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Sponsor:

The Soap and Detergent Association
New York, New York

FINAL REPORT

Study Title:

Primary Eye Irritation Study of Nonionic Surfactants in Rabbits (Low Volume Procedure)

Author:

Steven M. Glaza

Study Completion Date:

February 27, 1996

Performing Laboratory:

Hazleton Wisconsin, Inc.
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Laboratory Project Identification:

HWI 6310-105

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QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Hazleton Wisconsin, Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58.35 (b) (6) (7). The following inspections were conducted and findings reported to the Study Director and management. Written status reports of inspections and findings are issued to Hazleton management monthly according to standard operating procedures.

Inspection Dates			Date Reported to	Date to
From	To	Phase	Study Director	Management
09/10/94	09/10/94	Protocol Review	09/13/94	10/10/94
10/18/94	10/18/94	Dose Preparation	10/18/94	11/10/94
03/23/95	03/23/95	Protocol Amendment	03/23/95	04/10/95
06/19/95	06/20/95	Protocol Amendment	06/20/95	07/10/95
07/10/95	07/10/95	Data/Report Review	07/10/95	08/10/95

Lori Kong — 2-27-96

Representative, Quality Assurance Unit — Date

STUDY IDENTIFICATION

Primary Eye Irritation Study of Nonionic Surfactants in Rabbits
(Low Volume Procedure)

Test Materials	1. $A_{12-13}EO_{6.5}$ (Neodol 23-6.5) 2. $A_{12-14}EO_{7}$ (Alfonic 1412-7) 3. $A_{8-10}EO_{5}$ (Alfonic 810-5) 4. $A_{12-13}EO_{3}$ (Neodol 23-3) 5. $A_{12}EO_{23}$ (Brij-35) 6. Nonylphenol - $EO_{9.5}$ (Triton N101) 7. Sorbitan oleate - EO_{20} (Tween 85) 8. A_{12-16} - $glucose_{1.6}$ (Glucopon 625CS) 9. Lauramine oxide (Incromine Oxide L) 10. Cocamide DEA (Standamid KD)
Sponsor	The Soap and Detergent Association 475 Park Avenue New York, NY 10016
Sponsor's Representative	Richard I. Sedlak, PhD The Soap and Detergent Association 475 Park Avenue New York, NY 10016 (212) 725-1262
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Locations	Initial Test Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704 Additional Animals Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53707
Study Timetable	
Study Initiation Date	October 13, 1994
Experimental (In-life) Start Date	October 18, 1994
In-life End Date	April 7, 1995
Experimental Termination Date	April 7, 1995
Study Completion Date	February 27, 1996

KEY PERSONNEL

Acute Toxicology

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CONTENTS

OBJECTIVES

The objectives of this study were to develop a reliable, consistent database of eye irritation scores on nonionic surfactants to use for evaluating the ability of nonanimal tests to predict eye irritation and to determine if the eye irritation potential of nonionic surfactants can be predicted from structural or surfactant properties.

REGULATORY COMPLIANCE

This study was conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, 21 CFR 58 with the exception that analysis of the test material mixtures for concentration and stability was not conducted. All procedures used in this study were in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work.

TEST MATERIALS

The test materials were identified and described as follows:

	Test Material Identification (Trade Name)	Physical Description
1.	$A_{12-13}EO_{6.5}$ (Neodol 23-6.5)	Cloudy, white liquid
2.	$A_{12-14}EO_{7}$ (Alfonic 1412-7)	Viscous, cloudy, white liquid
3.	$A_{8-10}EO_{5}$ (Alfonic 810-5)	Clear, colorless liquid
4.	$A_{12-13}EO_{3}$ (Neodol 23-3)	Cloudy, white liquid
5.	$A_{12}EO_{23}$ (Brij-35)	White solid
6.	Nonylphenol - $EO_{9.5}$ (Triton N101)	Clear, colorless liquid
7.	Sorbitan oleate - EO_{20} (Tween 85)	Amber liquid
8.	A_{12-16} - $glucose_{1.6}$ (Glucopon 625CS)	Amber semisolid
9.	Lauramine oxide (Incromine Oxide L)	Clear, colorless liquid
10.	Cocamide DEA (Standamid KD)	Clear, light-yellow liquid

A Alcohol (subscript is the number of carbons in the alcohol chain).
EO Ethylene oxide units (subscript is the number of moles of ethylene oxide present).

Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions) of the undiluted materials. Homogeneity of the test mixtures was verified by visual inspection. Analysis of the test material mixtures for concentration and stability was not conducted or requested by the Sponsor. Samples of test material/vehicle mixtures for

physical and chemical analysis were taken before administration and sent to the individuals indicated in the Preparation of Test Materials section of this report. The analysis of these samples is the responsibility of the Sponsor.

Storage and Retention

The test materials were stored at room temperature. A reserve sample of each batch/lot of test material and each prepared test mixture was taken and will be retained in a freezer set to maintain a temperature of below 0° for 1 year. The Sponsor will be contacted after 1 year regarding the final disposition of the reserve samples. Any unused test material will be discarded 1 year after issuance of the final report according to Hazleton Wisconsin (HWI) Standard Operating Procedure (SOP).

Safety Precautions

The test material handling procedures were according to HWI SOPs and policies.

TEST SYSTEM

Test Animal

Adult albino rabbits of the Hra:(NZW)SPF strain were procured from HRP, Inc. The animals used in the initial test were received from Kalamazoo, Michigan on September 21, 1994. The additional animals treated with Alfonic 1412-7 (0.1-mL dose volume) were received from Denver, Pennsylvania on February 8, 1995.

Housing

After receipt, the animals were acclimated for a period of at least 7 days. During acclimation and throughout the study, the animals were individually housed in screen-bottom stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 19° to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided access to water *ad libitum* and a measured amount of Laboratory Rabbit Diet HF #5326, PMI Feeds, Inc. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed by HWI. There were no known contaminants in the feed or water at levels that would have interfered with or affected the results of the study.

Animal Selection

For the initial test, 16 male and 17 female healthy, acclimated animals, weighing from 2,550 to 3,010 g at initiation of treatment, were assigned to study groups (eleven groups of three animals each) using a computer-generated random numbers list. Six additional healthy, acclimated animals (three males and three females), weighing from 2,833 to 3,117 g, were selected at random and used for an additional group. The animals were identified by animal number and corresponding ear tag. All animals were chosen based on health and body weight requirements. The animals' eyes were examined on the day before test material administration using sodium fluorescein dye procedures. Only those animals with no sign of ocular injury or irritation were used.

Justification for Species Selection

Historically, the New Zealand White albino rabbit has been the animal of choice based on its large orbit and nonpigmented iris.

PROCEDURES

Preparation of Test Materials

Each test material was prepared as a 0.2 M solution/suspension by diluting the appropriate amount of test material with deionized water. The Lauramine oxide was prepared and tested in two groups of three rabbits, one group with a 0.2 M mixture with the pH adjusted to approximately 10.5 (Group 9) and the other group with a 0.2 M mixture with the pH adjusted to approximately 7.0 (Group 11). The pH of each solution/suspension was determined. The test material mixtures were prepared fresh on the day of dosing and stored at room temperature until administered.

A 110-mL subsample of each prepared test material mixture used in the initial test was obtained and stored at room temperature until being shipped 2 days after preparation. The subsamples were packed in insulated containers and shipped at ambient conditions to each of the following individuals for analysis of physical and/or chemical properties: Joseph Kwiatkowski, United States Testing Company, Inc., Chemical Services, 1415 Park Avenue, Hoboken, NJ 07030; Victor P. Janule, SensaDyne Instruments, 2855 East Brown Road, Suite #19, Mesa, AZ 85275; and Dewey Smith, Vista Chemical Company, 12025 Vista Park Drive, Austin, TX 78725-4050. The Sponsor is responsible for the conduct of these analyses, the retention of the data, and/or for reporting of the results.

Subsamples of the Alfonic 1412-7 test mixture used to treat the additional group of animals were shipped to the above individuals but analysis of these samples was not required by the Sponsor.

Treatment

Each 0.2 M test mixture was administered to a group of three animals in the initial test as follows:

Group	Test Material	Approximate pH of Test Mixture
1	$A_{12-13}EO_{6.5}$ (Neodol 23-6.5)	5.4
2	$A_{12-14}EO_{7}$ (Alfonic 1412-7)	5.8
3	$A_{8-10}EO_{5}$ (Alfonic 810-5)	6.0
4	$A_{12-13}EO_{3}$ (Neodol 23-3)	5.1
5	$A_{12}EO_{23}$ (Brij-35)	3.1
6	Nonylphenol - $EO_{9.5}$ (Triton N101)	6.1
7	Sorbitan oleate - EO_{20} (Tween 85)	7.4
8	A_{12-16} - $glucose_{1.6}$ (Glucopon 625CS)	9.0
9	Lauramine oxide (Incromine Oxide L)	10.5*
10	Cocamide DEA (Standamid KD)	10.0
11	Lauramine oxide (Incromine Oxide L)	7.0**

* The pH of the test mixture was adjusted to 10.5 using approximately 3 drops of a 50% w/v mixture of sodium hydroxide in deionized water. The pH of the original test mixture was 7.4.

** The pH of the test mixture was adjusted to 7.0 using approximately 2 drops of concentrated hydrochloric acid. The pH of the original test mixture was 7.4.

Each rabbit in the initial test received 10 µL of the respective test material mixture placed directly on the corneal surface of the right eye, with the left eye serving as the untreated control. The eyelids were released without forced blinking or manipulation.

An additional group of six animals was subsequently treated with a 0.2 M Alfonic 1412-7 test mixture. The test material mixture was prepared in the same manner used for the initial test. The pH of this test material mixture was determined to be 5.6. Each animal in the additional group received 0.1 mL of the test material mixture placed into the everted lower lid of the right eye, with the left eye serving as the untreated control. The upper and lower lids were gently held together for 1 second to prevent loss of material and then released.

The eyes of the rabbits in all groups remained unflushed immediately after treatment.

Reason for Route of Administration

Historically, the ocular route has been the route of choice based on the method of Draize.[1]

Observations

The treated eyes of all animals in each group were observed for ocular irritation at 1, 24, 48, and 72 hours after treatment. Additional observations were made at 96 hours for the animals in Groups 1, 3, and 6. The additional group of animals treated with Alfonic 1412-7 (0.1-mL dose volume) were also observed at 96 hours and Days 7, 10, 13, 16, 19, and 21 after treatment. Scoring was discontinued for each group once all treated eyes within that group cleared of irritation. After recording the 24-hour observations, sodium fluorescein was used to aid in revealing any possible corneal injury. Irritation was graded and according to the Draize technique using a penlight as the source of illumination. All eye abnormalities were recorded.

Animals were weighed just before test material administration and at weekly intervals throughout the study (when applicable).

Termination

At termination of the in-life phase for each group, the animals were designated to be euthanized and discarded.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of HWI in accordance with HWI SOP.

RESULTS/DISCUSSION

Average primary eye irritation scores are in Table 1, with the maximum average scores in Table 2. Individual body weights, individual eye irritation scores, and sodium fluorescein examination results are in Appendix A.

CONCLUSION

Based on the maximum average scores (MAS) the relative ranking of the materials from least to most irritating would be as follows:

Rank	Group	Test Material	MAS Score	Time of MAS Score (Hour)
1	4	$A_{12-13}EO_3$ (Neodol 23-3)	0.0	1
2	7	Sorbitan oleate - EO_{20} (Tween 85)	0.7	1
3	5	$A_{12}EO_{23}$ (Brij-35)	1.3	1
4	2	$A_{12-14}EO_7$ (Alfonic 1412-7)	4.7	1
5	1	$A_{12-13}EO_{6.5}$ (Neodol 23-6.5)	7.0	1
6	10	Cocamide DEA (Standamid KD)	7.3	1
7	8	A_{12-16} - $glucose_{1.6}$ (Glucopon 625CS)	8.7	1
8	3	$A_{8-10}EO_5$ (Alfonic 810-5)	11.3	1
9	11	Lauramine oxide (Incromine Oxide L)[a]	11.7	1
10	9	Lauramine oxide (Incromine Oxide L)[b]	13.0	1
11	6	Nonylphenol - $EO_{9.5}$ (Triton N101)	13.3	1
12	2[c]	$A_{12-14}EO_7$ (Alfonic 1412-7)	17.3	24

a pH adjusted to approximately 7.0.
b pH adjusted to approximately 10.5.
c Additional Group 2 animals (treated with 0.1 mL of test material mixture).

SIGNATURE

Steven M. Glaza
Study Director
Acute Toxicology

2-27-96
Date

REFERENCE

1. Draize, J. H., "Eye Mucosa," In: *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity*, Association of Food and Drug Officials of the U.S., pp. 49-50 (1959).

Table 1

Average Primary Eye Irritation Scores*

Group	Test Material	Hour					Day					
		1	24	48	72	96	7	10	13	16	19	21
1	$A_{12-13}EO_{6.5}$ (Neodol 23-6.5)	7.0	2.7	2.0	1.3	0.0	-	-	-	-	-	-
2	$A_{12-14}EO_{7}$ (Alfonic 1412-7)	4.7	2.7	2.0	0.0	-	-	-	-	-	-	-
2[c]	$A_{12-14}EO_{7}$ (Alfonic 1412-7)	10.3	17.3	10.7	8.8	7.0	3.2	2.8	2.2	2.2	2.2	1.8
3	$A_{8-10}EO_{5}$ (Alfonic 810-5)	11.3	4.0	0.7	0.7	0.0	-	-	-	-	-	-
4	$A_{12-13}EO_{3}$ (Neodol 23-3)	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
5	$A_{12}EO_{23}$ (Brij-35)	1.3	0.0	0.0	0.0	-	-	-	-	-	-	-
6	Nonylphenol - $EO_{9.5}$ (Triton N101)	13.3	11.7	3.3	2.0	0.0	-	-	-	-	-	-
7	Sorbitan oleate - EO_{20} (Tween 85)	0.7	0.0	0.0	0.0	-	-	-	-	-	-	-
8	A_{12-16} - glucose$_{1.6}$ (Glucopon 625CS)	8.7	0.0	0.0	0.0	-	-	-	-	-	-	-
9	Lauramine oxide (Incromine Oxide L)[b]	13.0	0.0	0.0	0.0	-	-	-	-	-	-	-
10	Cocamide DEA (Standamid KD)	7.3	0.0	0.0	0.0	-	-	-	-	-	-	-
11	Lauramine oxide (Incromine Oxide L)[a]	11.7	2.0	0.7	0.0	-	-	-	-	-	-	-

* The average primary eye irritation score is the total eye irritation score for all the animals divided by the number of animals in each group (3 or 6).

\- Not applicable.

a pH adjusted to approximately 7.0.

b pH adjusted to approximately 10.5.

c Additional Group 2 animals (treated with 0.1 mL of test material mixture).

Table 2

Maximum Average Scores

Test Group	Material	MAS*	MAS Time (Hour)	Irritation Clearance Times**	Median Days to Clear†	Source of Score‡
1	$A_{12-13}EO_{6.5}$ (Neodol 23-6.5)	7.0	1	72h, 96h, 96h	96h	IR, CJ
2	$A_{12-14}EO_{7}$ (Alfonic 1412-7)	4.7	1	72h, 72h, 72h	72h	CO, CJ
2[c]	$A_{12-14}EO_{7}$ (Alfonic 1412-7)	17.3	24	7d, 7d, 7d, 10d, 13d, 21+	8.5d	CO, IR, CJ
3	$A_{8-10}EO_{5}$ (Alfonic 810-5)	11.3	1	48h, 48h, 96h	48h	IR, CJ
4	$A_{12-13}EO_{3}$ (Neodol 23-3)	0.0	1	††, ††, ††	-	-
5	$A_{12}EO_{23}$ (Brij-35)	1.3	1	††, 24h, 24h	24h	CJ
6	Nonylphenol - $EO_{9.5}$ (Triton N101)	13.3	1	96h, 96h, 96h	96h	CO, IR, CJ
7	Sorbitan oleate - EO_{20} (Tween 85)	0.7	1	††, ††, 24h	<24h	CJ
8	A_{12-16} - glucose$_{1.6}$ (Glucopon 625CS)	8.7	1	24h, 24h, 24h	24h	IR, CJ
9	Lauramine oxide (Incromine Oxide L)[b]	13.0	1	24h, 24h, 24h	24h	IR, CJ
10	Cocamide DEA (Standamid KD)	7.3	1	24h, 24h, 24h	24h	IR, CJ
11	Lauramine oxide (Incromine Oxide L)[a]	11.7	1	48h, 48h, 72h	48h	IR, CJ

* The maximum average score (MAS) is the highest average primary eye irritation score recorded for any given observation period.

** The times presented are the individual clearance times, in ascending order, for each animal in the indicated group.

† The median days to clear is determined by ranking the individual animal irritation clearance times in ascending order and then either selecting the middle value or determining the mean of the two middle values (Group 2[c] only).

‡ CO - Corneal; IR - Iris; CJ - Conjunctivae

†† No irritation observed.

\- Not applicable.
h Hour.
d Day.
\+ Irritation still present.
a pH adjusted to approximately 7.0.
b pH adjusted to approximately 10.5.
c Additional Group 2 animals (treated with 0.1 mL of test material mixture).

APPENDIX A

Individual Body Weights
Individual Eye Irritation Scores
Sodium Fluorescein Examinations

Individual Body Weights (g)

Animal Number	Sex	Initial	Day 7	Day 14	Day 21
Group 1 - $A_{12-13}EO_{6.5}$ (Neodol 23-6.5)					
F52372	M	2,800	-	-	-
F52377	F	2,720	-	-	-
F52355	M	3,010	-	-	-
Group 2 (Initial Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)					
F52382	F	2,741	-	-	-
F52364	F	2,669	-	-	-
F52383	F	2,887	-	-	-
Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)					
F53845	M	3,117	3,223	3,314	3,309†
F53846	M	3,095	3,148	3,136†	3,234
F53853	M	2,833	2,918	2,942	3,065
F53848	F	2,905	2,973	3,051	3,118
F53849	F	3,053	3,131	3,199	3,307
F53850	F	2,917	2,972	3,039	3,140
Group 3 - $A_{8-10}EO_5$ (Alfonic 810-5)					
F52371	F	2,782	-	-	-
F52379	M	2,718	-	-	-
F52366	M	2,774	-	-	-
Group 4 - $A_{12-13}EO_3$ (Neodol 23-3)					
F52374	M	2,814	-	-	-
F52380	M	2,688	-	-	-
F52351	F	2,707	-	-	-
Group 5 - $A_{12}EO_{23}$ (Brij-35)					
F52352	F	2,776	-	-	-
F52367	M	2,637	-	-	-
F52368	M	2,626	-	-	-

- Not applicable.
† Denotes weight loss from previous weighing.

Individual Body Weights (g)

Animal Number	Sex	Initial	Day 7	Day 14	Day 21
Group 6 - Nonylphenol - $EO_{9.5}$ (Triton N101)					
F52357	F	2,771	-	-	-
F52365	F	2,754	-	-	-
F52363	F	2,780	-	-	-
Group 7 - Sorbitan oleate - EO_{20} (Tween 85)					
F52360	M	2,625	-	-	-
F52354	M	2,756	-	-	-
F52358	F	2,864	-	-	-
Group 8 - A_{12-16} - $glucose_{1.6}$ (Glucopon 625CS)					
F52373	M	2,581	-	-	-
F52362	M	2,966	-	-	-
F52361	M	2,610	-	-	-
Group 9 - Lauramine oxide (Incromine Oxide L); 10.5 pH					
F52370	F	2,875	-	-	-
F52359	F	2,910	-	-	-
F52356	M	2,726	-	-	-
Group 10 - Cocamide DEA (Standamid KD)					
F52369	F	2,736	-	-	-
F52375	F	2,676	-	-	-
F52353	F	2,585	-	-	-
Group 11 - Lauramine oxide (Incromine Oxide L); 7.0 pH					
F52378	M	2,933	-	-	-
F52384	M	2,622	-	-	-
F52376	F	2,550	-	-	-

- Not applicable.
† Denotes weight loss from previous weighing.

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Individual Eye Irritation Scores

Group 1 - $A_{12-13}EO_{6.5}$ (Neodol 23-6.5)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52372[t]	0	0	0	1	1	1[c]	6.0
F52377[t]	0	0	0	1	1	0	4.0
F52355[t]	0	0	1[i]	1	1	1[c]	11.0
					Mean		7.0
			24 Hours				
F52372	0	0	0	1	0	0	2.0
F52377	0	0	0	1	0	0	2.0
F52355	0	0	0	2	0	0	4.0
					Mean		2.7
			48 Hours				
F52372	0	0	0	1	0	0	2.0
F52377	0	0	0	1	0	0	2.0
F52355	0	0	0	1	0	0	2.0
					Mean		2.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
c Clear discharge.
i Injected.
t No pain response after test material instillation.

Individual Eye Irritation Scores

Group 1 - $A_{12-13}EO_{6.5}$ (Neodol 23-6.5) (Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			72 Hours				
F52372	0	0	0	1	0	0	2.0
F52377	0	0	0	1	0	0	2.0
F52355	0	0	0	0	0	0	0.0
					Mean		1.3
			96 Hours				
F52372	0	0	0	0	0	0	0.0
F52377	0	0	0	0	0	0	0.0
F52355	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period: Preinitiation	Observation Period: 72 Hour
F52372	NEG	NEG
F52377	NEG	NEG
F52355	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 2 (Initial Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52382[u]	0	0	0	1	1	0	4.0
F52364[t]	0	0	0	2	1	1[c]	8.0
F52383[u]	0[k]	0	0	1	0	0	2.0
					Mean		4.7
			24 Hours				
F52382	0	0	0	1	0	0	2.0
F52364	0	0	0	1	0	0	2.0
F52383	0	0	0	1	1	0	4.0
					Mean		2.7
			48 Hours				
F52382	0	0	0	1	0	0	2.0
F52364	0	0	0	1	0	0	2.0
F52383	0	0	0	1	0	0	2.0
					Mean		2.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
c Clear discharge.
k Corneal epithelial pitting; no area of opacity. Observation may have been test material adhered to the cornea.
t No pain response after test material instillation.
u Excessive pawing at the treated eye after test material instillation.

Individual Eye Irritation Scores

Group 2 (Initial Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7) (Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			72 Hours				
F52382	0	0	0	0	0	0	0.0
F52364	0	0	0	0	0	0	0.0
F52383	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period: Preinitiation	Observation Period: 72 Hour
F52382	NEG	NEG
F52364	NEG	NEG
F52383	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F53845[u]	0	0	0	2	1	1[c]	8.0
F53846[t]	0	0	0	2[b]	1	0	6.0
F53853[u]	0	0	0	2[b]	1	0	6.0
F53848[u]	0	0	1[i]	2[b]	1	0	11.0
F53849[t]	1	1	1[i]	2[b]	1	1[c]	18.0
F53850[t]	0	0	1[i]	2[b]	1	1[c]	13.0
					Mean		10.3
			24 Hours				
F53845	0	0	0	2[b]	1	1[c]	8.0
F53846	0	0	0	2[b]	1	2[c]	10.0
F53853	0	0	1[i]	2[b]	1	1[c]	13.0
F53848	0	0	1[i]	2[b]	2	3[c]	19.0
F53849	1[j]	4	1[i]	2[b]	3	3[c]	41.0
F53850	0	0	1[i]	2[b]	1	1[c]	13.0
					Mean		17.3

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
b Blanching.
c Clear discharge.
i Injected.
j Corneal epithelial peeling.
t No pain response after test material instillation.
u Excessive pawing at the treated eye after test material instillation.

Individual Eye Irritation Scores

Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)
(Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			48 Hours				
F53845	0	0	0	2	1	0	6.0
F53846	0	0	0	2	1	0	6.0
F53853	0	0	0	2	1	0	6.0
F53848	0	0	0	2	1	0	6.0
F53849	1[j]	3	1[i]	2[b]	3	2[d]	34.0
F53850	0	0	0	2	1	0	6.0
					Mean		10.7
			72 Hours				
F53845	0	0	0	2	1	0	6.0
F53846	0	0	0	1	1	0	4.0
F53853	0	0	0	2	1	0	6.0
F53848	0	0	0	2	1	0	6.0
F53849	1[j]	2	1[i]	2[b]	2	1[d]	25.0
F53850	0	0	0	2	1	0	6.0
					Mean		8.8

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
b Blanching.
d Purulent discharge.
i Injected.
j Corneal epithelial peeling.

Individual Eye Irritation Scores

Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)
(Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			96 Hours				
F53845	0	0	0	1	1	0	4.0
F53846	0	0	0	1	0	0	2.0
F53853	0	0	0	2	1	0	6.0
F53848	0	0	0	2	1	0	6.0
F53849	1[j]	2	0	2[b]	2	1[d]	20.0
F53850	0	0	0	1	1	0	4.0
					Mean		7.0
			Day 7				
F53845	0	0	0	0	0	0	0.0
F53846	0	0	0	0	0	0	0.0
F53853	0	0	0	1	0	0	2.0
F53848	0	0	0	0	0	0	0.0
F53849	1[j]	1	0	2	2	1[d]	15.0
F53850	0	0	0	1	0	0	2.0
					Mean		3.2

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
b Blanching.
d Purulent discharge.
j Corneal epithelial peeling.

Individual Eye Irritation Scores

Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)
(Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			Day 10				
F53845	0	0	0	0	0	0	0.0
F53846	0	0	0	0	0	0	0.0
F53853	0	0	0	1	0	0	2.0
F53848	0	0	0	0	0	0	0.0
F53849	1[j,n]	1	0	2	2	1[d]	15.0
F53850	0	0	0	0	0	0	0.0
					Mean		2.8
			Day 13				
F53845	0	0	0	0	0	0	0.0
F53846	0	0	0	0	0	0	0.0
F53853	0	0	0	0	0	0	0.0
F53848	0	0	0	0	0	0	0.0
F53849	1[j,n]	1	0	2	2	0	13.0
F53850	0	0	0	0	0	0	0.0
					Mean		2.2

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
d Purulent discharge.
j Corneal epithelial peeling.
n Corneal neovascularization.

Individual Eye Irritation Scores

Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)
(Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			Day 16				
F53845	0	0	0	0	0	0	0.0
F53846	0	0	0	0	0	0	0.0
F53853	0	0	0	0	0	0	0.0
F53848	0	0	0	0	0	0	0.0
F53849	1[j,n]	1	0	2	2	0	13.0
F53850	0	0	0	0	0	0	0.0
					Mean		2.2
			Day 19				
F53845	0	0	0	0	0	0	0.0
F53846	0	0	0	0	0	0	0.0
F53853	0	0	0	0	0	0	0.0
F53848	0	0	0	0	0	0	0.0
F53849	1[j,n]	1	0	2	2	0	13.0
F53850	0	0	0	0	0	0	0.0
					Mean		2.2

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
j Corneal epithelial peeling.
n Corneal neovascularization.

Individual Eye Irritation Scores

Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)
(Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			Day 21				
F53845	0	0	0	0	0	0	0.0
F53846	0	0	0	0	0	0	0.0
F53853	0	0	0	0	0	0	0.0
F53848	0	0	0	0	0	0	0.0
F53849	1[j,n]	1	0	2	1	0	11.0
F53850	0	0	0	0	0	0	0.0
					Mean		1.8

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
j Corneal epithelial peeling.
n Corneal neovascularization.

Sodium Fluorescein Examinations

Group 2 (Additional Animals) - $A_{12-14}EO_7$ (Alfonic 1412-7)

Animal Number	Observation Period				
	Preinitiation	24 Hour	48 Hour	72 Hour	96 Hour
F53845	NEG	NEG	NA	NA	NA
F53846	NEG	NEG	NA	NA	NA
F53853	NEG	NEG	NA	NA	NA
F53848	NEG	NEG	NA	NA	NA
F53849	NEG	POS (85%)	POS (70%)	POS (40%)	POS (35%)
F53850	NEG	NEG	NA	NA	NA

Animal Number	Observation Period					
	Day 7	Day 10	Day 13	Day 16	Day 19	Day 21
F53845	NA	NA	NA	NA	NA	NA
F53846	NA	NA	NA	NA	NA	NA
F53853	NA	NA	NA	NA	NA	NA
F53848	NA	NA	NA	NA	NA	NA
F53849	POS (20%)	POS (20%)	POS (20%)	POS (20%)	POS (20%)	POS (20%)
F53850	NA	NA	NA	NA	NA	NA

NA Not applicable.
NEG Negative stain retention.
POS Positive stain retention (area of cornea involved).

Individual Eye Irritation Scores

Group 3 - $A_{8-10}EO_5$ (Alfonic 810-5)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52371[t]	0	0	1[i]	2	2	2[c]	17.0
F52379[t]	0	0	0	2	1	1[c]	8.0
F52366[t]	0	0	1[i]	1	1	0	9.0
					Mean		11.3
			24 Hours				
F52371	0	0	0	2	1	0	6.0
F52379	0	0	0	1	1	0	4.0
F52366	0	0	0	1	0	0	2.0
					Mean		4.0
			48 Hours				
F52371	0	0	0	1	0	0	2.0
F52379	0	0	0	0	0	0	0.0
F52366	0	0	0	0	0	0	0.0
					Mean		0.7

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
c Clear discharge.
i Injected.
t No pain response after test material instillation.

Individual Eye Irritation Scores

Group 3 - $A_{8-10}EO_5$ (Alfonic 810-5)
(Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			72 Hours				
F52371	0	0	0	1	0	0	2.0
F52379	0	0	0	0	0	0	0.0
F52366	0	0	0	0	0	0	0.0
					Mean		0.7
			96 Hours				
F52371	0	0	0	0	0	0	0.0
F52379	0	0	0	0	0	0	0.0
F52366	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period: Preinitiation	Observation Period: 72 Hour
F52371	NEG	NEG
F52379	NEG	NEG
F52366	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 4 - $A_{12-13}EO_3$ (Neodol 23-3)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52374[t]	0	0	0	0	0	0	0.0
F52380[t]	0	0	0	0	0	0	0.0
F52351[t]	0	0	0	0	0	0	0.0
					Mean		0.0
			24 Hours				
F52374	0	0	0	0	0	0	0.0
F52380	0	0	0	0	0	0	0.0
F52351	0	0	0	0	0	0	0.0
					Mean		0.0
			48 Hours				
F52374	0	0	0	0	0	0	0.0
F52380	0	0	0	0	0	0	0.0
F52351	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
t No pain response after test material instillation.

Individual Eye Irritation Scores

Group 4 - $A_{12-13}EO_3$ (Neodol 23-3)
(Continued)

Animal Number	Cornea		Iris	Conjunctivae			Total Score*
	A	B	C	D	E	F	
			72 Hours				
F52374	0	0	0	0	0	0	0.0
F52380	0	0	0	0	0	0	0.0
F52351	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period	
	Preinitiation	72 Hour
F52374	NEG	NEG
F52380	NEG	NEG
F52351	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 5 - $A_{12}EO_{23}$ (Brij-35)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52352[t]	0	0	0	0	0	0	0.0
F52367[t]	0	0	0	1	0	0	2.0
F52368[t]	0	0	0	1	0	0	2.0
					Mean		1.3
			24 Hours				
F52352	0	0	0	0	0	0	0.0
F52367	0	0	0	0	0	0	0.0
F52368	0	0	0	0	0	0	0.0
					Mean		0.0
			48 Hours				
F52352	0	0	0	0	0	0	0.0
F52367	0	0	0	0	0	0	0.0
F52368	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
t No pain response after test material instillation.

Individual Eye Irritation Scores

Group 5 - $A_{12}EO_{23}$ (Brij-35) (Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			72 Hours				
F52352	0	0	0	0	0	0	0.0
F52367	0	0	0	0	0	0	0.0
F52368	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period: Preinitiation	Observation Period: 72 Hour
F52352	NEG	NEG
F52367	NEG	NEG
F52368	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 6 - Nonylphenol - $EO_{9.5}$ (Triton N101)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52357[u]	0	0	1[i]	2	3	3[c]	21.0
F52365[t]	0	0	0	1	2	1[c]	8.0
F52363[t]	0	0	1[i]	1	1	1[c]	11.0
					Mean		13.3
			24 Hours				
F52357	1[j]	1	1[i]	2	2	0	18.0
F52365	0	0	0	2	1	0	6.0
F52363	1	1	0	2	1	0	11.0
					Mean		11.7
			48 Hours				
F52357	0	0	0	2	1	0	6.0
F52365	0	0	0	1	0	0	2.0
F52363	0	0	0	1	0	0	2.0
					Mean		3.3

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
c Clear discharge.
i Injected.
j Corneal epithelial peeling.
t No pain response after test material instillation.
u Excessive pawing at the treated eye after test material instillation.

Individual Eye Irritation Scores

Group 6 - Nonylphenol - $EO_{9.5}$ (Triton N101) (Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			72 Hours				
F52357	0	0	0	1	0	0	2.0
F52365	0	0	0	1	0	0	2.0
F52363	0	0	0	1	0	0	2.0
					Mean		2.0
			96 Hours				
F52357	0	0	0	0	0	0	0.0
F52365	0	0	0	0	0	0	0.0
F52363	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period: Preinitiation	Observation Period: 72 Hour
F52357	NEG	NEG
F52365	NEG	NEG
F52363	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 7 - Sorbitan oleate - EO_{20} (Tween 85)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52360[t]	0	0	0	1	0	0	2.0
F52354[t]	0	0	0	0	0	0	0.0
F52358[t]	0	0	0	0	0	0	0.0
					Mean		0.7
			24 Hours				
F52360	0	0	0	0	0	0	0.0
F52354	0	0	0	0	0	0	0.0
F52358	0	0	0	0	0	0	0.0
					Mean		0.0
			48 Hours				
F52360	0	0	0	0	0	0	0.0
F52354	0	0	0	0	0	0	0.0
F52358	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
t No pain response after test material instillation.

Individual Eye Irritation Scores

Group 7 - Sorbitan oleate - EO_{20} (Tween 85)
(Continued)

Animal Number	Cornea		Iris	Conjunctivae			Total Score*
	A	B	C	D	E	F	
			72 Hours				
F52360	0	0	0	0	0	0	0.0
F52354	0	0	0	0	0	0	0.0
F52358	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period	
	Preinitiation	72 Hour
F52360	NEG	NEG
F52354	NEG	NEG
F52358	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 8 - A_{12-16} - $glucose_{1.6}$ (Glucopon 625CS)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52373[u]	0	0	1[i]	2	1	1[c]	13.0
F52362[u]	0	0	0	1	0	0	2.0
F52361[u]	0	0	1[i]	2	1	0	11.0
					Mean		8.7
			24 Hours				
F52373	0	0	0	0	0	0	0.0
F52362	0	0	0	0	0	0	0.0
F52361	0	0	0	0	0	0	0.0
					Mean		0.0
			48 Hours				
F52373	0	0	0	0	0	0	0.0
F52362	0	0	0	0	0	0	0.0
F52361	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
c Clear discharge.
i Injected.
u Excessive pawing at the treated eye after test material instillation.

Individual Eye Irritation Scores

Group 8 - A_{12-16} - $glucose_{1.6}$ (Glucopon 625CS)
(Continued)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			72 Hours				
F52373	0	0	0	0	0	0	0.0
F52362	0	0	0	0	0	0	0.0
F52361	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period: Preinitiation	Observation Period: 72 Hour
F52373	NEG	NEG
F52362	NEG	NEG
F52361	NEG	NEG

NEG Negative stain retention.

HWI 6310-105

Individual Eye Irritation Scores

Group 9 - Lauramine oxide (Incromine Oxide L); 10.5 pH

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52370[u]	0	0	1[i]	2	1	1[c]	13.0
F52359[u]	0	0	1[i]	2	1	1[c]	13.0
F52356[u]	0	0	1[i]	2	1	1[c]	13.0
					Mean		13.0
			24 Hours				
F52370	0	0	0	0	0	0	0.0
F52359	0	0	0	0	0	0	0.0
F52356	0	0	0	0	0	0	0.0
					Mean		0.0
			48 Hours				
F52370	0	0	0	0	0	0	0.0
F52359	0	0	0	0	0	0	0.0
F52356	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
c Clear discharge.
i Injected.
u Excessive pawing at the treated eye after test material instillation.

Individual Eye Irritation Scores

Group 9 - Lauramine oxide (Incromine Oxide L); 10.5 pH
(Continued)

Animal Number	Cornea		Iris	Conjunctivae			Total Score*
	A	B	C	D	E	F	
			72 Hours				
F52370	0	0	0	0	0	0	0.0
F52359	0	0	0	0	0	0	0.0
F52356	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period	
	Preinitiation	72 Hour
F52370	NEG	NEG
F52359	NEG	NEG
F52356	NEG	NEG

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 10 - Cocamide DEA (Standamid KD)

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52369[t]	0	0	1[i]	1	1	1[c]	11.0
F52375[t]	0	0	0	1	0	0	2.0
F52353[t]	0	0	1[i]	1	0	1[c]	9.0
					Mean		7.3
			24 Hours				
F52369	0	0	0	0	0	0	0.0
F52375	0	0	0	0	0	0	0.0
F52353	0	0	0	0	0	0	0.0
					Mean		0.0
			48 Hours				
F52369	0	0	0	0	0	0	0.0
F52375	0	0	0	0	0	0	0.0
F52353	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
i Injected.
c Clear discharge.
t No pain response after test material instillation.

Individual Eye Irritation Scores

Group 10 - Cocamide DEA (Standamid KD)
(Continued)

Animal Number	Cornea		Iris	Conjunctivae			Total Score*
	A	B	C	D	E	F	
			72 Hours				
F52369	0	0	0	0	0	0	0.0
F52375	0	0	0	0	0	0	0.0
F52353	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period	
	Preinitiation	72 Hour
F52369	NEG	NA
F52375	NEG	NA
F52353	NEG	NA

NEG Negative stain retention.

Individual Eye Irritation Scores

Group 11 - Lauramine oxide (Incromine Oxide L); 7.0 pH

Animal Number	Cornea A	Cornea B	Iris C	Conjunctivae D	Conjunctivae E	Conjunctivae F	Total Score*
			1 Hour				
F52378[u]	0	0	1[i]	2	1	1[c]	13.0
F52384[t]	0	0	1[i]	2	1	1[c]	13.0
F52376[u]	0	0	1[i]	1	0	1[c]	9.0
					Mean		11.7
			24 Hours				
F52378	0	0	0	1	0	0	2.0
F52384	0	0	0	1	0	0	2.0
F52376	0	0	0	1	0	0	2.0
					Mean		2.0
			48 Hours				
F52378	0	0	0	0	0	0	0.0
F52384	0	0	0	1	0	0	2.0
F52376	0	0	0	0	0	0	0.0
					Mean		0.7

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].
c Clear discharge.
i Injected.
t No pain response after test material instillation.
u Excessive pawing at the treated eye after test material instillation.

Individual Eye Irritation Scores

Group 11 - Lauramine oxide (Incromine Oxide L); 7.0 pH
(Continued)

Animal Number	Cornea		Iris	Conjunctivae			Total Score*
	A	B	C	D	E	F	
			72 Hours				
F52378	0	0	0	0	0	0	0.0
F52384	0	0	0	0	0	0	0.0
F52376	0	0	0	0	0	0	0.0
					Mean		0.0

Cornea

A - Degree of opacity
B - Area of involvement

Iris

C - Degree of iridal irritation

Conjunctivae

D - Redness
E - Chemosis
F - Discharge

* Total score = (A x B x 5) + (C x 5) + [(D + E + F) x 2].

Sodium Fluorescein Examinations

Animal Number	Observation Period	
	Preinitiation	72 Hour
F52378	NEG	NEG
F52384	NEG	NEG
F52376	NEG	NEG

NEG Negative stain retention.

APPENDIX B

Protocol
Protocol Amendment No. 1
Protocol Amendment No. 2

HAZLETON
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MADISON, WI 53707 7545

a CORNING Company

Sponsor:

The Soap and Detergent Association
New York, New York

PROTOCOL TP6360.F

Study Title:

Primary Eye Irritation Study of Nonionic Surfactants in Rabbits
(Low Volume Procedure)

Date:

October 13, 1994

Performing Laboratory:

Hazleton Wisconsin, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

HWI 6310-105

STUDY IDENTIFICATION

Primary Eye Irritation Study of Nonionic Surfactants in Rabbits
(Low Volume Procedure)

HWI No.	6310-105
Test Materials	1. $A_{12-13}EO_{6.5}$ (Neodol 23-6.5) 2. $A_{12-14}EO_{7}$ (Alfonic 1412-7) 3. $A_{8-10}EO_{5}$ (Alfonic 810-5) 4. $A_{12-13}EO_{3}$ (Neodol 23-3) 5. $A_{12}EO_{23}$ (Brij-35) 6. Nonylphenol - $EO_{9.5}$ (Triton N101) 7. Sorbitan oleate - EO_{20} (Tween 85) 8. A_{12-16} - glucose $_{1,6}$ (Glucopon 625CS) 9. Lauramine oxide (Incromine Oxide L) 10. Cocamide DEA (Standamid KD)
Sponsor	The Soap and Detergent Association 475 Park Avenue New York, NY 10016
Sponsor's Representative	Richard I. Sedlak, PhD The Soap and Detergent Association 475 Park Avenue New York, NY 10016 (212) 725-1262
Study Director	Steven M. Glaza Hazleton Wisconsin, Inc. P.O. Box 7545 Madison, WI 53707-7545 (608) 241-7292
Study Location	Hazleton Wisconsin, Inc. Building No. 3 3802 Packers Avenue Madison, WI 53704
Proposed Study Timetable	
Experimental Start Date	Week of October 17, 1994
Experimental Termination Date	Week of November 7, 1994
Final Report Date	Week of December 26, 1994

1. Study
Primary Eye Irritation Study in Rabbits (Low Volume Procedure)

2. Purpose
To 1) develop a reliable, consistant database of eye irritation scores on nonionic surfactants to use for evaluating the ability of non-animal tests to predict eye irritation: and 2) determine if the eye irritation potential of nonionic surfactants can be predicted from structural or surfactant properties.

3. Regulatory Compliance
This study will be conducted in accordance with the Food and Drug Administration Good Laboratory Practice Regulations as outlined in 21 CFR 58 with the exception that analyses of the test mixtures for concentration and stability will not be conducted.

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

4. Quality Assurance
The protocol, study conduct, and the final report will be audited by the Quality Assurance Unit in accordance with Hazleton Wisconsin (HWI) Standard Operating Procedures (SOPs) and policies.

5. Test Materials

A. Identification (Trade Name)
1. $A_{12\text{-}13}EO_{6.5}$ (Neodol 23-6.5)
2. $A_{12\text{-}14}EO_{7}$ (Alfonic 1412-7)
3. $A_{8\text{-}10}EO_{5}$ (Alfonic 810-5)
4. $A_{12\text{-}13}EO_{3}$ (Neodol 23-3)
5. $A_{12}EO_{23}$ (Brij-35)
6. Nonylphenol - $EO_{9.5}$ (Triton N101)
7. Sorbitan oleate - EO_{20} (Tween 85)
8. $A_{12\text{-}16}$ - glucose $_{1,6}$ (Glucopon 625CS)
9. Lauramine oxide (Incromine Oxide L)
10. Cocamide DEA (Standamid KD)

A = alcohol (Subcript is the number of carbons in the alcohol chain)
EO = ethylene oxide units (Subscript is the number of moles of ethylene oxide present)

B. Physical Description
To be documented in the raw data

C. Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions) of the undiluted materials. Homogeneity of the test mixtures will be verified by visual inspection. Analyses of the test mixtures for concentration and stability will not be conducted. Samples of test material/vehicle mixtures for physical and chemical analysis will be taken before administration and sent to the individuals listed in Section 6. B. (1). The dose analyses of these items is the responsibility of the Sponsor.

D. Storage
Room temperature

E. Reserve Samples
Reserve sample(s) of each batch/lot of test material and each prepared test mixture will be taken for this study.

The reserve samples of the test materials and the prepared test mixtures will be stored at HWI in a freezer set to maintain a temperature of below 0°C for 1 year. The Sponsor will be contacted after 1 year regarding the final disposition of these reserve samples.

F. Retention
Any unused test material will be discarded one year after issuance of the final report, unless directed otherwise by the Sponsor.

G. Safety Precautions
As required by HWI SOPs and policies

6. Experimental Design

A. Animals

(1) Species
Rabbit

(2) Strain/Source
Hra:(NZW)SPF/HRP, Inc.

(3) Age at Initiation
Adult

(4) Weight at Initiation
2.0 to 3.5 kg

(5) Number and Sex
33 (3 of any sex per test material mixture)
3 of any sex for any additional test material mixtures (if necessary)

(6) Identification
Individual numbered ear tag

(7) Husbandry

(a) Housing
Individually, in screen-bottom stainless steel cages (heavy gauge)

(b) Food
A measured amount of Laboratory Rabbit Diet HF #5326 (PMI Feeds, Inc.). The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from an automatic system. Samples of the water are analyzed by HWI for total dissolved solids, hardness, and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study.

(e) Environment
Environmental controls for the animal room will be set to maintain a temperature of 19 to 23°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 7 days

(8) Selection of Test Animals
Healthy animals meeting the body weight requirements and having no pre-existing eye irritation will be assigned to study groups using a random numbers list. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. The eyes will be examined using sodium fluorescein dye procedures on the day before test material administration. Only animals with no sign of corneal injury or eye abnormalities will be used.

(9) Justification for Species Selection
Historically, the New Zealand White albino rabbit has been the animal of choice based upon its large orbit and nonpigmented iris.

B. Dose Administration

(1) Test Material Preparation
Each test material will be prepared as a 0.2 M solution/suspension by diluting the appropriate amount of test material with deionized water. The Lauramine oxide will be prepared and tested in two groups of three rabbits, one group as a 0.2 M mixture (pH of approximately 10.5) and the other group as a 0.2 M mixture (pH of approximately 7.0). The Sponsor will be consulted if any problems are encountered in preparing the test mixtures. The pH of each solution/suspension will also be determined. If any test material solution/suspension results in a pH outside of the 6-9 pH range, then an additional solution/suspension adjusted to a pH of 7 will also be tested in the same manner as the other test material mixtures. All test mixtures will be prepared fresh on the day of dosing and stored at room temperature until administration. An adequate amount of each test mixture will be prepared, to allow at least 100 mL of each mixture to be shipped to the following individuals for analysis of physical and/or chemical properties:

Joseph Kwiatkowski
United States Tesing Company, Inc.
Chemical Services
1415 Park Avenue
Hoboken, NJ 07030

Victor P. Janule
SensaDyne Instruments
2855 East Brown Road Suite #19
Mesa, AZ 85275

Dewey Smith
Vista Chemical Company
12025 Vista Park Drive
Austin, TX 78725-4050

The subsamples of the test mixtures will be stored at room temperature until shipped within 1-3 days of preparation. The samples will be packed in insulated containers and shipped at ambient conditions. HWI does not accept any responsibility for these analyses, the retention of the data or for the reporting of the results. These items are the responsibility of the Sponsor.

(2) Dose Administration
Before administration of each test mixture, the pH of each prepared mixture will be determined (if possible). Each test mixture will be administered to a group of three animals as follows:

Group	Test Material
1	$A_{12-13}EO_{6.5}$ (Neodol 23-6.5)
2	$A_{12-14}EO_{7}$ (Alfonic 1412-7)
3	$A_{8-10}EO_{5}$ (Alfonic 810-5)
4	$A_{12-13}EO_{3}$ (Neodol 23-3)
5	$A_{12}EO_{23}$ (Brij-35)
6	Nonylphenol - $EO_{9.5}$ (Triton N101)
7	Sorbitan oleate - EO_{20} (Tween 85)
8	A_{12-16} - glucose $_{1,6}$ (Glucopon 625CS)
9	Lauramine oxide (Incromine Oxide L) - pH approx. 10.5
10	Cocamide DEA (Standamid KD)
11	Lauramine oxide (Incromine Oxide L) - pH approx. 7.0

Each animal will receive a single dose of 10 μL of respective test mixture. The test mixture will be placed onto the corneal surface of the rabbit's right eye. The upper and lower lids will then be released without forced blinking or manipulation. The eyes of the rabbits will remain unflushed for approximately 24 hours after instillation of the test material. After 24 hours, a washout may be used if considered appropriate based on the level of irritation observed. The right eye of each animal will be treated with the test material and the left eye will serve as the untreated control.

(3) Reason for Route of Administration
Historically, the ocular route has been the route of choice based on the method of Draize.

C. Observation of Animals

(1) Reading of Ocular Irritation
The treated eyes of all animals in each group will be examined for ocular irritation at approximately 1, 24, 48, and 72 hours after treatment. If no irritation or injury is present at 72 hours, the animals for that test material will be terminated. If irritation is present at 72 hours, additional observations will be made at approximately 96 hours and at 7, 10, 13, 16, 19, and 21 days, or until all irritation has cleared. Additional weekly observations may be requested by the Sponsor. After recording the 24-hour observations, sodium fluorescein may be used to aid in revealing possible corneal injury.

Irritation will be graded and scored using the Draize technique (Attachment 1). All eye abnormalities will be recorded. All animals that have a damaged eye producing undue stress or discomfort will be brought to the attention of the study director or designee according to HWI policy.

(2) Body Weights
Before test material administration and weekly thereafter (when applicable).

D. Pathology
Any animals dying during the study will be subjected to an abbreviated gross necropsy examination and all abnormalities will be recorded. After necropsy, the animals will be discarded and no tissues will be saved. At termination of the experimental phase, surviving animals will be designated to be sacrificed and discarded.

E. Statistical Analyses
No statistical analyses are required.

7. Report
A final report including those items listed below will be submitted:

Description of the test materials
Description of the test system
Dates of experimental initiation and termination
Summary tables showing the individual irritation data at each observation period, the maximum average score and the median days to recover for each test mixture

8. Location of Raw Data, Records, and Final Report
Original data, or copies thereof, will be available at HWI to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below will be retained in the archives of HWI according to HWI SOP.

Protocol and protocol amendments
Dose preparation records
In-life records
  Body weights
  Dose administration
  Observations
Anatomical pathology records (if applicable)
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at HWI but will not be archived with the study data.

Animal receipt/acclimation records
Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

Richard I. Sedlak, PhD
Sponsor's Representative
The Soap and Detergent Association

8/31/95
Date

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

10-13-94
Date

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

10.13.94
Date

(6310105.pr)HD

Attachment 1

SCALE FOR SCORING OCULAR LESIONS (DRAIZE TECHNIQUE)

(1) Cornea

(A) Opacity - Degree of density (area most dense taken for reading)
No opacity.....0
Scattered or diffuse area, details of iris clearly visible.....1*
Easily discernible translucent areas, details of iris slightly obscured.....2*
Opalescent areas, no details of iris visible, size of pupil barely discernible.....3*
Opaque, iris invisible.....4*

(B) Area of Cornea Involved
One-quarter (or less), but not zero.....1
Greater than one-quarter, but less than half.....2
Greater than half, but less than three-quarters.....3
Greater than three-quarters up to whole area.....4

A x B x 5 Total Maximum = 80

(2) Iris

(A) Values
Normal.....0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris is still reacting to light (sluggish reaction is positive).....1*
No reaction to light, hemorrhage, gross destruction (any or all of these).....2*

A x 5 Total Maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
Vessels normal.....0
Vessels definitely injected above normal.....1
More diffuse, deeper crimson red, individual vessels not easily discernible.....2*
Diffuse beefy red.....3*

(B) Chemosis
No swelling.....0
Any swelling above normal (includes nictitating membrane).....1
Obvious swelling with partial eversion of the lids.....2*
Swelling with lids about half closed.....3*
Swelling with lids about half closed to completely closed.....4*

(C) Discharge
No discharge.....0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals).....1
Discharge with moistening of the lids and hairs just adjacent to the lids.....2
Discharge with moistening of the lids and hairs, and considerable area around the eye...3

Score (A + B + C) x 2 Total Maximum = 20

The total score for the eye is the sum of all scores obtained for the cornea, iris, and conjunctivae.

* Indicates a positive effect. (FHSA Interpretation)

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PROTOCOL TP6360.F

Primary Eye Irritation Study of Nonionic Surfactants in Rabbits
(Low Volume Procedure)

HWI 6310-105

Sponsor	Contractor
The Soap and Detergent Association 475 Park Avenue New York , NY 10016	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	Study Director
Richard I. Sedlak, PhD	Steven M. Glaza

Amendment No. 1

This amendment modifies the following portion of the protocol:

Effective March 16, 1995

1. Page 7, 6. Experimental Design; B. Dose Administration; (2) Dose Administration. At the request of the Sponsor to treat six new Group 2 animals with the standard volume of test mixture (0.1 mL), add the following paragraph to this section:

> An additional group of six animals will be treated with $A_{12-14}EO_7$ (Alfonic 1412-7) prepared using the same procedure as for the initial testing. Each animal will receive a single dose of 0.1 mL of the test mixture. All other procedures in the protocol will be followed.

Amendment No. 1

Effective March 21, 1995

1. Page 7, 6. Experimental Design; B. Dose Administration; (2) Dose Administration. At the request of the Sponsor, the samples for analysis of physical and/or chemical properties taken from the test mixture prepared for the additional group of six animals treated with $A_{12-14}EO_7$ (Alfonic 1412-7) do not need to be analyzed. Modify the last sentence in the second paragraph of this section with the following underlined addition:

> All other procedures in the protocol will be followed, with the exception that the analysis of the test mixture samples for physical and chemical properties by the individuals listed in Section 6. B. (1). will not be required.

PROTOCOL AMENDMENT APPROVAL

Richard I. Sedlak, PhD
Sponsor's Representative
The Soap and Detergent Association

Date 8/31/95

Steven M. Glaza
Study Director
Acute Toxicology
Hazleton Wisconsin, Inc.

Date 4-18-95

Representative
Quality Assurance Unit
Hazleton Wisconsin, Inc.

Date 4.18.95

(6310-105.Aml.dsk3)

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PROTOCOL TP6360.F

Primary Eye Irritation Study of Nonionic Surfactants in Rabbits
(Low Volume Procedure)

HWI 6310-105

Sponsor	Contractor
The Soap and Detergent Association 475 Park Avenue New York , NY 10016	Hazleton Wisconsin, Inc. 3301 Kinsman Boulevard Madison, WI 53704
Sponsor's Representative	Study Director
Richard I. Sedlak, PhD	Steven M. Glaza

Amendment No. 2

This amendment modifies the following portion of the protocol:

Effective March 16, 1995

1. Page 6, 6. Experimental Design; B. Dose Administration; (1) Test Material Preparation. After discussion with the Sponsor it was determined that additional testing of those test mixtures that had pH values outside the 6-9 range would not be required due to the low level of irritation observed with the initial test mixtures. The fifth sentence of this section is thus to be deleted.

2. Page 2, Study Location. The testing of the additional group of animals with the 0.2 M Alfonic 1412-7 test mixture will require the animals to be treated at the Hazleton Wisconsin facility located at 3301 Kinsman Boulevard, Madison, Wisconsin. Add the following to this section of the protocol:

   Additional Animals

   Hazleton Wisconsin, Inc.
   3301 Kinsman Boulevard
   Madison, WI 53707