

**STUDY TITLE:**

ACUTE DERMAL TOXICITY 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

TABLE OF CONTENTS

	<u>Page Number</u>
SUMMARY .....	3
INTRODUCTION.....	4
MATERIALS .....	4
METHODS.....	4
RESULTS.....	6
CONCLUSION .....	6
RECORD STORAGE.....	6
TABLES	
I - DERMAL OBSERVATIONS .....	7
II - INDIVIDUAL OBSERVATIONS .....	8
APPENDIX	
1 - DRAIZE EVALUATION OF TOPICAL REACTIONS .....	9



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SUMMARY

The test article, CHIMAL SKIN SHIELD, 005-1, was evaluated for dermal toxicity in accordance with the guidelines of the Federal Hazardous Substances Act (FHSA) Regulations, 16 CFR 1500. A single test article dose of 2 g/kg of body weight was applied to the intact and abraded skin of 10 rabbits and left in place for 24 hours. The animals were then observed for up to 14 days for any signs of toxicity.

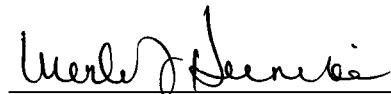
Under the conditions of this study, there was mortality observed in the rabbits. However, this mortality was not believed to be related to the single dermal dose of the test article. The test article would not be considered toxic at a dose of 2 g/kg by the dermal route in the rabbit.

Study and Supervisory

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INTRODUCTION

The test article identified below was evaluated for dermal toxicity in accordance with the guidelines of the Federal Hazardous Substances Act (FHSA) Regulations, 16 CFR 1500. The purpose of the study was to determine the potential for the material to cause systemic toxicity following a single application to intact and abraded skin of the rabbit. The test article was received on August 28, 2000. Animals were dosed on September 7, 2000, and the observations were concluded on September 21, 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: The test article was dosed as received. For this study, the density of the test article was determined to be 1.1 g/ml.

Sample Disposition: Any remaining sample was discarded.

METHODSTest System:

Species: Rabbit (*Oryctolagus cuniculus*)

Breed: New Zealand White

Source: Myrtle's Rabbitry, Inc.

Sex: Five male, five female

Body Weight Range: 2.3 kg to 3.0 kg at dosing

Age: No particular age was prescribed for this test

Acclimation Period: Minimum 5 days

Number of Animals: Ten

Identification Method: Ear tag

Justification of Test System:

The albino rabbit has historically been used to determine acute dermal toxicity. The test article may have intentional or accidental dermal exposure during manufacture or consumer use.

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 Office for Laboratory Animal Welfare.
- Personnel:** Associates involved were appropriately qualified and trained.
- Selection:** Only healthy animals free from irritation or other dermatological lesions that could interfere with the test 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 application of the test article, the upper back area of all animals was clipped free of fur with an electric clipper. Five rabbits received a series of epidermal abrasions with a sterile needle; the skin of five rabbits remained intact.

Animals were weighed. The test article was then applied to the skin at a dose of 2 g/kg. The trunk of each rabbit was wrapped with polyethylene plastic taped in place to form a reservoir over the test site. The rabbits were fitted with collars and returned to their respective cages. Wrappings were removed after 24 hours. Collars were removed when there was no significant test article residue that could be ingested. The test sites were wiped with gauze sponges soaked with deionized water in an attempt to remove any remaining test article residue.

The rabbits were observed immediately after treatment, at 4 hours, and daily for up to 14 days for signs of illness and mortality. Dermal reactions were scored daily for erythema and edema (Appendix 1). Body weights were recorded at dosing and at 14 days for the survivors. Animals found dead during the study and those euthanatized by intravenous injection of a sodium pentobarbital based euthanasia drug at termination of the study were subjected to a macroscopic examination of the viscera. Based on the FHSA Regulations, a substance is considered "toxic" if it produces death within 14 days in 50% of a group of rabbits receiving a single 200 mg/kg to 2 g/kg dose administered by continuous dermal contact for 24 hours.

## RESULTS

Individual observations appear in Tables I and II.

Dermal Observations: Erythema ranging from very slight to moderate was observed in 8 of the 10 rabbits on day 1 but lessened in severity and incidence over days 2 and 3. Slight to barely perceptible edema was also observed on days 1, 2 and 3 of the study in a small number of animals. On day 4, 3 rabbits were observed to have barely perceptible erythema after which all rabbits exhibited no erythema or edema for the remainder of the study.

Body Weight: Body weight data at both initiation and termination of the study were acceptable.

Mortality: One animal (21897) was euthanatized on day 9. A necropsy showed, however, that this mortality was not related to the single dermal dose of the test article.

Clinical Observations: Rabbit #21897 had diarrhea on day 7 and day 8 of the study. Shredded wheat and a water bowl were given on both days. The animal was euthanatized on day 9 and a necropsy was performed. No observations were noted at the necropsy attributing the illness to the single dermal dose of the test article. Otherwise, all animals appeared clinically normal throughout the study.

Necropsy: There were no macroscopic changes in the viscera at necropsy that could be attributed to the single dermal dose.

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 toxic at a dose of 2 g/kg by the dermal route in 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  
DERMAL OBSERVATIONS

Rabbit Number	Obs.	Dermal Reactions													
		Day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
21288*	ER	3	2	1	1	0	0	0	0	0	0	0	0	0	0
Intact	ED	1	1	0	0	0	0	0	0	0	0	0	0	0	0
21810	ER	2	1	0	0	0	0	0	0	0	0	0	0	0	0
Intact	ED	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21953*	ER	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Intact	ED	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21381*	ER	3	2	0	0	0	0	0	0	0	0	0	0	0	0
Intact	ED	1	0	0	0	0	0	0	0	0	0	0	0	0	0
21952	ER	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Intact	ED	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21925	ER	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abraded	ED	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21489*	ER	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Abraded	ED	1	0	0	0	0	0	0	0	0	0	0	0	0	0
21675	ER	1	1	0	1	0	0	0	0	0	0	0	0	0	0
Abraded	ED	2	0	0	0	0	0	0	0	0	0	0	0	0	0
21023*	ER	3	1	1	0	0	0	0	0	0	0	0	0	0	0
Abraded	ED	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21897*	ER	1	0	0	0	0	0	0	0	†	†	†	†	†	†
Abraded	ED	0	0	0	0	0	0	0	0	†	†	†	†	†	†

\*Previous use history traceable in laboratory records.

†Animal #21897 was euthanatized on day 9 of the study.

ER = Erythema

ED = Edema

TABLE II  
INDIVIDUAL OBSERVATIONS

Rabbit Number/ Sex	Body Weight (kg)		Clinical Observations (Days 0-14)	Necropsy (Day 14)
	Day 0	Day 14		
21288/ Female	3.0	3.4	Appeared normal	Macroscopically normal
21810/ Female	2.6	3.0	Appeared normal	Macroscopically normal
21953/ Female	2.3	2.6	Appeared normal	Macroscopically normal
21381/ Male	3.0	3.1	Appeared normal	Macroscopically normal
21952/ Female	2.3	2.5	Appeared normal	Macroscopically normal
21925/ Male	2.6	2.9	Appeared normal	Macroscopically normal
21489/ Female	2.6	3.0	Appeared normal	Macroscopically normal
21675/ Male	2.7	3.0	Appeared normal	Macroscopically normal
21023/ Female	3.0	3.2	Appeared normal	Macroscopically normal
21897/ Male	2.5	‡	Animal had diarrhea on days 7 and 8. A water bowl and shredded wheat were given both days. The animal was euthanatized on day 9 and a necropsy was performed.	Lower gastrointestinal tract distended by watery fluid, mesenteric lymph nodes enlarged and edematous. Definitive cause of diarrhea not determined.
Mean:	2.7	3.0		

‡Animal's weight not recorded at euthanazation.

APPENDIX 1

DRAIZE\* EVALUATION OF TOPICAL REACTIONS

SCORE

Erythema and Eschar Formation (Most predominant condition):

No erythema .....	0
Very slight erythema (barely perceptible) .....	1
Well-defined erythema .....	2
Moderate to severe erythema.....	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth).....	4

NOTE: Test sites assigned a "4" score for erythema require further description as to the extent of tissue injury.

Edema Formation (Most predominant condition):

No edema.....	0
Very slight edema (barely perceptible) .....	1
Slight edema (edges of area well defined by definite raising).....	2
Moderate edema (raised approximately 1 mm).....	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure).....	4

\*Draize, J.H. 1959. Dermal Toxicity. Pages 46-59 in Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States, Bureau of Food and Drugs, Austin, TX.