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中華民國實驗室認證體系
行政院環保署環境檢驗認證

Animal Acute Toxicity Test (LD_{50}) for BIO-ALPOSOL

Client	GreenTech Biotechnology Environmental Co., LTD	Report No.	SL92E9019m1
Client Address	3F, No. 97, Jingye Road, Section 1, Taipei, Taiwan, ROC		
Client Specimen I.D.	BIO-ALPOSOL	Date Received	June 3, 2003
Specimen I.D.	9019E01	Date Issued	July 18, 2003
Specimen description	Liquid form in can (stored at room temperature)	Specimen collector： ■ Research Contract	

Remarks：

1. This report includes：10 pages totally. It will be in vain if separated and/or partially copied.
2. The results in this report are valid only to the specimen sent by client.
3. All report content is used as references, not for advertising, sales promotion and notarial purpose.
4. The former report（No.SL92E9019）is replaced by this report.

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Abstract

The objective of this experiment is to evaluate the lethal dose which kill 50% tested animals (LD_{50}) by BIO-ALPOSOL. The experiment is so called " the animal acute toxicity test". The specimen no is SL92E9019 and provided by the GreenTech Biotechnology Environmental Co., Ltd. The tested animals (ICR strain; six-week old) were brought from the National Laboratory Animal Center, Taipei, Taiwan. The tested animals were examined by the veterinarian before conducting the experiment in order to make sure they are free from any specific pathogens (SPF). All the mice were divided into six groups and each group contained two mice, including one male one female. Another two mice were carried out as control group. Preliminary study was carried out in order to assure its dosage range of acute toxicity (LD_{50}). After that, the test of the lethal dose (i.e. LD_{50}) was determined. The result had showed that the LD_{50} for BIO-ALPOSOL (SL92E9019) was 246,305.4mg/kg or 246,305.4ppm. Based on the toxicity category of drug, we can conclude that it had no toxicity when LD_{50} was larger that 15,000 mg/kg. After biopsy for dead mice, we found that BIO-ALPOSOL (SL92E9019) can cause acute swelling for liver cell, swelling for epithelial cells of esophagus and inflammatory swelling for stomach mucosa epithelial cells, and also liver stasis, carbuncular esophagus and digestive and stomach mucosa could be seen by macroscopic observation. Acute toxic cellular swollen of liver cell, acute inflammatory and cell swollen of digestive epithelial cell could also be seen under microscopic observation. Therefore, the target organ of toxicity for BIO-ALPOSOL is liver and digestive system.

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Table 1. Categories of drug toxicity related to its dose.

Categories	LD_{50} (mg/kg)
Very Severe toxicity	< 5
Severe toxicity	5 ~ 50
High toxicity	50 ~ 500
Moderate toxicity	500 ~ 5,000
Low toxicity	5,000 ~ 15,000
No toxicity	> 15,000

Objective：

To determine the LD_{50} for BIO-ALPOSOL by the animal acute toxicity test.

Materials：

（1） ICR strain small mice： six-week in age, brought from the National Laboratory animal Center of the National Science Council, Executive Yuan, Taiwan. The purchase number is 92-005222.

（2） BIO-ALPOSOL：The test chemical was provided by the GreenTech Biotechnology Environmental Co., LTD.

（3） Apparatus for Biopsy.

（4） Sterile feeding needles.

（5） Ear labels for mice.

（6） Formalin and buffer solution for viscera fixation: purchased from Creative Microbiologicals Products Co., Ltd., Taipei, Taiwan.

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Methods：

（1） Specific pathogen-free(SPF)ICR strain small mice(six-weeks in age)were isolated for at least ten days, after that, physiological, clinical, and pathological examination were conducted to assure no specific pathogens in the tested animals（Table 2）.

（2） Establish the conditions for feeding environment, including temperature, humidity, feeding consuming dose, water intake amount etc.（Figure 1 and 2）.

（3） Preliminary study: After assuring the tested animals have no specific pathogen or injury, they were divided into two six groups, each group had contained two small mice, one for female and one for female. And the given doses of BIO-ALPOSOL for each group of mice are as follow: 0.5g/mice, 1.0g/mice, 2.0g/mice, 4.0g/mice, and 8.0g/mice. The mortality for ICR strains mice were calculated and determined. After that, all of the mice were forced to mercy killing, followed by histopathological examination, thus their drug toxicity can then be observed（Table 3）.

（4） LD_{50} Determination and Calculation:

The suitable range for given BIO-ALPOSOL doses of LD_{50} can be referred and determined from the results of preliminary study. The doses were given to all the six tested and control group, and their dose range between groups is increased individually by 1.2 fold. Each group contains 3 male and 3 female mice. The mortality of mice from each groups were observed and calculated after 24～48 hours（Table 4）. Finally, all of the mice were forced to mercy killing, followed by histopathological examination, thus, the target organ(s) of BIO-ALPOSOL toxicity can then be observed. The LD_{50} can be determined from the graph which drawn from the results of each tested group, and the value was then divided by the average weigh of ICR mice. The unit for LD_{50} is shown as mg/kg.

（5） Histopathological Examination and Explanation:

The histopathological examination was carried for all ICR strain mice, and the affected target organ(s) of drug was recorded.

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Results：

（1） Before experiment was carried out, the basic function of central nervous system, respiratory tract system, gastrointestinal tract system, cardiovascular system, skeletal system, muscular system, genitourinary tract system and parasitic examination were examined. The results were shown on Table 2.

Table 2. Results of the Examination on small mice before experiment

System or parasites Examined	Results
Central Nervous System	Normal
Respiratory Tract System	Normal
Gastro-intestinal System	Normal
Cardiovascular System	Normal
Skeletal System	Normal
Muscular System	Normal
Genitourinary Tract System	Normal
Parasites	Not found

*The Average of feeding doses=6.82.g ± 0.78g/mouse；
the average water intake=7.92mL ± 2.58 mL/mouse.

（2） Environmental control: the temperature and humidity for animal room were maintained around 22~26℃ and 45~60%, respectively.

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Figure 1：Control Chart for Temperature in Animal Room

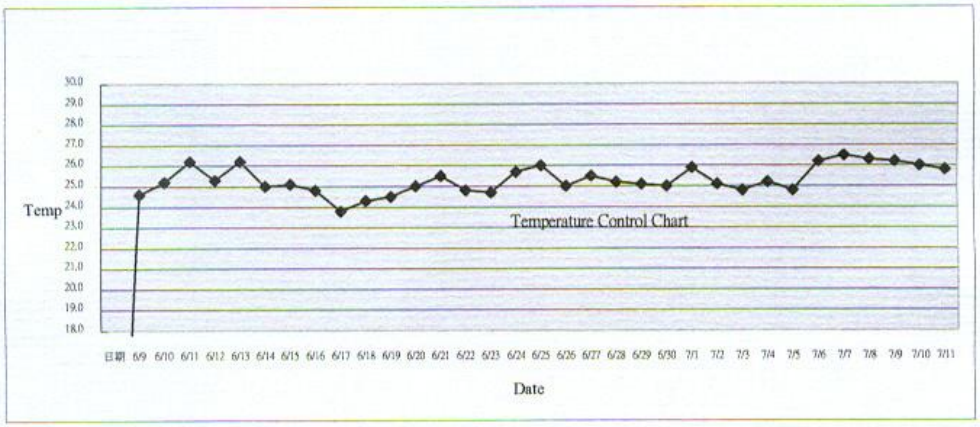
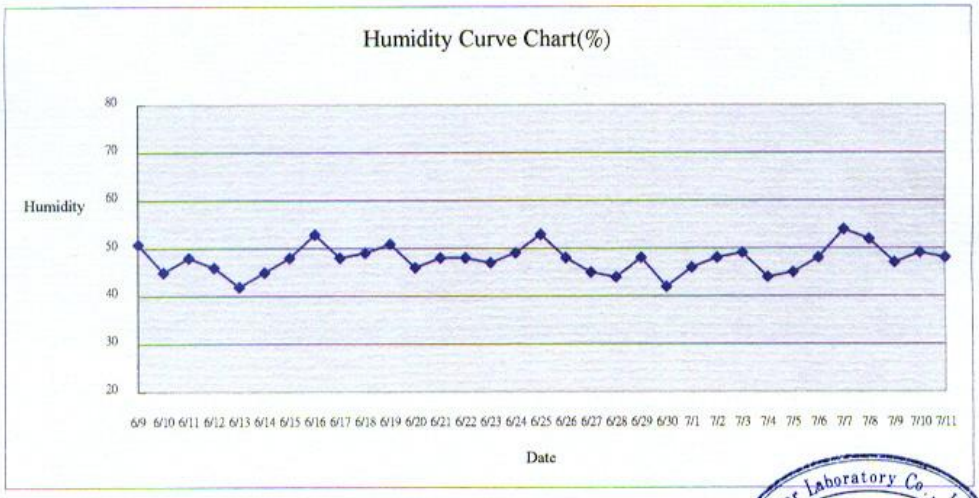


Figure 2：The humidity control chart for animal room



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Client：GreenTech Biotechnology Environmental Co., LTD ☐Preliminary Report ■ Final Report

Report No.：SL92E9019m1

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（3） Preliminary results of drug toxicity test:

The results have showed that the suitable range for feeding doses of lethal dose -50（LD_{50}）is 4g～8g/mouse（Table 3）.

Table 3：Preliminary test results for BIO-ALPOSOL

Dose / small mouse	8g/mouse	4g/mouse	2g/mouse	1g/mouse	0.5g/mouse	Control group**
Male	001alive*	002 alive	003 alive	004 alive	005 alive	006 alive
Female	025 death	026 alive	027 alive	028 alive	029 alive	030 alive

***The number of labels for ICR mice (six-week in age)；** control group : no dose was given.**

（4） Test for LD_{50} and Its Calculation:

According to the results showed in Table 4, The LD_{50} will be 7.5g/mouse（007～024 and 0.31～0.48）divided by body weight（30.45g±2.75g）of ICR mice and got a value of 246,305.4mg/kg or 246,305.4ppm.

Table 4：The Results of Lethal Dose 50 for BIO-ALPOSOL

Dose / Small mouse	8.0g/mouse	7.0g/mouse	6.0g/mouse	5.0g/mouse	4.0g/mouse	Control Group**
Male	*007 alive	008 death	009 alive	010 alive	011 alive	012 alive
Male	013 death	014 alive	015 alive	016 alive	017 alive	018 alive
Male	019 death	020 death	021 alive	022 alive	023 alive	024 alive
Female	031 alive	032 alive	033 alive	034 alive	035 alive	036 alive
Female	037 death	038 alive	039 alive	040 alive	041 alive	042 alive
Female	043 death	044 alive	045 alive	046 alive	047 alive	048 alive

***The number of labels for ICR mice(six-week in age)；**Control group : no dose was given.**

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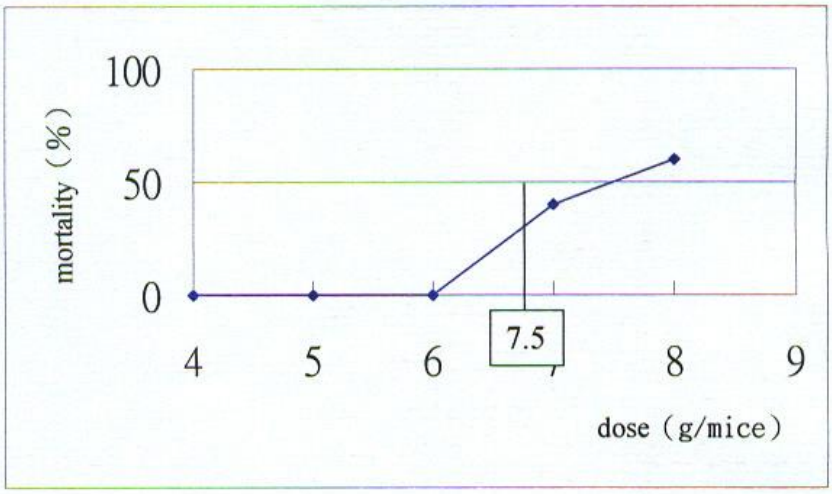
Figure 3：The Representative Chart of LD_{50} for BIO-ALPOSOL.



（5） Histopathological Examination：

After biopsy for dead mice, we found that BIO-ALPOSOL（SL92E9019） can cause (i) acute swelling for liver cell, (ii) swelling for epithelial cells of esophagus , (iii) acute inflammation and swelling for stomach mucosa epithelial cells, and (iv) blood clot and stasis in liver. Furthermore, we also found that carbuncular esophagus and swelling digestive and stomach mucosa by macroscopic observation. Microscopically, acute toxic cellular swollen of liver cell, acute inflammatory and cell swollen of digestive epithelial cell could also be observed. Therefore, the target organs of toxicity for BIO-ALPOSOL are liver and digestive system.

The toxic effect manifested by BIO-ALPOSOL（specimen number：SL92E9019）included acute cell swollen and steatosis of liver cell. The possible manifestation for toxic damage in animal and human included：(1) affect liver secretive function（e.g. cholesterol, phospholipid, bile salt and bile pigment）; (2) affect conjugating function（e.g. removal of drug and toxic material）; (3) affect

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metabolic function (e.g. proteins, carbonhydrates, and lipid biosynthesis and utilization) ; (4) cause acute inflammatory and cell swollen in digestive tract ; (5) cause burn injury for digestive tract, digestive obstruction and decrease in food intake.

Discussion and Conclusion :

From the above study in ICR mice, we found that the LD_{50} for BIO-ALPOSOL is 246,305.4mg/kg (or 246,305.4ppm) . According to the categories of acute toxic effect related to drug dose (Table 1) , when the dose of LD_{50} is greater than 15,000mg/kg, it is of non-toxicity. After comparing the LD_{50} of BIO-ALPOSOL with those of other various chemicals (Table 5) , the results have showed that the LD_{50} of BIO-ALPOSOL is greater than those of other chemicals listed on Table 5.

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Table 5. The LD_{50} for some other chemicals

Chemicals' name	Tested Animals	LD_{50} (mg/kg)
Ethanol	Mice	10,000
NaCl	Mice	4,000
$FeSO_4$	Mice	1,500
Morphine	Mice	900
Phenobarbital	Mice	150
DDT	Mice	100
Picrotoxin	Mice	5
Strychnine	Mice	2
Nicotine	Mice	1
d-Tubocurarine	Mice	0.5
Tetrodotoxin	Mice	0.1
Dioxin	Mice	0.001
Botulinus toxin	Mice	0.00001

Laboratory Director : Laboratory Director *Wen-cherng Tsai, Ph. D.* Researcher : RDS Manager *Michael Lin*

July 18, 2003

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