

## PRIMARY SKIN IRRITATION STUDY IN RABBITS

TEST METHOD NO: P201

STUDY NUMBER: 13267

SPONSOR: REACT-NTI LLC  
1 Whispering Spring Drive  
Freehold, NJ 07728

TEST ARTICLE IDENTIFICATION: ALL-Natural  
Batch #2161

TEST ARTICLE DESCRIPTION: White to pale yellow powder

DATE RECEIVED: February 11, 2003

PSL REFERENCE NO.: 030211-5H

DATES OF TEST: March 6 - 9, 2003

NOTEBOOK NO.: 03-07: pages 82-82A, 83-86

### 1. PURPOSE

To provide information on the skin irritation likely to arise from a single topical exposure to ALL-Natural.

### 2. PROCEDURE

A group of New Zealand albino rabbits was received from Davidson's Mill Farm, South Brunswick, NJ. The animals were singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cages and was changed at least three times per week. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animals were fed Purina Rabbit Chow #5326 and filtered tap water was supplied *ad libitum* by an automatic watering system.

Following acclimation to the laboratory, a group of animals was prepared by clipping (Oster model #A5-small) the flanks and the trunk of each animal free of hair. On the day after clipping, three healthy rabbits (2 males and 1 female) without pre-existing dermal irritation were selected for test. Two sites, each approximately 6 cm<sup>2</sup>, were delineated on each animal. One site was left intact and the other was abraded using a 23-gauge needle. The abraded site consisted of five vertical and five horizontal abrasions. Only the epidermal layer was abraded and care was taken to avoid causing bleeding when abrading the skin.



Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing 65% w/w mixture. Five tenths of a gram of the test substance (0.77 g of the test mixture) was applied to a 1 inch x 1 inch, 4-ply gauze pad and placed on both the abraded and intact dose site of all animals. The pads and entire trunk of each animal were then wrapped with 3-inch Durapore tape to avoid dislocation of the pads. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 24 hours of exposure to the test substance, the pads and collars were removed and the test sites were cleaned of residual test substance. Individual dose sites were scored according to the Draize<sup>1</sup> scoring system approximately 24 and 72 hours after application.

The erythema and edema scores for each animal were combined and the summation of the combined scores was divided by the number of animals tested. The result was then divided by the number of sites per animal and the number of evaluation intervals.

The resulting Primary Dermal Irritation Index (PDII) was classified as follows:

<u>PDII</u>	<u>Classification</u>
< 5.0	Not a primary irritant
≥ 5.0	Primary irritant

### 3. RESULTS

Individual skin irritation scores are presented in Table 1. Primary skin irritation scores used for calculation of the Primary Dermal Irritation Index are presented in Table 2. The Draize Primary Skin Irritation Scoring System is presented in Table 3.

All animals appeared active and healthy. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects or abnormal behavior.

The Primary Dermal Irritation Index for ALL-Natural is 0.

### 4. CONCLUSION

Based on the classification system used, ALL-Natural is not considered a primary skin irritant.

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<sup>1</sup> Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 1944; 82:377-390.



SIGNATURES

ALL-Natural

We, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

*George E. Moore*

George E. Moore, B.S.  
Study Director

*05/13/03*

Date

*Rhonda S. Krick*

Rhonda S. Krick, B.S.  
Quality Assurance Director

*05/13/03*

Date

**TABLE 1: INDIVIDUAL SKIN IRRITATION SCORES**
**ERYTHEMA/EDEMA**

Animal #	Sex	Time After Application			
		24 Hours		72 Hours	
		Abraded	Intact	Abraded	Intact
9090	M	0/0	0/0	0/0	0/0
9091	F	0/0	0/0	0/0	0/0
9092	M	0/0	0/0	0/0	0/0
<b>Total</b>		0/0	0/0	0/0	0/0
<b>Mean</b>		0/0	0/0	0/0	0/0

**TABLE 2: SUMMARY OF PRIMARY DERMAL IRRITATION (PDI) SCORES<sup>1</sup>**

	Scoring Interval	Average Exposure Unit
	Hours	Value <sup>2</sup>
<b>Erythema &amp; Eschar</b>		
Abraded	24	0
	72	0
Intact	24	0
	72	0
Subtotal		0
<b>Edema</b>		
Abraded	24	0
	72	0
Intact	24	0
	72	0
Subtotal		0
Total		0
Primary Dermal Irritation Index (PDII) <sup>3</sup>		0

<sup>1</sup> See Table 3 for scoring system.

<sup>2</sup> The "value" recorded for each reading represents the average of the three animals.

<sup>3</sup> Total "value" divided by 4 (2 scoring intervals x 2 sites, abraded & intact).



TABLE 3: PRIMARY SKIN IRRITATION SCORING SYSTEM

<u>Evaluation of Skin Reactions</u>	<u>Value</u>
Erythema and eschar formation:	
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
Edema formation:	
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 millimeter).....	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)..	4