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Dermal Sensitization Potential of  
Triethyleneglycol Dinitrate (TEGDN) in Guinea Pigs

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MAMMALIAN TOXICOLOGY BRANCH  
DIVISION OF TOXICOLOGY

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## ABSTRACT

Triethyleneglycol dinitrate (TEGDN) 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 of TEGDN-induced sensitization was obtained in the study.

**Key Words:** Dermal Sensitization, Mammalian Toxicology, Triethyleneglycol Dinitrate (TEGDN), Buehler Test, Guinea Pigs, Propellant, Munitions

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## **PREFACE**

**TYPE REPORT:** Dermal Sensitization GLP Study Report

**TESTING FACILITY:**

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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.:** 84046

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

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

**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:** Triethyleneglycol Dinitrate (TEGDN)

**INCLUSIVE STUDY DATES:** 11 February - 8 April 1985

**OBJECTIVE:**

The objective of the study was to evaluate the dermal sensitization potential of triethyleneglycol dinitrate in guinea pigs.

## **ACKNOWLEDGMENTS**

SP4 Paul B. Simboli, BS, SP4 John R.G. Ryabik, BS, Gerald F.S. Hiatt, PhD, and Yvonne C. Johnson, BS, assisted with the research. Richard D. Spieler, Charlotte Speckman, SP4 James J. Fisher, and SP4 Scott Schwebe provided animal care and managed the facilities. Colleen Kamiyama and Ann Wilkinson provided office management during the performance of the study and preparation of the report.

**SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY**

We, the undersigned, declare that GLP Study 84046 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. / 3 FEB 89

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

Larry D. Brown / 11 Jan 89

LARRY D. BROWN, DVM / DATE  
LTC VC  
Principal Investigator

Conrad Wheeler / 3 Feb 89

CONRAD R. WHEELER, PhD / DATE  
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Analytical Chemist



REPLY TO  
ATTENTION OF:

## DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH  
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4 January 1989

MEMORANDUM FOR RECORD

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1. This is to certify that the protocol for GLP Study 84346 was reviewed on 16 October 1984.
2. The institute report entitled "Dermal Sensitization Potential of Triethyleneglycol Dinitrate (TEGDN) in Guinea Pigs," Toxicology Series 141, was audited on 22 July 1987.

*Carolyn M Lewis*

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

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## **Dermal Sensitization Potential of Triethyleneglycol Dinitrate (TEGDN) in Guinea Pigs—Brown and Korte**

### **INTRODUCTION**

The Department of Defense is considering the use of either diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in new propellant formulations. However, considerable gaps in the toxicology data of the compounds were identified during a review of their health effects (1) conducted for the US Army Biomedical Research and Development Laboratory (USABRDL). Consequently, USABRDL 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 dermal sensitization potential of triethyleneglycol dinitrate (TEGDN) in guinea pigs.

### **MATERIALS**

#### Test Substance

Chemical Name: Triethyleneglycol Dinitrate (TEGDN)

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Physical State: Liquid

Brown and Korte-2

Chemical Structure:



Molecular Formula: C<sub>6</sub>H<sub>12</sub>N<sub>2</sub>O<sub>8</sub>

Source: Naval Ordnance Station  
Indian Head, MD

Other test substance information is presented in Appendix A.

Vehicle for Test Substance

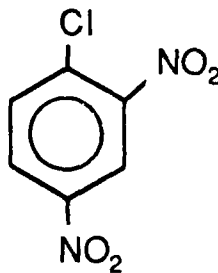
On 13 February 1985, a pilot study indicated that neat TEGDN (100%) was not a dermal irritant in the guinea pig. Therefore, neat TEGDN was used in this study and no vehicle or vehicle control group of animals was necessary.

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 weekly, on 4 March, 11 March, and 1 April 1985. The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485, Exp. Date

1991) was obtained from Certified Laboratories, Inc., (Philadelphia, PA). Sterile, isotonic saline (lot number 7C950X0, Exp. Date Oct 1985) was obtained from Travenol Laboratories, Inc., Deerfield, IL.

#### Animal Data

On 11 February 1985, three animals were transferred from GLP 84042 for a pilot study to determine a non-irritating dose level. The three were dosed with neat TEGDN. Since the neat TEGDN was non-irritating, a vehicle and vehicle control group were deemed unnecessary. Thirty-three male albino guinea pigs, Hartley strain (Charles River Breeding Laboratories, Wilmington, MA), from a shipment received on 20 February 1985 were assigned to this study. They were identified individually with ear tags. Two animals (85E0102, 85E0103) were selected for quality control necropsy evaluation on the day following receipt. The animals received for use as vehicle controls were not assigned to the study. Animal weights on the day following receipt (21 February 1985) ranged from 189 to 245 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<sup>®</sup> Diet 5026 or regular Purina Guinea Pig Chow<sup>®</sup> Diet 5025 (Ralston Purina Company, Checkerboard Square, St. Louis, MO), the lot of regular Chow<sup>®</sup> was certified by LAIR; water was provided by continuous drip from a central line. Temperature within the animal room was maintained in the range from 22.2 to 27.8°C. Relative humidity was maintained in the range of 41 to 50%, with occasional spikes as high as 63% during periods of room washing. 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. Ten animals were assigned to each of three groups by a randomization technique based on their animal identification number.

### Dose Levels

Three animal groups comprise the basis for this report. Dermal sensitization potential was evaluated in a test group receiving three weekly induction doses of 100% triethyleneglycol dinitrate and, after a two-week delay, a challenge dose at the same concentration. Dinitrochlorobenzene, a known potent sensitizing agent (4), was applied to another group, at a 0.1% concentration, as a positive control. A negative control group received 100% triethyleneglycol dinitrate only on the day of challenge dosing.

### Compound Preparation

TEGDN was received as a liquid in 10% ethanol. Rotoevaporation was performed to remove the ethanol, resulting in neat TEGDN. TEGDN was used neat (undiluted) in the study. 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 the appropriate compound/suspension applied topically under a 2.5-cm<sup>2</sup> gauze patch. This procedure was performed for three consecutive weeks (5, 12, and 19 March, except for one substitute animal, 85E0098, which was dosed on 6, 12, and 19 March due to the death of one animal dosed on 5 March 1985). 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<sup>®</sup> Model A5, size 40 blade, Sunbeam Corp., Milwaukee, WI) and then shaved with an electric razor (Norelco<sup>®</sup> Speed Razor Model HP1134/S, North American Phillips Corp., Stamford, CT). The patch was taped with Blenderm<sup>®</sup> hypoallergenic surgical tape (3M Corp., St. Paul, MN) to the same site each time, and the animal was wrapped several times with Vetrap<sup>®</sup> (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 (2 April) following the third induction dose. Test group and positive control group animals received two 0.5-ml doses each of TEGDN 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 TEGDN, 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 cage 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 the following exceptions: Ten animals, not 15, were used per group. One animal, 85E0074, died on 5 March 1985, the day of the first induction dose probably from a constrictive effect of the wrappings. Animal 85E0098 was substituted and dosed the following day. For the first week, observations on this animal were conducted one day following the observations for the remainder of the study animals. The remaining induction doses and challenge dose were synchronized with the other animals.

Animals were randomized by number rather than body weight. It is believed that these changes did not adversely affect the outcome of the 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. No reaction was observed in response to triethyleneglycol dinitrate after any of the induction doses or the challenge dose. This 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 triethyleneglycol dinitrate no responses were obtained; therefore, severity scores were zero at all times.

#### Positive Control

Dinitrochlorobenzene produced a marked response at all time points after the first induction dose (Table 1). Between 50% and 90% of the DNCB-treated animals exhibited a response 24 hours following the second or third induction and challenge doses. These reactions persisted, yielding scorable effects in 40-80% of the animals at 48 hours after dosing and 40-60% of the animals at 72 hours after dosing. Severity scores for these responses to DNCB ranged from 0.5 to 0.9 at the 24-hour scoring period (Table 2). The highest score, 0.9, was observed in response to the second induction dose. By 48 hours the reactions had subsided slightly; consequently, the severity range decreased to between 0.4 and 0.8. At 72 hours the reactions diminished further to a range of 0.4 to 0.6.



**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>Left</u>	<u>Right</u>
<b><u>24 Hours</u></b>					
TEGDN	0/10	0/10	0/10	0/10	0/10
Negative Control*	--	--	--	0/10	--
DNCB	0/10	9/10	7/10	5/10	8/10
<b><u>48 Hours</u></b>					
TEGDN	0/10	0/10	0/10	0/10	0/10
Negative Control*	--	--	--	0/10	--
DNCB	0/10	8/10	7/10	4/10	5/10
<b><u>72 Hours</u></b>					
TEGDN	0/10	0/10	0/10	0/10	0/10
Negative Control*	--	--	--	0/10	--
DNCB	0/10	4/10	6/10	4/10	5/10

\*The Negative Control Group received only a challenge dose of the test compound.

**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>Left</u>	<u>Right</u>
<b><u>24 Hours</u></b>					
TEGDN	0.0	0.0	0.0	0.0	0.0
Negative Control*	--	--	--	0.0	--
DNCB	0.0	0.9	0.7	0.5	0.8
<b><u>48 Hours</u></b>					
TEGDN	0.0	0.0	0.0	0.0	0.0
Negative Control*	--	--	--	0.0	--
DNCB	0.0	0.8	0.7	0.4	0.6
<b><u>72 Hours</u></b>					
TEGDN	0.0	0.0	0.0	0.0	0.0
Negative Control*	--	--	--	0.0	--
DNCB	0.0	0.5	0.6	0.4	0.6

\*The Negative Control Group received only a challenge dose of the test compound.

Negative Control

No response was observed in the negative control (challenge dose of TEGDN) group.

Individual 24-hour, 48-hour, and 72-hour dermal scores for all animals appear, by group, in Appendix D.

Clinical Signs

Except for animal 85E0074 which died, all animals were healthy and gained weight during the study. Individual body weight data are presented in Appendix E.

Pathology Findings

A necropsy was performed on all study animals. Quality control animals were diagnosed with the following conditions: 85E0102 had diffuse tracheitis, mild endocarditis, mild hepatitis, and diffuse pigment granules in the small intestine; 85E0103 had mild rhinitis, minimal mineral deposits of the kidney medulla, and minimal lymphocytic aggregates of the kidney pelvis. These morphologic observations are frequent subclinical findings in guinea pigs and have little experimental significance. Histopathology on animal 85E0074 which died revealed extensive liver necrosis. It was suggested that the abdominal wrap may have been too tight, compromising venous return and leading to shock, liver necrosis, and death. Coagulative liver necrosis was identified in 5 of 30 test animals at terminal sacrifice. This is a commonly observed incidental finding in guinea pigs. The complete pathology report is presented in Appendix E.

## 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, the intensity/severity of inflammation often is greater at 72 to 96 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 non-irritating 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.

### Triethyleneglycol Dinitrate

Triethyleneglycol dinitrate (TEGDN) was evaluated for its ability to elicit a delayed-hypersensitivity or cellular immunologic reaction via contact with the skin. TEGDN 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 TEGDN 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 within the Institute (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 TEGDN will not sensitize humans. However, it does indicate that TEGDN is unlikely to sensitize humans and its potential is low enough to permit its evaluation in man.

### **CONCLUSION**

Triethyleneglycol dinitrate (TEGDN) possesses minimal sensitizing potential, as it did not induce a dermal sensitization reaction under conditions of this study.

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10. Hiatt GFS, Morgan EW, Korte DW. Dermal sensitization potential of guanidine hydrochloride in guinea pigs. Toxicology Series 84. Presidio of San Francisco, CA: Letterman Army Institute of Research. Institute Report No. 210, January 1986.

Appendix A. Chemical Data .....	16
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Brown and Korte-16

**Appendix A: CHEMICAL DATA**

Chemical Name: Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, dinitrate

Alternate Chemical Names: Triethyleneglycol dinitrate, NOSET-A

Chemical Abstracts Service Registry No.: 111-22-8

LAIR Code Number: TA44

Chemical Structure:



Molecular Formula: C<sub>6</sub>H<sub>12</sub>N<sub>2</sub>O<sub>8</sub>

Molecular Weight: 240

Physical State: Yellow oil

Density: (g/cm<sup>3</sup>): 1.32\*

Manufacturer: Naval Ordnance Station  
Indian Head, MD

Lot No.: 130-84

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\* Holleman JW, Ross RH, Carroll JW. Problems definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1983, DTIC No. ADA 127846, p17.

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

Analytical data: The compound chromatographed as a single peak (retention time 5.8 min) by HPLC analysis under the following conditions: column, Brownlee RP-18 (4.6 x 250 mm); solvent system, 30% water, 70% methanol; flow rate 0.9 ml/min, detection wavelength, 215 nm.† No impurities were detectable by NMR.‡ NMR (80 MHz, CDC13): 3.65 (S, 4H, -CH<sub>2</sub>-O-CH<sub>2</sub>CH<sub>2</sub>-O-CH<sub>2</sub>-), 3.72-3.84 (Complex multiplet, 4H, terminal methylene groups). IR (KBr): 2900, 1630, 1280, 1130, 1030, 910, 860 cm.§

Stability: The compound was received as a 10% solution in ethanol. Periodic analysis of this solution by HPLC has shown no evidence of decomposition to date (4 months).† NMR analysis demonstrated that the neat compound is stable for at least 1 month.‡

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† Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.1, p26-30, 42-43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

‡ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p63. Letterman Army Institute of Research, Presidio of San Francisco, CA.

§ ibid. p64.

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

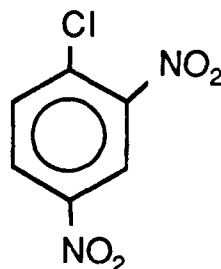
POSITIVE CONTROL

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, pp. 9-10. Letterman Army Institute of Research, Presidio of San Francisco, CA.

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

<sup>4</sup>*Ibid.* pp. 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 C.J. The Aldrich Library of NMR Spectra. Vol. 1, 2nd ed. Milwaukee: Aldrich Chemical Co., 1981:1173, spectrum D.

<sup>7</sup>Wheeler C.R. Toxicity Studies of Water Disinfectant. Laboratory Notebook #85-12-021, pp. 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  
Wilmington, MA

Sex: Male

Date of Birth: 2 February 1985

Method of randomization: Allocation by animal number

Animals in each group: 10 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tag, numbers 85E0068 to 85E0133 inclusive.

Pretest conditioning: Quarantine/acclimation 20 February - 3 March 1985

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 EVENTS**

<u>Date</u>	<u>Event</u>
20 Feb 85	Sixty-six animals arrived at LAIR. Animals were examined, weighed, placed in cages, and fed.
21 Feb 85	Animals were assigned ear tags. Two animals were submitted for necropsy quality control.
20 Feb - 8 Apr 85	Animals were checked daily.
21,25 Feb, 4,11,18 25 Mar, 1,8 Apr 85	Animals were weighed.
28 Feb 85	Thirty animals were randomized into three groups (experimental, positive control, negative control) of 10 animals each.
4,11,18 Mar 85	Study animals, except negative control group, were clipped and shaved.
5,12,19 Mar 85	Study animals, except negative control group, were given induction dose.
5 Mar 85	Animal 85E0074 died and was replaced by 85E0098.
6,13,20 Mar 85	Study animals, except negative control group and 85E0098 (first week only), were scored for 24-hr skin reaction. Animal 85E0098 was dosed on 6 Mar 85.
7 Mar 85	Thirty-one animals were transferred to GLP 85005. Animal 85E0098 was scored for 24-hr reaction.
7,14,21 Mar 85	Study animals, except negative control group and 85E0098 (first week only), were scored for 48-hr reaction.
8 Mar 85	Animal 85E0098 was scored for 48-hr reaction.
8,15,22 Mar 85	Study animals, except negative control group and 85E0098 (first week only), were scored for 72-hr reaction.
9 Mar 85	Animal 85E0098 was scored for 72-hr reaction.

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

<u>Date</u>	<u>Event</u>
1 Apr 85	Study animals were clipped and shaved.
2 Apr 85	Study animals were given challenge dose.
3 Apr 85	Study animals were scored for 24-hr reaction.
4 Apr 85	Study animals were scored for 48-hr reaction.
5 Apr 85	Study animals were scored for 72-hr reaction.
8 Apr 85	All animals were delivered to Necropsy Suite.

Appendix D: INDIVIDUAL ANIMAL SCORES

ANIMAL NUMBER	GROUP: ONE						COMPOUND: IEGDN								
	FIRST INDUCTION		SECOND INDUCTION		THIRD INDUCTION		CHALLENGE DOSE								
	24 H	48 H	72 H	24 H	48 H	72 H	24 H	48 H	72 H	24 H	48 H	72 H			
85E0070	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0073	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0081	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0085	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0086	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0091	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0092	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0093	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85E0097	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AVERAGES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

ANIMAL NUMBER	GROUP: IWO														
	COMPOUND: DNQB						CHALLENGE DOSE								
	FIRST INDUCTION		SECOND INDUCTION		THIRD INDUCTION		LEFT FLANK		RIGHT FLANK						
	24 H	48 H	72 H	24 H	48 H	72 H	24 H	48 H	72 H	24 H	48 H	72 H			
85E0071	0	0	0	1	1	1	1	1	1	1	1	1			
85E0074	DIED ON 5 MAR 85 REPLACED BY 85E0098														
85E0076	0	0	0	1	1	0	1	1	1	1	1	1			
85E0080	0	0	0	1	1	1	0	0	1	0	1	0			
85E0083	0	0	0	1	1	2	1	1	1	0	0	1			
85E0084	0	0	0	1	0	0	1	0	0	0	0	0			
85E0088	0	0	0	1	1	0	1	1	1	0	0	0			
85E0090	0	0	0	1	1	1	1	1	1	1	1	2			
85E0094	0	0	0	0	0	0	0	0	0	0	0	0			
85E0096	0	0	0	1	1	0	1	1	1	0	0	0			
85E0098	0	0	0	1	1	0	0	0	0	1	0	1			
AVERAGES	0	0	0	0.9	0.8	0.5	0.7	0.7	0.6	0.5	0.4	0.4	0.3	0.6	0.6

Appendix D (cont.): INDIVIDUAL ANIMAL SCORES

ANIMAL NUMBER	COMPOUND: <u>NEGATIVE CONTROL</u>																	
	FIRST INDUCTION						THIRD INDUCTION						CHALLENGE DOSE					
	24 H	48 H	72 H	24 H	48 H	72 H	24 H	48 H	72 H	24 H	48 H	72 H	LEFT FLANK	RIGHT FLANK				
85E0068	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	N/A	N/A	N/A
85E0069	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	N/A	N/A	N/A
85E0072	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	N/A	N/A	N/A
85E0075	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	N/A	N/A	N/A
85E0077	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	N/A	N/A	N/A
85E0079	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	N/A	N/A	N/A
85E0082	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	N/A	N/A	N/A
85E0087	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	N/A	N/A	N/A
85E0089	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	N/A	N/A	N/A
85E0095	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	N/A	N/A	N/A
AVERAGES	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	N/A	N/A	N/A

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

<u>Animal Number</u>	<u>DAY OF STUDY</u>							
	<u>Q*Q</u>	<u>Q4</u>	<u>Q11</u>	<u>6</u>	<u>13</u>	<u>20</u>	<u>27</u>	<u>33</u>
85E0070	208	233	292	321	369	424	487	480
85E0073	224	249	319	352	412	473	528	517
85E0078	224	250	325	368	435	513	581	582
85E0081	215	240	293	325	377	420	468	465
85E0085	215	245	292	327	356	396	445	423
85E0086	205	230	276	328	366	405	458	445
85E0091	190	224	280	324	367	428	487	472
85E0092	215	235	274	304	319	345	373	373
85E0093	197	240	308	349	387	443	501	486
85E0097	208	229	279	316	354	394	429	409
MEAN	210.0	237.5	293.8	331.4	374.2	424.1	475.7	465.2
Standard Deviation	10.9	8.8	18.0	19.1	32.0	45.9	56.5	58.6
Standard Error	3.4	2.8	5.7	6.0	10.1	14.5	17.9	18.5

\* Q represents quarantine period.

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

DNCB

Animal Number	DAY OF STUDY								
	<u>0*0</u>	<u>04</u>	<u>011</u>	<u>6</u>	<u>13</u>	<u>20</u>	<u>27</u>	<u>33</u>	
85E0071	210	249	307	363	424	490	553	527	
85E0074	221	243	289	DIED 5 MAR REPLACED BY 85E0098					
85E0076	211	248	309	346	404	474	545	535	
85E0080	229	273	341	375	426	470	532	537	
85E0083	223	247	322	367	421	497	564	558	
85E0084	218	243	298	350	386	429	485	471	
85E0088	217	239	296	320	361	419	469	469	
85E0090	215	249	312	354	401	447	489	463	
85E0094	212	242	304	344	380	430	477	470	
85E0096	214	247	301	342	382	428	488	475	
85E0098	210	241	308	342	396	444	490	482	
MEAN	216.4	247.4	307.9	350.3	398.1	452.8	509.2	498.7	
Standard Deviation	6.0	9.2	14.0	15.6	21.4	28.0	35.3	36.0	
Standard Error	1.8	2.8	4.2	4.9	6.8	8.8	11.2	11.4	

\* Q represents quarantine period.

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

Animal Number	DAY OF STUDY							
	<u>Q*0</u>	<u>Q4</u>	<u>Q11</u>	<u>6</u>	<u>13</u>	<u>20</u>	<u>27</u>	<u>33</u>
85E0068	218	246	285	319	364	399	450	437
85E0069	227	258	302	338	378	417	452	527
85E0072	215	246	304	353	409	464	506	499
85E0075	196	220	274	309	366	385	426	400
85E0077	241	273	321	365	423	491	540	530
85E0079	217	252	312	366	415	431	499	505
85E0082	226	255	307	364	415	472	515	493
85E0087	229	251	310	364	416	479	526	523
85E0089	240	276	329	390	411	484	541	531
85E0095	229	259	321	370	418	456	507	494
MEAN	223.6	253.6	306.5	353.8	401.5	447.8	496.2	493.9
Standard Deviation	13.1	15.6	16.7	24.8	22.8	37.4	40.0	43.2
Standard Error	4.1	4.9	5.3	7.8	7.2	11.8	12.7	13.7

\* Q represents quarantine period.

## Appendix F: PATHOLOGY REPORT

LAIR Gross Pathology Report  
GLP Study 84046

Study: GLP #84046, Toxicology Services Group

Test: Buehler Dermal Sensitization (TEGDN) in Guinea Pig.

Investigator: MAJ Larry Brown, DVM, VC

Test Substance: Triethyleneglycol Dinitrate (TEGDN).

History: Part of a shipment of 66 animals from Charles-River Laboratory. The study was conducted in accordance with SOP-OP-STX-82. Animals were killed with sodium pentobarbital anesthesia and axillary bleeding.

## Gross findings:

<u>Animal ID</u>	<u>LAIR Acc.</u>	<u>Sex</u>	<u>Gross Lesions</u>
Experimental: (0.5 ml TEGDN)			
85E0070	37165	M	Not remarkable (NR)
85E0073	37168	M	NR
85E0078	37172	M	See gross findings below
85E0081	37175	M	NR
85E0085	37179	M	NR
85E0086	37180	M	NR
85E0091	37185	M	NR
85E0092	37186	M	See gross findings below
85E0093	37187	M	NR
85E0097	37191	M	NR
Negative Control: (0.5 ml TEGDN)			
85E0068	37163	M	NR
85E0069	37164	M	NR
85E0072	37167	M	See gross findings below
85E0075	37169	M	See gross findings below
85E0077	37171	M	NR
85E0079	37173	M	NR
85E0082	37176	M	NR
85E0087	37181	M	NR
85E0089	37183	M	NR
85E0095	37189	M	NR

## Appendix F (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 84046

<u>Animal ID</u>	<u>LAIR Acc.</u>	<u>Sex</u>	<u>Gross Lesions</u>
Positive Control: (0.5 ml DNFB)			
85E0071	37166	M	NR
85E0076	37170	M	NR
85E0080	37174	M	NR
85E0083	37177	M	NR
85E0084	37178	M	NR
85E0088	37182	M	NR
85E0090	37184	M	NR
85E0094	37188	M	NR
85E0096	37190	M	See gross findings below
85E0098	37192	M	NR

## Gross findings:

85E0078 - The left lateral lobe of the liver (diaphragmatic surface), area of necrosis (0.5 cm x 0.1 cm).

85E0092 - The liver contained multifocal areas of necrosis, minimal.

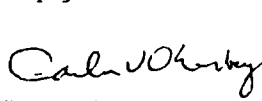
85E0072 - Left lateral lobe of the liver (visceral surface), area of necrosis (2 mm wide x 1.5 cm long).

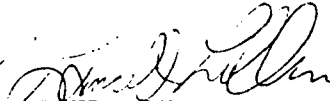
85E0075 - Right lateral lobe diaphragmatic surface of the liver had four (1 mm) areas of necrosis.

85E0096 - Left lateral visceral surface of the liver contained an area of necrosis (2mm x 1 cm).

Comments: There were no agent or positive control related gross alterations in the skin of these guinea pigs.

A number of guinea pigs in all 3 groups had focal areas of coagulation necrosis in the liver. This is a commonly observed incidental finding in guinea pigs.

  
CARLIN V. OKERBERG, DVM  
MAJ, VC  
Pathology Services Group

  
LANCE O. LOLLINI, DVM  
LTC, VC  
Chief, Pathology Services Group

6 June 1985

## Distribution List

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