



Leberco • Celsis Testing

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Nov. 5, 1996

SUBMITTED TO: ACTS Testing Labs
Buffalo, NY
Patty Dick

ASSAY NUMBER: 9620341

DATE RECEIVED: 10/25/96

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

DATE INITIATED: 10/29/96

COMPLETED: 11/1/96

PURPOSE OF ASSAY:

To determine the degree of eye irritation, if any, that the test material may produce when instilled to the eyes of rabbits, in accordance with the method described in the Federal Hazardous Substances Act, 16 CFR, Section 1500.42.

RESULTS: Not a primary eye irritant.

METHOD OF ASSAY:

A. ANIMALS

The albino rabbits used in this study were obtained from a USDA licensed dealer. The animals were housed individually and acclimated to laboratory conditions for at least four days prior to being placed on test.

During the acclimation and test period each animal was housed and maintained according to The Guide For The Care and Use of Laboratory Animals (NIH 86-23). Each animal was identified by an eartag number and a cage card.

The animals were provided with Agway Prolab High Fiber Rabbit feed and city water ad libitum.

B. PROCEDURE:

Six normal healthy albino rabbits were used for this study. On the day of the study prior to testing, each animal had their eyes examined for any irritation or corneal damage; any animal exhibiting abnormalities was excluded from testing.

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.

Each animal had 0.1 milliliters of the composited test material, as supplied, instilled into the conjunctival sac of the test eye. The contralateral untreated eye served as a control for that animal.

The treated and untreated eyes were examined and graded at 24, 48 and 72 hours post instillation. The criteria for scoring is shown in Table II.

The individual readings taken may be found on Table I.

C. INTERPRETATION OF RESULTS:

A test is interpreted as:

Positive - if four or more animals exhibit a positive response on the cornea, iris or conjunctiva.

Negative - if one or less animals exhibit a positive response on the cornea, iris or conjunctiva.

Inconclusive - if two or three animals exhibit a positive response on the cornea, iris or conjunctiva. The test may be repeated with a different group of six animals and is positive if three or more animals exhibit a positive response.

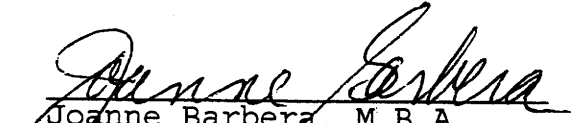
SUMMARY:

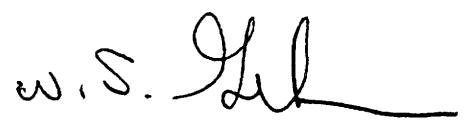
The submitted material, when tested as a composite in accordance with the above procedure, produced minimal conjunctival irritation in two of the six animals tested. Four animals had no irritation. All signs of irritation cleared by day 3 of the study.

Therefore, the test material would not be considered a primary eye irritant as defined by the Federal Hazardous Substance Act, 16 CFR, Section 1500.3 (c) (4).

Performed Under the
Supervision of:

LEBERCO-CELSIS TESTING


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Director
or
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TABLE I (Continued)

Sample ID #: 9620341

Primary Eye Irritation Scores

RABBIT #4 Left Eye

I. Cornea
 A. Opacity
 B. Area of Cornea Involved

 $Total\ Score = A \times B \times 5$
 II. Iris
 A. Values

 $Total\ Score = A \times 5$
 III. Conjunctivae
 A. Redness
 B. Chemosis
 C. Discharge

 $Total\ Score = (A + B + C) \times 2$

Total Irritation Score

1 day	2 day	3 day				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				

RABBIT #5 Left Eye

I. Cornea
 A. Opacity
 B. Area of Cornea Involved

 $Total\ Score = A \times B \times 5$
 II. Iris
 A. Values

 $Total\ Score = A \times 5$
 III. Conjunctivae
 A. Redness
 B. Chemosis
 C. Discharge

 $Total\ Score = (A + B + C) \times 2$

Total Irritation Score

1 day	2 day	3 day				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				

RABBIT #6 Left Eye

I. Cornea
 A. Opacity
 B. Area of Cornea Involved

 $Total\ Score = A \times B \times 5$
 II. Iris
 A. Values

 $Total\ Score = A \times 5$
 III. Conjunctivae
 A. Redness
 B. Chemosis
 C. Discharge

 $Total\ Score = (A + B + C) \times 2$

Total Irritation Score

1 day	2 day	3 day				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
0	0	0				
1	0	0				
0	0	0				
0	0	0				
2	0	0				
2	0	0				

AVERAGE IRRITATION SCORES 0.7 0.3 0.0

TABLE II

GRADES FOR OCULAR LESIONS

CORNEA

Opacity: Degree of density (area most dense taken for reading)

0	No ulceration or opacity
1*	Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible
2	Easily discernible translucent area, details of iris slightly obscured
3	Nacreous area, no details of iris visible, size of pupil barely discernible
4	Complete corneal opacity, iris not discernible

IRIS

0	Normal
1*	Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)
2	No reaction to light, hemorrhage, gross destruction (any or all of these)

CONJUNCTIVAE

Redness: refers to palpebral and bulbar conjunctivae excluding cornea and iris

0	Vessels normal
1	Some vessels definitely injected
2*	Diffuse, crimson red, individual vessels not easily discernible
3	Diffuse beefy red

Chemosis: lids and/or nictitating membranes

0	No swelling
1	Any swelling above normal (includes nictitating membrane)
2*	Obvious swelling with partial eversion of lids
3	Swelling with lids about half closed
4	Swelling with lids more than half closed

Discharge:

0	No discharge
1	Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)
2	Discharge with moistening of the lids and hairs just adjacent to lids
3	Discharge with moistening of the lids and hairs, and considerable area around the eye

* Starred figures indicate lowest grade considered positive under the Federal Hazardous Substances Act Regulation 16 CFR, 1500.42.