

Confidential  
TA006-300

Lab No. 93T 11849 00  
P.O. No. 7467

STUDY TITLE:

DELAYED CONTACT SENSITIZATION STUDY

(Maximization Method)

IN THE GUINEA PIG

(Saline Extract)

TEST ARTICLE:

Comb-18

Sponsor: \_\_\_\_\_

DEBASHISH CHAKRAVARTHY  
VARISEAL MANUFACTURING CORP  
P.O. BOX 710  
15547 MAIN MARKET ROAD  
PARKMAN, OH 44080

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## INTRODUCTION

A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. The method of Magnusson and Kligman, as reported in Allergic Contact Dermatitis in the Guinea Pig, 1970, was employed with adaptations for a test article extract. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at NAmSA with this method. Treatment began on September 30, 1993 and the observations were concluded October 31, 1993.

## MATERIALS

The sample provided by the sponsor was identified as follows:

Test Article:	Comb-18
Storage Conditions:	Controlled Room temperature
Vehicle:	0.9% sodium chloride USP solution (SC)
Preparation:	For each phase of this test, a ration of 30 cm <sup>2</sup> /80ml (surface area of test article to volume of vehicle) was used for the test extract. The test article was extracted in SC for 72 hours at 50° C. For the challenge phase, the vehicle (without test article) was similarly prepared to serve as the control.
Condition of Extracts:	SC test: slightly cloudy with particulates* (induction I); clear (induction II and challenge)  SC control: clear  *Pressure filtered with a 0.8 µm filter to yield a clear, particulate-free extract.
Additional Materials:	Freund's complete Adjuvant (FCA) was used at induction I, and a 10% (w/w) sodium lauryl sulfate (SLS) suspension in petroleum was used for induction II. These materials were provided by the test facility.
Sample disposition:	Discarded following completion of testing program.

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METHODSAnimal management:

A minimum of 15 healthy female albino guinea pigs of the Hartley strain were obtained from an approved supplier traceable in NAmSA records. The animals were acclimated to the laboratory for at least 5 days. The range of animals weights at first treatment was 356 g to 438 g ; general health was checked daily.

Guinea pigs, identified individually by ear punch at dosing, were group housed in conformance with husbandry guidelines and received a commercial guinea pig feed on a daily basis; tap water was freely available. No diet or water analysis was performed since there was no contaminants suspected that could interfere with this study. Animal husbandry and environmental conditions conformed to current NAmSA SOP's which are based on the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23.

Induction I :

The hair was removed with an electric clipper from an area of the back over the dorsoscapular region of ten guinea pigs designated as test animals. Three rows of intradermal injections (two per row) were given to each animal within an approximate 2 x 4 cm boundary of the fur clipped area as illustrated below:

4 cm

	a.		a.
2 cm	b.		b.
	c.		c.

- a. 0.1 ml of FCA
- b. 0.1 ml of SC test article extract
- c. 0.1 ml of a 1;1 suspension of the SC test article extract and FCA

Five untreated guinea pigs were simply maintained during the induction phases as controls to be used during the challenge phase. This group of animals was not induced so that any background or primary irritant response could be differentiated from true sensitization at the challenge phase.

Induction II:

One week after the injections, the same area used during induction I was reclipped with an electric clipper. The 10% SLS suspension in petroleum was massaged into the skin over the injection site to provoke a mild acute inflammation. The area was left uncovered. The day after the SLS administration, any remaining SLS residue was gently removed with a gauze pad. A 2 x 4 cm section of Whatman No 3MM filter paper, saturated with 0.3 ml of freshly prepared SC test article extract, was then topically applied to the previously injected sites of the animals. Each patch was secured with a non-reactive tape and the trunk of each animal was wrapped with an elastic bandage. At 48 hours, the binders and patches were removed.

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Challenge:

At 14 days after the induction patch, the hair of each test and previously untreated control guinea pig was clipped over the flank areas as needed. For each animal, the nonwoven cotton disk contained in a Hill Top Chamber® was saturated with 0.3 ml of the SC test article extract or control vehicle. All patches were topically applied as indicated below:

	Challenge	Site
TREATMENT GROUP (n)	LEFT FLANK	RIGHT FLANK
TEST (10)	Control Vehicle	Test Extract
CONTROL (5)	Control Vehicle	Test Extract

Each patch was secured to the skin with semioclusive hypoallergenic adhesive tape. The trunk of each animal was wrapped with an elastic bandage to maintain well-occluded sites for the 24 hour exposure.

Observation for dermal reactions were conducted at 24, 48, 72, and 96 hours after challenge patch removal. The sites were wiped gently with gauze after patch removal. Prior to scoring at each interval, sites were wiped with 70% isopropyl alcohol. Scores were recorded in accordance with the criteria shown below:

<u>Score</u>	<u>Observation</u>
0	No visible reaction
0.5	Background response
1	Mild or faint but distant reaction
2	Moderate erythema
3	Strong erythema with or without edema

The response, pattern, character, and duration of any test animal reactions were compared to any reactions in the control conditions. Any dermal inflammatory response at the test sites greater than that seen in any control background or artifactual reactions (0.5 score) were not counted as evidence of a sensitization response.

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Based on the number of guinea pigs considered as sensitized, the allergenicity rating was assessed as follows:

<u>% Reacting Test Animals</u>	<u>Classification</u>
0%	Not a Sensitizer
1-10%	Weak Sensitizer
11-30%	Mild Sensitizer
31-60%	Moderate Sensitizer
61-80%	Strong Sensitizer
81-100%	Extreme Sensitizer

Note: In the Mangnusson and Kligman model, weak sensitization is not regarded as significant

#### Results

Individual results of dermal scoring for the challenge appear in Table I. Only insignificant background responses (scores of 0.5) were observed. No evidence of sensitization was noted.

#### Conclusion

Under the conditions of this study, the SC test article extract showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

#### Record Storage

All raw data pertaining to this study and a copy of the final report are to be stored in the designated archive files at North American Science Associates, Inc. (NAmsA), 2261 Tracy Road, Northwood, OH 43619-1397.

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Table I

GUINEA PIG SENSITIZATION  
DERMAL REACTIONS – CHALLENGE

Test Article: Comb – 18

Animal No./ Group		Hours	Following	Patch	Removal			
1 Test	24	24	48	48	72	72	96	96
2 Test	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
1 Test	0.5	0.5	0.5	0.5	0	0.5		0.5
2 Test	0	0.5	0.5	0.5	0.5	0.5	0	0
3 Test	0.5	0.5	0	0.5	0	0.5	0	0
4 Test	0.5	0	0	0	0	0	0	0
5 Test	0	0.5	0	0	0	0	0	0
6 Test	0.5	0.5	0.5	0	0	0	0	0
7 Test	0.5	0.5	0	0	0	0	0	0
8 Test	0.5	0.5	0.5	0	0.5	0	0	0
9 Test	0.5	0	0	0	0	0	0	0
10 Test	0.5	0	0	0	0	0	0	0
11 Control	0	0	0.5	0	0.5	0	0	00
12 Control	0	0	0	0.5	0	0	0	0
13 Control	0	0	0.5	0.5	0.5	0.5	0	0
14 Control	0.5	0	0.5	0	0	0	0	0
15 Control	0.5	0	0	0	0	0	0	0

Site A = Left Flank = SC Control vehicle

Site B = Right Flank = SC test extract

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SUMMARY

A guinea pig maximization test of Comb – 18, was conducted to evaluate the potential for delayed dermal contact sensitization. The method of Magnusson and Kligman (1970) was adapted for a 0.9% sodium chloride USP solution (SC) test article extract.

The SC extract of the test article was intradermally injected and occlusively patched to ten test guinea pigs in an attempt to induce sensitization . Following a recovery period, the original ten test and five previously untreated control animals received a challenge patch of the test article extract and the control vehicle. All sites were scored at 24,48, 72, and 96 hours after patch removal.

Under the conditions of this study, the SC test article extract showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

## Study and Supervisory

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Date Completed 11-3-1993

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