

**STUDY TITLE:**

OCULAR IRRITATION STUDY IN THE RABBIT

(FHSA Method)

**TEST ARTICLE:**

CHIMAL SKIN SHIELD

**IDENTIFICATION NO.:**

005-1

**TEST FACILITY:**

NAMSA  
2261 Tracy Road  
Northwood, OH 43619-1397

**SPONSOR:**

DANN SCHWARTZ  
ESP LLC  
1069 S JEFF DAVIS PKWY  
NEW ORLEANS, LA 70125

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SUMMARY

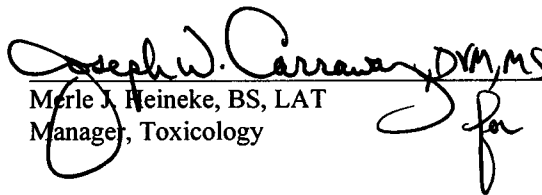
The test article, CHIMAL SKIN SHIELD, 005-1, was evaluated for primary ocular irritation in accordance with the guidelines of the Federal Hazardous Substances Act (FHSA) Regulations, 16 CFR 1500. A single 0.1 ml dose of the test article was placed in one eye each of six rabbits. Ocular reactions were evaluated at 24, 48, and 72 hours after sample instillation.

Under the conditions of this study, slight irritation was observed in the treated eyes as compared to the untreated control eyes of the animals. The test article would not be considered an irritant to the ocular tissue of the rabbit.

Study and Supervisory  
Personnel:

Beth A. Yeager  
Brian Brooks  
Merle J. Heineke, BS, LAT  
Martha Oswanski, BS, LAT

Approved by:

  
Merle J. Heineke, BS, LAT  
Manager, Toxicology

9-12-00  
Date Completed

/cmb

INTRODUCTION

The test article identified below was evaluated for ocular irritation in accordance with the guidelines of the Federal Hazardous Substances Act (FHSA) Regulations, 16 CFR 1500. The purpose of this study was to determine the potential for irritation of the material following a single instillation to the ocular tissue of the rabbit. The test article was received on August 28, 2000. Eyes were dosed on September 5, 2000, and the observations were concluded on September 8, 2000.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	CHIMAL SKIN SHIELD
Identification No.:	005-1
Storage Conditions:	Room temperature
Preparation:	Dosed as received.
Sample Disposition:	Any remaining sample was discarded.

METHODSTest System:

Species:	Rabbit ( <i>Oryctolagus cuniculus</i> )
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	Three male, three female
Body Weight Range:	No particular body weight range was prescribed for this test
Age:	No particular age was prescribed for this test
Acclimation Period:	Minimum 5 days
Number of Animals:	Six
Identification Method:	Ear tag

Justification of Test System:

The albino rabbit is suggested by various governmental guidelines as an appropriate model for evaluating potential eye irritants. The ocular tissue of the rabbit has traditionally been used to predict irritant properties of materials having potential contact with mucosal or ocular tissues during manufacture or use. Because of the lack of pigmentation in the eye, lack of excessive tearing, and ease of handling, the albino rabbit has historically been used for this study.

Animal Management:

- Husbandry:** Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
- Food:** PROLAB® High Fiber Rabbit Diet was provided daily.
- Water:** Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.
- Contaminants:** Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
- Housing:** Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.
- Environmental:** The room temperature was monitored daily. The temperature range for the room was within a range of 61-72°F.
- The room humidity was monitored daily. The humidity range for the room was 30-70%.
- The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
- Facility:** NAMSA is an AAALAC International accredited facility and is registered with the United States Department of Agriculture. Additionally, NAMSA maintains an approved Animal Welfare Assurance on file with the National Institutes of Health, Office for Laboratory Animal Welfare.
- Personnel:** Associates involved were appropriately qualified and trained.
- Selection:** Only healthy animals without significant ocular irritation were selected. To reduce the number of animals used for testing, and to comply with the directives of the NAMSA IACUC, rabbits on this study may have been used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations are identified in the report.

Experimental Procedure:

Prior to test article instillation, all test and control eyes were judged clinically normal for rabbits by gross examination with an auxiliary light source. To detect any pre-existing corneal injury, the eyes were treated with fluorescein stain, flushed with 0.9% sodium chloride USP solution, and observed with ultraviolet light in a darkened room.

A 0.1 ml dose of the test article was instilled into the lower conjunctival sac of one eye of each rabbit and the lid was gently held closed for 1 second. The opposite eye of each rabbit remained untreated and served as the comparative control. Animals were returned to their cages following treatment.

At 24, 48, and 72 hours after dosing, the test eye of each rabbit was examined with an auxiliary light source and appropriate magnification, compared to the untreated control eye, and graded for ocular irritation. To detect or confirm corneal injury, the test eyes were treated with fluorescein stain, flushed with 0.9% sodium chloride USP solution, and examined in darkened conditions with an ultraviolet lamp at 24 hours.

Reactions were scored in accordance with the FHSA-modified Draize scoring criteria (Appendix 1). As defined in 16 CFR 1500, a substance producing any significant positive reaction in one of six test eyes is not considered as an irritant to the eye. Two or three animals exhibiting a significant positive reaction is a borderline finding that may warrant a retest for conclusive results. At any time four or more animals exhibit a significant positive response, the test article is an irritant.

### RESULTS

Individual results of the ocular scoring appear in Table I. Slight irritation was observed in the treated eyes as compared to the untreated control eyes of the animals.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and EN45001 Quality Standards (TÜV Product Services 1/96).

### CONCLUSION

Under the conditions of this study, the test article would not be considered an irritant to the ocular tissue of the rabbit.

### RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

TABLE I

OCULAR IRRITATION SCORES

Animal Number	Items Scored	24 Hours	48 Hours	72 Hours	Results
22024†	Cornea:	0	0	0	(-)
	Iris:	0	0	0	
	Redness:*	0	0	0	
	Chemosis:*	0	0	0	
	Fluorescein Exam	(-)	NA	NA	
22023†	Cornea:	0	0	0	(-)
	Iris:	0	0	0	
	Redness:*	0	0	0	
	Chemosis:*	0	0	0	
	Fluorescein Exam	(-)	NA	NA	
22022†	Cornea:	0	0	0	(-)
	Iris:	0	0	0	
	Redness:*	0	1	0	
	Chemosis:*	0	0	0	
	Fluorescein Exam	(-)	NA	NA	
22032†	Cornea:	0	0	0	(-)
	Iris:	0	0	0	
	Redness:*	1	1	0	
	Chemosis:*	0	0	0	
	Fluorescein Exam	(-)	NA	NA	
22033†	Cornea:	0	0	0	(-)
	Iris:	0	0	0	
	Redness:*	0	0	0	
	Chemosis:*	0	0	0	
	Fluorescein Exam	(-)	NA	NA	
21848	Cornea:	0	0	0	(-)
	Iris:	0	0	0	
	Redness:*	0	1	0	
	Chemosis:*	0	0	0	
	Fluorescein Exam	(-)	NA	NA	

†Previous use history traceable in laboratory records.

\* = Conjunctival tissues

(-) = Negative

NA = Not applicable

APPENDIX 1

MODIFIED DRAIZE GRADES FOR OCULAR LESIONS - (FHSA)<sup>1</sup>

Cornea

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

Iris

Normal.....	0
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).....	(1)*
No reaction to light, hemorrhage, gross destruction (any or all of these) .....	2

Conjunctivae

Redness (Refers to palpebral and bulbar conjunctivae excluding cornea and iris)

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

Chemosis

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

\*Minimal positive reaction considered significant

<sup>1</sup>U.S. Food and Drug Administration 1965. Illustrated Guide for Grading Eye Irritation by Hazardous Substances. U.S. Government Printing Office, Washington, D.C.

NOTE: In the Federal Hazardous Substances Act (FHSA), the above method was adopted as the official method for eye irritancy evaluation. The Consumer Product Safety Commission functions under the FHSA (CFR Title 16, Part 1500.42).