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

LETTERMAN ARMY INSTITUTE OF RESEARCH
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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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

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

TESTING FACILITY:

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

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development 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
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: SPC John R.G. Ryabik, BS

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PATHOLOGIST: LTC Lance D. Lollini, DVM, MS, VC, Diplomate,
American College of Veterinary Pathologists

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.

Don W. Korte Jr 27 APR 89
DON W. KORTE JR, PhD / DATE
LTC, MSC
Study Director

Larry D. Brown 11 Jan 89
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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

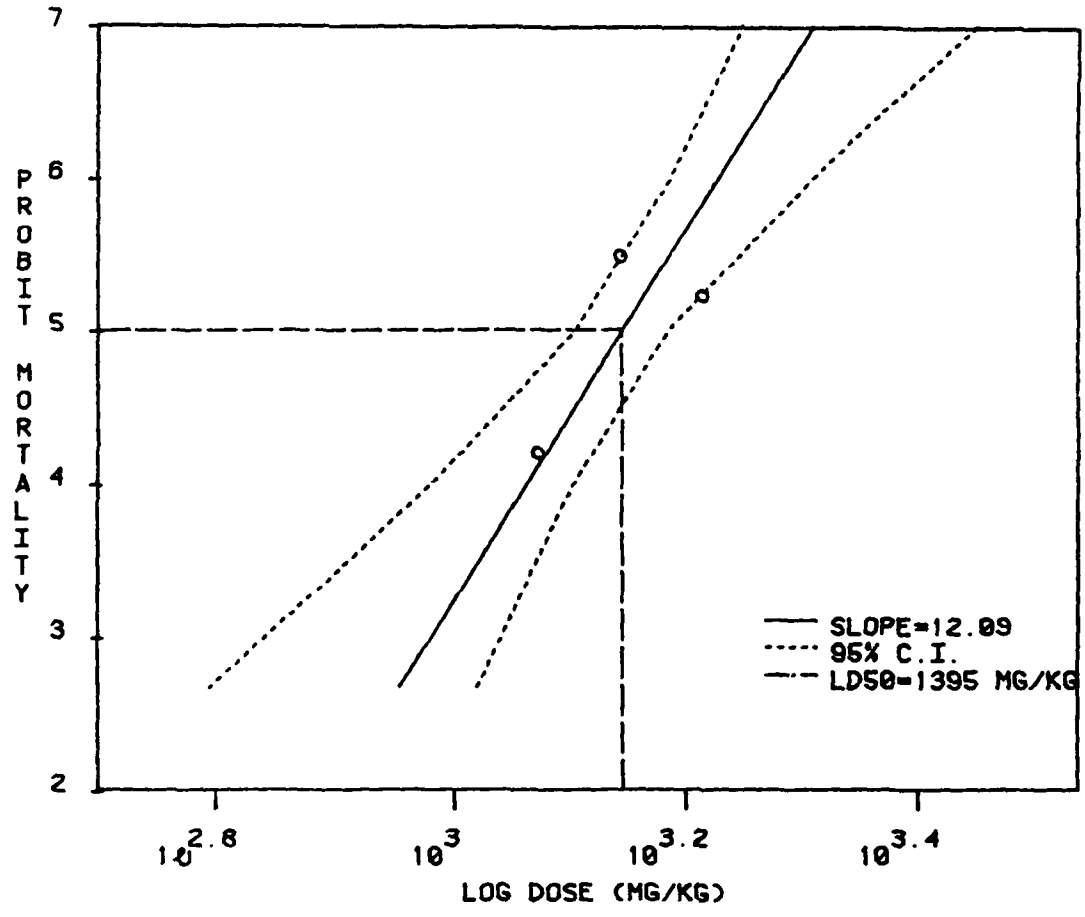
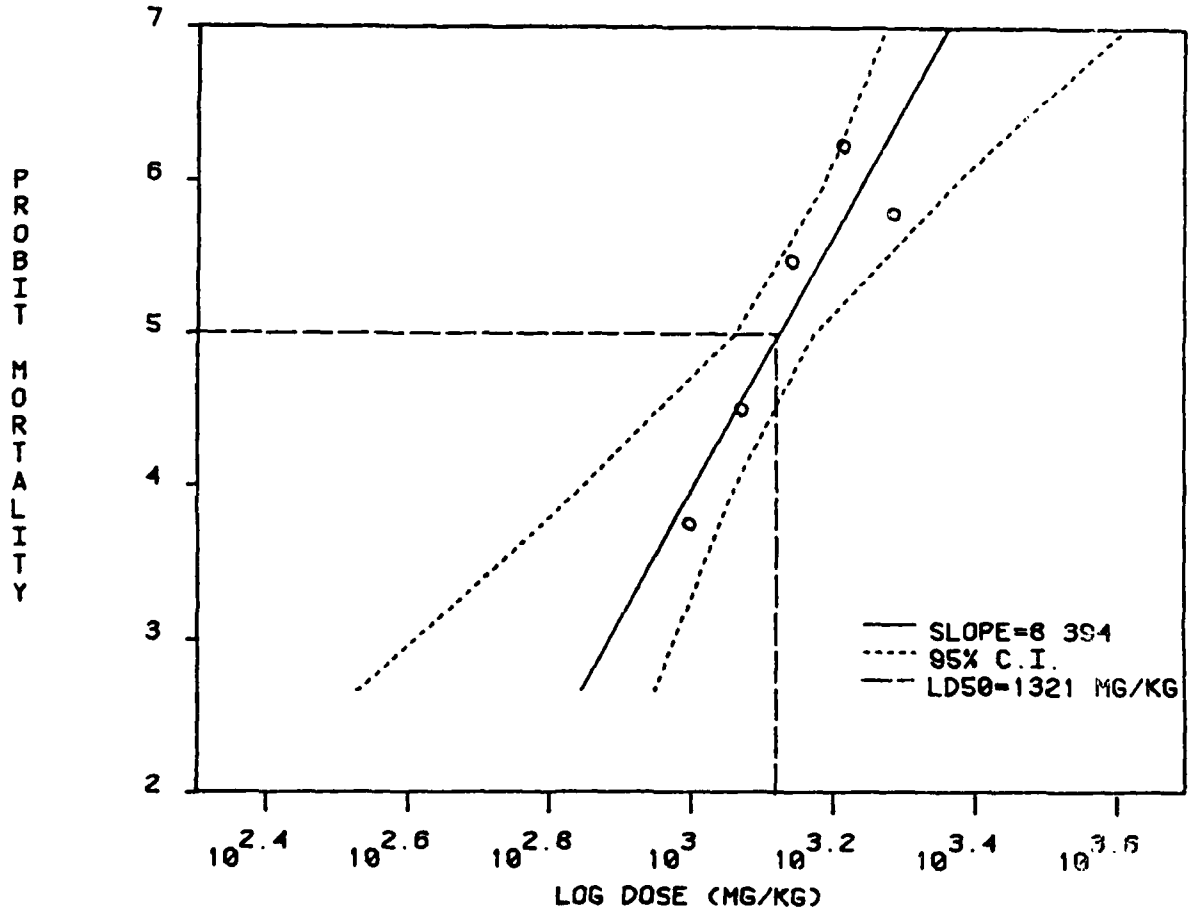


Figure 2

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.

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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:

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

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 δ (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, 572cm^{-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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