HAZLETON LABORATORIES AMERICA INC
Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

FINAL REPORT

ROGER PERKINS
MINNESOTA MINING & MANUFACTURING COMPANY
TOXICOLOGY SERVICES
ST. PAUL, MN 55101

SAMPLE NUMBER: 70100355
SAMPLE ENTERED: 01/07/87
REPORT PRINTED: 01/27/87

SAMPLE: 1-4016

PURCHASE ORDER NUMBER: T837389-410 712

ENCLOSED: PRIMARY EYE IRRITATION STUDY IN RABBITS - METHOD, SUMMARY
RAW DATA APPENDIX

SIGNED: Steven M. Glaza ..... 1-28-87 .....
STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY
DATE

BY AND FOR HAZLETON LABORATORIES AMERICA, INC.

RAW DATA FOR THIS STUDY ARE KEPT ON FILE AT HAZLETON LABORATORIES AMERICA, INC., MADISON, WISCONSIN.

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SAMPLE NUMBER: 70100355

SAMPLE: T-4016

EYE IRRITATION

Objective: To determine the level of irritation produced following a single exposure of a test material to one eye of albino rabbits according to the U.S. Environmental Protection Agency's Guidelines for Testing Pesticides and Toxic Substances.

Test Material: T-4016
Physical Description: Thick brown liquid
Purity and Stability: Sponsor assumes responsibility for purity and stability determinations.

Test Animal: Young adult rabbits of the New Zealand White strain were procured, maintained individually in screen-bottom cages in temperature- and humidity-controlled quarters, provided access to water ad libitum and a measured amount of Purina High Fiber Rabbit Chow, and held for an acclimation period of at least 7 days.

Six acclimated animals, weighing from 2354 to 2514 g, were chosen at random for the test. The animals' eyes were examined within 24 hours prior to test material administration using sodium fluorescein dye procedures. Only those animals with no sign of ocular injury or irritation were used on the test. Test animals were identified by animal number and corresponding ear tag.

Reason for Species Selection: The New Zealand White albino rabbit is the animal of choice based upon its large orbit and nonpigmented iris.

Preparation of Test Material: The sample was dosed as received. The pH was not determined.

Treatment: Each rabbit received 0.1 ml of the liquid test material placed on the everted lower lid of one eye, with the contralateral eye serving as the untreated control. The upper and lower lids were gently held together for one second to prevent loss of material and then released. The eyes of the rabbits remained unflushed.

Reason for Route of Administration: Historically, the route of choice based on the method of Draize.

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SAMPLE NUMBER: 70100355

SAMPLE: T-4016

EYE IRRITATION (CONTINUED)

Observations: The treated eyes were observed for ocular irritation at 1, 24, 48, 72 and 96 hours after treatment.

At the 72-hour reading, sodium fluorescein was used to aid in revealing possible corneal injury. Irritation was graded and scored according to the Draize technique.

Animals were weighed just prior to test material administration.

Pathology: At study termination all animals were euthanatized and discarded.

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SAMPLE NUMBER: 70100355

SAMPLE: T-4016

EYE IRRITATION (CONTINUED)

SUMMARY

Test Animal: Albino rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 12/16/86

Date Test Started: 01/08/87 Date Test Completed: 01/12/87

PRIMARY EYE IRRITATION SCORES*

OBSERVATION PERIOD	6 Rabbit Mean 0.1 ml (Unwashed)
1 Hour:	22.7
24 Hours:	25.7
48 Hours:	12.5
72 Hours:	5.7
96 Hours:	0.0

* The Primary Eye Irritation Score is the total eye irritation score for all the animals divided by the number of animals (6) at each observation period.

SAMPLE NUMBER: 70100355

SAMPLE: T-4016

EYE IRRITATION (CONTINUED)

Table 1
Individual Eye Irritation Scores

Animal Number	Observation Period	Cornea A	Cornea B	Score AXBX5	Iris A	Score A X 5	Conjunctivae A	Conjunctivae B	Conjunctivae C	Score (A+B+C)2
F16208	1 Hour	0	0	0	1	5	3	4	3	20
	24 Hours	1	3	15	1	5	2	3	2	14
	48 Hours	1	1	5	0	0	2	2	0	8
	72 Hours	0	0	0	0	0	2	1	0	6
	96 Hours	0	0	0	0	0	0	0	0	0
F16219	1 Hour	0	0	0	1	5	3	4	2	18
	24 Hours	1	4	20	1	5	3	4	3	20
	48 Hours	1	1	5	1	5	3	3	1	14
	72 Hours	0	0	0	0	0	2	2	1	10
	96 Hours	0	0	0	0	0	0	0	0	0
F16220	1 Hour	0	0	0	1	5	3	3	2	16
	24 Hours	1	1	5	1	5	3	2	1	12
	48 Hours	0	0	0	1	5	2	1	1	8
	72 Hours	0	0	0	0	0	2	0	1	6
	96 Hours	0	0	0	0	0	0	0	0	0
F16221	1 Hour	0	0	0	1	5	3	4	2	18
	24 Hours	1	1	5	1	5	3	4	1	16
	48 Hours	0	0	0	1	5	2	1	1	8
	72 Hours	0	0	0	0	0	2	1	0	6
	96 Hours	0	0	0	0	0	0	0	0	0
F16222	1 Hour	0	0	0	1	5	3	4	2	18
	24 Hours	0	0	0	0	0	3	2	2	14
	48 Hours	0	0	0	0	0	2	1	1	8
	72 Hours	0	0	0	0	0	1	1	0	4
	96 Hours	0	0	0	0	0	0	0	0	0
F16223	1 Hour	0	0	0	1	5	3	3	2	16
	24 Hours	0	0	0	1	5	2	2	0	8
	48 Hours	0	0	0	0	0	1	1	0	4
	72 Hours	0	0	0	0	0	1	0	0	2
	96 Hours	0	0	0	0	0	0	0	0	0

SAMPLE NUMBER: 70100355

SAMPLE: T-4016

EYE IRRITATION (CONTINUED)

Table 2
Sodium Fluorescein Examination

Animal Number	Observation Period Pre-initiation	72 Hours
F16208	NEG	NEG
F16219	NEG	NEG
F16220	NEG	NEG
F16221	NEG	NEG
F16222	NEG	NEG
F16223	NEG	NEG

NEG = No stain retention
POS = Positive stain retention (area of cornea involved).

Comments:

No pain response (vocalization) was elicited from any animal following instillation of the test material.

Blanching of the conjunctivae was seen in all six animals at 1 and 24 hours, in three animals at 48 hours and in two animals at 72 hours.

Corneal epithelial peeling was exhibited by four animals at 24 hours and by two animals at 48 hours.

References:

1. Hitch, R.K., "Primary Eye Irritation Study," Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, U.S. Environmental Protection Agency Office of Pesticide and Toxic Substances Series 81-4, pp. 51-55 (November 1982).

2. Draize, J.H., "Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity." Association of Food and Drug Officials of the U.S., pp. 46-59 (1975).

3. 21 CFR 58.

4. DHHS Publication No. (NIH 85-23 1985) Guide for the Care and Use of Laboratory Animals.

70100355

PROTOCOL - APPENDIX I

(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 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 conjunctivae only)

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 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 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.

* Starred figures indicate positive effect.

PRIMARY EYE IRRITATION TEST

HLA No. 70100355

Initial Sodium Fluorescein Exam and Animal Body Weights

Test Material: T-4016

Physical Description: Thick Brown liquid

pH result: U with NA pH meter No. NA

Dose: 0.1 ml

Review of folder preparation by: MN Date: 1-8-87

Room Number: 161

Date Animals Received: 10-16-86

Species: RABBIT

Strain: NEW ZEALAND WHITE

Source: HAZLETON RESEARCH PRODUCTS, INC.

All animals appeared normal just prior to dosing.

Technician: BB Date: 1-8-87

Animal No.	Sex	Initial SFe	Vocalization Following Dosing	Animal Body Weights (g) Initiation	Day 7	Day 14	Day 21
FL 60208	♂	Neg	N	2499			
60219	♂	Neg	N	2418			
60220	♂	Neg	N	2514			
60221	♀	Neg	N	2478			
60222	♀	Neg	N	2354			
60223	♀	Neg	N	2423			
TECHNICIAN	BB	Lmc	BB	BB			
DATE 1987	1-8	1/7	1-8	1-8			
SCALE USED			Ktron	15019			

✓ Dosed in the conjunctival sac. The upper and lower lids were gently held together for one second.

NA Dosed directly on the cornea. The eyelids were released immediately without forced blinking or manipulation.

* - Sodium Fluorescein Examination.
NEG - Negative
POS - Positive
NA - Not Applicable
U - Unable to determine pH
Y - Yes
N - No
M - Male
F - Female

Time of Dosing: 9:15Am

Tech: BB Date: 1-8-87

Time of First Observation: 10:15 am

Tech: BB Date: 1-8-87

Reviewed by: MN Date: 1-21-87

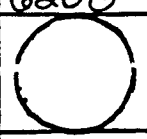
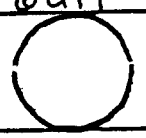
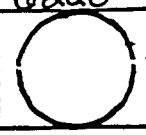
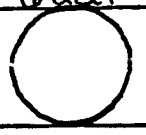
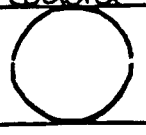
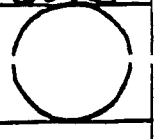
Primary Eye Irritation Test Observations

Test Material: T-4016 HLA No. 70100355

Test Eye: Right Group: NA

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 1 hour

Animal No. FI-	6208	6219	6220	6221	6222	6223
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	0	0	0	0	0	0
Area	0	0	0	0	0	0
Iris	1 I	1 I	1 I	1 I	1 I	1 I
Conjunctivae - Redness	3 B	3 B	3 B	3 B	3 B	3 B
Chemosis	4	4	3	4	4	3
Discharge	3 D	2 D	2 D	2 D	2 D	2 D
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician Sam

Date 1-8-87

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: mm Date: 1-21-87 Eye Irritation Score: 22.7 ✓ mm 1-22-87
slu 1-8-87

(4253A)

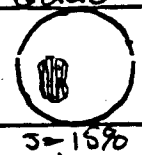
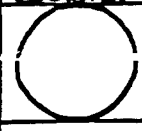
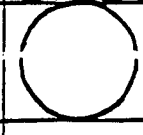
Primary Eye Irritation Test Observations

Test Material: T-4016 HLA No. 70100355

Test Eye: Right Group: NA

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 24 hours

Animal No. FI-	6208	6219	6220	6221	6222	6223
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	J=60% / 1	J=85% / 1	J=15% / 1	J=15% / 1	0	0
Area	3	4	1	1	0	0
Iris	1I	1I	1I	1I	0	1I
Conjunctivae - Redness	2B	3B	3B	3B	3B	2B
Chemosis	3	4	2	4	2	2
Discharge	2D	3D	1C	1D	2D	0
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician NP

Date 1-9-87

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MM Date: 1-21-87 Eye Irritation Score: 25.7

slw 1-9-87
MM 1-21-87

(4253A)

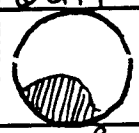
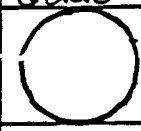
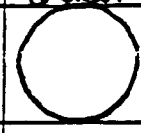
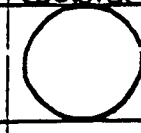
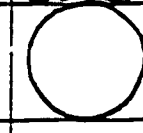
Primary Eye Irritation Test Observations

Test Material: T-4016 HLA No. 70100355

Test Eye: Right Group: NA

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 48 hours

Animal No. FI-	6208	6219	6220	6221	6222	6223
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	J=5% 1	J=20% 1	0	0	0	0
Area	1	1	0	0	0	0
Iris	0	1I	1I	1I	0	0
Conjunctivae - Redness	2B	3B	2	2B	2	1
Chemosis	2	3	1	1	1	1
Discharge	0	1C	1C	1D	1D	0
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician IP

Date 1-10-87

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MH Date: 1-21-87 Eye Irritation Score: 12.5

Slw 1-11-87
MH 1-21-87

(4253A)

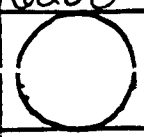
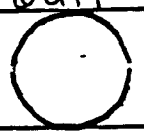
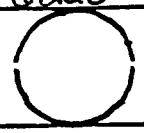
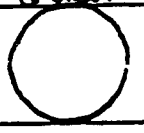
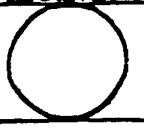
Primary Eye Irritation Test Observations

Test Material: T-4016 HLA No. 70100355

Test Eye: Right Group: NA

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 72 hours

Animal No. FI-	6208	6219	6220	6221	6222	6223
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	0	0	0	0	0	0
Area	0	0	0	0	0	0
Iris	0	0	0	0	0	0
Conjunctivae - Redness	2 B	2	2	2 B	1	1
Chemosis	1	2	0	1	1	0
Discharge	0	1 C	1 C	0	0	0
Sodium Fluorescein Exam	Neg	Neg	Neg	Neg	Neg	Neg

Technician MD

Date 1-11-87

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: MD Date: 1-21-87 Eye Irritation Score: 5.7

(4253A)

1-11-87
MD 1-21-87

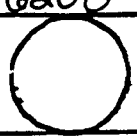
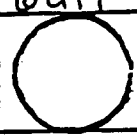
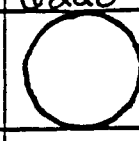
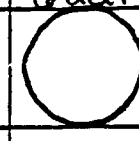
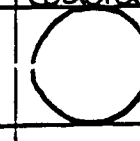
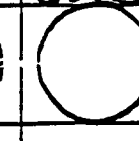
Primary Eye Irritation Test Observations

Test Material: T-4016 HLA No. 70100355

Test Eye: Right Group: NA

NA Washed: NA seconds following instillation of test material, the test eye was washed with NA mL of lukewarm tap water for NA seconds. ✓ Unwashed

OBSERVATION PERIOD: 96 hours

Animal No. FI-	6208	6219	6220	6221	6222	6223
Location of Corneal Lesions Tail ------ Head						
Cornea - Opacity	0	0	0	0	0	0
Area	0	0	0	0	0	0
Iris	0	0	0	0	0	0
Conjunctivae - Redness	0	0	0	0	0	0
Chemosis	0	0	0	0	0	0
Discharge	0	0	0	0	0	0
Sodium Fluorescein Exam	NA	NA	NA	NA	NA	NA

Technician Sam

Date 1-12-87

NA - Not Applicable
A - Petite hemorrhage
B - Blanching
C - Clear discharge
D - Purulent discharge
E - Hair loss around the eye
F - Necrotic Areas
G - Unable to visualize due to severe swelling
H - No reaction to light
I - Injected
J - Corneal epithelial damage, peeling
K - Corneal epithelial damage, piling
L - Corneal epithelial damage, pitting
M - Hypopyon
N - Corneal neovascularization
P - Pannus
R - Unable to visualize due to severe opacity
S - Granulation scar tissue
POS - Positive stain retention
NEG - Negative stain retention

Reviewed By: mm Date: 1-21-87 Eye Irritation Score: 0.0

(4253A)

Slw HZ-87
✓ mm 1-21-87