PRIMARY SKIN IRRITATION STUDY IN RABBITS

TEST METHOD NO:	P201
STUDY NUMBER:	8822
SPONSOR:	SYNTROLEUM CORPORATION 1350 South Boulder, Suite 1100 Tulsa, OK 74119-3295
TEST SUBSTANCE IDENTIFICATION:	RDIL 0486
TEST SUBSTANCE DESCRIPTION:	Clear colorless liquid
DATE RECEIVED:	February 18, 2000
PSL REFERENCE NO.:	E00218-1D
DATES OF TEST:	February 24-27, 2000
NOTEBOOK NO.:	00-05; pages 322-325

1. PURPOSE

To provide information on the skin irritation likely to arise from a single topical exposure to RDIL 0486.

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 #A2-small) the dorsal area of each animal's trunk free of hair. On the day after clipping, six healthy rabbits (3 male and 3 female) without pre-existing dermal irritation were selected for test. One intact test site, approximately 6 cm^2, was delineated on each animal.

Five-tenths of a milliliter of the test substance was applied to one 6 cm^2 intact dose site on each animal and covered with a 1 inch x 1 inch, 4-ply gauze pad. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3 inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and collars were removed and the test sites gently wiped with water and a clean towel to remove any residual test substance. Individual dose sites

were scored according to the Draize scoring system (See Table 3)[1] at approximately 1, 24, 48 and 72 hours after patch removal.

The classification of irritancy was obtained by adding the average erythema and edema scores for the 1, 24, 48 and 72 hour scoring intervals and dividing by the number of evaluation intervals (4).

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

PDII	Classification
< 0.5	Non-irritating
0.5 - 2.0	Slightly irritating
2.1 - 5.0	Moderately irritating
> 5.0	Severely irritating

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 during the study. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.

Two rabbits were free from dermal irritation throughout the study. One hour after patch removal, very slight erythema was observed at four treated sites. The incidence of irritation decreased with time. Within 72 hours, all affected treated sites were free from erythema.

The Primary Dermal Irritation Index for RDIL 0486 is 0.3.

4. CONCLUSION

Based on the classification system used, RDIL 0486 is classified as non-irritating to the skin.

[1] 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

RDIL 0486

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.

Daniel J. Merkel, B.S. Study Director	Date
Christopher Day, B.S. Principal Toxicology Technician	Date
Frank Fielder, B.S. Quality Assurance Supervisor	Date

TABLE 1: INDIVIDUAL SKIN IRRITATION SCORES

ERYTHEMA/EDEMA

Animal No.	Sex	Hours After Patch Removal			
		1	24	48	72
919	M	0/0	0/0	0/0	0/0
920	F	1/0	0/0	0/0	0/0
921	M	1/0	0/0	0/0	0/0
922	F	1/0	0/0	0/0	0/0
923	F	1/0	1/0	1/0	0/0
924	M	0/0	0/0	0/0	0/0
TOTAL		4/0	1/0	1/0	0/0
MEAN		0.7/0.0	0.2/0.0	0.2/0.0	0.0/0.0

TABLE 2: SUMMARY OF PRIMARY DERMAL IRRITATION (PDI) SCORES[1]

	Hours			
	1	24	48	72
Erythema	0.7	0.2	0.2	0.0
Edema	0.0	0.0	0.0	0.0
TOTAL (PDI)[2]	0.7	0.2	0.2	0.0

Primary Dermal Irritation Index (PDII): $\frac{\text{PDI for 1, 24, 48 and 72 hrs}}{4} = 0.3$

Classification: Non-irritating

CLASSIFICATION SYSTEM

PDII	Classification
< 0.5	Non-irritating
0.5 - 2.0	Slightly irritating
2.1 - 5.0	Moderately irritating
> 5.0	Severely irritating

[1] Average values for six rabbits.

[2] PDI = Average Erythema + Average Edema

TABLE 3: PRIMARY SKIN IRRITATION SCORING SYSTEM

Evaluation of Skin Reactions	Value
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