

EXPERIMENT REPORT
by
Japan Food Research Laboratories
Tama Laboratory

6-11-10 Eizan Tama-City Tokyo-To Japan 206-0025
Examination#: 205040685-001
06/20/2005

Requested by: Litanial Bio Science. Co.,Ltd
2-26 Kitamachi Shinnobe Beppu-cho Kakogawa City Hyogo-Ken
Japan Zip 675-0121

Subject sample: LBS Culture Extract (sterilized)

Title: Acute Oral Toxicity examination to male mouse.

Results are in the following pages

Acute Oral Toxicity examination to male mouse

Summary

Feeding LBS Culture Extract (sterilized) to male mouse examined acute oral toxicity. Examination group is given 2,000 mg/kg dosage orally by injection with LBS Extract solvent with water to male mouse group in single dosage a day for 14 days. During the examination period, there were no mouse became abnormal or dead recognized. As a result, We found LD50 level is above 2,000mg/kg to male mouse by a single dosage application.

Client:	Litanial Bio Science. Co.,Ltd
Subject sample:	LBS Culture Extract (sterilized)
Examination period	5/23/2005 – 6/20/2005
Examination taken place	Japan Food Research Laboratories Tama Laboratory 6-11-10 Eizan Tama-City Tokyo-To Japan 206-0025
Examination in charge	Shinichi Katsuta Japan Food Research Laboratories Tama Laboratory 6-11-10 Eizan Tama-City Tokyo-To Japan 206-0025
Examination Performed by	Tomoko Shimazaki, Takesi Nagai, Jun Fukai, Yasuharu Kawamoto

1. Examination purpose

Examine the sample about a possibility of Acute Oral Toxicity examination using male mouse.

2. Sample

LBS culture Extract (sterilized)

Appearance: Light yellowish liquid with white particles.

3. Method of making sample liquid

Sample is made thinner with injected water to 100mg/mL

4. Tested Animal

5 weeks old 10 of ICR line male mouse purchased from Japan SLC Co., Ltd. Spend a week to observing the testing animals are in normal condition to be available for our test. 5 mouse as one group and kept each group in separate polycarbonate cage in room temperature 23 degree Celsius (73.4 degree Fahrenheit) +/- 2 degrees, Lighting time 12 hours/ day in breeding room. They were fed with mouse, rat sold feed, LaboMRStock by Japan Agricultural industrial Company along with tap water as freely they like.

5. Test Method

One group of 5 mouse is given 2,000/mg/kg dosage and the other group is given only water. Before testing, they are fasted for 4 hours and weighted each. One group was given LBS solvent and the other was given only water. Each dosage given are scaled 20 ml/kg in volume by injecting into their each mouse through stomach sondel tube at single time. Observation period was 14 days, and inspected several times on the first day dosage given, and inspected once a day in the following day. After 7th day and 14th day, each weight is taken. Applied t-official approved method and considered significance level 5% between the groups to make comparison. After testing, all mouse are dissected for observation.

6. Testing Result

1) Resulted Death

Either group found no death during examining.

2) General State

Either group found no abnormality during examination

3) Weight change (see chart-1)

After the test over, there is no difference in weight gain.

4) Autopsy finding

After the test over, there is no abnormality found to all tested mouse.

5) Consideration

After testing the sample about a possibility of Acute Oral Toxicity examination using male mouse, providing 2,000 mg/kg dosage in single oral application, there no abnormal or dead mouse observed. Therefore, LD50 level to male mouse single dosage oral application is determined more than 2,000 mg/kg.

6) References

ODCD Guidelines for the Testing of Chemicals 420(2001).

Chart -1 Weight shift

2 Groups	Dosage before	Dosage after	
		7 th day	14th day
Dosaged group	25.6+-0.6 (5)	27.6+-0.9 (5)	30.1+-1.8 (5)
Non dosaged group	25.5+-0.6 (5)	26.9+-1.1 (5)	29.5+-2.4 (5)

Weight is listed as Average +- Standard deviation (Unit: gram).

Inside of a parenthesis shows number of animal