Reviewed By: Y.M. Ioannou 12-6-88
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M. Copley
Section II, Toxicology Branch I (IRS)

DATA EVALUATION REPORT

Study Type: Delayed Contact Hypersensitivity (81-6)

TOX Chem No.: 716A

Test Material: Pyridate

MRID No.: 408774-01

Synonyms: CL11344

Study Number: 046866

Sponsor: Gilmore, Inc., Memphis, TN

Testing Facility: Research and Consulting Company AG, Itingen, Switzerland

Title of Report: Test for Delayed Contact Hypersensitivity in the Albino Guinea Pig with CL11344/216EC (Tough 3.75 EC).

Author: L. Ullmann

Report Issued: June 27, 1985

Conclusions:

Data presented here indicate that Pyridate, CL11344/216EC, is a strong sensitizing agent in male and female guinea pigs.

Classification: Core-Minimum

Materials and Methods:

Fifteen male and fifteen female Dunkin-Hartley albino guinea pigs (from Kleintierfarm Madoerin AG, Switzerland) 8 to 9 weeks old and weighing 307 to 458 g (males) or 360 to 429 g (females) were used in this study for assessing the allergenic potential of CL11344/216EC. The animals were housed individually in Makrolon Type-3 cages and kept in an air-conditioned room with a temperature of 22 ± 2 °C, a relative humidity of 55 ± 10 percent, 10 to 15 air changes/hour, and a 12-hour light/dark cycle. Food (pelleted standard Kliba 342) and water were available to all animals ad libitum.

The test article, CL11344/216EC (Tough 3.75 EC), EC liquid (Batch No. 2431768), with a purity of 457 g/l ai, was used in this study. The animals were divided into two groups/sex, five animals for the vehicle control group, and 10 animals for the test article group. For detection of delayed contact hypersensitivity the guinea pig maximization test described by Magnusson and Kligmann (1970) was employed. Preliminary investigations established the test article concentration suitable for the induction phase as well as a nonirritant concentration for the challenge application. (Note: Results of the preliminary range-finding study were not reported.)

The procedure used was carried out in two steps:

A. Induction Phase:

1. Intradermal Injections - Animals were prepared for treatment by clipping free of hair an area (6 x 8 cm) of the dorsal skin from the scapular region. Three pairs of intradermal injections (0.1 mL/site) were made in a 4 x 4 cm area in the clipped region as follows:

a. Freund's Complete Adjuvant 50:50 with physiological saline;

b. The test article, diluted to 0.5 percent with physiological saline; and

c. The test article at 0.5 percent, emulsified in 50:50 in Freund's Complete Adjuvant and physiological saline.

2. Topical Applications - Seven days after the intradermal injections, the treated scapular area was again clipped free of hair and a 4 x 4 cm patch (filter paper) saturated with the test article (100%) was placed over the injection sites. The patch was covered with aluminum foil and firmly secured with elastic plaster and impervious adhesive tape for 48 hours.

Animals of the control groups (male and female) received identical treatment for the induction phase but without the test article.

B. Challenge Phase:

All animals (test and control) were challenged two weeks after the topical induction application by applying a 2 x 2 cm patch of filter paper, saturated with a nonirritant concentration (100%) of the test article, on an area (5 x 5 cm) of the left flank of each guinea pig clipped free of hair. The patch was held in place (for 24 hours) as described earlier (topical induction application). A second challenge (rechallenge) was performed 2 weeks after the first challenge as described above using the right flanks of each animal. The challenge site was evaluated immediately after application, and at 24 and 48 hours after removal of the patch.

Reactions were scored according to the following arbitrary scales:

Erythema and Eschar Formation

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

Edema Formations

No edema	0
Slight edema (barely perceptable)	1
Well-defined 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

Allergenicity was rated as follows:

Sensitivity Rate (%)	Grade	Classification
0-8	1	Weak
9-28	2	Mild
29-64	3	Moderate
65-80	4	Strong
81-100	5	Extreme

Clinical Observations - In addition to the sensitizing reactions, all animals were observed daily for mortality and toxic symptoms. Body weights were recorded at pretreatment, start of application, and at study termination.

No necropsy was performed on the animals at study termination.

Results and Discussion:

No mortality was reported in male or female animals in control or treated groups throughout the study. Mean body weights at study termination (39 days) were comparable between the treated and control groups in both sexes.

The authors reported that all animals in treated and control groups (both sexes) developed erythema and edema 2 to 5 days after the induction application (intradermal injections). Between days 6 and 10 on test, animals showed edema and crusts. Between days 11 to 19 only crusts were observed, and between days 20 to 36 exfoliation was seen. No data were presented to substantiate these findings.

Sensitivity Effects - Results presented in the attached Table 1 (abstracted from the original report) indicate that all animals that received the test article (during the induction phase) exhibited sensitization after the first challenge application, at all three time points examined (immediately, 24 hours, and 48 hours after removal of dressing). Although the authors did not report separately in Table 1 the incidence and severity of erythema and/or edema, it is apparent that Pyridate is a sensitizer in guinea pigs and based on the fact that 100 percent of the animals tested exhibited sensitization reaction, Pyridate is rated as an extreme sensitizing agent in guinea pigs. Further indications that Pyridate is a potent sensitizer was provided by the results obtained from a second challenge application as shown in the attached Table 2 (abstracted from the original report).

Conclusions:

CL11344/216EC (Tough 3.75 EC) was found to be a very strong (extreme) skin sensitizing agent in male and female guinea pigs.

Classification: Core-Minimum

006966

TABLE 1 SKIN RESPONSE AFTER 1ST -CHALLENGE PROCEDURE
CL 11344/216 EC

Animal Number	sex	Readings after removal of bandage immediately	24 hours	48 hours
106	male	3	3	2
107	male	2	2	2
108	male	2	1	1
109	male	2	2	2
110	male	1	1	1
111	male	3	3	3
112	male	2	2	2
113	male	2	2	2
114	male	2	1	1
115	male	2	2	1
121	female	2	2	2
122	female	2	2	2
123	female	2	2	2
124	female	1	1	1
125	female	2	2	1
126	female	1	1	1
127	female	2	1	1
128	female	3	2	2
129	female	2	2	1
130	female	2	2	2

5

53036:I:Ioannou:C.Disk:KENCO:11/15/88:de:VO:EK:aw

TABLE 2 SKIN RESPONSE AFTER 2ND -CHALLENGE PROCEDURE
CL 11344/216 EC

Animal Number	sex	Readings after removal of bandage: immediately	24 hours	48 hours
106	male	3	2	1
107	male	2	1	1
108	male	1	1	0
109	male	2	1	1
110	male	2	1	1
111	male	3	2	1
112	male	2	2	1
113	male	1	1	1
114	male	2	1	0
115	male	2	1	1
121	female	2	2	1
122	female	2	2	2
123	female	2	2	2
124	female	1	1	1
125	female	1	1	1
126	female	1	1	1
127	female	2	1	1
128	female	2	2	2
129	female	2	2	2
130	female	2	2	2