	**0,44**			OM	acc. to §64 LFGB L00.00-34
Profenofos	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34
Sulfotep	mg/kg	**<0,010**			OM	acc. to §64 LFGB L00.00-34

Explanation: "<", n.d.: not detected, below limit of detection .
The actual limit of detection can be different to the standard value for a particular analysis due to matrix effects or insufficient sample volume.
Remark: OM=original matter, DM=dry matter

LUFA - ITL Dr. Reutter, Tel. 0431/1228-230

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ADVINUS THERAPEITICS PRIVATE LIMITED

The analytical results are valid for the delivered sample material only. The testing period is the time between the receipt of the sample and the reporting date. Validation of results is not possible for samples of unknown origin .

ANNEXURE 4. Analysis Report - Water Sample

ADVINUS THERAPEUTICS PRIVATE LIMITED
21 & 22, PEENYA INDUSTRIAL AREA, II PHASE
BANGALORE 560 058

ANALYSIS REPORT - WATER SAMPLE

From: Analytical R&D Department
Advinus, Bangalore-560 058

To: Department of Safety Assessment.
Advinus, Bangalore-560 058

Our Ref. No: WC/TWS/237 Date: 10/08/2009

Sample Details: Source of Collection: Outlet of the Aquaguard (At use point)

Date of Collection: 10/07/2009

ANALYSIS RESULTS

Sl. No.	Parameter	Content
1.	Colour	Colourless
2.	Odour	Odourless
3.	Turbidity	Clear
4.	PH	7.81
5.	Electrical Conductivity, dSm^{-1}	1.476
6.	Total solids, (ppm)	935
7.	Suspended solids, (ppm)	12
8.	Dissolved solids, (ppm)	923
9.	Dissolved oxygen (ppm)	4.4
10.	Biochemical Oxygen Demand 5 days at 20°C, (ppm)	1.8
11.	Chemical Oxygen Demand (ppm)	8.2

Sl. No.	Parameter	Content (ppm)
12.	Total hardness as $CaCO_3$	213
13.	Calcium as Ca^{2+}	34
14.	Magnesium as Mg^{2+}	31
15.	Chlorides as Cl^-	297
16.	Sulphates as SO_4^{2-}	74
17.	Carbonates as CO_3^{2-}	NIL
18.	Bicarbonates as HCO_3^-	378
19.	Sodium as Na	152
20.	Potassium as K	2.5

10/08/2009
Analytical R&D Department

ANNEXURE 5. Contaminant Analysis Report for Water Sample

UCL

UCL Umwelt Control Labor GmbH · Postfach 2063 · 44510 Lünen

ADVINUS THERAPEUTICS PRIVATE LIMITED
- Herr Dr. Shivaram -
No. 21&22 Phasel 2 Peenya Industrial Area
560058 BANGALORE
INDIEN

Wertemitteilung

Auftragsnummer	:	09-02877
Verantwortlicher	:	Dipl.-Chem. Jelena Spanig
Telefon	:	02306/2409-9302
Freigabe Bericht	:	19.02.2009
Prüfzeitraum	:	12.02.2009 - 19.02.2009
Berichtsnummer	:	09-02877/1

Water sample for analysis — Proben-Nr.: 09-02877-001
Water — Eingangsdatum: 12.02.2009

Analysenparameter	Einheit	Ergebnis	Best. - Grenze	Methode
PCB				
PCB-028	µg/l	n.n.	0,02	DIN 38407 F2
PCB-052	µg/l	n.n.	0,02	DIN 38407 F2
PCB-101	µg/l	n.n.	0,02	DIN 38407 F2
PCB-138	µg/l	n.n.	0,02	DIN 38407 F2
PCB-153	µg/l	n.n.	0,02	DIN 38407 F2
PCB-180	µg/l	n.n.	0,02	DIN 38407 F2
Summe PCB 028-180	µg/l	n.n.	0,02	DIN 38407 F2
PCB ges.	µg/l	n.n.	0,1	DIN 38407 F2
Organochlorpestizide				
Hexachlorbenzol (HCB)	µg/l	< 0,001		DIN 38407 Teil 2,FV
Aldrin	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
o,p-DDD	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
p,p-DDD	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
o,p-DDE	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
p,p-DDE	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
o,p-DDT	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
p,p-DDT	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
Dieldrin	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
alpha-Endosulfan	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
beta-Endosulfan	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
Endrin	µg/l	< 0,001		DIN EN ISO 11369 F12,FV
alpha-HCH	µg/l	< 0,001		DIN 38407 - F2,FV

20090424-1858265

ANNEXURE 5 contd. Contaminant Analysis Report for Water Sample

UCL

Water sample for analysis				Proben-Nr.:	09-02877-001
Water				Eingangsdatum:	12.02.2009
Analysenparameter	Einheit	Ergebnis	Best. - Grenze	Methode	
Organochlorpestizide					
beta-HCH	µg/l	< 0,001		DIN 38407 - F2,FV	
gamma-HCH (Lindan)	µg/l	< 0,001		DIN 38407 - F2, FV	
delta-HCH	µg/l	< 0,001		DIN 38407 - F2,FV	
Heptachlor	µg/l	< 0,001		DIN EN ISO 11369 F12,FV	
cis-Heptachlorepoxid	µg/l	< 0,001		DIN EN ISO 11369 F12,FV	
trans-Heptachlorepoxid	µg/l	< 0,001		DIN EN ISO 11369 F12,FV	
Methoxychlor	µg/l	< 0,001		DIN EN ISO 11369 F12,FV	

n.n. = kleiner Bestimmungsgrenze n.b. = nicht bestimmbar - = nicht bestimmt ° = nicht akkreditiert FV = Fremdvergabe

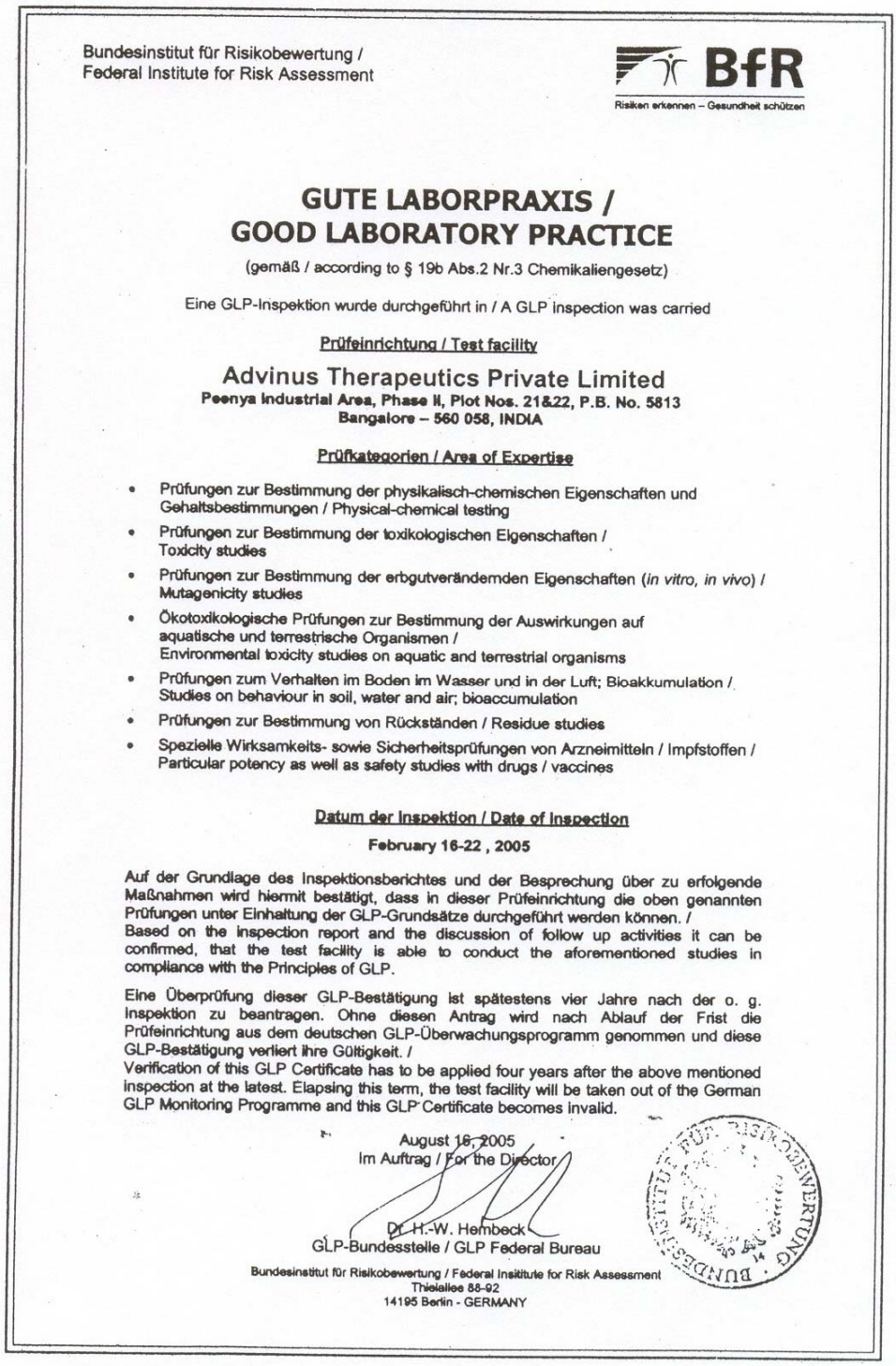
Mit freundlichen Grüßen

UCL GmbH

i. A. Cordula Althaus

Dipl.-Lab.Chem. Cordula Althaus

ANNEXURE 6. GLP Certificate – Germany



Bundesinstitut für Risikobewertung /
Federal Institute for Risk Assessment

BfR
Risiken erkennen – Gesundheit schützen

GUTE LABORPRAXIS / GOOD LABORATORY PRACTICE

(gemäß / according to § 19b Abs.2 Nr.3 Chemikaliengesetz)

Eine GLP-Inspektion wurde durchgeführt in / A GLP inspection was carried

Prüfeinrichtung / Test facility

Advinus Therapeutics Private Limited
Peenya Industrial Area, Phase II, Plot Nos. 21&22, P.B. No. 5813
Bangalore – 560 058, INDIA

Prüfkategorien / Area of Expertise

- Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen / Physical-chemical testing
- Prüfungen zur Bestimmung der toxikologischen Eigenschaften / Toxicity studies
- Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (*in vitro, in vivo*) / Mutagenicity studies
- Ökotoxikologische Prüfungen zur Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen / Environmental toxicity studies on aquatic and terrestrial organisms
- Prüfungen zum Verhalten im Boden im Wasser und in der Luft; Bioakkumulation / Studies on behaviour in soil, water and air; bioaccumulation
- Prüfungen zur Bestimmung von Rückständen / Residue studies
- Spezielle Wirksamkeits- sowie Sicherheitsprüfungen von Arzneimitteln / Impfstoffen / Particular potency as well as safety studies with drugs / vaccines

Datum der Inspektion / Date of Inspection

February 16-22 , 2005

Auf der Grundlage des Inspektionsberichtes und der Besprechung über zu erfolgende Maßnahmen wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können. /
Based on the inspection report and the discussion of follow up activities it can be confirmed, that the test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Eine Überprüfung dieser GLP-Bestätigung ist spätestens vier Jahre nach der o. g. Inspektion zu beantragen. Ohne diesen Antrag wird nach Ablauf der Frist die Prüfeinrichtung aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bestätigung verliert ihre Gültigkeit. /
Verification of this GLP Certificate has to be applied four years after the above mentioned inspection at the latest. Elapsing this term, the test facility will be taken out of the German GLP Monitoring Programme and this GLP Certificate becomes invalid.

August 16, 2005
Im Auftrag / For the Director

Dr. H.-W. Hembeck
GLP-Bundesstelle / GLP Federal Bureau

Bundesinstitut für Risikobewertung / Federal Institute for Risk Assessment
Thielallee 88-92
14195 Berlin - GERMANY

ANNEXURE 7. GLP Certificate – The Netherlands

voedsel en waren autoriteit

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF
GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 4 – 7 May 2009 at

Advinus Therapeutics Private Limited
21 & 22 Phase II, Peenya Industrial Area
Bangalore - 560 058 INDIA

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: physical-chemical testing, toxicity studies, mutagenicity studies, environmental studies on aquatic and terrestrial animals, and analytical and clinical chemistry.

The Hague, 27 August 2009

Dr Th. Helder, DVM
Manager GLP Compliance Monitoring Program

Food and Consumer Product Safety Authority (VWA)
Prinses Beatrixlaan 2, 2595 AL Den Haag
Postbus 19506, 2500 CM Den Haag, The Netherlands

ANNEXURE 8. GLP Certificate – India

सत्यमेव जयते

NATIONAL GLP COMPLIANCE MONITORING AUTHORITY

GOOD LABORATORY PRACTICE

GLP CERTIFICATE

GLP Inspection was carried out at **Advinus Therapeutics Private Limited, Plot Nos. 21 & 22, Phase-II , Post Box No. 5813, Peenya Industrial Area, Bangalore-560 058, India** in the following areas of expertise:

- **physical-chemical testing**
- **toxicity studies**
- **mutagenicity studies**
- **environmental toxicity studies on aquatic & terrestrial organisms**
- **studies on behaviour in water, soil and air, bioaccumulation**
- **residue studies**
- **analytical and clinical chemistry testing**

Based on the Inspection Report and the follow-up actions taken by the test facility, it is confirmed that the test facility is capable of conducting the above-mentioned tests/studies in compliance with **OECD Principles of Good Laboratory Practice (GLP) and Norms**, as adopted by the National GLP Compliance Monitoring Authority.

This GLP Certificate is valid for a period of **three years** from November 7, 2008 subject to the condition that the test facility complies with the **Terms & Conditions of the National GLP Compliance Monitoring Authority's Document Number GLP-101.**

Certificate No.: GLP/C-019

Issue Date : 24-12-2008

(R.SAHA)
Head
National GLP Compliance Monitoring Authority
Department of Science & Technology
Technology Bhavan New Delhi-110016

Annex 1 Certificate of Analysis of enzyme preparation

Certificate of Analysis of amylomaltase from *Bacillus amyloliquefaciens* updated with analytical data from GLP studies, TNO report V8791

DSM Food Specialties B.V.
R&D/RFG

DSM

CERTIFICATE OF ANALYSIS

Name of the product	**Meltamase**
Batch no	MEG GRZ 0905
Status	ocUF for toxicity study
Date of manufacture	February 2009
Date of expiration	12 months (provisionally) , extended 4 months
Active component	Amylomaltase or 4-α-glucanotransferase
Date of issue	29 October 2009, updated 17 August 2010

Parameter	Method	Unit	Result
	Characterization data		
Amylomaltase activity	B1903	ATU / g	2130
Dry Matter	60405	% (w/w)	6.70
Ash	60328	% (w/w)	1.26
TOS	Calculation	% (w/w)	5.50
Proteins by Kjeldahl Nitrogen x 6.25	61804	% (w/w)	2.20
	Stability data; > 90% residual activity		
Stability at 4°C, undiluted	B1903	Days	7
Stability at 4°C, 91 mg / ml	B1903	Days	7
Stability at 4°C, 273 mg / ml	B1903	Days	7
Stability at 4°C, 909 mg / ml	B1903	Days	7
Stability at RT, undiluted	B1903	Hours	4
Stability at RT, 91 mg / ml	B1903	Hours	4
Stability at RT, 273 mg / ml	B1903	Hours	4
Stability at RT, 909 mg / ml	B1903	Hours	4

Signature R&D QESH:	Remarks (if any): CoA drafted for toxicity study. Analyses performed under GLP