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Elf Atochem North America, Inc.

2000 Market Street
Philadelphia, PA 19103-3222
Tel.: 215.419.7000

8EHQ-1195-13508

ORIGINAL

October 4, 1995

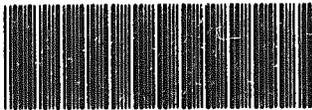
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Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460
Attn: 8(e) Submission



89960000005

Contains No CBI

Dear Sir/Madam:

Elf Atochem North America, Inc. (Elf Atochem) has received the final report of a primary eye irritation study in rabbits and is submitting it to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e). Preliminary results from this study were submitted to the Agency by Elf Atochem on September 12, 1995. This study provides information on 2,5-dimethylhexane-2,5-dihydroperoxide (CAS No. 30254-88-5) and does not involve effects in humans. The title of the study is *A Primary Eye Irritation Study in Rabbits with Luperox 2,5-2,5*.

Nothing in this letter or the enclosed study report is considered confidential business information of Elf Atochem.

The results of this study showed the test material to be corrosive to rabbit eyes. In light of the corrosive nature of several other organic hydroperoxides towards the eye, and the currently recommended hygiene practices in our material safety data sheet for the material, it is the opinion of Elf Atochem that the effects noted in this study do not, therefore, necessarily support a conclusion of substantial health risk, but are being submitted in response to the EPA 8(e) reporting standards.

Best Regards,

C.H. Farr, PhD, DABT
Manager, Product Stewardship
and Toxicology

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95 NOV - 6 PM 7:50

Springborn Laboratories, Inc.

Life Sciences Division

640 N. Elizabeth Street • Spencerville, Ohio 45887 • (419) 647-4196 • Telex 4436041 • Facsimile 419-647-6560

**A PRIMARY EYE IRRITATION STUDY
IN RABBITS WITH LUPEROX 2,5-2,5**

FINAL REPORT

Author

Deborah A. Douds, M.S.

Study Completed on

September 18, 1995

Performing Laboratory

Springborn Laboratories, Inc. (SLS)
Life Sciences Division
640 North Elizabeth Street
Spencerville, OH 45887

SLS Study No.

3255.58

Contains No CBI

Submitted to

Elf Atochem North America, Inc.
2000 Market Street
Philadelphia, PA 19103

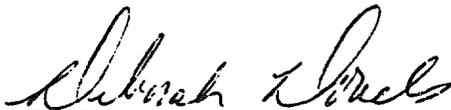
Page 1 of 37

SLS Study No. 3255.58

(2)

COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Regulations as described by the FDA (21 CFR Part 58), the EPA (40 CFR Part 792) and the OECD Annex 2 C(81)30.



Deborah A. Douds, M.S.
Study Director/Author
Springborn Laboratories, Inc.

Date 9/18/95

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the study director in accordance with SLS's Standard Operating Procedures as follows:

<u>Phase</u>	<u>Date</u>
Animal Receipt	06/19/95
Dose Preparation	06/29/95
Data Audit	08/28/95
Draft Report Review	08/30/95
Final Report Review	09/18/95
Reports to Study Director and Management	07/11/95, 08/30/95, 09/18/95

This study was conducted in compliance with the Good Laboratory Practice Regulations as described by the FDA (21 CFR Part 58), the EPA (40 CFR Part 792) and the OECD Annex 2 C(81)30.

Amy L. Sizemore
Amy L. Sizemore, B.S.
Quality Assurance Auditor I

Date 0-18-95

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SUMMARY

The potential eye irritant and/or corrosive effects of Luperox 2,5-2,5 were evaluated on New Zealand White rabbits. Each of six rabbits received a 0.0410 g dose (0.1 mL weight equivalent) of the test article in the conjunctival sac of the right eye. The contralateral eye of each animal remained untreated and served as a control. Test and control eyes were examined for signs of irritation for up to 14 days following dosing.

Due to the severity of swelling observed at the 1 hour scoring interval, 5/6 test eyes could only be scored for swelling and discharge. However, exposure to the test article produced corneal opacity in 6/6 test eyes by the 24 hour scoring interval which was confirmed by positive fluorescein dye retention. The corneal opacity could not be scored for density in 1/6 or area in 2/6 test eyes on study day 14 due to severe vascularization and apparent scar tissue growth. The corneal opacity did not resolve in any of the remaining test eyes on day 14. Iritis was noted in 6/6 test eyes at the 24 hour scoring interval. The iridal irritation either persisted or progressed in 3/6 test eyes throughout the test period. Iridal irritation could not be determined on day 14 due to the opacity density for 2/6 test eyes. However, one of the animals had cleared previously for iritis on study day 7. Conjunctivitis (redness, swelling and discharge) was noted in 6/6 test eyes by the 24 hour scoring interval. The conjunctival irritation did not resolve in any test eyes by study day 14. Additional ocular findings of sloughing of the corneal epithelium, vascularization, opaque areas within the interior chamber, corneal edema and head tilt were observed in 1/6, 6/6, 3/6, 6/6 and 2/6, respectively. Due to the severity of the reactions, the study was terminated on study day 14.

Under the conditions of this test, Luperox 2,5-2,5 is considered to be corrosive to the ocular tissue of the rabbit.

I. INTRODUCTION

This study was performed to assess the irritant and/or corrosive effects of Luperox 2,5-2,5 in New Zealand White rabbits when administered by a single ocular dose. This study is intended to provide information on the potential health hazards of the test article with respect to ocular exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed at Springborn Laboratories, Inc., 640 N. Elizabeth Street, Spencerville, Ohio.

II. MATERIALS AND METHODS

Study Dates

Study Initiation: June 13, 1995
Experimental Initiation: June 29, 1995
Experimental Completion: July 13, 1995

Protocol

The study protocol is presented in Appendix A.

Test Article

Sponsor I.D.: Luperox 2,5-2,5
Lot No.: 8258511503
Springborn I.D.: S95.006.3255
Receipt Date: May 15, 1995
Physical Description: White granules
Storage Conditions: Room temperature
Expiration Date: October 31, 1995

The Sponsor is responsible for any necessary evaluations related to chemical composition, purity, strength, stability and other data required by 21 CFR Part 58.105 and 40 CFR Part 792.105.

Test Article Preparation

The test article was ground in a mortar with a pestle and passed through a No. 40 mesh sieve prior to dose administration. The weight equivalent of the test article (0.1 mL) was determined to be 0.0410 g.

Animals and Animal Husbandry

Description: Adult, New Zealand White rabbits were received at SLS from Myrtle's Rabbitry, Thompson Station, TN.

Method of Identification: Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage.

Housing: The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

Environment: The animal room temperature and relative humidity ranges were 69-71° F and 46-70%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle. There were 10-15 air changes in the animal room per hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

Food: Purina Certified Rabbit Chow #5322 was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLS.

Water: Municipal tap water treated by reverse osmosis was available to the animals ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants was conducted by SLS and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study.

Acclimation: Upon receipt, animals were examined, identified with plastic ear tags and then acclimated for a minimum of five days.

Animal Selection: The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant.

III. EXPERIMENTAL PROCEDURES

Preliminary Examination: Prior to dosing on day 0, both eyes of each animal provisionally selected for test use were examined macroscopically for ocular irritation with the aid of an auxiliary light source. In addition, the corneal surface was examined using fluorescein sodium dye. One drop of fluorescein/physiological saline was applied to the superior sclera of each eye. Following an approximate 15 second exposure, the eyes were thoroughly rinsed with physiological saline. The corneal surface was then examined for dye retention under a long-wave UV light source. Animals exhibiting ocular irritation, preexisting corneal injury or fluorescein dye retention (other than normal background retention) were not used on study. All animals found to be acceptable for test use were returned to their cages until dosing.

Dosing: A minimum of one hour after the preliminary ocular examination, the test article was instilled as follows:

Group	Concentration (%)	Amount Instilled	No. of Animals	
			Males	Females
No Rinse	100	0.0410 g	0	6

The test article was instilled into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eye. Following instillation, the eyelids were gently held together for approximately one second in order to limit test article loss and the animal was returned to its cage. The contralateral eye remained untreated to serve as a control.

Ocular Observations: The eyes were macroscopically examined with the aid of an auxiliary light source for signs of irritation at 1, 24, 48 and 72 hours and up to 14 days after dosing according to the Ocular Grading System presented in Protocol Appendix A which is based on Draize [2]. Following macroscopic observations at the 24 hour scoring interval, the fluorescein examination procedure was repeated on all test and control eyes and any residual test article was gently rinsed from the eye at this time (if possible) using 0.9% physiological saline. If fluorescein dye retention was noted at 24 hours, a fluorescein exam was conducted on the affected eyes at each subsequent interval until a negative response was obtained.

Clinical Observations: Any unusual observations and/or mortality were recorded. Mortality checks were performed twice daily, in the morning and afternoon.

Body Weights: Individual body weights were obtained for each animal prior to dosing on study day 0.

Gross Necropsy: Each animal was euthanized (intravenous injection of sodium pentobarbital) following its final observation interval. Gross necropsy examinations were not required for these animals.

Protocol Deviations

No protocol deviations occurred during this study.

IV. ANALYSIS OF DATA

The ocular irritation score for each parameter (i.e., corneal opacity x area, iritis and conjunctival redness + swelling + discharge) was multiplied by the appropriate factor (i.e., corneal injury x 5, iritis x 5, conjunctivitis x 2) and the totals added for each animal/interval. The group mean irritation score was then calculated for each scoring interval based on the number of animals initially dosed in each group. The data was analyzed and summarized based on the definitions presented below:

1. Non Irritant - Following instillation of the test article, none of the test eyes show a positive effect as defined in the Ocular Grading System in Protocol Appendix A.
2. Irritant - Following instillation of the test article, one or more test eyes exhibit a positive effect, but the changes are reversible.
3. Corrosive - One or more test eyes exhibit irreversible changes (ex., necrosis or ulceration) following instillation of the test article.

V. MAINTENANCE OF RAW DATA AND RECORDS

The remaining test article was returned to the Sponsor following completion of the in-life phase of the study. Where necessary, the Sponsor was responsible for maintaining a retention sample of the test article. All original paper data, the final report and magnetically encoded records were transferred to the SLS archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

VI. RESULTS**Ocular Observations:**

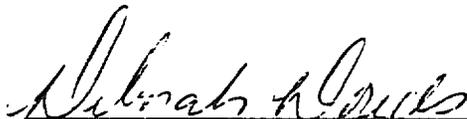
Individual Data: Table 1

Due to the severity of swelling observed at the 1 hour scoring interval, 5/6 test eyes could only be scored for swelling and discharge. However, exposure to the test article produced corneal opacity in 6/6 test eyes by the 24 hour scoring interval which was confirmed by positive fluorescein dye retention. The corneal opacity could not be scored for density in 1/6 or area in 2/6 test eyes on study day 14 due to severe vascularization and apparent scar tissue growth. The corneal opacity did not resolve in any of the remaining test eyes on day 14. Iritis was noted in 6/6 test eyes at the 24 hour scoring interval. The iridal irritation either persisted or progressed in 3/6 test eyes throughout the test period. Iridal irritation could not be determined on day 14 due to the opacity density for 2/6 test eyes. However, one of the animals had cleared previously for iritis on study day 7. Conjunctivitis (redness, swelling and discharge) was noted in 6/6 test eyes by the 24 hour scoring interval. The conjunctival irritation did not resolve in any test eyes by study day 14. Additional ocular findings of sloughing of the corneal epithelium, vascularization, opaque areas within the interior chamber, corneal edema and head tilt were observed in 1/6, 6/6, 3/6, 6/6 and 2/6, respectively. Due to the severity of the reactions, the study was terminated on study day 14.

No corneal opacity, iritis or conjunctivitis was observed in the control eyes.

VII. CONCLUSION

Under the conditions of this test, Luperox 2,5-2,5 is considered to be corrosive to the ocular tissue of the rabbit.



Deborah A. Douds, M.S.
Study Director

Date

9/8/95

VIII. REPORT REVIEW

Todd N. Merriman
Todd N. Merriman, B.S., LATG
Toxicologist

Date 9/18/95

Kimberly L. Bonnette
Kimberly L. Bonnette, M.S., LATG
Manager of Acute Toxicology

Date 9/18/95

IX. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 86-23, 1985.
2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

TABLE 1
A PRIMARY EYE IRRITATION STUDY IN RABBITS
INDIVIDUAL OCULAR IRRITATION SCORES

Animal No./Sex Body Weight (kg)	Scoring Interval	Cornea		Iris		Conjunctivae			Total	Test Eye*		Control Eye*		
		O	A	OxAv5	I	k5	R	S		D	(R+S+D)2	Fluorescein Examination	Secondary Findings	Fluorescein Examination
53180/F 2.558	1 Hour	a	a	-	a	-	a	4	2	-	-	-	-	
	24 Hours	2	4	40	1	5	3	3	2	16	61	FAO	TAE	[-]
	48 Hours	2	4	40	1	5	3	2	2	14	59	FAO	OA	
	72 Hours	2	4	40	1	5	3	2	2	14	59	FAO	OA	
	7 Days	2	4	40	0	0	2	1	1	8	48	FAO	OA, CE, VAS-2	
	10 Days	2	4	40	1	5	2	1	0	6	51	FAO	OA, CE, VAS-2	
53181/F 2.690	1 Hour	a	a	-	a	-	a	4	3	-	-	-	-	
24 Hours	2	4	40	1	5	3	3	2	16	61	FAO	TAE	[-]	
48 Hours	2	4	40	1	5	3	2	2	14	59	FAO	SCE, OA		
72 Hours	2	4	40	1	5	3	2	2	14	59	FAO	OA		
7 Days	2	4	40	2	10	3	2	2	14	64	FAO	VAS-2, RAC, YOD		
10 Days	2	4	40	2	10	2	2	1	10	60	FAO	VAS-3, RAC, YOD		
14 Days	c	c	-	-	c	-	2	2	1	10	-	c	VAS-4, CE	
53182/F 2.605	1 Hour	a	a	-	a	-	a	4	2	-	-	-	-	
	24 Hours	2	4	40	1	5	3	3	1	14	59	FAO	HT, TAE	[-]
	48 Hours	2	4	40	1	5	3	2	1	12	57	FAO	HT, OA	
	72 Hours	2	4	40	1	5	3	2	1	12	57	FAO	OA	
	7 Days	3	4	60	1	5	3	2	1	12	77	FAO	CE	
	10 Days	3	4	60	1	5	2	2	0	8	73	FAO	VAS-1, CE	
53183/F 2.627	1 Hour	a	a	-	a	-	a	4	2	-	-	-	-	
24 Hours	2	4	40	1	5	3	3	3	18	63	FAO	TAE	[-]	
48 Hours	2	4	40	1	5	3	2	2	14	59	FAO	TAE		
72 Hours	2	4	40	1	5	3	2	2	14	59	FAO	VAS-2, CE		
7 Days	2	4	40	1	5	2	2	1	10	55	FAO	VAS-1, CE		
10 Days	2	4	40	1	5	2	2	0	8	53	FAO	VAS-1, CE		
14 Days	2	4	40	1	5	2	2	0	8	53	FAO	VAS-2, CE		

* Due to degree of swelling, unable to grade parameter.

* Pupil has no reaction to light and iris has hemorrhaging on right and left side.

* Cannot score due to severe vascularization and apparent scar tissue growth.

* See Protocol Appendix A for definition of codes.

TABLE 1
 A PRIMARY EYE IRRITATION STUDY IN RABBITS
 INDIVIDUAL OCULAR IRRITATION SCORES

Animal No./Sex Body Weight (kg)	Swelling Interval	Cornea		Iris			Conjunctivae			Total	Test Eye*		Control Eye*			
		O	A	OxAv5	I	Ik5	R	S	D		(R+S+D)/2	Fluorescein Examination	Secondary Findings	Fluorescein Examination	Secondary Findings	
53184/F 2.441	1 Hour	a	a	-	a	-	a	4	3	-	-	-	-	-	-	-
	24 Hours	3	4	60	1	5	3	3	2	16	81	FAO	HT	FAO	[-]	[-]
	48 Hours	3	4	60	1	5	3	2	2	14	79	FAO		FAO		
	72 Hours	3	4	60	1	5	3	2	1	12	77	FAO		FAO		
	7 Days	3	4	60	0	0	2	1	0	6	66	FAO	VAS-1, CE	FAO		
	10 Days	2	4	40	0	0	2	1	0	6	46	FAO	VAS-1, CE, HL:ARE	FAO		
14 Days	2	4	40	1	5	2	1	0	6	51	FAO	VAS-1, CE, HL:ARE	FAO			
53185/F 2.604	1 Hour	a	a	-	1	5	a	4	3	-	-	-	-	-	-	-
	24 Hours	2	4	40	1	5	3	3	2	16	61	FAO	TAE	FAO	[-]	[-]
	48 Hours	2	4	40	1	5	3	2	2	14	59	FAO		FAO		
	72 Hours	2	4	40	1	5	3	2	2	14	59	FAO		FAO		
	7 Days	3	4	60	0	0	2	2	1	10	70	FAO	VAS-2, CE	FAO		
	10 Days	3	4	60	0	0	2	1	0	6	66	FAO	VAS-3, CE	FAO		
14 Days	3	4	60	b	-	2	1	0	6	-	-	FAO	VAS-2, CE	FAO		

* Due to degree of swelling, unable to grade parameter.
 † Unable to grade iris due to opacity density.

Additional codes: OA = Opaque area approximately 1 mm in length in interior chamber of the eye; YOD = An approximate 4 mm x 2 mm yellowish opaque deposit observed in front of the iris at the 4 to 8 o'clock position; YOI = An approximate 4 mm x 3 mm yellowish opaque deposit observed in front of the iris at the 6 to 9 o'clock position; HL:ARE = hairloss around right eye.

*See Protocol Appendix A for definition of codes.

Group Mean Irritation Scores

Group	Mean Irritation Score
1 Hour	a
24 Hours	64.33
48 Hours	62.00
72 Hours	61.67
7 Days	63.33
10 Days	58.17
14 Days	a

* A mean was not calculated due to inability to score parameters.

APPENDIX A

Protocol

A PRIMARY EYE IRRITATION STUDY IN RABBITS WITH LUPEROX 2,5-2,5

Springborn Study No. 3255.58
Protocol No.: ELFATO/EI-1
Issue Date: May, 1995

Springborn Laboratories, Inc. (SLS)
Life Sciences Division
640 North Elizabeth Street
Spencerville, Ohio 45887

Deborah A. Douds, M.S.
Study Director

For

Elf Atochem North America, Inc.
2000 Market Street
Philadelphia, PA 19103

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I. PURPOSE

To assess the irritant and/or corrosive effects of a test article in rabbits when administered by a single ocular dose. This study is intended to provide information on the potential health hazards of the test article with respect to ocular exposure. Data from this study may serve as a basis for classification and/or labeling of the test article.

II. SPONSOR

Elf Atochem North America, Inc.
2000 Market Street
Philadelphia, PA 19103

III. SPONSOR'S REPRESENTATIVE

Roy M. Bannister
Phone: (215) 419-5875
FAX: (215) 419-5800

IV. TESTING LOCATION

Springborn Laboratories, Inc.
Life Sciences Division
640 North Elizabeth Street
Spencerville, OH 45887
Phone: (419) 647-4196
FAX: (419) 647-6560

V. SPRINGBORN PERSONNEL RESPONSIBILITIES

Deborah A. Douds, M.S.
Study Director/Toxicologist

Kimberly L. Bonnette, M.S., LATG
Alternate Contact/Manager of Acute Toxicology

Dean E. Rodwell
President

Malcolm Blair, Ph.D.
Vice President/Director of Research

Rusty E. Rush, M.S., LAT, DABT
Associate Director of Toxicology

Todd N. Merriman, B.S., LATG
Toxicologist

Patricia K. Jenkins, A.A.S., LATG, RILAM
Acute Toxicology Supervisor

Pamela S. Smith, ALAT
Unit Leader

Jan K. Severt, B.S., ALAT
Acute Report Preparation Supervisor

Delores P. Knippen
Pharmacy Supervisor

Steven H. Magness, B.S., LATG
Supervisor of Gross & Fetal Pathology

Anita M. Bosau
Director of Regulatory Affairs

Raymond V. Karcher, B.A., LAT
Manager of Quality Assurance

J. Dale Thurman, D.V.M., M.S.
Diplomate, ACVP
Director of Pathology

VI. PROPOSED STUDY SCHEDULE

A. Experimental Initiation: June 1995

B. Experimental Completion: July 1995

C. Audited Report Date: Eight weeks following experimental completion

VII. TEST ARTICLE IDENTIFICATION**A. Sponsor's Identification**

Luperox 2,5-2,5

B. SLS Test Article Identification Number

S95.006.3255

C. Characteristics

The Sponsor is responsible for any necessary evaluations related to chemical composition, purity, strength, stability and other data required by 21 CFR Part 58.105 and 40 CFR Part 792.105. Any special storage conditions for the test article will be supplied by the Sponsor.

D. Handling Precautions

Safety data regarding the test article should be provided by the Sponsor (Material Safety Data Sheet or equivalent, if available). Technical personnel should review this information prior to handling the test article. In addition, any special handling precautions will be provided by the Sponsor/Study Director.

E. Method of Test Article Preparation

Liquids, gels and pastes are generally administered as received from the Sponsor. Solids and powders are generally ground and sieved prior to test use. This may be accomplished by grinding the material in a mortar and pestle and passing the material through a No. 40 mesh sieve. The weight of processed test article that occupies a volume of 0.1 ml will be determined by measuring a convenient volume (at least 2 ml) of the powder in a suitable volumetric container. The powder will be gently compacted by tapping the measuring container. The test article dose per eye will then be calculated (weight equivalent of 0.1 ml, not to exceed 100 mg). The test article will be prepared and/or dispensed fresh on the day of dosing. The method of preparation will be documented in the raw data and presented in the final report.

VIII. TEST SYSTEM

A. Justification of the Test System

1. The rabbit is the preferred species for primary eye irritation testing by various U.S. and International regulatory agencies.
2. The New Zealand White rabbit has been shown to be sensitive to the irritant/corrosive effects of a variety of drugs and chemicals. Therefore, this species and strain is a reasonable alternative to larger mammals for primary eye irritation testing of drugs and chemicals for human safety assessment.
3. The New Zealand White rabbit has been used extensively for eye irritation testing. Thus, data from this study may be compared and contrasted to other studies performed in New Zealand White rabbits.
4. Historical information concerning New Zealand White rabbits is available at SLS and in the published literature.
5. Healthy, outbred New Zealand White rabbits may be obtained from reliable, USDA approved and regulated suppliers.
6. The laboratory rabbit may be safely handled and manipulated by trained technical personnel.

B. Justification of the Route of Exposure and Number of Animals

1. Ocular administration of the test substance was selected since this is a potential route of human exposure.
2. Since New Zealand White rabbits have no pigment and have an easily accessible ocular area, substances may be accurately instilled and any resulting effects easily observed.
3. The number of animals used on this study will be consistent with the guidelines published by a number of U.S. and International regulatory agencies including EPA-FIFRA, EPA-TSCA, FDA, CPSC-FHSA, DOT, IMO, EEC, OECD, MAFF and MOHW.

C. Description1. Species

Rabbit

2. Strain

New Zealand White

3. Source

Myrtle's Rabbitry or another USDA approved supplier

4. Age and Body Weight Range

Adult, approximately 2.0 to 3.5 kg (prior to dosing on day 0)

5. Number and Sex

6 rabbit test (males and/or females)

D. Method of Identification

Plastic ear tags displaying unique identification numbers will be used to individually identify the animals. Cage cards displaying at least the study number, animal number, and sex will be affixed to each cage.

IX. ANIMAL HUSBANDRY AND EXPERIMENTAL DESIGNA. Animal Housing1. Housing

The animals will be housed individually in suspended stainless steel cages. All housing and care will be based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

2. Environment

The environmental conditions in the animal room will be controlled. The desired animal room temperature and relative humidity ranges are 61-73° F and 40-70%, respectively. Environmental control equipment will be monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers will be set to maintain a 12-hour light/12-hour dark cycle. There will be 10-15 air changes in the animal room per hour. The animal room temperature and relative humidity will be recorded a minimum of once daily.

3. Food

Purina Certified Rabbit Chow #5322 will be provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study will be recorded. The feed is analyzed by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may be present are not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These will be maintained by the testing laboratory.

4. Water

Municipal tap water treated by reverse osmosis will be available to the animals ad libitum throughout the study. The purified water will be supplied by an automatic watering system. Monitoring of the drinking water for contaminants will be conducted by the testing laboratory and the records will be available for inspection. Within generally accepted limits, contaminants which may be present are not expected to compromise the purpose of this study.

B. Acclimation

Upon receipt, the animals will be examined, identified with plastic ear tags, and then acclimated for a minimum of 5 days.

C. Animal Selection

The animals chosen for study use will be arbitrarily selected from healthy stock animals to avoid potential bias. All animals will receive a detailed pretest observation prior to dosing. Only healthy animals will be chosen for study use. Females will be nulliparous and nonpregnant.

D. Experimental Design [2]

A six rabbit test will be performed. Materials which are determined by the Sponsor to be strong acids ($\text{pH} \leq 2.0$), strong alkalis ($\text{pH} \geq 11.5$) or a material which produces severe dermal irritation need not be tested in a full number of animals due to their predictive corrosive properties. However, at the request of the Sponsor, these materials will be administered to one animal. If no corrosive response is seen during the first 72 hours, the material will be tested on the remaining five animals.

X. EXPERIMENTAL PROCEDURES

A. Preliminary Examination

On day 0 prior to dosing, both eyes of each animal provisionally selected for test use will be examined macroscopically for ocular irritation with the aid of an auxiliary light source. In addition, the corneal surface will be examined using fluorescein sodium dye. One drop of fluorescein/physiological saline will be applied to the superior sclera of each eye. Following an approximate 15 second exposure, the eyes will be thoroughly rinsed with physiological saline. The corneal surface will then be examined for dye retention under a long-wave UV light source. Animals exhibiting ocular irritation, preexisting corneal injury or fluorescein dye retention (other than normal background retention) will not be used on study. All animals found to be acceptable for test use will be returned to their cages until dosing.

B. Dosing

A minimum of one hour after preliminary ocular examination, the test article will be instilled into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eye. Liquids, gels and pastes will be administered at a volume of 0.1 ml. Solids and powders will be administered at a weight equivalent to 0.1 ml volume, not to exceed 0.1 g. Following instillation, the eyelids will be gently held together for approximately

one second in order to limit test article loss and the animal returned to its cage. The contralateral eye will remain untreated to serve as a control. Following dosing, the Study Director will be notified by the technician if severe local reactions occur or if the animals exhibit overt clinical indications of pain/distress immediately postdose. If such is noted, the Sponsor will be contacted to see if the animals should be humanely euthanized.

C. Body Weights

Individual body weights will be obtained for each animal prior to dosing on study day 0.

D. Ocular Observations

The eyes will be macroscopically examined with the aid of an auxiliary light source for signs of irritation at 1, 24, 48 and 72 hours after dosing according to the Ocular Grading System presented in Protocol Appendix A which is based on Draize [2]. At the discretion of the study director, a bimicroscopic slit-lamp may be utilized to further examine and clarify ocular lesions. Following macroscopic observations at the 24 hour scoring interval, the fluorescein examination procedure will be repeated on all test and control eyes and any residual test article should be gently rinsed from the eye at this time (if possible) using 0.9% physiological saline. If fluorescein dye retention is noted at 24 hours, a fluorescein exam will be conducted on the affected eyes at each subsequent interval until a negative response is obtained. If there is no evidence of treatment related ocular irritation at the 72 hour scoring interval, the study will be terminated. If ocular irritation persists in any test eye, the observation period may be extended for the affected animals (scored on days 7, 10, 14 and 21). Animals requiring an extended observation period will remain on test (up to and including 21 days post-dose) until the irritation has resolved, permanent injury is evident or the Study Director/Sponsor determines that additional scoring intervals are unnecessary.

E. Clinical Observations

Any unusual observations and mortality will be recorded. Mortality checks will be performed twice daily, in the morning and afternoon.

F. Unscheduled Deaths

Any animals dying or euthanized (due to a possible accidental injury) during the study period will be necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) will be opened and examined. No tissues will be retained.

G. Scheduled Euthanasia

Each surviving animal will be euthanized by intravenous injection of sodium pentobarbital following its final observation interval. A gross necropsy examination will not be required for surviving animals.

XI. DATA REPORTING

One unbound copy of the draft report will be submitted to the Sponsor. Two copies of the final report (one bound and one unbound) will be submitted to the Sponsor. The final report will include all information necessary to provide a complete and accurate description and evaluation of the experimental procedures and results.

The report will include at least the following information and data:

- Table of Contents
- Regulatory Compliance
- Summary
- Introduction
- Experimental Design and Test Procedures
- Presentation and Discussion of Results
- Conclusion
- References
- Data Tables
- Protocol and Amendments
- SLS Personnel Responsibilities

XII. ANALYSIS OF DATA

For each group, the ocular irritation score for each parameter (i.e., corneal opacity x area, iritis and conjunctival redness + swelling + discharge) will be multiplied by the appropriate factor (i.e., corneal injury x 5, iritis x 5, conjunctivitis x 2) and the totals added for each animal/interval. The group mean irritation

score will then be calculated for each scoring interval based on the number of animals initially dosed in each group. If an animal dies during the study, the total animals in that group will be reduced (by the number of animals dead) for each subsequent scoring interval for the purpose of calculating the mean ocular irritation score for each interval. The data will be analyzed and summarized in the report based on the definitions presented below:

1. Non Irritant - Following instillation of the test article, none of the test eyes show a positive effect as defined in the Ocular Grading System in Protocol Appendix A.
2. Irritant - Following instillation of the test article, one or more test eyes exhibit a positive effect, but the changes are reversible.
3. Corrosive - One or more test eyes exhibit irreversible changes (ex., necrosis or ulceration) following instillation of the test article.

XIII. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS

All original data, magnetically encoded records, specimens and reports from this study are the property of the Sponsor. These materials shall be available at SLS to facilitate auditing of the study during its progress and prior to acceptance of the final report. The remaining test article(s) will be returned to the Sponsor following completion of the in-life phase of the study. Where necessary, the Sponsor will be responsible for maintaining a retention sample of the test article. All original paper data, the final report, magnetically encoded records, and any specimens will be transferred to the SLS archives for a period of 10 years. The Sponsor will be contacted prior to the final disposition of these items.

XIV. REGULATORY COMPLIANCE

This study may be submitted to and will be performed in general compliance with EPA-TSCA, OECD and EEC guidelines; the principles of the Good Laboratory Practice regulations as described by the FDA (21 CFR Part 58), the EPA (40 CFR Part 792), and the OECD Annex 2 C(81)30. Changes may be made in this protocol prior to, during, and/or following study completion. A protocol amendment will be prepared for such changes and will be signed by the Study Director, SLS Quality Assurance Unit and the Sponsor. The Sponsor shall be notified as soon as practical whenever an event occurs that is unexpected and may have an effect on the study.

XV. QUALITY ASSURANCE

The study will be inspected at least once during the in-life phase by the Springborn Laboratories, Inc., Life Sciences Division's Quality Assurance Unit to assure compliance with Good Laboratory Practice regulations, SLS's Standard Operating Procedures and for conformance with the protocol and protocol amendments. The final report will be audited prior to submission to the Sponsor to ensure that it completely and accurately describes the test procedures and results of the study.

XVI. USDA ANIMAL WELFARE COMPLIANCE STATEMENT

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Public Health Service Policy on Humane Care and Use of Laboratory Animals (OPRR, NIH, 1986). Wherever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress and pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures. These procedures are based on the most currently available technologies concerning proper laboratory animal use and management. Methods of euthanasia used during this study are in conformance with the above referenced regulations and the American Veterinary Medical Association Panel on Euthanasia [3]. This protocol has been reviewed and approved by Springborn Laboratories, Inc. Institutional Animal Care and Use Committee (IACUC) for a maximum of 18 animals.

This study is being conducted to evaluate potential irritant effects of the test article and potential reversibility of such effects. Following dosing, the Study Director will be notified by the technician if severe local reactions occur or if the animals exhibit overt clinical indications of pain/distress post-dose or if delayed severe ocular or clinical changes subsequently develop. If severe reactions are noted, the Study Director will contact the Facility Veterinarian and Sponsor to consider an appropriate course of action. In the event that the Sponsor cannot be contacted, the Study Director and Facility Veterinarian may authorize treatment or euthanasia of the animals. In general, the ocular tissue will not be anesthetized prior to or following dosing since these substances have been shown to inhibit the blink and/or tear response which may alter the irritation response. Furthermore, anesthetic agents may interact with and/or dilute the test article and thereby alter the experimental results. However, if it is suspected that the test article may induce more than transient pain/distress based on existing information, preanesthesia will be considered. In such circumstances, the Study Director and/or Facility Veterinarian will consult with the Sponsor to devise an appropriate study plan.

XVII. GENERIC PROTOCOL APPROVAL

The Sponsor's signature below documents that there are no acceptable non-animal alternatives for this study, and that since this study is required by the relevant supervising government agency, it does not unnecessarily duplicate any previous experiments.

Kimberly L. Bonnette Date 5/23/95
Kimberly L. Bonnette, M.S., LATG
Manager of Acute Toxicology (SLS)

Raymond V. Karcher Date 5-23-95
Raymond V. Karcher, B.A., LAT
Quality Assurance Unit (SLS)

Roy Banister Date 5/31/95
Roy Banister, Ph.D.
Sponsor's Representative
(Principal Investigator)

XVIII. STUDY SPECIFIC PROTOCOL APPROVAL

Abraham Grubbs Date 6/13/95
Study Director (SLS)

Christopher W. Wilson Date 6/17/95
Quality Assurance Unit (SLS)

Jan K. Sweet Date 10/13/95
IACUC Representative (SLS)

XIX. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 86-23, 1985.
2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.
3. 1993 Report of the American Veterinary Medical Assoc. Panel on Euthanasia, JAVMA, Vol. 202, No. 2, pp. 229-249, January 15, 1993.

PROTOCOL APPENDIX A
OCULAR GRADING SYSTEM

(O) CORNEAL OPACITY--DEGREE OF DENSITY (AREA MOST DENSE TAKEN FOR READING)	
OBSERVATION	CODE
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Nacreous (opalescent) area, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through opacity	4*

(A) AREA OF CORNEA INVOLVED (TOTAL AREA EXHIBITING ANY OPACITY, REGARDLESS OF DEGREE)	
OBSERVATION	CODE
No ulceration or opacity	0
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

Cornea Score = O x A x 5

Total Maximum = 80

(I) IRITIS	
OBSERVATION	CODE
Normal	0
Markedly deepened rugae (folds above normal), congestion, swelling, moderate circumcorneal hyperemia or 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*

Iris Score = I x 5

Total Maximum = 10

*Positive Effect.

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PROTOCOL APPENDIX A--(Continued)
OCULAR GRADING SYSTEM

(R) CONJUNCTIVAL REDNESS (REFERS TO PALPEBRAL AND BULBAR CONJUNCTIVAE EXCLUDING CORNEA AND IRIS)	
OBSERVATION	CODE
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected) above normal (slight erythema)	1
Diffuse, crimson color, individual vessels not easily discernible (moderate erythema)	2*
Diffuse beefy red (marked erythema)	3*

(S) CONJUNCTIVAL SWELLING (LIDS AND/OR NICTATING MEMBRANE)	
OBSERVATION	CODE
No swelling	0
Any swelling above normal (includes nictitating membrane, slightly swollen)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*

(D) CONJUNCTIVAL DISCHARGE	
OBSERVATION	CODE
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

Conjunctival Score = (R + S + D) x 2

Total Maximum = 20

*Positive Effect.

PROTOCOL APPENDIX A--(Continued)
OCULAR GRADING SYSTEM

CORNEAL NEOVASCULARIZATION		
OBSERVATION	CODE	DEFINITION
Neovascularization - Very Slight	VAS-1	Total area of vascularized corneal tissue is < 10% of corneal surface
Neovascularization - Mild	VAS-2	Total area of vascularized corneal tissue is > 10% but < 25% of corneal surface
Neovascularization - Moderate	VAS-3	Total area of vascularized corneal tissue is > 25% but < 50% of corneal surface
Neovascularization - Severe	VAS-4	Total area of vascularized corneal tissue is > 50% of corneal surface

SECONDARY OCULAR FINDINGS		
OBSERVATION	CODE	DEFINITION
Sloughing of the corneal epithelium	SCE	Corneal epithelial tissue is observed to be peeling off the corneal surface.
Corneal bulging	CB	The entire corneal surface appears to be protruding outward further than normal.
Slight dulling of normal luster of the cornea	SDL	The normal shiny surface of the cornea has a slightly dulled appearance.
Raised area on the corneal surface	RAC	A defined area on the corneal surface that is raised above the rest of the cornea. This area is generally associated with neovascularization and has a off-white to yellow color.
Corneal edema	CE	The cornea has a swollen appearance.
Test article present in eye	TAE	Apparent residual test article is observed on the eye or in the conjunctival sac/inner canthus.
Observation confirmed by slit lamp	OCS	A slit lamp examination was performed to confirm the initial observation.
Corneal mineralization	CM	Small white or off-white crystals that are observed in the corneal tissue.

PROTOCOL APPENDIX A--(Continued)
OCULAR GRADING SYSTEM

FLUORESCEIN EXAMINATION OF CORNEA	
OBSERVATION	CODE
<u>Fluorescein Dye Retention</u>	
Fluorescein dye retention associated with the area of corneal opacity	FAO
Fluorescein dye retention is not associated with any other finding	FNF
<u>Negative Results</u>	
No fluorescein retention is observed	(-)
<u>Secondary Ocular Findings</u>	
Superficial mechanical abrasion to the cornea observed during the fluorescein examination period	MI
Fine stippling on the cornea observed during the fluorescein examination procedure	ST
Superficial desquamation of the cornea observed during the fluorescein examination procedure	DES

POSTDOSE CLINICAL OBSERVATIONS	
OBSERVATION	CODE
Animal vocalized following dosing	VOC
Animal excessively pawed test eye following dosing	PAW
Animal exhibited excessive hyperactivity following dosing	HYP
Animal exhibited excessive head tilt following dosing	HT
Animal exhibited excessive squinting of test eye following dosing	SQ

Any additional findings will be noted in the raw data and in the final report.

APPENDIX B

SLS Personnel Responsibilities

SLS PERSONNEL RESPONSIBILITIES

Deborah A. Douds, M.S.	Study Director/Toxicologist
Kimberly L. Bonnette, M.S., LATG	Alternate Contact/Manager of Acute Toxicology
Dean E. Rodwell	President
Malcolm Blair, Ph.D.	Vice President/Director of Research
Rusty E. Rush, M.S., LAT, DABT	Associate Director of Toxicology
Todd N. Merriman, B.S., LATG	Toxicologist
Patricia K. Jenkins, A.A.S., LATG, RILAM	Supervisor of Acute Toxicology
Pamela S. Smith, ALAT	Unit Leader
Jan K. Severt, B.S., ALAT	Supervisor of Acute Report Preparation
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Supervisor of Gross and Fetal Pathology
Anita M. Bosau	Director of Regulatory Affairs
J. Dale Thurman, D.V.M., M.S. DACVP	Director of Pathology

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