TEST REPORT : 7191147889-CHM16-01-LX

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1. GENERAL

1.1 STUDY TITLE

Acute Oral Toxicity Study of EAT THE GREASE in Rats

1.2 TEST ITEM IDENTIFICATION (AS DECLARED BY SPONSOR)

Test Item Name	:	EAT THE GREASE
Lot No.	:	A03216
Sterilization Condition	:	N.A.
Quantity	:	1 packet
Date of Manufacture	:	18082016
Date of Expiry	:	17082019

1.2.1 Material Composition

Composition	:	Active constituent: Crude protein 20.45%; moisture 7.95%; lipid (crude fat) 0.71%; crude fiber 6.30%, ash 12.30% (as labeled) Raw material: Rice bran 74.0% w/w; rice flour 10.0% w/w; soybean extract 10.0% w/w; skim milk powder 3.0% w/w; citric acid 1.0% w/w; sodium carbonate 1.0% w/w; trace nutrient 1.0% w/w (as labeled)
Homogeneity	:	N.A.
Purity	:	N.A.

1.2.2 Physical Features / Properties

Colour / state	:	Solid, brown color powder
Density	:	N.A.
pH	:	N.A.
Solubility	:	Soluble in water

1.3 TEST ITEM SUBMISSION DATE

03 Oct 2016

2. SPONSOR

Sentinel Solution (Thailand) Co.,Ltd
388 Exchange Tower, 42nd Floor,
Sukhumvit road, Klongtoey,
Bangkok 10110

3. TESTING FACILITY, TESTING SITES AND STAFF

3.1 TESTING FACILITY

Chemical and Materials
Testing Services
TÜV SÜD PSB Pte Ltd
No 1 Science Park Drive
Singapore 118221

3.2 STAFF

Study Director	:	Mr Lin Xi
Study Personnel	:	Ms Li Yang Dr Lim Chiaw Hwee Dr Li Baihong
The above staff are located at	:	Chemical and Materials Testing Services TÜV SÜD PSB Pte Ltd 1 Science Park Drive Singapore 118221

4. STUDY SCHEDULE AND GUIDELINES

4.1 STUDY SCHEDULE

Experimental commencement date	13 Oct 2016
Experimental completion date	03 Nov 2016

4.2 STUDY GUIDELINES

4.2.1 OECD Guideline For Testing of Chemicals 423: Acute Oral Toxicity- Acute Toxic Class Method, adopted on 17th December 2001

4.2.2 Globally Harmonized System of Classification and Labelling of Chemicals (GHS), fourth revised edition, United Nations, New York and Geneva, 2011, Part 3 Health Hazards, Chapter 3.1: Acute Toxicity.

5. MATERIAL AND METHODS

5.1 TEST ANIMALS

Species	Rats
Strain	SD
Microbiological status	Murine Pathogen Free (MPF)
Age	8-12 weeks old
Sex	Female
Number	6
Source	InVivos Pte Ltd 9 Perahu Road, Lim Chu Kang, Singapore 718793
Animal Holding Facility	Animal Holding Unit TÜV SÜD PSB Pte Ltd No 1 Science Park Drive Singapore 118221
Housing Condition	OptiMICE Caging System
Temperature	19 - 25ºC
Humidity	30 - 70%
Food	Altromin Maintenance Diet #1324
Water	Tap water
Animal ID	7191147889-01-G1-1~3 7191147889-01-G2-1~3

5.2 TEST CONDITIONS

5.2.1 Preparation of test animals

5.2.1.1 The test animals were acclimatised for at least 5 days before the test was conducted.

5.2.1.2 The test animals were fasted overnight before dosing. Feed, but not water was withheld. The animals were weighed prior to dosing. The test substance was then administered by pasteur pipette at a starting dose level of 2000 mg/kg body weight based on the body weight of each test animal.

5.2.2 Rationale of selection of starting dose

According to OECD Guideline for the testing of Chemicals 423, the limit dose of 2000 mg/kg is used for the test item which the toxicity or mortality is not expected. Thus, starting dose of 2000 mg/kg was selected according to the requirement of sponsor.

5.2.3 Preparation of test substance

Test item was suspended in distill water at concentration of 200 mg/ml. The suspension was used as the test substance. A single dose was administered orally to each animal based on the body weight using pasteur pipette.

5.2.4 Details of administration

Administration route	Oral route by pasteur pipette
Dose level	2000 mg/kg body weight
Dose Interval	Single dose

The details of dose for each animal are as follows:

Group	Animal ID	Dosing date	Body weight (g)	Amount of test item used for dosing (mg)
Group 1	7191147889-01-G1-1	19 Oct 16	204	408
	7191147889-01-G1-2		210	420
	7191147889-01-G1-3		180	360

Group	Animal ID	Dosing date	Body weight (g)	Amount of test item used for dosing (mg)
Group 2	7191147889-01-G2-1	20 Oct 16	198	396
	7191147889-01-G2-2		208	416
	7191147889-01-G2-3		208	416

5.2.5 Feed and water frequency

Animals were fasted overnight before dosing. Feed, but not water was withheld.

Feed was given about 3 hours after dosing and throughout the observation period. Feed was given in the chamber in the cage.

Water was given *ad libitum* during dosing and observation period. Water was given through plastic bottle.

5.2.6 Observation, body weight measurement and necropsy

The observation was conducted on each animal during the first 30 minutes, 1, 2 and 4 hours, and daily thereafter to 14 days.

The body weight of each animal was measured once a week.

On the termination day, all the test animals were euthanized by CO_2 inhalation. Gross necropsy was conducted on each test animal.

6. TEST RESULTS

6.1 OBSERVATION OF EACH TEST ANIMAL

Group	Animal ID	Observation during 14-day period
Group 1	7191147889-01-G1-1	No adverse effects observed
	7191147889-01-G1-2	No adverse effects observed
	7191147889-01-G1-3	No adverse effects observed

Group	Animal ID	Observation during 14-day period
Group 2	7191147889-01-G2-1	No adverse effects observed
	7191147889-01-G2-2	No adverse effects observed
	7191147889-01-G2-3	No adverse effects observed

6.2 BODY WEIGHT (BW, IN GRAM) AND BODY WEIGHT CHANGES (CH, IN GRAM) OF EACH ANIMAL

Group	Animal ID	Day 0 (before dosing) (19 Oct 16)	Day 7 (26 Oct 16)		Day 14 (Termination Day) (02 Nov 16)	
		BW	BW	CH	BW	CH
Group 1	7191147889-01-G1-1	204	234	+30	240	+6
	7191147889-01-G1-2	210	248	+38	258	+10
	7191147889-01-G1-3	180	210	+30	226	+16

Group	Animal ID	Day 0 (before dosing) (20 Oct 16)	Day 7 (27 Oct 16)		Day 14 (Termination Day) (03 Nov 16)	
		BW	BW	CH	BW	CH
Group 2	7191147889-01-G2-1	198	236	+38	236	0
	7191147889-01-G2-2	208	240	+32	258	+18
	7191147889-01-G2-3	208	250	+42	256	+6

No body weight loss was found in all animals.

6.3 DEATH PRIOR TO ENDPOINT

No animals died before the endpoint, i.e. 14 days after dosing.

6.4 ONSET OF TOXICITY AND REVERSAL

No toxicity effect was observed on all the test animals during dosing and observation period.

6.5 NECROPSY FINDINGS

No abnormality was observed on all the test animals.

7. DISCUSSION

Based on the above study,

a) No animal died during the study.

b) No adverse effect was observed on all the test animals during the study.

c) No body weight loss was observed during the study.

d) No abnormality was observed on all animals during necropsy.

8. CONCLUSION

Under the condition of this study and based on Global Harmonised Classification System (GHS) for acute toxicity hazard categories, the acute oral toxicity of the test item – EAT THE GREASE, Lot No: A03216, is considered as Category 5 or unclassified; the LD_{50} cut-off value of the test item – EAT THE GREASE, Lot No A03216, is 5000 mg/kg body weight or unclassified.

REMARKS:

1. The above test results relate to the sample of test item as received.

DR LIM CHIAW HWEE
STUDY PERSONNEL
CHEMICAL AND MATERIALS
TESTING SERVICES

MR LIN XI
STUDY DIRECTOR
CHEMICAL AND MATERIALS
TESTING SERVICES

PSB Singapore

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July 2011