

**HAZLETON**

LABORATORIES AMERICA, INC.

Chemical & BioMedical Sciences Division

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FINAL REPORT



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

SAMPLE NUMBER: 70905395

SAMPLE ENTERED: 09/23/87

REPORT PRINTED: 01/17/88

SAMPLE: T-4101

PURCHASE ORDER NUMBER: T837389-410 753

ENCLOSED: PRIMARY EYE IRRITATION/CORROSION STUDY IN RABBITS
(OECD GUIDELINES)

- o Key Personnel
- o Method
- o Summary
- o References
- o Raw Data Appendix

SIGNED:

STEVEN M. GLAZA
STUDY DIRECTOR
ACUTE TOXICOLOGY

.....1-18-88.....
DATE

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KEY PERSONNEL

Acute ToxicologySteven M. Glaza
Study DirectorCalvin L. Horton
Group Leader
Support ServicesSharen L. Howery
Report CoordinatorQuality AssuranceDebra Curley Arndt
ManagerLaboratory Animal VeterinarianCindy J. Cary, DVM
Diplomate, ACLAM



SAMPLE NUMBER: 70905395

SAMPLE: T-4101

OECD EYE IRRITATION

Objective: To determine the level of ocular irritation produced following a single exposure of a test substance to one eye of albino rabbits according to the Organization for Economic Cooperation and Development's Guidelines for Testing Chemicals.

Test Material: T-4101

Physical Description: Clear yellow liquid
Purity and Stability: Sponsor assumes responsibility for purity and stability determinations.
Storage and Retention: The test material was stored at room temperature. Any unused material will be discarded according to HLA Standard Operating Procedure.
Safety Precautions: Normal handling procedures were used according to HLA Standard Operating Procedure.

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. Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals". If variations from the prescribed environmental conditions existed, they were documented and considered to have no effect on the study outcome. No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

Three acclimated animals, weighing from 2069 to 2146 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. 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 and Administration of Test Material: The sample was dosed as received. The pH was determined to be 12.9.

Treatment: Each rabbit received 0.1 ml of the liquid test material placed into 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.

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OECD EYE IRRITATION

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

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

Statistical Methods: Other than average eye irritation scores, no other statistical method was performed.

Location of Raw Data and Final Report: The raw data and a copy of the final report will be retained in the archives of HLA.

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OECD EYE IRRITATION

(CONTINUED)

SUMMARY

Test Animal: Albino rabbits - New Zealand White
Source: Hazleton Research Products, Inc., Denver PA
Date Animals Received: 10/20/87

Date Test Started: 11/06/87

Date Test Completed: 11/09/87

PRIMARY EYE IRRITATION SCORES*

OBSERVATION PERIOD	3 Rabbit Mean
	0.1 ml (Unwashed)
1 Hour:	6.3
24 Hours:	6.7
48 Hours:	0.7
72 Hours:	0.0

* The Primary Eye Irritation Score is the total eye irritation score for all the animals divided by the number of animals (3) at each observation period.



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OECD EYE IRRITATION

(CONTINUED)

Table 1
Individual Eye Irritation Scores

Animal Number	Observation Period	Cornea		Score A X B X 5	Iris		Score A X 5	Conjunctivae			Score (A+B+C) 2
		A	B		A			A	B	C	
F20334	1 Hour	0	0	0	0		0	1	1	0	4
	24 Hours	1	1	5	0		0	1	1	0	4
	48 Hours	0	0	0	0		0	1	0	0	2
	72 Hours	0	0	0	0		0	0	0	0	0
F20326	1 Hour	0	0	0	0		0	1	1	0	4
	24 Hours	1	1	5	0		0	1	1	0	4
	48 Hours	0	0	0	0		0	0	0	0	0
	72 Hours	0	0	0	0		0	0	0	0	0
F20329	1 Hour	0	0	0	1		5	2	1	0	6
	24 Hours	0	0	0	0		0	1	0	0	2
	48 Hours	0	0	0	0		0	0	0	0	0
	72 Hours	0	0	0	0		0	0	0	0	0

Cornea

A = Degree of opacity
B = Area of involvement

Conjunctivae

A = Redness
B = Chemosis
C = DischargeTable 2
Sodium Fluorescein Examination

Animal Number	Observation Period	
	Pre-initiation	72 Hours
F20334	NEG	NEG
F20326	NEG	NEG
F20329	NEG	NEG

NEG = No stain retention

POS = Positive stain retention (area of cornea involved).



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OECD EYE IRRITATION

(CONTINUED)

Comments:

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

Corneal epithelial peeling was observed in two animals at 24 hours.

References:

1. Organisation for Economic Cooperation and Development's Guidelines for Testing of Chemicals, Section 405, Acute Eye Irritation/Corrosion, adopted May 12, 1981.
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 United States, pp. 46-59 (1975).
3. OECD's Principles of Good Laboratory Practice, Annex 2, C(81)30 (Final).
4. NIH Publication No. 86-23 (Revised 1985).

HLA No. 70905395

**Personnel Signature Sheet
Acute Toxicology**

<u>Name</u>	<u>Job Title</u>	<u>Signature</u>	<u>Initials</u>
Becky Beckwith	Sr. Lab. Animal Asst.	<u>Becky Beckwith</u>	<u>BB</u>
Steve Beloungy	Lab. Animal Asst.	<u>Steve Beloungy</u>	<u>SB</u>
Ken Bridges	Sr. Lab. Animal Asst.	<u>Ken Bridges</u>	<u>KB</u>
Pat Crary	Sr. Lab. Animal Asst.	<u>Pat Crary</u>	<u>PC</u>
Shane Eith	Lab Animal Asst.	<u>Shane Eith</u>	<u>SE</u>
Shawn Frazier	Lab Animal Asst.	<u>Shawn Frazier</u>	<u>SF</u>
Steven M. Glaza	Group Leader	<u>Steven M. Glaza</u>	<u>SG</u>
Molly Hahn	Admin. Clerk	<u>Molly Hahn</u>	<u>MH</u>
Kevin Hamilton	Lab. Animal Asst.	<u>Kevin Hamilton</u>	<u>KH</u>
Jeff Hicks	Sr. Lab. Animal Asst.	<u>Jeff Hicks</u>	<u>JH</u>
Calvin Horton	Group Leader	<u>Calvin Horton</u>	<u>CH</u>
Sharon L. Howery	Administrative Asst.	<u>Sharon L. Howery</u>	<u>SH</u>
Gregory Johnson	Lab. Animal Tech.	<u>Gregory Johnson</u>	<u>GJ</u>
Paul Krebs	Lab. Animal Asst.	<u>Paul Krebs</u>	<u>P.K.</u>
Wayne Madison	Section Supervisor	<u>Wayne A. Madison</u>	<u>WAM</u>
Scott McConnell	Lab. Animal Tech.	<u>Scott McConnell</u>	<u>SMC</u>
Shelley McConnell	Lab. Animal Tech.	<u>Shelley McConnell</u>	<u>SMC</u>
Dawn Conant	Sr. Lab. Animal Asst.	<u>Dawn Conant</u>	<u>DC</u>
Don Navis	Lab. Animal Asst.	<u>Don Navis</u>	<u>DN</u>
Robin Olson	Lab. Animal Asst.	<u>Robin Olson</u>	<u>RO</u>
Patricia Padgham	Team Leader	<u>Patricia Padgham</u>	<u>TP</u>
Joseph J. Daum	Lab. Animal Asst.	<u>Joseph J. Daum</u>	<u>JD</u>
Michael Patzka	Lab. Animal Asst.	<u>Michael Patzka</u>	<u>MP</u>
John Paulson	Sr. Lab. Animal Asst.	<u>John Paulson</u>	<u>JP</u>
Jane Polnow	Lab. Animal Tech.	<u>Jane Polnow</u>	<u>JP</u>
Dennis B. Steiner	Lab. Animal Tech.	<u>Dennis B. Steiner</u>	<u>DS</u>
Michael Thesing	Lab. Animal Asst.	<u>Michael Thesing</u>	<u>MT</u>
Paula G. Vangen	Administrative Asst.	<u>Paula G. Vangen</u>	<u>PV</u>
Albert Olson	Manpower	<u>Albert J. Olson</u>	<u>AO</u>
Jim Jirschele	LTE	<u>Jim Jirschele</u>	<u>JJ</u>
Ben Haley	Sr. Lab Animal Asst.	<u>Ben Haley</u>	<u>BH</u>
Eileen M. McConnell	Admin. Clerk	<u>Eileen M. McConnell</u>	<u>EMC</u>
Annette R. Turner	Manpower	<u>Annette R. Turner</u>	<u>AT</u>

HLA: 10905395

BODY WEIGHT/DOSE RECORD

Study title: Primary Eye Irritation
Test material: T-4101
Physical description: Clear, yellow liquid
pH result: 12.9 with Hanna Meter No. 6890
Dose: 0.1 ml/0.1 g (0.1 ml equivalent)
Individually drawn up with a 1-ml disposable syringe/0.1 ml each dose
Date animals received: 10-20-87
Species/strain: Rabbit/New Zealand White
Source: Hazleton Research Products, Inc.
Room No: 161-E
Review of folder preparation by: NC Date: 11-6-87

Pain Response

Animal No.	Group	Sex	Initial SF*	Following Dosing		Animal Body Weights (g)		All animals appeared normal just prior to dosing.
				Initiation	Day 7	Day 14	Day 21	
E2 0324	1	♀	NEG	N	206.9			Technician: KB Date: 11/6/87
E2 0326	1	♀	NEG	N	214.6			* - Sodium fluorescein examination. NEG - Negative. POS - Positive. NA - Not applicable. U - Unable to determine pH. V - Vocalization. E - Excessive pawing of treated eye. N - None
E2 0328	1	♀	NEG	N	210.4			NA Dosed directly on the cornea. The eyelids were released immediately without forced blinking or manipulation.
								✓ Dosed in the conjunctival sac. The upper and lower lids were gently held together for one second.
Technician				KB/gw.	KB			
Date	1987/11-5		11-5	11/6	11/6			Time of dosing: 2:45 PM Technician: KB/gw Date: 11/6/87
Scale Used				Kron	15019			Time of first observation: 3:45 PM Technician: N Date: 11/6/87

Surviving animals designated for sacrifice and discard. Technician NC Date 11-10-87

Reviewed by: NC Date: 12-8-87
(00261/vmt)

HLA: 70905395

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
 Test material: T-410
 Test eye: Right

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.	F2-0334	F2-0326	F2-0329			
Location of corneal lesions						
Tail ----- Head						
Cornea - Opacity	0	0	0			
Area	0	0	0			
Iris	0	0	I ¹			
Conjunctivae - Redness	1	1	2			
Chemosis	1	1	1			
Discharge	0	0	0			
Sodium fluorescein exam	NA	NA	NA			

Technician: KB / MP
 Date: 11/1/87

EYE IRRITATION SCORE: 6.3
 Calculated By: SP Date 11-8-87
 Verified By: MP Date 12-8-87

NA - Not applicable.
 A - Petite hemorrhaging.
 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: MP Date: 12-8-87
 (00261/vmt)

HLA: 70905395

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
 Test material: T-4101
 Test eye: Right

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

Observation Period: 24 hours

Animal No.	F2-0334	F2-0326	F2-0329			
Location of corneal lesions						
Tail ----- Head						
Cornea - Opacity	<u>S=15%</u>	<u>S=15%</u>	<u>0</u>			
Area	<u>1</u>	<u>1</u>	<u>0</u>			
Iris	<u>0</u>	<u>0</u>	<u>0</u>			
Conjunctivae - Redness	<u>1</u>	<u>1</u>	<u>1</u>			
Chemosis	<u>1</u>	<u>1</u>	<u>0</u>			
Discharge	<u>BLD</u>	<u>0</u>	<u>0</u>			
Sodium fluorescein exam	<u>NA</u>	<u>NA</u>	<u>NA</u>			

(Correct error 11-7-87)

Technician: JDDate: 11-7-87EYE IRRITATION SCORE: 6.7Calculated By: JD Date 11-9-87Verified By: JD Date 12-8-87

NA - Not applicable.
 A - Petite hemorrhaging.
 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.

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 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: JDDate: 12-8-87

(00261/vmt)

HLA: 70905395

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
 Test material: T-4101
 Test eye: Right

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

Observation Period: 48 hours

Animal No.	F2-0334	F2-0326	F2-0328			
Location of corneal lesions						
Tail ----- Head						
Cornea - Opacity						
Area						
Iris						
Conjunctivae - Redness						
Chemosis						
Discharge						
Sodium fluorescein exam	NA	NA	NA			

Technician: Sam
 Date: 11-8-87

EYE IRRITATION SCORE: 0.7
 Calculated By: SPH Date 11-9-87
 Verified By: NP Date 12-8-87

NA - Not applicable.
 A - Petite hemorrhaging.
 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.

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 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: NP Date: 12-8-87

(00261/vmt)

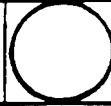
HLA: 70905395

PRIMARY EYE IRRITATION TEST OBSERVATIONS

Group: 1
 Test material: T-410
 Test eye: Right

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

Observation Period: 72 hours

Animal No.	<u>F2-0334</u>	<u>F2-0326</u>	<u>F2-0328</u>			
Location of corneal lesions						
Tail ----- Head						
Cornea - Opacity						
Area						
Iris						
Conjunctivae - Redness						
Chemosis						
Discharge						
Sodium fluorescein exam	<u>Neg</u>	<u>Neg</u>	<u>Neg</u>			

EYE IRRITATION SCORE: 0.0
 Calculated By: SPH Date 11-9-87
 Verified By: MP Date 12-8-87

Technician: SPH
 Date: 11-9-87

NA - Not applicable.
 A - Petite hemorrhaging.
 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.
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 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: MP Date: 12-8-87

(00261/vmt)

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 d 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 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 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.

* Indicates positive effect. (FHSA Interpretation)

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

(00261/vmt)