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Institute Report No. 409

**Acute Oral Toxicity of
DIGL-RP Solid Propellant in Sprague-Dawley Rats**

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and
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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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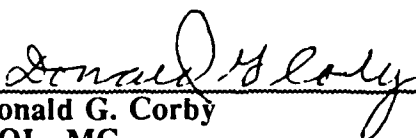
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ABSTRACT

The acute oral toxicity of DIGL-RP solid propellant was determined in male and female Sprague-Dawley rats by using an oral gavage split-dose method. The MLD was 2946.1 ± 39.2 mg/kg for male rats and 2636.4 ± 117.8 mg/kg for female rats. DIGL-RP produced clinical signs that were attributed to its nitrate ester component, diethyleneglycol dinitrate. These signs included tremors, depression of reflexes, cyanosis, increases in respiratory rate and depth, and decreased body temperature. Other clinical signs observed were associated with the general malaise of the animals and dosing and included hunched posture, squinting, reddish stains around the eyes and nose, and perianal staining. Most animals exhibited signs by 4 hours after dosing and either had died or the signs had cleared by 96 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place DIGL-RP in the slightly toxic class.

Key Words: Acute Oral Toxicity, DIGL-RP Solid Propellant, Diethyleneglycol Dinitrate, Mammalian Toxicology, Propellant, Rat



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PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

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Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

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Project Officer: Gunda Reddy, PhD

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

GLP STUDY NUMBER: 85021

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DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT: A copy of the final report,
study protocol, 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: DIGL-RP Solid Propellant

INCLUSIVE STUDY DATES: 30 Dec 85 - 11 Jun 86

OBJECTIVE: The objective of this study was to determine the
acute oral toxicity of DIGL-RP Solid Propellant
in male and female Sprague-Dawley rats.

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

We, the undersigned, declare that GLP Study 85021 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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REPLY TO
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SGRD-ULZ-QA

29 November 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85021

1. This is to certify that the protocol for LAIR GLP Study 85021 was reviewed on 10 May 1985.
2. The institute report entitled "Acute Oral Toxicity (MLD) of DIGL-RP Solid Propellant in Rats," Toxicology Series 167, was audited on 21 July 1987.

Carolyn M. Lewis

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

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Acute Oral Toxicity of DIGL-RP Solid Propellant in Sprague-Dawley Rats--Brown et al.

INTRODUCTION

The Department of Defense is considering the use of diethyleneglycol dinitrate (DEGDN), triethyleneglycol dinitrate (TEGDN), or trimethylolethane trinitrate (TMETN) as a replacement for nitroglycerin in munition formulations. A "health effects" review conducted for the US Army Biomedical Research and Development Laboratory (USABDRL) identified numerous gaps in the toxicology database of these compounds. (1). Consequently, USABDRL 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, acute dermal toxicity tests 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 DIGL-RP Solid Propellant in male and female Sprague-Dawley rats.

MATERIALS

Test Substance

Chemical Name: DIGL-RP Solid Propellant

LAIR Code No.: TP57

Description: Solid black cylinders (stick configuration)

Lot Number: RAD83M001S169

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

Vehicle

The vehicle for DIGL-RP was 1% gum tragacanth (Lot 91044A2, Spectrum Chemical Manufacturing Corporation, Gardena, CA) made up in sterile water for injection (Lot 65-914-DM-03, Abbott Laboratories, North Chicago, IL). The expiration date was Nov 1995 for the gum tragacanth and Jun 1986 for the sterile water for injection.

Animal Data

Seventy-nine male and 80 female Sprague-Dawley rats were obtained from Bantin-Kingman Inc. (Fremont, CA) for this study. They were identified individually with ear tags. Twenty (10 of each sex) were used as approximate lethal dose (ALD) animals and two males and two females were submitted as necropsy quality controls. Fifty females were dosed with DIGL-RP and 10 were dosed with the vehicle. Fifty-four males were dosed with DIGL-RP and 10 were dosed with the vehicle. Extra animals were transferred to another study. The animal weights on receipt ranged from 123 to 174 g. Additional animal data appear in Appendix B.

Husbandry

Rats were caged individually in stainless steel wire mesh cages in racks equipped with automatic 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); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 17.8°C to 24.4°C with a relative humidity range of 44 to 88 percent. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Male and female rats were randomized separately into five dose groups and a vehicle control group using a stratified, weight-biased computer program (Beckman TOXSYS® Animal Allocation Program run on a Beckman TOXSYS® Data Collection Terminal). The animals were acclimated for 9-19 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of an approximate lethal dose (ALD) determination suggested that the median lethal dose (MLD) was between 2000 and 3500 mg/kg. Based on these data, test doses were selected (Table 1).

TABLE 1: DIGL-RP Solid Propellant Doses

<u>Male</u> <u>Dose Levels</u> (mg/kg)	<u>Female</u> <u>Dose Levels</u> (mg/kg)
2510	1590
2820	2000
2985	2510
3160	3160
3550	3550
Vehicle	Vehicle

Compound Preparation

The DIGL-RP solid propellant (stick configuration) was ground into a fine powder before dosing using a Spex Model 6700 liquid nitrogen freezer/mill (Spex Industries, Inc., Edison, NJ). After passing through an 80-mesh sieve, this finely ground powder was weighed and mixed with appropriate volumes of a 1% solution of gum tragacanth to make dosing suspensions. Homogeneity was assured by mixing these suspensions with a Brinkman homogenizer. Because the homogenization process created numerous small air bubbles in

the suspensions, they were prepared, placed overnight under refrigeration at 4°C, warmed and remixed gently the next morning, and then used.

Chemical Analyses of Dosing Suspensions

DIGL-RP was stable in the gum tragacanth vehicle for at least 24 hrs (Appendix A). This was sufficient since dosing was begun and completed within 20 hrs. Tests for homogeneity and concentration verification of the test compound in the gum tragacanth vehicle were conducted as outlined in Appendix A. The deviation of individual values from the mean of each set of 3 samples (top, middle, bottom) from each suspension did not exceed 3.1% for any suspension. The DIGL-RP dosing suspensions used in this study were within 92.5 - 105.5% of target.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP OP-STX-36 (3). Animals were fasted overnight before dosing. The volume of dosing solution each animal received was based upon the desired dose level, the compound concentration in suspension, and the animal's weight. Dosing was performed using the oral gavage method without animal sedation or anesthesia. Since the test compound was viscous and thus difficult to administer at high concentrations, the animals were administered a split dose one hour apart to achieve the desired dose level. The dose level was increased by varying the concentration of each suspension. Split dose volumes ranged from 2.28 to 3.45 ml in the males and 1.53 to 2.03 ml in the females. The vehicle control (1% gum tragacanth) group received 1.95 to 2.17 ml (males) and 1.51 to 1.74 ml (females). The total volume administered to each animal can be obtained by multiplying the split-dose volume by 2. The volumes given were based on a rate of 10 ml/kg for each split dose. Sterile disposable syringes (Monoject, Sherwood Medical, St. Louis, MO and Becton, Dickenson & Co., Rutherford, NJ) fitted with 14-18 gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized. Animals in Phase I (females and vehicle control males) were dosed between 0930 and 1207 hrs on 9 Jan 1986. Phase II animals were dosed between 0930 and 1156 on 13 May 1986 (2985 mg/kg, 3160 mg/kg, and 3550 mg/kg males) and between 0841 and 1059 on 20 May 1986 (2510 mg/kg and 2820 mg/kg males) after analysis of the preliminary data.

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 2 and 4 hours after the initial 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 once 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 submitted for necropsy immediately after receiving a barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD₁₀, LD₅₀, and LD₉₀ 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 plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable addenda with the following exceptions: The cage control group was not run as historical cage control data was available. Temperature and relative humidity fluctuated outside the specified ranges due to problems in the central heating unit (30 Dec 85-2 Jan 86) and two power outages (29-30 May and 25 Jun 86). Males that received 2820 mg/kg were sacrificed on Day 13 (not Day 14) after dosing due to an error in preparation of the study schedule. The DIGL-RP suspensions were administered as a split dose one hour apart because of their high viscosity which made concentrations greater than 200 mg/ml impossible to administer via the feeding tubes. Consequently, the first

of 3 scheduled observations (1 hr after dosing) was deleted because the split-dosing procedure required 2 hrs instead of the normal 1 hr to complete. These deviations did not significantly affect the outcome of the study.

Storage of Raw Data and Final Report

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

RESULTS

Mortality

Forty-six of 92 animals (25/45 males, 21/47 females) dosed with DIGL-RP died as a result of its toxicity. Ten (21.7%) deaths occurred within 24 hrs of dosing. An additional 19 (41.3%) deaths occurred by 48 hrs after dosing, and the remaining 17 (37.0%) deaths occurred within the first week after dosing. Table 2 lists the compound-related deaths by dose group with percent mortality. Appendix D is a tabular presentation of the cumulative mortality data.

TABLE 2: Compound-Related Deaths by Group

<u>Dose Level</u> (mg/kg)	<u>Deaths/</u> <u>Group</u>	<u>Percent</u> <u>Mortality</u>
Males		
2510	0/9*	0.0
2820	1/9*	11.1
2985	6/8*	75.0
3160	9/10	90.0
3550	9/9*	100.0
Vehicle	0/7*	0.0
Females		
1590	0/9*	0.0
2000	1/10	10.0
2510	2/9*	22.2
3160	8/9*	88.9
3550	10/10	100.0
Vehicle	0/10	0.0

*Reduced numbers in groups were due to one or more animals which were removed from the study.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis and the equations for the probit regression line were: $Y = -173.56 + 51.47 \log X$ (males); $Y = -44.92 + 14.59 \log X$ (females), where X is the dose and Y the corresponding probit value. Animals removed from the study were not included in the calculations. Figures 1 and 2 graphically present the actual data points and the regression line. Lethal doses calculated from the equation for the probit regression line are presented in Table 3.

TABLE 3: Calculated Lethal Doses (LD) of DIGL-RP Solid Propellant in Sprague-Dawley Rats

Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
Males		
LD10	2782.0 ± 65.0	(2508.0, 2872.0)
LD50	2946.1 ± 39.2	(2842.3, 3035.5)
LD90	3120.0 ± 64.9	(3029.8, 3410.8)
Females		
LD10	2153.7 ± 150.2	(1708.5, 2389.1)
LD50	2636.4 ± 117.8	(2372.3, 2894.8)
LD90	3227.3 ± 200.8	(2932.2, 3940.0)

* Calculated dose ± standard error.

Clinical Observations

The most frequently observed category of clinical observations was behavioral disturbances (86 of 92 animals dosed). Behavioral signs exhibited by the animals included tremors, inactivity, twitching, irritability, hyperactivity, and ataxia. They were first observed 4 to 24 hours after dosing and were generally no longer observed 4 to 5 days after dosing. All animals that died exhibited one or more behavioral signs. Behavioral signs were present in all animals of all dose groups except the 1590 mg/kg female group which exhibited a noticeably lower incidence. Other clinical signs attributed directly to the administration of DIGL-RP were changes in reflexes (44 of 92), cyanosis (67 of 92), changes in respiration (11 of 92), and decreased body temperature (24 of 92). The decreased body temperature was based on the animals feeling cold and clammy to the observer.

Figure 1

DIGL-RP Dose Response Curve for Male Rats

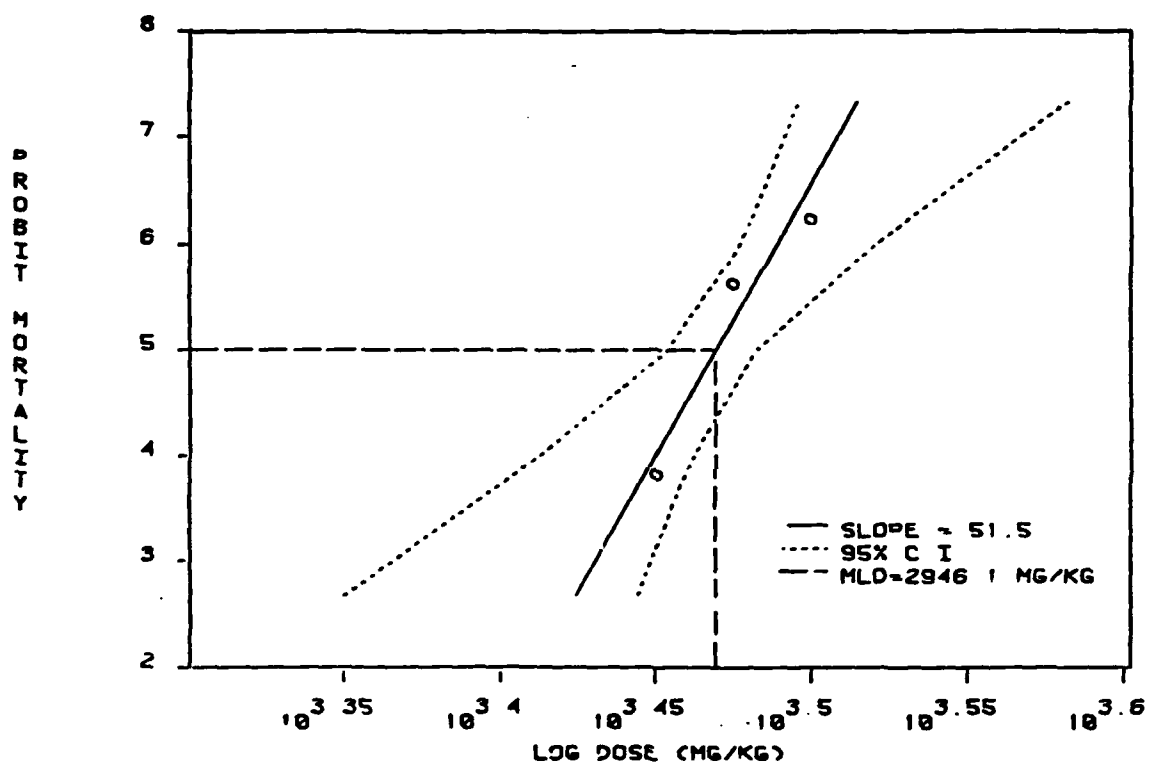
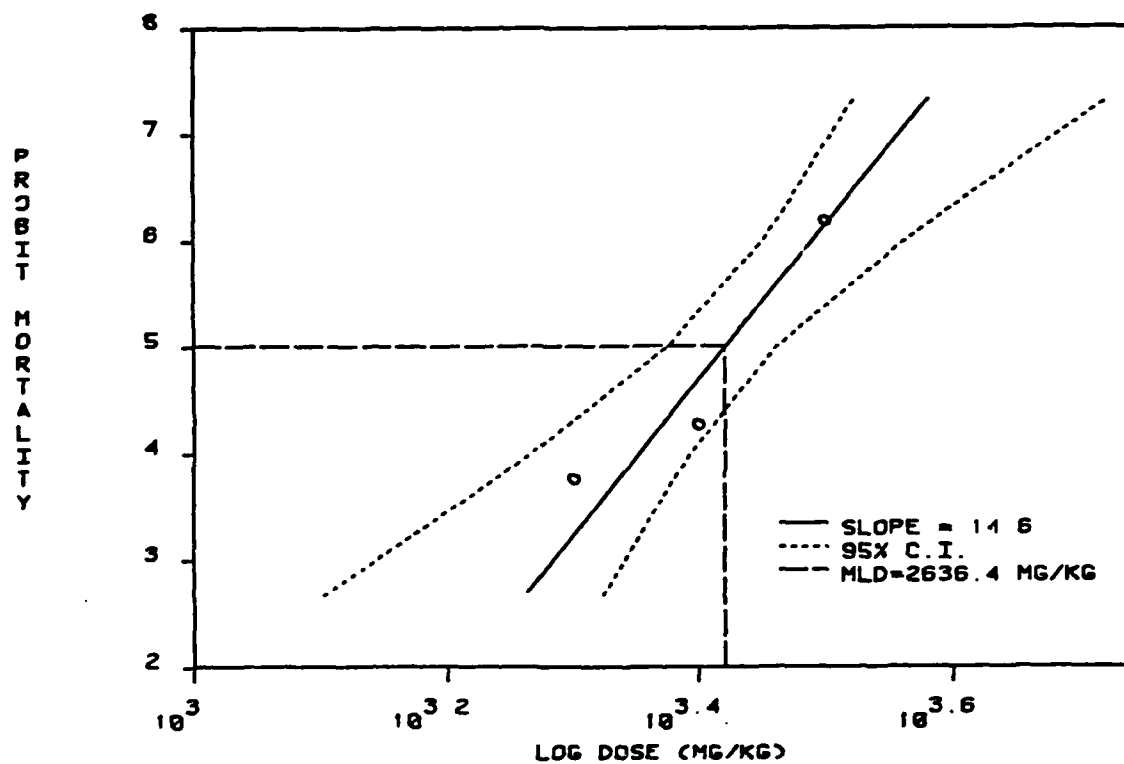


Figure 2
DIGL-RP Dose Response Curve for Female Rats



Most other clinical signs were attributable to general malaise associated with the administration of DIGL-RP. Included with these signs were the categories of skin signs (rough coat) observed in 42 of 92, ocular signs (squinting, lacrimation, and chromodacryorrhea) in 61 of 92, hunched posture in 64 of 92, and various stains in 42 of 92.

Nine female and 4 male vehicle control animals were normal throughout the study. A variety of signs was observed in 3 male vehicle control animals. These included hunched posture, inactivity, and rough coat and could be attributed to the dosing procedure and/or the volume of the gum tragacanth vehicle administered. Table 4 contains a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not affected by administration of DIGL-RP. Table 5 presents the mean body weights by groups. Appendix F contains individual weight tables.

Pathology Findings

Gastroenteropathy, used to categorize multifocal petechiation (hemorrhage) and necrosis of the stomach as well as bloody contents, was observed in 17 animals. Hepatic pallor was present in 13 males and 6 females distributed throughout all except the lowest dose group. A diffuse hepatic vacuolation from fatty degeneration was identified microscopically as the probable cause of the liver paleness. Renal glomerular and tubular casts were observed in 5 of 12 animals examined microscopically. Pulmonary congestion was observed primarily in the high dose female group. Other lesions observed were considered incidental or were also observed in control animals. The veterinary pathologist's report appears in Appendix G.

**TABLE 4: Incidence Summary for Clinical Observations
in Rats Administered DIGL-RP Solid Propellant**

MALES	Dose (n=)	<u>Vehicle</u>	<u>2510</u>	<u>2820</u>	<u>2985</u>	<u>3160</u>	<u>3550</u>
		7	9	9	8	10	9
Respiratory ^a		0	1	0	1	2	1
Behaviorial ^b		2	9	9	8	10	9
Opisthotonus		0	0	1	0	0	0
Skin/Hair ^c		2	2	6	1	3	0
Gastrointestinal ^d		1	1	2	1	1	0
Stains ^e		1	1	5	5	8	5
Ocular ^f		0	1	5	8	10	9
Hunched posture		2	7	8	8	9	1
Reflex ^g		1	1	0	5	7	7
Cyanosis		1	5	9	8	10	8
Decreased temperature		0	0	0	2	1	2
Prostrate/Moribund		0	2	3	6	9	8
Normal throughout		4	0	0	0	0	0
Deaths		0	0	1	6	9	9
FEMALES	Dose (n=)	<u>Vehicle</u>	<u>1590</u>	<u>2000</u>	<u>2510</u>	<u>3160</u>	<u>3550</u>
		10	9	10	9	9	10
Respiratory ^a		0	2	0	1	2	1
Behaviorial ^b		0	3	10	9	9	10
Skin/Hair ^c		1	2	9	8	8	3
Gastrointestinal ^d		0	1	0	0	0	0
Stains ^e		0	7	5	3	1	2
Ocular ^f		0	0	2	8	8	10
Hunched posture		0	9	10	7	3	2
Reflex ^g		0	2	7	7	4	4
Cyanosis		0	0	4	9	6	8
Decreased temperature		0	0	0	6	5	8
Prostrate/Moribund		0	0	0	5	8	10
Normal throughout		9	0	0	0	0	0
Deaths		0	0	1	2	8	10

^a Includes changes in rate or depth, gasping, wheezing, or irregular rate.

^b Includes tremors, inactivity, irritability, hyperactivity, ataxia, and twitching.

^c Includes rough coat and alopecia on head.

^d Includes diarrhea, tarry feces, emaciation, and dehydration.

^e Includes yellow urine/fecal stains/material on tail, abdomen, or perineum and red, brown, or yellow stains on mouth, eyes, head, neck, or nose.

^f Includes squinting, lacrimation, chromodacryorrhea, and mucous discharge.

^g Includes changes in grasping, righting, and startle reflexes.

TABLE 5: Mean Body Weights in Grams $\pm$ S.E (N)

<u>Dose Groups</u> (mg/kg)	<u>At</u> <u>Receipt</u>	<u>Dosing</u> <u>Day</u>	<u>Midtrial</u> <u>Day</u>	<u>Termination</u> <u>Day*</u>
MALES				
2510	145.3 ± 3.7 (9)	293.8 ± 4.6 (9)	329.2 ± 6.3 (9)	360.6 ± 10.1 (9)
2820	145.9 ± 4.0 (9)	342.3 ± 9.2 (9)	348.6 ± 11.7 (8)	366.0 ± 12.2 (8)
2985	150.8 ± 2.3 (8)	266.2 ± 6.9 (8)	284.0 ± 27.0 (2)	342.5 ± 25.5 (2)
3160	147.8 ± 1.6 (10)	255.0 ± 4.1 (10)	240.0 (1)	286.0 (1)
3550	145.8 ± 1.6 (9)	254.3 ± 4.1 (9)	N/A	N/A
Vehicle	165.9 ± 0.9 (7)	204.4 ± 1.7 (7)	246.1 ± 7.2 (7)	243.1 ± 18.2 (7)
FEMALES				
1590	147.4 ± 2.7 (9)	185.8 ± 2.7 (9)	209.3 ± 10.7 (9)	207.8 ± 6.4 (9)
2000	145.6 ± 2.4 (10)	167.9 ± 4.0 (10)	193.7 ± 6.0 (9)	196.7 ± 7.4 (9)
2510	146.7 ± 2.0 (9)	162.9 ± 2.6 (9)	185.3 ± 7.6 (7)	200.0 ± 4.6 (7)
3160	148.3 ± 3.0 (9)	169.4 ± 3.8 (9)	195.0 (1)	205.0 (1)
3550	144.1 ± 2.2 (9)	182.3 ± 3.2 (9)	N/A	N/A
Vehicle	144.6 ± 2.0 (10)	158.6 ± 2.1 (10)	184.6 ± 4.4 (10)	189.2 ± 5.0 (10)

* Weight after overnight fast.

DISCUSSION

The calculated median lethal dose (MLD) for DIGL-RP was 2946.1 mg/kg in male rats and 2636.4 mg/kg in female rats. These values place DIGL-RP within the slightly toxic classification (5).

DIGL-RP has as its major constituents, nitrocellulose and diethyleneglycol dinitrate (DEGDN) (Appendix A). Nitrocellulose is relatively nontoxic (MLD >5000 mg/kg) to rats (6) while a MLD of 750-1000 mg/kg for DEGDN in rats has been reported (7). The spectrum of clinical signs (tremors, cyanosis, depressed reflexes, etc.) observed following DIGL-RP administration supports the assumption that DEGDN (8) is the toxic component of DIGL-RP. The relative contribution of nitrocellulose and DEGDN to the MLD of DIGL-RP can be determined using their percentage compositions by weight (DIGL-RP is 62.5% nitrocellulose and 36.7% DEGDN). The calculated composition for the MLD of DIGL-RP is approximately 1875 mg/kg nitrocellulose and 1000 mg/kg DEGDN. These calculations reveal that a quantity of DEGDN present in DIGL-RP is equivalent to its MLD while the quantity of nitrocellulose present is less than 40% of its MLD. Thus it is highly unlikely that nitrocellulose contributes significantly to the toxicity of DIGL-RP.

CONCLUSION

DIGL-RP Solid Propellant is a slightly toxic compound that produces tremors, cyanosis, and depressed reflexes. Calculated MLD values were 2946.1 ± 39.2 mg/kg in male and 2636.4 ± 117.8 mg/kg in female Sprague-Dawley rats.

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Appendix A: CHEMICAL DATA

Chemical Name: DIGL-RP Solid Propellant

Physical State: Solid black cylinders (stick configuration)

Chemical Analysis:

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

Table 1. Analysis of Standards

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

*Average of 2 determinations

Equation for line by linear regression analysis:

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

¹ Wheeler CW. Toxicity testing of propellents. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.

² Wheeler CW. Nitrocellulose-nitroguanidine projects. Laboratory Notebook #84-05-010.3, p. 58. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

Table 2. Analysis of DIGL Powder

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

*Calculated using the following equation for the standard curve:
 $= \{[\text{Peak Area} - 3.51 \times 10^5] / 5.62 \times 10^4\} + \text{wgt DIGL (mg)} \times 10.$

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

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

Stability:

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

Source: Radford Army Ammunition Plant, Radford, VA
 (prime contractor: Hercules, Inc., Wilmington, DE)

LAIR Code Number: TP57

Lot No.: RAD83M001S169

³ Wheeler CW. Toxicity testing of propellents. Laboratory Notebook #85-12-023, p. 24-42. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont): CHEMICAL ANALYSIS

Homogeneity⁴: Suspensions (20 ml) of DIGL-RP powder were prepared in 1% gum tragacanth at concentrations of approximately 50, 100 and 200 mg/ml. After withdrawing one ml from the top, middle, and bottom of each suspension and diluting with ethanol, the samples were analyzed by HPLC for DEGDN content. The suspensions were homogeneous since no individual value deviated more than 10% from the mean value for each concentration tested.

Table 3. Analysis of DEGDN Standards

Concentration of DEGDN (µg/ml)	Peak Area* (x 10 ⁶)
76.9	4.46
95.8	5.59
158.2	9.18
195.0	11.26
279.6	16.21
306.9	17.75
340.2	19.50
413.1	23.70

*Average of standards run before and after samples.

Equation for line by linear regression analysis: $Y = 5.7 \times 10^4 X - 1.1 \times 10^5$,
 $r^2 = 0.999$

Table 4. Analysis of DIGL Samples for Homogeneity

Concentration (mg/ml)	Dilution Factor (D.F.)	Peak Area x 10 ⁶	Conc. of DIGL* (mg/ml)
50.6 T	100	11.39	51.5
50.6 M	100	11.21	50.6
50.6 B	100	11.76	53.1
100.5 T	250	8.90	100.2
100.5 M	250	9.02	101.6
100.5 B	250	8.85	99.7
198.9 T	500	8.12	182.7
198.9 M	500	7.99	179.7
198.9 B	500	8.26	185.9

*Conc. of DIGL-RP (mg/kg) = [(peak area - 1.1×10^5)/ 5.7×10^4] x D.F. x 2.60/1000 µg/mg

⁴ Wheeler CW. Toxicity testing of propellents. Laboratory Notebook #85-12-023.1, p. 2-5. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL ANALYSIS

Analysis of Dosing Suspensions⁵: DIGL dosing suspensions were analyzed by HPLC to determine concentration accuracy. As with the homogeneity determinations, this was accomplished by analyzing for the DEGDN component of DIGL. The results given in Table 5 indicate that all suspensions were within 10% of the target concentration.

Table 5. Concentration of DIGL in Dosing Suspensions

Target Conc. (mg/ml)	Date Prepared	Date Analyzed	Dilution Factor	Peak Area	Conc. of DIGL (mg/ml)	% Target Conc.
FEMALES						
79.6	14/1/86	10/2/86 ^a	100	1.553x10 ⁷	75.9	95.5
100.0	9/1/86	10/2/86	100	2.163x10 ⁷	105.5	105.5
125.5	9/1/86	10/2/86	100	2.688x10 ⁷	131.0	104.4
158.0	9/1/86	10/2/86	200	1.496x10 ⁷	146.2	92.5
177.5	14/1/86	10/2/86	200	1.812x10 ⁷	176.9	99.7
MALES						
125.5	19/5/86	20/5/86 ^b	100	5279	120.1	95.7
141.0	27/5/86	8/6/86 ^c	100	5729	132.2	93.8
149.25	12/5/86	16/5/86 ^d	100	6327	144.7	97.0
158.0	12/5/86	16/5/86	100	6484	148.4	93.9
177.5	12/5/86	16/5/86	100	7379	169.6	95.5

Equations for the standard curves and the %DEGDN in DIGL-RP:

^a Y (peak area) = $5.741 \times 10^4 X$ ($\mu\text{g/ml}$) - 8.194×10^4 , $R^2 = 0.999$.
%DEGDN = 35.9

^b Y (peak area) = $10.70 X$ ($\mu\text{g/ml}$) + 229.6, $R^2 = 0.999$. Assuming
%DEGDN = 39.3.

^c Y (peak area) = $10.64 X$ ($\mu\text{g/ml}$) + 216.1, $R^2 = 0.999$. %DEGDN = 39.2

^d Y (peak area) = $10.74 X$ ($\mu\text{g/ml}$) + 218.7, $R^2 = 0.999$. Assuming
%DEGDN = 39.3.

⁵ Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023.1, p. 6-11, 45-68. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL ANALYSIS

Manufacturer's Data Sheet for DIGL-RP Formulation

<u>Finished Propellant Ingredients</u>	<u>Percentage</u>
Nitrocellulose (13.05 $\pm$ 0.05% Nitrogen) (6-12 seconds viscosity)	62.5 $\pm$ 2.00
Diethyleneglycol Dinitrate (DEGDN)	36.70 $\pm$ 1.50
	0.25
Ethyl Centralite (EC)	0.25 $\pm$ 0.05
	0.25
Akardit II	0.45 $\pm$ 0.15
Magnesium Oxide	0.05 Max
Graphite (Chg 5)	<u>0.05 Max</u> 100.00

Appendix B: ANIMAL DATA

Species: *Rattus norvegicus*

Strain: Albino, Sprague-Dawley

Source: Bantin & Kingman, Inc.
Fremont, CA

Sex: Male and female

Date of Birth: DIGL-RP Males - 9 Mar 1986
Vehicle Males - 19 Nov 1985
Females - 14 Nov 1985

Method of randomization: Weight bias, stratified animal
allocation using the Toxsys
Software Package (Standard
Procedures for Assigning Animals to
Treatment Groups, SOP OP-ISG-24).

Animals in each group: 10 males and 10 females, except Group
8 and Group 13 were assigned 12 males

Condition of animals at start of study: Normal

Body weight range at dosing: 151-324 g

Identification procedures: Ear tag

Conditioning: Quarantine/acclimation 31 Dec 85 - 8 Jan 86
for females and male vehicle controls and 25
Apr 86 - 12 May 86 for males.

Justification: The laboratory rat has proven to be a
sensitive and reliable animal model for
acute toxicity determinations.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

MALE VEHICLE CONTROLS AND FEMALES

<u>Date</u>	<u>Event</u>
30 Dec 85	Animals were received and checked for physical condition, sexed, and individually caged.
31 Dec 85	Animals were ear tagged and weighed. Two female and two male rats were submitted for necropsy quality control.
31 Dec 85-8 Jan 86	Animals were observed daily.
8 Jan 86	Animals were weighed, randomized into dose groups, and removed from quarantine.
7-8 Jan 86	ALD animals were weighed, dosed, and observed at 2 and 4 hours after dosing.
9 Jan 86	Groups 1-3 and 6 animals were weighed, dosed, and observed at 2 and 4 hours after dosing.
10-22 Jan 86	Groups 1-3 and 6 animals were observed daily in the am and pm.
14 Jan 86	Groups 4 and 5 animals were weighed, dosed, and observed at 2 and 4 hours after dosing.
15 Jan 86	All animals not weighed on 14 Jan were weighed.
15-27 Jan 86	Group 4 and 5 animals were observed daily in the am and pm.
21 Jan 86	Groups 4 and 5 animals were weighed.
23 Jan 86	Groups 1-3 and 6 animals were weighed, observed, and submitted to necropsy.
28 Jan 86	Groups 4 and 5 were weighed, observed, and submitted to necropsy.

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

<u>Date</u>	<u>Event</u>
24 Apr 86	Rats were received and checked for physical condition, weighed, ear-tagged, and individually caged.
25 Apr 86	Two rats were submitted for necropsy quality control.
25 Apr-12 May 86	Animals were observed daily.
12 May 86	Animals were weighed, randomized into dose groups, and removed from quarantine.
13 May 86	Groups 8-10 animals were weighed, dosed, and observed at 2 and 4 hours after dosing.
14-27 May 86	Groups 8-10 animals were observed daily in am and pm.
20 May 86	Groups 8-10 animals were weighed.
20 May 86	Group 11 animals were weighed, dosed and observed at 2 and 4 hours after dosing.
21 May-3 Jun 86	Group 11 animals were observed daily in am and pm.
27 May 86	Group 11 animals were weighed.
27 May 86	Groups 8-10 animals were weighed, observed, and submitted to necropsy.
28 May 86	Group 13 animals were weighed, dosed, and observed at 1 and 4 hours after dosing.
28 May-9 Jun 86	Group 13 animals were observed daily in am and pm.
3 Jun 86	Group 13 animals were weighed.
3 Jun 86	Group 11 animals were weighed, observed, and submitted to necropsy.
10 Jun 86	Group 13 animals were weighed, observed, and submitted to necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

Dose mg/kg	Animals/ Group	Time After Dosing									
		Hours		Days							
		2	4	1	2	3	4	5	6	7	8-14
MALES											
2510	9	0	0	0	0	0	0	0	0	0	0
2820	9	0	0	0	1	1	1	1	1	1	1
2985	8	0	0	0	1	2	6	6	6	6	6
3160	10	0	0	0	3	5	7	8	8	9	9
3550	9	0	0	3	7	9	9	9	9	9	9
Vehicle	7	0	0	0	0	0	0	0	0	0	0
TOTAL*	45	0	0	3	12	17	23	24	24	25	25
FEMALES											
1590	9	0	0	0	0	0	0	0	0	0	0
2000	10	0	0	0	1	1	1	1	1	1	1
2510	9	0	0	0	0	2	2	2	2	2	2
3160	9	0	0	2	6	8	8	8	8	8	8
3550	10	0	0	5	10	10	10	10	10	10	10
Vehicle	10	0	0	0	0	0	0	0	0	0	0
TOTAL*	47	0	0	7	17	21	21	21	21	21	21

* TOTAL reflects only animals receiving DIGL-RP.

APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 2510 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00202	Hunched Posture	May 20-22	Marked
	Tremors	May 20	Moderate
	Inactive	May 22	Marked
86D00203	Hunched Posture	May 20	Marked
	Inactive	May 20	Moderate
	Tremors	May 20	Slight
	Stain, Neck, Red	May 24	Slight
	Irritable	June 1	Slight
86D00216	Hunched Posture	May 20	Moderate
	Ataxia	May 20	Marked
	Tremors	May 20	Slight
	Rough Coat	June 1	Slight
86D00218	Hunched Posture	May 20	Moderate
	Inactive	May 20	Moderate
	Irritable	May 26	Slight
	Diarrhea	May 26	Slight
86D00223	Prostrate	May 20	N/A
	Cyanosis	May 20	Slight
	Ataxia	May 21	Marked
	Inactive	May 22	Moderate
86D00230	Hunched Posture	May 20	Moderate
	Ataxia	May 20	Slight
	Cyanosis	May 20, 21	Slight
	Irritable	May 24, 31, June 1	Slight
	Decr. Respiratory Rate	May 31	Slight
	Tachypnea	June 1	Slight

APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 2510 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00244	Cyanosis	May 20,21	Slight
	Twitching	May 20	Slight
	Squinting	May 20	Moderate
	Prostrate	May 20	N/A
	Depr. Grasping Reflex	May 20	Moderate
	Hunched Posture	May 21	Marked
	Inactive	May 21,22	Moderate
86D00246	Hunched Posture	May 20,23-26	Moderate
	Tremors	May 20	Slight
	Cyanosis	May 20,21	Slight
	Ataxia	May 21-24	Slight
	Hyperactive	May 21,27-June 2	Slight
	Irritable	May 23-June 2	Moderate
	Rough Coat	May 25,26, June 1	Slight
86D00249	Misdosed	N/A	N/A
86D00255	Tremors	May 20	Slight
	Cyanosis	May 20,21	Slight
	Ataxia	May 20,21	Moderate
	Inactive	May 20,21	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2820 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00207	Hunched Posture	May 28-June 3	Slight
	Tremors	May 28	Slight
	Cyanosis	May 28,29	Slight
	Inactive	May 28-June 5	Moderate
	Rough Coat	May 30	Slight
	Stain, Nose, Brown	May 28	Slight
86D00210	Misdosed	N/A	N/A
86D00217	Prostrate	May 28	N/A
	Cyanosis	May 28,29,31	Slight
	Tremors	May 28	Slight
	Hunched Posture	May 28-June 1	Slight
	Inactive	May 28,29,31, June 1	Marked
	Ataxia	May 29, June 2	Slight
86D00235	Rough Coat	May 30-June 1	Slight
	Hunched Posture	May 28,29	Slight
	Cyanosis	May 28,29	Slight
	Tremors	May 28,29	Slight
	Squinting	May 28,29	Slight
	Twitching	May 28	Slight
86D00240	Stain, Nose, Red	May 28	Slight
	Death	May 30	1.9 days
	Hunched Posture	May 28-June 2	Moderate
	Cyanosis	May 28,29,31	Slight
	Squinting	May 28	Slight
	Inactive	May 28-June 1,3-5	Moderate
86D00241	Ataxia	May 28-30	Moderate
	Stain, Nose, Brown	May 28	Slight
	Irritable	June 1,2	Slight
	Twitching	May 28	Slight
	Cyanosis	May 28,29,31	Slight
	Prostrate	May 28,29	N/A
86D00241	Squinting	May 28,29	Moderate
	Stain, Eye, Red	May 28	Slight
	Stain, Nose, Brown	May 28	Slight
	Rough Coat	May 29-June 1	Slight
	Hunched Posture	May 31, June 1	Slight
	Irritable	June 1	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2820 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00241 (cont.)	Tarry Feces Inactive	May 31 May 31-June 1	Slight Moderate
86D00250	Misdosed	N/A	N/A
86D00251	Prostrate Tremors Cyanosis Squinting Stain, Nose, Red Rough Coat Ataxia Stain, Ear, Brown Scab, Ear Ulceration, Ear Irritable	May 28 May 28 May 28, 29 May 28 May 28 May 28-June 1 May 28 May 29 May 30, 31 June 1 May 31, June 1	N/A Moderate Slight Marked Slight Slight Marked Moderate Slight Slight Slight
86D00257	Ataxia Inactive Squinting Cyanosis Rough Coat Hunched Posture	May 28, 29 May 28 May 28 May 28, 29, 31 May 29-June 2 May 30-June 2	Slight Moderate Slight Slight Slight Slight
86D00272	Hunched Posture Ataxia Tremors Cyanosis Opisthotonos Rough Coat Emaciated Inactive	May 28-June 2 May 28, 29 May 28 May 29 May 30 May 30-June 2 May 30 May 31, June 1	Moderate Slight Slight Slight N/A Slight Moderate Slight
86D00276	Removed From Study	N/A	N/A
86D00279	Ataxia Tremors Cyanosis Hunched Posture Irritable Inactive Hyperactive	May 28 May 28 May 28, 29 May 28 May 29, 31, June 1 June 1 May 30	Moderate Moderate Slight Slight Slight Slight Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2985 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00201	Misdosed	N/A	N/A
86D00212	Tremors	May 13	Moderate
	Inactive	May 13	Marked
	Ataxia	May 13-16	Marked
	Squinting	May 13, 16	Moderate
	Prostrate	May 13	N/A
	Depr. Righting Reflex	May 13	Slight
	Cyanosis	May 13, 14, 16	Slight
	Hunched Posture	May 14-16	Slight
	Lacrimation	May 14-16	Moderate
	Stain, Abdomen, Urine	May 15, 16	Slight
	Death	May 17	3.9 days
86D00213	Inactive	May 13, 14	Moderate
	Squinting	May 13	Slight
	Hunched Posture	May 13, 14	Moderate
	Cyanosis	May 13-16	Slight
	Ataxia	May 15, 16	Slight
86D00219	Inactive	May 13	Slight
	Tremors	May 13	Slight
	Prostrate	May 13-16	N/A
	Hunched Posture	May 13	Slight
	Squinting	May 13	Slight
	Cyanosis	May 13-16	Moderate
	Lacrimation	May 14, 16	Moderate
	Stain, Mouth, Red	May 14	Slight
	Stain, Nose, Red	May 14	Slight
	Stain, Abdomen, Urine	May 14-16	N/A
	Death	May 16	3.1 days
86D00220	Misdosed	N/A	N/A
86D00225	Misdosed	N/A	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2985 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00228	Prostrate	May 13	N/A
	Tremors	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Moderate
	Cyanosis	May 13,14	Slight
	Hunched Posture	May 14	Slight
	Ataxia	May 14	Moderate
	Lacrimation	May 14	Slight
	Death	May 15	1.9 days
86D00237	Prostrate	May 13,15	N/A
	Tremors	May 13	Slight
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Moderate
	Lacrimation	May 13	Slight
	Cyanosis	May 13,15	Slight
	Hunched Posture	May 14	Slight
	Ataxia	May 14	Marked
	Death	May 16	2.9 days
86D00245	Misdosed	N/A	N/A
86D00253	Prostrate	May 13,14	N/A
	Tremors	May 13	Moderate
	Cyanosis	May 13-16,21-23	Moderate
	Decr. Temperature	May 13	Moderate
	Squinting	May 13-17	Slight
	Lacrimation	May 14,16	Moderate
	Stain, Nose, Yellow	May 14	Slight
	Ataxia	May 15-17	Moderate
	Hunched Posture	May 16-21	Moderate
	Stain, Eye, Yellow	May 17,18	Marked
	Inactive	May 17-20	Moderate
	Tarry feces	May 17,18	Marked
	Decr. Respiratory Depth	May 19	N/A
	Rough Coat	May 19-26	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2985 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00260	Hunched Posture	May 13-15	Moderate
	Tremors	May 13	Moderate
	Inactive	May 13	Moderate
	Depr. Righting Reflex	May 13	Slight
	Squinting	May 13,16	Moderate
	Cyanosis	May 13-16	Slight
	Stain, Nose, Red	May 13	Slight
	Ataxia	May 14,15	Moderate
	Prostrate	May 16	N/A
	Death	May 17	3.9 days
86D00275	Inactive	May 13,15	Marked
	Ataxia	May 13-16	Marked
	Cyanosis	May 13-16	Moderate
	Decr. Temperature	May 13	Moderate
	Squinting	May 13	Moderate
	Lacrimation	May 13-16	Moderate
	Dep. Righting Reflex	May 13	Slight
	Hunched Posture	May 14-16	Moderate
	Stain, Nose, Red	May 16	Slight
	Stain, Abdomen, Urine	May 16	Slight
	Death	May 17	3.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3160 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00209	Prostrate	May 13	N/A
	Tremors	May 13	Moderate
	Depr. Righting Reflex	May 13,14	Marked
	Squinting	May 13,16	Marked
	Twitching	May 13	Slight
	Cyanosis	May 13-16	Moderate
	Ataxia	May 14	N/A
	Stain, Abdomen, Urine	May 16	N/A
	Death	May 16	3.2 days
86D00211	Prostrate	May 13	N/A
	Tremors	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Moderate
	Cyanosis	May 13-15	Slight
	Stain, Nose, Red	May 13-15	Slight
	Hunched Posture	May 14,15	Slight
	Ataxia	May 14,15	Moderate
	Lacrimation	May 14	Slight
	Death	May 16	2.9 days
86D00221	Hunched Posture	May 13-21	Slight
	Rough Coat	May 13	Slight
	Inactive	May 13,17-21	Moderate
	Ataxia	May 13-16	Marked
	Prostrate	May 13	N/A
	Squinting	May 13	Slight
	Lacrimation	May 13,14,16	Moderate
	Cyanosis	May 13-16	Slight
	Twitching	May 13	Slight
	Stain, Head, Red	May 14	Slight
	Stain, Nose, Red	May 16,17	Slight
86D00224	Prostrate	May 13,14,16	N/A
	Tremors	May 13	Moderate
	Squinting	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Cyanosis	May 13-16	Moderate
	Lacrimation	May 14	Slight
	Stain, Mouth, Red	May 14-16	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3160 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00224 (cont.)	Hunched Posture	May 15	Slight
	Ataxia	May 15	Slight
	Stain, Abdomen, Urine	May 16	Slight
	Death	May 17	3.9 days
86D00229	Hunched Posture	May 13,14	Moderate
	Ataxia	May 13	Moderate
	Rough Coat	May 13	Moderate
	Tachypnea	May 13	Slight
	Cyanosis	May 13,14	Slight
	Squinting	May 13	Slight
	Stain, Nose, Red	May 13	Slight
	Stain, Mouth, Red	May 14	Moderate
	Incr. Startle Reflex	May 13	Moderate
	Death	May 15	1.9 days
86D00238	Hunched Posture	May 13-17	Marked
	Tremors	May 13	Moderate
	Rough Coat	May 13	Moderate
	Stain, Nose, Brown	May 13	Slight
	Squinting	May 13	Moderate
	Cyanosis	May 13,15,16	Slight
	Prostrate	May 13	N/A
	Ataxia	May 14-17	Moderate
	Stain, Mouth, Yellow	May 15-17	Moderate
	Stain, Abdomen, Urine	May 16	Slight
	Stain, Perianal, Yellow	May 17	Marked
	Twitching	May 17	Moderate
	Death	May 17	4.2 days
86D00256	Inactive	May 13	Marked
	Tremors	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Marked
	Prostrate	May 13,19	N/A
	Cyanosis	May 13-16	Slight
	Lacrimation	May 13,14	Slight
	Hunched Posture	May 14-18	Moderate
	Ataxia	May 14-17	Moderate
	Stain, Mouth, Yellow	May 14	Slight
	Stain, Perianal, Yellow	May 17-19	Marked

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3160 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00256 (cont.)	Tarry Feces	May 18	Slight
	Gasping	May 19	N/A
	Urine, Abdomen	May 19	Slight
	Death	May 19	6.2 days
86D00258	Hunched Posture	May 13	Moderate
	Tremors	May 13	Marked
	Ataxia	May 13,14	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Moderate
	Prostrate	May 13	N/A
	Cyanosis	May 13,14	Slight
	Lacrimation	May 13	Slight
	Death	May 15	1.9 days
86D00261	Prostrate	May 13	N/A
	Tremors	May 13	Slight
	Cyanosis	May 13,15	Moderate
	Decr. Temperature	May 13	Moderate
	Squinting	May 13	Moderate
	Lacrimation	May 13	Moderate
	Hunched Posture	May 14,15	Moderate
	Ataxia	May 14,15	Moderate
	Inactive	May 14,15	Slight
	Death	May 15	2.2 days
86D00265	Prostrate	May 13	N/A
	Tremors	May 13,14	Moderate
	Stain, Nose, Brown	May 13,14	Slight
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Slight
	Lacrimation	May 13	Slight
	Cyanosis	May 13,14	Slight
	Hunched Posture	May 14	Slight
	Chromodacryorrhea	May 14	Slight
	Ataxia	May 14	Moderate
	Death	May 15	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3550 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00205	Prostrate	May 13, 14	N/A
	Tremors	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Marked
	Twitching	May 13	Slight
	Cyanosis	May 13, 14	Slight
	Lacrimation	May 14	Marked
	Death	May 15	2.0 days
86D00215	Prostrate	May 13	N/A
	Tremors	May 13	Marked
	Squinting	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Cyanosis	May 13	Slight
	Incr. Startle Reflex	May 13	Slight
	Death	May 14	20.3 h
86D00222	Prostrate	May 13	N/A
	Tremors	May 13	Marked
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13	Moderate
	Twitching	May 13	Moderate
	Cyanosis	May 13	Slight
	Incr. Startle Reflex	May 13	Slight
	Death	May 14	20.3 h
86D00239	Misdosed	N/A	N/A
86D00263	Prostrate	May 13	N/A
	Tremors	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Cyanosis	May 13, 14	Moderate
	Squinting	May 13, 14	Moderate
	Lacrimation	May 13, 14	Marked
	Moribund	May 14	N/A
	Stain, Abdomen, Urine	May 14	Slight
	Death	May 15	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3550 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00264	Inactive	May 13	Marked
	Tremors	May 13	Moderate
	Chromodacryorrhea	May 13	Moderate
	Prostrate	May 13	N/A
	Lacrimation	May 13,14	Marked
	Cyanosis	May 13,14	Slight
	Squinting	May 13,14	Moderate
	Moribund	May 14	N/A
	Stain, Mouth, Clear	May 14	Slight
	Death	May 14	1.2 days
86D00267	Prostrate	May 13,14	N/A
	Tremors	May 13	Moderate
	Cyanosis	May 13,14	Moderate
	Decr. Temperature	May 13	Moderate
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13,14	Marked
	Lacrimation	May 13,14	Moderate
	Stain, Nose, Red	May 14	Slight
	Ataxia	May 14	Moderate
	Death	May 16	2.9 days
86D00273	Prostrate	May 13	N/A
	Tremors	May 13	Slight
	Depr. Righting Reflex	May 13	Moderate
	Squinting	May 13,14	Moderate
	Twitching	May 13	Moderate
	Cyanosis	May 13,14	Slight
	Lacrimation	May 13,14	Moderate
	Moribund	May 14	N/A
	Stain, Nose, Red	May 14	Slight
	Stain, Mouth, Brown	May 14	Slight
	Death	May 16	2.9 days
86D00277	Inactive	May 13	Marked
	Tremors	May 13	Marked
	Hunched Posture	May 13	Moderate
	Ataxia	May 13	Moderate
	Squinting	May 13	Marked
	Depr. Righting Reflex	May 13	Slight
	Death	May 14	21.5 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3550 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
86D00278	Prostrate	May 13	N/A
	Tremors	May 13	Moderate
	Cyanosis	May 13,14	Moderate
	Decr. Temperature	May 13	Moderate
	Squinting	May 13,14	Moderate
	Gasping	May 13,14	Slight
	Moribund	May 14	N/A
	Lacrimation	May 14	Moderate
	Stain, Mouth, Yellow	May 14	Slight
	Death	May 15	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01164	Normal	N/A	N/A
85D01168	Normal	N/A	N/A
85D01169	Misdosed	N/A	N/A
85D01171	Normal	N/A	N/A
85D01175	Normal	N/A	N/A
85D01185	Hunched Posture	Jan. 9,10	Slight
	Inactive	Jan. 9,10	Moderate
	Depr. Grasping Reflex	Jan. 10	Slight
	Cyanosis	Jan. 10	Slight
85D01197	Removed From Study	N/A	N/A
85D01198	Material, Tail, Brown	Jan. 22	Moderate
	Rough Coat	Jan. 22	Slight
	Dehydrated	Jan. 23	Moderate
85D01202	Hunched Posture	Jan. 9	Moderate
	Inactive	Jan. 9	Slight
	Rough Coat	Jan. 9	Slight
85D01218	Misdosed	N/A	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1590 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01251	Misdosed	N/A	N/A
85D01260	Stain, Nose, Red	Jan 14	Slight
	Rough Coat	Jan 14,15	Slight
	Hunched Posture	Jan 14-18	Moderate
	Inactive	Jan 14,15	Slight
85D01262	Hunched Posture	Jan 14-18	Moderate
	Stain, Nose, Red	Jan 14	Slight
	Inactive	Jan 15	Slight
	Rough Coat	Jan 15	Slight
	Hyperactive	Jan. 25	Moderate
	Irritable	Jan 26,27	Moderate
85D01265	Hunched Posture	Jan 14-18	Moderate
	Stain, Nose, Red	Jan 14	Slight
85D01267	Hunched Posture	Jan 14-18	Slight
	Stain, Head, Red	Jan 14	Slight
	Incr. Startle Reflex	Jan 15	Slight
85D01272	Hunched Posture	Jan 14-18	Moderate
	Depr. Grasping Reflex	Jan 14	Slight
	Stain, Nose, Red	Jan 14	Slight
	Stain, Perianal, Urine	Jan 14,15	Slight
	Dehydrated	Jan 26,27	Slight
85D01280	Hunched Posture	Jan 14-18	Moderate
	Stain, Nose	Jan 14	Slight
	Incr. Respiratory Rate	Jan 15	N/A
85D01285	Hunched Posture	Jan 14-18	Slight
	Inactive	Jan 14	Slight
85D01286	Hunched Posture	Jan 14-18	Moderate
85D01294	Hunched Posture	Jan 14-18	Moderate
	Stain, Nose, Red	Jan 14,23	Slight
	Wheezing	Jan 14	Slight
	Material, Neck, Red	Jan 15-28	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2000 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01244	Incr. Startle Reflex	Jan 9,10	Marked
	Hunched Posture	Jan 9-11	Marked
	Inactive	Jan 9	Marked
	Tremors	Jan 9	Moderate
	Rough Coat	Jan 9-11	Moderate
	Depr. Grasping Reflex	Jan 9,10	Slight
85D01261	Inactive	Jan 9	Marked
	Rough Coat	Jan 9-11	Moderate
	Hunched Posture	Jan 9,10	Marked
	Tremors	Jan 9	Slight
	Chromodacryorrhea	Jan 9	Moderate
	Squinting	Jan 9,10	Moderate
	Lacrimation	Jan 10	Slight
85D01268	Hunched Posture	Jan 9-12	Marked
	Inactive	Jan 9-11	Marked
	Tremors	Jan 9	Slight
	Dep. Grasping Reflex	Jan 9	Moderate
	Rough Coat	Jan 9-11	Moderate
	Stain, Perianal, Yellow	Jan 10,11	Moderate
85D01269	Hunched Posture	Jan 9-11	Moderate
	Inactive	Jan 9	Moderate
	Tremors	Jan 9	Moderate
	Depr. Grasping Reflex	Jan 9	Moderate
	Rough Coat	Jan 9-11	Slight
	Cyanosis	Jan 10	Slight
85D01292	Inactive	Jan 9	Marked
	Hunched Posture	Jan 9,10	Marked
	Rough Coat	Jan 9-11	Marked
	Tremors	Jan 9	Marked
	Cyanosis	Jan 9	Moderate
	Stain, Perianal, Yellow	Jan 10	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2000 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01293	Inactive	Jan 9,11	Marked
	Hunched Posture	Jan 9,10	Marked
	Tremors	Jan 9	Marked
	Rough Coat	Jan 9-11	Moderate
	Depr. Grasping Reflex	Jan 9,10	Marked
	Cyanosis	Jan 9,10	Slight
85D01295	Hunched Posture	Jan 9,10	Moderate
	Inactive	Jan 9	Slight
	Rough Coat	Jan 9	Slight
	Ataxia	Jan 9	N/A
	Tremors	Jan 9	Slight
	Depr. Grasping Reflex	Jan 10	Slight
	Stain, Perianal, Yellow	Jan 10	Slight
85D01300	Hunched Posture	Jan 9,10	Marked
	Inactive	Jan 9	Moderate
	Tremors	Jan 9	Slight
	Depr. Grasping Reflex	Jan 9	Slight
	Rough Coat	Jan 10	Slight
	Stain, Perianal, Yellow	Jan 10	Moderate
85D01305	Hunched Posture	Jan 9,10	Marked
	Inactive	Jan 9,10	Marked
	Tremors	Jan 9	Moderate
	Depr. Grasping Reflex	Jan 9,10	Marked
	Rough Coat	Jan 9,10	Moderate
	Lacrimation	Jan 9,10	Marked
	Incr. Startle Reflex	Jan 10	Moderate
	Cyanosis	Jan 10	Slight
	Ataxia	Jan 10	Slight
	Death	Jan 11	1.9 days
85D001310	Hunched Posture	Jan 9-11	Marked
	Inactive	Jan 9	Moderate
	Ataxia	Jan 9	Slight
	Stain, Perianal, Urine	Jan 10,11	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2510 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01247	Hunched Posture	Jan 9-11	Moderate
	Rough Coat	Jan 9,10	Slight
	Tremors	Jan 9	Marked
	Decr. Temperature	Jan 10	N/A
	Cyanosis	Jan 10	Slight
85D01254	Prostrate	Jan 9	N/A
	Tremors	Jan 9	Moderate
	Chromodacryorrhea	Jan 9	Marked
	Squinting	Jan 9	Marked
	Ataxia	Jan 10	Moderate
	Depr. Grasping Reflex	Jan 10	Moderate
	Lacrimation	Jan 10	Slight
	Decr. Temperature	Jan 10	N/A
	Cyanosis	Jan 10	Slight
	Hunched Posture	Jan 10,11	Slight
	Rough Coat	Jan 11	Moderate
85D01263	Inactive	Jan 9	Marked
	Hunched Posture	Jan 9-11	Marked
	Depr. Grasping Reflex	Jan 9,10	Marked
	Tremors	Jan 9	Moderate
	Squinting	Jan 9,10	Moderate
	Lacrimation	Jan 9,10	Slight
	Decr. Temperature	Jan 10	N/A
	Cyanosis	Jan 10	Slight
85D01290	Prostrate	Jan 9,11	N/A
	Rough Coat	Jan 9-12	Marked
	Tremors	Jan 9	Marked
	Lacrimation	Jan 9,10	Moderate
	Squinting	Jan 9,10	Marked
	Cyanosis	Jan 9-11	Marked
	Ataxia	Jan 10	Moderate
	Hunched Posture	Jan 10-12	Marked
	Dec. Temperature	Jan 10	N/A
	Stain, Perianal, Yellow	Jan 13	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2510 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01299	Misdosed	N/A	N/A
85D01303	Inactive	Jan 9,10	Marked
	Hunched Posture	Jan 9-11	Marked
	Depr. Grasping Reflex	Jan 9,10	Marked
	Rough Coat	Jan 9-11	Moderate
	Tremors	Jan 9	Slight
	Squinting	Jan 9	Marked
	Stain, Perianal, Yellow	Jan 10	Slight
	Cyanosis	Jan 10	Slight
85D01308	Hunched Posture	Jan 9-12	Marked
	Inactive	Jan 9,11,12	Marked
	Tremors	Jan 9	Moderate
	Depr. Grasping Reflex	Jan 9	Marked
	Rough Coat	Jan 9,10	Moderate
	Squinting	Jan 9,10	Marked
	Prostrate	Jan 10	N/A
	Lacrimation	Jan 10	Slight
	Cyanosis	Jan 10	Slight
	Decr. Respiratory Rate	Jan 10	N/A
85D01309	Hunched Posture	Jan 9-13	Marked
	Inactive	Jan 9-12	Marked
	Tremors	Jan 9	Moderate
	Depr. Grasping Reflex	Jan 9,10	Marked
	Rough Coat	Jan 9	Moderate
	Lacrimation	Jan 9,10	Marked
	Squinting	Jan 9	Moderate
	Cyanosis	Jan 10	Moderate
	Ataxia	Jan 10	Moderate
	Stain, Nose, Red	Jan 10	Slight
	Decr. Temperature	Jan 10	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2510 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01315	Prostrate	Jan 9	N/A
	Tremors	Jan 9	Marked
	Rough Coat	Jan 9-11	Moderate
	Squinting	Jan 9	Marked
	Lacrimation	Jan 9,10	Moderate
	Cyanosis	Jan 10,11	Moderate
	Depr. Grasping Reflex	Jan 10,11	Moderate
	Ataxia	Jan 10	Moderate
	Inactive	Jan 11	Moderate
	Death	Jan 12	3.0 days
85D01316	Prostrate	Jan 9-11	N/A
	Tremors	Jan 9	Marked
	Rough Coat	Jan 9-11	Moderate
	Cyanosis	Jan 9-11	Moderate
	Squinting	Jan 9-11	Marked
	Lacrimation	Jan 9,10	Moderate
	Decr. Temperature	Jan 10	N/A
	Depr. Grasping Reflex	Jan 10,11	Moderate
	Death	Jan 12	2.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3160 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01238	Prostrate	Jan 9	N/A
	Tremors	Jan 9	Marked
	Rough Coat	Jan 9,10	Moderate
	Squinting	Jan 9,10	Marked
	Lacrimation	Jan 9,10	Moderate
	Cyanosis	Jan 9,10	Moderate
	Moribund	Jan 10	N/A
	Decr. Temperature	Jan 10	N/A
	Death	Jan 11	2.0 days
85D01249	Prostrate	Jan 9	N/A
	Depr. Righting Reflex	Jan 9	Marked
	Tremors	Jan 9	Marked
	Incr. Startle Reflex	Jan 9	Marked
	Rough Coat	Jan 9	Moderate
	Squinting	Jan 9,10	Slight
	Lacrimation	Jan 9,10	Marked
	Moribund	Jan 10	N/A
	Cyanosis	Jan 10	Moderate
	Decr. Temperature	Jan 10	N/A
	Death	Jan 10	1.1 days
85D01257	Misdosed	N/A	N/A
85D01266	Hunched Posture	Jan 9	Marked
	Inactive	Jan 9	Marked
	Twitching	Jan 9	Marked
	Depr. Grasping Reflex	Jan 9	Marked
	Rough Coat	Jan 9	Moderate
	Prostrate	Jan 9	N/A
	Tremors	Jan 9	Marked
	Twitching	Jan 9	Slight
	Squinting	Jan 9	Marked
	Death	Jan 10	24.0 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3160 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01279	Prostrate	Jan 9	N/A
	Tremors	Jan 9	Marked
	Rough Coat	Jan 9,10	Marked
	Cyanosis	Jan 9,10	Marked
	Squinting	Jan 9,10	Marked
	Lacrimation	Jan 9,10	Marked
	Moribund	Jan 9,10	N/A
	Decr. Temperature	Jan 10	N/A
	Death	Jan 11	1.9 days
85D01283	Inactive	Jan 9	Marked
	Hunched Posture	Jan 9-11	Marked
	Rough Coat	Jan 9,10	Marked
	Depr. Grasping Reflex	Jan 9,10	Slight
	Tremors	Jan 9	Moderate
	Stain, Perianal, Yellow	Jan 10	Slight
85D01287	Moribund	Jan 9,10	N/A
	Tremors	Jan 9	Marked
	Twitching	Jan 9	Marked
	Squinting	Jan 9,10	Marked
	Lacrimation	Jan 9,10	Moderate
	Cyanosis	Jan 9,10	Moderate
	Decr. Temperature	Jan 10	N/A
	Death	Jan 11	1.9 days
85D01297	Moribund	Jan 9	N/A
	Prostrate	Jan 9-11	N/A
	Tremors	Jan 9	Marked
	Twitching	Jan 9	Slight
	Squinting	Jan 9	Marked
	Lacrimation	Jan 9,10	Marked
	Rough Coat	Jan 10,11	Slight
	Decr. Respiratory Rate	Jan 10,11	N/A
	Cyanosis	Jan 10,11	Slight
	Death	Jan 11	2.2 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3160 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01302	Prostrate	Jan 9	N/A
	Tremors	Jan 9	Marked
	Rough Coat	Jan 9	Marked
	Lacrimation	Jan 9	Marked
	Death	Jan 10	22.2 h
85D01312	Inactive	Jan 9	Marked
	Hunched Posture	Jan 9	Marked
	Depr. Grasping Reflex	Jan 9	Marked
	Rough Coat	Jan 9-11	Moderate
	Tremors	Jan 9	Moderate
	Prostrate	Jan 9-11	N/A
	Squinting	Jan 9	Marked
	Lacrimation	Jan 9-11	Marked
	Decr. Temperature	Jan 10	N/A
	Cyanosis	Jan 10,11	Moderate
	Decr. Respiratory Rate	Jan 10,11	N/A
	Death	Jan 12	2.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3550 mg/kg DIGL-RP

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01240	Moribund	Jan 14	N/A
	Decr. Temperature	Jan 14	N/A
	Tremors	Jan 14	Moderate
	Squinting	Jan 14	Moderate
	Lacrimation	Jan 14	Slight
	Cyanosis	Jan 14	Moderate
	Twitching	Jan 14	Moderate
	Chromodacryorrhea	Jan 14	Moderate
	Death	Jan 15	22.1 h
85D01243	Moribund	Jan 14,15	N/A
	Tremors	Jan 14	Moderate
	Decr. Temperature	Jan 14,15	N/A
	Squinting	Jan 14,15	Marked
	Lacrimation	Jan 14	Slight
	Cyanosis	Jan 14,15	Moderate
	Twitching	Jan 14,15	Moderate
	Chromodacryorrhea	Jan 15	Moderate
	Death	Jan 16	2.0 days
85D01253	Prostrate	Jan 14	N/A
	Decr. Temperature	Jan 14,15	N/A
	Squinting	Jan 14,15	Marked
	Lacrimation	Jan 14,15	Moderate
	Depr. Grasping Reflex	Jan 14	Marked
	Twitching	Jan 14,15	Slight
	Moribund	Jan 14,15	N/A
	Cyanosis	Jan 15	Moderate
	Death	Jan 16	1.9 days
85D01255	Moribund	Jan 14,15	N/A
	Tremors	Jan 14	Slight
	Twitching	Jan 14	Moderate
	Decr. Temperature	Jan 14,15	N/A
	Squinting	Jan 14,15	Marked
	Lacrimation	Jan 14,15	Moderate
	Cyanosis	Jan 15	Moderate
	Death	Jan 16	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3550 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01256	Moribund	Jan 14	N/A
	Twitching	Jan 14	Moderate
	Decr. Temperature	Jan 14	N/A
	Squinting	Jan 14	Moderate
	Lacrimation	Jan 14	Slight
	Tremors	Jan 14	Slight
	Death	Jan 15	22.8 h
85D01284	Moribund	Jan 14	N/A
	Tremors	Jan 14	Marked
	Decr. Temperature	Jan 14	N/A
	Squinting	Jan 14	Slight
	Rough Coat	Jan 14	Slight
	Death	Jan 15	21.8 h
85D01296	Twitching	Jan 14	Moderate
	Tremors	Jan 14	Moderate
	Prostrate	Jan 14	N/A
	Rough Coat	Jan 14	Slight
	Depr. Grasping Reflex	Jan 14	Marked
	Squinting	Jan 14	Marked
	Mucus Discharge, Eyes	Jan 14	Moderate
	Chromodacryorrhea	Jan 14	Moderate
	Cyanosis	Jan 14	Moderate
	Decr. Temperature	Jan 14	N/A
	Death	Jan 15	21.8 h
85D01304	Moribund	Jan 14, 15	N/A
	Twitching	Jan 14, 15	Moderate
	Tremors	Jan 14	Slight
	Squinting	Jan 14, 15	Moderate
	Stain, Nose, Red	Jan 14	Slight
	Lacrimation	Jan 14, 15	Moderate
	Irregular Respiration	Jan 14	N/A
	Cyanosis	Jan 15	Moderate
	Death	Jan 15	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3550 mg/kg DIGL-RP (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01313	Prostrate	Jan 14	N/A
	Twitching	Jan 14	Slight
	Tremors	Jan 14	Moderate
	Depr. Grasping Reflex	Jan 14	Marked
	Squinting	Jan 14	Moderate
	Lacrimation	Jan 14	Moderate
	Hunched Posture	Jan 14	Moderate
	Cyanosis	Jan 14	Slight
	Death	Jan 15	21.8 h
85D01317	Moribund	Jan 14,15	N/A
	Hunched Posture	Jan 14	Slight
	Tremors	Jan 14	Moderate
	Depr. Grasping Reflex	Jan 14	Marked
	Stain, Perianal, Yellow	Jan 14	Slight
	Squinting	Jan 14,15	Moderate
	Decr. Temperature	Jan 14,15	N/A
	Rough Coat	Jan 14,15	Moderate
	Stain, Nose, Red	Jan 14	Slight
	Cyanosis	Jan 14,15	Moderate
	Lacrimation	Jan 15	Moderate
	Death	Jan 16	1.9 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85D01250	Normal	N/A	N/A
85D01273	Normal	N/A	N/A
85D01276	Normal	N/A	N/A
85D01277	Normal	N/A	N/A
85D01278	Normal	N/A	N/A
85D01288	Normal	N/A	N/A
85D01289	Normal	N/A	N/A
85D01301	Normal	N/A	N/A
85D01306	Normal	N/A	N/A
85D01314	Alopecia, Head	Jan 22,23	Slight

Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 2510 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86D00202	152	296	330	320
86D00203	133	303	340	379
86D00216	167	297	323	343
86D00218	149	292	324	368
86D00223	130	276	307	336
86D00230	141	315	366	418
86D00244	147	277	303	336
86D00246	148	307	342	379
86D00255	141	281	328	366
Mean	145.3	293.8	329.2	360.6
Standard Deviation	11.0	13.6	19.0	30.0
Std. Error of Mean	3.7	4.6	6.3	10.1

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 2820 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 5	Termination Day 13
86D00207	134	308	320	325
86D00217	153	336	327	344
86D00235	150	323	Dead	---
86D00240	155	376	377	399
86D00241	151	333	318	348
86D00251	139	332	348	363
86D00257	123	331	337	355
86D00272	162	344	346	360
86D00279	146	398	416	434
Mean	145.9	342.3	348.6	366.0
Standard Deviation	12.0	27.7	33.2	34.6
Std. Error of Mean	4.0	9.2	11.7	12.2

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 2985 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86D00212	158	275	Dead	---
86D00213	159	288	311	368
86D00219	156	293	Dead	---
86D00228	147	267	Dead	---
86D00237	145	265	Dead	---
86D00253	144	261	257	317
86D00260	153	236	Dead	---
86D00275	144	245	Dead	---
Mean	150.8	266.2	284.0	342.5
Standard Deviation	6.5	19.5	38.2	36.1
Std. Error of Mean	2.3	6.9	27.0	25.5

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 3160 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86D00209	142	259	Dead	---
86D00211	146	265	Dead	---
86D00221	154	228	240	286
86D00224	143	258	Dead	---
86D00229	152	270	Dead	---
86D00238	149	239	Dead	---
86D00256	146	243	Dead	---
86D00258	142	268	Dead	---
86D00261	156	267	Dead	---
86D00265	148	253	Dead	---
Mean	147.8	255.0	240	286
Standard Deviation	5.0	14.1	N/A	N/A
Std. Error of Mean	1.6	4.5	N/A	N/A

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 3550 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
86D00205	154	263	Dead	---
86D00215	144	273	Dead	---
86D00222	137	246	Dead	---
86D00263	150	244	Dead	---
86D00264	144	243	Dead	---
86D00267	148	267	Dead	---
86D00273	146	255	Dead	---
86D00277	145	261	Dead	---
86D00278	144	237	Dead	---
Mean	145.8	254.3	----	---
Standard Deviation	4.7	12.4	N/A	N/A
Std. Error of Mean	1.6	4.1	N/A	N/A

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS**Males: Vehicle Control**

Animal No.	Receipt	Dosing	Day 6	Termination
				Day 14
85D01164	168	195	232	249
85D01168	168	207	233	190
85D01171	162	204	255	267
85D01175	165	206	261	274
85D01185	165	207	258	277
85D01198	168	204	216	161
85D01202	165	208	268	284
Mean	165.9	204.4	246.1	243.1
Standard Deviation	2.3	4.4	19.1	48.2
Std. Error of Mean	0.9	1.7	7.2	18.2

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 1590 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination
				Day 14
85D01260	140	185	220	210
85D01262	142	189	210	201
85D01265	149	188	225	216
85D01267	153	193	235	221
85D01272	136	170	134	161
85D01280	152	178	185	207
85D01285	143	184	218	223
85D01286	163	198	236	223
85D01294	149	187	221	208
Mean	147.4	185.8	209.3	207.8
Standard Deviation	8.2	8.2	32.0	19.2
Std. Error of Mean	2.7	2.7	10.7	6.4

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 2000 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 6	Termination Day 14
85D01244	147	163	198	201
85D01261	132	171	188	191
85D01268	146	162	181	186
85D01269	151	195	192	209
85D01292	144	161	164	153
85D01293	146	153	183	181
85D01295	148	162	210	221
85D01300	145	168	200	201
85D01305	137	160	Dead	---
85D01310	160	184	227	227
Mean	145.6	167.9	193.7	196.7
Standard Deviation	7.5	12.6	18.1	22.4
Std. Error of Mean	2.4	4.0	6.0	7.4

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 2510 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 6	Termination Day 14
85D01247	139	154	182	184
85D01254	156	174	203	209
85D01263	152	169	152	209
85D01290	145	153	166	182
85D01303	151	164	198	208
85D01308	138	163	190	199
85D01309	146	170	206	209
85D01315	143	154	Dead	---
85D01316	150	165	Dead	---
Mean	146.7	162.9	185.3	200.0
Standard Deviation	6.1	7.7	20.1	12.2
Std. Error of Mean	2.0	2.6	7.6	4.6

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 3160 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 6	Termination	
				Day 14	
85D01238	142	163	Dead	---	
85D01249	147	170	Dead	---	
85D01266	170	194	Dead	---	
85D01279	151	175	Dead	---	
85D01283	138	167	195	205	
85D01287	147	158	Dead	---	
85D01297	146	157	Dead	---	
85D01302	147	165	Dead	---	
85D01312	147	176	Dead	---	
Mean	148.3	169.4	195	205	
Standard Deviation	8.9	11.4	N/A	N/A	
Std. Error of Mean	3.0	3.8	N/A	N/A	

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: 3550 mg/kg DIGL-RP

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01240	144	182	Dead	---
85D01243	141	203	Dead	---
85D01253	137	178	Dead	---
85D01255	133	191	Dead	---
85D01256	146	182	Dead	---
85D01284	146	167	Dead	---
85D01304	158	179	Dead	---
85D01313	151	184	Dead	---
85D01317	145	187	Dead	---
Mean	144.1	182.3	---	---
Standard Deviation	7.1	10.2	---	---
Std. Error of Mean	2.2	3.2	---	---

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS IN GRAMS

Females: Vehicle Control

Animal No.	Receipt	Dosing	Day 6	Termination Day 14
85D01250	147	158	189	195
85D01273	152	164	191	197
85D01276	144	153	187	189
85D01277	145	157	185	189
85D01278	155	174	213	217
85D01288	138	162	169	155
85D01289	140	156	185	196
85D01301	146	158	161	189
85D01306	145	153	190	188
85D01314	134	151	176	177
Mean	144.6	158.6	184.6	189.2
Standard Deviation	6.2	6.7	14.0	15.8
Std. Error of Mean	2.0	2.1	4.4	5.0

Appendix G: PATHOLOGY REPORT

GLP Study #85021
Principal Investigator: MAJ Brown

I. INTRODUCTION

Study: Oral LD50/DIGL-RP
Animal: Rat (Sprague-Dawley)/2 months old/both sexes
Reference: SOP OP-STX-36

II. SUMMARY OF PROCEDURES

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

III. GROSS FINDINGS

Males - Vehicle Control
(Live animals indicated by '*' / Histopathology by '\$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 38970	85D01164	14 Days	Not remarkable (NR)
* 38972	85D01168	14 Days	NR
* 38973	85D01171	14 Days	NR
* 38976	85D01175	14 Days	NR
* 38979	85D01185	14 Days	NR
*\$38981	85D01198	14 Days	Thymus--atrophic Peritoneal cavity--sparse body fat Liver, rt lateral lobe-- 10 to 15 discrete white foci (<1 mm)
*\$38983	85D01202	14 Days	Mesenteric lymph node-- enlarged

Appendix G (cont.): PATHOLOGY REPORT

Males - 2510 mg/kg DIGL-RP
(Live animals indicated by '*' / Histopathology by '\$')

<u>ACCESSION NUMBER</u>	<u>ANIMAL ID NUMBER</u>	<u>DOSE-DEATH INTERVAL</u>	<u>OBSERVATIONS</u>
* 39695	86D00202	14 Days	NR
* 39696	86D00203	14 Days	NR
* 39699	86D00216	14 Days	NR
* 39700	86D00218	14 Days	NR
* 39701	86D00223	14 Days	NR
* 39703	86D00230	14 Days	NR
* 39706	86D00244	14 Days	NR
* 39707	86D00246	14 Days	NR
* 39710	86D00255	14 Days	NR

Males - 2820 mg/kg DIGL-RP

* 39741	86D00207	13 Days	NR
* 39736	86D00217	13 Days	NR
39692	86D00235	2 Days	Liver--pale
* 39739	86D00240	13 Days	NR
* 39737	86D00241	13 Days	NR
* 39742	86D00251	13 Days	NR
* 39738	86D00257	13 Days	NR
39740	86D00272	13 Days	NR
* 39743	86D00279	13 Days	NR

Males - 2985 mg/kg DIGL-RP

39621	86D00212	4 Days	Gastric mucosa--min. petechiae
* 39687	86D00213	14 Days	NR
39620	86D00219	3 Days	Gastric mucosa--min. petechiae Kidneys--light brown Liver--pale
39547	86D00228	2 Days	Liver--pale
39556	86D00237	3 Days	Liver--pale
39689	86D00253	14 Days	NR
39624	86D00260	4 Days	Gastric mucosa--min. petechiae
39625	86D00275	4 Days	Liver--pale Gastric mucosa--min. petechiae

Appendix G (cont.): PATHOLOGY REPORT

Males - 3160 mg/kg DIGL-RP
(Live animals indicated by '*' / Histopathology by '\$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
\$ 39619	86D00209	3 Days	Gastric mucosa--petechiae Liver, Kidneys--pale
39555	86D00211	3 Days	Liver--pale
* 39688	86D00221	14 Days	NR
\$ 39622	86D00224	4 Days	Gastric mucosa--petechiae Lungs--congested Liver--pale
\$ 39548	86D00229	2 Days	Stomach--test compound Kidney, rt--single 1 mm depressed gray focus
39623	86D00238	4 Days	Gastric mucosa--min. petechiae
\$ 39686	86D00256	6 Days	Lung, rt--focus of brown discoloration Liver--focus (1.5 cm) of discoloration
39549	86D00258	2 Days	NR
\$ 39554	86D00261	2 Days	Liver, Kidneys--pale
39551	86D00265	2 Days	Gastric mucosa--min. petechiae

Males - 3550 mg/kg DIGL-RP

\$ 39553	86D00205	2 Days	Gastric mucosa--hemorrhage Liver, Kidneys--pale
39542	86D00215	1 Day	Gastric mucosa--min. petechiae
39544	86D00222	1 Day	Gastric mucosa--min. petechiae
39550	86D00263	2 Days	Gastric mucosa--min. petechiae
\$ 39546	86D00264	1 Day	Lungs--brown discoloration Kidneys--brown discoloration Liver--brown discoloration Heart--multiple, minute red foci
39557	86D00267	3 Days	Liver--pale
39558	86D00273	3 Days	Liver--pale
39545	86D00277	1 Day	Liver--pale Lungs--brown discoloration Blood--brown discoloration Gastric mucosa--mod. petechiae
\$ 39552	86D00278	2 Days	Stomach--test compound Heart--brown discolored focus

Appendix G (cont.): PATHOLOGY REPORT

Females - Vehicle Controls
(Live animals indicated by '*' / Histopathology by '\$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 38996	85D01250	14 Days	NR
* 39003	85D01276	14 Days	NR
* 39004	85D01277	14 Days	NR
* 39005	85D01278	14 Days	NR
* 39007	85D01288	14 Days	Abdominal cavity--absence of body fat
* 39008	85D01289	14 Days	NR
* 39014	85D01301	14 Days	NR
* 39016	85D01306	14 Days	NR
*\$39002	85D01273	14 Days	Mesenteric lymph node-- enlarged 2-3x
*\$39020	85D01314	14 Days	Lung lt--depressed gray foci

Females - 1590 mg/kg DIGL-RP

* 39062	85D01260	14 Days	NR
* 39063	85D01262	14 Days	NR
* 39064	85D01265	14 Days	NR
* 39065	85D01267	14 Days	NR
* 39066	85D01272	14 Days	NR
* 39067	85D01280	14 Days	NR
* 39068	85D01285	14 Days	NR
* 39069	85D01286	14 Days	NR
* 39070	85D01294	14 Days	NR

Females - 2000 mg/kg DIGL-RP

* 38994	85D01244	14 Days	NR
* 38998	85D01261	14 Days	NR
* 39000	85D01268	14 Days	NR
* 39001	85D01269	14 Days	NR
* 39010	85D01292	14 Days	NR
* 39011	85D01293	14 Days	NR
* 39012	85D01295	14 Days	NR
* 39013	85D01300	14 Days	NR
38865	85D01305	2 Days	Liver--pale
* 39019	85D01310	14 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

Females - 2510 mg/kg DIGL-RP
(Live animals indicated by '*' / Histopathology by '\$')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 38995	85D01247	14 Days	NR
* 38997	85D01254	14 Days	NR
* 38999	85D01263	14 Days	Mesen. lymph nodes--enlarged
* 39009	85D01290	14 Days	Abdomen--absence of body fat
* 39015	85D01303	14 Days	NR
* 39017	85D01308	14 Days	NR
* 39018	85D01309	14 Days	NR
38870	85D01315	3 Days	Gastric mucosa--hyperemic Jejunum--bloody contents Liver--pale
38871	85D01316	3 Days	Stomach--multifocal petechiae Liver--pale

Females - 3160 mg/kg DIGL-RP

38866	85D01238	2 Days	NR
38861	85D01249	1 Day	NR
38862	85D01266	1 Day	NR
38863	85D01279	2 Days	NR
* 39006	85D01283	14 Days	NR
38864	85D01287	2 Days	NR
38867	85D01297	2 Days	Stomach--multifocal necrosis and petechiae Liver--pale
38859	85D01302	1 Day	Lungs--severe congestion
38869	85D01312	3 Days	Stomach--multifocal petechiae Liver--pale

Females - 3550 mg/kg DIGL-RP

38895	85D01240	1 Day	Lungs--mild congestion
38933	85D01243	2 Days	NR
38934	85D01253	2 Days	Lungs--severe congestion
38935	85D01255	2 Days	NR
38896	85D01256	1 Day	Lungs, liver--pale Blood--brown discoloration
38897	85D01284	1 Day	Lungs--mild congestion
38898	85D01296	1 Day	Lungs--mild congestion
38936	85D01304	2 Days	NR
38899	85D01313	1 Day	NR
38937	85D01317	2 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

IV. SUMMARY OF GROSS FINDINGS

The following table outlines the noteworthy necropsy findings by incidence.

LESION	MALES				FEMALES			
	<u>2820</u>	<u>2985</u>	<u>3160</u>	<u>3550</u>	<u>2000</u>	<u>2510</u>	<u>3160</u>	<u>3550</u>
Gastroenter- opathy*		4/8	4/10	5/9		2/9	2/9	
Pale Liver	1/9	4/8	4/10	4/9	1/10	2/9	2/9	1/10
Pulmonary Congestion			1/10				1/9	4/10

* Includes gastric hemorrhage and necrosis

The incidence of lesions, generally speaking, was slightly higher in males than in females. Longevity appeared dose-dependent for both sexes. The vehicle control groups and the low dose groups had only incidental background lesions.

V. MICROSCOPIC FINDINGS

Females - Vehicle Controls

<u>ACCESSION NUMBER</u>	<u>ANIMAL ID NUMBER</u>	<u>DOSE-DEATH INTERVAL</u>	<u>OBSERVATIONS</u>
39002	85D01273	14 Days	Lymphoid hyuperplasia (physiologic response)
39020	85D01314	14 Days	Focally extensive granulomatous pneumonia (etiology unknown)

Males - Vehicle Controls

38981	85D01198	14 Days	Liver--hepatitis, necro- granulomatous, multifocal, mild to moderate (etiology unknown)
38983	85D01202	14 Days	Lymphoid hyperplasia (physiologic response)

Appendix G (cont.): PATHOLOGY REPORT

Males - 3160 mg/kg DIGL-RP

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
39548	86D00229	2 Days	Kidney--protein cast formation, cortical tubules
39554	86D00261	2 Days	Kidney--protein droplet and cast formation, cortical tubules and glomeruli
39619	86D00209	3 Days	Liver--diffuse vacuolation Kidney--protein droplet and cast formation, glomeruli and cortical tubules Liver--diffuse vacuolation
39686	86D00256	6 Days	Stomach--multifocal, acute, necrotizing gastritis, minimal, gastric mucosa Lung--minimal peri-bronchiolar edema and parenchymal congestion Liver--multifocal, moderate, acute, necrotizing hepatitis (etiology unknown)
39622	86D00224	4 Days	Lung--minimal edema and congestion Liver--diffuse vacuolation

Males - 3550 mg/kg DIGL-RP

39553	86D00205	2 Days	Spleen†--minimal congestion Liver--NR Kidney--protein droplet and cast formation, glomeruli and cortical tubules Stomach--minimal multifocal necrosis and hemorrhage, gastric mucosa
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†Comment: The congestion occurred in a circular zone around the white pulp at the point where penicilliary arteries joined the sinusoids.

39546	86D00264	2 Days	Kidney--protein cast formation, cortical tubules Lungs--minimal multifocal lymphocytic bronchiolitis
39552	86D00278	2 Days	Heart, Liver--NR Heart-- NR

Appendix G (cont.): PATHOLOGY REPORT

VI. SUMMARY OF MICROSCOPIC FINDINGS

The choice of tissues examined histologically was biased by gross evaluation. Indications of renal protein loss were noted in five animals (casts and protein droplet formation). Diffuse hepatic vacuolation, although not confirmed by special stains, was interpreted to be fatty degeneration and probably accounts for much of the hepatic pallor seen in many animals. The term gastroenteropathy is used to categorize multifocal petechiation (hemorrhage) and necrosis of the stomach as well as bloody intestinal contents. Some findings implicate various infectious processes, but were not frequent enough to warrant concern for the entire experiment.

VII. CONCLUSIONS

The most obvious target tissue is the stomach and proximal small intestine. Less consistent are liver and kidney changes. It is entirely possible that the fatty change in the liver is due to renal protein loss or to direct hepatotoxic mechanisms, both of which could compromise fat mobilization. A contributing feature could be the strong possibility that the animals may have stopped eating, although this by itself probably would not cause fatty changes in such a short time frame. All other pathologic findings are considered background or incidental changes.



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