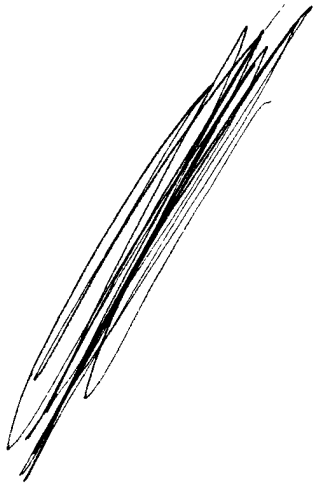
file

DERMAL SENSITIZATION STUDY OF 10% FORMALDEHYDE SOLUTION IN GUINEA PIGS

FINAL REPORT

IITRI Project No. L8100
Study No. 696
Test Article No. 100



Contractor:

IIT Research Institute
Life Sciences Research
10 West 35th Street
Chicago, IL 60616

FILE COPY

Sponsor:

Standard Oil Co. (Ind)
200 E. Randolph Drive
Chicago, IL 60601

July 31, 1984

IIT RESEARCH INSTITUTE

DERMAL SENSITIZATION STUDY OF
10% FORMALDEHYDE SOLUTION IN GUINEA PIGS
Study No. 696
Test Article No. 100

SUMMARY

FORMALDEHYDE SOLUTION at a concentration of 10% in water was applied in 0.5 ml doses to the shaved backs of ten guinea pigs once per week during an induction period of 3 weeks. Two and three weeks following the induction period, the treated guinea pigs along with ten control guinea pigs received a challenge dose of 0.5 ml of the dosage formulation at a different site on the back. All guinea pigs were scored for edema and erythema 24 and 48 hours following application of each challenge dose.

Positive erythema reactions, usually accompanied by edema, were observed in the majority of treated guinea pigs during the study. No positive reactions were noted in the control group. Statistically, the main effects of treatment (treated vs. control) and time of scoring (24 vs. 48 hours) were significant factors, whereas the main effect of challenge (1st vs. 2nd) was not a significant factor.

STUDY PARTICIPANTS:

Vladislava S. Rac, D.V.M., Study Pathologist
Christopher Lord, Technician
Calvin Reaves, Animal Care
This report was prepared by Marcia Reckers

Marcia Reckers 7/31/84
Marcia Reckers, B.S. Date
Study Director
Life Sciences Research

Nabil Hatoum 7/31/84
Nabil Hatoum, Ph.D. Date
Director, Standard Oil Toxicology Program
Life Sciences Research

Josephine M. Reed 7/31/84
Josephine M. Reed, M.M., M.S. Date
Supervisor, Quality Assurance
Life Sciences Research

TABLE OF CONTENTS

DERMAL SENSITIZATION STUDY OF 10% FORMALDEHYDE SOLUTION IN GUINEA PIGS

I. INTRODUCTION

The purpose of this study was to determine the dermal sensitizing potential of 10% FORMALDEHYDE SOLUTION in guinea pigs following repeated skin application.

II. MATERIALS AND METHODS

a. Test Article: FORMALDEHYDE SOLUTION, 37% (Sargent-Welch Scientific Co., lot no. KJKH) was received April 14, 1981. The test article was a clear liquid with a viscosity similar to water.

b. Dosage Formulation: A 10% concentration (v/v) of FORMALDEHYDE SOLUTION in NANOpure water was used in this study. This concentration was intended to produce a Draize erythema score of 1 or less in the control guinea pigs.

c. Animals: Adult male Hartley Guinea Pigs weighing approximately 325 g were purchased from Charles River Breeding Labs Inc., Portage, MI for use in this study. Upon arrival (10/12/83), the guinea pigs were held in quarantine for approximately 3 weeks and examined carefully to insure their health and suitability as test subjects. Guinea pigs selected for the study were identified by a unique numbered metal tag inserted through the pinna of the right ear and by a cage card.

d. Food and Water: Guinea Pig Chow 5025 (Ralston Purina Co., St. Louis, MO) and water supplied from a reverse-osmosis purifier by an automatic watering system were available **ad libitum.**

e. Environment: The guinea pigs were housed individually in stainless steel cages measuring 23.9 x 17.8 x 39.8 cm. Alfalfa pellets (Research Industries, Monee, IL) were placed in the pan below the stainless steel mesh floor of each animal cage to absorb liquids. Air conditioned animal rooms were maintained at approximately 22°C and 40% relative humidity. Fluorescent lighting was provided for 12 hours followed by 12 hours of darkness.

f. Methods:

1. Animals: Guinea pigs selected for testing were assigned at random to two groups of ten males each.

2. Skin Preparation: Approximately 24 hours prior to test article application the backs of the treated guinea pigs were clipped free of

hair. The upper left quadrant was clipped for the induction applications and the lower left quadrant was clipped for the challenge applications. Hair from the lower left quadrant was clipped from the backs of the control guinea pigs 24 hours prior to the challenge applications.

3. Dosing:

   a) Induction: A 0.5 ml quantity of the dosage formulation was applied to the upper left quadrant of ten guinea pigs once/week for a period of 3 weeks beginning on November 2, 1983. The test article was covered by a 1" x 1" patch (Webril^R Appli-Pad, the Kendall Co., Boston, MA), held in place with impermeable plastic tape (Blenderm^R, 3 M Company, St. Paul, MN) and elastic adhesive bandage (Elastoplast^R, Beiersdorf Inc., S. Norwalk, CN). All wrapping materials were removed 6 hours after each application.

   b) Challenge: Two weeks following the application of the last induction phase dose a 0.5 ml quantity of the dosage formulation was applied to the lower left quadrant of the backs of the ten treated and ten control guinea pigs (11/30/83). The test article was applied as described in Section 3a. All wrapping materials were removed 6 hours after the application. A second challenge dose was applied in an identical manner 1 week later (12/7/83).

4. Skin Examination: Twenty-four and 48 hours after removal of each challenge patch, the test sites were scored for edema and erythema according to the method of Draize (Appendix I). Guinea pigs were clipped, if necessary, prior to scoring.

5. Observations: All guinea pigs were observed daily for mortality and morbidity.

6. Body Weights: Body weights were not measured.

7. Animal Disposition: After the final observation (12/9/83), the guinea pigs were sacrificed by carbon dioxide asphyxiation and discarded without necropsy.

g. Evaluation: A reaction with a Draize erythema score of 2 or greater in the treated animals is considered a positive response. The concentration of test article used was intended to produce a Draize erythema reaction of 1 or less in the control animals.

h. Statistical Analysis: A three-factor log-linear model (Bishop, Fineberg and Holland, **Discrete Multivariate Analysis,** 1975) was used to assess the effect of treatment (treated vs. control), challenge (1st vs. 2nd), and time of scoring (24 hr vs. 48 hr) on erythema reactions. For the purpose of interpretation, the log-linear model is an analogue of the analysis of variance procedures where a qualitative response, such as a rating scale of erythema, is the focus of investigation. A Chi-square statistic is generated to test the significance of overall treatment vs. control differences and for specific combinations of treatment, challenge, and time of scoring. In the presence of a significant effect individual contingency tables are displayed.

III. RESULTS

a. Mortality: No deaths were observed during the study.

b. Skin Effects: The individual irritation scores are presented in Appendix II. Positive erythema reactions, usually accompanied by edema, were observed in eight of the treated guinea pigs during the study. Severe erythema to slight eschar formation was evident in these guinea pigs 48 hours following the 2nd challenge. No positive reactions were noted in the control group, although very slight erythema was observed in three control guinea pigs during the study. A summary of the overall differences in various erythema scores between the control and treated guinea pigs is shown in Table 2.

IV. EVALUATION

Results of the log-linear model are presented in Table 1. Based on this statistical analysis, the main effects of treatment and time of scoring were significant, whereas the main effect of challenge was not a significant factor.

V. QUALITY ASSURANCE

Laboratory operations were inspected on November 2 and December 1 and 8, 1983 by Josephine M. Reed. The final draft report was audited on July 6, 1984 by Josephine M. Reed. All operations were found to be in compliance with Life Sciences Quality Assurance criteria. Original data generated during the course of the study are retained in the IITRI Life Sciences Archives as specified by standard operating procedures.

VI. TABLES

TABLE 1
STATISTICAL ANALYSIS

SKIN SENSITIZATION STUDY OF 10% FORMALDEHYDE SOLUTION IN GUINEA PIGS

Group	Challenge	Time of Scoring: 24 hour Erythema Score 0	1	2	3	4	48 hour Erythema Score 0	1	2	3	4
Treated	1	3	4	2	1	0	4	1	5	0	0*
Treated	2	2	0	2	3	3*	1	1	0	0	8*
Control	1	8	2	0	0	0	10	0	0	0	0
Control	2	9	1	0	0	0	10	0	0	0	0

The following are the results of the log-linear model:

The main effect of treatment was significant:
(Chi-square = 72.137451, df = 1, p = <.005)

Challenge was not a significant factor:
(Chi-square = 1.985351, df = 1, p = ns)

A significant treatment x time interaction was found:
(Chi-square = 4.613992, df = 1, p = <.05)
This finding indicates that the treatment effect was different for the two time points.

It should be noted that in some cases individual comparisons on the accompanying table may be significant (denoted by an *), but the overall main effect or interaction is not significant (i.e., p = ns). In all cases significant individual comparisons should only be interpreted in the presence of a significant main effect of treatment or interaction.

TABLE 2
SUMMARY OF ERYTHEMA SCORE DIFFERENCES

Erythema Scores of 2 and Greater

Time Interval		Group: Treated (%)	Group: Control (%)	Difference (%)
Challenge 1	24 Hours	30.00	0.0	30.00
Challenge 1	48 Hours	50.00	0.0	50.00
Challenge 2	24 Hours	80.00	0.0	80.00
Challenge 2	48 Hours	80.00	0.0	80.00
Overall Mean		60.00	0.0	60.00

Erythema Scores of 3 and Greater

Time Interval		Group: Treated (%)	Group: Control (%)	Difference (%)
Challenge 1	24 Hours	10.00	0.0	10.00
Challenge 1	48 Hours	0.0	0.0	0.0
Challenge 2	24 Hours	60.00	0.0	60.00
Challenge 2	48 Hours	80.00	0.0	80.00
Overall Mean		37.50	0.0	37.50

Erythema Scores of 4

Time Interval		Group: Treated (%)	Group: Control (%)	Difference (%)
Challenge 1	24 Hours	0.0	0.0	0.0
Challenge 1	48 Hours	0.0	0.0	0.0
Challenge 2	24 Hours	30.00	0.0	30.00
Challenge 2	48 Hours	80.00	0.0	80.00
Overall Mean		27.50	0.0	27.50

VII. APPENDICES

APPENDIX I

SCALE FOR SCORING SKIN LESIONS*

Evaluation of Skin Reactions

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 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4

*Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, Assoc. Food and Drug Officials of the U.S., Austin, Texas, 1959.

APPENDIX II

INDIVIDUAL IRRITATION SCORES (TREATED GUINEA PIGS)

Animal Number	Challenge 1				Challenge 2			
	24 hrs		48 hrs		24 hrs		48 hrs	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
321	0	0	0	0	2	1	4	2
322	2	1	2	1	3	2	4	2
323	0	0	0	0	0	0	0	0
324	3	1	2	1	4	2	4	3
325	1	0	0	0	0	0	1	1
326	1	0	1	0	3	2	4	2
327	0	0	0	0	2	1	4	2
328	2	1	2	1	3	2	4	2
329	1	1	2	1	4	2	4	3
330	1	0	2	1	4	3	4	3

L8100SN696

APPENDIX II (cont'd)

INDIVIDUAL IRRITATION SCORES (CONTROL GUINEA PIGS)

Animal Number	Challenge 1				Challenge 2			
	24 hrs		48 hrs		24 hrs		48 hrs	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
331	0	0	0	0	1	0	0	0
332	1	0	0	0	0	0	0	0
333	0	1	0	0	0	0	0	0
334	0	0	0	0	0	0	0	0
335	0	0	0	0	0	0	0	0
336	0	0	0	0	0	0	0	0
337	0	1	0	0	0	0	0	0
338	1	0	0	0	0	0	0	0
339	0	0	0	0	0	0	0	0
340	0	0	0	0	0	0	0	0