TEST REPORT: 219127054-01-00

Date: 30 MAY 2011 Tel: +65 68851323 Fax: +65 67784301

Client's Ref: OE1000076 Email: Yang.LI@tuv-sud-psb.sg

1. GENERAL

1.1 STUDY TITLE

Acute Oral Toxicity Study of Bio-X Lullaby

1.2 TEST ITEM IDENTIFICATION

Test Item Name	:	Bio-X Lullaby
Lot No	:	20110201
Sterilization Condition	:	Non-Sterile
Quantity	:	220 ml per bottle, 2 bottles
Date of Manufacture	:	1 Feb 2011
Expiry Date	:	1 Feb 2014

1.2.1 Active Ingredients
(based on Material Handling Form provided by sponsor)

Composition	:	Contains Etofenprox
Purity	:	2.50%w/w

1.2.2. Physical features/properties
(based on Material Handling Form provided by sponsor)

Colour/state	:	Milky White Emulsion
Density	:	0.997
pH	:	5.83
Solubility	:	Soluble in water

1.3 REFERENCE ITEM IDENTIFICATION

Nil

PSB Singapore

2. SPONSOR

OKADA ECOTECH PTE LTD
55, Ayer Rajah Crescent
#03-19/23
Singapore 139949

3. TESTING FACILITY, TESTING SITES AND STAFF

3.1 TESTING FACILITY AND TESTING SITES

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

3.2 STAFF

Study Director	Ms Li Yang
Study Personnel	Mr Chong Koon Chiang
	Mr Tang Xiao Hua
The above staffs are located at	Chemical and Materials Testing Services TÜV SÜD PSB Pte Ltd No 1 Science Park Drive Singapore 118221

4. STUDY SCHEDULE AND GUIDELINES

4.1 STUDY SCHEDULE

Experimental commencement date	25 Apr 2011
Experimental completion date	11 May 2011

4.2 STUDY GUIDELINES

4.2.1 Organisation for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals No 423: Acute Oral Toxicity – Acute Toxic Class Method, adopted on 17th Dec 2001.

5. QUALITY ASSURANCE STATEMENT

This study was audited by Quality Assurance personnel of Chemical and Materials Testing Services as follows:

1. The study plan was audited on 21 Apr 2011 and the audit report was submitted to Study Director and Test Facility Management on the same day.

2. A process-based audit was conducted on 26 Apr 2011 and 10 May 2011 and the audit report was submitted to Study Director and Test Facility Management on the same day.

3. The raw data and study report were audited on 30 May 2011 and the audit report was submitted to Study Director and Test Facility Management on the same day.

The final report has been found to reflect the raw data obtained.

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30 May 2011

CHEW WAN YU **DATE**
QUALITY ASSURANCE
CHEMICAL AND MATERIALS
TESTING SERVICES

PSB Singapore

6. STATEMENT OF COMPLIANCE

6.1 The procedure of the study complies with the study plan as agreed by the sponsor. The conclusion of the test report is based on the raw data obtained from the study.

6.2 This study is in compliance with the regulation of National Advisory Committee on Laboratory Animal Research (NACLAR) of Singapore.

6.3 This study is conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Environmental Health and Safety Publications ENV/MC/CHEM(98)17, Series on Principles of Good Laboratory Practice and Compliance Monitoring No.1, OECD Principles of Good Laboratory Practice (as revised in 1997), Paris 1998.

30 May 2011

MS LI YANG **DATE**
STUDY DIRECTOR
CHEMICAL AND MATERIALS
TESTING SERVICES

7. MATERIAL AND METHODS

7.1 PRE-TREATMENT OF TEST ITEM

The test item was used for oral administration directly without pre-treatment.

7.2 PREPARATION OF TEST SUBSTANCE AND NEGATIVE CONTROL

The test item was used for oral administration directly. So, in this study, the test substance was the test item.

7.3 TEST ANIMALS

Species	Rats
Strain	Wistar, albino
Microbiological status	Specific Pathogen Free (SPF)
Age	6 to 8 weeks old
Sex	Female
Number	6
Source	National University of Singapore Centre for Animal Resources (CARE) 7 Perahu Road Singapore 718836
Housing Condition	Individual Ventilated Cage System
Temperature	18 - 22^{o}C
Humidity	30 - 70%
Food	PicoLab® Rodent Diet 20 5053
Water	Tap water

7.4 TEST CONDITIONS

7.4.1. Preparation of test animals

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

7.4.1.2 The test animals were fasted overnight before dosing, water was not withheld. The animals were weighed prior to dosing. The test substance was then administered by gavage feeding needle at a starting dose level of 5000 mg/kg body weight based on the body weight of each test animal.

7.4.2 Rationale of selection of starting dose

The starting dose level was 5000 mg/kg body weight as required by sponsor based on the dose level of limit test in OECD Guideline 423

7.4.3 Administration level at 5000 mg/kg body weight

Administration	Oral route by gavage feeding needle
Dose level	5000 mg/kg body weight based on product weight
Dose Interval	Single dose

The details of dose for each animal are as follows:

Group No	Animal ID No	Dosing date	Dose volume (ml)	Dose level (based on the product weight, mg/kg) by body weight
Group 1	219127054-01-00-G-1-1	26-Apr-2010	1.1	5000
	219127054-01-00-G-1-2		1.2	5000
	219127054-01-00-G-1-3		1.1	5000

Group No	Animal ID No	Dosing date	Dose volume (ml)	Dose level (based on the product weight, mg/kg) by body weight
Group 2	219127054-01-00-G-2-1	27-Apr-2010	1.2	5000
	219127054-01-00-G-2-2		1.2	5000
	219127054-01-00-G-2-3		1.1	5000

7.4.4 Feed and water frequency

Feed was given 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.

7.4.5 Observation

The observation of adverse effects was conducted on each animal during the first 30 minutes, periodically during the first 24 hours (with special attention during the first 4 hours), and daily thereafter, up to 14 days. The body weight of each animal was measured every 3 to 7 days.

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

8. TEST RESULTS

8.1. Dose level of each test animal and adverse effects

Group No	Animal ID	Body weight (g)	Dose volume (ml)	Dose level (based on the product weight, mg/kg) by body weight	Adverse effects after dosing till endpoint date
Group 1	219127054-01-00-G-1-1	210.9	1.1	5000	No adverse effects observed
	219127054-01-00-G-1-2	245.4	1.2	5000	No adverse effects observed
	219127054-01-00-G-1-3	220.8	1.1	5000	No adverse effects observed
Group 2	219127054-01-00-G-2-1	242.2	1.2	5000	No adverse effects observed
	219127054-01-00-G-2-2	232.3	1.2	5000	No adverse effects observed
	219127054-01-00-G-2-3	223.0	1.1	5000	No adverse effects observed

8.2. Body weight (bw, in gram) and body weight changes (change, in gram) of each animal

Group No	Animal ID	26-Apr-11 Dosing day (Day 0)	27-Apr-11 (Day 1)		03-May-11 (Day 7)		10-May-11 (Day 14, Termination Day)	
			Bw	Change	Bw	Change	Bw	Change
Group 1	219127054-01-00-G-1-1	210.9	217.9	+7.0	227.9	+10.0	232.8	+4.9

	219127054-01-00-G-1-2	245.4	249.9	+4.5	257.0	+7.1	282.5	+25.5
	219127054-01-00-G-1-3	220.8	234.5	+13.7	239.2	+4.7	245.8	+6.6

Group No	Animal ID	27-Apr-11 Dosing day (Day 0)	28-Apr-11 (Day 1)		04-May-11 (Day 7)		11-May-11 (Day 14, Termination Day)	
			Bw	Change	Bw	Change	Bw	Change
Group 2	219127054-01-00-G-2-1	242.2	248.5	+6.3	263.1	+14.6	278.2	+15.1
	219127054-01-00-G-2-2	232.3	237.1	+4.8	252.0	+14.9	267.8	+15.8
	219127054-01-00-G-2-3	223.0	237.4	+14.4	240.3	+2.9	266.7	+26.4

8.3. Death prior to endpoint

No death was observed in all the test animals prior to endpoint.

8.4. Onset of toxicity and reversal

No adverse effect was observed in all animals during dosing and observation period.

8.5. Necropsy findings

Necropsy was conducted on all the test animals on the termination day. No abnormality was observed on all the test animals.

The body weight of each animal at necropsy date (in gram)

Group No	Animal ID No	Body weight at necropsy date/ g
Group 1	219127054-01-00-G-1-1	232.8
	219127054-01-00-G-1-2	282.5
	219127054-01-00-G-1-3	245.8

Group 2	219127054-01-00-G-2-1	278.2
	219127054-01-00-G-2-2	267.8
	219127054-01-00-G-2-3	266.7

9. DISCUSSION

Based on the above study,

a) No adverse effects observed in all the test animals in dosing and observation period.
b) Necropsy findings were normal in all the test animals.

Hence, based on Global Harmonised Classification System (GHS) for acute toxicity hazard categories, the LD_{50} cut-off value of Bio-X Lullaby, Lot No. 20110201 is 5000 mg/kg body weight or unclassified.

10. CONCLUSION

Based on the above results and Global Harmonised Classification System (GHS) for acute toxicity hazard categories, the acute oral toxicity of Bio-X Lullaby, Lot No. 20110201 is considered as Category 5 or unclassified; the LD_{50} cut-off value of Bio-X Lullaby, Lot No. 20110201 is 5000 mg/kg body weight or unclassified.

11. DEVIATIONS OF THE STUDY PROCEDURES FROM THE STUDY PROTOCOL

11.1 The animals used in the study were 6-8 weeks old instead of 8 -12 weeks old stated in the study plan. The deviation is due to the animals supplied by local supplier. The deviation does not impact the validity of the study.

11.2 Six animals were used for dosing in the study instead of three animals as stated in the study plan. The deviation is due to the confirmation test of the study. The deviation does not affect the validity of the study.

12. ARCHIVAL

The study protocol, study plan, study schedule, all the raw data of experiment, audit report of quality assurance unit and other related documentations and the final test report are stored in the archive of TÜV SÜD PSB Pte Ltd.

REMARKS:

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

Tang

MR TANG XIAO HUA
STUDY PERSONNEL
CHEMICAL AND MATERIALS
TESTING SERVICES

MS LI YANG
STUDY DIRECTOR
CHEMICAL AND MATERIALS
TESTING SERVICES

PSB Singapore

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March 2010