*Institute Report No. 336*

# Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in ICR Mice

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY





May 1989

Toxicology Series: 137

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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in ICR Mice (Toxicology Series 137) --Ryabik *et al.*

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

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

Edwin S. Beatrice 24 May 91

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The acute oral toxicity of diethyleneglycol dinitrate (DEGDN) was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose (MLD) ± S.E. for male mice was 1395 ± 59 mg/kg and 1321 ± 74 mg/kg for female mice. Clinical signs produced by DEGDN included inactivity, twitching, tremors, hypertonia, squinting, hunched posture, depression of grasping and righting reflexes, rough coat, increased startle reflex, hyperactivity, moribund condition/prostration, and various stains in the perianal and abdominal areas. The duration of clinical signs was acute. Most animals were exhibiting signs by 2 hours after dosing and hac either died or cleared by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place DEGDN in the slightly toxic class.

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## ABSTRACT

The acute oral toxicity of diethyleneglycol dinitrate (DEGDN) was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose (MLD) ± S.E. for male mice was 1395 ± 59 mg/kg and 1321 ± 74 mg/kg for female mice. Clinical signs produced by DEGDN included inactivity, twitching, tremors, hypertonia, squinting, hunched posture, depression of grasping and righting reflexes, rough coat, increased startle reflex, hyperactivity, moribund condition/prostration, and various stains in the perianal and abdominal areas. The duration of clinical signs was acute. Most animals were exhibiting signs by 2 hours after dosing and had either died or cleared by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place DEGDN in the slightly toxic class.



KEY WORDS: Acute Oral Toxicity, Diethyleneglycol Dinitrate, DEGDN, Mammalian Toxicology, Mouse, Propellant

## PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

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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 Command
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

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

GLP STUDY NUMBER: 84018

STUDY DIRECTOR: LTC Don W. Korte Jr., PhD, MSC
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DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT:

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

TEST SUBSTANCE: Diethyleneglycol Dinitrate

INCLUSIVE STUDY DATES: 8 May - 6 June 1985

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of diethyleneglycol dinitrate in ICR mice.

## ACKNOWLEDGMENTS

SP4 James Fischer and PFC Scott Schwebe provided research assistance; SP4 Paul B. Simboli, BS, provided chemical preparation and assisted in the chemical analysis; Richard A. Spieler and Charlotte L. Speckman provided animal care and facility management; Colleen S. Kamiyama, Ann L. Wilkinson, and Julie Peacock provided secretarial assistance.

## SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that study number 84018 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

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DEPARTMENT OF THE ARMY
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REPLY TO
ATTENTION OF

SGRD-ULZ-QA

23 May 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 84018

1. This is to certify that in relation to LAIR GLP Study 84018 the following inspections were made:

| | |
|---|---|
| 06 March 1984 | - Protocol Review |
| 23 May 1985 | - Dosing |

2. The institute report entitled "Acute Oral Toxicity of Diethyleneglycol Dinitrate (DEGDN) in ICR Mice," Toxicology Series 137, was audited on 19 May 1989.

Walter G. Bell

WALTER G. BELL
SFC, USA
Quality Assurance Auditor

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## 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 munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABRDL) identified numerous gaps in the toxicology database of these compounds (1). Consequently, USABRDL has tasked the Division of Toxicology, LAIR, to conduct an initial health effects evaluation of DEGDN, TMETN, TEGDN, and two DEGDN-based propellants, JA-2 and DIGL-RP. This initial evaluation includes the Ames mutagenicity assay, acute oral toxicity tests in rats and mice, a dermal toxicity test 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 acute oral toxicity of DEGDN in male and female ICR mice.

## MATERIALS

### Test Substance

Chemical name: Diethyleneglycol Dinitrate

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

Chemical structure:

$$O_2N\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}CH_2CH_2\text{-}O\text{-}NO_2$$

Molecular formula: $C_4H_8N_2O_7$

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for DEGDN was corn oil (Sigma Chemical Co, St Louis, MO, Lot No. 13F-0705). The expiration date was April 1995.

Animal Data

ICR mice (Harlan Sprague-Dawley, Inc, Indianapolis, IN) from a shipment that arrived 8 May 85 were used for this study. They were identified individually. Two males and 2 females were selected randomly for quality control necropsy evaluation at receipt. The animal weights on receipt ranged from 23 to 36 g. Additional animal data are presented in Appendix B.

Husbandry

Mice were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow Diet 5002 (Ralston Purina Company, St. Louis, MO, Lot Nos.: FEB15851D and MAR26852A); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 20°C to 23.4°C with a relative humidity range of 50 to 64 percent. The photoperiod was 12 hours of light per day.

**METHODS**

Group Assignment/Acclimation

Study mice were randomized into 5 dose groups of 10 males and 10 females each and a vehicle control group of 5 males and 5 females each. Allocation was accomplished using a computer-based stratified, weight-biased method. The Beckman TOXSYS Animal Allocation Program was used in conjunction with a Beckman TOXSYS Data Collection Terminal. The animals were acclimated for 12 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of the approximate lethal dose (ALD) determination suggested that the median lethal dose (MLD) was between 1500 and 2000 mg/kg. Based on these data, test doses for Phase I were selected. Results of Phase I dosing were

used to select doses for Phase II. Dosing was carried out in two phases for a more accurate MLD determination. Test doses are given in Table 1.

**TABLE 1: Diethyleneglycol Dinitrate Doses**

| Group | Dosage Level (mg/kg) |
|---|---|
| 1 | 1000 |
| 2 | 1180 |
| 3 | 1390 |
| 4 | 1640 |
| 5 | 1930 |
| 6 (vehicle control) | 10 ml/kg(corn oil) |

Compound and Dosing Suspension Preparation

DEGDN was received as a solution containing 18% acetone. The acetone was removed with a rotary evaporator. Since DEGDN is miscible in corn oil, all dosing suspensions/ emulsions were prepared by mixing appropriate quantities of DEGDN and corn oil in a vial. Emulsification was accomplished and homogeneity maintained during the dosing procedures by use of a stir plate and intermittent vortexing.

Chemical Analysis of DEGDN and Dosing Suspensions

NMR analysis demonstrated that the neat DEGDN was stable for at least one year (Appendix A). An emulsion of DEGDN in corn oil was stable for at least 24 hours. Tests for the accuracy and homogeneity of the DEGDN dosing emulsions were conducted. Analysis of the emulsions determined that all emulsions were within 2.6% of the target concentration and no sample from a particular suspension varied more than 5% from the mean value for the suspension.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP-OP-STX-36 (3).

Food was removed from the animals' cage at approximately 0600 hours the day of dosing. The volume of dosing suspension each animal received was based upon the desired dose level, the compound concentration in suspension, and the weight of the animal. The dose level was increased by varying the concentration of each suspension. Volumes administered ranged from 0.31 to 0.39 ml in the males and 0.23 to 0.31 ml in females. The vehicle control group animals received volumes of corn oil ranging from 0.32 to 0.36 ml in the males and 0.25 to 0.27 ml in the females. Dosing was performed using the oral gavage single-dose method without animal sedation or anesthesia. Sterile disposable syringes (Sherwood Medical, St Louis, MO) fitted with 20 gauge, 1.5-inch, ball-tipped feeding tubes (Popper & Sons, Inc, New Hyde Park, NY) were utilized. Animals were dosed on two days, 21 May (Phase I) and 23 May 85 (Phase II), between 1014 and 1128 hours.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed approximately 1, 2, and 4 hours after dosing and daily for the remainder of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those that survived the 14-day study period were also submitted for necropsy immediately following administration of a barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The MLD and associated lethal doses were derived by

probit analysis using the maximum likelihood method, as described by Finney (4). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to determine the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The study was conducted in accordance with the protocol and associated addenda. No changes or deviations were necessary.

Storage of Raw Data and Final Report

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

## RESULTS

Mortality

Fifty-three mice died from administration of DEGDN. Thirty-five (66%) of the deaths occurred between 4 and 27 hours after dosing. Seventeen (32.1%) of the deaths occurred between 27 and 45 hours after dosing. One additional animal was found dead on the morning of the fourth day. Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis and the equations for the probit regression line were: $Y= -33.0 + 12.1 \log X$ (males), $Y= -21.2 + 8.4 \log X$ (females), where X is the dose and Y the corresponding probit value. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

**TABLE 2: Compound-Related Deaths by Group**

| Group | Dose Level (mg/kg) | Compound-Related Death/ Number in Group | Percent Mortality |
|---|---|---|---|
| | | MALE | |
| 1 | 1000 | 0/10 | 0 |
| 2 | 1180 | 2/10 | 20 |
| 3 | 1390 | 7/10 | 70 |
| 4 | 1640 | 6/10 | 60 |
| 5 | 1930 | 10/10 | 100 |
| 6 | Vehicle Control | 0/5 | 0 |
| | | FEMALE | |
| 1 | 1000 | 1/10 | 10 |
| 2 | 1180 | 3/10 | 30 |
| 3 | 1390 | 7/10 | 70 |
| 4 | 1640 | 9/10 | 90 |
| 5 | 1930 | 8/10 | 80 |
| 6 | Vehicle Control | 0/5 | 0 |

**TABLE 3: Calculated Lethal Doses (LD) of DEGDN in ICR Mice**

| Effect Level | Calculated Dose* (mg/kg) | 95% Confidence Limits (mg/kg) |
|---|---|---|
| | MALES | |
| LD10 | 1092.7 ± 76.4 | (871.8, 1212.6) |
| LD50 | 1394.7 ± 59.3 | (1270.8, 1513.1) |
| LD90 | 1780.1 ± 124.9 | (1603.8, 2233.0) |
| | FEMALES | |
| LD10 | 929.3 ± 106.1 | (602.7, 1087.9) |
| LD50 | 1320.7 ± 73.5 | (1150.5, 1485.4) |
| LD90 | 1877.1 ± 190.3 | (1627.5, 2736.9) |

* Calculated dose ± standard error.

**Figure 1**

**DEGDN Dose Response Curve in Male ICR Mice**

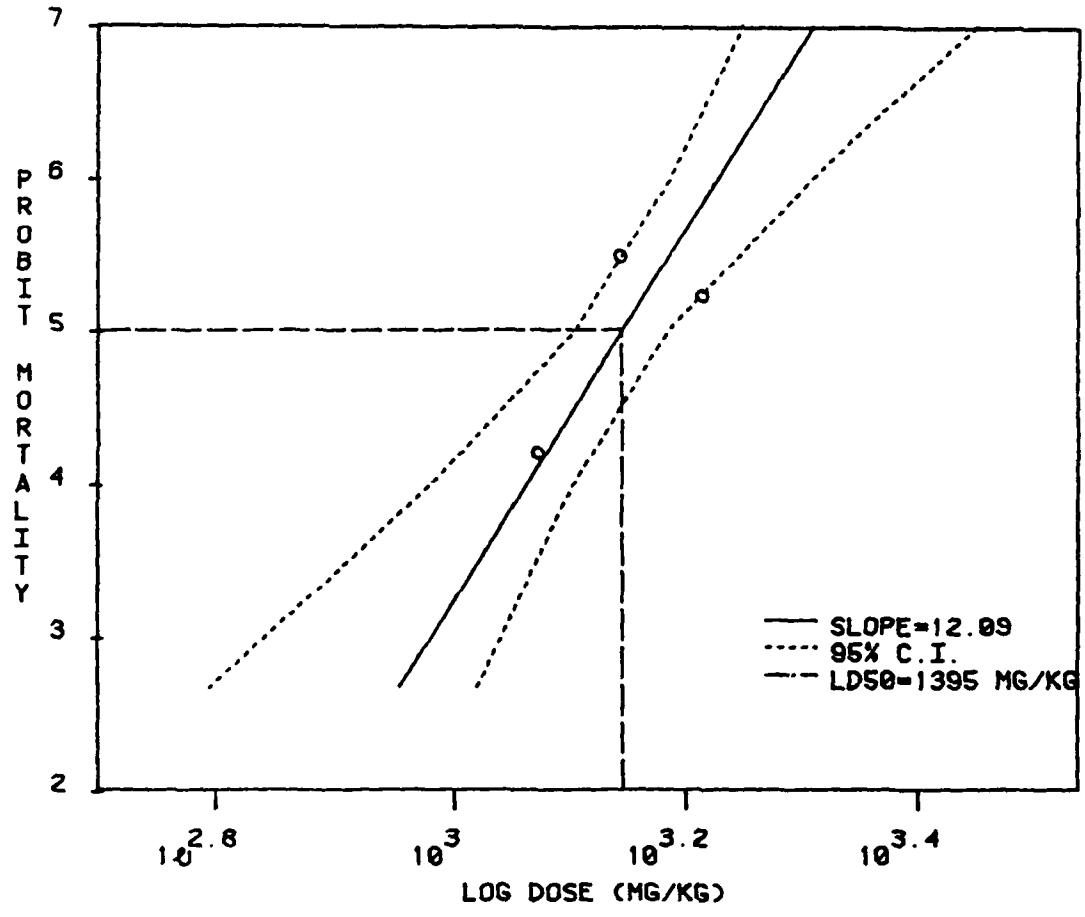
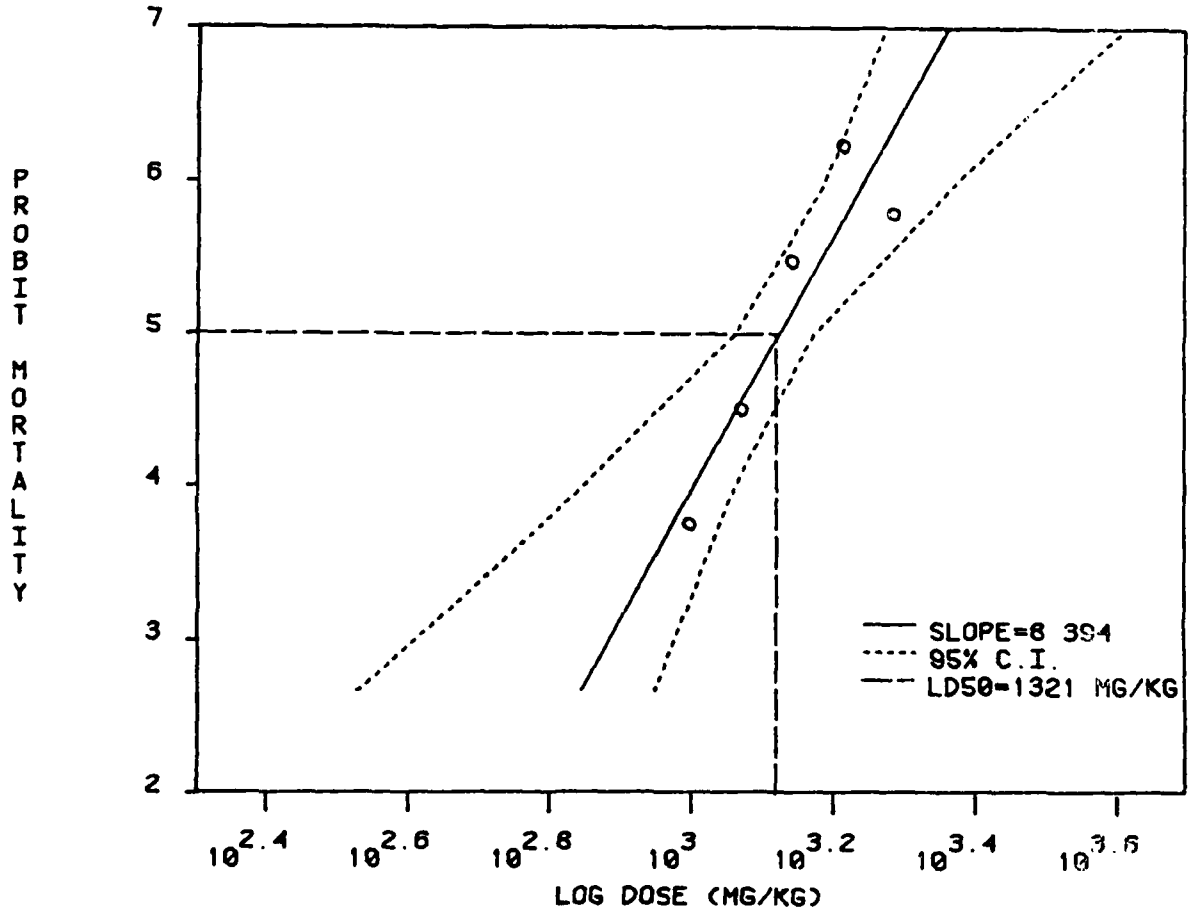


**Figure 2**

**DEGDN Dose Response Curve in Female ICR Mice**



Clinical Observations

The most frequently observed category of clinical signs was behavioral disturbances (64 of 100 animals dosed with DEGDN). Behavioral signs exhibited by the animals included inactivity, twitching, tremors, hypertonia, and hyperactivity. The second major category of clinical observations was general signs (62 of 100 animals), and included hunched posture, prostration, and moribund condition. Hunched posture was the most frequently observed general sign. The observations of prostration and moribund condition were associated with those animals that subsequently died. The incidences for these observations would undoubtably have been higher but many of the animals died at night, thus precluding the chance for observing these signs. Squinting was observed in a total of 56 of 100 DEGDN-dosed animals. Another frequently observed category of clinical observations was reflexive signs, observed in 46 of 100 DEGDN-dosed animals. Reflexive signs included depressed grasping or righting reflexes and increased startle reflex.

These clinical signs were observed at each dose level; however, there was an increasing prevalence and complexity of the clinical signs at the higher dose levels. The clinical signs were first noted in most animals within 2 hours of dosing and most had resolved by 72 hours after dosing. Initial observations generally were of signs of ill health: inactivity, squinting, and hunched posture. An increased startle reflex and hyperactivity were observed in some animals. As the dose was increased these signs were more often compounded with the presence of tremors, twitching, and depressed grasping and righting reflexes. Severity and time of onset of clinical signs exhibited a dose-response relationship.

Other clinical signs frequently observed were rough coat (20 of 100 animals) and miscellaneous signs (22 of 100 animals) which included urine stains on the abdomen and fecal stains/material in the perianal region. Clinical signs had resolved within 4 days after dosing. Tables 4 and 5 contain a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not significantly affected by dosing. Table 6 presents the mean body weights by groups. Appendix F contains individual weight tables.

**TABLE 4: Incidence Summary for Clinical Observations in Male Mice Administered DEGDN**

| Group | 5 | 4 | 1 | 2 | 3 | 6 |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 1000 | 1180 | 1390 | 1640 | 1930 | Control |
| Clinical Signs (N=) | 10 | 10 | 10 | 10 | 10 | 5 |
| General[a] | 4 | 3 | 7 | 7 | 10 | 0 |
| Behavioral[b] | 1 | 5 | 9 | 7 | 10 | 0 |
| Miscellaneous[c] | 0 | 2 | 5 | 4 | 4 | 0 |
| Rough Coat | 0 | 2 | 1 | 6 | 5 | 0 |
| Squinting | 4 | 7 | 7 | 6 | 8 | 0 |
| Reflexes[d] | 2 | 2 | 7 | 5 | 7 | 0 |
| Normal Throughout | 4 | 1 | 1 | 1 | 0 | 5 |

[a] Includes moribund, hunched posture, and prostration.
[b] Includes inactivity, hypertonia, tremors, and twitching.
[c] Includes urine/fecal stains on abdominal/perianal areas.
[d] Includes depressed grasping and righting reflexes and increased startle reflex.

**TABLE 5: Incidence Summary for Clinical Observations in Female Mice Administered DEGDN**

| Group | 5 | 4 | 1 | 2 | 3 | 6 |
|---|---|---|---|---|---|---|
| Dose (mg/kg) | 1000 | 1180 | 1390 | 1640 | 1930 | Control |
| Clinical Signs (N=) | 10 | 10 | 10 | 10 | 10 | 5 |
| General[a] | 2 | 3 | 7 | 9 | 10 | 0 |
| Behavioral[b] | 1 | 3 | 9 | 9 | 10 | 0 |
| Miscellaneous[c] | 0 | 0 | 1 | 3 | 3 | 0 |
| Rough Coat | 0 | 0 | 2 | 1 | 3 | 0 |
| Squinting | 1 | 3 | 6 | 7 | 7 | 0 |
| Reflexes[d] | 1 | 3 | 5 | 7 | 9 | 0 |
| Normal Throughout | 8 | 7 | 0 | 1 | 0 | 5 |

[a] Includes moribund, hunched posture, and prostration.
[b] Includes inactivity, hypertonia, hyperactivity, tremors, and twitching.
[c] Includes urine stains on the abdomen.
[d] Includes depressed grasping and righting reflexes and increased startle reflex.

**TABLE 6: Mean Body Weights in Grams ± S.E.(n)**

| Dose | Receipt | Dosing Day | Day 7 | Day 14 |
|---|---|---|---|---|
| | | MALES | | |
| 1000 | 31.7 ±0.5(10) | 35.7 ±0.8(10) | 37.5 ±0.9(10) | 38.8 ±0.8(10) |
| 1180 | 30.6 ±0.4(10) | 32.9 ±0.5(10) | 35.9 ±0.6(8) | 37.5 ±0.6(8) |
| 1390 | 32.1 ±0.5(10) | 34.0 ±0.4(10) | 34.7 ±0.3(3) | 35.3 ±0.3(3) |
| 1640 | 31.0 ±0.9(10) | 34.2 ±0.6(10) | 33.3 ±2.2(4) | 35.3 ±0.5(4) |
| 1930 | 31.0 ±0.8(10) | 34.0 ±0.5(10) | - | - |
| Vehicle Control | 31.8 ±0.7(5) | 34.0 ±0.7(5) | 37.2 ±0.9(5) | 37.4 ±1.1(5) |
| | | FEMALES | | |
| 1000 | 26.4 ±0.5(10) | 27.2 ±0.7(10) | 29.4 ±0.6(9) | 29.7 ±0.6(9) |
| 1180 | 26.1 ±0.5(10) | 27.2 ±0.6(10) | 28.4 ±0.8(7) | 28.4 ±0.7(7) |
| 1390 | 26.1 ±0.7(10) | 26.3 ±0.6(10) | 28.7 ±1.2(3) | 30.0 ±1.7(3) |
| 1640 | 24.7 ±0.4(10) | 25.7 ±0.4(10) | 26.0(1) | 29.0(1) |
| 1930 | 25.5 ±0.7(10) | 26.0 ±0.8(10) | 28.5 ±1.5(2) | 30.5 ±1.5(2) |
| Vehicle Control | 26.4 ±0.2(5) | 26.6 ±0.4(5) | 29.6 ±0.5(5) | 29.4 ±0.5(5) |

*Dosing to termination

Gross Pathological Observations

There were no gross lesions attributable to the test compound; however, the test compound was the most likely cause of death in all cases, as there was a clear dose-response relationship in both male and female mice. The veterinary pathologist's report is presented in Appendix G.

## DISCUSSION

The calculated MLD and standard error (S.E.) for DEGDN are 1395 ± 59 mg/kg in male and 1321 ± 74 mg/kg in female ICR mice. These MLD values are within the slightly toxic range (5) and are very similiar to published reports that the MLD of DEGDN in white mice is 1250 mg/kg (6).

DEGDN produced a variety of behavioral and reflexive signs in the mouse following oral administration. These signs included inactivity, depressed grasping and righting reflexes, hypertonia, tremors, twitching, hyperactivity, and increased startle reflex. Other signs observed included squinting, hunched posture, rough coat and abdominal and perianal staining. These signs were interpreted as indicators of general ill health following DEGDN administration rather than a direct manifestation of DEGDN toxicity. The incidence, severity, and onset of these clinical signs exhibited a dose-response effect.

The clinical signs observed in this study are similiar to those reported for DEGDN in rats (7) and other nitrate esters of military importance such as triethyleneglycol dinitrate (8) with the exception that cyanosis was not observed. Krasovsky *et al.* (6) have reported that cyanosis was observed in rats and mice following acute oral administration of DEGDN. The failure to observe cyanosis in the animals in this study most probably reflects the difficulty in detecting cyanotic changes in mice under the artifical (fluorescent) light conditions of the animal facility and/or in coordinating the scheduled observation periods with the kinetics in the mouse of methemoglobin formation and reduction following DEGDN administration.

## CONCLUSION

DEGDN is a slightly toxic compound that appears to produce primarily behavioral and reflexive clinical signs. The calculated MLD and standard error for DEGDN are 1395 ± 59 mg/kg in male and 1321 ± 74 mg/kg in female ICR mice.

## REFERENCES

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7. Frost DF, Brown LD, Morgan EW, Korte DW. Acute toxicity of diethyleneglycol dinitrate (DEGDN) and two DEGDN-containing solid propellants, DIGL-RP and JA-2. Laurel, MD: Chemical Propulsion Information Agency, 1988; CPIA Publication 485, pp. 305-314.

8. Andersen M, Koppenhaver, RE, Jenkins LJ, Jr. Some neurotoxic properties of triethylene glycol dinitrate: a comparison with decamethonium. Toxicol Appl Pharmacol 1976; 36:585-594.

## Appendix A: CHEMICAL DATA

Chemical Name: Ethanol, 2,2'-oxybisdinitrate

Alternate Chemical Name: Diethyleneglycol dinitrate (DEGDN)

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

LAIR Code No.: TP47

Chemical Structure:

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

Molecular Formula: $C_4H_8N_2O_7$

Molecular Weight: 196

Physical State: Pale yellow liquid

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

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

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[1] Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland; US Army Medical Bioengineering Research and Development Laboratory, 1983; DTIC No. AD A127846, p. 17.

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

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

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

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

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

Lot No.: RAD84M001S214

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[3] *Ibid.* pp. 44-48.

[4] *Ibid.* pp. 49-50.

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

## Appendix A (cont.): CHEMICAL DATA

### Analysis of DEGDN/Corn Oil Emulsions for Stability, Homogeneity, and Concentration

INTRODUCTION

Emulsions of diethyleneglycol dinitrate (DEGDN) in corn oil were prepared by shaking or stirring mixtures of the two components. The emulsions were subsequently used for dosing animals in the GLP Studies #84017 (acute oral toxicity in rats) and #84018 (acute oral toxicity in mice). After dosing, the remainder of each emulsion was stored at 4°C for analysis. Determination of the DEGDN concentration was accomplished by reverse-phase liquid chromatography.

MATERIALS

Chromatographic analysis was performed using a Hewlett-Packard 1090 high pressure liquid chromatography (HPLC) system with diode array detector (Hewlett-Packard, Palo Alto, CA). Separations were obtained on a Brownlee RP-18 column (4.6 x 250 mm, Brownlee Labs, Inc., Santa Clara, CA). HPLC grade acetonitrile and water were obtained from the J.T. Baker Chemical Co., Phillipsburg, NJ.

METHODS

Analysis of DEGDN solutions was accomplished under the following HPLC conditions: solvent, 70% acetonitrile-30% water; solvent flow, 0.9 ml/min; injection volume, 10 µL; detector wavelength, 205 nm. The HPLC mobile phase was used to prepare standards as well as to extract the DEGDN/corn oil mixtures. Standard solutions of DEGDN ranging in concentrations from 80 to 670 mg DEGDN/ml were prepared in 70% acetonitrile. A set of 12 standards covering this range was analyzed both before and after each set of samples (diluted dosing emulsions).

To measure the effect of corn oil on DEGDN analysis, a series of DEGDN solutions in 70% acetonitrile were prepared with and without the inclusion of corn oil.[6] Eight solutions of DEGDN at 300 µg/ml were prepared by adding 6 ml aliquots

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[6] Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 43-48. Letterman Army Institute of Research, Presidio of San Francisco, CA.

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

of stock solution (50 mg DEGDN/ml) to 50 ml volumetric flasks. Corn oil (1 ml) was then added to 4 of the flasks before filling all to volume with 70% acetonitrile. One ml from each volumetric flask was transferred to a second volumetric flask for a further dilution prior to analysis.

To determine if the emulsions of DEGDN in corn oil prepared for dosing were homogenous, a series of emulsions was prepared with DEGDN concentrations that spanned the range of concentrations employed in the dosing preparations.[7] Emulsions (15 ml each) containing 50, 150, and 300 mg of DEGDN per ml were prepared in 20 ml scintillation vials. After stirring with a magnetic stir bar for at least 5 min, aliquots from the top, middle, and bottom of the emulsions were removed and transferred to tared 25 ml volumetric flasks. The exact weight of the aliquot was recorded and the flask filled to volume. One ml of this solution was transferred to a second volumetric flask for further dilution prior to HPLC analysis.

To determine the stability of DEGDN in corn oil, an emulsion (100 mg DEGDN/ml corn oil) was prepared.[8] Eight 1-ml aliquots were removed and transferred to individual tared volumetric flasks. The weights of the aliquots were recorded and the flasks divided into two equal groups. The first set of four was analyzed immediately and the second set 24 h after preparation of the emulsion. For analysis, the flasks were filled to volume with 70% acetonitrile. One ml from each flask was transferred to a second volumetric flask for further dilution prior to analysis by HPLC.

To prepare the dosing emulsions for analysis the DEGDN/corn oil mixtures were removed from the refrigerator and warmed to room temperature. After rapidly stirring each sample for a minimum of 5 min, an aliquot of approximately one ml was removed and transferred to a tared 50 ml volumetric flask. The weight of each aliquot transferred was

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[7] *Ibid.* pp. 30-40.

[8] Wheeler CR. Toxicology Testing of Propellants. Laboratory Notebook #85-12-023, pp. 74-75. Letterman Army Institute of Research, Presidio of San Francisco, CA.

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

recorded and the flask filled to volume. A second dilution was required prior to analysis by HPLC.[9]

RESULTS

Under the conditions of the analysis DEGDN eluted with a retention time of 4.2 min. A plot of the DEGDN concentration versus peak area was linear within the range of concentrations (80.2-855.5 µg/ml) employed as standards. The differences in peak areas between corresponding standards run before and after the samples were less than 1%. As shown at the bottom of Tables 1, 2, 3, and 4, the equation for the standard plot was virtually identical from assay to assay.

Extraction of the dosing emulsions with 70% acetonitrile-30% water resulted in a very clean chromatogram with no peaks from corn oil. To evaluate the effect of corn oil on DEGDN quantitation, the data obtained from analysis of solutions prepared with and without corn oil (Table 1) was analyzed using the t-test. The concentration of DEGDN in the two sets of samples was not significantly different ($p = 0.91$).[10] This demonstrated that corn oil does not affect the results of the assay under the conditions described, and extraction is therefore quantitative.

The data from the assessment of emulsion homogeneity are presented in Table 2. For each emulsion the deviation of concentration determined for the top, middle, and bottom of the emulsion was less than 5% of the mean. Analysis of DEGDN/corn oil emulsions showed that the concentration of DEGDN in an emulsion stored for 24 h at room temperature was 97.3% of value determined immediately after preparations (Table 3). The data obtained from the analysis of dosing emulsions are presented in Table 4. All but two of the values were within 10% of the target. The two values that fall outside this range do so by only 2.3% and 2.5%.

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[9] Wheeler CR. Nitrocellulose - Nitroguanidine Projects. Laboratory Notebook #85-01-006, pp. 48-56. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[10] Ryan T, Joiner B. Ryan B. Minitab Computer Program for the Data General MV/8000, University Park, PA: Pennsylvania State University, 1982.

## Appendix A (cont.): CHEMICAL DATA

**Table 1.** Analysis of DEGDN with and without corn oil. The target concentration of DEGDN was 300 mg/ml.

| | [DEGDN] by Analysis (mg/ml)* | |
|---|---|---|
| | Corn Oil | Without Corn oil |
| | 292 | 294 |
| | 296 | 301 |
| | 296 | 294 |
| | 297 | 293 |
| Average | 295.2 | 295.5 |

Equation of the standard plot, Y = 0.055X + 0.025; r = 0.9998

**Table 2.** Assessment of homogenity for DEGDN/corn oil emulsions. Aliquots of approximately 1 ml were withdrawn from the top (T), middle (M), and bottom (B) of the emulsions and analyzed.*

| Target [DEGDN] (mg/ml) | Site of Sampling | [DEGDN] Determined by Analysis | Mean [DEGDN] (T+M+B)/3 | Deviation from Mean [DEGDN] |
|---|---|---|---|---|
| | T | 48.8 | | 99.6 |
| 49.7 | M | 49.0 | 49.0 | 100.0 |
| | B | 49.2 | | 100.4 |
| | T | 140.3 | | 96.4 |
| 150.0 | M | 145.0 | 145.5 | 99.7 |
| | B | 151.2 | | 103.9 |
| | T | 279.1 | | 96.1 |
| 299.7 | M | 301.2 | 290.3 | 103.8 |
| | B | 290.5 | | 100.1 |

*Equation of standard plot: Y = 0.057 X - 0.309; r = 0.9998

## Appendix A (cont.): CHEMICAL DATA

**Table 3.** Determination of DEGDN stability in corn oil. An emulsion of DEGDN in corn oil was prepared and analyzed immediately after preparation and 24 h later (4 samples were analyzed each time).

| | Concentration Determined by Analysis (mg/ml) | |
|---|---|---|
| | Time: 0 Hour* | Time: 24 Hours† |
| | 97.9 | 96.2 |
| | 98.9 | 95.3 |
| | 98.1 | 95.0 |
| | 96.8 | 94.7 |
| Average: | 97.9 | 95.3 |

*Equation of standard plot: Y = 0.058 X -0.138; r = 0.9999
†Equation of standard plot: Y = 0.058 X -0 187; r = 0.9998

**Table 4.** Concentration of DEGDN in dosing emulsions prepared for GLP Studies 84017 and 84018. Samples that were analyzed a second time for verification have been denoted with an R (Reanalyzed) in front of the target concentration. In each case, reanalysis yielded a value for concentration that was within 3% of the initial concentration.

| Study No. | Target (mg/ml) | Date Prepared (1985) | Date Analyzed (1985) | Actual (mg/ml) | % Target |
|---|---|---|---|---|---|
| | 50.0 | 07 May | 22 Nov* | 49.1 | 98.2 |
| | 100.0 | 07 May | 22 Nov | 102.1 | 102.1 |
| 84017 | (R) 150.0 | 07 May | 19 Nov† | 168.5 | 112.3 |
| | (R) 126.0 | 14 May | 22 Nov | 110.3 | 87.5 |
| | 79.4 | 14 May | 22 Nov | 81.7 | 102.9 |
| | 100.0 | 14 May | 22 Nov | 96.4 | 96.4 |
| | 193.0 | 20 May | 19 Nov | 194.5 | 100.8 |
| 84018 | 164.0 | 20 May | 19 Nov | 167.4 | 102.1 |
| | 139.0 | 20 May | 19 Nov | 138.0 | 99.3 |
| | 118.0 | 23 May | 22 Nov | 121.1 | 102.6 |
| | 100.0 | 23 May | 19 Nov | 95.0 | 95.0 |

* Equation of standard plot: Y = 0.059X - 1.449; r = 0.9986
† Equation of standard plot: Y = 0.056X + 0.010; r = 0.9999

## Appendix B: ANIMAL DATA

Species: *Mus musculus*

Strain: ICR

Source: Harlan Sprague-Dawley
Indianapolis, IN

Sex: Male and female.

Date of birth: Male: 29 March 1985
Female: 22 March 1985

Method of randomization: Weight bias, stratified animal allocation

Animal allocation: 10 male and 10 female per test group
5 male and 5 female in the control group

Condition of animals at start of study: Normal

Body weight range at dosing: 23 - 39 g

Identification procedures: Ear tag

Pretest conditioning: Quarantine/acclimation 9 May - 20 May 1985

Justification: The laboratory mouse has proven to be a sensitive and reliable system for lethal dose determinations.

## Appendix C: HISTORICAL LISTING OF STUDY EVENTS

| Date | Event |
|---|---|
| 8 May 85 | ICR mice for GLP protocol 84018 arrived. Mice were checked for physical condition, sexed, individually caged, and fed. |
| 9 May 85 | Animals were weighed and tagged, and four mice (2 male and 2 female) were submitted for necropsy quality control. |
| 9 -20 May 85 | Animals were observed daily while in quarantine. |
| 13,17 May 85 | Animals were weighed and randomized into dose groups. |
| 14,16 May 85 | Food was removed from ALD animals at approximately 0600 hrs. ALD animals were weighed, dosed, and observed. |
| 20 May 85 | Animals were weighed and removed from quarantine. |
| 21 May 85 | Food was removed from Phase I animals at approximately 0600 hrs. Animals were weighed and dosed at approximately 1000 hrs. Observations were conducted at approximately 1, 2, and 4 hrs after dosing. |
| 22 May-3 Jun 85 | Phase I animals were observed daily for clinical signs in a.m. and p.m. |
| 23 May 85 | Food was removed from Phase II animals at approximately 0600 hrs. Animals were weighed and dosed at approximately 1000 hrs. Observations conducted at approximately 1, 2, and 4 hrs after dosing. |
| 24 May-5 Jun 85 | Phase II animals were observed daily for clinical signs in a.m. and p.m. |
| 28 May 85 | Phase I animals were weighed. |
| 30 May 85 | Phase II animals were weighed. |

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

| | |
|---|---|
| 4 Jun 85 | Phase I animals had food removed at approximately 0600 hrs. Animals were weighed and observed for clinical signs at approximately 0730 hrs. Animals were delivered to the Necropsy Suite for gross necropsy. |
| 6 Jun 85 | Phase II animals had food removed at approximately 0600 hrs. Animals were weighed and observed for clinical signs at approximately 0730 hrs. Animals were delivered to the Necropsy Suite for gross necropsy. |

**Appendix D: CUMULATIVE MORTALITY DATA (Deaths/Group) (10 Animals/Group)**

| Dose (mg/kg) | Time After Dosing: Hours 2 | 4 | 6 | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10-14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MALES | | | | | | | | | |
| 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1180 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 1390 | 0 | 0 | 0 | 3 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 1640 | 0 | 0 | 0 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 1930 | 0 | 1 | 1 | 8 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Vehicle* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | FEMALES | | | | | | | | | |
| 1000 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1180 | 0 | 0 | 0 | 1 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 1390 | 0 | 0 | 1 | 3 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 1640 | 0 | 0 | 0 | 6 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 1930 | 0 | 2 | 2 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Vehicle* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0 | 3 | 4 | 35 | 52 | 52 | 53 | 53 | 53 | 53 | 53 | 53 | 53 |

*5 animals per group

## Appendix E: INDIVIDUAL ANIMAL HISTORIES

MALE: VEHICLE CONTROLS

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00441 | NORMAL | 23 MAY-6 JUN | |
| 85C00403 | NORMAL | 23 MAY-6 JUN | |
| 85C00399 | NORMAL | 23 MAY-6 JUN | |
| 85C00386 | NORMAL | 23 MAY-6 JUN | |
| 85C00376 | NORMAL | 23 MAY-6 JUN | |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: VEHICLE CONTROLS

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00494 | NORMAL | 23 MAY-6 JUN | |
| 85C00490 | NORMAL | 23 MAY-6 JUN | |
| 85C00484 | NORMAL | 23 MAY-6 JUN | |
| 85C00478 | NORMAL | 23 MAY-6 JUN | |
| 85C00449 | NORMAL | 23 MAY-6 JUN | |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1000 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00442 | SQUINTING | 23 MAY | SLIGHT |
| | HUNCHED POSTURE | 23 MAY | SLIGHT |
| | INCREASED STARTLE REFLEX | 23 MAY | SLIGHT |
| 85C00424 | NORMAL | 23 MAY-6 JUN | |
| 85C00421 | SQUINTING | 23 MAY | SLIGHT |
| 85C00419 | HUNCHED POSTURE | 23 MAY | SLIGHT |
| 85C00413 | NORMAL | 23 MAY-6 JUN | |
| 85C00410 | NORMAL | 23 MAY-6 JUN | |
| 85C00406 | INACTIVE | 23 MAY | SLIGHT |
| | SQUINTING | 23 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 23 MAY | MODERATE |
| 85C00404 | SQUINTING | 23 MAY | SLIGHT |
| | HUNCHED POSTURE | 23 MAY | SLIGHT |
| 85C00377 | HUNCHED POSTURE | 23 MAY | SLIGHT |
| 85C00369 | NORMAL | 23 MAY-6 JUN | |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1000 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00512 | NORMAL | 23 MAY-6 JUN | |
| 85C00505 | HUNCHED POSTURE | 23 MAY | SLIGHT |
| 85C00496 | NORMAL | 23 MAY-6 JUN | |
| 85C00495 | NORMAL | 23 MAY-6 JUN | |
| 85C00487 | NORMAL | 23 MAY-6 JUN | |
| 85C00483 | NORMAL | 23 MAY-6 JUN | |
| 85C00481 | NORMAL | 23 MAY-6 JUN | |
| 85C00471 | NORMAL | 23 MAY-6 JUN | |
| 85C00466 | NORMAL | 23 MAY-6 JUN | |
| 85C00456 | INACTIVE | 23-24 MAY | MARKED |
| | HUNCHED POSTURE | 23 MAY | MODERATE |
| | SQUINTING | 23-24 MAY | MARKED |
| | TREMORS | 23-24 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 23-24 MAY | MARKED |
| | DEATH | 25 MAY | 69.4 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1180 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00440 | NORMAL | 23 MAY-6 JUNE | |
| 85C00439 | INACTIVE | 23 MAY | SLIGHT |
| | ROUGH COAT | 23 MAY | SLIGHT |
| | URINE, ABDOMEN | 23-24 MAY | SLIGHT |
| 85C00433 | ROUGH COAT | 23 MAY | SLIGHT |
| 85C00431 | SQUINTING | 23 MAY | SLIGHT |
| | HUNCHED POSTURE | 23 MAY | SLIGHT |
| 85C00426 | HUNCHED POSTURE | 23 MAY | MARKED |
| | INACTIVE | 23 MAY | MARKED |
| | SQUINTING | 23 MAY | MARKED |
| | TREMORS | 23 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 23 MAY | MARKED |
| | PROSTRATE | 23 MAY | |
| | DEATH | 24 MAY | 21.8 HOURS |
| 85C00409 | INACTIVE | 23 MAY | SLIGHT |
| | SQUINTING | 23 MAY | SLIGHT |
| 85C00401 | INACTIVE | 23 MAY | SLIGHT |
| | SQUINTING | 23 MAY | SLIGHT |
| 85C00392 | SQINTING | 23 MAY | SLIGHT |
| 85C00381 | HUNCHED POSTURE | 23 MAY | SLIGHT |
| | SQUINTING | 23 MAY | SLIGHT |
| 85C00368 | INACTIVE | 23-24 MAY | MARKED |
| | SQUINTING | 23-24 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 23-24 MAY | MARKED |
| | URINE, ABDOMEN | 23-24 MAY | MARKED |
| | TREMORS | 24 MAY | SLIGHT |
| | DEATH | 24 MAY | 27.2 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1180 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00513 | INACTIVE | 23-24 MAY | MARKED |
| | TREMORS | 23-24 MAY | SLIGHT |
| | HUNCHED POSTURE | 23 MAY | SLIGHT |
| | SQUINTING | 23-24 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 23-24 MAY | MARKED |
| | DEATH | 24 MAY | 25.9 HOURS |
| 85C00509 | NORMAL | 23 MAY-6 JUNE | |
| 85C00504 | HUNCHED POSTURE | 23 MAY | SLIGHT |
| | SQUINTING | 23-24 MAY | MARKED |
| | TREMORS | 23-24 MAY | MODERATE |
| | INACTIVE | 23-24 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 24 MAY | MARKED |
| | DEATH | 25 MAY | 45.8 HOURS |
| 85C00503 | NORMAL | 23 MAY-6 JUNE | |
| 85C00488 | NORMAL | 23 MAY-6 JUNE | |
| 85C00480 | SQUINTING | 23-26 MAY | SLIGHT |
| | HUNCHED POSTURE | 23 MAY | SLIGHT |
| | INACTIVE | 23-26 MAY | MARKED |
| | TREMORS | 23-25 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 25-26 MAY | MARKED |
| | DEATH | 27 MAY | 93.9 HOURS |
| 85C00473 | NORMAL | 23 MAY-6 JUNE | |
| 85C00469 | NORMAL | 23 MAY-6 JUNE | |
| 85C00467 | NORMAL | 23 MAY-6 JUNE | |
| 85C00450 | NORMAL | 23 MAY-6 JUNE | |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1390 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00437 | TREMORS | 22 MAY | SLIGHT |
| | INACTIVE | 21-23 MAY | MODERATE |
| | HUNCHED POSTURE | 22 MAY | SLIGHT |
| | SQUINTING | 21,23 MAY | SLIGHT |
| 85C00430 | NORMAL | 21 MAY-4 JUN | |
| 85C00423 | INACTIVE | 21 MAY | MARKED |
| | SQUINTING | 21-22 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | TREMORS | 22 MAY | SLIGHT |
| | URINE, ABDOMEN | 22 MAY | SLIGHT |
| | PROSTRATE | 22 MAY | |
| | DEATH | 23 MAY | 45.1 HOURS |
| 85C00412 | INACTIVE | 21 MAY | MARKED |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | TREMORS | 21-22 MAY | MARKED |
| | PROSTRATE | 22 MAY | |
| | DEATH | 23 MAY | 45.1 HOURS |
| 85C00396 | INACTIVE | 21-22 MAY | MARKED |
| | SQUINTING | 22 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 22 MAY | MODERATE |
| | TREMORS | 22 MAY | MODERATE |
| | URINE, ABDOMEN | 22 MAY | SLIGHT |
| | DEATH | 23 MAY | 45.2 HOURS |
| 85C00389 | INACTIVE | 21 MAY | SLIGHT |
| | ROUGH COAT | 22-23 MAY | SLIGHT |
| | FECES, YELLOW, PERIANAL | 22 MAY | SLIGHT |
| 85C00387 | INACTIVE | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | TREMORS | 21 MAY | MARKED |
| | DEATH | 22 MAY | 21.8 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1390 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00384 | INACTIVE | 21-22 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 22 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21-22 MAY | MODERATE |
| | TREMORS | 22 MAY | MODERATE |
| | URINE, ABDOMEN | 22 MAY | SLIGHT |
| | DEATH | 23 MAY | 45.2 HOURS |
| 85C00383 | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | INACTIVE | 21-22 MAY | MARKED |
| | SQUINTING | 21-22 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21-22 MAY | MARKED |
| | TREMORS | 22 MAY | MODERATE |
| | URINE, ABDOMEN | 22 MAY | MODERATE |
| | DEATH | 22 MAY | 27.7 HOURS |
| 85C00370 | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | TWITCHING | 21 MAY | SLIGHT |
| | TREMORS | 22 MAY | MODERATE |
| | PROSTRATE | 22 MAY | |
| | DEATH | 22 MAY | 27.7 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1390 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00510 | ROUGH COAT | 23 MAY | SLIGHT |
| 85C00508 | INACTIVE | 22 MAY | SLIGHT |
| 85C00507 | TREMORS | 21-22 MAY | MODERATE |
| | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | TWITCHING | 21 MAY | SLIGHT |
| | PROSTRATE | 22 MAY | |
| | DEATH | 22 MAY | 27.4 HOURS |
| 85C00506 | TREMORS | 21-22 MAY | MODERATE |
| | INACTIVE | 21 MAY | SLIGHT |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | SLIGHT |
| | ROUGH COAT | 22 MAY | MARKED |
| | PROSTRATE | 22 MAY | |
| | DEATH | 23 MAY | 44.9 HOURS |
| 85C00502 | TREMORS | 21 MAY | MARKED |
| | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | DEATH | 22 MAY | 21.7 HOURS |
| 85C00499 | TREMORS | 22 MAY | MODERATE |
| | INACTIVE | 21 MAY | SLIGHT |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | SLIGHT |
| | MORIBUND | 22 MAY | |
| | DEATH | 23 MAY | 44.0 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1390 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00492 | TREMORS | 21 MAY | MODERATE |
| | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | INCREASED STARTLE REFLEX | 21 MAY | SLIGHT |
| | MORIBUND | 21 MAY | |
| | DEATH | 21 MAY | 5.6 HOURS |
| 85C00475 | HYPERACTIVE | 21 MAY | SLIGHT |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | TREMORS | 22 MAY | SLIGHT |
| | URINE, ABDOMEN | 22 MAY | SLIGHT |
| | PROSTRATE | 22 MAY | |
| | DEATH | 22 MAY | 45.0 HOURS |
| 85C00462 | TREMORS | 22 MAY | MODERATE |
| | INACTIVE | 22-23 MAY | MARKED |
| 85C00446 | TREMORS | 21-22 MAY | MODERATE |
| | INACTIVE | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | HYPERTONIA | 21 MAY | SLIGHT |
| | PROSTRATE | 22 MAY | |
| | DEATH | 22 MAY | 45.1 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1640 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00444 | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | DEPRESSED RIGHTING REFLEX | 21 MAY | MARKED |
| | TREMORS | 21 MAY | MODERATE |
| | PROSTRATE | 21 MAY | |
| | DEATH | 22 MAY | 21.4 HOURS |
| 85C00443 | SQUINTING | 21 MAY | SLIGHT |
| | ROUGH COAT | 22 MAY | SLIGHT |
| | HUNCHED POSTURE | 22 MAY | SLIGHT |
| | URINE, PERIANAL | 22 MAY | SLIGHT |
| 85C00436 | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | INACTIVE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | SLIGHT |
| | DEATH | 22 MAY | 21.3 HOURS |
| 85C00427 | INACTIVE | 21-23 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21-23 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 22 MAY | MODERATE |
| | ROUGH COAT | 25-26 MAY | SLIGHT |
| 85C00425 | INACTIVE | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | ROUGH COAT | 21 MAY | SLIGHT |
| | TWITCHING | 21 MAY | SLIGHT |
| | TREMORS | 21 MAY | MARKED |
| | HYPERTONIA | 21 MAY | MODERATE |
| | PROSTRATE | 21 MAY | |
| | DEATH | 22 MAY | 21.4 HOURS |
| 85C00418 | NORMAL | 21 MAY-4 JUN | |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1640 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00405 | SQUINTING | 21 MAY | SLIGHT |
| | URINE, PERIANAL | 22 MAY | SLIGHT |
| | TREMORS | 22 MAY | MARKED |
| | PROSTRATE | 22 MAY | |
| | DEATH | 22 MAY | 27.2 HOURS |
| 85C00394 | URINE, ABDOMEN | 21-22 MAY | MARKED |
| | ROUGH COAT | 21-23 MAY | MODERATE |
| 85C00378 | INACTIVE | 21-22 MAY | MARKED |
| | ROUGH COAT | 21-22 MAY | MARKED |
| | URINE, ABDOMEN | 21 MAY | SLIGHT |
| | SQUINTING | 21-22 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 22 MAY | MODERATE |
| | FECES, BROWN, PERIANAL | 22 MAY | MARKED |
| | DEATH | 23 MAY | 44.8 HOURS |
| 85C00374 | INACTIVE | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | MODERATE |
| | ROUGH COAT | 21 MAY | SLIGHT |
| | TREMORS | 21 MAY | MODERATE |
| | PROSTRATE | 22 MAY | |
| | DEATH | 22 MAY | 27.3 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1640 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00500 | INACTIVE | 21-22 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21-22 MAY | SLIGHT |
| | TREMORS | 22 MAY | SLIGHT |
| | ROUGH COAT | 22 MAY | SLIGHT |
| | URINE, PERIANAL | 22 MAY | SLIGHT |
| | DEATH | 23 MAY | 44.3 HOURS |
| 85C00468 | INACTIVE | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | TWITCHING | 21 MAY | SLIGHT |
| | TREMORS | 21 MAY | MARKED |
| | INCREASED STARTLE REFLEX | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 22.7 HOURS |
| 85C00465 | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | MARKED |
| | TREMORS | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 22.4 HOURS |
| 85C00464 | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | DEPRESSED RIGHTING REFLEX | 21 MAY | MARKED |
| | TREMORS | 21 MAY | MARKED |
| | PROSTRATE | 21 MAY | |
| | DEATH | 22 MAY | 21.4 HOURS |
| 85C00461 | INACTIVE | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | TREMORS | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 21.4 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1640 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00457 | TREMORS | 22 MAY | SLIGHT |
| | PROSTRATE | 22 MAY | |
| | DEATH | 23 MAY | 20.5 |
| 85C00454 | INACTIVE | 22 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | INCREASED STARTLE REFLEX | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 22 MAY | MARKED |
| | DEPRESSED RIGHTING REFLEX | 22 MAY | SLIGHT |
| | TREMORS | 21 MAY | SLIGHT |
| | SQUINTING | 21-22 MAY | SLIGHT |
| | URINE, PERIANAL | 22 MAY | MODERATE |
| | DEATH | 22 MAY | 27.0 HOURS |
| 85C00453 | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | TREMORS | 21 MAY | SLIGHT |
| | DEATH | 22 MAY | 21.4 HOURS |
| 85C00451 | NORMAL | 21 MAY-4 JUN | |
| 85C00445 | INACTIVE | 21-22 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21-22 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21-22 MAY | MARKED |
| | URINE, ABDOMEN | 22 MAY | SLIGHT |
| | TREMORS | 21 MAY | MODERATE |
| | DEATH | 23 MAY | 44.6 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1930 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00438 | INACTIVE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEATH | 22 MAY | 21.1 HOURS |
| 85C00429 | INACTIVE | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | SLIGHT |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | ROUGH COAT | 21 MAY | MODERATE |
| | URINE, ABDOMEN | 21 MAY | SLIGHT |
| | TREMORS | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 20.9 HOURS |
| 85C00417 | INACTIVE | 21-22 MAY | MARKED |
| | SQUINTING | 22 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | ROUGH COAT | 21 MAY | SLIGHT |
| | DEPRESSED RIGHTING REFLEX | 22 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 22 MAY | MARKED |
| | TREMORS | 21 MAY | SLIGHT |
| | DEATH | 23 MAY | 44.3 HOURS |
| 85C00411 | INACTIVE | 21 MAY | MARKED |
| | SQUINTING | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | DEATH | 22 MAY | 20.9 HOURS |
| 85C00408 | INACTIVE | 21 MAY | MARKED |
| | SQUINTING | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | TREMORS | 21 MAY | SLIGHT |
| | DEATH | 22 MAY | 20.9 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

MALE: 1930 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00398 | INACTIVE | 21 MAY | MARKED |
| | TREMORS | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | DEPRESSED RIGHTING REFLEX | 21 MAY | MARKED |
| | MORIBUND | 21 MAY | |
| | DEATH | 21 MAY | 4.6 HOURS |
| 85C00390 | INACTIVE | 21 MAY | MODERATE |
| | URINE, ABDOMEN | 21 MAY | SLIGHT |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | ROUGH COAT | 21 MAY | SLIGHT |
| | TREMORS | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 20.9 HOURS |
| 85C00385 | INACTIVE | 21-22 MAY | MARKED |
| | URINE, PERIANAL | 22 MAY | SLIGHT |
| | SQUINTING | 21-22 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 22 MAY | MODERATE |
| | ROUGH COAT | 22 MAY | MODERATE |
| | TREMORS | 22 MAY | MODERATE |
| | DEATH | 23 MAY | 45.3 HOURS |
| 85C00382 | INACTIVE | 21 MAY | MODERATE |
| | URINE, ABDOMEN | 21 MAY | SLIGHT |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MODERATE |
| | ROUGH COAT | 21 MAY | SLIGHT |
| | TREMORS | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 20.9 HOURS |
| 85C00365 | INACTIVE | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | SQUINTING | 21 MAY | MARKED |
| | TREMORS | 21 MAY | MODERATE |
| | PROSTRATE | 21 MAY | |
| | DEATH | 22 MAY | 20.9 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1930 mg/kg DIETHYLENEGLYCOL DINITRATE

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00489 | INACTIVE | 21 MAY | MODERATE |
| | TREMORS | 21 MAY | MARKED |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED RIGHTING REFLEX | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | PROSTRATE | 21 MAY | |
| | DEATH | 22 MAY | 20.9 HOURS |
| 85C00482 | INACTIVE | 21 MAY | MARKED |
| | TREMORS | 21 MAY | MARKED |
| | SQUINTING | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | PROSTRATE | 21 MAY | |
| | DEATH | 22 MAY | 20.9 HOURS |
| 85C00477 | INACTIVE | 21 MAY | MODERATE |
| | TREMORS | 21 MAY | MODERATE |
| | TWITCHING | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | SLIGHT |
| | DEPRESSED RIGHTING REFLEX | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | PROSTRATE | 21 MAY | |
| | DEATH | 21 MAY | 4.6 HOURS |
| 85C00476 | INACTIVE | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | TREMORS | 21 MAY | MODERATE |
| | HYPERTONIA | 21 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | PROSTRATE | 21 MAY | |
| | DEATH | 21 MAY | 4.6 HOURS |
| 85C00472 | INACTIVE | 21 MAY | MARKED |
| | TREMORS | 21 MAY | MARKED |
| | SQUINTING | 21 MAY | MARKED |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | DEPRESSED RIGHTING REFLEX | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MARKED |
| | DEATH | 22 MAY | 21.0 HOURS |

**Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES**

FEMALE: 1930 mg/kg DIETHYLENEGLYCOL DINITRATE (cont.)

| Animal Number | Clinical Signs | Dates Observed (1985) | Severity |
|---|---|---|---|
| 85C00460 | INACTIVE | 21 MAY | MODERATE |
| | ROUGH COAT | 21 MAY | SLIGHT |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| 85C00459 | SQUINTING | 22 MAY | SLIGHT |
| | ROUGH COAT | 22 MAY | SLIGHT |
| | HUNCHED POSTURE | 22 MAY | MODERATE |
| 85C00458 | INACTIVE | 22 MAY | MARKED |
| | TREMORS | 21-22 MAY | MODERATE |
| | SQUINTING | 21-22 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | SLIGHT |
| | ROUGH COAT | 22 MAY | SLIGHT |
| | DEPRESSED GRASPING REFLEX | 21-22 MAY | MODERATE |
| | DEATH | 23 MAY | 44.1 HOURS |
| 85C00455 | HYPERACTIVE | 21 MAY | SLIGHT |
| | SQUINTING | 21 MAY | MODERATE |
| | HUNCHED POSTURE | 21 MAY | MODERATE |
| | INCREASED STARTLE REFLEX | 21 MAY | MODERATE |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 21.0 HOURS |
| 85C00447 | INACTIVE | 21 MAY | MODERATE |
| | SQUINTING | 21 MAY | MARKED |
| | DEPRESSED GRASPING REFLEX | 21 MAY | MODERATE |
| | TREMORS | 21 MAY | MODERATE |
| | DEATH | 22 MAY | 21.0 HOURS |

**Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS**
**1000 mg/kg**

**Males**

| Animal No. | Receipt | Dosing | Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00369 | 35 | 39 | 42 | 42 | 3 |
| 85C00377 | 30 | 36 | 38 | 38 | 2 |
| 85C00404 | 30 | 35 | 37 | 37 | 2 |
| 85C00406 | 33 | 39 | 41 | 43 | 4 |
| 85C00410 | 32 | 32 | 32 | 36 | 4 |
| 85C00413 | 30 | 35 | 37 | 38 | 3 |
| 85C00419 | 32 | 32 | 34 | 35 | 3 |
| 85C00421 | 32 | 37 | 38 | 39 | 2 |
| 85C00424 | 33 | 35 | 38 | 40 | 5 |
| 85C00442 | 30 | 37 | 38 | 40 | 3 |
| Mean | 31.7 | 35.7 | 37.5 | 38.8 | 3.10 |
| Standard Deviation | 1.70 | 2.45 | 2.92 | 2.53 | 0.99 |
| Std. Error of the Mean | 0.54 | 0.76 | 0.92 | 0.80 | 0.31 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1000 mg/kg**

**Females**

| Animal No. | Receipt | Dosing | Termination Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00456 | 24 | 24 | | | |
| 85C00466 | 24 | 27 | 29 | 28 | 1 |
| 85C00471 | 26 | 27 | 31 | 32 | 5 |
| 85C00481 | 28 | 29 | 31 | 30 | 1 |
| 85C00483 | 28 | 28 | 29 | 30 | 2 |
| 85C00487 | 28 | 31 | 31 | 31 | 0 |
| 85C00495 | 26 | 25 | 27 | 27 | 2 |
| 85C00496 | 27 | 29 | 32 | 32 | 3 |
| 85C00505 | 27 | 27 | 28 | 30 | 3 |
| 85C00512 | 26 | 25 | 27 | 27 | 2 |
| Mean | 26.4 | 27.2 | 29.4 | 29.7 | 2.11 |
| Standard Deviation | 1.51 | 2.15 | 1.88 | 1.94 | 1.45 |
| Std. Error of the Mean | 0.48 | 0.68 | 0.63 | 0.65 | 0.48 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1180 mg/kg**

**Males**

| Animal No. | Receipt | Dosing | Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00368 | 29 | 32 | | | |
| 85C00381 | 30 | 31 | 35 | 36 | 5 |
| 85C00392 | 31 | 32 | 34 | 36 | 4 |
| 85C00401 | 33 | 34 | 37 | 39 | 5 |
| 85C00409 | 31 | 35 | 37 | 37 | 2 |
| 85C00426 | 31 | 32 | | | |
| 85C00431 | 31 | 32 | 35 | 37 | 5 |
| 85C00433 | 30 | 35 | 39 | 41 | 6 |
| 85C00439 | 28 | 34 | 36 | 37 | 3 |
| 85C00440 | 32 | 32 | 34 | 37 | 5 |
| Mean | 30.6 | 32.9 | 35.9 | 37.5 | 4.38 |
| Standard Deviation | 1.43 | 1.45 | 1.73 | 1.69 | 1.30 |
| Std. Error of the Mean | 0.45 | 0.46 | 0.61 | 0.60 | 0.46 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1180 mg/kg**

**Females**

| Animal No. | Receipt | Dosing | Termination Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00450 | 25 | 24 | 27 | 28 | 4 |
| 85C00467 | 27 | 28 | 30 | 28 | 0 |
| 85C00469 | 25 | 27 | 29 | 31 | 4 |
| 85C00473 | 26 | 27 | 28 | 28 | 1 |
| 85C00480 | 27 | 28 | | | |
| 85C00488 | 28 | 31 | 32 | 31 | 0 |
| 85C00503 | 25 | 26 | 27 | 27 | 1 |
| 85C00504 | 24 | 26 | | | |
| 85C00509 | 25 | 26 | 26 | 26 | 0 |
| 85C00513 | 29 | 29 | | | |
| Mean | 26.1 | 27.2 | 28.4 | 28.4 | 1.43 |
| Standard Deviation | 1.60 | 1.93 | 2.07 | 1.90 | 1.81 |
| Std. Error of the Mean | 0.50 | 0.61 | 0.78 | 0.72 | 0.69 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1390 mg/kg**

**Males**

| Animal No. | Receipt | Dosing | Termination Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00370 | 33 | 33 | | | |
| 85C00383 | 30 | 33 | | | |
| 85C00384 | 30 | 33 | | | |
| 85C00387 | 33 | 34 | | | |
| 85C00389 | 31 | 33 | 35 | 36 | 3 |
| 85C00396 | 33 | 34 | | | |
| 85C00412 | 31 | 35 | | | |
| 85C00423 | 33 | 37 | | | |
| 85C00430 | 33 | 34 | 35 | 35 | 1 |
| 85C00437 | 34 | 34 | 34 | 35 | 1 |
| Mean | 32.1 | 34.0 | 34.7 | 35.3 | 1.67 |
| Standard Deviation | 1.45 | 1.25 | 0.58 | 0.58 | 1.15 |
| Std. Error of the Mean | 0.46 | 0.39 | 0.33 | 0.33 | 0.67 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1390 mg/kg**

**Females**

| Animal No. | Receipt | Dosing | Termination Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00446 | 23 | 25 | | | |
| 85C00462 | 27 | 30 | 31 | 33 | 3 |
| 85C00475 | 25 | 23 | | | |
| 85C00492 | 26 | 27 | | | |
| 85C00499 | 30 | 28 | | | |
| 85C00502 | 24 | 24 | | | |
| 85C00506 | 29 | 27 | | | |
| 85C00507 | 26 | 26 | | | |
| 85C00508 | 25 | 26 | 27 | 27 | 1 |
| 85C00510 | 26 | 27 | 28 | 30 | 3 |
| Mean | 26.1 | 26.3 | 28.7 | 30.0 | 2.33 |
| Standard Deviation | 2.13 | 2.00 | 2.08 | 3.00 | 1.15 |
| Std. Error of the Mean | 0.67 | 0.63 | 1.20 | 1.73 | 0.67 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1640 mg/kg**

**Males**

| Animal No. | Receipt | Dosing | Termination Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00374 | 32 | 31 | | | |
| 85C00378 | 34 | 38 | | | |
| 85C00394 | 30 | 33 | 35 | 36 | 3 |
| 85C00405 | 30 | 35 | | | |
| 85C00418 | 33 | 33 | 34 | 34 | 1 |
| 85C00425 | 31 | 33 | | | |
| 85C00427 | 32 | 34 | 27 | 36 | 2 |
| 85C00436 | 32 | 35 | | | |
| 85C00443 | 32 | 34 | 37 | 35 | 1 |
| 85C00444 | 24 | 36 | | | |
| Mean | 31.0 | 34.2 | 33.3 | 35.3 | 1.75 |
| Standard Deviation | 2.75 | 1.93 | 4.35 | 0.96 | 0.96 |
| Std. Error of the Mean | 0.87 | 0.61 | 2.17 | 0.48 | 0.48 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1640 mg/kg**

**Females**

| Animal No. | Receipt | Dosing | Termination Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00445 | 25 | 25 | | | |
| 85C00451 | 23 | 25 | 26 | 29 | 4 |
| 85C00453 | 23 | 24 | | | |
| 85C00454 | 27 | 27 | | | |
| 85C00457 | 24 | 25 | | | |
| 85C00461 | 25 | 25 | | | |
| 85C00464 | 25 | 28 | | | |
| 85C00465 | 24 | 26 | | | |
| 85C00468 | 24 | 25 | | | |
| 85C00500 | 27 | 27 | | | |
| Mean | 24.7 | 25.7 | 26.0 | 29.0 | 4.00 |
| Standard Deviation | 1.42 | 1.25 | 0.00 | 0.00 | 0.00 |
| Std. Error of the Mean | 0.45 | 0.40 | 0.00 | 0.00 | 0.00 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1930 mg/kg**

**Males**

| Animal No. | Receipt | Dosing | Termination Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00365 | 31 | 35 | | | |
| 85C00382 | 35 | 37 | | | |
| 85C00385 | 30 | 33 | | | |
| 85C00390 | 31 | 33 | | | |
| 85C00398 | 32 | 34 | | | |
| 85C00408 | 32 | 35 | | | |
| 85C00411 | 33 | 32 | | | |
| 85C00417 | 31 | 35 | | | |
| 85C00429 | 25 | 33 | | | |
| 85C00438 | 30 | 33 | | | |
| Mean | 31.0 | 34.0 | - | - | - |
| Standard Deviation | 2.58 | 1.49 | - | - | - |
| Std. Error of the Mean | 0.82 | 0.47 | - | - | - |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS 1930 mg/kg**

**Females**

| Animal No. | Receipt | Dosing | Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00447 | 25 | 25 | | | |
| 85C00455 | 23 | 23 | | | |
| 85C00458 | 24 | 25 | | | |
| 85C00459 | 26 | 27 | 30 | 32 | 5 |
| 85C00460 | 23 | 23 | 27 | 29 | 6 |
| 85C00472 | 26 | 28 | | | |
| 85C00476 | 27 | 25 | | | |
| 85C00477 | 30 | 31 | | | |
| 85C00482 | 24 | 25 | | | |
| 85C00489 | 27 | 28 | | | |
| Mean | 25.5 | 26.0 | 28.5 | 30.5 | 5.50 |
| Standard Deviation | 2.17 | 2.49 | 2.12 | 2.12 | 0.71 |
| Std. Error of the Mean | 0.69 | 0.79 | 1.50 | 1.50 | 0.50 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS (Vehicle Control) 10 ml/kg**

**Males**

| Animal No. | Receipt | Dosing | Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00376 | 30 | 34 | 37 | 36 | 2 |
| 85C00386 | 34 | 36 | 40 | 41 | 5 |
| 85C00399 | 32 | 35 | 38 | 39 | 4 |
| 85C00403 | 32 | 32 | 35 | 35 | 3 |
| 85C00441 | 31 | 33 | 36 | 36 | 3 |
| Mean | 31.8 | 34.0 | 37.2 | 37.4 | 3.40 |
| Standard Deviation | 1.48 | 1.58 | 1.92 | 2.51 | 1.14 |
| Std. Error of the Mean | 0.66 | 0.71 | 0.86 | 1.12 | 0.51 |

*Weight change is from day of dosing to termination.

**Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS (Vehicle Control) 10 ml/kg**

**Females**

| Animal No. | Receipt | Dosing | Day 7 | Termination Day 14 | Weight* Change |
|---|---|---|---|---|---|
| 85C00449 | 26 | 27 | 30 | 31 | 4 |
| 85C00478 | 26 | 27 | 29 | 29 | 2 |
| 85C00484 | 27 | 25 | 28 | 28 | 3 |
| 85C00490 | 26 | 27 | 31 | 30 | 3 |
| 85C00494 | 27 | 27 | 30 | 29 | 2 |
| Mean | 26.4 | 26.6 | 29.6 | 29.4 | 2.8 |
| Standard Deviation | 0.55 | 0.89 | 1.14 | 1.14 | 0.84 |
| Standard Error of the Mean | 0.24 | 0.40 | 0.51 | 0.51 | 0.37 |

*Weight change is from day of dosing to termination.

## Appendix G: PATHOLOGY REPORT

GLP Study 84018 Investigator: SP4 John Ryabik

History: This study is designed to determine the median lethal dose (MLD) in mice of diethyleneglycol dinitrate (DEGDN) in corn oil, (CAS No. 693-21-0). Ten males and 10 females were dosed by oral gavage at each of the following dose levels:

| DOSAGE GROUP | DOSE LEVEL (mg DEGDN/kg BW) |
|---|---|
| 1 | 1390 |
| 2 | 1640 |
| 3 | 1930 |
| 4 | 1180 |
| 5 | 1000 |
| 6 | 0 |

Gross Necropsy Results (Males): The individual animal gross findings are as follows:

**DOSE GROUP 1 - 1390 mg/kg**
**MALES**

| LAIR ACCESSION # | ID # | GROSS FINDINGS |
|---|---|---|
| 37617 | 85C00370 | Dead - NR |
| 37619 | 85C00383 | Dead - NR |
| 37625 | 85C00384 | Dead - NR |
| 37593 | 85C00387 | Dead - NR |
| 37761 | 85C00389 | Live - NR |
| 37627 | 85C00396 | Dead - NR |
| 37628 | 85C00412 | Dead - NR |
| 37630 | 85C00423 | Dead - NR |
| 37765 | 85C00430 | Live - NR |
| 37766 | 85C00437 | Live - NR |

**DOSE GROUP 2 - 1640 mg/kg**
**MALES**

| | | |
|---|---|---|
| 37618 | 85C00374 | Dead - NR |
| 37624 | 85C00378 | Dead - NR |
| 37762 | 85C00394 | Live - NR |
| 37620 | 85C00405 | Dead - NR |
| 37763 | 85C00418 | Live - NR |
| 37598 | 85C00425 | Dead - NR |
| 37764 | 85C00427 | Live - NR |
| 37600 | 85C00436 | Dead - NR |
| 37767 | 85C00443 | Live - NR |
| 37602 | 85C00444 | Dead - NR |

## Appendix G (cont.): PATHOLOGY REPORT

### DOSE GROUP 3 - 1930 mg/kg
### MALES

| LAIR ACCESSION # | ID # | GROSS FINDINGS |
|---|---|---|
| 37591 | 85C00365 | Dead - NR |
| 37592 | 85C00382 | Dead - NR |
| 37626 | 85C00385 | Dead - NR |
| 37594 | 85C00390 | Dead - NR |
| 37595 | 85C00398 | Dead - NR |
| 37596 | 85C00408 | Dead - NR |
| 37597 | 85C00411 | Dead - NR |
| 37629 | 85C00417 | Dead - NR |
| 37599 | 85C00429 | Dead - NR |
| 37601 | 85C00438 | Dead - NR |

### DOSE GROUP 4 - 1180 mg/kg
### MALES

| | | |
|---|---|---|
| 37655 | 85C00368 | Dead - NR |
| 37827 | 85C00381 | Live - NR |
| 37829 | 85C00392 | Live - NR |
| 37831 | 85C00401 | Live - NR |
| 37835 | 85C00409 | Live - NR |
| 37656 | 85C00426 | Dead - NR |
| 37841 | 85C00431 | Live - NR |
| 37842 | 85C00433 | Live - NR |
| 37843 | 85C00439 | Live - NR |
| 37844 | 85C00440 | Live - NR |

### DOSE GROUP 5 - 1000 mg/kg
### MALES

| | | |
|---|---|---|
| 37824 | 85C00369 | Live - NR |
| 37826 | 85C00377 | Live - NR |
| 37833 | 85C00404 | Live - NR |
| 37834 | 85C00406 | Live - NR |
| 37836 | 85C00410 | Live - NR |
| 37837 | 85C00413 | Live - NR |
| 37838 | 85C00419 | Live - NR |
| 37839 | 85C00421 | Live - NR |
| 37840 | 85C00424 | Live - NR |
| 37846 | 85C00442 | Live - NR |

## Appendix G (cont.): PATHOLOGY REPORT

### DOSE GROUP 6 - VEHICLE CONTROL
### MALES

| LAIR ACCESSION # | ID # | GROSS FINDINGS |
|---|---|---|
| 37825 | 85C00376 | Live - NR |
| 37828 | 85C00386 | Live - NR |
| 37830 | 85C00399 | Live - NR |
| 37832 | 85C00403 | Live - NR |
| 37845 | 85C00441 | Live - NR |

All deaths occured within 72 hours after dosing and the test compound was the most likely cause of death in all cases. All survivors were killed by sodium pentobarbital injection 2 weeks after dosing.

Gross Necropsy Results (Females): The individual animal gross findings are as follows:

### DOSE GROUP 1 - 1390 mg/kg
### FEMALES

| | | |
|---|---|---|
| 37632 | 85C00446 | Dead - NR |
| 37771 | 85C00462 | Live - NR |
| 37635 | 85C00475 | Dead - NR |
| 37615 | 85C00492 | Dead - NR |
| 37636 | 85C00499 | Dead - NR |
| 37616 | 85C00502 | Dead - NR |
| 37638 | 85C00506 | Dead - NR |
| 37622 | 85C00507 | Dead - NR |
| 37772 | 85C00508 | Live - NR |
| 37773 | 85C00510 | Live - NR |

### DOSE GROUP 2 - 1640 mg/kg
### FEMALES

| | | |
|---|---|---|
| 37631 | 85C00445 | Dead - NR |
| 37768 | 85C00451 | Live - NR |
| 37604 | 85C00453 | Dead - NR |
| 37621 | 85C00454 | Dead - NR |
| 37633 | 85C00457 | Dead - NR |
| 37606 | 85C00461 | Dead - NR |
| 37607 | 85C00464 | Dead - NR |
| 37608 | 85C00465 | Dead - NR |
| 37609 | 85C00468 | Dead - NR |
| 37637 | 85C00500 | Dead - NR |

## Appendix G (cont.): PATHOLOGY REPORT

### DOSE GROUP 3 - 1930 mg/kg
### FEMALES

| LAIR ACCESSION # | ID # | GROSS FINDINGS |
|---|---|---|
| 37603 | 85C00447 | Dead - NR |
| 37605 | 85C00455 | Dead - NR |
| 37634 | 85C00458 | Dead - NR |
| 37769 | 85C00459 | Live - NR |
| 37770 | 85C00460 | Live - NR |
| 37610 | 85C00472 | Dead - NR |
| 37611 | 85C00476 | Dead - NR |
| 37612 | 85C00477 | Dead - NR |
| 37613 | 85C00482 | Dead - NR |
| 37614 | 85C00489 | Dead - NR |

### DOSE GROUP 4 - 1180 mg/kg
### FEMALES

| | | |
|---|---|---|
| 37804 | 85C00450 | Live - NR |
| 37806 | 85C00467 | Live - NR |
| 37807 | 85C00469 | Live - NR |
| 37809 | 85C00473 | Live - NR |
| 37661 | 85C00480 | Dead - NR |
| 37815 | 85C00488 | Live - NR |
| 37820 | 85C00503 | Live - NR |
| 37662 | 85C00504 | Dead - NR |
| 37822 | 85C00509 | Live - NR |
| 37657 | 85C00513 | Dead - NR |

### DOSE GROUP 5 - 1000 mg/kg
### FEMALES

| | | |
|---|---|---|
| 37660 | 85C00456 | Dead-Post mortem autolysis - severe |
| 37805 | 85C00466 | Live - NR |
| 37808 | 85C00471 | Live - NR |
| 37811 | 85C00481 | Live - NR |
| 37812 | 85C00483 | Live - NR |
| 37814 | 85C00487 | Live - NR |
| 37818 | 85C00495 | Live - NR |
| 37819 | 85C00496 | Live - NR |
| 37821 | 85C00505 | Live - NR |
| 37823 | 85C00512 | Live - NR |

## Appendix G (cont.): PATHOLOGY REPORT

### DOSE GROUP 6 - VEHICLE CONTROL
### FEMALES

| LAIR ACCESSION # | ID # | GROSS FINDINGS |
|---|---|---|
| 37803 | 85C00449 | Live - NR |
| 37810 | 85C00478 | Live - NR |
| 37813 | 85C00484 | Live - NR |
| 37816 | 85C00490 | Live - NR |
| 37817 | 85C00494 | Live - NR |

All deaths occured within 72 hours after dosing and the test compound was the most likely cause of death in all cases. All survivors were killed by sodium pentobarbital injection 2 weeks after dosing.

Microscopic Findings: No tissues were taken for microscopic examination.

Results Summary: A clear dose response effect is apparent in both male and female mice.

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