Leberco • Celsis Testing

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Nov. 5, 1996

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

ASSAY NUMBER: 9620342

SUBJECT: Primary Dermal Irritation in Rabbits

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

DATE RECEIVED: 10/25/96

OBJECTIVE: To ascertain the potential for dermal irritation of a test substance using rabbits.

DATE INITIATED: 10/29/96 COMPLETED: 11/1/96

RESULTS: Primary Irritation Score: 0.00
(Not a primary dermal irritant.)

PURPOSE OF ASSAY:

This test is designed to identify substances which are primary irritants to rabbit skin, in accordance with the procedure described by the Federal Hazardous Substances Act, 16 CFR, Section 1500.41.

METHOD OF ASSAY:

Six (6) New Zealand white rabbits, about three months of age, weighing approximately 2-3 kilograms, were obtained from a USDA licensed dealer. Animals were checked upon receipt for diarrhea and dehydration, respiratory difficulties, postural deficiencies, and general conditions of health. During the acclimation and testing period, each animal was housed and maintained according to The Guide For The Care and Use of Laboratory Animals (NIH 86-23).

Animals were acclimated for at least four days prior to initiation of the study. They were housed in clean cages, in a temperature controlled environment. Diet consisted of Agway Prolab High Fiber Rabbit feed, alfalfa cubes and city water, ad libitum. Each animal was identified by an individual eartag number on the right ear, as well as a corresponding cage card.

Twenty-four hours prior to test initiation, the animals were reexamined and any found in poor condition, particularly those with skin eruptions or dermal lesions, were not used. Animals were prepared for testing by close-clipping the skin of the mid-dorsal area of the trunk, between the scapulae and the pelvis, using a small animal clipper equipped with a #40 (surgical) head.

Two test sites, each 2.5 centimeters square, were chosen on opposite sides of the vertebral column. The one on the left side of the animal was maintained intact and the test site on the right side was prepared by abrading with a sterile 21 gauge hypodermic needle. The abrasions are longitudinal epidermal incisions, sufficiently deep to penetrate the stratum corneum, but not so deep as to destroy the integrity of the derma.

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, $NaHCO_3$, 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.

A single dose of 0.5 milliliters of the composited test material was placed on each site. Each site was then covered with a one-inch square gauze patch held in place with Micropore tape.

After both test sites were treated, the entire trunk of each animal was encased in an impermeable occlusive wrapping held in place with Elastikon tape. This aided in maintaining the test material and patches in position and prevents the evaporation of possible volatile components of the test article.

METHOD OF ASSAY (continued)

The wrapping and test article were removed 24 hours following application, any remaining test material was gently wiped from the skin. Each test site was individually examined and scored at twenty-four and seventy-two hours post dosing for erythema and edema using the Draize skin scoring scale. The presence of effects not listed in the scoring scale are also noted.

INTERPRETATION OF ASSAY:

Following the seventy-two hour reading, the scores for twenty-four and seventy-two hour gradings were averaged to determine the primary irritation index. A score of 5.0 or more indicates a primary dermal irritant.

SUMMARY:

The submitted material, when dosed as a composite in accordance with the above procedure, produced no erythema and no edema. The Primary Irritation Score was 0.00.

The test material would not be classified as a primary dermal irritant as defined by the Federal Hazardous Substances Act, 16 CFR, Section 1500.3 (c) (4).

Work Performed Under the Supervision of:

Joanne Barbera, M.B.A.
Manager, Toxicology

LEBERCO-CELSIS TESTING

Edwin C. Rothstein, Ph.D.
Director
or
William Gilman, M.S.
Associate Director

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SUBMITTED TO: ACTS TESTING LABS, INC.

ASSAY NUMBER: 9620342
RECEIVED: 05-Nov-96
TEST MATERIAL: Renilla Luciderase & Coelenterazine
Lot/ID #: none

Interpretation of Primary Dermal Irritation Indices
(Based on Draize Score)

Score	*Interpretation*
C	Corrosive - highly dangerous, warning labels must be used
5.0 & Up	Primary Dermal Irritant - highly dangerous, warning label must be used
3.0 - 4.9	Potential for severe irritation - warning label would be advised
2.0 - 2.9	Potential for moderate irritation - may be irritating to humans under conditions similar to test (may consider warning label)
1.0 - 1.9	Potential for mild irritation - possible irritant to some people under occlusive wrap conditions - usually no warning required
0.1 - 0.9	Potential for slight irritation - rarely irritating to people - no warning required
0.0	No irritation potential - no warning required

NOTE: The above interpretation is based on a fully occlusive test patch which may not be indicative of the end-use of the test material.

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TABLE 1

Draize Scoring Table for Skin Reactions

Erythema and Eschar Formation	
0	No erythema
1	Very slight erythema (barely preceptible)
2	Well-defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)
4	Total possible erythema score

Edema Formation	
0	No edema
1	Very slight edema (barely preceptible)
2	Well-defined edema (edges of area well-defined by definite raising)
3	Moderate edema (area raised approximately 1 mm
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
4	Total possible edema score
8	Total possible primary irritation score

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