



Leberco • Celsis Testing

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Nov. 25, 1996

SUBMITTED TO: ACTS Testing Labs, Inc.
Buffalo, NY
Patty Dick

ASSAY NUMBER: 9619784

DATE RECEIVED: 10/25/96

TEST MATERIAL: Renilla Luciferase & Coelenterazine
w/Table Salt & Sodium Carbonate - (composite)
Log #: 6B-15193

DATE INITIATED: 10/29/96 COMPLETED: 11/12/96

PROCEDURE: Acute Oral Toxicity in Rats, Federal Hazardous
Substances Act (FHSA Sept. 27, 1973 and Nov.
1, 1973)

SUMMARY:

The test material, as supplied, was administered to a group of 10 white rats (5 males, 5 females), to evaluate its toxic characteristics in accordance with Federal requirements as listed in 16 CFR 1500.3 (c) (2) (i). A single oral dose (5 gm per kg body weight) was given and the animals were observed for 14 days thereafter. Any and all behavioral/clinical abnormalities including mortalities were recorded. All animals appeared normal throughout the study period.

No mortalities occurred during the observation period. Animals were sacrificed at the conclusion of the study. There were no gross abnormalities observed in any of the animals at necropsy.

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OBJECTIVE:

To determine the degree of toxicity that the test substance may produce when administered orally to white rats in a single oral dose of 5 grams per kilogram of body weight.

TEST ANIMALS:

Species: Rattus Norvegicus
Strain: Sprague Dawley (Ace Animals, Boyertown, PA)
No./Sex: 1 group of 10 (5 males, 5 females)
Weight: 200-300 grams

During the acclimation and testing periods, each animal was housed and maintained according to the "Guide For The Care and Use of Laboratory Animals" (NIH 86-23). All animals were identified through eartags and cage cards which provided the individual animal numbers and the project number. The animals were either single or double housed and fed Agway Prolab Rodent pellets and city water, ad libitum. All animals were acclimated for at least 4 days prior to testing.

PROCEDURE:

1. Animals were fasted overnight, approximately 18 hours prior to dosing.
2. Prior to dosing, the test materials were composited. Five-tenths ml of DMSO (Prolume Ltd.) was added to 1 tube of Coelenterazine, then inverted 5 times to dissolve. To one tube of Prolume Ltd. #520-367-6047 which contained Renilla L.Ase, NaCl, NaHCO₃, tap water was added to the 45 ml line, then inverted 10 times to dissolve. To the tube of Coelenterazine & DMSO mixture, 1.5 ml of Prolume Ltd. "Gin" was added, inverted 5 times to mix; then added to the Renilla L.ASE & tap water solution. This resulted in a yellow liquid.

The composited test material was administered orally as a single dose via syringe and suitable intubation tube. The single dose of 5 g/kg of body weight was based on the density of the composited test materials which was 1.03 g/kg.

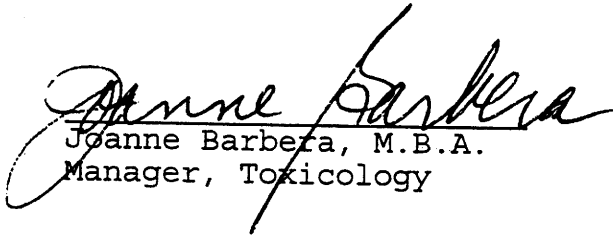
3. The animals were examined for signs of toxicity, including mortality, immediately after dosing, four hours after dosing, and then daily for a maximum of 14 days. All observations were recorded and included the following:
 - A. Behavioral abnormalities
 - B. Gross necropsy
 - C. Body weight changes
 - D. Mortality
 - E. Any other toxicological effects


CONCLUSION:

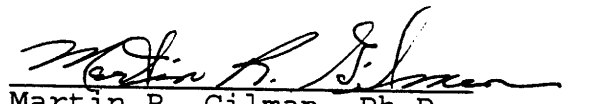
The submitted test materials when administered as a composite at a single oral dosage level of 5 g/kg body weight, did not produce compound related mortality in half or more of the animals; therefore, the LD₅₀ is greater than 5 g/kg and the test material is not considered to be toxic according to definitions listed in 16 CFR 1500.3 (c) (2) (i).

Work Performed Under the
Supervision of:

LEBERCO-CELSIS TESTING


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SUBMITTED TO: ACTS TESTING LABS, INC.

ASSAY NUMBER: 9619784

RECEIVED: 25-Oct-96

TEST MATERIAL: Composite Table & Sodium Bicarbonate

Lot/ID #: RECOMBINANT RENILLA...

Acute Oral Toxicity in Rats

Animal # and Sex	Body Weight in Grams		
	Initial Weight	Final Weight	Body Weight change
Rat #1 (Female)	252	266	+14
Rat #2 (Female)	259	294	+35
Rat #3 (Female)	248	264	+16
Rat #4 (Female)	241	272	+31
Rat #5 (Female)	236	267	+31
Rat #6 (Male)	255	364	+109
Rat #7 (Male)	256	332	+76
Rat #8 (Male)	274	378	+104
Rat #9 (Male)	257	367	+110
Rat #10 (Male)	245	317	+72

Dose:	Number	
	Fed	Dead
Gm/kg Rat		
5 g/kg	10	0

Necropsy:

No gross abnormalities.