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REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *ASPERGILLUS NIGER* (GEP44) IN WISTAR RATS

CRO REPORT NUMBER 3715/03

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Signature author: PM Satish, Rallis Research Centre, Banalore, India Date manuscript August 21, 2003	Signature department manager:

RALLIS RESEARCH CENTRE
Peenya, Bangalore - 560 058.

REPORT

(COPY No. 2/3)

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

TEST ITEM: ENZYME PREPARATION OF *Aspergillus niger* (GEP44)

STUDY No.: 3715/03

STUDY DIRECTOR AND AUTHOR: Mr.P.M.SATHISH

SPONSORED BY

DSM FOOD SPECIALTIES
A. FLEMINGLAAN 1
P.O. BOX 1, 2600MA DELFT
THE NETHERLANDS

TEST FACILITY

TOXICOLOGY DEPARTMENT
RALLIS RESEARCH CENTRE
RALLIS INDIA LIMITED
POST BOX No. 5813, PLOT Nos. 21 & 22
PEENYA II PHASE, BANGALORE - 560 058
INDIA

DATE OF SUBMISSION: AUGUST 21, 2003

TABLE OF CONTENTS

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TABLE OF CONTENTS contd. **PAGE**

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TABLE OF CONTENTS contd.

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TABLE OF CONTENTS contd.

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TABLE OF CONTENTS contd.

PAGE

RALLIS RESEARCH CENTRE
Peenya, Bangalore - 560 058.

QUALITY ASSURANCE STATEMENT

The Study No.: 3715/03, entitled "Repeated dose (14-day) oral toxicity study by gavage with enzyme preparation of *Aspergillus niger* (GEP44) in Wistar rats" has been inspected in accordance with the OECD Principles of Good Laboratory Practice (GLP) for the testing of chemicals [OECD, {C(97)186/Final} [ENV/MC/CHEM(98)/17] adopted on 26th November, 1997].

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

INSPECTIONS DATE	PHASE	REPORTING DATE
	INITIATION PHASE	
23.06.2003	Study plan review	23.06.2003
	IN LIFE PHASE	
30.06.2003	Acclimatization	07.07.2003
04.07.2003	Test item preparation, initial body weight, veterinary examination, food input and test item administration as gavage	07.07.2003
19.07.2003	Terminal sacrifice	21.07.2003

contd.

QUALITY ASSURANCE STATEMENT contd.

INSPECTIONS DATE	PHASE	REPORTING DATE
	REPORTING PHASE	
04.08.2003 to 11.08.2003	Report Review - I	11.08.2003
18.08.2003 and 19.08.2003	Report Review - II	19.08.2003

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: 20/08/2003

Satish Murthy. V

(Mr.SATISH MURTHY. V)
Head, Quality Assurance Unit
Rallis Research Centre, Bangalore

STATEMENT OF CONFIDENTIALITY

The report contains **confidential** and **proprietary** information of DSM Food Specialties, A. Fleminglaan 1, P.O. Box 1, 2600MA Delft, THE NETHERLANDS, which would 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, A. Fleminglaan 1, P.O. Box 1, 2600MA Delft, THE NETHERLANDS.

STATEMENT OF GLP COMPLIANCE

The study was performed in compliance with the OECD Principles of Good Laboratory Practice (GLP) for the testing of chemicals as specified by EU Legislation (enacted in the German Chemical Law, dated July 25, 1994 Appendix 1 to § 19a, Bundesgesetzblatt, Part I of July 29, 1994) and International [OECD, {C(97) 186/Final} [ENV/MC/CHEM(98)/17] adopted on 26th November, 1997] Legislation and also in accordance with the Standard Operating Procedures and the Study Plan.

This study was conducted as per the OECD Guideline No. 407 for testing of chemicals, "Repeated Dose 28-day Oral Toxicity Study in Rodents" adopted on July 27, 1995 and in compliance with Annex to Commission Directive 96/54/EEC of July 30, 1996, Part B. Methods for the determination of toxicity: B.7. "Repeated Dose (28 Days) Toxicity (Oral)" with suitable modifications to meet the sponsors requirement and objective of the study. However, this study is a 14-day pre-study which did not include recovery groups for the control and high dose groups. Further clinical examination, haematology, clinical chemistry and histopathology had also not been done. However, tissue samples have been stored for histopathological examination if required. This pre-study is to select suitable doses for a subsequent 90-day toxicity study in rats. This study was also conducted as per the mutually agreed Study Plan signed by Study Director on 26.06.2003 and by Monitoring Scientist on 01.07.2003.

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 might 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 accept overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

Date: 20/08/2003

(Mr.P.M.SATHISH)
Study Director

STUDY DETAILS

Study Title	:	Repeated dose (14-day) oral toxicity study by gavage with enzyme preparation of *Aspergillus niger* (GEP44) in Wistar rats
Test Item	:	Enzyme preparation of *Aspergillus niger* (GEP44)
Study Number	:	3715/03
Study Director	:	Mr.P.M.Sathish
Sponsor	:	DSM Food Specialties A. Fleminglaan 1 Pobox 1 2600MA Delft THE NETHERLANDS
Monitoring Scientist	:	Dr.Ir Mariëlla Kuilman-Wahls DSM Food Specialties Regulatory Affairs Postpoint 001-0490 P.O.Box 1, 2600MA Delft THE NETHERLANDS
Test Facility	:	Toxicology Department Rallis Research Centre Rallis India Limited Post Box No. 5813, Plot Nos. 21 & 22 Peenya II Phase Bangalore - 560 058, INDIA
Experimental period		
Acclimatization	:	Start: 29-06-2003 End: 03-07-2003
First day of treatment	:	04-07-2003
Last day of treatment	:	17-07-2003
Date of sacrifice	:	19-07-2003

RALLIS RESEARCH CENTRE
Peenya, Bangalore - 560 058.

STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Signature	Date
Mr.P.M.SATHISH M.Sc., Study Director Chronic Toxicity Section		20/08/03
Mr.B.N.VISHWANATH M.Sc., Technical Co-ordinator Chronic Toxicity Section		20/08/03
Dr.S.GANIGER B.V.Sc., Study veterinarian Chronic Toxicity Section		20/08/03
Dr.B.S.MADHUKAR B.V.Sc., Necropsy and Histotechniques Histopathology Section		20/8/03
Mr. H.S.ANAND M.Sc.,(Agri), Analyst Residue/Analytical Department		20/08/2003
Mr.M.VENKATESULU B.Sc, Data entry, Data analyses and Report compilation EDP Section		20.8.03

LIST OF COMMONLY USED ABBREVIATIONS AND SYMBOLS

App	Appendix / Appendices	NA	Not Applicable
		NAD	No Abnormality Detected
Bwt	Body weight		
		No.	Number
cm	centimetre		
contd.	continued	Ref	Reference
Epididym	Epididymides	s	seconds
		SD	Standard Deviation
F	Female		
		U	Units
g	gram		
G.	Group	V	Volume
kg	kilogram	W	Width
		%	per cent
M	Male	°C	Degree centigrade

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY

Enzyme preparation of *Aspergillus niger* (GEP44) was tested for its toxic potential in a 14-day repeated dose oral toxicity study in Wistar rats. The test item was dissolved in double distilled water and administered by oral gavage at doses of 2000 and 7000 mg/kg Bwt/day to low (G2) and mid (G3) dose groups of rats. For high dose (G4) group rats, the undiluted test item was administered at a dose volume of 18.54 ml/kg Bwt/day which corresponds to a dose of 20000 mg/kg Bwt/day. .

The concurrent control group (G1) received double distilled water without the test item. 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. The results of the stability of the test item were provided by the sponsor before start of the treatment. The gavage solutions were analysed for protein content twice i.e. on the initial day of the treatment and on 8th day of the treatment.

All rats were observed for clinical signs, eye affections, physical abnormalities, changes in body weights, food consumption and pre-terminal deaths. The rats were subjected to detailed necropsy at terminal sacrifice.

Under the experimental conditions described in the material and method section, the following results were obtained:

A. SUMMARY OF RESULTS

1. Veterinary and Ophthalmological examinations, Clinical signs and Pre-terminal deaths

   Veterinary and ophthalmological examination did not reveal any abnormalities. No treatment related clinical signs were observed in any of the tested doses and there were no pre-terminal deaths.

2. Body weights and cumulative net weight gains

   There were no significant changes in the body weights and net body weight gains at any of the tested doses in both the sexes.

3. Food consumption

   There were no significant changes in the food consumption at any of the tested doses in both the sexes.

4. Terminal fasting body weights, organ weights and organ weight ratios

   There were no treatment related changes in fasting body weights, organ weights and their ratios to body weights at any of the tested doses in both the sexes.

5. Gross pathology

   No treatment related gross pathological changes were observed at any of the tested doses in both the sexes.

B. NO OBSERVED EFFECT LEVEL (NOEL)

The results of this study indicate that the oral administration of Enzyme preparation of *Aspergillus niger* (GEP44) in Wistar rats at concentrations of 2000, 7000 and 20000 mg/kg Bwt/day did not reveal any effect on general health, growth and food consumption. No treatment related changes were observed in the fasting body weights, organ weights and their ratios and gross pathology.

In light of the results discussed above, as no changes of toxicological significance were observed among all the animals that received a concentration of 20000 mg/kg Bwt/day, this level is considered to be the No Observed Effect Level (NOEL) of Enzyme preparation of *Aspergillus niger* (GEP44) in Wistar rats under the test conditions and doses employed.

Date: 26|08|2003

(Mr.P.M.SATHISH)
Study Director

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INTRODUCTION

The purpose of this Repeated Dose (14-day) Oral Toxicity Study was to assess the systemic toxic potential of the test item when administered by gavage to rats and also to provide information for selection of dose levels for a subsequent 90-day gavage study. The animals were observed during the entire experimental schedule.

MATERIAL AND METHOD

1. TEST SPECIES : HsdCpb: WU rats conventionally bred (in-house random bred)

Source : Toxicology Department
Rallis Research Centre
Rallis India Limited
Bangalore - 560 058, INDIA

No. of groups : Four groups; vehicle control, low, mid and high dose groups

No. of rats/group : Twelve rats (6 males and 6 females)

Age of rats at the start of the treatment : 7 weeks

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

	Males	Females
G1:	221 ± 10.5	158 ± 12.6
G2:	218 ± 8.3	161 ± 13.6
G3:	220 ± 11.1	158 ± 11.8
G4:	220 ± 8.4	158 ± 13.3

Identification	:	By accession number, cage card and crystal violet solution body marking.
Acclimatization	:	After veterinary examination, for good health and the suitability for the study, the rats were acclimatized for five days before the start of the treatment. Females used in the study were nulliparous and non-pregnant. During acclimatization animals were temporarily identified using turmeric solution body marking.

2. HUSBANDRY

Room number: Subchronic laboratory room No. SC - 16.

Conditions:

Rats were housed under standard laboratory conditions; air conditioned with 12 - 15 filtered fresh air changes per hour. The maximum and minimum temperature in the experimental room was recorded once daily in the morning hours and it ranged from : 21 - 24°C; relative humidity in the experimental room was calculated once daily from dry and wet bulb temperature recordings and it ranged from 30 - 70%. Further the experimental room had 12 hour fluorescent light and 12 hour dark cycle.

Housing:

Two rats of the same sex were housed in a sterilized suspended polypropylene rat cage (size: L 410 mm x W 282 mm x H 150 mm) with stainless steel mesh bottom and stainless steel top grill having facilities for holding pellet food and for drinking water in a glass bottle with stainless steel sipper tube.

Food:

Ssniff rats/mice pellet food - maintenance meal - low in germs manufactured by Ssniff Spezialdiäten GmbH, Ferdinand-Gabriel-Weg 16, D-59494 Söest, GERMANY was provided ad libitum. Animal diet sample analysis report and feed contaminant analysis report for Ssniff rats/mice diet - maintenance meal are given in Annexures 1 and 2, respectively.

Water:

Protected water: Deep borewell 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 in collaboration with Electrolux, SWEDEN) was provided in glass bottles with stainless steel sipper tube ad libitum. The analysis report and contaminant analysis report for water samples are given in Annexures 3 and 4, respectively.

3. GROUPING

Grouping was done on the last day of the acclimatization period. Grouping was by in-house method of body weight stratification and distribution as follows: the rats procured for the study were weighed and grouped into the following body weight ranges (The selected ranges were : 191 – 220 g for males and 131 – 170 g for females). These body weight stratified rats were distributed to all the study groups in equal numbers. The rats with extreme body weights were discarded and the mode of disposal was recorded in the raw data.

DOSE LEVELS

Three dose levels 2000, 7000 and 20000 mg/kg Bwt/day had been selected for the study in consultation with the sponsor. In addition to the test doses, a concurrent control group was also included. Rats in the control group were handled in a manner similar to the treatment groups, however no test item was administered and only vehicle was administered.

GROUP ALLOCATION AND NUMBER OF ANIMALS

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

Group No.	Group	Colour of cage card	Dose (mg/kg Bwt/day)	No. of rats	Sex	Rat Numbers	
						From	To
G1	Vehicle control	White	0	6	M	Rf2261	Rf2266
				6	F	Rf2285	Rf2290
G2	Low dose	Yellow	2000	6	M	Rf2267	Rf2272
				6	F	Rf2291	Rf2296
G3	Mid dose	Green	7000	6	M	Rf2273	Rf2278
				6	F	Rf2297	Rf2302
G4	High dose	Pink	20000	6	M	Rf2279	Rf2284
				6	F	Rf2303	Rf2308

ROUTE OF ADMINISTRATION

Oral through gavage, dose was expressed as mg/kg Bwt/day.

TEST ITEM INFORMATION: (as furnished by the study sponsor)

Common name (active ingredient)	:	Prolyl-oligo peptidase
Chemical name (IUPAC)	:	Enzyme protein
Name to be used in report	:	Enzyme preparation of *Aspergillus niger* (GEP44)
Code by test facility	:	052/7-GEP44
Batch No.	:	JLL03006IDF
Manufactured by	:	DSM Food Specialties 15, Rue des Comtesses Pobox 239 59472 Seclin Cedex FRANCE
Supplied by	:	DSM Food Specialties A. Fleminglaan 1 Pobox 1 2600MA Delft THE NETHERLANDS
Date of manufacture	:	March 2003
Date of expiry	:	1 Year after production (March 2004)
Date of receipt at test facility	:	14-06-2003
Storage conditions	:	Deep freezer (-68°C to -76°C)
Physical appearance	:	Dark brown liquid
Hazards and precautions	:	Material may be sensitizing by inhalation, so avoid skin and inhalatory contact

ANALYSES OF THE TEST ITEM

1. 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 of the test item rests with the sponsor.

2. Stability of the test item

As per the certificate of analysis provided by the sponsor, the undiluted test item and the test item in water at concentrations of 100 mg/ml and 350 mg/ml is stable at 4°C for 7 days and also at room temperature (21°C) for 48 hours.

3. Gavage sample analysis

Gavage solutions on days 1 and 8 were analysed for protein content by Micro-Kjeldahl method. In brief, 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 sulphuric acid and digestion mixture. A known volume of digested sample was distilled by using Micro-Kjeldahl distillation unit and the 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.

4. Test item preparation and administration

The test item solutions were prepared on first day of the treatment and at 3 – 4 day intervals thereafter (within the stability period). The volume of test item was measured based on density (density: 1.0785 g/cm^3). To prepare the test item solution, approximate quantities of 40 g (G2: 37.1 ml) and 140 g (G3: 129.8 ml) of test item were separately measured and volume of G2 and G3 was made up to 400 ml with double distilled water to get the test item concentrations of 100 mg (G2) and 350 mg (G3)/ml respectively.

For Group G4, the undiluted test item was administered at a dose volume of 18.54 ml/kg Bwt/day. The dose volume was calculated for individual animals on the first day of the treatment and the same was adjusted according to the body weights recorded during different intervals of the treatment period.

The prepared test item solutions for groups G2 and G3 were made into required number of aliquots depending on daily requirement. The remaining aliquots were stored lower than 4°C and these were used daily. The required volume of test item for G4 was made into different aliquots depending on daily requirement. The prepared aliquots for G4 were stored lower than 4°C and these were used daily. The prepared test item solutions and the undiluted test item were stored in deep freezer in Chronic toxicity facility. Control animals were administered double distilled water at an equivolume dose of 20.0 ml/kg Bwt/day. 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. The difference between the nominal and actual concentrations had not exceed ± 5%. Test item was administered at an equivolume dose of 20.0 ml/kg Bwt/day except for G4 which was administered at a dose volume of 18.54 ml/kg Bwt/day.

TREATMENT

The test item solutions were administered to the specific group rats once daily at approximately the same time (± 1 hour) each day once for 14 consecutive days. Similarly, the double distilled water (vehicle) was administered to vehicle control rats for 14 consecutive days at an equivolume dose (20.0 ml/kg Bwt/day). When the total dose volume exceeded the capacity of the syringe (3 ml), then the volume was administered in two portions.

OBSERVATIONS

1. VETERINARY AND OPHTHALMOLOGICAL EXAMINATIONS, CLINICAL SIGNS AND PRE-TERMINAL DEATHS:

a. Veterinary examination:

Veterinary examination was carried out prior to test item administration and at weekly intervals.

b. Ophthalmological examination:

Ophthalmological examination was carried out with an ophthalmoscope before start of the treatment and at the end of the treatment for all animals prior to sacrifice. Mydriasis was induced before examination using 1% Tropicamide solution.

c. Clinical signs and pre-terminal deaths:

Rats were observed for clinical signs of toxicity once daily and for pre-terminal deaths twice daily.

2. BODY WEIGHTS:

Individual body weights were recorded at the beginning (on day 1) and on days 5, 8, 12 and 15 of the treatment period.

3. FOOD INTAKE:

The following method was adopted for the measurement of weekly food consumption:

I. Day 1[@] Food input: 400 g Food output on Day 5

II. Day 5 Food input: 400 g Food output on Day 8

[@]: Day 1 denotes food input at the start of each week.

The weekly cagewise food consumption was calculated by adding the food consumed in I (4 days) and II (3 days) and the total divided by the number of rats per cage to determine the food intake/rat/week. The visual estimation of the food spillage by the animals was determined and the recorded food spillage data was taken into consideration (i.e., the food spillage data/cage/week was added to food output data) in the calculation of the weekly food consumption. The weekly food consumption was divided by the number of days (7) and the food consumption was expressed as g/rat/day. This was repeated during the second week of the treatment period.

4. PATHOLOGY:

a. Gross necropsy:

All rats in the study were subjected to gross necropsy and the findings were recorded. The rats to be sacrificed at term were fasted overnight (water allowed), anaesthetised as per random numbers generated for the study, weighed and exsanguinated and were subjected to detailed necropsy by a pathologist.

b. Organ weights:

The following organs were collected and weighed: thymus, epididymides, brain, heart, adrenals, gonads, spleen, liver and kidneys. The organ weight ratios as percentage of body weights were determined and presented in the report.

The following organs and tissues were preserved in 10% buffered neutral formalin and archived:

1. All gross lesions
2. Brain (Cerebrum, Cerebellum and Pons)
3. Spinal cord
4. Stomach
5. Duodenum
6. Jejunum
7. Ileum (with Peyer's patches)
8. Cecum
9. Colon
10. Rectum
11. Liver
12. Kidneys
13. Adrenals
14. Spleen
15. Heart
16. Thymus
17. Thyroid
18. Trachea
19. Lungs#
20. Testes
21. Epididymides
22 Ovaries
23. Uterus
24. Seminal vesicles
25. Coagulating glands
26. Prostate
27. Urinary bladder
28. Mandibular lymph node
29. Mesenteric lymph node
30. Sciatic nerves
31. Bone marrow(Femoral bone)
32. Axillary lymph node

#: Inflated with fixative and then immersed in formalin.
Note: Histopathological examination was not performed.

STATISTICAL ANALYSES

Using specific computer programmes, body weights, food consumption, organ weights and organ weight ratios data were compared by Bartlett's test for homogeneity of intra group variances. When the variances proved to be heterogeneous, the data were transformed using appropriate transformation. The data with homogeneous intragroup variances were subjected to one-way analyses of variance (ANOVA - Snedecor and Cochran, 1987).

Following ANOVA, when 'F' value was found significant, Dunnett's pairwise comparison (Scheffe, 1953) of means of treated groups with control group mean was done individually. Following a significant difference of a test group from the control group, the Dose response correlation was estimated by including the control and all the treated groups by employing 't' test.

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

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

The statistical analysis for clinical signs and ophthalmological examinations was not carried out as it was not needed.

References:

1. *Games, P.A. and Howell, J. K. (1976).J. Ed, Statist.I :113-125.*
2. *Scheffe, H. (1953), Biometrika, 40 : 87-104.*
3. *Snedecor,G.W. and Cochran, W.G. (1987): Statistical Methods, 7th ed.(Reprint) Iowa State University Press, Ames, IA.*
4. *Sokal, R.R. and Rohlf, F.F. (1981) : Biometry, Freeman, San Francisco.*

ARCHIVING

Rallis will archive at the archives of Rallis Research Centre, Peenya II phase, Bangalore - 560058, INDIA the following for 30 years after completion of the study: study plan, raw data and report. A sample of test item had been sent from test item stores to the archives at the time of receipt of test item. This sample shall be stored for a period of 2 years from the date of this report or till the next GLP inspection, whichever is later, however not beyond 30 years.

RESULTS AND DISCUSSION

Details of experimental layout and treatment schedule are furnished in Table 1.

A. ANALYSES OF THE TEST ITEM:

a. Identity: App. 13

The identity of the test item was provided by the sponsor by a Certificate of analysis. The responsibility for the correct identity of the test item rests with the sponsor.

b. Stability of the test item: App. 13

As per the certificate of analysis provided by the sponsor, the undiluted test item and the test item in water at concentrations of 100 mg/ml and 350 mg/ml is stable at 4°C for 7 days and also at room temperature (21°C) for 48 hours.

c. Gavage sample analysis: App. 14

The concentrations of test item in the gavage solutions were determined analytically twice during treatment period based on the protein value of test item as per the certificate of analysis provided by the sponsor. The results showed mean concentrations of 99.4 ± 0.85 and 348.7 ± 1.84 mg of the test item/ml as against the nominal concentrations of 100 and 350 mg/ml for G2 and G3 groups respectively and for G4 group, the result showed a mean concentration of 1075.3 ± 2.55 mg of test item/ml.

B. IN-LIFE DATA

a. Veterinary and ophthalmological examinations, clinical signs and pre-terminal deaths : Table 2; App. 1 and 2

Veterinary examination carried out during acclimatization period did not reveal any clinical signs.

Veterinary examination carried out prior to test item administration and at weekly intervals thereafter did not reveal any treatment related clinical signs.

Incidences of hair thinning with hair regrowth were observed one each in control and mid and three in low dose group females and were considered incidental as they appeared as isolated incidences.

Ophthalmological examination carried out during acclimatization period and at the end of the treatment period did not reveal any eye abnormalities.

There were no pre-terminal deaths in any of the groups.

b. Body weights and cumulative net weight gains:
Tables 3 - 6 and App. 3 - 6, Figures 1 and 2

Males and females:

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

c. Food intake: Tables 7 and 8, App. 7 and 8, Figures 3 and 4

Males and females:

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

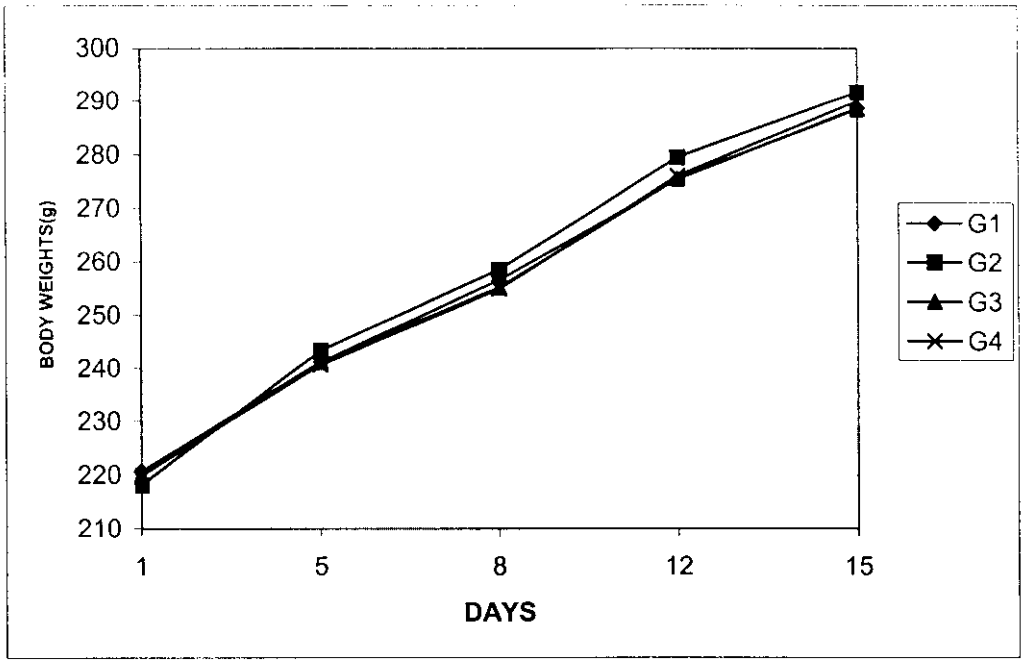

FIGURE 1: BODY WEIGHT AND GROWTH CURVES-MALES

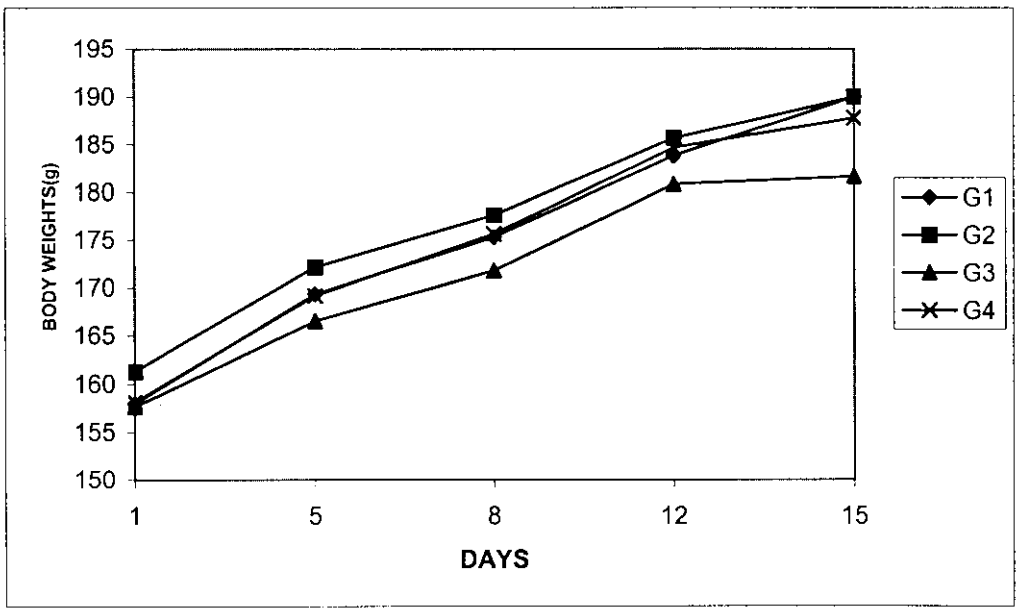

FIGURE 2: BODY WEIGHT AND GROWTH CURVES-FEMALES

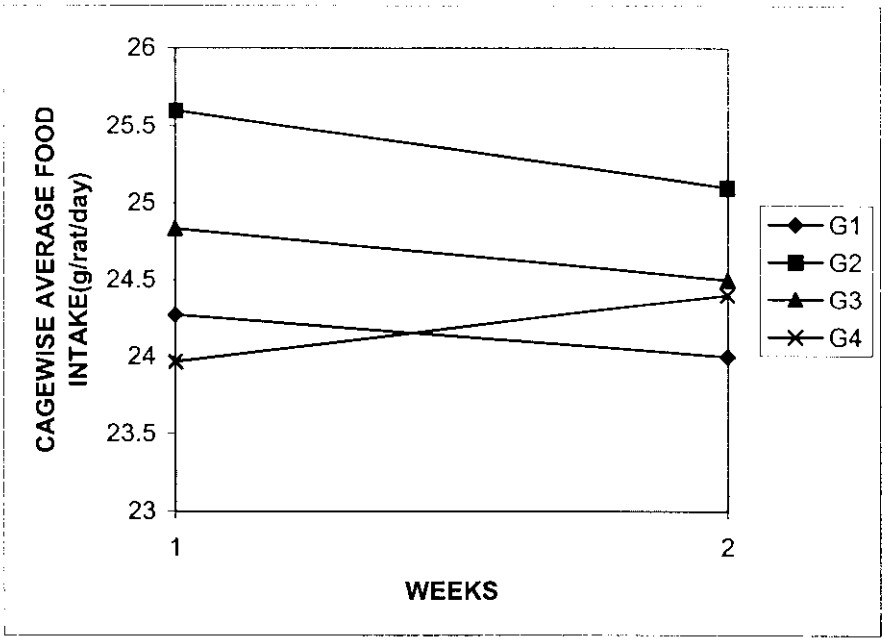

FIGURE 3: FOOD CONSUMPTION CURVES-MALES

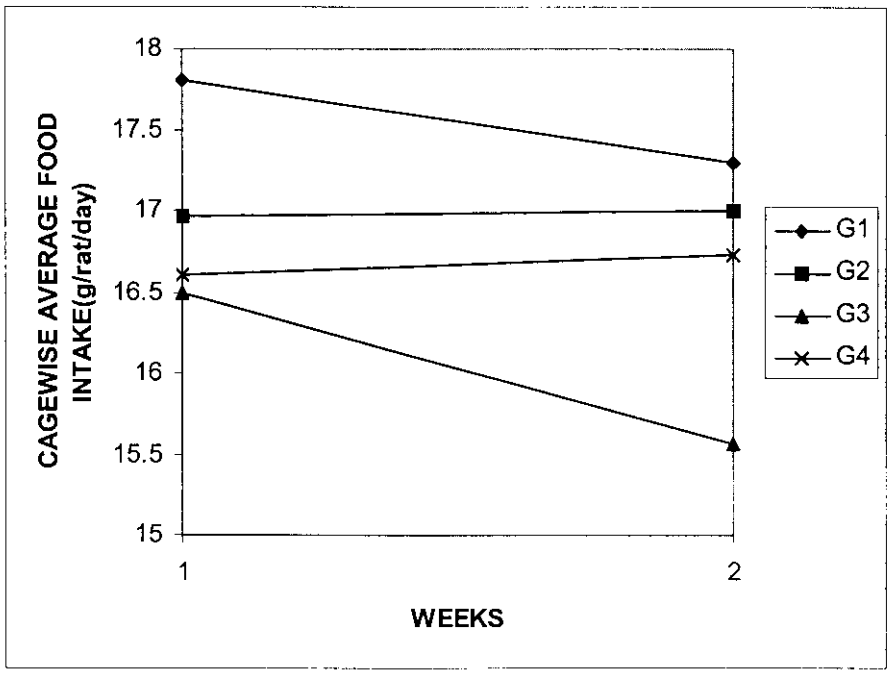

FIGURE 4: FOOD CONSUMPTION CURVES-FEMALES

d. Terminal fasting body weights, organ weights and organ weight ratios: Tables 9 and 10; App. 9 and 10

Males and females:

No treatment related changes were observed in fasting body weights, organ weights and their ratios to body weights at any of the tested doses in either sex.

e. Pathology: Table 11; App. 11 and 12

There were no treatment related gross pathological changes. Incidences of skin-hair thinning with hair regrowth were observed one in control, one in mid and three in low dose group females. Kidneys with unilateral cystic and uterus-horn (unilateral) not attached to the body in one control female and kidneys with dilated pelvis in one female at low dose were observed.

These changes were considered incidental and were not attributed to treatment.

CONCLUSION

The results of this study indicated that oral administration of Enzyme preparation of *Aspergillus niger* (GEP44) in Wistar rats at concentrations of 2000, 7000 and 20000 mg/kg Bwt/day did not reveal any effect on general health, growth and food consumption. No treatment related changes were observed in the fasting body weights, organ weights and their ratios and gross pathology.

In light of the results discussed above, as no changes of toxicological significance were noted among the animals that received a concentration of 20000 mg/kg Bwt/day, this level is considered to be the No Observed Effect Level (NOEL) of Enzyme preparation of *Aspergillus niger* (GEP44) in Wistar rats, under the test conditions and doses employed.

TABLE 1

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

DETAILS OF EXPERIMENTAL LAYOUT, TREATMENT AND SACRIFICE SCHEDULE

Group No.	Dose (mg/kg Bwt/day)	No. of rats per group		Treatment period (days)	Pathology	Sacrifice Schedule	
		Males	Females		Gross pathology and organ weights	16th day	
						Males	Females
G1	0	6	6	14	+	+	+
G2	2000	6	6	14	+	+	+
G3	7000	6	6	14	+	+	+
G4	20000	6	6	14	+	+	+

+: Yes

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TABLE 2

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF VETERINARY, OPHTHALMOLOGICAL EXAMINATIONS AND PRE-TERMINAL DEATHS DATA

Ref. App.: 1 - 2

PARAMETERS	Sex	Males				Females			
	Group No.	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (mg/kg Bwt/day)	0	2000	7000	20000	0	2000	7000	20000
	No. of rats	6	6	6	6	6	6	6	6
1. GENERAL AFFECTIONS		0	0	0	0	0	0	0	0
2. RESPIRATORY AFFECTIONS		0	0	0	0	0	0	0	0
3. EYE AFFECTIONS		0	0	0	0	0	0	0	0
4. GASTRO INTESTINAL AFFECTIONS		0	0	0	0	0	0	0	0
5. SKIN AFFECTIONS									
- Hair thinning with hair regrowth		0	0	0	0	1	3	1	0
6. UROGENITAL AFFECTIONS		0	0	0	0	0	0	0	0
7. PRE-TERMINAL DEATHS		0	0	0	0	0	0	0	0

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TABLE 3

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF BODY WEIGHTS (g) - MALES

Values: Mean ± SD Ref.App.: 3

G. No. Dose (mg/kg Bwt/day)	No. of rats	Days 1	5	8	12	15
G1	6	221	241	257	275	289
0		10.5	10.6	13.9	14.2	17.5
G2	6	218	243	259	280	292
2000		8.3	8.4	7.2	7.0	6.5
G3	6	220	241	255	276	289
7000		11.1	13.1	14.0	15.5	17.8
G4	6	220	241	255	276	290
20000		8.4	10.3	8.7	10.2	8.9

TABLE 4

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF CUMULATIVE NET BODY WEIGHT GAINS (g) - MALES

Values: Mean ± SD — Ref.App.: 4

G. No. Dose (mg/kg Bwt/day)	No. of rats	Days 5	8	12	15
G1	6	21	36	55	68
0		4.0	7.6	8.8	12.7
G2	6	25	40	61	74
2000		2.3	3.1	3.1	5.1
G3	6	21	35	56	69
7000		3.1	5.0	6.0	7.5
G4	6	21	35	56	70
20000		3.8	3.3	6.1	7.3

TABLE 5

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF BODY WEIGHTS (g) - FEMALES

Values: Mean ± SD — Ref.App.: 5

G. No. Dose (mg/kg Bwt/day)	No. of rats	Days 1	5	8	12	15
G1	6	158	169	175	184	190
0		12.6	12.2	15.7	16.1	15.4
G2	6	161	172	178	186	190
2000		13.6	12.9	13.1	14.9	15.3
G3	6	158	167	172	181	182
7000		11.8	15.0	15.3	15.0	16.4
G4	6	158	169	176	185	188
20000		13.3	13.1	13.4	13.5	13.9

TABLE 6

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF CUMULATIVE NET BODY WEIGHT GAINS (g) - FEMALES

Values: Mean ± SD Ref.App.: 6

G. No. Dose (mg/kg Bwt/day)	No. of rats	Days 5	8	12	15
G1	6	11	17	26	32
0		1.4	3.6	5.5	5.0
G2	6	11	16	24	29
2000		2.6	2.0	3.7	4.7
G3	6	9	14	23	24
7000		4.6	6.9	6.0	8.9
G4	6	11	18	27	30
20000		5.4	4.6	5.0	8.0

TABLE 7

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF CAGEWISE AVERAGE FOOD INTAKE (g/rat/day) - MALES

Values: Mean ± SD — Ref.App.: 7

G. No. Dose (mg/kg Bwt/day)	No. of cages	No. of rats / cage	Weeks 1	Weeks 2
G1 0	3	2	24.3 1.26	24.0 1.35
G2 2000	3	2	25.6 0.89	25.1 0.44
G3 7000	3	2	24.8 1.01	24.5 0.85
G4 20000	3	2	24.0 0.84	24.4 0.75

TABLE 8

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF CAGEWISE AVERAGE FOOD INTAKE (g/rat/day) - FEMALES

Values: Mean ± SD

Ref.App.: 8

G. No. Dose (mg/kg Bwt/day)	No. of cages	No. of rats / cage	Weeks 1	Weeks 2
G1 0	3	2	17.8 0.70	17.3 0.53
G2 2000	3	2	17.0 0.12	17.0 0.72
G3 7000	3	2	16.5 0.61	15.6 0.64
G4 20000	3	2	16.6 0.87	16.7 0.72

TABLE 9

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF TERMINAL FASTING BODY WEIGHTS, ORGAN WEIGHTS AND ORGAN WEIGHT RATIOS - MALES

Values: Mean ± SD

Ref.App.: 9

G. No. Dose (mg/kg Bwt/day)	No. of rats	Fasting Bwt (g)	Organ weights(g)									Organ weight ratios(%)								
			Adrenals	Testes	Kidneys	Liver	Heart	Brain	Epididym	Thymus	Spleen	Adrenals	Testes	Kidneys	Liver	Heart	Brain	Epididym	Thymus	Spleen
G1	6	264	0.048	3.318	2.124	9.101	0.999	1.869	0.885	0.645	0.640	0.018	1.263	0.808	3.456	0.380	0.711	0.337	0.246	0.244
0		21.92	0.008	0.295	0.163	0.798	0.077	0.077	0.115	0.137	0.073	0.003	0.166	0.095	0.363	0.041	0.071	0.057	0.056	0.039
G2	6	278	0.046	3.258	2.184	9.235	1.022	1.843	0.838	0.646	0.601	0.017	1.171	0.786	3.322	0.367	0.664	0.301	0.232	0.216
2000		7.80	0.003	0.411	0.102	0.676	0.130	0.061	0.114	0.090	0.103	0.001	0.139	0.031	0.212	0.039	0.032	0.040	0.026	0.035
G3	6	275	0.045	3.175	1.984	9.085	1.041	1.897	0.810	0.643	0.637	0.016	1.154	0.723	3.295	0.379	0.692	0.294	0.233	0.230
7000		16.37	0.005	0.281	0.142	1.012	0.098	0.025	0.085	0.157	0.143	0.001	0.078	0.058	0.203	0.034	0.041	0.027	0.051	0.039
G4	6	273	0.049	3.219	2.128	9.186	0.995	1.873	0.857	0.615	0.672	0.018	1.179	0.779	3.363	0.364	0.687	0.314	0.226	0.246
20000		7.67	0.006	0.199	0.288	0.592	0.094	0.057	0.122	0.106	0.084	0.002	0.055	0.095	0.145	0.029	0.031	0.038	0.043	0.030

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TABLE 10

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF TERMINAL FASTING BODY WEIGHTS, ORGAN WEIGHTS AND ORGAN WEIGHT RATIOS - FEMALES

Values: Mean ± SD

Ref.App.: 10

G. No. Dose (mg/kg Bwt/day)	No. of rats	Fasting Bwt (g)	Organ weights(g)								Organ weight ratios(%)							
			Adrenals	Ovaries	Kidneys	Liver	Heart	Brain	Thymus	Spleen	Adrenals	Ovaries	Kidneys	Liver	Heart	Brain	Thymus	Spleen
G1	6	179	0.062	0.111	2.044	5.629	0.652	1.711	0.502	0.516	0.035	0.062	1.195	3.126	0.364	0.956	0.279	0.284
0		15.30	0.005	0.024	1.759	0.796	0.084	0.090	0.105	0.156	0.004	0.014	1.160	0.183	0.044	0.039	0.048	0.064
G2	6	181	0.063	0.100	1.261	5.633	0.681	1.774	0.458	0.443	0.035	0.056	0.700	3.105	0.376	0.986	0.254	0.244
2000		16.94	0.004	0.019	0.093	0.706	0.091	0.081	0.065	0.101	0.004	0.009	0.071	0.137	0.039	0.090	0.033	0.045
G3	6	172	0.058	0.100	1.230	5.306	0.655	1.731	0.426	0.421	0.033	0.058	0.716	3.094	0.382	1.013	0.246	0.243
7000		15.64	0.008	0.008	0.131	0.544	0.057	0.094	0.098	0.092	0.004	0.003	0.037	0.196	0.007	0.078	0.041	0.037
G4	6	177	0.059	0.108	1.300	5.840	0.709	1.688	0.430	0.456	0.033	0.061	0.735	3.297	0.401	0.956	0.244	0.258
20000		12.25	0.005	0.010	0.116	0.550	0.067	0.036	0.055	0.033	0.003	0.002	0.068	0.221	0.033	0.053	0.039	0.021

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TABLE 11

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

SUMMARY OF GROSS PATHOLOGY FINDINGS

Ref. App.: 11 and 12

PARAMETERS	Sex	Males				Females			
	Group No.	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (mg/kg Bwt/day)	0	2000	7000	20000	0	2000	7000	20000
	No. of rats	6	6	6	6	6	6	6	6
1. No. dead during medication		0	0	0	0	0	0	0	0
2. No. of moribund sacrifice		0	0	0	0	0	0	0	0
3. No. finally 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	2	3	1	0
A. No. showing external pathology		0	0	0	0	1	3	1	0
i. Skin hair thinning with hair regrowth focal/multifocal		0	0	0	0	1	3	1	0
B. No. showing visceral organ pathology		0	0	0	0	1	1	0	0
i. Kidney - unilateral cystic		0	0	0	0	1	0	0	0
- unilateral pelvis dilated		0	0	0	0	0	1	0	0
ii. Uterus – Horn unilateral not attached to the body		NA	NA	NA	NA	1	0	0	0

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APPENDIX 1

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL VETERINARY AND OPHTHALMOLOGICAL EXAMINATIONS, CLINICAL SIGNS AND PRE-TERMINAL DEATHS - MALES

Group No. Dose (mg/kg Bwt/day)	Rat No.	Clinical signs	Ophthalmological findings (End of treatment period)
G1	Rf2261	NAD	NAD
0	Rf2262	NAD	NAD
	Rf2263	NAD	NAD
	Rf2264	NAD	NAD
	Rf2265	NAD	NAD
	Rf2266	NAD	NAD
G2	Rf2267	NAD	NAD
2000	Rf2268	NAD	NAD
	Rf2269	NAD	NAD
	Rf2270	NAD	NAD
	Rf2271	NAD	NAD
	Rf2272	NAD	NAD
G3	Rf2273	NAD	NAD
7000	Rf2274	NAD	NAD
	Rf2275	NAD	NAD
	Rf2276	NAD	NAD
	Rf2277	NAD	NAD
	Rf2278	NAD	NAD
G4	Rf2279	NAD	NAD
20000	Rf2280	NAD	NAD
	Rf2281	NAD	NAD
	Rf2282	NAD	NAD
	Rf2283	NAD	NAD
	Rf2284	NAD	NAD

NAD: No Abnormality Detected

Note: Veterinary and ophthalmological examination during acclimatization period for all animals did not reveal any abnormalities.

APPENDIX 2

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL VETERINARY AND OPHTHALMOLOGICAL EXAMINATIONS, CLINICAL SIGNS AND PRE-TERMINAL DEATHS - FEMALES

Group No. Dose (mg/kg Bwt/day)	Rat No.	Clinical signs	Ophthalmological findings (End of treatment period)
G1	Rf2285	Hair thinning with hair regrowth	NAD
0	Rf2286	NAD	NAD
	Rf2287	NAD	NAD
	Rf2288	NAD	NAD
	Rf2289	NAD	NAD
	Rf2290	NAD	NAD
G2	Rf2291	Hair thinning with hair regrowth	NAD
2000	Rf2292	NAD	NAD
	Rf2293	NAD	NAD
	Rf2294	Hair thinning with hair regrowth	NAD
	Rf2295	NAD	NAD
	Rf2296	Hair thinning with hair regrowth	NAD
G3	Rf2297	NAD	NAD
7000	Rf2298	NAD	NAD
	Rf2299	NAD	NAD
	Rf2300	NAD	NAD
	Rf2301	NAD	NAD
	Rf2302	Hair thinning with hair regrowth	NAD
G4	Rf2303	NAD	NAD
20000	Rf2304	NAD	NAD
	Rf2305	NAD	NAD
	Rf2306	NAD	NAD
	Rf2307	NAD	NAD
	Rf2308	NAD	NAD

NAD: No Abnormality Detected

Note: Veterinary and ophthalmological examination during acclimatization period for all animals did not reveal any abnormalities.

APPENDIX 3

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL BODY WEIGHTS (g) - MALES

G. No. Dose (mg/kg Bwt/day)	Rat No.	Days 1	5	8	12	15
G1	Rf2261	226	249	268	285	304
0	Rf2262	215	240	259	278	295
	Rf2263	236	251	265	281	291
	Rf2264	206	224	231	250	258
	Rf2265	225	249	265	289	304
	Rf2266	216	234	251	269	280
G2	Rf2267	220	244	258	280	289
2000	Rf2268	224	247	261	281	295
	Rf2269	226	255	269	289	301
	Rf2270	204	231	249	270	287
	Rf2271	213	237	252	273	283
	Rf2272	222	246	262	284	295
G3	Rf2273	234	257	269	292	309
7000	Rf2274	225	245	257	277	291
	Rf2275	216	237	250	270	281
	Rf2276	203	219	232	250	261
	Rf2277	227	250	270	291	306
	Rf2278	214	239	253	273	283
G4	Rf2279	208	226	243	264	280
20000	Rf2280	218	243	258	285	302
	Rf2281	231	255	267	288	296
	Rf2282	223	238	253	273	288
	Rf2283	214	234	248	265	280
	Rf2284	226	248	260	281	294

APPENDIX 4

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL CUMULATIVE NET BODY WEIGHT GAINS (g) - MALES

G. No. Dose (mg/kg Bwt/day)	Rat No.	Days 5	8	12	15
G1	Rf2261	23	42	59	78
0	Rf2262	25	44	63	80
	Rf2263	15	29	45	55
	Rf2264	18	25	44	52
	Rf2265	24	40	64	79
	Rf2266	18	35	53	64
G2	Rf2267	24	38	60	69
2000	Rf2268	23	37	57	71
	Rf2269	29	43	63	75
	Rf2270	27	45	66	83
	Rf2271	24	39	60	70
	Rf2272	24	40	62	73
G3	Rf2273	23	35	58	75
7000	Rf2274	20	32	52	66
	Rf2275	21	34	54	65
	Rf2276	16	29	47	58
	Rf2277	23	43	64	79
	Rf2278	25	39	59	69
G4	Rf2279	18	35	56	72
20000	Rf2280	25	40	67	84
	Rf2281	24	36	57	65
	Rf2282	15	30	50	65
	Rf2283	20	34	51	66
	Rf2284	22	34	55	68

APPENDIX 5

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL BODY WEIGHTS (g) - FEMALES

G. No. Dose (mg/kg Bwt/day)	Rat No.	Days 1	5	8	12	15
G1	Rf2285	159	170	174	180	183
0	Rf2286	173	182	193	199	206
	Rf2287	138	149	149	160	167
	Rf2288	169	181	189	201	207
	Rf2289	158	170	177	191	194
	Rf2290	151	164	170	172	183
G2	Rf2291	163	170	177	183	189
2000	Rf2292	153	166	172	178	181
	Rf2293	174	187	192	203	210
	Rf2294	175	185	190	202	205
	Rf2295	164	173	179	184	186
	Rf2296	139	152	156	164	169
G3	Rf2297	168	180	183	191	191
7000	Rf2298	155	158	160	169	165
	Rf2299	165	172	176	189	187
	Rf2300	157	168	172	180	186
	Rf2301	136	141	149	158	159
	Rf2302	165	180	191	198	202
G4	Rf2303	151	165	170	179	182
20000	Rf2304	173	190	197	207	213
	Rf2305	138	150	157	168	176
	Rf2306	155	165	171	179	180
	Rf2307	160	172	177	182	181
	Rf2308	172	173	182	193	195

APPENDIX 6

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL CUMULATIVE NET BODY WEIGHT GAINS (g) - FEMALES

G. No. Dose (mg/kg Bwt/day)	Rat No.	Days 5	8	12	15
G1	Rf2285	11	15	21	24
0	Rf2286	9	20	26	33
	Rf2287	11	11	22	29
	Rf2288	12	20	32	38
	Rf2289	12	19	33	36
	Rf2290	13	19	21	32
G2	Rf2291	7	14	20	26
2000	Rf2292	13	19	25	28
	Rf2293	13	18	29	36
	Rf2294	10	15	27	30
	Rf2295	9	15	20	22
	Rf2296	13	17	25	30
G3	Rf2297	12	15	23	23
7000	Rf2298	3	5	14	10
	Rf2299	7	11	24	22
	Rf2300	11	15	23	29
	Rf2301	5	13	22	23
	Rf2302	15	26	33	37
G4	Rf2303	14	19	28	31
20000	Rf2304	17	24	34	40
	Rf2305	12	19	30	38
	Rf2306	10	16	24	25
	Rf2307	12	17	22	21
	Rf2308	1	10	21	23

APPENDIX 7

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

CAGEWISE AVERAGE FOOD INTAKE (g/rat/day) - MALES

G. No. Dose (mg/kg Bwt/day)	Rat Nos. From	To	Cage No.	Weeks 1	2
G1	Rf2261	Rf2262	1	25.6	25.3
0	Rf2263	Rf2264	2	23.1	22.6
	Rf2265	Rf2266	3	24.1	24.1
G2	Rf2267	Rf2268	4	26.3	24.9
2000	Rf2269	Rf2270	5	25.9	25.6
	Rf2271	Rf2272	6	24.6	24.8
G3	Rf2273	Rf2274	7	26.0	25.3
7000	Rf2275	Rf2276	8	24.2	23.6
	Rf2277	Rf2278	9	24.3	24.6
G4	Rf2279	Rf2280	10	24.4	24.5
20000	Rf2281	Rf2282	11	23.0	23.6
	Rf2283	Rf2284	12	24.5	25.1

APPENDIX 8

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

CAGEWISE AVERAGE FOOD INTAKE (g/rat/day) - FEMALES

G. No. Dose (mg/kg Bwt/day)	Rat Nos. From	To	Cage No.	Weeks 1	2
G1	Rf2285	Rf2286	13	17.8	16.9
0	Rf2287	Rf2288	14	18.5	17.9
	Rf2289	Rf2290	15	17.1	17.1
G2	Rf2291	Rf2292	16	16.9	16.4
2000	Rf2293	Rf2294	17	16.9	17.8
	Rf2295	Rf2296	18	17.1	16.8
G3	Rf2297	Rf2298	19	16.1	15.1
7000	Rf2299	Rf2300	20	17.2	16.3
	Rf2301	Rf2302	21	16.2	15.3
G4	Rf2303	Rf2304	22	17.6	17.2
20000	Rf2305	Rf2306	23	16.0	15.9
	Rf2307	Rf2308	24	16.2	17.1

APPENDIX 9

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL TERMINAL FASTING BODY WEIGHTS, ORGAN WEIGHTS AND ORGAN WEIGHT RATIOS - MALES

G.No.	Rat	Fasting	Organ weights(g)									Organ weight ratios(%)								
Dose (mg/kg Bwt/day)	No.	Bwt (g)	Adrenals	Testes	Kidneys	Liver	Heart	Brain	Epididym	Thymus	Spleen	Adrenals	Testes	Kidneys	Liver	Heart	Brain	Epididym	Thymus	Spleen
G1	Rf2261	287.93	0.041	3.245	2.182	9.571	0.984	1.979	0.869	0.560	0.570	0.014	1.127	0.758	3.324	0.342	0.687	0.302	0.194	0.198
0	Rf2262	277.85	0.048	3.065	2.120	8.991	1.009	1.777	0.796	0.487	0.694	0.017	1.103	0.763	3.236	0.363	0.640	0.286	0.175	0.250
	Rf2263	279.48	0.063	3.662	2.237	10.182	1.081	1.859	1.037	0.866	0.698	0.023	1.310	0.800	3.643	0.387	0.665	0.371	0.310	0.250
	Rf2264	246.73	0.040	2.912	1.840	8.168	0.858	1.813	0.754	0.560	0.556	0.016	1.180	0.746	3.311	0.348	0.735	0.306	0.227	0.225
	Rf2265	230.82	0.044	3.579	2.302	9.473	1.051	1.942	1.011	0.694	0.720	0.019	1.551	0.997	4.104	0.455	0.841	0.438	0.301	0.312
	Rf2266	263.90	0.050	3.444	2.063	8.222	1.013	1.843	0.841	0.705	0.599	0.019	1.305	0.782	3.116	0.384	0.698	0.319	0.267	0.227
G2	Rf2267	275.20	0.041	2.469	2.125	9.473	0.850	1.738	0.708	0.604	0.477	0.015	0.897	0.772	3.442	0.309	0.632	0.257	0.219	0.173
2000	Rf2268	283.02	0.048	3.338	2.074	10.091	1.053	1.858	0.912	0.648	0.503	0.017	1.179	0.733	3.565	0.372	0.656	0.322	0.229	0.178
	Rf2269	289.94	0.047	3.619	2.343	9.726	1.245	1.818	0.794	0.804	0.710	0.016	1.248	0.808	3.354	0.429	0.627	0.274	0.277	0.245
	Rf2270	270.48	0.043	3.225	2.216	9.113	0.960	1.844	0.737	0.532	0.603	0.016	1.192	0.819	3.369	0.355	0.682	0.272	0.197	0.223
	Rf2271	269.61	0.048	3.366	2.103	8.788	1.005	1.916	0.864	0.630	0.584	0.018	1.248	0.780	3.260	0.373	0.711	0.320	0.234	0.217
	Rf2272	279.30	0.050	3.531	2.243	8.216	1.021	1.882	1.012	0.659	0.728	0.018	1.264	0.803	2.942	0.366	0.674	0.362	0.236	0.261
G3	Rf2273	296.03	0.045	3.516	1.928	10.345	1.032	1.926	0.950	0.697	0.881	0.015	1.188	0.651	3.495	0.349	0.651	0.321	0.235	0.298
7000	Rf2274	278.16	0.042	3.104	2.166	9.005	1.166	1.894	0.815	0.608	0.612	0.015	1.116	0.779	3.237	0.419	0.681	0.293	0.219	0.220
	Rf2275	263.80	0.046	2.797	2.000	8.376	1.005	1.888	0.800	0.501	0.510	0.017	1.060	0.758	3.175	0.381	0.716	0.303	0.190	0.193
	Rf2276	251.30	0.039	2.981	1.785	7.526	0.904	1.914	0.744	0.449	0.483	0.016	1.186	0.710	2.995	0.360	0.762	0.296	0.179	0.192
	Rf2277	288.95	0.053	3.170	1.905	9.625	0.995	1.905	0.705	0.732	0.672	0.018	1.097	0.659	3.331	0.344	0.659	0.244	0.253	0.233
	Rf2278	272.58	0.047	3.479	2.122	9.635	1.144	1.855	0.843	0.873	0.665	0.017	1.276	0.778	3.535	0.420	0.681	0.309	0.320	0.244
G4	Rf2279	265.54	0.053	2.836	1.744	8.449	0.882	1.807	0.763	0.585	0.688	0.020	1.068	0.657	3.182	0.332	0.681	0.287	0.220	0.259
20000	Rf2280	279.66	0.051	3.329	2.138	9.994	1.105	1.900	0.788	0.481	0.762	0.018	1.190	0.764	3.574	0.395	0.679	0.282	0.172	0.272
	Rf2281	283.06	0.045	3.405	2.331	9.395	1.025	1.795	1.091	0.672	0.583	0.016	1.203	0.824	3.319	0.362	0.634	0.385	0.237	0.206
	Rf2282	273.24	0.049	3.265	1.980	9.410	1.096	1.903	0.799	0.609	0.756	0.018	1.195	0.725	3.444	0.401	0.696	0.292	0.223	0.277
	Rf2283	263.36	0.040	3.217	2.010	8.525	0.942	1.903	0.818	0.789	0.564	0.015	1.222	0.763	3.237	0.358	0.723	0.311	0.300	0.214
	Rf2284	273.25	0.056	3.260	2.564	9.343	0.919	1.932	0.884	0.551	0.677	0.020	1.193	0.938	3.419	0.336	0.707	0.324	0.202	0.248

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APPENDIX 10

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL TERMINAL FASTING BODY WEIGHTS, ORGAN WEIGHTS AND ORGAN WEIGHT RATIOS - FEMALES

G.No.	Rat	Fasting	Organ weights(g)								Organ weight ratios(%)							
Dose	No.	Bwt																
(mg/kg Bwt/day)		(g)	Adrenals	Ovaries	Kidneys	Liver	Heart	Brain	Thymus	Spleen	Adrenals	Ovaries	Kidneys	Liver	Heart	Brain	Thymus	Spleen
G1	Rf2285	171.18	0.061	0.068	1.264	5.253	0.632	1.718	0.524	0.413	0.036	0.040	0.738	3.069	0.369	1.004	0.306	0.241
0	Rf2286	198.70	0.067	0.128	1.403	6.757	0.555	1.826	0.678	0.798	0.034	0.064	0.706	3.401	0.279	0.919	0.341	0.402
	Rf2287	157.87	0.068	0.121	5.620	4.649	0.565	1.559	0.475	0.343	0.043	0.077	3.560	2.945	0.358	0.988	0.301	0.217
	Rf2288	192.00	0.061	0.126	1.602	6.330	0.767	1.760	0.460	0.530	0.032	0.066	0.834	3.297	0.399	0.917	0.240	0.276
	Rf2289	185.61	0.055	0.098	1.205	5.701	0.721	1.726	0.516	0.536	0.030	0.053	0.649	3.071	0.388	0.930	0.278	0.289
	Rf2290	171.07	0.059	0.125	1.171	5.081	0.670	1.676	0.358	0.478	0.034	0.073	0.685	2.970	0.392	0.980	0.209	0.279
G2	Rf2291	176.46	0.065	0.102	1.269	5.405	0.595	1.851	0.362	0.482	0.037	0.058	0.719	3.063	0.337	1.049	0.205	0.273
2000	Rf2292	171.92	0.056	0.089	1.294	5.296	0.736	1.653	0.494	0.481	0.033	0.052	0.753	3.081	0.428	0.961	0.287	0.280
	Rf2293	201.83	0.066	0.132	1.297	6.624	0.786	1.791	0.555	0.593	0.033	0.065	0.643	3.282	0.389	0.887	0.275	0.294
	Rf2294	200.43	0.060	0.100	1.355	6.413	0.763	1.758	0.464	0.441	0.030	0.050	0.676	3.200	0.381	0.877	0.232	0.220
	Rf2295	177.28	0.068	0.074	1.082	5.104	0.575	1.869	0.428	0.319	0.038	0.042	0.610	2.879	0.324	1.054	0.241	0.180
	Rf2296	158.50	0.062	0.105	1.267	4.956	0.633	1.719	0.445	0.343	0.039	0.066	0.799	3.127	0.399	1.085	0.281	0.216
G3	Rf2297	183.86	0.056	0.101	1.280	5.089	0.685	1.796	0.367	0.512	0.030	0.055	0.696	2.768	0.373	0.977	0.200	0.278
7000	Rf2298	158.12	0.065	0.094	1.067	5.069	0.592	1.645	0.365	0.381	0.041	0.059	0.675	3.206	0.374	1.040	0.231	0.241
	Rf2299	173.91	0.057	0.110	1.342	5.781	0.673	1.648	0.478	0.485	0.033	0.063	0.772	3.324	0.387	0.948	0.275	0.279
	Rf2300	173.57	0.058	0.100	1.303	5.526	0.668	1.875	0.455	0.405	0.033	0.058	0.751	3.184	0.385	1.080	0.262	0.233
	Rf2301	149.27	0.044	0.089	1.058	4.452	0.581	1.667	0.309	0.265	0.029	0.060	0.709	2.983	0.389	1.117	0.207	0.178
	Rf2302	191.18	0.065	0.105	1.328	5.919	0.729	1.753	0.579	0.480	0.034	0.055	0.695	3.096	0.381	0.917	0.303	0.251
G4	Rf2303	170.34	0.058	0.101	1.244	5.149	0.680	1.714	0.384	0.397	0.034	0.059	0.730	3.023	0.399	1.006	0.225	0.233
20000	Rf2304	199.48	0.060	0.125	1.357	6.728	0.800	1.717	0.430	0.477	0.030	0.063	0.680	3.373	0.401	0.861	0.216	0.239
	Rf2305	165.94	0.050	0.098	1.148	5.776	0.638	1.621	0.506	0.476	0.030	0.059	0.692	3.481	0.384	0.977	0.305	0.287
	Rf2306	170.21	0.060	0.104	1.472	6.107	0.786	1.694	0.466	0.470	0.035	0.061	0.865	3.588	0.462	0.995	0.274	0.276
	Rf2307	174.84	0.058	0.106	1.225	5.425	0.691	1.679	0.355	0.436	0.033	0.061	0.701	3.103	0.395	0.960	0.203	0.249
	Rf2308	182.27	0.065	0.112	1.355	5.853	0.661	1.703	0.441	0.482	0.036	0.061	0.743	3.211	0.363	0.934	0.242	0.264

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APPENDIX 11

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL GROSS PATHOLOGY FINDINGS - MALES

Group No. Dose (mg/kg Bwt/day)	Rat No.	Changes observed
G1	Rf2261	NAD
0	Rf2262	NAD
	Rf2263	NAD
	Rf2264	NAD
	Rf2265	NAD
	Rf2266	NAD
G2	Rf2267	NAD
2000	Rf2268	NAD
	Rf2269	NAD
	Rf2270	NAD
	Rf2271	NAD
	Rf2272	NAD
G3	Rf2273	NAD
7000	Rf2274	NAD
	Rf2275	NAD
	Rf2276	NAD
	Rf2277	NAD
	Rf2278	NAD
G4	Rf2279	NAD
20000	Rf2280	NAD
	Rf2281	NAD
	Rf2282	NAD
	Rf2283	NAD
	Rf2284	NAD

NAD: No Abnormality Detected

APPENDIX 12

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

INDIVIDUAL GROSS PATHOLOGY FINDINGS - FEMALES

Group No. Dose (mg/kg Bwt/day)	Rat No.	Changes observed
G1	Rf2285	Skin thoracic hair thinning with hair regrowth focal
0	Rf2286	NAD
	Rf2287	Kidney unilateral cystic 2.6 cms
		Uterus, Horn unilateral not attached to the body
	Rf2288	NAD
	Rf2289	NAD
	Rf2290	NAD
G2	Rf2291	Skin thoracic hair thinning with hair regrowth focal
2000	Rf2292	NAD
	Rf2293	NAD
	Rf2294	Skin hair thinning with hair regrowth multifocal
		Kidney unilateral pelvis dilated
	Rf2295	NAD
	Rf2296	Skin hair thinning with hair regrowth multifocal
G3	Rf2297	NAD
7000	Rf2298	NAD
	Rf2299	NAD
	Rf2300	NAD
	Rf2301	NAD
	Rf2302	Skin hair thinning with hair regrowth multifocal
G4	Rf2303	NAD
20000	Rf2304	NAD
	Rf2305	NAD
	Rf2306	NAD
	Rf2307	NAD
	Rf2308	NAD

NAD: No Abnormality Detected

APPENDIX 13

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

CERTIFICATE OF ANALYSIS

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

DSM

F-12202 version 1
Page 1 of 1

CERTIFICATE OF ANALYSIS

Name of the product	Enzyme preparation from *Aspergillus niger* GEP44
Batch no	JLL 03 006 IDF
Study no.	ANA/03/D68
GLP-archive no.	GLP-0302
Status	ISO 9002
Date of manufacture	March 2003
Date of expiration	March 2004 (provisional)
Active component	Endoprotease
Date of issue	17 June 2003

Analysis type	Method number	Dimension	Result
Endoprotease activity	62186	PPU/g	11.0
Dry matter	60485	% (w/w)	25.9
Ash	60328	% (w/w)	0.7
Total organic solids (TOS)	W-10850NLv2	% (w/w)	25.2
Proteins by Kjeldahl Nitrogen x 6.25	62186	% (w/w)	13.9
Stability in water 21°C, 100 mg/ml	62186	hours	48
Stability in water 21°C, 350 mg/ml	62186	hours	48
Stability in water 21°C, undiluted	62186	hours	48
Stability in water 4°C, 100 mg/ml	62186	days	7
Stability in water 4°C, 350 mg/ml	62186	days	7
Stability in water 4°C, undiluted	62186	days	7

Signature Study Director: P.P.J.M. Snuverink

Date

Remarks (if any):

REG/DOC#4:685

APPENDIX 14

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

TEST ITEM PREPARATION DATA

Date of test item preparation	Group No.	Dose (mg/kg Bwt/day)	Test Item (ml)	Total volume (ml) with double distilled water
04.07.2003	G1	0	-	400
	G2	2000	37.1	400
	G3	7000	129.8	400
	G4	20000	220	#
07.07.2003	G1	0	-	400
	G2	2000	37.1	400
	G3	7000	129.8	400
	G4	20000	220	#
11.07.2003	G1	0	-	250
	G2	2000	23.2	250
	G3	7000	81.1	250
	G4	20000	200	#
14.07.2003	G1	0	-	250
	G2	2000	23.2	250
	G3	7000	81.1	250
	G4	20000	210	#

Note: '-' Not applicable
#: For G4 group, undiluted test item was administered at a dose volume of 18.54 ml/kg Bwt/day.

BATCH ANALYSIS DATA

Date	Mean protein content in the sample (mg/ml)				Test item concentration in the sample (mg/ml)			
	G1	G2	G3	G4	G1	G2	G3	G4
04.07.2003	-	13.90	48.65	149.72	-	100.0	350.0	1077.1
11.07.2003	-	13.74	48.29	149.21	-	98.8	347.4	1073.5
Mean ± SD	-	13.82 ± 0.11	48.47 ± 0.25	149.5 ± 0.36	-	99.4 ± 0.85	348.7 ± 1.84	1075.3 ± 2.55

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APPENDIX 15

REPEATED DOSE (14-DAY) ORAL TOXICITY STUDY BY GAVAGE WITH ENZYME PREPARATION OF *Aspergillus niger* (GEP44) IN WISTAR RATS

DEVIATION FROM THE APPROVED STUDY PLAN

AS IN STUDY PLAN		AS IN REPORT	
TEST ITEM INFORMATION: (as furnished by the study sponsor) **(PAGE No. 10/17)**			
Storage conditions :	Deep freezer (-10°C to –20°C)	Storage conditions :	Deep freezer (-68°C to -76°C)

This deviation from the approved study plan did not affect the outcome of the study or the interpretation of the study results.

Date: 20/08/2003

(Mr.P.M.SATHISH)
Study Director

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ANNEXURE 1

RALLIS RESEARCH CENTRE
21 & 22, PEENYA INDUSTRIAL AREA, II PHASE
BANGALORE 560 058
ANALYSIS REPORT - ANIMAL DIET SAMPLE

FROM Residue/Analytical Department, RRC, Bangalore-560 058 — TO Toxicology Department, RRC, Bangalore-560 058

Our Ref. No : SS/TF/1319 — Date: 24.06.2003

Sample Details Name: Ssniff Rats/Mice (pellet) Feed Maintenance — Sampling Date: 02.06.2003

Batch No : 4893120

Supplier : Ssniff Spezialdiäten GmbH, D-59494, Soest Germany

Manufacturer: Ssniff Spezialdiäten GmbH, D-59494, Soest Germany

ANALYSIS RESULTS
(Analysis on "as is basis")

No.	PARAMETER	(%)
1	Moisture	12.3
2.	Crude protein (Nx6.25)	18.9
3	Crude fat (Ether extract)	2.9
4.	Crude fibre	3.5
5.	Total ash	5.8
6	Acid insoluble ash	0.6
7	Nitrogen free extract	56.6
8	Calcium (Ca)	1.23
9	Phosphorus (P)	0.59

Residue/Analytical Dept.,
24/6/2003

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ANNEXURE 2

TOXICOLOGY DEPARTMENT

FEED CONTAMINANT ANALYSIS REPORT FOR SSNIFF RATS/MICE DIET - MAINTENANCE MEAL

ANALYSED BY: Landwirtschaftliche Untersuchungs - und Forschungsanstalt
Institut für Tiergesundheit und Lebensmittelqualität
GmbH KIEL, Germany

AGRICULTURAL EXPERIMENTAL RESEARCH STATION AND
INSTITUTE FOR ANIMAL HEALTH AND FOOD STUFF QUALITY
GmbH KIEL, GERMANY

REFERENCE: SSNIFF RATS/MICE DIET - MAINTENANCE MEAL
Date of Sampling : 06.09.2002
Batch No. : 5362331

ANALYSIS REPORT
Date of Analysis: 17.10.2002
Reference No. : AN-55494 Li

I. CHLORINATED HYDROCARBONS		**(mg/kg)**
a.	Aldrine	n.d. < 0.005
b.	α - Chlordane	n.d. < 0.005
c.	χ - Chlordane	n.d. < 0.005
d.	Oxy - Chlordane	n.d. < 0.005
e.	p,p-DDD	n.d. < 0.005
f.	p,p-DDE	n.d. < 0.005
g.	o,p-DDT	n.d. < 0.005
h.	p,p-DDT	n.d. < 0.005
i.	Dieldine	n.d. < 0.005
j.	α - Endosulfane	n.d. < 0.005
k.	β - Endosulfane	n.d. < 0.005
l.	Endosulfansulfate	n.d. < 0.005
m.	Endrine	n.d. < 0.005
n.	HCB (Hexachlorbenzole)	n.d. < 0.005
o.	α - HCH	n.d. < 0.005
p.	β - HCH	n.d. < 0.005
q.	δ - HCH	n.d. < 0.005
r.	Epsilon - HCH	n.d. < 0.005
s.	χ - HCB (Lindan)	n.d. < 0.005
t.	Heptachlor	n.d. < 0.005
u.	cis - Heptachlorepoxide	n.d. < 0.005
v.	trans - Heptachlorepoxide	n.d. < 0.005
w.	Methoxychlor	n.d. < 0.005
x.	Quintozene	n.d. < 0.005
y.	Tecnazen	n.d. < 0.005
z.	Tetradifon	n.d. < 0.005

II. PHOSPHORIC ACID ESTERS		**(mg/kg)**
a.	Bromophos (-ethyle)	n.d. < 0.010
b.	Bromophos (-methyle)	n.d. < 0.010
c.	Chlorfenvinphos	n.d. < 0.010
d.	Chlorpyriphos (-ethyle)	n.d. < 0.010
e.	Chlorpyriphos (-methyle)	n.d. < 0.010
f.	Chlorthione	n.d. < 0.010
g.	Diazinone	n.d. < 0.010
h.	Dichlorvos	n.d. < 0.010
i.	Dimethoate	n.d. < 0.010
j.	Ethione	n.d. < 0.010
k.	Fenithrothione	n.d. < 0.010
l.	Fenthione	n.d. < 0.010
m.	Malathione	0.037
n.	Mecarbame	n.d. < 0.010
o.	Methidathione	n.d. < 0.010
p.	Parathion (-ethyle)	n.d. < 0.010
q.	Parathion (-methyle)	n.d. < 0.010
r.	Pirimiphos (-ethyle)	n.d. < 0.010
s.	Pirimiphos (-methyle)	0.312
t.	Profenofos	n.d. < 0.010
u.	Sulfotep	n.d. < 0.010

III. POLYCHLORIERTE BIPHENYLS (PCB)		**mg/kg**
a.	PCB EK 28	n.d. < 0.005
b.	PCB EK 52	n.d. < 0.005
c.	PCB EK 101	n.d. < 0.005
d.	PCB EK 118	n.d. < 0.005
e.	PCB EK 138	n.d. < 0.005
f.	PCB EK 153	n.d. < 0.005
g.	PCB EK 180	n.d. < 0.005

IV. AFLATOXINS		**µg/kg**
a.	Aflatoxine B1	n.d. < 1µg/kg
b.	Aflatoxine B2	n.d. < 1µg/kg
c.	Aflatoxine G1	n.d. < 1µg/kg
d.	Aflatoxine G2	n.d. < 1µg/kg

n.d: Not detected

Sd/-
Dr. Wehage

RALLIS RESEARCH CENTRE
Peenya, Bangalore - 560 058.

ANNEXURE 3

RALLIS RESEARCH CENTRE, BANGALORE - 560 058

ANALYSIS REPORT - WATER SAMPLE

FROM: Residue/Analytical Dept.
RRC, Bangalore-560 058

TO: Toxicology Dept.
RRC, Bangalore-560 058

Our Ref. No: SS/TW/155

Date: 30.05.2003

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

Date of Collection : 02.05.2003

ANALYSIS RESULTS

Sl. No.	Parameter	Content
1.	Colour	colourless
2.	Odour	odourless
3.	Turbidity	clear
4.	pH	7.15
5.	Electrical Conductivity, dSm^{-1}	1.726
6.	Total solids, (ppm)	980
7.	Suspended solids, (ppm)	11
8.	Dissolved solids, (ppm)	969
9.	Dissolved oxygen, (ppm)	6.00
10.	Biochemical Oxygen Demand 5 days at 20°C, (ppm)	2.07
11.	Chemical Oxygen Demand (ppm)	6.72

Sl. No.	Parameter	Content (ppm)
12.	Total hardness as $CaCo_3$	446
13.	Calcium as Ca^{2+}	85
14.	Magnesium as Mg^{2+}	57
15.	Chlorides as Cl^-	272
16.	Sulphates as SO_4^{2-}	71
17.	Carbonates as CO_3^{2-}	Nil
18.	Bicarbonates as HCO_3^-	405
19.	Sodium as Na	89
20.	Potassium as K	10

30/5/2003

Residue/Analytical Dept.,

RALLIS RESEARCH CENTRE
Peenya, Bangalore - 560 058.

ANNEXURE 4

TOXICOLOGY DEPARTMENT
CONTAMINANT ANALYSIS REPORT FOR WATER SAMPLE

ANALYSED BY: **UMWELT CONTROL LABOR GmbH**
EUPENER STRASSE, 150
D-50933 KÖLN, GERMANY

REFERENCE : **WATER SAMPLE - FROM OUTLET OF AQUAGUARD WATER FILTER**

Sample No. : WATER; W-13
Date of Sampling : 06.09.2002
Date of receipt : 16.10.2002

Sl. No.	PARAMETERS	VALUES µg/l	Sl. No.	PARAMETERS	VALUES µg/l
ORGANOCHLORPESTICIDES					
1.	Hexachlorbenzol (HCB)	< 0.001	12.	Endrine	< 0.001
2.	Aldrine	< 0.001	13.	α - HCH	< 0.001
3.	o.p-DDD	< 0.001	14.	β - HCH	< 0.001
4.	p.p-DDD	< 0.001	15.	δ - HCH	< 0.001
5.	o.p-DDE	< 0.001	16.	χ - HCB (Lindan)	< 0.001
6.	p.p-DDE	< 0.001	17.	Heptachlor	< 0.001
7.	o.p-DDT	< 0.001	18.	cis - Heptachlorepoxide	< 0.001
8.	p.p-DDT	< 0.001	19.	trans - Heptachlorepoxide	< 0.001
9.	Dieldrin	< 0.001	20.	Methoxychlor	< 0.001
10.	α - Endosulfane	< 0.001	21.	Quintozene	< 0.001
11.	β - Endosulfane	< 0.001			< 0.001
III. POLYCHLORIERTE BIPHENYLS (PCB) µg/l					
22..	PCB EK 28	< 0.02			
23.	PCB EK 52	< 0.02			
24.	PCB EK 101	< 0.02			
25.	PCB EK 118	< 0.02			
26.	PCB EK 138	< 0.02			
27.	PCB EK 153	< 0.02			
28.	PCB EK 180	< 0.02			

Sd/-
Head of Laboratory
Cologne

ANNEXURE 5

GLP CERTIFICATE - GERMANY

Bundesinstitut
für gesundheitlichen Verbraucherschutz und Veterinärmedizin

bgvv

GUTE LABORPRAXIS / GOOD LABORATORY PRACTICE

GLP-Bestätigung / GLP Certificate

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

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

Prüfeinrichtung / Test facility

Rallis India, Ltd.
Rallis Research Centre
Peenya Industrial Area
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; Prüfungen zur Bioakkumulation und zur Metabolisierung / Studies on behaviour in water, soil and air, bioaccumulation
Prüfungen zur Bestimmung von Rückständen / Residue studies

Datum der Inspektion / Date of Inspection

13. - 20. December 2000

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.)

20. November 2001
Im Auftrag / For the Director

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

contd.

ANNEXURE 5 contd.

GLP CERTIFICATE - THE NETHERLANDS

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF
GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 88/320/EEC the conformity with the OECD Principles of GLP was assessed on 24-28 March 2003 at

Rallis Research Centre
Rallis India Limited
Plot 21&22 Phase II Peenya Industrial Area, PO Box 5813
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.

NEDERLAND
STATISTOEZICHT OP DE VOLKSGEZONDHEID

The Hague, 26 May 2003

Dr Th. Helder
GLP Compliance Monitoring Department

Inspectorate for Health Protection and Veterinary Public Health
Ministry of Health, Welfare and Sport