TEST REPORT

DANAK
TEST Reg. No 145

Scantox

Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265]

ACUTE ORAL TOXICITY STUDY IN THE RAT THE FIXED DOSE PROCEDURE

LAB Scantox Study No:	62123
Date:	19 September 2006
Author:	Marianne M Jochumsen, DVM
Number of pages:	19
Sponsor:	Genencor International Inc. (A Danisco Company) 925 Page Mill Road Palo Alto, CA 94304 USA

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report "Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265] – Acute oral toxicity study in the rat, the fixed dose procedure" was conducted under my supervision and responsibility and in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), which are in conformity with other international GLP regulations.

This report is a complete and accurate account of the methods employed and the data obtained.

Marianne M Jochumsen, DVM
Study Director
LAB Scantox

22 September 2006
Date

QUALITY ASSURANCE STATEMENT

Study number: 62123

Study title: Acyltransferase BL1 (B. licheniformis strain BML 780-KLM3' CAP50) (GICC 3265) - Acute oral toxicity study in the rat - the fixed dose procedure

A review of the study plan has been performed and reported to the Study Director:

Date of review:	**Reporting date:**
03 April 2006	03 April 2006

This study performed by LAB Scantox has been inspected by the Quality Assurance Unit in compliance with the principles of Good Laboratory Practice. Inspection reports have been communicated to the Study Director and to management on the dates stated in the table below. Process and facility inspections are performed on a regular basis in accordance with LAB Scantox procedures. Study-based inspection dates and the most recent inspection dates of the processes applicable to this study are stated in the below table.

Inspection type	**Inspection item(s)**	**Inspection date(s)**	**Reporting date(s)**
Study-based	Registration, handling and documentation of test item	11 April 2006	11 April 2006
	Preparation of dose formulation	11 April 2006	11 April 2006
	Dosing	11 April 2006	11 April 2006
	Observation 15 minutes after dosing	11 April 2006	11 April 2006
	Raw data	11 April 2006	11 April 2006
	Anaesthesia of animals	25 April 2006	25 April 2006
	Necropsy	25 April 2006	25 April 2006
Process-based	Arrival and allocation of animals	16 February 2006	16 February 2006
	Weighing of animal	14 February 2006	14 February 2006
	Necropsy	25 January 2006 26 April 2006	25 January 2006 26 April 2006

The study report has been audited. As far as can be reasonably established, the methods, procedures and observations have been accurately described, and the results and data presented in the study report accurately reflect the raw data generated during the study.

The study report gives an accurate account of the methods and procedures outlined in the study plan and in LAB Scantox Standard Operating Procedures.

Audit date(s) of Draft Report and data: 11 September 2006	**Reporting date (Study Director and management):** 11 September 2006
Audit date of Final Report: 19 September 2006	**No report**

Pauline Sylvest Salanti
Head of Quality Assurance
LAB Scantox

19 September 2006
Date

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SUMMARY

This study was conducted at LAB Scantox, Hestehavevej 36A, Ejby, DK-4623 Lille Skensved, Denmark.

The objective of this study was to assess the acute toxicity of Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265] total protein content 30.40 mg/ml, when administered as a single oral dose followed by an observation period of 14 days and thereby obtain information both about hazard assessment purposes and for ranking items. A preliminary sighting study, using a female animal at each dose level, was included in order to estimate the dose effect for toxicity and to provide information on dose selection for the main study. The study was conducted in accordance with the OECD Guideline No 420, "Acute Oral Toxicity - Fixed Dose Procedure", December 2001. The following dose levels were considered: 300 mg and 600 mg total protein/kg. Due to a total protein content of 30.40 mg/ml, no higher dose level was possible as the maximal dose volume for a rat is 20 ml/kg. The starting dose level was chosen by the Sponsor.

The test item was supplied by the Sponsor as a brown liquid.

The study was initiated with a sighting study, in which 300 mg total protein/kg, 20 ml/kg, vehicle 3%NaCl and 600 mg total protein/kg 20 ml/kg, undiluted, was examined in a female rat at each dose level in order to provide information on dose selection for the main study.

On the basis of the results of the sighting study, the main study was performed in four additional female rats given a dose of 600 mg total protein/kg body weight, dose volume 20 ml/kg in 4 out of 5 main study animals.

Slight signs of toxicity (piloerection) were observed at the 3 hours observation, in some of the animals treated with 600 mg total protein/ml, 20 ml/kg on the day of treatment. However, no signs of toxicity were observed among the animals at any other observation.

From Day 2 to 3, two of the animals in the main study (Nos 7 and 17) lost between 3 and 4 g body weight, respectively. However, all animals had an overall acceptable body weight gain from treatment until termination of the study.

The post-mortem inspection revealed no abnormalities.

In conclusion under the experimental conditions described in this report, no signs of severe toxicity were observed at neither 300 or 600 mg total protein per kg, 20 ml/kg Transient weight losses of 3-4 g were observed in 2 out of 5 animals treated with 600 mg total protein/kg body weight (20 ml/kg).

INTRODUCTION

The objective of this study was to assess the acute toxicity of Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265] total protein content 30.40 mg/ml, when administered as a single oral dose followed by an observation period of 14 days and thereby obtain information both about hazard assessment purposes and for ranking items. A preliminary sighting study, using a female animal at each dose level, was included in order to estimate the dose effect for toxicity and to provide information on dose selection for the main study. The study was conducted in accordance with the OECD Guideline No 420, "Acute Oral Toxicity - Fixed Dose Procedure", December 2001. The following dose levels were considered: 300 mg and 600 mg total protein/kg. Due to a total protein content of 30.40 mg/ml, no higher dose level was possible as the maximal dose volume for a rat is 20 ml/kg. The starting dose level was chosen by the Sponsor.

The rat was selected as the test model because of its proven suitability in toxicological studies.

This study was conducted at LAB Scantox, Hestehavevej 36A, Ejby, DK-4623 Lille Skensved, Denmark.

The treatment was performed on 11 April 2006. The study was terminated on 16 May 2006. This report describes the procedures used and the results obtained.

Personnel involved in the study

Study Director: Marianne M Jochumsen, DVM

MATERIALS AND METHODS

Test item

Test item name:	Acyltransferase BL1 (*B. Licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265]
Lot number:	20068010
Expiry date:	At least 1 years from issuance of Certificate of Analysis 08/02/2006
Description:	Clear brown liquid
Stability:	Stable.
Protein content:	30.40 mg total protein/ml
Specific gravity:	1.021
pH:	6.30

The test item was received from the Sponsor on 15 March 2006.

Test item characterisation was the responsibility of the Sponsor. The test item was labelled with the Study No (62123) and kept in a freezer at -18°C in the dark. The data obtained during the conduct of this study was related to the test item supplied by the Sponsor.

Vehicle

Vehicle name:	3% NaCl
Manufacturer:	LAB Scantox
Production date:	11 April 2006
Expiry:	12 April, 2008

Dose formulations

The test item dose formulation 300 mg total protein/kg was prepared by dissolving the test item in the vehicle. The dose formulation 300 mg total protein/kg was prepared on the day of treatment and stored at room temperature, protected from light, until use. The test item dose formulation 600 mg total protein/kg was used undiluted.

The calculations on which the preparations were based were checked by two persons independently before each preparation of the dose formulations. The dose formulation was weighed before and after dose administration and the weight was recorded in order to verify the amount of dose formulation used.

Animals

The sighting study was performed in two healthy young female rat (ordered as SPF) of the strain HanTac:WH (Wistar Hannover GALAS) from Taconic Europe A/S, DK-8680 Ry, Denmark. The main study was performed in four additional female healthy young rats of the same strain and breeder criteria as mentioned previously. On the day of treatment, the animals in both the sighting study and main study weighed from 142 to 148 g.

An acclimatisation period of 5 days was allowed.

For the animal welfare of the sighting study animals, a female companion animal (animal Nos 14 and 18) was housed with the sighting study animal. The companion animal was observed for clinical behaviour and subjected to gross necropsy. However, the results are not included in the report. The companion animal was euthanised at termination of the study.

Housing

The study took place in animal room No 110 provided with filtered air at a temperature of 21°C ± 3°C, relative humidity of 55% ± 15%. The temperature and relative humidity in the animal room were recorded hourly during the study and the records are retained. No significant deviations of the temperature or relative humidity occurred during the study. The ventilation system has been designed to give 10 air changes per hour. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. Light was on from 06:00 hours to 18:00 hours.

The rats were kept in transparent polycarbonate cages (macrolone type III, floor area 810 cm^2). The animals were housed two in each cage. The cages were cleaned and the bedding changed at least twice a week.

Before the animals arrived, the animal room was cleaned. During the study, the animal room was cleaned regularly.

Bedding

Bedding was softwood sawdust "Jeluxyl" from Jelu Werk GmbH, Josef Ehrler GmbH & Co KG, D-73494 Rosenberg, Germany. Analyses for relevant possible contaminants are performed regularly. Certificates of analysis are retained.

Diet

A pelleted complete rodent diet "Altromin 1314 fortified" from ALTROMIN Gesellschaft für Tierernährung mbH, D-32791 Lage, Germany, was available *ad libitum*. Analyses for major nutritive components and relevant possible contaminants are performed regularly. Certificates of analysis are retained.

Drinking water

The animal had free access to bottles with domestic quality drinking water acidified with hydrochloric acid to pH 2.5 in order to prevent microbial growth. Analyses for relevant possible contaminants are performed regularly on the drinking water prior to acidification. Certificates of analysis are retained.

Environmental enrichment

The animals had access to an autoclaved brick of Aspen wood from Tapvei Oy, FIN-73620 Kortteinen, Finland. Analyses for relevant possible contaminants are performed regularly. Certificates of analysis are retained.

The animals were offered a supply of Aspen Wood Wool from Tapvei Oy, FIN-73620 Kortteinen, Finland, at each change of bedding. Analyses for relevant possible contaminants are performed regularly. Certificates of analysis are retained.

Animal identification and grouping

On the day of arrival, each animal was identified by punched earmarks indicating a unique number for each animal in this study and allocated to the groups (i.e. sighting or main study).

Each cage was identified by a colour coded cage card marked with the study number, group identification, dose level, administration route, sex, cage and animal ear numbers.

Two females were used for the sighting study.

On the basis of the results of the sighting study, the main study was performed in four additional rats given a dose of 600 mg total protein/kg (dose volume 20 ml/kg). The main study included the animal treated in the sighting study, treated with 600 mg total protein/kg in order to obtain a total of 5 animals.

Treatment procedure

The test item was administered as a single oral dose by gavage using a stomach tube to animals that had been fasted overnight. After treatment, the feed was withheld for a further three hours.

The study was initiated with a **sighting study** in order to select the appropriate dose level for the main study:

Based on information from the Sponsor, an initial dose level of 300 mg total protein/kg 20 ml/kg body weight and thereafter 600 mg total protein/kg 20 ml/kg body weight was investigated in the sighting study using one female at each dose level. As no signs of severe toxicity were observed in the sighting study animals treated with 300 or 600 mg total protein/kg body weight, respectively, the **main study** was carried out at the dose level of 600 mg/kg body weight.

The dose volume administered was 20 ml/kg body weight both in the sighting study and in the main study. Thus, the test item in the main study was used undiluted, whereas the sighting study animals were dosed with the test item in a 3% NaCl solution.

Clinical signs

Each rat was observed 15 minutes and 1, 3 and 6 hours after administration and thereafter daily for a period of 14 consecutive days.

Body weight

Body weights (b.wt.) were recorded prior to treatment on Day 1 and on Days 2, 3, 8 and 15. The group mean body weight was calculated.

Necropsy

Fourteen days (on Day 15) after treatment, all animals were anaesthetised and killed by an intraperitoneal injection of a barbiturate (2.5% Pentothal® Natrium). All animals were subjected to a gross necropsy examination.

ARCHIVES

For a period of 10 years, the following material relating to the study will be retained in the archives of LAB Scantox:

Study plan, Study plan amendments and correspondence
Test material receipts
Animal records
All original data
Final report

At the end of the storage period LAB Scantox will contact the Sponsor for instructions whether the material should be transferred, retained or destroyed.

RESULTS

The group mean body weight; body weight gain and individual values are presented in Table 1. A key to daily observations is listed in Table 2. The clinical signs of the animals observed daily throughout the study are presented in Table 3.

Sighting study

One female – 300 mg total protein/kg 20 ml/kg body weight

The animal appeared normal at all observations.

From Day 2 to 3, the animal lost 1 g body weight. However, the animal had an overall acceptable body weight gain from treatment until termination of the study.

The post-mortem inspection of the animal revealed no abnormalities.

One female – 600 mg total protein/kg 20 ml/kg body weight

The animal appeared normal at all observations.

From Day 2 to 3, the animal lost 4 g body weight. However, the animal had an overall acceptable body weight gain from treatment until termination of the study.

The post-mortem inspection of the animal revealed no abnormalities.

Main study

Four females – 600 mg total protein/kg 20 ml/kg body weight

The sighting study animal treated with 600 mg/kg is considered as part of the main study. However, please see above for details regarding observations and reactions for this animal.

At the observation 3 hours after treatment, all other main study animals had piloerection. At all other observations the four animals appeared normal.

From Day 2 to 3, animal No 7 lost 3 g body weight. However, this animal as well as the other three animals had an overall acceptable body weight gain from treatment until termination of the study.

The post-mortem inspection of the four animals revealed no abnormalities.

CONCLUSION

Under the experimental conditions described in this report, no signs of severe toxicity were observed at neither 300 or 600 mg total protein per kg, 20 ml/kg Transient weight losses of 3-4 g were observed in 2 out of 5 animals treated with 600 mg total protein/kg body weight (20 ml/kg).

Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265]

ACUTE ORAL TOXICITY STUDY IN THE RAT
THE FIXED DOSE PROCEDURE

Body weight and body weight gain (g)
Individual values

Sighting study

Rat No	Dose (mg/kg b.wt.)	Sex	Day 1	Day 2	Day 3	Day 8	Day 15	Body weight gain (g)
13	300	♀	142	159	158	172	191	49
17	600	♀	148	168	164	185	195	47

Main study

Rat No	Dose (mg/kg b.wt.)	Sex	Day 1	Day 2	Day 3	Day 8	Day 15	Body weight gain (g)
5	600	♀	145	160	162	175	185	40
6	600	♀	142	154	160	172	193	51
7	600	♀	144	164	161	176	188	44
8	600	♀	147	160	165	177	190	43

Dose (mg/kg b.wt.)	Sex	Day 1			Day 2			Day 3		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
600	♀	145.2	2.4	5	161.2	5.2	5	162.4	2.1	5

Dose (mg/kg b.wt.)	Sex	Day 8			Day 15		
		Mean	SD	N	Mean	SD	N
600	♀	177.0	4.8	5	190.2	4.0	5

N = Number of animals
SD = Standard deviation

Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265]

ACUTE ORAL TOXICITY STUDY IN THE RAT
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Key to daily observations

N	Normal
P	Piloerection

Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265]

ACUTE ORAL TOXICITY STUDY IN THE RAT
THE FIXED DOSE PROCEDURE

Daily observations

Sighting study

Rat No	Dose (mg/kg b.wt.)	Sex	Day 1 after start of treatment				Day					
			15 min	1 hr	3 hrs	6 hrs	2	3	4	5	6	7
13	300	♀	N	N	N	N	N	N	N	N	N	N
17	600	♀	N	N	N	N	N	N	N	N	N	N

Rat No	Dose (mg/kg b.wt.)	Sex	Day							
			8	9	10	11	12	13	14	15
13	300	♀	N	N	N	N	N	N	N	N
17	600	♀	N	N	N	N	N	N	N	N

Acyltransferase BL1 (*B. licheniformis* strain BML 780-KLM3'CAP50)[GICC 3265]

ACUTE ORAL TOXICITY STUDY IN THE RAT
THE FIXED DOSE PROCEDURE

Daily observations

Main study

Rat No	Dose (mg/kg b.wt.)	Sex	Day 1 after start of treatment				Day					
			15 min	1 hr	3 hrs	6 hrs	2	3	4	5	6	7
5	600	♀	N	N	P	N	N	N	N	N	N	N
6	600	♀	N	N	P	N	N	N	N	N	N	N
7	600	♀	N	N	P	N	N	N	N	N	N	N
8	600	♀	N	N	P	N	N	N	N	N	N	N

Rat No	Dose (mg/kg b.wt.)	Sex	Day							
			8	9	10	11	12	13	14	15
5	600	♀	N	N	N	N	N	N	N	N
6	600	♀	N	N	N	N	N	N	N	N
7	600	♀	N	N	N	N	N	N	N	N
8	600	♀	N	N	N	N	N	N	N	N

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CERTIFICATE OF ANALYSIS

Name of Test Article: ACYLTRANSFERASE BL1

Production/Strain Name: *Bacillus licheniformis* BML780-KLM3' CAP50.

Production Site: Rochester, USA

Genencor International Culture Collection Number: GICC 3265

Designation of Lot Tested: 20068010

Description: Clear brown liquid

Expiration Date: Stable for at least 1 year from date of issuance when stored frozen

All of the analytical studies listed below were conducted in accordance with GLP regulations and ISO 9002 standards.

RESULTS:

1. Activity: 1156 U/ml
2. Total and TCA Protein
   The samples were measured for TCA and total protein by nitrogen analysis (with a KLM3' conversion factor of 5.96 g protein/g nitrogen).

Total Proteins:	30.40 mg/ml
TCA Proteins:	14.13 mg/ml
% Total Organic Solids: (100% – moisture% – ash%)	8.67%

3. Specific gravity: 1.021 g/ml
4. pH: 6.30
5. Inorganic materials

% Ash:	1.05%
% moisture:	90.28%

6. Microbial analysis: Microbial analysis conducted by GCOR, Rochester, NY

Analysis	Results
Total viable count	< 1CFU/ml
Coliform	< 1CFU/ml

E. Coli	negative/25 ml
Salmonella	negative/25 ml
Staphylococcus aureus	< 1 CFU/ml
Production strain	negative
Anaerobic sulfite reducers	negative
Antibiotic activity assay	negative

7. Mycotoxin analysis: Not applicable
8. Heavy metals analysis (conducted at Silliker Laboratories)

Analysis	Results
Heavy metals as Pb	< 30 ppm
Arsenic	< 3 ppm
Lead	< 0.5 ppm
Mercury	< 0.5 ppm
Cadmium	< 5 ppm

9. Stability Data :

Room Temperature (all activity units are reported in U/ml)

Sample ID	Dilution	T = 0	T = 5 hours	% of T = 0
20068010	straight	1094	1088	99.5
20068010	1/2	542	554	102.2
20068010	1/4	274	270	98.5

Refrigerator (4C): Undiluted Material

Sample ID	Dilution	*T=0*	*T = 7 days*	% of T = 0
20068010	straight	1094	851	77.8
20068010	1/2	542	441	81.3
20068010	1/4	274	220	80.2

Frozen (-20°C) : Undiluted Material

Sample ID	Dilution	T = 0	T = 30 days	% of T = 0	T = 60 days	% of T = 0	T = 90 days	% of T = 0
20068010	straight	1094	1097	100.3	1120	102.4	1106	101.1
20068010	1/2	542	557	102.8	551	101.7	529	97.6
20068010	1/4	274	271	98.9	277	101.1	229	83.6

Bio-Analytical Representatives:

John Smith Date: 7/31/06

John Smith

Annette Diaz Date: 7/31/06

Annette Diaz

Study Sponsor's Representative

Hanne Valsted Thygesen Date: 25 July 2006

Hanne Valsted Thygesen

Study Monitor's Representative:

Quang Q. Bui Date: 08/02/2006

Quang Q. Bui