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*Institute Report No. 411*

**Dermal Sensitization Potential of  
DIGL-RP Solid Propellant in Guinea Pigs**

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and  
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MAMMALIAN TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY

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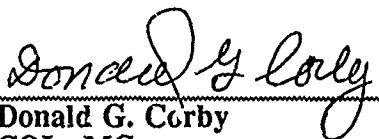
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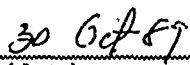
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# ABSTRACT

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DIGL-RP Solid Propellant was evaluated for its potential to produce dermal sensitization in male guinea pigs. The Buehler test, which utilizes repeated closed patch inductions with the test compound, was used for this evaluation. No evidence that DIGL-RP Solid Propellant induced sensitization was obtained in the study. Keywords:

Key Words: Dermal Sensitization, Mammalian Toxicology, DIGL RP Solid Propellant, Diethyleneglycol Dinitrate, Buehler Test, Guinea Pigs, Munition. (AW)

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## **PREFACE**

**TYPE REPORT:** Dermal Sensitization 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/TLBO

**GLP STUDY NO.:** 85026

**STUDY DIRECTOR:** Don W. Korte, Jr., PhD, LTC, MSC  
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**PRINCIPAL INVESTIGATOR:** Yvonne C. LeTellier, BS

**CO-INVESTIGATOR:** Larry D. Brown, DVM, LTC, VC, Diplomate,  
American College of Veterinary Preventive Medicine,  
American Board of Toxicology.

**PATHOLOGIST:** Michael V. Slayter, DVM, MAJ, VC

**REPORT AND DATA MANAGEMENT:**

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.

**TEST SUBSTANCE:** DIGL-RP Solid Propellant

**INCLUSIVE STUDY DATES:** 4 April - 16 May 1986

**OBJECTIVE:**

The objective of the study was to evaluate the dermal sensitization potential of DIGL-RP Solid Propellant in guinea pigs.

## **ACKNOWLEDGMENTS**

SP4 Gregory A. Rothhammer, SP4 Scott Schwebe, SP4 Theresa L. Polk, Obie Goodrich, and Richard Spieler provided technical assistance, animal care, and facilities management.

## SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85026 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. 30 Oct 89*

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

*Yvonne C. Letellier 30 Oct 89*

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DEPARTMENT OF THE ARMY

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26 October 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85026

1. This is to certify that the protocol for LAIR GLP Study 85026 was reviewed on 10 May 1985.
2. The institute report entitled "Dermal Sensitization Potential of DGL-RP Solid Propellant in Guinea Pigs," Toxicology Series 179, was audited on 25 October 1989.

*Carolyn M. Lewis*

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



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# **Dermal Sensitization Potential of DIGL-RP Solid Propellant in Guinea Pigs- LeTellier 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 studies in rats and mice, acute dermal toxicity study 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 dermal sensitization potential of DIGL-RP Solid Propellant in guinea pigs.

## **MATERIALS**

### **Test Substance**

Chemical Name: DIGL-RP Solid Propellant

LAIR Code Number: TP57

Description: Solid black cylinders (stick configuration)

Lot Number: RAD83M001S169

DIGL RP Solid Propellant was received in the stick configuration. It was ground into a fine powder for this study (Appendix A). Other test substance information is presented in Appendix A.

#### Vehicle

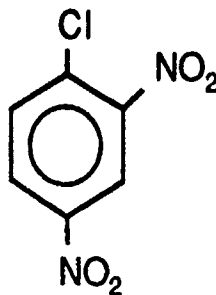
Isotonic saline (Viaflex<sup>®</sup>, Sodium Chloride Injection, USP; Travenol Laboratories, Inc., Deerfield, IL) was used as the vehicle for the test compound and as a component of the positive control vehicle.

#### Positive Control

Chemical Name: Dinitrochlorobenzene (DNCB)

Chemical Abstracts Service Registry No.: 97-00-7

Chemical Structure:



Molecular Formula: C<sub>6</sub>H<sub>3</sub>N<sub>2</sub>O<sub>4</sub>Cl

Other positive control substance information is presented in Appendix A.

#### Vehicle for Positive Control

A 0.1% solution of DNCB was prepared on 14, 21, and 28 April and 12 May 1986. The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Dow Chemical Company (Freeport, TX).

### Animal Data

Sixty male albino guinea pigs, Hartley strain (Charles River Breeding Laboratories, Kingston, NY), from a shipment received on 4 April 1986 were assigned to this study. They were identified individually with ear tags. Two animals (86E0130, 86E0147) were selected for quality control necropsy evaluation on receipt. Animal weights on the day of receipt ranged from 178 to 235 g. Additional animal data appear in Appendix B.

### Husbandry

Guinea pigs assigned to this study were caged individually in stainless steel, wire mesh cages in racks equipped with automatically flushing dump tanks. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow® Diet 5026 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. Temperature within the animal room was maintained in the range from 21.6° to 25.6°C. Relative humidity was maintained in the range of 40% to 59% with spikes to 72% associated with room cleaning. The photoperiod was 12 hours of light per day.

## **METHODS**

This study was conducted in accordance with LAIR SOP-OP-STX 82 "Buehler Dermal Sensitization Test" (2) and EPA guidelines (3).

### Group Assignment/Acclimation

The guinea pigs were quarantined for 12 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Fifteen animals were assigned to each of four groups by a stratified randomization technique based on their body weights.

### Dose Levels

Dermal sensitization potential was evaluated in a test group receiving three weekly induction doses of 100% (w/v) DIGL-RP and, after a two-week delay, a challenge dose at the same concentration. Dinitrochlorobenzene, a known potent sensitizing agent (4), was used as the positive control. It was applied to another group, at a 0.1% concentration, in the same dosing sequence as the test compound. The vehicle control group received isotonic saline only for the three weekly induction doses and the challenge dose. The negative control group received 100% (w/v) DIGL-RP only on the day of challenge dosing.

### Compound Preparation

DIGL-RP was received in the stick configuration and ground in a Spex Industries freezer mill to a fine powder. The ground DIGL-RP was sieved through a 80-mesh screen before mixing with saline to form a 100% (w/v) concentration for testing. A 0.5 g dose of this concentration of DIGL-RP was shown to be non-irritating in pilot studies. The dinitrochlorobenzene (DNCB) dosing solution was prepared by first adding 30 mg DNCB to 1.0 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

### Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (5-7) to mimic the repeated-insult patch test for humans. Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site.

To distinguish between reactions from primary irritation and sensitization, a negative control group was added which received only the challenge dose.

During the induction phase, the test and positive control groups were dosed with 0.5 ml of 100% (w/v) DIGL-RP applied topically under a 2.5-cm<sup>2</sup> gauze patch. This procedure was performed for three consecutive weeks (15, 22, and 29 Apr 86). Twenty-four hours before each dosing, a 7.6-cm<sup>2</sup> area on the left flank of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT). The patch was taped with Blenderm® hypoallergenic surgical tape (3M Corp., St. Paul, MN) to the same site each time, and the animal was wrapped several times with Vet Wrap® (3M Corp., St. Paul, MN). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was gently wiped of any excess compound using a saline-moistened gauze and the site was marked for scoring.

Animals were challenged two weeks (13 May 86) following the third induction dose. Test group and positive control group animals received two 0.5-ml doses each of DIGL-RP or DNCB, respectively, one applied to the old site on the left flank and the other to a new site on the right flank. Negative control animals received only a single 0.5-ml dose of DIGL-RP, applied to the left flank. Vehicle control animals received only a single 0.5-ml dose of saline, applied to the left flank. Procedures for clipping, shaving, and wrapping and the exposure period remained the same.

In Buehler's procedure, skin reactions are scored 24 and 48 hours after the challenge dose only. In the present study, skin reactions were scored 24, 48, and 72 hours after each induction dose as well as 24, 48, and 72 hours after the challenge dose. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema), and 3 (marked erythema). Results are expressed in terms of both incidence (the number of animals showing responses of 1 or greater at either 24, 48, or 72 hours) and severity (the sum of the test scores

divided by the number of animals tested). Results from the left flank are compared with right flank and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-hour exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cages during the exposure period. Buehler and Griffith (7) also recommended depilating the day before the challenge dose. For consistency with induction procedures, this step was replaced by clipping the animals.

The animals were observed daily for clinical signs and weight gain was monitored during the study. At the conclusion of the study, a necropsy was performed on each animal. A historical listing of study events appears in Appendix C.

#### Changes/Deviations

This study was conducted in accordance with the protocol and applicable amendments with two exceptions. Animal 86E0183 of the negative control group had its wrappings in place 24 hrs instead of 6 hrs as scheduled for the challenge dose. Animal 86E0152 of the DIGL-RP treatment group was not shaved the night before the challenge dose and therefore was removed from the study. It is believed that these deviations had no effect on the outcome of this study.

#### 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**

### **Experimental**

Table 1 summarizes the incidence of reactions 24, 48, and 72 hours after each dose. Slight erythema was observed in two animals after the first induction dose of DIGL-RP and in two other animals after the second induction dose of DIGL-RP. No other reactions were observed in the animals in the DIGL-RP test group. This relative lack of response is reflected in Table 2 which depicts the severity of skin reactions. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. For DIGL-RP, the maximum severity scores were 0.13 and 0.07, obtained after the first and second induction doses, respectively.

### **Positive Control**

Dinitrochlorobenzene produced a marked response at all time points (Table 1). All DNCB-treated animals exhibited a response 24 hours following the second or third induction and challenge doses. These reactions persisted, yielding scorable effects in all the animals at 48 hours and at 72 hours after dosing. Severity scores for these responses to DNCB ranged from 0.53 to 1.6 at the 24-hour scoring period (Table 2). By 48 hours the reactions had peaked, ranging from 0.53 to 1.87. At 72 hours the reactions had subsided to the same score as for the 24-hour observation.

### **Negative and Vehicle Controls**

No response was observed in the negative control (challenge dose of DIGL-RP) group or the vehicle control (isotonic saline) group. Individual 24 hour, 48-hour, and 72-hour dermal scores for all animals appear, by group, in Appendix D.

### **Clinical Signs**

All animals were healthy and gained weight during the study. Individual body weight data are presented in Appendix E.

**TABLE 1: Incidences of Skin Reactions**

| <u>Test Group</u>      | <u>Induction</u> |               |              | <u>Challenge</u> |             |
|------------------------|------------------|---------------|--------------|------------------|-------------|
|                        | <u>First</u>     | <u>Second</u> | <u>Third</u> | <u>Right</u>     | <u>Left</u> |
| <b><u>24 Hours</u></b> |                  |               |              |                  |             |
| DNCB                   | 8/15             | 15/15         | 15/15        | 14/15            | 15/15       |
| Vehicle Control        | 0/15             | 0/15          | 0/15         | ---              | 0/15        |
| DIGL RP                | 0/15             | 1/15          | 0/15         | 0/14             | 0/14        |
| Negative Control       | ---              | ---           | ---          | ---              | 0/14        |
| <b><u>48 Hours</u></b> |                  |               |              |                  |             |
| DNCB                   | 8/15             | 15/15         | 15/15        | 14/15            | 15/15       |
| Vehicle Control        | 0/15             | 0/15          | 0/15         | ---              | 0/15        |
| DIGL RP                | 0/15             | 1/15          | 0/15         | 0/14             | 0/14        |
| Negative Control       | ---              | ---           | ---          | ---              | 0/15        |
| <b><u>72 Hours</u></b> |                  |               |              |                  |             |
| DNCB                   | 8/15             | 15/15         | 15/15        | 14/15            | 15/15       |
| Vehicle Control        | 0/15             | 0/15          | 0/15         | ---              | 0/15        |
| DIGL-RP                | 2/15             | 0/15          | 0/15         | 0/14             | 0/14        |
| Negative Control       | ---              | ---           | ---          | ---              | 0/15        |

**TABLE 2: Severity of Skin Reactions**

| <u>Test Group</u>      | <u>Induction</u> |               |              | <u>Challenge</u> |             |
|------------------------|------------------|---------------|--------------|------------------|-------------|
|                        | <u>First</u>     | <u>Second</u> | <u>Third</u> | <u>Right</u>     | <u>Left</u> |
| <b><u>24 Hours</u></b> |                  |               |              |                  |             |
| DNCB                   | 0.53             | 1.47          | 1.47         | 1.33             | 1.6         |
| Vehicle Control        | 0.0              | 0.0           | 0.0          | ---              | 0.0         |
| DIGL-RP                | 0.0              | 0.07          | 0.0          | 0.0              | 0.0         |
| Negative Control       | ---              | ---           | ---          | ---              | 0.0         |
| <b><u>48 Hours</u></b> |                  |               |              |                  |             |
| DNCB                   | 0.53             | 1.47          | 1.2          | 1.47             | 1.87        |
| Vehicle Control        | 0.0              | 0.0           | 0.0          | ---              | 0.0         |
| DIGL-RP                | 0.0              | 0.07          | 0.0          | 0.0              | 0.0         |
| Negative Control       | ---              | ---           | ---          | ---              | 0.0         |
| <b><u>72 Hours</u></b> |                  |               |              |                  |             |
| DNCB                   | 0.53             | 1.53          | 1.4          | 1.33             | 1.6         |
| Vehicle Control        | 0.0              | 0.0           | 0.0          | ---              | 0.0         |
| DIGL-RP                | 0.13             | 0.0           | 0.0          | 0.0              | 0.0         |
| Negative Control       | ---              | ---           | ---          | ---              | 0.0         |

## **Pathology Findings**

A necropsy was performed on all study animals. Lobular liver necrosis was identified in at least half of the study animals. This is a common finding of unknown etiology in otherwise healthy guinea pigs. The complete pathology report is presented in Appendix F.

## **DISCUSSION**

### **Dermal Irritation and Sensitization**

Most skin reactions occurring from contact with chemicals can be classified as either irritation or sensitization. Both reactions present as inflammation of the skin; the difference between irritation and sensitization is the mechanism responsible for this inflammation. Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild reversible inflammation to severe ulceration progressing to necrosis. Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical (8). Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. Although both types of reactions can appear grossly similar in experimental animals and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, whereas sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, whereas only a fraction of the population normally becomes sensitized to the same chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction

(a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, whereas the reaction to a sensitizer often increases over 2 to 4 exposures after the initial contact. An irritant produces inflammation of rapid onset with short duration, whereas a sensitization reaction is somewhat delayed and prolonged. The inflammatory response to an irritant may spread beyond the area of contact, whereas sensitization reactions are usually circumscribed.

The features of irritation and sensitization have been used to establish guidelines for differentiation between the two (5-8). In evaluating a dermal sensitization study it is recommended that the results from a challenge dose in the experimental group (sensitization) be compared with those for the negative control group (irritation) in accordance with the following criteria:

**Irritative Responses:**

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- usually fade within 24 to 48 hours, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

**Sensitization Reactions:**

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly, with the intensity/severity of inflammation often greater at 72 to 96 hours than at 24 to 48 hours.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy potential is evaluated by the method of Draize et al. (9) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory reaction is graded. Evaluation of sensitizing potential is accomplished by repeated application, at lower nonirritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow

the immune system to elaborate and increase its specific response to the chemical. A challenge dose is then given, and the resulting inflammatory response is graded. Analysis of the incidence, severity, and timing of the response to the challenge dose estimates the sensitizing potential of the study compound.

#### DIGL-RP Solid Propellant

DIGL-RP Solid Propellant was evaluated for its ability to elicit a delayed hypersensitivity or cellular immunologic reaction via contact with the skin. DIGL-RP produced no response indicative of the potential to elicit dermal sensitization when evaluated according to the method of Buehler and Griffith (5-7).

Sensitization produced by DIGL-RP would have been detected by this study. A hypersensitivity-type response was reliably elicited by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously at the Letterman Army Institute of Research (10). Although DNCB is capable of producing primary irritation, the characteristics of the responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also, the response to DNCB was observed primarily after two or more exposures.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than does man, this result does not guarantee that DIGL-RP will not sensitize humans. However, it does indicate that DIGL-RP Solid Propellant is unlikely to sensitize humans and its potential is low enough to permit its evaluation in man.

#### **CONCLUSION**

DIGL-RP Solid Propellant possesses minimal sensitizing potential, as it did not induce a dermal sensitization reaction under conditions of this study.

## REFERENCES

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**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 Analyzed (mg) | Dilution Factor | Peak Area ( $\times 10^{-7}$ ) | Conc. of DEGDN in 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.

**Stability:**

The aqueous stability of the DEGDN component in the DIGL powder was examined.<sup>3</sup> The amount of DEGDN in aqueous DIGL suspensions was determined immediately after preparation of a suspension and again 24 hrs later. The study was conducted as follows. A suspension of DIGL in 1% gum tragacanth (200 mg/ml) was prepared. Three 1 ml aliquots were removed from the suspension immediately after preparation and again 24 hrs later. The 1 ml samples were transferred to individual 100 ml volumetric flasks. After diluting to volume with ethanol, the flasks were shaken well. A sample from each was analyzed by HPLC as described above. The average of the peak area values was  $4.03 \pm 0.12$  for the 0 time samples and  $4.10 \pm 0.14$  for the 24-hour samples. These results indicate that there was no decomposition of DEGDN in 1% gum tragacanth for a period of 24 hours.

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

Lot No.: RAD83M001S159

<sup>1</sup> Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>2</sup> Wheeler CW. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p. 58. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>3</sup> Wheeler CW. Toxicity Testing of Propellents. Laboratory Notebook #85-12-023, p. 24-42. Letterman Army Institute of Research, Presidio of San Francisco, CA.

**Appendix A (cont.): CHEMICAL ANALYSIS****Manufacturer's Data Sheet for DIGL-RP Formulation**

| <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                              |
| Ethyl Centralite (EC)  | 0.25<br>0.25 $\pm$ 0.05                       |
| Akardit II   | 0.25<br>0.45 $\pm$ 0.15                       |
| Magnesium Oxide  | 0.05 Max                                      |
| Graphite<br>(Chg 5)  | <u>0.05 Max</u><br>100.00                     |

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

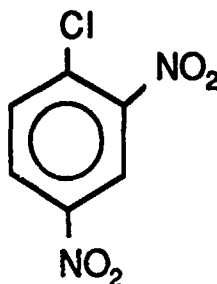
**POSITIVE CONTROL**

Chemical Name: 1-Chloro-2,4-dinitrobenzene

Alternate Chemical Name: 2,4-Dinitrochlorobenzene

Chemical Abstracts Service Registry Number: 97-00-7

Chemical Structure:



Molecular Formula: C<sub>6</sub>H<sub>3</sub>N<sub>2</sub>O<sub>4</sub>Cl

Molecular Weight: 202.6

Physical State: Yellow crystals

Melting Point: 52-54° C<sup>1</sup>

Purity: The compound was designated as 95% pure by source.

Analytical Data: Chemical analysis was performed as follows: Infrared spectra were obtained with a Perkin-Elmer 983 spectrometer.<sup>2</sup> Proton magnetic resonance (NMR) spectra were recorded on a Varian XL300 instrument with tetramethylsilane as the internal standard and chemical shifts expressed as parts per million (d).<sup>3</sup> Low resolution GC-MS analysis was performed with a Kratos MS-25RFA (30 m DB-1 capillary column).<sup>4</sup>

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<sup>1</sup> Windholz M, ed. The Merck Index. 10th ed. Rahway, NJ: Merck and Co., Inc., 1983:300.

<sup>2</sup> Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, p. 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>3</sup> *Ibid.* p. 11-12.

<sup>4</sup> *Ibid.* p. 13-16.

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

The following data were obtained: IR (KBr): 3443, 3104, 2877, 1963, 1829, 1801, 1756, 1705, 1604, 1591, 1542, 1349, 1246, 1156, 1046, 917, 902, 850, 835, 749, 732  $\text{cm}^{-1}$ . The IR spectrum was very close to the Sadtler reference spectrum.<sup>5</sup> Differences were due to the much finer spectral resolution obtained on the P-E 983 instrument. NMR ( $\text{CDCl}_3$ ): d 7.78 (1 H, d,  $J = 8.7$  Hz), 8.38 (1 H, q,  $J_{\text{ortho}} = 8.7$  Hz,  $J_{\text{meta}} = 3.6$  Hz), 8.74 (1 H, d,  $J_{\text{meta}} = 2.4$  Hz). The spectrum of DNCB was identical to the Aldrich reference spectrum.<sup>6</sup> GC-MS Analysis: A plot of the total ion current versus scan number showed one major peak for DNCB with only traces of other compounds (not identified). Molecular ion masses ( $m/z$ ) of 202 and 204 confirmed the identity of the major peak as DNCB.<sup>7</sup>

Lot Number: 11F-0543

Source: Sigma Chemical Co.  
St. Louis, MO

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<sup>5</sup> Sadtler Research Laboratory, Inc., Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared spectrogram #964.

<sup>6</sup> Pouchert CJ. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

<sup>7</sup> Wheeler CR. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, p. 13-15. Letterman Army Institute of Research, Presidio of San Francisco, CA.

**Appendix B: ANIMAL DATA**

**Species:** *Cavia porcellus*

**Strain:** Hartley, albino

**Source:** Charles River Breeding Laboratories  
Kingston, NY

**Sex:** Male

**Date of Birth:** 17 March 1986

**Method of randomization:** Weight bias, stratified animal allocation

**Animals in each group:** 15 male animals

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

**Identification procedures:** Ear tag.

**Pretest conditioning:** Quarantine/acclimation 4 - 15 April 1986

**Body weight at dosing:** 252 - 361 g

**Justification:** The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

**Appendix C: HISTORICAL LISTING OF STUDY EVENTS**

| <u>Date</u>                         | <u>Event</u>  |
|-------------------------------------|---|
| 4 Apr 86                            | Animals arrived at LAIR. Animals were examined, weighed, placed in cages, and fed. Animals were assigned ear tags. Two animals were submitted for necropsy quality control. |
| 4-15 Apr 86                         | Animals were checked daily.   |
| 4,7,14,21,28 Apr,<br>5,12,16 May 86 | Animals were weighed.   |
| 14 Apr 86                           | Animals were randomized into four groups (experimental, positive control, negative control, vehicle control) of 15 animals each.  |
| 14,21,28 Apr 86                     | Study animals, except negative control group, were clipped and shaved.  |
| 15,22,29 Apr 86                     | Study animals, except negative control group, were given induction dose.  |
| 16,23,30 Apr 86                     | Study animals, except negative control group , were scored for 24-hr skin reaction.   |
| 17,24 Apr, 1 May 86                 | Study animals, except negative control group , were scored for 48-hr reaction.  |
| 18,25 Apr, 2 May 86                 | Study animals, except negative control group , were scored for 72-hr reaction.  |
| 12 May 86                           | Study animals were clipped and shaved.  |
| 13 May 86                           | Study animals were given challenge dose.  |
| 14 May 86                           | Study animals were scored for 24-hr reaction.   |
| 15 May 86                           | Study animals were scored for 48-hr reaction.   |
| 16 May 86                           | Study animals were scored for 72-hr reaction. All animals were delivered to Necropsy Suite.   |

## Appendix D: INDIVIDUAL ANIMAL SCORES

| GROUP: ONE    |  | COMPOUND: DNCE  |      |      |   |                  |      |      |   |                 |      |      |   |
|---------------|--|-----------------|------|------|---|------------------|------|------|---|-----------------|------|------|---|
|               |  | CHALLENGE DOSE  |      |      |   |                  |      |      |   |                 |      |      |   |
|               |  | FIRST INDUCTION |      |      |   | SECOND INDUCTION |      |      |   | THIRD INDUCTION |      |      |   |
| ANIMAL NUMBER |  | 24 H            | 48 H | 72 H |   | 24 H             | 48 H | 72 H |   | 24 H            | 48 H | 72 H |   |
|               |  |                 |      |      |   |                  |      |      |   |                 |      |      |   |
| 86E0131       |  | 1               | 1    | 1    | 1 | 2                | 2    | 2    | 1 | 2               | 1    | 2    | 1 |
| 86E0133       |  | 1               | 1    | 1    | 1 | 2                | 1    | 1    | 1 | 2               | 2    | 2    | 1 |
| 86E0138       |  | 0               | 0    | 0    | 0 | 2                | 2    | 2    | 1 | 2               | 2    | 1    | 2 |
| 86E0140       |  | 1               | 0    | 0    | 0 | 2                | 2    | 2    | 2 | 1               | 1    | 1    | 2 |
| 86E0143       |  | 1               | 1    | 1    | 1 | 2                | 2    | 2    | 2 | 1               | 2    | 2    | 2 |
| 86E0150       |  | 0               | 0    | 1    | 1 | 1                | 1    | 1    | 1 | 1               | 1    | 1    | 1 |
| 86E0151       |  | 1               | 1    | 0    | 0 | 1                | 1    | 2    | 1 | 1               | 1    | 1    | 1 |
| 86E0155       |  | 0               | 1    | 1    | 1 | 1                | 1    | 1    | 1 | 2               | 1    | 1    | 1 |
| 86E0165       |  | 1               | 1    | 1    | 1 | 1                | 1    | 1    | 1 | 2               | 1    | 1    | 1 |
| 86E0167       |  | 0               | 0    | 1    | 1 | 2                | 2    | 2    | 1 | 1               | 2    | 1    | 2 |
| 86E0187       |  | 1               | 1    | 0    | 0 | 1                | 2    | 2    | 2 | 2               | 2    | 2    | 2 |
| 86E0191       |  | 0               | 0    | 0    | 0 | 1                | 1    | 1    | 1 | 1               | 1    | 1    | 1 |
| 86E0192       |  | 1               | 1    | 1    | 1 | 1                | 1    | 1    | 1 | 1               | 2    | 2    | 1 |
| 86E0193       |  | 0               | 0    | 0    | 0 | 2                | 1    | 2    | 2 | 1               | 0    | 0    | 2 |
| 86E0195       |  | 0               | 0    | 0    | 0 | 1                | 2    | 1    | 1 | 1               | 2    | 2    | 2 |



## Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

| GROUP: TWQ    |  | COMPOUND: ISOTONIC SALINE |      |      |                  |      |      |                 |      |      |             |      |      |
|---------------|--|---------------------------|------|------|------------------|------|------|-----------------|------|------|-------------|------|------|
|               |  | CHALLENGE DOSE            |      |      |                  |      |      |                 |      |      |             |      |      |
|               |  | FIRST INDUCTION           |      |      | SECOND INDUCTION |      |      | THIRD INDUCTION |      |      | RIGHT FLANK |      |      |
| ANIMAL NUMBER |  | 24 H                      | 48 H | 72 H | 24 H             | 48 H | 72 H | 24 H            | 48 H | 72 H | 24 H        | 48 H | 72 H |
| 86E0145       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0148       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0156       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0159       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0164       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0166       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0168       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0170       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0174       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0177       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0180       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0181       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0182       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0185       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |
| 86E0186       |  | 0                         | 0    | 0    | 0                | 0    | 0    | 0               | 0    | 0    | N/A         | N/A  | N/A  |

## Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

| GROUP: THREE  |  | COMPOUND: DIGL-RP |      |      |  |                  |      |      |  |                 |      |      |  |
|---------------|--|-------------------|------|------|--|------------------|------|------|--|-----------------|------|------|--|
|               |  | CHALLENGE DOSE    |      |      |  |                  |      |      |  |                 |      |      |  |
|               |  | FIRST INDUCTION   |      |      |  | SECOND INDUCTION |      |      |  | THIRD INDUCTION |      |      |  |
| ANIMAL NUMBER |  | 24 H              | 48 H | 72 H |  | 24 H             | 48 H | 72 H |  | 24 H            | 48 H | 72 H |  |
|               |  |                   |      |      |  |                  |      |      |  |                 |      |      |  |
| 86E0134       |  | 0                 | 0    | 1    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0135       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0144       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0149       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0152       |  | 0                 | 0    | 1    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0161       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0162       |  | 0                 | 0    | 0    |  | 1                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0163       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0169       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0171       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0178       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0184       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0188       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0190       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |
| 86E0194       |  | 0                 | 0    | 0    |  | 0                | 0    | 0    |  | 0               | 0    | 0    |  |

\* Animal not shaved as scheduled, so removed from study.

## Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

| GROUP: <u>FOUR</u>   |      | COMPOUND: <u>NEGATIVE CONTROL</u> |      |      |                         |      |      |                        |      |      |                    |      |                   |      |      |   |
|----------------------|------|-----------------------------------|------|------|-------------------------|------|------|------------------------|------|------|--------------------|------|-------------------|------|------|---|
|                      |      | <u>CHALLENGE DOSE</u>             |      |      |                         |      |      |                        |      |      |                    |      |                   |      |      |   |
|                      |      | <u>FIRST INDUCTION</u>            |      |      | <u>SECOND INDUCTION</u> |      |      | <u>THIRD INDUCTION</u> |      |      | <u>RIGHT FLANK</u> |      | <u>LEFT FLANK</u> |      |      |   |
| <u>ANIMAL NUMBER</u> | 24_H | 48_H                              | 72_H | 24_H | 48_H                    | 72_H | 24_H | 48_H                   | 72_H | 24_H | 48_H               | 72_H | 24_H              | 48_H | 72_H |   |
| 86E0136              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0137              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0141              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0142              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0146              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0153              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0154              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0157              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0158              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0160              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0173              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0176              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0179              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |
| 86E0183              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | N/A† | 0    | 0 |
| 86E0189              | N/A  | N/A                               | N/A  | N/A  | N/A                     | N/A  | N/A  | N/A                    | N/A  | N/A  | N/A                | N/A  | N/A               | 0    | 0    | 0 |

† Wrappings were left in place 24 hours, so animal was not scored.

**Appendix E: INDIVIDUAL BODY WEIGHTS (grams)****DNCB**

| <b>Animal<br/>Number</b> | <b>DAY OF STUDY</b> |           |            |          |           |           |           |           |
|--------------------------|---------------------|-----------|------------|----------|-----------|-----------|-----------|-----------|
|                          | <b>0*0</b>          | <b>06</b> | <b>013</b> | <b>7</b> | <b>14</b> | <b>21</b> | <b>28</b> | <b>32</b> |
| 86E0131                  | 192                 | 223       | 275        | 307      | 337       | 373       | 406       | 398       |
| 86E0133                  | 217                 | 248       | 310        | 350      | 399       | 451       | 505       | 510       |
| 86E0138                  | 194                 | 223       | 266        | 292      | 324       | 348       | 384       | 384       |
| 86E0140                  | 190                 | 223       | 263        | 307      | 337       | 386       | 425       | 432       |
| 86E0143                  | 229                 | 284       | 361        | 420      | 481       | 558       | 613       | 630       |
| 86E0150                  | 196                 | 220       | 260        | 300      | 347       | 371       | 411       | 406       |
| 86E0151                  | 199                 | 229       | 290        | 320      | 358       | 400       | 433       | 430       |
| 86E0155                  | 206                 | 232       | 280        | 318      | 364       | 414       | 459       | 461       |
| 86E0165                  | 197                 | 232       | 285        | 318      | 353       | 396       | 437       | 425       |
| 86E0167                  | 214                 | 248       | 284        | 312      | 348       | 381       | 415       | 414       |
| 86E0187                  | 210                 | 249       | 302        | 347      | 384       | 422       | 478       | 484       |
| 86E0191                  | 210                 | 252       | 296        | 334      | 374       | 429       | 479       | 485       |
| 86E0192                  | 217                 | 245       | 287        | 304      | 340       | 380       | 426       | 425       |
| 86E0193                  | 195                 | 229       | 269        | 312      | 329       | 367       | 399       | 398       |
| 86E0195                  | 217                 | 248       | 282        | 313      | 354       | 395       | 435       | 441       |
| MEAN                     | 205.5               | 239.0     | 287.3      | 323.6    | 361.9     | 404.7     | 447.0     | 448.2     |
| Standard<br>Deviation    | 11.7                | 16.9      | 24.8       | 31.2     | 38.7      | 50.1      | 56.5      | 61.8      |
| Standard<br>Error        | 3.0                 | 4.4       | 6.4        | 8.0      | 10.0      | 12.9      | 14.6      | 16.0      |

\* Q represents quarantine period.

**Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)****ISOTONIC SALINE**

| <b>Animal<br/>Number</b> | <b>DAY OF STUDY</b> |                  |                   |                 |                  |                  |                  |                  |
|--------------------------|---------------------|------------------|-------------------|-----------------|------------------|------------------|------------------|------------------|
|                          | <b><u>0*0</u></b>   | <b><u>06</u></b> | <b><u>013</u></b> | <b><u>7</u></b> | <b><u>14</u></b> | <b><u>21</u></b> | <b><u>28</u></b> | <b><u>32</u></b> |
| 86E0145                  | 217                 | 258              | 313               | 356             | 391              | 446              | 501              | 519              |
| 86E0148                  | 227                 | 253              | 302               | 340             | 373              | 398              | 440              | 444              |
| 86E0156                  | 187                 | 218              | 274               | 328             | 368              | 412              | 466              | 471              |
| 86E0159                  | 212                 | 242              | 290               | 322             | 362              | 392              | 439              | 445              |
| 86E0164                  | 194                 | 228              | 275               | 332             | 377              | 415              | 461              | 470              |
| 86E0166                  | 184                 | 210              | 255               | 292             | 320              | 360              | 397              | 404              |
| 86E0168                  | 226                 | 263              | 282               | 324             | 362              | 405              | 454              | 450              |
| 86E0170                  | 221                 | 276              | 333               | 376             | 431              | 474              | 533              | 537              |
| 86E0174                  | 199                 | 240              | 295               | 338             | 375              | 427              | 460              | 468              |
| 86E0177                  | 197                 | 226              | 284               | 337             | 366              | 414              | 458              | 483              |
| 86E0180                  | 197                 | 222              | 265               | 315             | 356              | 403              | 457              | 460              |
| 86E0181                  | 203                 | 230              | 260               | 288             | 319              | 338              | 370              | 366              |
| 86E0182                  | 189                 | 236              | 279               | 335             | 380              | 420              | 469              | 479              |
| 86E0185                  | 192                 | 226              | 290               | 331             | 371              | 405              | 445              | 453              |
| 86E0186                  | 209                 | 243              | 283               | 321             | 346              | 391              | 423              | 426              |
| MEAN                     | 203.6               | 238.1            | 285.3             | 329.0           | 366.5            | 406.7            | 451.5            | 458.3            |
| Standard<br>Deviation    | 14.2                | 18.2             | 20.2              | 21.8            | 27.0             | 31.9             | 38.4             | 41.7             |
| Standard<br>Error        | 3.7                 | 4.7              | 5.2               | 5.6             | 7.0              | 8.2              | 9.9              | 10.8             |

\* Q represents quarantine period.

**Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)**

**DIGL-RP SOLID PROPELLANT**

| <u>Animal<br/>Number</u> | <u>DAY OF STUDY</u> |           |            |          |           |           |           |           |
|--------------------------|---------------------|-----------|------------|----------|-----------|-----------|-----------|-----------|
|                          | <u>Q*Q</u>          | <u>Q6</u> | <u>Q13</u> | <u>7</u> | <u>14</u> | <u>21</u> | <u>28</u> | <u>32</u> |
| 86E0134                  | 203                 | 234       | 264        | 300      | 335       | 375       | 397       | 405       |
| 86E0135                  | 188                 | 218       | 274        | 299      | 349       | 380       | 437       | 440       |
| 86E0144                  | 192                 | 230       | 282        | 324      | 369       | 415       | 471       | 478       |
| 86E0149                  | 228                 | 270       | 318        | 357      | 398       | 417       | 467       | 475       |
| 86E0152                  | 195                 | 229       | 274        | 312      | 348       | 398       | 480       | 459       |
| 86E0161                  | 203                 | 230       | 280        | 326      | 373       | 410       | 462       | 471       |
| 86E0162                  | 205                 | 224       | 267        | 327      | 386       | 441       | 491       | 497       |
| 86E0163                  | 180                 | 212       | 252        | 301      | 338       | 376       | 420       | 426       |
| 86E0169                  | 210                 | 256       | 298        | 340      | 386       | 440       | 486       | 488       |
| 86E0171                  | 204                 | 233       | 292        | 337      | 388       | 425       | 473       | 484       |
| 86E0178                  | 220                 | 254       | 305        | 343      | 378       | 411       | 460       | 468       |
| 86E0184                  | 182                 | 221       | 286        | 332      | 374       | 429       | 490       | 495       |
| 86E0188                  | 212                 | 236       | 286        | 335      | 384       | 436       | 490       | 479       |
| 86E0190                  | 235                 | 272       | 301        | 334      | 370       | 401       | 441       | 445       |
| 86E0194                  | 211                 | 247       | 280        | 320      | 341       | 362       | 392       | 400       |
| MEAN                     | 204.5               | 237.7     | 283.9      | 325.8    | 367.8     | 407.7     | 457.1     | 460.7     |
| Standard<br>Deviation    | 15.8                | 18.2      | 17.1       | 16.9     | 20.4      | 25.3      | 32.9      | 31.0      |
| Standard<br>Error        | 4.1                 | 4.7       | 4.4        | 4.4      | 5.3       | 6.5       | 8.5       | 8.0       |

\* Q represents quarantine period.

**Appendix E (cont.): INDIVIDUAL BODY WEIGHTS (grams)****Negative Control**

| <u>Animal<br/>Number</u> | <u>DAY OF STUDY</u> |           |            |          |           |           |           |           |
|--------------------------|---------------------|-----------|------------|----------|-----------|-----------|-----------|-----------|
|                          | <u>0*0</u>          | <u>06</u> | <u>013</u> | <u>7</u> | <u>14</u> | <u>21</u> | <u>28</u> | <u>32</u> |
| 86E0136                  | 185                 | 217       | 254        | 301      | 334       | 369       | 409       | 410       |
| 86E0137                  | 195                 | 226       | 282        | 330      | 369       | 399       | 437       | 440       |
| 86E0141                  | 207                 | 252       | 297        | 351      | 393       | 445       | 484       | 485       |
| 86E0142                  | 230                 | 265       | 305        | 363      | 413       | 468       | 512       | 520       |
| 86E0146                  | 190                 | 227       | 282        | 332      | 375       | 440       | 486       | 487       |
| 86E0153                  | 222                 | 254       | 305        | 333      | 389       | 428       | 474       | 469       |
| 86E0154                  | 206                 | 245       | 289        | 335      | 386       | 433       | 478       | 477       |
| 86E0157                  | 188                 | 227       | 280        | 327      | 381       | 440       | 486       | 495       |
| 86E0158                  | 211                 | 262       | 321        | 380      | 432       | 486       | 542       | 546       |
| 86E0160                  | 208                 | 232       | 279        | 326      | 375       | 416       | 469       | 461       |
| 86E0173                  | 193                 | 227       | 267        | 318      | 364       | 409       | 451       | 469       |
| 86E0176                  | 178                 | 217       | 260        | 319      | 363       | 410       | 467       | 463       |
| 86E0179                  | 211                 | 238       | 285        | 338      | 388       | 422       | 479       | 478       |
| 86E0183                  | 200                 | 243       | 291        | 341      | 379       | 418       | 454       | 437       |
| 86E0189                  | 183                 | 218       | 266        | 319      | 354       | 404       | 449       | 452       |
| MEAN                     | 200.5               | 236.7     | 284.2      | 334.2    | 379.7     | 425.8     | 471.8     | 472.6     |
| Standard<br>Deviation    | 14.9                | 16.1      | 18.2       | 19.3     | 23.4      | 28.5      | 31.2      | 33.2      |
| Standard<br>Error        | 3.8                 | 4.2       | 4.7        | 5.0      | 6.0       | 7.4       | 8.1       | 8.6       |

\* Q represents quarantine period.

## Appendix F: PATHOLOGY REPORT

GLP Study # 85026

Principal Investigator: Y. Johnson APC# LLE0

### I. INTRODUCTION

Study: Buehler Dermal Sensitization  
 Animals: Guinea Pig (Hartly-Albino)/2 months/male  
 Reference: SOP-OP-STX-84

### II. SUMMARY OF PROCEDURES

Euthanasia: Sodium Pentobarbital.  
 Fixative: 10% buffered formalin.  
 Histopathology: None  
 Clinical Lab: None

### III. GROSS FINDINGS

DOSE GROUP 1 - POSITIVE CONTROL (DNCH)  
 (All live animals)

| LAIR ACC# | ANIMAL ID# | OBSERVATION                            |
|-----------|------------|--|
| 39559     | 86D00131   | Not remarkable (NR)                    |
| 39560     | 86D00133   | Liver - multiple white foci, minimal.  |
| 39565     | 86D00138   | NR                                     |
| 39566     | 86D00140   | Liver - multiple white foci, mild.     |
| 39569     | 86D00143   | Liver - multiple white foci, minimal.  |
| 39575     | 86D00150   | Liver - multiple white foci, minimal.  |
| 39576     | 86D00151   | NR                                     |
| 39580     | 86D00155   | Liver - multiple white foci, minimal.  |
| 39590     | 86D00165   | Liver - multiple white foci, minimal.  |
| 39592     | 86D00167   | Liver - multiple white foci, moderate. |
| 39610     | 86D00187   | Liver - multiple white foci, minimal.  |
| 39614     | 86D00191   | NR                                     |
| 39615     | 86D00192   | Liver - multiple white foci, minimal.  |
| 39616     | 86D00193   | Liver - multiple white foci, mild.     |
| 39618     | 86D00195   | NR                                     |



## Appendix F (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 85026

DOSE GROUP 2 - SALINE CONTROL  
(All live animals)

| LAIR ACC# | ANIMAL ID# | OBSERVATION                           |
|-----------|------------|---------------------------------------|
| 39571     | 86D00145   | Liver - Multiple white foci, minimal. |
| 39573     | 86D00148   | Liver - multiple white foci, minimal. |
| 39581     | 86D00156   | Liver - multiple white foci, minimal. |
| 39584     | 86D00159   | Liver - multiple white foci, minimal. |
| 39589     | 86D00164   | NR                                    |
| 39591     | 86D00166   | Liver - multiple white foci, minimal. |
| 39593     | 86D00168   | NR                                    |
| 39595     | 86D00170   | Liver - multiple white foci, minimal. |
| 39598     | 86D00174   | Liver - multiple white foci, minimal. |
| 39600     | 86D00177   | Liver - multiple white foci, minimal. |
| 39603     | 86D00180   | Liver - multiple white foci, minimal. |
| 39604     | 86D00181   | Liver - multiple white foci, minimal. |
| 39605     | 86D00182   | NR                                    |
| 39608     | 86D00185   | NR                                    |
| 39609     | 86D00186   | Liver - multiple white foci, minimal. |

DOSE GROUP 3 - DIGL-RP  
(All live animals)

| LAIR ACC# | ANIMAL ID# | OBSERVATION                           |
|-----------|------------|---------------------------------------|
| 39561     | 86D00134   | NR                                    |
| 39562     | 86D00135   | NR                                    |
| 39570     | 86D00144   | Liver - multiple white foci, minimal. |
| 39574     | 86D00149   | NR                                    |
| 39577     | 86D00152   | NR                                    |
| 39586     | 86D00161   | NR                                    |
| 39587     | 86D00162   | NR                                    |
| 39588     | 86D00163   | Liver - multiple white foci, minimal. |

## Appendix F (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 85026

## DOSE GROUP 3 (Continued)

|       |          |                                       |
|-------|----------|---------------------------------------|
| 39594 | 86D00169 | NR                                    |
| 39596 | 86D00171 | NR                                    |
| 39601 | 86D00178 | NR                                    |
| 39607 | 86D00184 | NR                                    |
| 39611 | 86D00188 | Liver - minimal white foci.           |
| 39613 | 86D00190 | Liver - multiple white foci, minimal. |
| 39617 | 86D00194 | NR                                    |

DOSE GROUP 4 - NEGATIVE CONTROL  
(All live animals)

| LAIR ACC# | ANIMAL ID# | OBSERVATION                           |
|-----------|------------|---------------------------------------|
| -----     | -----      | -----                                 |
| 39563     | 86D00136   | NR                                    |
| 39564     | 86D00137   | NR                                    |
| 39567     | 86D00141   | Liver - multiple white foci, minimal. |
| 39568     | 86D00142   | NR                                    |
| 39572     | 86D00146   | Liver - multiple white foci, minimal. |
| 39578     | 86D00153   | Liver - multiple white foci, minimal. |
| 39579     | 86D00154   | NR                                    |
| 39582     | 86D00157   | Liver - multiple white foci, mild.    |
| 39583     | 86D00158   | NR                                    |
| 39585     | 86D00160   | Liver - multiple white foci, minimal. |
| 39597     | 86D00173   | Liver - multiple white foci, minimal. |
| 39599     | 86D00176   | NR                                    |
| 39602     | 86D00179   | NR                                    |
| 39606     | 86D00183   | Liver - multiple white foci, minimal. |
| 39612     | 86D00189   | NR                                    |

## Appendix F (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 85026

IV. SUMMARY COMMENTS: Lobular liver necrosis in guinea pigs is not an uncommon finding in normal animals and, as yet, has an unexplained etiology. No significant gross lesions were present in any of the animals included in this study.



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C, Comparative Pathology Branch

5 June 1986

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