

2503020409E

TTIC FILE COPY

2

Institute Report No. 403

**Primary Eye Irritation Potential of  
DIGL-RP Solid Propellant in Rabbits**

AD-A216 138

**Gerald F.S. Hiatt, PhD  
James D. Justus, MPA, SSG, USA  
and  
Don W. Korte, Jr., PhD, LTC, MSC**

**MAMMALIAN TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY**

**DTIC  
ELECTE  
DEC 12 1989  
S & E D**

October 1989

Toxicology Series: 153

**LETTERMAN ARMY INSTITUTE OF RESEARCH  
PRES'DIO OF SAN FRANCISCO, CALIFORNIA 94129**

**89 12 11 081**

Primary Eye Irritation Potential of DGL-RP Solid Propellant in Rabbits (Toxicology Series 245)-Blatt et al.

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the 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)

  
Donald G. Corby  
COL, MC  
Commanding

  
(date)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

| REPORT DOCUMENTATION PAGE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |       |                                                    |                                                                                                         | Form Approved<br>OMB No. 0704-0188                    |                                |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|----------------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------------------------------------------------|--------------------------------|
| 1a. REPORT SECURITY CLASSIFICATION<br>UNCLASSIFIED                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |       |                                                    | 1b. RESTRICTIVE MARKINGS                                                                                |                                                       |                                |
| 2a. SECURITY CLASSIFICATION AUTHORITY                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |       |                                                    | 3. DISTRIBUTION / AVAILABILITY OF REPORT                                                                |                                                       |                                |
| 2b. DECLASSIFICATION / DOWNGRADING SCHEDULE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |       |                                                    | APPROVED FOR PUBLIC RELEASE;<br>DISTRIBUTION IS UNLIMITED.                                              |                                                       |                                |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S)<br><br>Institute Report No.: 408                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |       |                                                    | 5. MONITORING ORGANIZATION REPORT NUMBER(S)                                                             |                                                       |                                |
| 6a. NAME OF PERFORMING ORGANIZATION<br>Mammalian Toxicology<br>Division of Toxicology                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |       | 6b. OFFICE SYMBOL<br>(If applicable)<br>SGRD-ULE-T | 7a. NAME OF MONITORING ORGANIZATION<br>US Army Biomedical Research<br>and Development Laboratory        |                                                       |                                |
| 6c. ADDRESS (City, State, and ZIP Code)<br>Letterman Army Institute of Research<br>Presidio of San Francisco, CA 94129-6800                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |       |                                                    | 7b. ADDRESS (City, State, and ZIP Code)<br>Fort Detrick<br>Frederick, MD 21701-5010                     |                                                       |                                |
| 8a. NAME OF FUNDING / SPONSORING<br>ORGANIZATION US Army Medical<br>Research & Development Command                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |       | 8b. OFFICE SYMBOL<br>(If applicable)               | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER                                                         |                                                       |                                |
| 8c. ADDRESS (City, State, and ZIP Code)<br>Fort Detrick<br>Frederick, Maryland 21701-5012                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |       |                                                    | 10. SOURCE OF FUNDING NUMBERS                                                                           |                                                       |                                |
| PROGRAM<br>ELEMENT NO.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |       | PROJECT<br>NO.                                     | TASK<br>NO.                                                                                             | WORK UNIT<br>ACCESSION NO.                            |                                |
| 62720                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |       | A835                                               | AB                                                                                                      | DA303913                                              |                                |
| 11. TITLE (Include Security Classification)<br>(U) Primary Eye Irritation Potential of DIGL-RP Solid Propellant in Rabbits                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |       |                                                    |                                                                                                         |                                                       |                                |
| 12. PERSONAL AUTHOR(S)<br>GFS Hiatt, JD Justus, and DW Korte, Jr.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |       |                                                    |                                                                                                         |                                                       |                                |
| 13a. TYPE OF REPORT<br>Institute                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |       | 13b. TIME COVERED<br>FROM 14 NOV 85 to 3 DEC 85    |                                                                                                         | 14. DATE OF REPORT (Year, Month, Day)<br>October 1989 |                                |
| 15. PAGE COUNT<br>20                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |       |                                                    |                                                                                                         |                                                       |                                |
| 16. SUPPLEMENTARY NOTATION<br><br>Toxicology Series No. 165                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |       |                                                    |                                                                                                         |                                                       |                                |
| 17. COSATI CODES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |       |                                                    | 18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)                       |                                                       |                                |
| FIELD                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | GROUP | SUB-GROUP                                          | DIGL-RP Solid Propellant, Diethyleneglycol<br>Dinitrate, Mammalian Toxicology, Munition,<br>Rabbit. (S) |                                                       |                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |       |                                                    |                                                                                                         |                                                       |                                |
| 19. ABSTRACT (Continue on reverse if necessary and identify by block number)<br>→ The potential for DIGL-RP Solid Propellant to produce primary eye irritation was evaluated in male New Zealand White rabbits by using a modified Draize method. DIGL-RP produced slight conjunctival vasodilation (indicative of mild inflammation), and slight chemosis of the conjunctival membranes. However, these responses were not sufficiently severe to be classified as a positive response. Therefore, the results indicate that DIGL-RP Solid Propellant is not a primary eye irritant under conditions of this study.<br><br><i>Keywords:</i> |       |                                                    |                                                                                                         |                                                       |                                |
| 20. DISTRIBUTION / AVAILABILITY OF ABSTRACT<br><input checked="" type="checkbox"/> UNCLASSIFIED / UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS                                                                                                                                                                                                                                                                                                                                                                                                                                                        |       |                                                    | 21. ABSTRACT SECURITY CLASSIFICATION<br>UNCLASSIFIED                                                    |                                                       |                                |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>DONALD G. CORBY, COL, MC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |       |                                                    | 22b. TELEPHONE (Include Area Code)<br>(415) 561-3600                                                    |                                                       | 22c. OFFICE SYMBOL<br>SGRD-ULZ |

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

## ABSTRACT

The potential for DIGL-RP Solid Propellant to produce primary eye irritation was evaluated in male New Zealand White rabbits by using a modified Draize method. DIGL-RP produced slight conjunctival vasodilation (indicative of mild inflammation), and slight chemosis of the conjunctival membranes. However, these responses were not sufficiently severe to be classified as a positive response. Therefore, the results indicate that DIGL-RP Solid Propellant is not a primary eye irritant under conditions of this study.

**Key Words:** DIGL-RP Solid Propellant, Ocular Irritation, Mammalian Toxicology, Rabbit, Diethyleneglycol Dinitrate, Munition

|                    |                                     |
|--------------------|-------------------------------------|
| Accession For      |                                     |
| NTIS GRA&I         | <input checked="" type="checkbox"/> |
| DTIC TAB           | <input type="checkbox"/>            |
| Unannounced        | <input type="checkbox"/>            |
| Justification      |                                     |
| By _____           |                                     |
| Distribution/      |                                     |
| Availability Codes |                                     |
| Dist               | Avail and/or<br>Special             |
| A-1                |                                     |

## **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, MD 21701-5010  
Project Officer: Gunda Reddy, PhD

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

**GLP STUDY NUMBER:** 85023

**STUDY DIRECTOR:** LTC Don W. Korte, Jr., PhD, MSC  
Diplomate, American Board of Toxicology

**PRINCIPAL INVESTIGATOR:** Gerald F.S. Hiatt, PhD

**CO-PRINCIPAL INVESTIGATOR:** SSG James D. Justus, MPA

**PATHOLOGIST:** MAJ G. Tracy Makovec, DVM, VC, Diplomate  
American College of Veterinary Pathologists

**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:** DIGL-RP Solid Propellant

**INCLUSIVE STUDY DATES:** 14 Nov 1985 - 13 Dec 1985

**OBJECTIVE:**

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

### **ACKNOWLEDGMENTS**

MAJ Larry D. Brown, DVM, and SP4 Gayle A. Omer, BS, provided technical assistance. SP4 James J. Fisher, SP4 Scott L. Schwebe, SP4 Theresa L. Polk, Obie B. Goodrich, and Diane Arevalo provided care for the animals. Colleen Kamiyama provided administrative and clerical support during the performance of this study and preparation of the report.

# SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85023 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. 7 Sep 89  
DON W. KORTE, JR., PhD / DATE  
LTC, MSC  
Study Director

Gerald F.S. Hiatt 13 Jan 86  
GERALD F.S. HIATT, PhD / DATE  
DAC  
Principal Investigator

James D. Justus 2 Aug 89  
JAMES D. JUSTUS, MPA / DATE  
SSG, USA  
Co-Principal Investigator

Conrad R. Wheeler 5 Sep 89  
CONRAD R. WHEELER, PhD / DATE  
DAC  
Analytical Chemist



DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH  
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6000

REPLY TO  
ATTENTION OF:

SGRD-ULZ-QA

26 October 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85023

1. This is to certify that the protocol for LAIR GLP Study 85023 was reviewed on 10 May 1985.
2. The institute report entitled "Primary Eye Irritation Potential of DIGL-RP Solid Propellant in Rabbits," Toxicology Series 165, was audited on 2 October 1989.

*Carolyn M. Lewis*  
CAROLYN M. LEWIS  
Diplomate, American Board of  
Toxicology  
Quality Assurance Auditor

## TABLE OF CONTENTS

|                                           |       |
|-------------------------------------------|-------|
| Abstract .....                            | i     |
| Preface .....                             | iii   |
| Acknowledgments.....                      | iv    |
| Signatures of Principal Scientists.....   | v     |
| Report of Quality Assurance Unit.....     | vi    |
| Table of Contents.....                    | vii   |
| <br>INTRODUCTION.....                     | <br>1 |
| Objective of Study.....                   | 1     |
| <br>MATERIALS.....                        | <br>1 |
| Test Substance.....                       | 1     |
| Animal Data .....                         | 2     |
| Husbandry.....                            | 2     |
| <br>METHODS .....                         | <br>2 |
| Group Assignment/Acclimation.....         | 2     |
| Dose Levels and Administration .....      | 3     |
| Compound Preparation.....                 | 3     |
| Test Procedures .....                     | 3     |
| Ocular Examination/Grading .....          | 3     |
| Duration of Study .....                   | 5     |
| Changes/Deviations .....                  | 5     |
| Storage of Raw Data and Final Report..... | 5     |
| <br>RESULTS.....                          | <br>6 |
| Cornea .....                              | 6     |
| Iris/Anterior Chamber.....                | 6     |
| Lens.....                                 | 6     |
| Conjunctiva .....                         | 6     |
| Control Eyes.....                         | 7     |
| Pathology Report.....                     | 7     |
| <br>DISCUSSION .....                      | <br>7 |
| <br>CONCLUSION .....                      | <br>8 |
| <br>REFERENCES .....                      | <br>9 |

## TABLE OF CONTENTS (cont.)

|                                                     |    |
|-----------------------------------------------------|----|
| APPENDICES .....                                    | 10 |
| Appendix A. Chemical Data .....                     | 11 |
| Appendix B. Animal Data .....                       | 14 |
| Appendix C. Historical Listing of Study Events..... | 15 |
| Appendix D. Tabulated Ocular Data.....              | 16 |
| Appendix E. Summary of Ocular Observations .....    | 18 |
| Appendix F. Pathology Report.....                   | 19 |
| OFFICIAL DISTRIBUTION LIST.....                     | 20 |

**Primary Eye Irritation Potential of DIGL-RP Solid Propellant in Rabbits -  
Hiatt et al.**

**INTRODUCTION**

The Department of Defense is considering the use of 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 has 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 DIGL-RP in male New Zealand White rabbits.

**MATERIALS**

**Test Substance**

Chemical Name: DIGL-RP Solid Propellant

LAIR Code Number: TP57

Physical State: Solid black cylinders (stick configuration)

Lot No.: RAD83M001S169

Other test substance information is presented in Appendix A.

#### Animal Data

Six male New Zealand White rabbits (Elkhorn Rabbitry, Watsonville, CA) were identified individually with ear tattoos numbered 85F309 to 85F314 inclusive. Animal weights on dosing day ranged from 2.6 to 2.9 kg. Additional animal data appear in Appendix B.

#### Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dumptanks. The diet consisted of approximately 150 g/day of Certified Purina Chow<sup>®</sup> 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 16.2°C to 20.0°C and relative humidity ranged from 51% to 69%, except for occasional humidity spikes as high as 78% (room washing). The photoperiod was 12 hours of light per day.

#### **METHODS**

Conduct of this study was in accordance with the LAIR Standard Operating Procedure OP-STX-33, "Primary Eye Irritation Study," and guidelines promulgated by the EPA for ocular irritation testing (2,3).

#### Group Assignment/Acclimation

Study rabbits were assigned by numerical sequence to two dose groups of 3 males each. These animals were quarantined in the Division of Animal Care and Services for 14 days and acclimated for 7 days in the GLP Suite before dosing. During these periods they were observed daily for signs of illness.

### Dose Levels and Administration

Approximately 83 mg (0.1 ml) of DIGL-RP was administered one time to one 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 second to prevent loss of material. Group 1 was dosed on 3 Dec 85 and Group 2 was dosed on 10 Dec 85.

### Compound Preparation

DIGL-RP was received as pellets and was ground in a liquid nitrogen freezer mill (Spex Industries, Edison, NJ) to a fine gray powder which required no further preparation.

### Test Procedures

On 2 Dec 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 nearest normal appearance was designated for treatment, the contralateral eye serving as an untreated control. On 3 Dec 85, approximately 83 mg of DIGL-RP was placed in the designated eye of each rabbit in this group. Group 2 rabbits underwent the same procedures on 9 and 10 Dec 85, respectively.

### Ocular Examination/Grading

Initially each eye was observed unaided in a darkened room with focal illumination (open 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 (4). During the observations, each eye was also 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

**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.

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, NY) 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. Examination and grading of ocular reactions were performed in this fashion at 1, 4, 24, 48, and 72 hours after dosing. Fluorescein staining was omitted from the 1- and 4-hour observations. Due to an almost total lack of reaction during the 72 hours after dosing, the study was terminated after this observation in accordance with the protocol. All animals were submitted for necropsy. 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.

#### Changes/Deviations

Slit lamp examination was added to the standard observation procedures. The slit lamp enables one to detect subtle reactions not grossly observable and to evaluate more thoroughly those abnormalities which are grossly observable. Color photographic documentation was not performed due to lack of significant response to the test compound. Animal 85F312 was removed from the study after sustaining a broken back on 9 Dec 85. With these exceptions, this study was completed in accordance with the appropriate protocol and addenda. It is believed that none of these changes/deviations affected the performance of the study or the validity of the results.

#### Storage of 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

Tabulation of the Draize-type ocular grading results is presented in Appendix D and a summary of the ocular observations in Appendix E.

### Cornea

DIGL-RP produced no grossly observable effects in the cornea. All treated eyes were assigned zero scores for both opacity and area involvement at all observations after dosing.

Slit lamp examination revealed no corneal reactions attributable to the test compound. Slit lamp observations revealed corneas of normal thickness, indicating lack of edema, and smooth surfaces, indicating epithelial integrity. No staining of corneal epithellum was observed at any of the fluorescein examinations in the treated eyes.

### Iris/Anterior Chamber

No grossly observable reactions were produced in the iris by DIGL-RP. Iridial scores were consistently zero at all observation times.

No iridial abnormalities were detected by slit lamp examination of the treated eyes. Circumiridial vessels and surface morphology were normal at all times after dosing. Close examination of anterior chamber fluid revealed no evidence of the presence of protein or cells (signs of iridial inflammation).

### Lens

The lens was 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.

### Conjunctiva

In this study, DIGL-RP produced only two grossly observable responses: slight conjunctival redness and chemosis. At 1 and 4 hours after dosing, all

of the treated eyes exhibited slight vasodilatation in the bulbar (sclera) or semiunar (nictitating membrane) conjunctiva. Conjunctival redness scores of 1 were assigned to 5 of 5 treated eyes and slit lamp examination confirmed the presence of dilated vessels within the outer layers of the sclera and the nictitating membrane. Chemosis scores of 1 were assigned to 4 of 5 treated eyes. Animal 85F309 continued to have conjunctival redness at 24 hours. Chemosis was not present in any treated eyes at 24 hours.

#### Control Eyes

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

#### Pathology Report

Lesions observed were considered incidental and in no way related to the treatment. The pathologist's report is presented in Appendix F.

### **DISCUSSION**

The primary goal of ocular toxicity testing is to determine the potential for ocular damage resulting from accidental contact of the test compound with the eye. For this purpose the Draize-type irritation test, used in the present study, is especially well-suited. An important feature of this test is that the route and type of exposure (ocular instillation followed by a forced blink) closely mimics potential human exposures.

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 present study (6). These Interagency Regulatory Liaison Group (IRLG) guidelines state: "[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, DIGL-RP produced no positive reactions, as defined by the IRLG. Slight conjunctival redness and slight chemosis were the only responses observed. These reactions, although scorable, did not achieve sufficient severity to warrant consideration as a "positive response." Due to this lack of positive response, DIGL-RP is classified as a nonirritant by the results of the present study.

#### **CONCLUSION**

DIGL-RP exhibited minimal potential to produce ocular irritation under conditions of this study.

## REFERENCES

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, MD: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846.
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. Recommended guidelines for acute eye irritation testing. January 1985.

|                                                     |    |
|-----------------------------------------------------|----|
| Appendix A. Chemical Data .....                     | 11 |
| Appendix B. Animal Data .....                       | 14 |
| Appendix C. Historical Listing of Study Events..... | 15 |
| Appendix D. Tabulated Ocular Data.....              | 16 |
| Appendix E. Summary of Ocular Observations .....    | 18 |
| Appendix F. Pathology Report .....                  | 19 |

**Appendix A: CHEMICAL DATA**

**Chemical Name:** DIGL-RP Solid Propellant

**LAIR Code Number:** TP57

**Physical State:** Solid black cylinders (stick configuration)

**Preparation of test substance for dosing:** The cylinders of DIGL-RP were ground under liquid nitrogen using a Spex freezer mill. After grinding, the powder was sieved through an 80-mesh screen.

**Chemical analysis:**

DEGDN was the only major component of DIGL which could be easily analyzed. For analysis, samples of DIGL powder were added to individual 100 ml volumetric flasks.<sup>1</sup> After dilution to volume with 90% ethanol, a second 1:100 dilution was performed. These solutions were analyzed by HPLC. Standards consisted of solutions of DEGDN in ethanol, ranging in concentration from 164.5 to 670.5 µg/ml. Analysis of DEGDN by HPLC was performed under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA); solvent system, 40% water - 60% acetonitrile; flow rate, 0.9 ml/min; wavelength monitored, 210 nm.<sup>2</sup> Under these conditions, DEGDN eluted with a retention time of approximately 5.4 min. The results from the analysis of standards and DIGL powder samples are presented in Tables 1 and 2.

**Table 1. Analysis of Standards**

| Concentration of<br>Standard (µg/ml) | Peak Area*<br>(x 10 <sup>-7</sup> ) |
|--------------------------------------|-------------------------------------|
| 164.5                                | 0.94                                |
| 191.0                                | 1.09                                |
| 275.5                                | 1.60                                |
| 299.4                                | 1.74                                |
| 362.0                                | 2.08                                |
| 399.6                                | 2.31                                |
| 444.4                                | 2.52                                |
| 539.8                                | 3.07                                |
| 585.0                                | 3.32                                |
| 670.5                                | 3.79                                |

\*Average of 2 determinations

Equation for line by linear regression analysis:

$$Y = 5.62 \times 10^4 X + 3.51 \times 10^5, r^2 = 0.9999$$

**Appendix A (cont.): CHEMICAL DATA**

**Table 2. Analysis of DIGL Powder**

| Weight of DIGL<br>Analyzed (mg) | Dilution<br>Factor | Peak Area<br>( $\times 10^{-7}$ ) | Conc. of DEGDN in<br>DIGL (weight %)* |
|---------------------------------|--------------------|-----------------------------------|---------------------------------------|
| 111.7                           | 100                | 2.45                              | 38.5                                  |
| 112.6                           | 100                | 2.46                              | 38.3                                  |
| 100.1                           | 100                | 2.21                              | 38.7                                  |

\*Calculated using the equation for the standard curve as follows:  

$$= \{[(\text{Peak Area} - 3.51 \times 10^5) / 5.62 \times 10^4] + \text{wgt DIGL (mg)} \times 10.$$

The average value for the concentration of DEGDN in DIGL was 38.5% and this agrees closely with the value of  $36.70 \pm 1.50$  reported in the manufacturer's data sheet.

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

Lot No.: RAD83MO01S169

- 1 Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.
- 2 Wheeler CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p. 58. Letterman Army Institute of Research, Presidio of San Francisco, CA.

**Appendix A (cont.): CHEMICAL DATA****CHEMICAL ANALYSIS FOR DIGL-RP  
(Information from the Manufacturer's Data Sheet)**

| <u>Ingredients</u>                                                         | <u>Finished<br/>Propellant<br/>Percentage</u> |
|----------------------------------------------------------------------------|-----------------------------------------------|
| Nitrocellulose<br>(13.05 $\pm$ 0.05% Nitrogen)<br>(6-12 seconds viscosity) | 62.5 $\pm$ 2.00                               |
| Diethyleneglycol Dinitrate (DEGDN)                                         | 36.70 $\pm$ 1.50                              |
|                                                                            | 0.25                                          |
| Ethyl Centralite (EC)                                                      | 0.25 $\pm$ 0.05                               |
|                                                                            | 0.25                                          |
| Akardit II                                                                 | 0.45 $\pm$ 0.15                               |
| Magnesium Oxide                                                            | 0.05 Max                                      |
| Graphite<br>(Chg 5)                                                        | <u>0.05 Max</u>                               |
| TOTAL                                                                      | 100.0                                         |

**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:** 3 males

**Condition of animals at start of study:** Normal

**Body weight range at dosing:** 2.6 - 2.9 kg

**Identification procedures:**

Ear tattoo: numbers 85F309, 85F310, 85F311, 85F312, 85F313,  
85F314.

**Pretest conditioning:**

1. Quarantine from 14 Nov 85 - 27 Nov 85.
2. Acclimation from 28 Nov 85 - 3 Dec 85.
3. 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**

| <u>Date</u>               | <u>Event</u>                                                                                                           |
|---------------------------|------------------------------------------------------------------------------------------------------------------------|
| 14 Nov 85                 | Animals arrived at LAIR. They were examined for illness and placed under a two-week quarantine.                        |
| 14 - 27 Nov 85            | Animals were checked daily by Division of Animal Care and Services personnel.                                          |
| 15,27 Nov,<br>3,13 Dec 85 | Animals were weighed.                                                                                                  |
| 18 Nov 85                 | They were tattooed and given one application of Canex®/mineral oil into ears for ear mite prevention.                  |
| 27 Nov 85                 | Rabbits were certified healthy by a staff veterinarian removed from quarantine, and assigned to test groups.           |
| 2 Dec 85                  | Animals were checked for preexisting ocular injury (Group 1).                                                          |
| 3 Dec 85                  | Group 1 rabbits were dosed. Eyes were scored 1 and 4 hours after exposure.                                             |
| 4 Dec 85                  | Eyes were scored 24 hours after exposure (Group 1).                                                                    |
| 5 Dec 85                  | Eyes were scored 48 hours after exposure (Group 1).                                                                    |
| 6 Dec 85                  | Eyes were scored 72 hours after exposure. Study was terminated (Group 1).                                              |
| 9 Dec 85                  | Animals were checked for preexisting ocular injury (Group 2). Group 1 animals were weighed and submitted for necropsy. |
| 10 Dec 85                 | Group 2 rabbits were dosed and weighed. Eyes were scored 1 and 4 hours after exposure.                                 |
| 11 Dec 85                 | Eyes were scored 24 hours after exposure (Group 2).                                                                    |
| 12 Dec 85                 | Eyes were scored 48 hours after exposure (Group 2).                                                                    |
| 13 Dec 85                 | Eyes were scored 72 hours after exposure. Study was terminated and animals were submitted for necropsy (Group 2).      |

# Appendix D: TABULATED OCULAR DATA

## CORNEAL OPACITY (score by animal)

| Rabbit<br>Number | Base-<br>Line | <u>1 hr</u> | <u>4 hr</u> | <u>24 hr</u> | <u>48 hr</u> | <u>72 hr</u> |
|------------------|---------------|-------------|-------------|--------------|--------------|--------------|
| 85F309           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F310           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F311           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F313           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F314           | 0             | 0           | 0           | 0            | 0            | 0            |

## IRIS (score by animal)

| Rabbit<br>Number | Base-<br>Line | <u>1 hr</u> | <u>4 hr</u> | <u>24 hr</u> | <u>48 hr</u> | <u>72 hr</u> |
|------------------|---------------|-------------|-------------|--------------|--------------|--------------|
| 85F309           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F310           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F311           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F313           | 0             | 0           | 0           | 0            | 0            | 0            |
| 85F314           | 0             | 0           | 0           | 0            | 0            | 0            |

## Appendix D (cont.): TABULATED OCULAR DATA

CONJUNCTIVA (CHEMOSIS)  
(score by animal)

| Rabbit<br>Number | Base-<br>Line | 1 hr | 4 hr | 24 hr | 48 hr | 72 hr |
|------------------|---------------|------|------|-------|-------|-------|
| 85F309           | 0             | 1    | 1    | 0     | 0     | 0     |
| 85F310           | 0             | 1    | 1    | 0     | 0     | 0     |
| 85F311           | 0             | 0    | 0    | 0     | 0     | 0     |
| 85F313           | 0             | 1    | 1    | 0     | 0     | 0     |
| 85F314           | 0             | 1    | 1    | 0     | 0     | 0     |

CONJUNCTIVA (REDNESS)  
(score by animal)

| Rabbit<br>Number | Base-<br>Line | 1 hr | 4 hr | 24 hr | 48 hr | 72 hr |
|------------------|---------------|------|------|-------|-------|-------|
| 85F309           | 0             | 1    | 1    | 1     | 0     | 0     |
| 85F310           | 0             | 1    | 1    | 0     | 0     | 0     |
| 85F311           | 0             | 1    | 1    | 0     | 0     | 0     |
| 85F313           | 0             | 1    | 1    | 0     | 0     | 0     |
| 85F314           | 0             | 1    | 1    | 0     | 0     | 0     |

## **Appendix E: SUMMARY OF OCULAR OBSERVATIONS**

### **One Hour After Dosing**

Slight hyperemia was present in all 5 test rabbits. This hyperemia was confined to the lower bulbar and palpebral conjunctiva and the nictitating membrane. Chemosis was present in 4 of the 5 test rabbits in the lower conjunctiva and the nictitating membranes. Both the vasodilatation and swelling were visible with the unaided eye. All other structures appeared normal.

### **Four Hours After Dosing**

Slight hyperemia was present in the conjunctiva of all 5 rabbits. Chemosis was present in 4 of the 5 rabbits. All other structures appeared normal.

### **Twenty-four Hours After Dosing:**

Slight hyperemia persisted in 1 rabbit. The chemosis was no longer present. All other structures appeared normal.

### **Forty-eight Hours After Dosing**

All structures examined by slit lamp appeared normal and no fluorescein staining was present.

### **Seventy-two Hours After Dosing**

All structures examined by slit lamp appeared normal and no fluorescein staining was present.

Appendix F: PATHOLOGY REPORT

LAIR Gross Pathology Report  
GLP Study 85023

Test: Eye Irritation

Investigator: Dr. Hiatt, Toxicology Branch

Test Substance: DIGL-RP

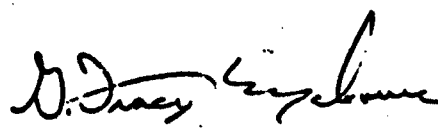
History: Study IAW OP-STX-33. Number of animals: 5. Sex: Male.  
Species: Rabbit, New Zealand White.

Gross findings:

| <u>ANIMAL ID #</u> | <u>LAIR ACCESSION #</u> | <u>FINDINGS</u>     |
|--------------------|-------------------------|---------------------|
| 85F309             | 38672                   | Not remarkable (NR) |
| 85F310             | 38673                   | Pinworm - cecum     |
| 85F311             | 38674                   | NR                  |
| 85F313             | 38679                   | Pinworm - cecum     |
| 85F314             | 38680                   | Pinworm - cecum     |

Comment: The lesions noted were incidental and not related to the treatment.

  
MICHAEL S. SLAYTER, DVM  
MAJ, VC  
Comparative Pathology Branch

  
G. TRACY MAKOVEC, DVM  
CPT, VC  
Diplomate, ACVP  
Comparative Pathology Branch

23 December 1985

## Distribution List

**Commander**

US Army Biomedical Research and  
Development Laboratory (12)  
ATTN: SGRD-UBZ-C  
Fort Detrick, Frederick, MD 21701-5010

**Defense Technical Information Center  
(DTIC) (2)**

ATTN: DTIC-DLA  
Cameron Station  
Alexandria, VA 22304-6145

**US Army Medical Research and  
Development Command (2)**

ATTN: SGRD-RMI-S  
Fort Detrick, Frederick, MD 21701-5012

**Commandant**

Academy of Health Sciences, US Army  
ATTN: AHS-CDM  
Fort Sam Houston, TX 78234

**Chief**

USAEHA Regional Division, West  
Fitzsimmons AMC  
Aurora, CO 80045

**Chief**

USAEHA Regional Division, North  
Fort George G. Meade, MD 20755

**Chief**

USAEHA Regional Division, South  
Bldg. 180  
Fort McPherson, GA 30330

**Commander**

USA Health Services Command  
ATTN: HSPA-P  
Fort Sam Houston, TX 78234

**Commander US Army Materiel  
Command**

ATTN: AMSCG  
5001 Eisenhower Avenue  
Alexandria, VA 22333

**Commander**

US Army Environmental Hygiene  
Agency  
ATTN: Librarian, HSDH-AD-L  
Aberdeen Proving Ground, MD 21010

**Dean**

School of Medicine  
Uniformed Services University of the  
Health Sciences  
4301 Jones Bridge Road  
Bethesda, MD 20014

**Commander**

US Army Materiel Command  
ATTN: AMCEN-A  
5001 Eisenhower Avenue  
Alexandria, VA 22333

**HQDA**

ATTN: DASG-PSP-E  
Falls Church, VA 22041-3258

**HQDA**

ATTN: DAEN-RDM  
20 Massachusetts, NW  
Washington, D.C. 20314

**CDR, US Army Toxic and Hazardous  
Material Agency**

ATTN: DRXTH/ES  
Aberdeen Proving Ground, MD 21010

**Commandant**

Academy of Health Sciences  
United States Army  
ATTN: Chief, Environmental  
Quality Branch  
Preventive Medicine Division  
(HSHA-IPM)  
Fort Sam Houston, TX 78234