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Primary Eye Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

October 1988

Toxicology Series: 153

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Primary Eye Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits (Toxicology Series 153)--Hiatt and Korte

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S. Beatrice 31 Oct 88

Edwin S. Beatrice (date)
COL, MC
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The potential for diethyleneglycol dinitrate (DEGDN) to produce primary eye irritation was evaluated in six male New Zealand White rabbits by using a modified Draize method. DEGDN produced no response indicative of a potential to cause irritation upon direct conta with the eye. Slight iridial vasodilation (one of six rabbits) and slight conjunctival vasodilation and swelling, indicative of mild inflammation, (three of six rabbits) were th most serious responses observed. DEGDN was classified as a non-irritant under conditions of this study.

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ABSTRACT

The potential for diethyleneglycol dinitrate (DEGDN) to produce primary eye irritation was evaluated in six male New Zealand White rabbits by using a modified Draize method. DEGDN produced no response indicative of a potential to cause irritation upon direct contact with the eye. Slight iridial vasodilation (one of six rabbits) and slight conjunctival vasodilation and swelling, indicative of mild inflammation, (three of six rabbits) were the most serious responses observed. DEGDN was classified as a non-irritant under conditions of this study.

Key Words: Diethyleneglycol dinitrate, DEGDN, Ocular Irritation, Mammalian Toxicology, Rabbits

PREFACE

TYPE REPORT: Primary Eye Irritation GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, Maryland 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 85002

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: Gerald F.S. Hiatt, PhD

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Diethyleneglycol dinitrate (DEGDN)

INCLUSIVE STUDY DATES: 22 Aug - 24 Sep 85

OBJECTIVE: The objective of this study was to determine the primary eye irritation potential of DEGDN in male New Zealand White rabbits.

ACKNOWLEDGMENTS

SSG James D. Justus, SP4 James J. Fischer, and SP4 Theresa L. Polk provided technical assistance in the conduct of the study. Michael J. Pearce provided assistance in the preparation of the report. SP4 Scott L. Schwebe, Richard D. Spieler, Obie B. Goodrich, and Diane Arevalo provided care for the animals. Colleen S. Kamiyama and Ann L. Wilkinson provided administrative and clerical support during the performance of this study and preparation of the report. MAJ Larry D. Brown, VC, served as the LAIR director of the research project for the acute toxicity studies of DEGDN.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, declare that GLP Study 85002 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr 18 OCT 88

DON W. KORTE JR., PhD / DATE
MAJ, MSC
Study Director

Gerald F.S. Hiatt

GERALD F.S. HIATT, PhD / DATE
DAC
Principal Investigator

Conrad Wheeler 18 Oct 88

CONRAD WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

25 October 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85ØØ2

1. This is to certify that in relation to LAIR GLP Study 85ØØ2, the following inspections were made:

Ø5 March 1988	-	Protocol Review
1Ø September 1988	-	Dosing
11 September 1988	-	24-hr Observations

2. The institute report entitled "Primary Eye Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits," Toxicology Series 153, was audited on 14 October 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

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Primary Eye Irritation Potential of Diethyleneglycol Dinitrate (DEGDN) in Rabbits -- Hiatt and Korte

INTRODUCTION

The Department of Defense is considering the use of either diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL was tasked the Division of Toxicology, Letterman Army Institute of Research (LAIR), to conduct an initial health effects evaluation of the proposed replacement nitrate esters. This initial evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP, includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, acute dermal toxicity in rabbits, dermal and ocular irritation studies in rabbits, and dermal sensitization studies in guinea pigs.

Objective of Study

The objective of this study was to determine the primary eye irritation potential of DEGDN in male New Zealand White rabbits.

MATERIALS

Test Substance

Chemical name: Diethylenglycol dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

Molecular Structure:

$$O_2N-O-CH_2CH_2-O-CH_2CH_2-O-NO_2$$

Empirical Formula: $C_4H_8N_2O_2$

Other test substance information is presented in Appendix A.

Animal Data

Six male New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA) were identified individually with ear tattoos numbered 85F162, 85F171, 85F172, 85F173, 85F174, and 85F175. Animal weights on dosing day ranged from 2.4 to 2.8 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, battery-type cages with screened floors and automatically flushing dumptanks. The diet consisted of approximately 150 g/day of Certified Purina Chow Diet 5322 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained at 18.9 to 21.1°C and relative humidity ranged from 44 to 58 percent. The photo period was 12 hours of light per day.

METHODS

Conduct of this study was in accordance with the LAIR Standard Operating Procedure "Primary Eye Irritation Study" (2) and guidelines promulgated by the EPA for ocular irritation testing (3).

Group Assignment/Acclimation

Study rabbits were divided into a group of two males and a group of four males. These animals were quarantined by the Division of Animal Care and Services for 14 days and acclimated for 5-12 days in the GLP room before dosing. During these periods they were observed daily for signs of illness.

Dosage Levels and Administration

One-tenth milliliter of DEGDN was administered once to the "treated" eye of each rabbit by gently pulling the lower lid away from the conjunctival cul-de-sac to form a cup into which the compound was instilled. Upper and lower lids were then held gently together for one to two seconds to prevent loss of material. Group 1 was dosed on 10 Sep 85 and Group 2 was dosed on 17 Sep 85.

Compound Preparation

DEGDN is a liquid and was administered neat (undiluted).

Test Procedures

On 9 Sep 85, both eyes of each Group 1 animal were examined, for any preexisting abnormalities, by the procedure detailed below. For each animal, the eye with the most normal appearance was designated for treatment; the contralateral eye served as an untreated control. On 10 Sep 85, 0.1 ml DEGDN was placed in the treated eye of each rabbit in this group. Group 2 rabbits underwent the same procedures on 16 and 17 Sep 85, respectively.

Ocular Examination/Grading

Initially each eye was observed unaided in a darkened room with focal illumination (pen light). Structures examined included: the lids and surrounding fur, the conjunctiva (semilunar, palpebral, and bulbar), the cornea, and the iris. Grading of the cornea, iris and conjunctiva was performed according to Table 1 (modified from Ref 4). Each eye was also then examined with a slit lamp. Special attention was given to integrity of the corneal surface, thickness of the corneal stroma, clarity of anterior chamber fluid, iridial morphology, clarity of the lens, and lenticular surface morphology (5). Additionally, any areas appearing grossly abnormal were examined under high magnification. All observations, including normal appearance, were detailed on the grading sheet. Following this, fluorescein dye (Fluor-I-Strips, Ayerst Laboratories Inc., New York, N.Y. 10017) was introduced into the eye, which was then observed under ultraviolet light. Any corneal areas reacting with the dye (a sign of discontinuity of the corneal epithelium) were described with respect to area and intensity of fluorescence. Ocular reactions were examined and graded in this fashion at 1, 4, 24, 48, and 72 hours after dosing. Fluorescein staining was omitted from the observations at 1 and 4 hours. Due to an almost total lack of reaction during the 72 hours after dosing, the study was terminated, according to protocol, after this observation. Therefore no scoring or observations were performed at 7, 14 or 21 days.

Duration of Study

Appendix C is a complete listing of historical events.

TABLE 1: GRADES FOR OCULAR LESIONS†

CORNEA

Opacity: degree of density (area of greatest density taken for reading)

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 areas, details of iris slightly obscured.....2
Nacreous areas, no details of iris visible, size of pupil barely discernible.....3
Opaque cornea, iris not discernible through opacity.....4

IRIS

Normal.....0
Markedly deepened rugae, congestion, swelling, moderate circumiridial hyperemia or injection, any of these or any combination thereof, iris still reacting to light (sluggish reaction is positive).....1*
No reaction to light, hemorrhage, gross destruction (any or all of these).....2

CONJUNCTIVA

Redness: (refers to palpebral and bulbar conjunctiva, excluding cornea and iris)

Blood vessels normal.....0
Some blood vessels definitely hyperemic (injected).....1
Diffuse, crimson color, individual vessels not easily discernible.....2*
Diffuse, beefy red.....3

Chemosis: (lids and/or nictitating membranes)

No swelling.....0
Any swelling above normal including nictitating membranes.....1
Obvious swelling with partial eversion of lids.....2*
Swelling with lids about half-closed.....3
Swelling with lids more than half-closed.....4

† Adapted from Table 6 in Draize *et al.* (4).
* Indicates minimum level for a positive response.

Changes/Deviations

Slit lamp examination (procedure detailed above) was added to the standard observation procedures. Use of the slit lamp enables detection of subtle reactions not grossly observable and better evaluation of those abnormalities which are grossly observable. Color photographic documentation was not performed due to lack of significant response to test compound.

During an ocular pre-exam conducted on 9 Sep 85, rabbit 85F170, initially assigned to this study, apparently sustained a back injury while struggling in the restrainer. The animal was normal before being placed in the restrainer and the full extent of the injury was not apparent upon visual examination by the Suite Veterinarian immediately following return of the rabbit to its cage. In response to worsened symptoms the next morning (the day of dosing) the animal was again examined by the Suite Veterinarian and was removed from the study. Another rabbit (85F162), which was excess to GLP study 85003, was substituted.

With these exceptions, this study was completed in accordance with the appropriate protocol and addenda.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Ocular grading results and slit lamp observations for each rabbit are presented in Appendices D and E.

DEGDN produced no grossly observable effects in the cornea. All treated eyes were assigned zero scores for both opacity and area of involvement at all observations after dosing. Slit lamp examination revealed no corneal reactions referable to the test compound. One rabbit (85F162) displayed a small mucoid globule on the corneal surface one hour after dosing. All other slit lamp observations revealed corneas of normal thickness, indicating lack of edema, with smooth surfaces, indicating epithelial integrity. No staining of the corneal epithelium was observed upon any of the fluorescein examinations in the treated eyes.

One rabbit (85F171) presented slight iridial vasodilation (score = 1) at the four-hour examination. This was primarily limited to the circumiridial vessels. No other grossly observable reactions were produced in the iris by DEGDN. Other iridial scores were consistently zero at all observation times. No additional iridial abnormalities were detected by slit lamp examination of the other treated eyes. Except for the vasodilation noted grossly in rabbit 85F171, circumiridial vessels and surface morphology were normal in all eyes at all times after dosing. Close examination of anterior chamber fluid revealed no evidence of the presence of protein or cells (another sign of iridial inflammation) in any of the treated eyes.

In this study, DEGDN produced slight conjunctival redness and swelling. At the observation at one hour, slight vasodilation (score = 1) was present in the conjunctiva of one rabbit (85F171); although it cleared in this animal, redness developed in two others (85F162, 85F173) by 4 hours. Another rabbit (85F174) exhibited slight conjunctival swelling (score = 1) at both 1 and 4 hours after dosing. All of these signs of mild conjunctival inflammation cleared by 24 hours. Slit lamp examination confirmed the presence of dilated vessels within the outer layers of the sclera, the nictitating membrane, and on the undersides of the eyelids.

The lens is not scored under the Draize-type grading system because of the difficulty in making unaided observations. At all times after dosing, the lens appeared normal during slit lamp examination. No changes were observed in clarity or surface morphology.

At no time during the study did the contralateral untreated eyes exhibit any change from their normal condition on the day of dosing.

With the exception of the ocular signs reported above, all animals appeared normal throughout the study and gained weight. Body weight data are presented in Appendix F.

The gross necropsy findings for the six rabbits in the study were considered unremarkable. A copy of the pathology report is presented in Appendix G.

DISCUSSION

Consumer Product Safety Commission Guidelines, which the EPA recommends for ocular irritation testing, state that an animal has exhibited a positive reaction if the test substance produces one or more of the following signs: ulceration of the cornea (other than a fine stippling), opacity of the cornea (other than a slight dulling of the normal luster), inflammation of the iris (other than a slight deepening of the rugae or a slight hyperemia of the circumcorneal blood vessels), an obvious swelling in the conjunctiva with partial eversion of the lids, or a diffuse crimson-red coloration in the conjunctiva with individual vessels not easily discernible (2). Guidelines for classification of chemicals as ocular irritants or nonirritants have been published and form the basis for evaluation in the the present study (6). These Interagency Regulatory Liaison Group (IRLG) guidelines state that for an initial evaluation: "[a] test result is considered positive if four or more animals exhibit a positive reaction. If only one animal exhibits a positive reaction, the test result is regarded as negative."

In this study, DEGDN produced only one positive reaction, as defined by the IRLG. Slight conjunctival redness and swelling (score = 1), indicating mild inflammation, were observed in three rabbits. These reactions, although scorable, did not achieve sufficient severity to warrant consideration as a "positive response." One observation of slight iridial vasodilation was made 4 hours after compound instillation; this response qualified for a score of 1. Although this was a "positive response," DEGDN was still classified as a <u>non-irritant</u> since at least two of six animals must respond positively for any consideration of classification as an irritant.

CONCLUSION

DEGDN did not produce sufficient irritation under conditions of this study to be classified as an ocular irritant.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethyloiethane trinitrate and their respective combustion products. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127816.

2. Primary Eye Irritation Study. LAIR Standard Operating Procedure OP-STX-33, Letterman Army Institute of Research, Presidio of San Francisco, CA, 15 June 1984.

3. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Primary Eye Irritation. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.

4. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82:377-390.

5. MacDonald TO, Baldwin HA, Beasley CH. Slit lamp examination of experimental animal eyes. I. Techniques of illumination and the normal animal eye. J Soc Cosmet Chem 1973; 24:163-180.

6. Interagency Regulatory Liaison Group. Testing Standards and Guidelines Work Group. Recommended guidelines for acute eye irritation testing. January 1981.

Appendix A: CHEMICAL DATA

Chemical name: Ethanol, 2,2'-oxybisdinitrate

Alternate chemical name: Diethyleneglycol dinitrate (DEGDN)

Chemical Abstracts Service Registry No.: 693-21-0

LAIR Code No.: TP047

Chemical structure:

$$O_2N-O-CH_2CH_2-O-CH_2CH_2-O-NO_2$$

Molecular formula: $C_4H_8N_2O_7$

Molecular weight: 196

Physical state: Pale yellow liquid

Density (g/cm^3): 1.38[1]

Analytical data: Refer to the attached data sheet, ARRCOM Form 213R. The compound chromatographed as a single peak (retention time 5.4 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% acetonitrile; flow rate, 0.9 ml/min; detection wavelength, 205 nm.[2] NMR (300 MHz, CD_3CN): 3.75 δ (complex multiplet, 4H, $-CH_2-O-CH_2-$), 4.61 δ complex

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[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland; US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. ADA127846, p. 17.

[2] Wheeler CR. Toxicity Testing of Propellants. Laboratory Notebook #85-12-023, p. 31. Letterman Army Institute of Research, Presidio of San Francisco, California.

Appendix A (cont.): CHEMICAL DATA

multiplet, 4H,-CH_2ONO_2).[3] Additional singlet signals of approximately equal intensity were observed at 2.08 δ and were due to sample impurities. Integration of all signals in the spectrum demonstrated that the sample contained 96.6% DEGDN. The impurities were not identified. IR(KBr): 2896, 1632, 1429, 1390, 1373, 1279, 1139, 1032, 909, 857, 758, 707, 655, 572 cm^{-1}.[4]

Stability: The DEGDN was shipped containing 18% acetone (a desensitizer) and arrived at LATR on 12 December 1984. The acetone was removed by rotary evaporation prior to studies with the propellant. Analysis of the compound one year after it was received gave the results described above. Stability of the compound in corn oil (the dosing vehicle) was examined. As determined by HPLC, the concentration of DEGDN in corn oil emulsions 24 h after preparation was within 1% of the target value.[5]

Source: Radford Army Ammunition Plant, Radford, Virginia (prime contractor: Hercules Inc., Wilmington, Delaware).

Lot No.: RAD84M001S214

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[3] Ibid. pp. 44-48.

[4] Ibid. pp. 49-50.

[5] Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 57-60. Letterman Army Institute of Research, Presidio of San Francisco, California.

Appendix A (cont): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC

REPORTS CONTROL SYMBOL: EXEMPT-Para 7-2a AR 335-15 | PAGE 1 OF 1 Copy

TO:

FROM:

DATE: December 5, 1984

MATERIAL: Diethylene Glycol Dinitrate (DEGDN)

MANUFACTURER: HERCULES INCORPORATED, RADFORD ARMY AMMUNITION PLANT

CONTRACT NO.: DAAA09-77-C-4007

SECTION A - DESCRIPTION OF LOTS

FROM NUMBER	THRU NUMBER	TOTAL NO. LOTS	TOTAL NET AMOUNT ACCEPTED
RAD84M0015214	-	1	5 lbs

PLACE MANUFACTURED: RADFORD ARMY AMMUNITION PLANT, RADFORD, VIRGINIA

SPECIFICATION AND AMENDMENT/DRAWING NO.: DOD-D-64015

SECTION B - DESCRIPTION OF MATERIAL

Requirements	Limit	Results
82.2°C Potassium Iodide Starch Paper Heat Test (KI)	10 minutes minimum	12
Nitrogen, %	14.10 minimum	14.15
Water, %	Info Only	0.43
Acidity	None	None
Alkalinity	None	None

REMARKS: DEGDN is desensitized with 15% or more of acetone for a total weight of 5 lbs, and packed in a DOT 6D 5 gallon drum with a DOT 2S liner, overpacked in a DOT-6J 30 gallon capacity drum with vermiculite as a cushioning agent around the 5 gallon drum and contained in the 30 gallon drum. Requested by shipping Order AMCCOM and COR letter SMCRA dated November 28, 1984 (DOT Exemption 5704).

SECTION C - CERTIFICATION

SAMPLING CONDUCTED BY: HERCULES INCORPORATED

TESTING CONDUCTED BY: HERCULES INCORPORATED

THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.

12-5-84 (DATE) F.A. Walker (SIGNATURE) F.A.WALKER

THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED

Dec 6, 1984 (DATE) _____ (TITLE) FOR THE COMMANDER _____ (SIGNATURE)

ARRCOM Form 213-R, 10 Aug 77

SEQUENCE No. 374

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male

Age: Young Adults

Animals in each group: Group I: 2 males
Group II: 4 males

Condition of animals at start of study: Normal

Body weight range at dosing: 2.4 - 2.8 kg

Identification procedures: Ear tattoo numbers 85F162, 85F171, 85F172, 85F173, 85F174, 85F175.

Pretest conditioning: Quarantine from 22 Aug - 5 Sep 1985 Animal eyes were examined 24 hours before dosing using slit lamp, fluorescein dye, and ultraviolet light.

Justification: Laboratory rabbits are a proven sensitive animal model for ocular testing.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
22 Aug 85	Animals arrived at LAIR. They were tattooed, weighed, examined for illness, placed under a two-week quarantine.
22 Aug-5 Sep 85	Animals were checked daily by Division of Animal Care and Services (DAC&S). No animals died during the quarantine period.
5 Sep 85	Rabbits were certified healthy by DAC&S Staff Veterinarian and removed from quarantine, separated into test groups and weighed.
9 Sep 85	Animals were checked for preexisting ocular injury (Group 1).
10 Sep 85	Group 1 rabbits were dosed according to test chemical group and weighed. Eyes were scored 1 and 4 hours after exposure.
11 Sep 85	Eyes were scored 24 hours after exposure (Group 1).
12 Sep 85	Eyes were scored 48 hours after exposure (Group 1).
13 Sep 85	Eyes were scored 72 hours after exposure. Study was terminated (Group 1).
16 Sep 85	Animals were weighed and sent to Necropsy Suite for sacrifice and necropsy (Group 1). Animals were checked for preexisting ocular injury (Group 2).
17 Sep 85	Group 2 Rabbits were dosed according to test chemical group and weighed. Eyes were scored 1 and 4 hours after exposure.
18 Sep 85	Eyes were scored 24 hours after exposure (Group 2).

Appendix C (cont.): HISTORICAL LISTING OF STUDY EVENTS

Date	Event
19 Sep 85	Eyes were scored 48 hours after exposure (Group 2).
20 Sep 85	Eyes were scored 72 hours after exposure (Group 2).
24 Sep 85	Study was terminated, animals were weighed and sent to Necropsy Suite for sacrifice and necropsy (Group 2).

Appendix D: TABULAR SCORING DATA ON ACUTE EYE IRRITATION SUMMARY FORMS

APPENDIX D-1: Cornea Opacity

Score by Animal

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F162	0	0	0	0	0	0
85F171	0	0	0	0	0	0
85F172	0	0	0	0	0	0
85F173	0	0	0	0	0	0
85F174	0	0	0	0	0	0
85F175	0	0	0	0	0	0

APPENDIX D-2: Iridial Scores

Score by Animal

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F162	0	0	0	0	0	0
85F171	0	0	1	0	0	0
85F172	0	0	0	0	0	0
85F173	0	0	0	0	0	0
85F174	0	0	0	0	0	0
85F175	0	0	0	0	0	0

APPENDIX D-3: Conjunctiva (Redness)

Score by Animal

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F162	0	0	1	0	0	0
85F171	0	1	0	0	0	0
85F172	0	0	0	0	0	0
85F173	0	0	1	0	0	0
85F174	0	0	0	0	0	0
85F175	0	0	0	0	0	0

APPENDIX D-4: Conjunctiva (Chemosis)

Score by Animal

Rabbit Number	Base-Line	1 hr	4 hr	24 hr	48 hr	72 hr
85F162	0	0	0	0	0	0
85F171	0	0	0	0	0	0
85F172	0	0	0	0	0	0
85F173	0	0	0	0	0	0
85F174	0	1	1	0	0	0
85F175	0	0	0	0	0	0

Appendix E: SUMMARY OF OCULAR OBSERVATIONS

One Hour After Dosing

85F162	Small "mucous-like" globule on cornea surface.
85F171	Slight conjunctival redness.
85F173	Hyperemic retina.
85F174	Slight swelling of the nictitating membrane. Slight bulging of the eye.

Four Hours After Dosing

85F162	Slight conjunctival redness.
85F171	Slight redness in the iris.
85F172	Small blister (1-2 mm) on nictitating membrane.
85F173	Slight conjunctival redness. Hyperemic retina.
85F174	Slight swelling of the nictitating membrane. Slight bulging of the eye.

Twenty-four Hours After Dosing

85F172	Small blister (1-2 mm) on nictitating membrane.
85F173	Hyperemic retina.
85F174	Slight bulging of the eye.

Forty-eight Hours After Dosing

85F172	Small blister (1-2 mm) on nictitating membrane.
85F173	Hyperemic retina.
85F174	Excess mucus on the eye.

Seventy-two Hours After Dosing

85F171	Very slight increased thickness in corneal surface.
85F173	Hyperemic retina.

APPENDIX F: Body Weight Data

Animal Number	Baseline	Termination	Change
	Group 1		
85F171	2399*	2479	80
85F172	2519	2576	57
	Group 2		
85F162	2654	2711	57
85F173	2782	2804	22
85F174	2774	2804	30
85F175	2748	2872	124

* Body weights recorded in grams.

APPENDIX G: Pathology Report

LAIR Gross Pathology Report
GLP Study 85002

Study: GLP #85002

Test: Primary Ocular Irritation Test

Investigator: Dr. Gerald Hiatt

Test Substance: DEGDN (CAS No. 693-21-0)

History: Six male rabbits (NZW) were exposed to 0.1 ml of the test compound in the conjunctival sac of one eye, the other eye being a control. After observation (IAW LAIR SOP-OP-STX-33), for an applicable period, the animals were euthanized with 4.0 ml sodium pentobarbital and necropsied.

Gross findings:

ANIMAL ID #	LAIR PATH #	MORPHOLOGIC DX
85F162	38239	Cecum - Pinworms Liver - Focal tan lesion, 2 x 0.2 cm.
85F171	38234	Not remarkable (NR)
85F172	38235	Lungs - Diffuse red mottled discoloration.
85F173	38240	NR
85F174	38241	NR
85F175	38242	NR

Comment: None of the gross findings were considered remarkable.

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