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*Institute Report No. 412*

AD-A217 347

**Acute Oral Toxicity of  
JA-2 Solid Propellant in Sprague-Dawley Rats**

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December 1989

Toxicology Series: 161

**DISTRIBUTION STATEMENT A**  
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LETTERMAN ARMY INSTITUTE OF RESEARCH  
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90 01 30 087

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.		
4. PERFORMING ORGANIZATION REPORT NUMBER(S)  Institute Report No.: 412			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology		6b. OFFICE SYMBOL (if applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Biomedical Research and Development Laboratory		
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800			7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5010		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION US Army Medical Research & Development Command		8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012			10. SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO 62720	PROJECT NO. A835	TASK NO. AB
			WORK UNIT ACCESSION NO. DA303913		
11. TITLE (Include Security Classification) (U) Acute Oral Toxicity of JA-2 Solid Propellant in Sprague-Dawley Rats					
12. PERSONAL AUTHOR(S) LD Brown, JD Justus, CR Wheeler, and DW Korte, Jr.					
13a. TYPE OF REPORT Institute		13b. TIME COVERED FROM 2NOV85 TO 9DEC85	14. DATE OF REPORT (Year, Month, Day) December 1989		15. PAGE COUNT 65
16. SUPPLEMENTARY NOTATION  Toxicology Series No. 161					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	JA-2 Solid Propellant, Diethyleneglycol Dinitrate; Nitroglycerin; Mammalian Toxicology; Rat; Propellant; Acute Oral Toxicity		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) - The acute oral toxicity of JA-2 Solid Propellant was determined in male and female Sprague-Dawley rats by using an oral gavage split-dose method. The MLD was 3990.6 ± 349.7 mg/kg for male rats and 2545.9 ± 421.1 mg/kg for female rats. JA-2 produced clinical signs that were attributed to its nitrate ester components, diethyleneglycol dinitrate and nitroglycerin. These signs included tremors and twitching, cyanosis, and increases in respiratory rate and depth. Other clinical signs observed were associated with the general malaise of the animals following dosing and included hunched posture, rough coat, 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 JA-2 in the slightly toxic class.					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED		
22a. NAME OF RESPONSIBLE INDIVIDUAL DONALD G. CORBY, COL, MC			22b. TELEPHONE (Include Area Code) (415) 561-3600		22c. OFFICE SYMBOL SGRD-UL2

### ABSTRACT

The acute oral toxicity of JA-2 Solid Propellant was determined in male and female Sprague-Dawley rats by using an oral gavage split-dose method. The MLD was  $3990.6 \pm 349.7$  mg/kg for male rats and  $2545.9 \pm 421.1$  mg/kg for female rats. JA-2 produced clinical signs that were attributed to its nitrate ester components, diethyleneglycol dinitrate and nitroglycerin. These signs included tremors and twitching, cyanosis, and increases in respiratory rate and depth. Other clinical signs observed were associated with the general malaise of the animals following dosing and included hunched posture, rough coat, 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 JA-2 in the slightly toxic class.

Key Words: Acute Oral Toxicity, JA-2 Solid Propellant, Diethyleneglycol Dinitrate, Nitroglycerin, Mammalian Toxicology, Propellant, Rat



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

GLP STUDY NUMBER: 85015

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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: JA-2 Solid Propellant

INCLUSIVE STUDY DATES: 12 November 85 - 19 December 85

OBJECTIVE: The objective of this study was to determine the  
acute oral toxicity of JA-2 Solid Propellant  
in male and female Sprague-Dawley rats.

## ACKNOWLEDGMENTS

SP4 James J. Fischer, SP4 Scott L. Schwebe, SP4 Theresa L. Polk, and Mr. Obie Goodrich provided research assistance and animal care. SP4 Paul B. Simboli, BS, provided chemical preparation and analysis. Colleen S. Kamiyama and Marie Rogers provided secretarial assistance.

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

We, the undersigned, declare that GLP Study 85015 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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SGRD-ULZ-QA

12 December 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85015

1. This is to certify that the protocol for LAIR GLP Study 85015 was reviewed on 10 May 1985.
2. The institute report entitled "Acute Oral Toxicity of JA-2 Solid Propellant in Sprague-Dawley Rats," Toxicology Series 161, was audited on 11 December 1989.

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**Acute Oral Toxicity of JA-2 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 JA-2 Solid Propellant in male and female Sprague-Dawley rats.

**MATERIALS**

Test Substance

Chemical Name: JA-2 Solid Propellant

LAIR Code No.: TP56

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Description: Solid black cylinders (stick configuration)

Lot Number: RAD83R001S153

JA-2 Solid Propellant was received in the stick configuration. It was ground into a fine powder for this study. Other test substance information is presented in Appendix A.

#### Vehicle

The vehicle for JA-2 was 1% gum tragacanth (Lot 91044A2, Spectrum Chemical Manufacturing Corp., Gardena, CA) made up in sterile water for injection (Lot 62-354-DM-03, Abbott Laboratories, North Chicago, IL). The expiration date was 11 Dec 1985 for the gum tragacanth and 1 Mar 1986 for the sterile water for injection.

#### Animal Data

Seventy-three male and 73 female Sprague-Dawley rats were obtained from Bantin-Kingman Inc. (Fremont, CA) for this study. They were identified individually with ear tags. Two males and two females were submitted as necropsy quality controls. Twenty-two animals were used in a limit test and ten additional animals were used in an approximate lethal dose (ALD) determination. The remaining 110 animals were placed on study. The animal weights on receipt ranged from 123 to 162 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<sup>®</sup> 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 20.0°C to 25.5°C with a relative humidity range of 33% to 50% with spikes to 75% during room cleaning. The photoperiod was 12 hours of light per day.

**METHODS**Group Assignment/Acclimation

Study animals were randomized separately into five dose groups of 10 males and 10 females each and a vehicle control group containing 5 males and 5 females with a stratified, weight-biased computer program (Beckman TOXSYS<sup>®</sup> Animal Allocation Program run on a Beckman TOXSYS<sup>®</sup> Data Collection Terminal). The animals were acclimated for 20-22 days before the day of dosing. During this period they were observed daily for signs of illness.

Dose Levels

The results of the limit test and the approximate lethal dose (ALD) determination suggested that the median lethal dose (MLD) was between 2000 and 4000 mg/kg. Based on these data, test doses were selected (Table 1).

**TABLE 1: JA-2 Solid Propellant Doses**

<u>Male</u> <u>Dose Levels</u> (mg/kg)	<u>Female</u> <u>Dose Levels</u> (mg/kg)
2370	2370
3160	2740
4220	3160
4880	4220
5620	4880
Vehicle	Vehicle

Compound Preparation

The JA-2 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, the 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.

### Chemical Analyses of Dosing Suspensions

The DEGDN component of JA-2 was used to verify the stability of dosing suspensions. JA-2 was stable in the gum tragacanth vehicle for at least 24 hrs (Appendix A). This was sufficient since dosing was begun and completed within 3 hrs of preparing the suspensions. 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) for each suspension did not exceed 3.6% for any suspension. The JA-2 dosing suspensions used in this study were within 103.2 - 109.3% of their target concentrations.

### 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 2.13 to 2.95 ml in the males and 1.62 to 2.15 ml in the females. The vehicle control (1% gum tragacanth) group received 2.28 to 2.84 ml (males) and 1.90 to 1.95 ml (females). The total volume administered 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 3-ml syringes (Monoject, Sherwood Medical, St. Louis, MO) fitted with 14-18 gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were utilized. Animals in Phase I (males, females at 2370 mg/kg, 3160 mg/kg, 4220 mg/kg, and controls) were dosed between 0915 and 1204 hrs on 3 Dec 1985. Phase II animals were dosed between 0940 and 1105 on 5 Dec 1985 (4880 mg/kg, 5620 mg/kg males; 2740 mg/kg, 4880 mg/kg females) after analysis of the preliminary Phase I 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 approximately 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 MLD was 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 historical listing of study 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. The JA-2 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-five of 80 animals (17/38 males, 28/42 females) dosed with JA-2 died as a result of its toxicity. Seven (15.5%) deaths occurred within 4 hrs of dosing. An additional 30 (66.7%) deaths occurred by 24 hrs after dosing, and the remaining 8 (17.8%) deaths occurred within 96 hrs 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>
<b>Males</b>		
2370	1/9*	11.1
3160	2/9*	22.2
4220	3/7*	42.9
4880	6/6*	100.0
5620	5/7*	71.4
Vehicle	0/4*	0.0
<b>Females</b>		
2370	3/8*	37.5
2740	6/9*	66.7
3160	5/8*	62.5
4220	5/7*	71.4
4880	9/10	90.0
Vehicle	0/3*	0.0

\* Reduced numbers in groups were due to one or more animals that 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:  $Y = -16.8 + 6.07 \log X$  (males);  $Y = -8.3 + 3.91 \log X$  (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 JA-2 Solid Propellant in Sprague-Dawley Rats**

Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
<b>Males</b>		
LD10	2453.7 ± 402.9	(1186.6, 3061.6)
LD50	3990.6 ± 349.7	(3272.2, 5026.4)
LD90	6490.4 ± 1165.1	(5115.9, 14556.0)
<b>Females</b>		
LD10	1197.0 ± 576.1	(.01198, 1986.6)
LD50	2545.9 ± 421.1	( 145.1, 3237.9)
LD90	5414.6 ± 1476.0	(3977.5, 2331300)

\* Calculated dose ± standard error.

Clinical Observations

The most frequently observed category of clinical observations was behavioral disturbances (66 of 80 animals dosed). Behavioral signs exhibited by the animals included tremors, inactivity, twitching, irritability, and ataxia. Skin changes included cyanosis, pallor, scabbing, and scaling. Respiratory changes included changes in rate, regularity or depth, and wheezing. Although clinical signs were observed at each dose level, there was no clear dose-response relationship observed. This was due to the fact that animals receiving higher doses of JA-2 died more rapidly than animals receiving lower doses of JA-2, precluding the observation of the entire spectrum of changes.

Figure 1  
JA-2 Dose Response Curve for Male Rats

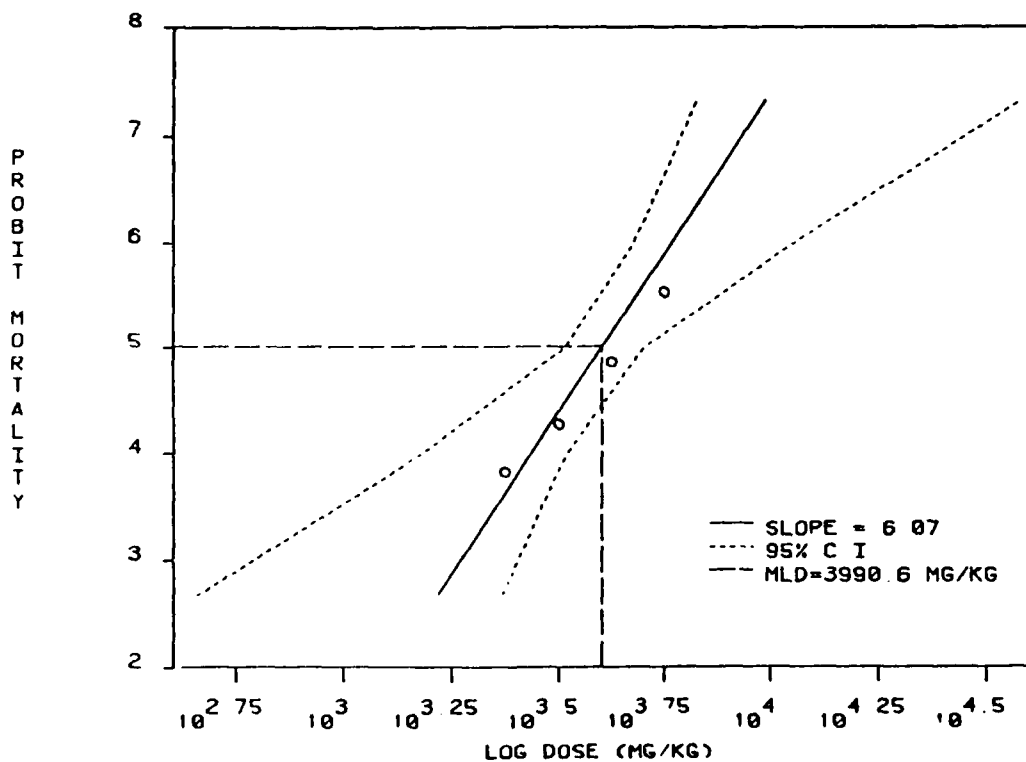
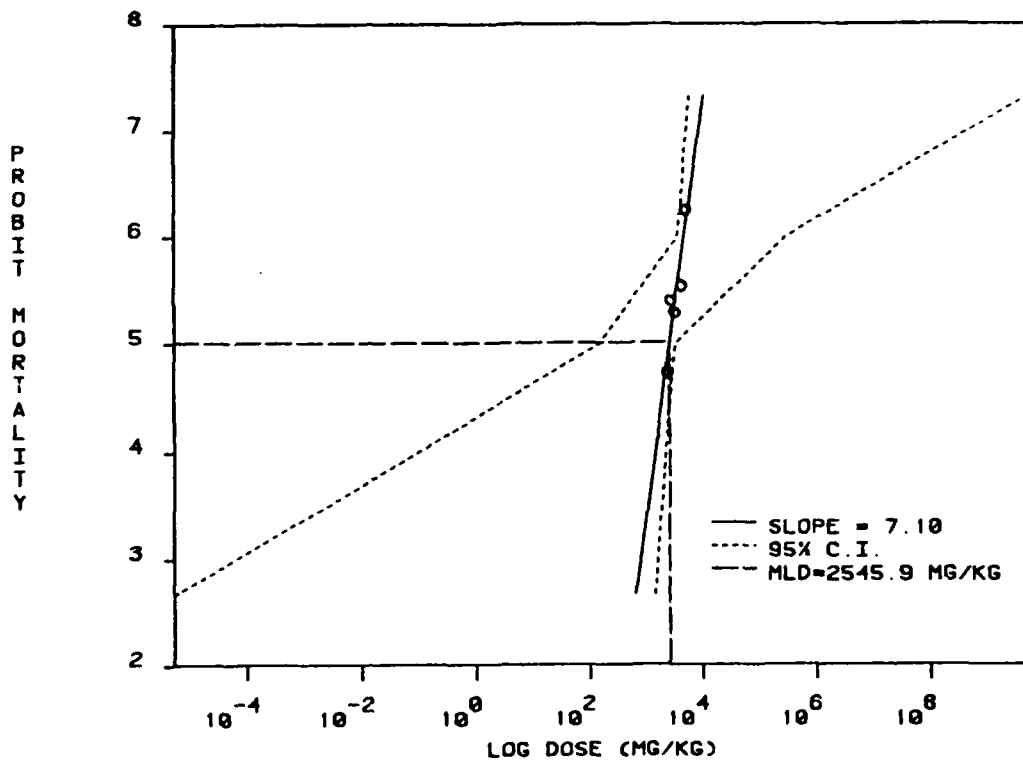


Figure 2  
JA-2 Dose Response Curve for Female Rats



Most other clinical signs were attributable to general malaise associated with the administration of JA-2. Included with these signs were rough coat, hunched posture, and various stains.

A variety of signs were observed in vehicle control animals. These included hunched posture, behavioral and respiratory signs, and various stains which were 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 JA-2. Table 5 presents the mean body weights by groups. Appendix F contains individual weight tables.

#### Pathology Findings

Eight males and seven females administered JA-2 presented with a brownish discoloration of blood and/or selected organs (lungs, liver) consistent with a diagnosis of methemoglobinemia. The lungs of two of these animals were examined microscopically and appeared normal except for a few peribronchiolar lymphoid nodules (evidence of subclinical mycoplasma infection) not related to the test compound. Three animals had multiple petechiae of the stomach mucosa and two animals had hemorrhage of the small intestine. Hydronephrosis was observed in two males. The veterinary pathologist's report appears in Appendix G.

#### **DISCUSSION**

The calculated median lethal dose (MLD) for JA-2 was 3990.6 mg/kg in male rats and 2545.9 mg/kg in female rats. These values place JA-2 within the slightly toxic classification (5).

JA-2 has as its major constituents, nitrocellulose, diethyleneglycol dinitrate (DEGDN), and nitroglycerin (Appendix A). Nitrocellulose is relatively nontoxic (MLD >5000 mg/kg) to rats (6) while a MLD for DEGDN of 750-1000 mg/kg in rats has been reported (7). The oral MLD for nitroglycerin in rats is 525 mg/kg (8). The spectrum of clinical signs observed following JA-2 administration supports the assumption that the nitrate esters, nitroglycerin (8) and/or DEGDN (9), are the toxic components of JA-2. The calculated quantity of DEGDN or nitroglycerin contributing to the oral MLD of JA-2 in male

**TABLE 4: Incidence Summary for Clinical Observations in Rats Administered JA-2 Solid Propellant**

<b>MALES</b>	Dose (n=)	<u>Vehicle</u>	<u>2370</u>	<u>3160</u>	<u>4220</u>	<u>4880</u>	<u>5620</u>
Respiratory <sup>a</sup>		4	9	9	7	6	7
Behavioral <sup>b</sup>		2	3	4	3	1	4
Skin <sup>c</sup>		4	9	8	7	2	5
Gastrointestinal <sup>d</sup>		0	7	8	3	2	7
Rough coat		0	1	1	0	1	0
Ocular <sup>e</sup>		0	3	3	5	0	3
Hunched posture		0	1	0	0	0	0
Reflex <sup>f</sup>		4	9	9	6	2	5
Prostrate/Moribund		0	1	0	0	0	0
Miscellaneous <sup>g</sup>		0	1	0	1	1	3
Deaths		4	7	5	2	0	1
		0	1	2	3	6	5

<b>FEMALES</b>	Dose (n=)	<u>Vehicle</u>	<u>2370</u>	<u>2740</u>	<u>3160</u>	<u>4220</u>	<u>4880</u>
Respiratory <sup>a</sup>		3	8	9	8	7	10
Behavioral <sup>b</sup>		1	7	7	3	4	8
Skin <sup>c</sup>		2	6	9	7	7	6
Ocular <sup>e</sup>		1	7	9	5	6	9
Hunched posture		0	2	3	0	0	3
Prostrate/Moribund		3	6	9	6	4	4
Miscellaneous <sup>g</sup>		0	4	4	2	4	8
Deaths		3	7	8	3	2	3
		0	3	6	5	5	9

<sup>a</sup> Includes changes in rate or depth, wheezing, or irregular rate.

<sup>b</sup> Includes tremors, inactivity, irritability, ataxia, and twitching.

<sup>c</sup> Includes cyanosis, pallor, scaling, and scabbing.

<sup>d</sup> Includes diarrhea.

<sup>e</sup> Includes lacrimation.

<sup>f</sup> Includes depressed grasping and righting reflexes.

<sup>g</sup> Includes material in urine, and stains on head and neck, tail, and perineum.

**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>
<b>MALES</b>				
2370	150.9 $\pm 1.6$ (9)	262.8 $\pm 6.9$ (9)	300.5 $\pm 4.0$ (8)	306.8 $\pm 4.5$ (8)
3160	151.3 $\pm 1.4$ (9)	270.6 $\pm 7.2$ (9)	317.7 $\pm 5.9$ (7)	328.6 $\pm 9.1$ (7)
4220	147.6 $\pm 2.2$ (7)	276.6 $\pm 4.9$ (7)	311.8 $\pm 13.3$ (4)	323.0 $\pm 16.0$ (4)
4880	153.0 $\pm 3.6$ (6)	264.2 $\pm 5.4$ (6)	N/A	N/A
5620	144.4 $\pm 3.6$ (7)	261.3 $\pm 3.9$ (4)	292.5 $\pm 22.5$ (2)	297.0 $\pm 21.0$ (2)
Vehicle	155.8 $\pm 1.6$ (4)	262.5 $\pm 12.0$ (4)	321.5 $\pm 10.3$ (4)	327.3 $\pm 6.8$ (4)
<b>FEMALES</b>				
2370	147.4 $\pm 2.4$ (8)	195.0 $\pm 3.4$ (8)	222.0 $\pm 6.8$ (5)	218.8 $\pm 5.8$ (5)
2740	146.6 $\pm 1.2$ (9)	198.4 $\pm 5.0$ (9)	241.0 $\pm 4.0$ (3)	241.0 $\pm 3.8$ (3)
3160	147.8 $\pm 1.3$ (8)	193.0 $\pm 3.9$ (8)	230.0 $\pm 2.3$ (3)	226.3 $\pm 4.1$ (3)
4220	145.4 $\pm 2.3$ (7)	189.6 $\pm 3.7$ (7)	221.0 $\pm 14.0$ (2)	219.5 $\pm 16.5$ (2)
4880	145.4 $\pm 2.1$ (10)	188.5 $\pm 4.4$ (10)	240 (1)	228 (1)
Vehicle	146.7 $\pm 3.0$ (3)	192.0 $\pm 1.5$ (3)	222.7 $\pm 0.9$ (3)	216.3 $\pm 3.3$ (3)

\* Weight after overnight fast.

rats is 1000 mg/kg and 640 mg/kg, respectively. These data suggest there is no additive relationship between the toxicity of DEGDN and nitroglycerin. However, based on their similar mechanisms of action as nitrate esters, more plausible explanations would be a temporal difference in their maximal effects or that the bioavailability of DEGDN or nitroglycerin is decreased by its presence in the JA-2 formulation. These data also suggest that nitrocellulose does not contribute to the toxicity of the JA-2 formulation. The MLD of JA-2 in male rats contains approximately 2335 mg/kg nitrocellulose, which is less than 50% of the MLD for nitrocellulose.

The major clinical signs associated with JA-2 administration were cyanosis and a temporally related increased depth of respiration. The health effects of DEGDN, nitroglycerin, and related nitrate esters have been evaluated in several animal models (9). In all species evaluated, acute poisoning was characterized by cyanosis that was attributable to methemoglobin formation. Cyanosis, if present, was observed in the first 24 hours after dosing and was no longer obvious at the 96-hour observation. A frequent finding at necropsy during the first 72 hours after dosing was a light-brown discoloration of the blood and a brownish discoloration of various organs, especially the liver and lungs. Chocolate-brown appearing blood is typical of methemoglobinemia (10). The increased depth of respiration was temporally related to the presence of cyanosis and represents a compensatory response. Nitrate esters have been reported to produce central nervous system toxicity, especially tremors and convulsions (9). Ataxia, tremors, and twitching were observed in rats administered JA-2; however, the incidence was approximately 30%, which may be a function of the scheduled observation periods versus the time of onset of these signs.

## CONCLUSION

JA-2 Solid Propellant is a slightly toxic compound that produces cyanosis, twitching, tremors, and increased respiration. Calculated MLD values were  $3990.6 \pm 349.6$  mg/kg in male and  $2545.9 \pm 421.1$  mg/kg in female Sprague-Dawley rats.

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**Appendix A: CHEMICAL DATA**

Test Substance: JA-2 Solid Propellant

LAIR Code Number: TP56

Physical State: Solid black cylinders (stick configuration)

Preparation of Test Substance for Dosing: The cylinders of JA-2 were ground to a fine powder under liquid nitrogen using a Spex freezer mill. The powder was then sieved through an 80-mesh screen. Aqueous suspensions were prepared with 1% gum tragacanth as the vehicle, using a Brinkman homogenizer.

pH of Dosing Suspensions: 4.5 - 5.0<sup>1</sup>

Chemical Analysis:

DEGDN was the only major component of JA-2 that could be easily analyzed.<sup>2,3</sup> To determine the percent DEGDN in the JA-2 propellant, samples of JA-2 powder were placed in individual 100 ml volumetric flasks to which 1 ml of 1% gum tragacanth was added. After dilution to volume with 95% 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 154.5 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. Under these conditions, DEGDN eluted with a retention time of approximately 5.4 min. The results from the analysis of standards and JA-2 powder samples are presented in Tables 1 and 2.

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<sup>1</sup> Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 43. Letterman Army Institute of Research, Presidio of San Francisco, CA.

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

<sup>3</sup> Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 51-61. Letterman Army Institute of Research, Presidio of San Francisco, CA.

## Appendix A (cont.): CHEMICAL DATA

Table 1. Analysis of Standards

Concentration of DEGDN ( $\mu\text{g/ml}$ )	Peak Area* ( $\times 10^6$ )
76.88	4.452
95.81	5.567
158.20	9.176
195.00	11.219
279.64	16.113
306.88	17.686
340.20	19.530
413.08	23.554
449.48	25.838
531.80	30.562
581.08	33.362
637.00	36.522
701.20	40.010

Equation for line by linear regression analysis:  
 $Y = 0.057(X) + 0.109, r^2 = 0.9999$

Table 2. Analysis of JA-2 Powder

Weight of JA-2 Analyzed (mg)	Dilution Factor	Peak Area ( $\times 10^6$ )	Conc. of DEGDN in JA-2 (weight %)*
101.8	100	15.667	26.8
98.6	100	15.119	26.7
102.1	100	15.745	26.9
103.5	100	15.956	26.9

\*Calculated using the standard curve equation as follows:  
 $= \{ [\text{Peak Area}(\times 10^6) - 0.109] / 0.57 \} + \text{wgt JA-2}(\text{mg}) \times 10.$

The average value for the concentration of DEGDN in JA-2 was 26.8% and this agrees closely with the value of  $24.82 \pm 1.50\%$  reported in the data sheet provided by the source.

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

Lot Number: RAD83K001S153

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

Stability: The aqueous stability of the DEGDN component of JA-2 propellant was determined.<sup>4</sup> The amount of DEGDN in JA-2 suspensions was determined immediately after preparation of a suspension and again 24 hours later. The study was conducted as follows: A suspension of JA-2 in 1% gum tragacanth (200 mg/ml) was prepared. Three 1 ml aliquots were removed from the suspension immediately after preparation and again 24 hours later. The 1 ml samples were transferred to individual 100 ml volumetric flasks. After diluting to volume with ethanol, the solutions were analyzed by HPLC as described above.

Table 3. Stability of JA-2 Samples\*

<u>Aliquot</u>	<u>0 Hours</u>	<u>24 Hours</u>
1	$2.79 \times 10^7$	$2.83 \times 10^7$
2	$2.94 \times 10^7$	$2.96 \times 10^7$
3	$3.02 \times 10^7$	$3.05 \times 10^7$
Mean( $\times 10^7$ ) $\pm$ S.D.	$2.91 \pm 0.12$	$2.95 \pm 0.11$

\* Peak area values from the analysis of DEGDN in JA-2 samples

These results indicate that there was no decomposition of DEGDN in 1% gum tragacanth for a period of 24 hours.

Homogeneity<sup>5</sup>: Suspensions (20 ml) of JA-2 powder were prepared in 1% gum tragacanth at concentrations of 100, 200 and 300 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 considered to be homogeneous since no individual value deviates more than 10% from the mean value for each concentration tested.

<sup>4</sup> Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 27, 35, 41. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>5</sup> Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 7-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

## Appendix A (cont.): CHEMICAL DATA

Table 4. Analysis of DEGDN Standards

Concentration of DEGDN ( $\mu\text{g/ml}$ )	Peak Area* ( $\times 10^6$ )
191	9.7
276	14.1
299	15.4
362	18.5
400	20.3
444	22.5
558	27.2
585	32.5
670	37.1
774	43.2
856	47.5
943	52.2

\*Average of standards run before and after samples.

Equation for line by linear regression:

$$Y = 5.8 \times 10^4 X - 2.27 \times 10^6, r^2 = 0.992$$

Table 5. Analysis of JA-2 Suspensions for Homogeneity

Concentration (mg/ml)	Dilution Factor (D.F.)	Peak Area $\times 10^6$	Conc. of JA-2* (mg/ml)
100T	100	16.1	118.1
100M	100	16.7	122.0
100B	100	17.4	126.5
200T	100	34.6	237.1
200M	100	35.9	245.5
200B	100	36.4	248.7
300T	250	17.1	311.4
300M	250	17.7	321.1
300B	250	18.3	330.7

\*Conc. = [(peak area +  $2.27 \times 10^6$ ) /  $5.8 \times 10^4$ ]  $\times$  D.F.  $\times$  3.73/1000  $\mu\text{g/mg}$

## Appendix A (cont.): CHEMICAL DATA

Concentration: Samples of the dosing suspensions were analyzed for accuracy of concentration by HPLC as described above for studies 85015<sup>6</sup> and 85016<sup>7</sup>. The samples were analyzed using the previously determined value of 26.8% as the percentage of DEGDN in JA-2. The results given in Table 6 indicate that all suspensions were within 10% of their target concentration.

Table 6. Concentration of JA-2 in Dosing Suspensions

Study Number	Target Conc. (mg/ml)	Date Prepared	Dilution Factor	Peak Area	Conc. of JA-2 (mg/ml)	% Target Conc.
85015*	118.5	3 Dec 85	100	$1.883 \times 10^7$	122.3	103.2
	158.0	3 Dec 85	100	$2.561 \times 10^7$	168.0	106.3
	211.25	3 Dec 85	100	$3.350 \times 10^7$	221.2	104.7
	137.0	5 Dec 85	100	$2.290 \times 10^7$	149.7	109.3
	244.0	5 Dec 85	250	$1.607 \times 10^7$	259.2	106.2
	281.0	5 Dec 85	250	$1.889 \times 10^7$	306.7	109.1
85016†	223.0	30 Dec 85	250	$1.357 \times 10^7$	219.1	98.3
	250.0	30 Dec 85	250	$1.476 \times 10^7$	238.9	95.6
	141.0	2 Jan 86	125	$1.586 \times 10^7$	128.6	91.2
	177.5	2 Jan 86	125	$2.278 \times 10^7$	186.0	104.8
	199.0	2 Jan 86	125	$2.477 \times 10^7$	202.6	101.8

\* Equation for the standard curve (Study #85015):<sup>6</sup>  
 $Y$  (peak area) =  $5.531 \times 10^4 X$  ( $\mu\text{g/ml}$ ) +  $7.028 \times 10^5$ ,  $R^2 = 0.999$ .

† Equation for the standard curve (Study #85016):<sup>7</sup>  
 $Y$  (peak area) =  $5.617 \times 10^4 X$  ( $\mu\text{g/ml}$ ) +  $3.74 \times 10^5$ ,  $R^2 = 0.999$ .

<sup>6</sup> Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 1-7. Letterman Army Institute of Research, Presidio of San Francisco, CA.

<sup>7</sup> Ibid. p. 51-63.

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

CHEMICAL ANALYSIS FOR . . .  
(Information from the Manufacturer's Data Sheet)

<u>Ingredient</u>	<u>Finished Propellant Percentage</u>
Nitrocellulose (13.8% $\pm$ 0.05% Nitrogen) (6-12 seconds viscosity)	58.5 $\pm$ 2.00
Nitroglycerin	15.88 $\pm$ 1.00
Diethyleneglycol dinitrate (DEGDN)	24.82 $\pm$ 1.50
Akardit II	0.70 $\pm$ 0.20
Magnesium Oxide	0.04 Max
Graphite	<u>0.04 Max</u>
Total	100.00%*

\*Data provided as listed; total actually equals 99.98%.

**Appendix B: ANIMAL DATA**

Species: *Rattus norvegicus*

Strain: Albino, Sprague-Dawley

Source: Bantin & Kingman, Inc.  
Fremont, CA

Sex: Male and female

Date of Birth: Males - 2 October 1985  
Females - 25 September 1985

Method of randomization: Weight bias, stratified animal  
allocation using the TOXSYS®  
Software Package (SOP OP-1SG-24).

Animals in each group: 10 males and 10 females, except for  
5 males and 5 females in the control

Condition of animals at start of study: Normal

Body weight range at dosing: 162-295 g

Identification procedures: Ear tag

Conditioning: Quarantine/acclimation 13 Nov 85 - 2,4 Dec 85

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**

<u>Date</u>	<u>Event</u>
12 Nov 85	Rats were received and checked for physical condition and individually caged.
13 Nov 85	Rats were weighed and ear-tagged. Four rats were submitted for necropsy quality control.
13 Nov-2 Dec 85	Animals were observed daily.
18 Nov 85	Animals were weighed and randomized into dose groups.
19 Nov 85	Twenty-two limit test animals were weighed, dosed, and observed.
21 Nov 85	Ten ALD animals were weighed, dosed, and observed.
26 Nov 85	Animals were weighed.
2 Dec 85	Animals were weighed and removed from quarantine (Phase I animals*).
3 Dec 85	Phase I animals were weighed and dosed at approximately 0900 hours. Observations were conducted approximately 2 and 4 hours after dosing.
4-16 Dec 85	Phase I animals were observed daily for clinical signs in am and pm.
4 Dec 85	Animals were weighed and removed from quarantine (Phase II animalst).
5 Dec 85	Phase II animals were weighed and dosed at approximately 0900 hours. Observations were conducted approximately 2 and 4 hours after dosing.
6-18 Dec 85	Phase II animals were observed daily for clinical signs in am and pm.

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

<u>Date</u>	<u>Event</u>
10 Dec 85	Phase I animals were weighed.
12 Dec 85	Phase II animals were weighed.
17 Dec 85	Phase I animals were weighed, observed, and submitted to necropsy.
19 Dec 85	Phase II animals were weighed, observed, and submitted to necropsy.

\* Phase I = 2370 mg/kg, 3160 mg/kg, 4220 mg/kg, controls

† Phase II = Males 4880 mg/kg, 5620 mg/kg  
Females 2740 mg/kg, 4880 mg/kg

**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
<b>MALES</b>											
2370	9	0	0	0	0	1	1	1	1	1	1
3160	9	0	0	2	2	2	2	2	2	2	2
4220	7	0	0	3	3	3	3	3	3	3	3
4880	6	0	3	6	6	6	6	6	6	6	6
5620	7	0	2	5	5	5	5	5	5	5	5
Vehicle	4	0	0	0	0	0	0	0	0	0	0
TOTAL*	38	0	5	16	16	17	17	17	17	17	17
<b>FEMALES</b>											
2370	8	0	0	0	0	2	3	3	3	3	3
2740	9	0	0	2	3	5	6	6	6	6	6
3160	8	0	0	5	5	5	5	5	5	5	5
4220	7	0	1	5	5	5	5	5	5	5	5
4880	10	0	1	9	9	9	9	9	9	9	9
Vehicle	3	0	0	0	0	0	0	0	0	0	0
TOTAL*	42	0	2	21	22	26	28	28	28	28	28

\* TOTAL reflects only animals receiving JA-2.

## APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 2370 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01003	Misdosed	N/A	N/A
85D01012	Hunched Posture	Dec 3-6	Marked
	Irritable	Dec 3,11,12	Moderate
	Inactive	Dec 3	Moderate
	Cyanosis	Dec 6	Slight
	Tachypnea	Dec 6	Slight
	Stain, Red, Nose	Dec 10-12	Slight
85D01013	Hunched Posture	Dec 3-10	Moderate
	Inactive	Dec 3,5,6	Slight
85D01018	Hunched Posture	Dec 3	Moderate
	Cyanosis	Dec 3	Slight
	Inactive	Dec 5,6,8	Slight
	Rough Coat	Dec 3-8	Moderate
	Tachypnea	Dec 6	Moderate
	Stain, Red, Nose	Dec 11	Slight
85D01023	Hunched Posture	Dec 3	Marked
	Cyanosis	Dec 3-5	Moderate
	Tremors	Dec 3	Moderate
	Inactive	Dec 3	Marked
	Depr. Grasping Reflex	Dec 3	Marked
	Prostrate	Dec 3	N/A
	Depr. Righting Reflex	Dec 3	Moderate
	Ataxia	Dec 3	Marked
	Moribund	Dec 4,5	N/A
	Lacrimation	Dec 4	Marked
	Death	Dec 5	2.2 days
85D01028	Hunched Posture	Dec 3-8	Slight
	Inactive	Dec 3,8	Moderate
	Stain, Red, Nose	Dec 10-14	Slight
	Diarrhea	Dec 13	Slight
85D01037	Hunched Posture	Dec 3-12	Slight
	Inactive	Dec 3,6-12	Slight
	Incr. Respiration Depth	Dec 3	Slight
	Stain, Red, Nose	Dec 11,12	Slight

**APPENDIX E: INDIVIDUAL ANIMAL HISTORIES**

MALE: 2370 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01043	Hunched Posture	Dec 3-9,11,16	Moderate
	Inactive	Dec 3,6-8	Moderate
	Cyanosis	Dec 3	Slight
	Missing Tip of Tail	Dec 12-14	N/A
	Stain, Red, Nose	Dec 13,14	Slight
	Urine	Dec 15	Marked
85D01058	Hunched Posture	Dec 3-7	Moderate
	Inactive	Dec 3,4,7,8,10	Slight
	Cyanosis	Dec 3	Slight
	Ataxia	Dec 3	N/A
	Rough Coat	Dec 7,8	Slight
	Stain, Red, Nose	Dec 10	Slight
	Material, White, Urine	Dec 15	N/A
	Irritable	Dec 14,16	Moderate
85D001067	Hunched Posture	Dec 3,8,9	Moderate
	Inactive	Dec 3,6-11	Marked
	Cyanosis	Dec 3	Moderate
	Rough Coat	Dec 6-8	Slight
	Scab, Tail	Dec 6-16	Slight
	Stain, Red, Nose	Dec 11,13,14	Slight

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3160 mc/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D00999	Misdosed	N/A	N/A
85D01005	Hunched Posture	Dec 3	Moderate
	Cyanosis	Dec 3	Moderate
	Inactive	Dec 3	Slight
	Incr. Respiration Depth	Dec 3	Slight
	Twitching	Dec 3	Slight
	Death	Dec 4	21.6 h
85D01007	Hunched Posture	Dec 3,4	Marked
	Inactive	Dec 3,4	Marked
	Rough Coat	Dec 3,4	Moderate
	Cyanosis	Dec 3,4	Moderate
	Death	Dec 5	21.8 h
85D01011	Hunched Posture	Dec 3	Moderate
	Inactive	Dec 3	Moderate
	Cyanosis	Dec 3	Slight
	Stain, Red, Nose	Dec 3	Slight
	Missing Tip of Tail	Dec 6,11-16	N/A
85D01014	Hunched Posture	Dec 3,6,9	Slight
	Tachypnea	Dec 6-8	Marked
	Irritable	Dec 6-8	Moderate
	Scab, Left Ankle	Dec 12-14	Slight
85D01026	Hunched Posture	Dec 3	Slight
	Tachypnea	Dec 6	Slight
	Inactive	Dec 8	Slight
	Scaling, Purple, Tail	Dec 6-8,11-16	Slight
85D01036	Hunched Posture	Dec 3-9	Moderate
	Tachypnea	Dec 6	Slight
	Stain, Red, Nose	Dec 11-14	Slight

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3160 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01044	Hunched Posture	Dec 3-9	Moderate
	Inactive	Dec 3,5-8	Moderate
	Irritable	Dec 3	Moderate
	Cyanosis	Dec 3	Slight
	Diarrhea	Dec 4	Slight
	Stain, Red, Nose	Dec 12-15	Slight
	Scaling, Tail	Dec 12	Slight
85D01054	Hunched Posture	Dec 3-7	Moderate
	Scaling, Tail	Dec 6-8,10-13	Slight
	Inactive	Dec 7,8	Moderate
	Rough Coat	Dec 8	Slight
	Irritable	Dec 10-16	Slight
	Stain, Red, Nose	Dec 11,12	Slight
85D01070	Hunched Posture	Dec 3-9	Marked
	Inactive	Dec 3,8,9	Marked
	Rough Coat	Dec 3-4,8,10-14	Marked
	Cyanosis	Dec 3	Slight
	Irritable	Dec 3	Slight
	Stain, Red, Nose	Dec 11-14	Slight

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4220 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01002	Hunched Posture	Dec 3	Slight
	Inactive	Dec 3	Slight
	Rough Coat	Dec 12	Slight
85D01019	Inactive	Dec 3	Marked
	Hunched Posture	Dec 3	Marked
	Cyanosis	Dec 3	Moderate
	Rough Coat	Dec 3	Slight
	Death	Dec 3	5.6 h
85D01022	Hunched Posture	Dec 3	Moderate
	Inactive	Dec 3	Marked
	Rough Coat	Dec 3	Moderate
	Tremors	Dec 3	Slight
	Cyanosis	Dec 3	Slight
	Death	Dec 4	21.1 h
85D01033	Prostrate	Dec 3	N/A
	Tremors	Dec 3	Slight
	Cyanosis	Dec 3	Marked
	Incr. Respiration Depth	Dec 3	Moderate
	Death	Dec 3	4.1 h
85D01035	Hunched Posture	Dec 3-9	Moderate
	Inactive	Dec 3, 8, 9	Moderate
	Inc. Respiration Depth	Dec 3	N/A
	Stain, Red, Nose	Dec 3	Slight
	Tremors	Dec 3	Slight
	Rough Coat	Dec 6-8	Slight
85D01046	Hunched Posture	Dec 3-9	Moderate
	Inactive	Dec 3, 5-8	Slight
	Incr. Respiration Depth	Dec 3	N/A
	Tachypnea	Dec 10-13	Slight

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

MALE: 4220 mg/kg JA-2

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Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01050	Inactive	Dec 3,10	Slight
	Hunched Posture	Dec 3,4	Moderate
	Irritable	Dec 3,13	Slight
	Rough Coat	Dec 6	Slight
	Stain, Red, Right Eye	Dec 13	Slight
85D01055	Misdosed	N/A	N/A
85D01061	Misdosed	N/A	N/A
85D01065	Misdosed	N/A	N/A

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## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4880 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01006	Hunched Posture	Dec 5	Moderate
	Inactive	Dec 5	Marked
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Moribund	Dec 5	N/A
	Death	Dec 6	21.8 h
85D01016	Death	Dec 5	3.6 h
85D01024	Hunched Posture	Dec 5	Moderate
	Inactive	Dec 5	Marked
	Cyanosis	Dec 5	Slight
	Death	Dec 6	21.8 h
85D01029	Misdosed	N/A	N/A
85D01030	Death	Dec 5	3.6 h
85D01040	Misdosed	N/A	N/A
85D01045	Misdosed	N/A	N/A
85D01056	Death	Dec 5	3.4 h
85D01060	Misdosed	N/A	N/A
85D01062	Death	Dec 5	4.8 h

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 5620 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01009	Moribund	Dec 5	N/A
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Death	Dec 5	4 h
85D01010	Hunched Posture	Dec 5	Moderate
	Inactive	Dec 5	Marked
	Cyanosis	Dec 5	Moderate
	Incr. Respiration Rate	Dec 5	N/A
	Ataxia	Dec 5	N/A
	Death	Dec 6	20.9 h
85D01017	Hunched Posture	Dec 5,6	Moderate
	Inactive	Dec 5-11	Moderate
	Rough Coat	Dec 5-11	Slight
	Cyanosis	Dec 6	Moderate
85D01041	Hunched Posture	Dec 5	Moderate
	Rough Coat	Dec 5	Slight
	Inactive	Dec 5	Slight
	Cyanosis	Dec 5	Moderate
	Death	Dec 6	20.8 h
85D01047	Hunched Posture	Dec 5-7,9	Moderate
	Rough Coat	Dec 5-8	Slight
	Inactive	Dec 5-9	Slight
	Cyanosis	Dec 6,7	Moderate
	Stain, Yellow, Perianal	Dec 6	Slight
	Stain, Red, Nose	Dec 10	Slight
	Scaling, Tail	Dec 12,13	Slight
85D01051	Inactive	Dec 5	Marked
	Hunched Posture	Dec 5	Marked
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Moribund	Dec 5	N/A
	Death	Dec 6	20.8 h

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

MALE: 5620 mg/kg JA-2 (cont.)

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Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01052	Misdosed	N/A	N/A
85D01053	Misdosed	N/A	N/A
85D01068	Misdosed	N/A	N/A
85D01069	Prostrate	Dec 5	N/A
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Death	Dec 5	3.2 h

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## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01042	Hunched Posture	Dec 3-11	Slight
	Inactive	Dec 3,4,6-11	Moderate
	Irreg. Respiration	Dec 3	N/A
	Irritable	Dec 3	Marked
	Wheezing	Dec 3	Slight
	Stain, Red, Nose	Dec 10-12	Slight
85D01049	Hunched Posture	Dec 3-9	Slight
	Inactive	Dec 6	Slight
	Irreg. Respiration	Dec 3	N/A
	Irritable	Dec 3	Slight
	Tachypnea	Dec 8	Moderate
	Material, White, Urine	Dec 15	N/A
85D01059	Hunched Posture	Dec 3-8	Moderate
	Irritable	Dec 3	Slight
	Inactive	Dec 3,9	Moderate
	Stain, Red, Nose	Dec 11	Slight
85D01063	Misdosed	N/A	N/A
85D01066	Irritable	Dec 3	Moderate
	Hunched Posture	Dec 3-6,8	Slight
	Inactive	Dec 3,4,6-8	Slight
	Stain, Red, Nose	Dec 4,6,10-12	Slight
	Scaling, Tail	Dec 11	Slight
	No Sensation, Tip of Tail	Dec 13,14	N/A

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2370 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01072	Hunched Posture	Dec 3, 4, 6	Moderate
	Inactive	Dec 3-10	Moderate
	Ataxia	Dec 3	N/A
	Cyanosis	Dec 3	Moderate
	Stain, Orange, Perianal	Dec 4, 5	Moderate
	Irritable	Dec 6-8	Moderate
	Stain, Red, Neck	Dec 6, 13	Slight
	Material, White, Urine	Dec 15	N/A
85D01079	Hunched Posture	Dec 3-6	Marked
	Inactive	Dec 3	Marked
	Cyanosis	Dec 3	Marked
	Incr. Respiration Depth	Dec 3	N/A
	Prostrate	Dec 3, 4	N/A
	Tremors	Dec 4	Moderate
	Pallor	Dec 4	Moderate
	Stain, Yellow, Perianal	Dec 4, 5	Slight
	Irritable	Dec 6-8	Slight
	Material, White, Urine	Dec 15	N/A
85D01085	Hunched Posture	Dec 3-6	Marked
	Inactive	Dec 3, 4	Marked
	Cyanosis	Dec 3	Moderate
	Incr. Respiration Depth	Dec 3	N/A
	Tremors	Dec 3	Moderate
	Prostrate	Dec 3	N/A
	Stain, Yellow, Perianal	Dec 4-6	Marked
	Stain, Yellow, Eyes	Dec 6	Slight
	Stain, Yellow, Nose	Dec 6	Slight
	Death	Dec 6	3.1 days
85D01090	Hunched Posture	Dec 3, 4	Moderate
	Inactive	Dec 3	Moderate
	Incr. Respiration Depth	Dec 3	N/A
	Stain, Red, Nose	Dec 10	Slight
	Stain, Red, Neck	Dec 12	Slight

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2370 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01098	Hunched Posture	Dec 3-6	Moderate
	Inactive	Dec 3	Marked
	Irritable	Dec 3,5	Slight
	Cyanosis	Dec 3,6	Moderate
	Incr. Respiration Rate	Dec 3	N/A
	Stain, Yellow, Perianal	Dec 4	Marked
	Tachypnea	Dec 6	Slight
	Material, White, Urine	Dec 15	N/A
85D01102	Hunched Posture	Dec 3,4	Slight
	Inactive	Dec 3	Slight
	Inc. Respiration Depth	Dec 3	N/A
	Scaling, Tail	Dec 10-12	Slight
	Tachypnea	Dec 10,11	Slight
	Irritable	Dec 10,11	Slight
	Stain, Red, Nose	Dec 12	Slight
85D01110	Prostrate	Dec 3,5	N/A
	Cyanosis	Dec 3,5	Marked
	Incr. Respiration Depth	Dec 3	N/A
	Moribund	Dec 3,4	N/A
	Pallor	Dec 4	Moderate
	Lacrimation	Dec 4,5	Moderate
	Death	Dec 5	2.2 days
85D01122	Misdosed	N/A	N/A
85D01129	Misdosed	N/A	N/A
85D01133	Prostrate	Dec 3,5	N/A
	Cyanosis	Dec 3	Marked
	Incr. Respiration Depth	Dec 3	N/A
	Moribund	Dec 3,4	N/A
	Stain, Yellow, Perianal	Dec 4,5	Moderate
	Lacrimation	Dec 5	Slight
	Death	Dec 6	2.9 days

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2740 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01076	Hunched Posture	Dec 5	Marked
	Inactive	Dec 5,6	Moderate
	Cyanosis	Dec 5,6	Slight
	Tachypnea	Dec 6	Moderate
	Material, White, Urine	Dec 15	N/A
85D01083	Hunched Posture	Dec 5	Moderate
	Inactive	Dec 5	Slight
	Cyanosis	Dec 5-8	Slight
	Incr. Respiration Depth	Dec 5	N/A
	Stain, Brown, Perianal	Dec 6-8	Moderate
	Prostrate	Dec 6-8	N/A
	Stain, Orange, Mouth	Dec 6-8	Slight
	Ataxia	Dec 7	Marked
	Stain, Red, Nose	Dec 8	Slight
Death	Dec 9	3.9 days	
85D01086	Hunched Posture	Dec 5	Marked
	Inactive	Dec 5	Marked
	Cyanosis	Dec 5,6	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Prostrate	Dec 5	N/A
	Moribund	Dec 6	N/A
	Lacrimation	Dec 6	Slight
	Stain, Brown, Perianal	Dec 6	Moderate
	Stain, Brown, Nose	Dec 6	Slight
	Stain, Clear, Mouth	Dec 6	Slight
Death	Dec 6	26.6 h	
85D01093	Inactive	Dec 5-7	Marked
	Hunched Posture	Dec 5-7	Marked
	Cyanosis	Dec 5-7	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Ataxia	Dec 6,7	Marked
	Stain, Red, Nose	Dec 6,7	Slight
	Stain, Yellow, Perianal	Dec 7	Slight
	Death	Dec 8	2.9 days

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2740 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01103	Hunched Posture	Dec 5	Marked
	Inactive	Dec 5	Marked
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Death	Dec 6	21.1 h
85D01107	Misdosed	N/A	N/A
85D01112	Hunched Posture	Dec 5-12	Moderate
	Inactive	Dec 5-8	Moderate
	Cyanosis	Dec 5-8	Slight
	Incr. Respiration Depth	Dec 5	N/A
	Ataxia	Dec 5-8	Slight
	Stain, Yellow, Perianal	Dec 6-8	Moderate
	Stain, Orange, Mouth	Dec 6-8	Moderate
	Stain, Red, Nose	Dec 6-8	Slight
Scaling, Tail	Dec 12	Slight	
85D01126	Hunched Posture	Dec 5	Moderate
	Inactive	Dec 5	Slight
	Cyanosis	Dec 5	Slight
	Ataxia	Dec 5	Slight
	Moribund	Dec 6,7	N/A
	Stain, Brown, Perianal	Dec 6,7	Moderate
	Lacrimation	Dec 6,7	Moderate
	Stain, Orange, Mouth	Dec 6,7	Slight
	Stain, Red, Nose	Dec 6,7	Slight
	Death	Dec 8	2.9 days
85D01137	Hunched Posture	Dec 5	Marked
	Inactive	Dec 5	Marked
	Cyanosis	Dec 5,6	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Incr. Respiration Rate	Dec 5	N/A
	Ataxia	Dec 5	Slight
	Urine, Brown, Slight	Dec 5	Slight
	Moribund	Dec 6	N/A
	Stain, Yellow, Perianal	Dec 6	Slight
	Stain, Red, Nose	Dec 6	Slight
	Lacrimation	Dec 6	Marked
	Death	Dec 7	1.9 days

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

FEMALE: 2740 mg/kg JA-2 (cont.)

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Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01139	Hunched Posture	Dec 5-10	Moderate
	Inactive	Dec 5-8	Slight
	Irritable	Dec 5	Slight
	Cyanosis	Dec 5-8	Moderate
	Material, White, Urine	Dec 15	N/A

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## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3160 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01077	Inactive	Dec 3	Marked
	Hunched Posture	Dec 3	Moderate
	Cyanosis	Dec 3	Slight
	Death	Dec 4	21.4 h
85D01080	Prostrate	Dec 3	N/A
	Cyanosis	Dec 3	Marked
	Twitching	Dec 3	Slight
	Incr. Respiration Depth	Dec 3	N/A
	Moribund	Dec 3	N/A
	Decr. Respiration Rate	Dec 3	Moderate
	Death	Dec 4	21.4 h
85D01088	Hunched Posture	Dec 3	Moderate
	Inactive	Dec 3	Marked
	Cyanosis	Dec 3	Moderate
	Twitching	Dec 3	Slight
	Ataxia	Dec 3	Slight
	Death	Dec 4	23.6 h
85D01094	Hunched Posture	Dec 3,4	Moderate
	Inactive	Dec 3	Moderate
	Stain, Red, Neck	Dec 10-12	Slight
	Stain, Red, Nose	Dec 11,12	Slight
85D01105	Prostrate	Dec 3	N/A
	Tremors	Dec 3	Moderate
	Twitching	Dec 3	Slight
	Cyanosis	Dec 3	Moderate
	Moribund	Dec 3	N/A
	Incr. Respiration Rate	Dec 3	Slight
	Death	Dec 4	21.3 h

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3160 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01109	Hunched Posture	Dec 3,4	Moderate
	Inactive	Dec 3	Moderate
	Stain, Red, Nose	Dec 11,12	Slight
	Stain, Red, Right Ear	Dec 12	Slight
85D01114	Hunched Posture	Dec 3	Marked
	Inactive	Dec 3	Marked
	Cyanosis	Dec 3	Moderate
	Incr. Respiration Depth	Dec 3	N/A
	Death	Dec 4	21.2 h
85D01138	Hunched Posture	Dec 3,4	Slight
	Stain, Red, Nose	Dec 11,12	Slight
85D01140	Misdosed	N/A	N/A
85D01143	Misdosed	N/A	N/A

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4220 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01074	Moribund	Dec 3	N/A
	Cyanosis	Dec 3	Marked
	Incr. Respiration Rate	Dec 3	N/A
	Incr. Respiration Depth	Dec 3	N/A
	Twitching	Dec 3	Moderate
	Death	Dec 3	4.0 h
85D01075	Prostrate	Dec 3	N/A
	Hunched Posture	Dec 3	Marked
	Cyanosis	Dec 3	Marked
	Tremors	Dec 3	Moderate
	Twitching	Dec 3	Moderate
	Death	Dec 3	5.5 h
85D01078	Hunched Posture	Dec 3-6	Slight
	Inactive	Dec 3-8	Slight
	Cyanosis	Dec 3	Slight
	Tremors	Dec 3	Slight
	Ataxia	Dec 3	Slight
	Stain, Yellow, Perianal	Dec 4,5	Moderate
	Irritable	Dec 6-8	Moderate
	Stain, Red, Nose	Dec 11	Slight
	Urine, Cloudy White	Dec 15	N/A
85D01087	Moribund	Dec 3	N/A
	Twitching	Dec 3	Marked
	Cyanosis	Dec 3	Moderate
	Incr. Respiration Depth	Dec 3	N/A
	Death	Dec 3	5.4 h
85D01100	Prostrate	Dec 3	N/A
	Twitching	Dec 3	Marked
	Incr. Respiration Depth	Dec 3	N/A
	Cyanosis	Dec 3	Moderate
	Moribund	Dec 3	N/A
	Decr. Respiration Depth	Dec 3	Moderate
	Death	Dec 4	20.9 h

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4220 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01106	Hunched Posture	Dec 3-8	Moderate
	Inactive	Dec 6-8	Slight
	Material, White, Urine	Dec 15	N/A
85D01115	Hunched Posture	Dec 3	Marked
	Inactive	Dec 3	Marked
	Incr. Respiration Depth	Dec 3	N/A
	Decr. Respiration Depth	Dec 3	N/A
	Cyanosis	Dec 3	Marked
	Death	Dec 4	20.9 h
85D01127	Misdosed	N/A	N/A
85D01130	Misdosed	N/A	N/A
85D01142	Misdosed	N/A	N/A

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4880 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01071	Death	Dec 5	3.6 h
85D01084	Hunched Posture	Dec 5	Moderate
	Inactive	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Tremors	Dec 5	Slight
	Moribund	Dec 5	N/A
	Cyanosis	Dec 5	Marked
	Stain, Yellow, Perianal	Dec 5	Slight
	Death	Dec 6	21.4 h
85D01089	Prostrate	Dec 5	N/A
	Twitching	Dec 5	Slight
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Moribund	Dec 5	N/A
	Stain, Brown, Perianal	Dec 5	Slight
	Death	Dec 6	21.4 h
85D01092	Hunched Posture	Dec 5-8	Moderate
	Inactive	Dec 5-8	Slight
	Cyanosis	Dec 5-7	Slight
	Stain, Yellow, Perianal	Dec 6,7	Slight
	Material, Cream, Urine	Dec 5	Slight
85D01095	Moribund	Dec 5	N/A
	Twitching	Dec 5	Marked
	Tremors	Dec 5	Moderate
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Death	Dec 6	21.3 h
85D01097	Prostrate	Dec 5	N/A
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Death	Dec 5	4.4 h

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4880 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01104	Hunched Posture	Dec 5	Marked
	Tremors	Dec 5	Marked
	Inactive	Dec 5	Marked
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	N/A
	Moribund	Dec 5	N/A
	Lacrimation	Dec 5	Moderate
	Death	Dec 6	21.2 h
85D01108	Prostrate	Dec 5	N/A
	Moribund	Dec 5	N/A
	Cyanosis	Dec 5	Marked
	Incr. Respiration Depth	Dec 5	Slight
	Lacrimation	Dec 5	Slight
	Death	Dec 6	21.2 h
	85D01117	Hunched Posture	Dec 5
Inactive		Dec 5	Marked
Incr. Respiration Depth		Dec 5	N/A
Lacrimation		Dec 5	Slight
Cyanosis		Dec 5	Moderate
Moribund		Dec 5	N/A
Tachypnea		Dec 5	Slight
Death		Dec 6	21.2 h
85D01141	Moribund	Dec 5	N/A
	Cyanosis	Dec 5	Moderate
	Incr. Respiration Depth	Dec 5	N/A
	Death	Dec 5	4.4 h

## APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

## FEMALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1985)	Severity
85D01118	Hunched Posture	Dec 3,4	Moderate
	Ataxia	Dec 3	N/A
	Stain, Red, Nose	Dec 4,10,11	Slight
	Stain, Red, Neck	Dec 6,7,10,11	Moderate
	Scaling, Tail	Dec 11,12	Slight
85D01124	Misdosed	N/A	N/A
85D01132	Hunched Posture	Dec 3,4	Moderate
	Inactive	Dec 3	Slight
	Stain, Red, Nose	Dec 10,11	Slight
	Tachypnea	Dec 11,12	Slight
85D01134	Misdosed	N/A	N/A
85D01136	Hunched Posture	Dec 3-8	Slight
	Stain, Red, Nose	Dec 10-12	Slight
	Stain, Red, Neck	Dec 6-12	Slight
	Material, White, Urine	Dec 15	N/A

**Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS**

Males: 2370 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01012	150	264	293	305
85D01013	151	274	320	323
85D01018	151	274	304	308
85D01023	151	213	Dead	
85D01028	159	256	282	281
85D01037	158	276	309	318
85D01043	147	262	296	304
85D01058	144	284	297	313
85D01067	147	262	303	302
Mean	150.9	262.8	300.5	306.8
Standard Deviation	4.9	20.6	11.4	12.7
Std. Error of Means	1.6	6.9	4.0	4.5

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

Males: 3160 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01005	146	226	Dead	
85D01007	153	295	Dead	
85D01011	146	261	299	305
85D01014	154	267	310	315
85D01026	157	291	326	320
85D01036	148	273	324	355
85D01044	157	273	329	344
85D01054	152	292	338	360
85D01070	149	257	298	301
Mean	151.3	270.6	317.7	328.6
Standard Deviation	4.3	21.7	15.5	24.1
Std. Error of Means	1.4	7.2	5.9	9.1

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

Males: 4220 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01002	147	283	290	292
85D01019	146	279	Dead	
85D01022	147	266	Dead	
85D01033	152	273	Dead	
85D01035	140	288	330	342
85D01046	143	255	288	300
85D01050	158	292	339	358
Mean	147.6	276.6	311.8	323.0
Standard Deviation	5.9	13.0	26.5	32.0
Std. Error of Means	2.2	4.9	13.3	16.0

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

Males: 4880 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01006	146	255	Dead	
85D01016	155	267	Dead	
85D01024	149	250	Dead	
85D01030	161	277	Dead	
85D01056	154	254	Dead	
85D01062	153	282	Dead	
Mean	153.0	264.2		
Standard Deviation	5.2	13.3		
Std. Error of Means	2.1	5.4		

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

Males: 5620 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01009	144	266	Dead	
85D01010	150	276	Dead	
85D01017	147	255	270	276
85D01041	145	261	Dead	
85D01047	152	270	315	318
85D01051	124	245	Dead	
85D01069	149	256	Dead	
Mean	144.4	261.3	292.5	297.0
Standard Deviation	9.4	10.4	31.8	29.7
Std. Error of Means	3.6	3.9	22.5	21.0

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

Females: 2370 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01072	151	199	206	208
85D01079	152	208	232	222
85D01085	148	193	Dead	
85D01090	145	190	209	210
85D01098	145	192	221	214
85D01102	133	209	242	240
85D01110	155	183	Dead	
85D01133	150	186	Dead	
Mean	147.4	195.0	222.0	218.8
Standard Deviation	6.7	9.6	15.2	13.0
Std. Error of Means	2.4	3.4	6.8	5.8

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

Females: 2740 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01076	152	211	238	234
85D01083	143	202	Dead	
85D01086	151	201	Dead	
85D01093	143	180	Dead	
85D01103	149	181	Dead	
85D01112	148	209	236	242
85D01126	145	177	Dead	
85D01137	145	210	Dead	
85D01139	143	215	249	247
Means	146.6	198.4	241.0	241.0
Standard Deviation	3.5	15.0	7.0	6.6
Std. Error of Means	1.2	5.0	4.0	3.8

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

Females: 3160 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01077	147	175	Dead	
85D01080	153	208	Dead	
85D01088	147	181	Dead	
85D01094	148	201	230	220
85D01105	142	188	Dead	
85D01109	152	197	226	225
85D01114	149	194	Dead	
85D01138	144	200	234	234
Mean	147.8	193.0	230.0	226.3
Standard Deviation	3.7	11.0	4.0	7.1
Std. Error of Means	1.3	3.9	2.3	4.1

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

Females: 4220 mg/kg JA-2

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Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01074	153	189	Dead	
85D01075	151	177	Dead	
85D01078	150	206	235	236
85D01087	138	184	Dead	
85D01100	144	188	Dead	
85D01106	144	184	207	203
85D01115	138	199	Dead	
Mean	145.4	189.6	221.0	219.5
Standard Deviation	6.1	9.8	19.8	23.3
Std. Error of Means	2.3	3.7	14.0	16.5

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

Females: 4880 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85D01071	146	187	Dead	
85D01084	148	194	Dead	
85D01089	148	198	Dead	
85D01092	145	212	240	228
85D01095	130	186	Dead	
85D01097	144	184	Dead	
85D01104	151	174	Dead	
85D01108	142	162	Dead	
85D01117	155	198	Dead	
85D01141	145	190	Dead	
Mean	145.4	188.5		
Standard Deviation	6.6	13.8		
Std. Error of Means	2.1	4.4		

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

## Vehicle Control

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
<b>MALES</b>				
85D01042	158	268	322	339
85D01049	159	228	302	316
85D01059	154	284	350	339
85D01066	152	270	312	315
Mean	155.8	262.5	321.5	327.3
Standard Deviation	3.3	24.1	20.7	13.6
Std. Error of Mean	1.6	12.0	10.3	6.8
<b>FEMALES</b>				
85D01118	151	195	221	216
85D01132	141	191	223	216
85D01136	148	190	224	217
Mean	146.7	192.0	222.7	216.3
Standard Deviation	5.1	2.6	1.5	5.8
Std. Error of Means	3.0	1.5	0.9	3.3

## Appendix G: PATHOLOGY REPORT

LD<sub>50</sub> Oral Toxicity  
GLP Study 85015

Study: GLP #85015, Toxicology Services Group

Test substance/vehicle: JA2/ Gum tragacanth

Species: Rattus norvegicus (Both sexes, 6 weeks old).

Method of euthanasia: Sodium Pentobarbital (IP).

Reference: LAIR SOP-OP-STX-36.

Investigator: MAJ Larry Brown.

## Gross Necropsy Findings:

GROUP 1 MALE  
4880 mg/kg - All animals found dead

LAIR ACC#	ANIMAL ID#	DOSE TO DEATH INTERVAL	GROSS FINDINGS
38653	85D01006	< 1 day	Not remarkable (NR)
38643	85D01016	3.5 hrs	Lungs & liver - Minimal brown discoloration
38655	85D01024	< 1 day	NR
38645	85D01030	3.5 hrs	Lungs & liver - Minimal brown discoloration
38646	85D01056	3.5 hrs	Lungs & liver - Minimal brown discoloration
38647	85D01062	5 hrs	Lungs & liver - Minimal brown discoloration

GROUP 2 MALE  
2370 mg/kg - Live animals indicated by asterick (\*)

38691*	85D01012	14 days	NR
38692*	85D01013	14 days	NR
38694*	85D01018	14 days	NR
38644	85D01023	2 days	Stomach - multiple petechial hemorrhages in mucosa. Small intestine - anterior half filled with dark hemorrhagic contents.
38696*	85D01028	14 days	NR
38699*	85D01037	14 days	NR
38701*	85D01043	14 days	NR
38707*	85D01058	14 days	NR
38710*	85D01067	14 days	NR

## Appendix G (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 85015

## GROUP 3 MALE

3100 mg/kg - Live animals indicated by asterick (\*)

LABR ACC#	ANIMAL ID#	DOSE TO DEATH INTERVAL	GROSS FINDINGS
38620	85D01005	< 1 day	Lungs - diffuse light brown discoloration
38641	85D01007	< 1 day	Not remarkable (NR)
38690*	85D01011	14 days	NR
38672*	85D01014	14 days	NR
38695*	85D01026	14 days	NR
38628*	85D01026	14 days	NR
38702*	85D01044	14 days	NR
38708*	85D01054	14 days	NR
38711*	85D01070	14 days	NR

## GROUP 4 MALE

4220 mg/kg - Live animals indicated by asterick (\*)

38670*	85D01002	14 days	NR
38670	85D01019	5.5 hrs	Right kidney - mild hydronephrosis
38671	85D01022	< 1 day	Left eye - severe keratitis
38627	85D01033	4 hrs	Lungs - diffuse light brown discoloration. Blood - light brown discoloration
38697*	85D01035	14 days	NR
38703*	85D01046	14 days	NR
38705*	85D01050	14 days	NR

## GROUP 5 MALE

5620 mg/kg - Live animals indicated by asterick (\*)

38642	85D01009	3.5 hrs	Lungs & liver - diffuse pale brown discoloration
38654	85D01010	< 1 day	Right kidney - mild hydronephrosis
38752*	85D01017	14 days	NR
38656	85D01041	< 1 day	NR
38752*	85D01047	14 days	NR
38657	85D01051	< 1 day	NR
38649	85D01069	3.5 hrs	Lungs & liver - pale brown discoloration

## Appendix G (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 85015

GROUP 6 MALE  
Vehicle control - All live animals

LAIR ACC#	ANIMAL ID#	DOSE TO DEATH INTERVAL	GROSS FINDINGS
38700	85D01042	14 days	Not remarkable (NR)
38704	85D01049	14 days	NR
38708	85D01059	14 days	NR
38709	85D01066	14 days	NR

GROUP 1 FEMALE  
2740 mg/kg - Live animals indicated by asterick (\*)

38754*	85D01076	14 days	NR
38671	85D01083	4 days	NR
38667	85D01086	26.5 hrs	Liver & lungs - mild brown discoloration
38669	85D01093	3 days	NR
38661	85D01103	< 1 day	Liver & lungs - mild brown discoloration
38756*	85D01112	14 days	NR
38670	85D01126	3 days	NR
38669	85D01137	2 days	Stomach - Multiple petechial hemorrhages on glandular mucosa.
38757	85D01139	14 days	NR

GROUP 2 FEMALE  
2370 mg/kg - Live animals indicated by asterick (\*)

38712*	85D01072	14 days	NR
38714*	85D01079	14 days	NR
38666	85D01085	3 days	Liver - moderate pale discoloration
38715*	85D01090	14 days	NR
38717*	85D01098	14 days	NR
38718*	85D01102	14 days	NR
38651	85D01110	2 days	Liver - diffuse pale tan discoloration, enlarged. Lung - Pale brown discoloration Small intestine - empty, dilated with gas
38665	85D01133	3 days	Liver - Pale yellow/red discoloration. Stomach - multiple petechiae in glandular mucosa. Small intestine - completely filled with red-black mucoid material.

## Appendix G (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 85015GROUP 3 FEMALE  
3160 mg/kg - Live animals indicated by asterick (\*)

LAIR ACC#	ANIMAL ID#	DOSE TO DEATH INTERVAL	GROSS FINDINGS
38633	85D01077	< 1 day	Not remarkable (NR)
38634	85D01080	< 1 day	NR
38640	85D01088	< 1 day	NR
38716*	85D01094	14 days	NR
38637	85D01105	< 1 day	NR
38720*	85D01109	14 days	NR
38638	85D01114	< 1 day	NR
38724	85D01138	14 days	NR

GROUP 4 FEMALE  
4220 mg/kg - Live animals indicated by asterick (\*)

38628	85D01074	4 hrs	Blood & lungs - pale brown discoloration
38632	85D01075	5.5 hrs	NR
38713*	85D01078	14 days	NR
38635	85D01087	5.5 hrs	NR
38636	85D01100	< 1 day	NR
38719*	85D01106	14 days	NR
38639	85D01115	< 1 day	NR

GROUP 5 FEMALE  
4880 mg/kg - Live animals indicated by asterick (\*)

38649	85D01071	3.5 hrs	Liver & lungs - diffuse pale brown discoloration
38658	85D01084	< 1 day	NR
38659	85D01089	< 1 day	NR
38755*	85D01092	14 days	NR
38650	85D01097	4.5 hrs	Liver & lungs - mild brown discoloration
38660	85D01095	< 1 day	NR
38662	85D01104	< 1 day	NR
38663	85D01108	< 1 day	Left eye - two corneal opacities
38664	85D01117	< 1 day	NR
38652	85D01141	4.5 hrs	Reddish nasal discharge Liver - dark red and swollen Blood - brownish discoloration within all tissues.

## Appendix G (cont.): PATHOLOGY REPORT

Pathology Report  
GLP Study 85015

GROUP 6 FEMALE  
Vehicle control - All Live Animals

<u>LAIR ACC#</u>	<u>ANIMAL ID#</u>	<u>DOSE TO DEATH INTERVAL</u>	<u>GROSS FINDINGS</u>
38721	85D01118	14 days	Not remarkable (NR)
38722	85D01132	14 days	NR
38723	85D01136	14 days	NR

## Gross Summary:

<u>GROUP</u>	<u>SEX</u>	<u>DOSE (mg/kg)</u>	<u>TOTAL #DEAD</u>	<u>TOTAL MORTALITY(%)</u>
1	M	4880	6/6	100
2	M	2370	1/9	11.11
3	M	3160	2/9	22.22
4	M	4220	3/7	42.86
5	M	5620	5/7	71.42
6	M	Vehicle	0/4	0
1	F	2740	6/9	66.67
2	F	2370	3/8	37.5
3	F	3160	5/8	62.5
4	F	4220	5/7	71.43
5	F	4880	9/10	90
6	F	Vehicle	0/3	0

Gross Comments: Most animals which exhibited brownish discoloration of blood and organs (primarily liver and lung) died in less than 1 day (except for #85D01085 - 3 days, #85D01086 - 26.5 hrs, #85D01110 - 2 days). Animals with segmental hemorrhage of the gastrointestinal tract died beyond 1 day post-dosing. Animals found dead with incidental lesions were: #85D01010 (group 5 male), #85D01019, #85D01022 (group 4 males), and #85D01108 (group 5 female). Deaths appear to be roughly dose related.

## Microscopic Finding:

85D01033: Lungs - Multifocally a few peribronchiolar lymphoid nodules contain small numbers of cells which have migrated into the submucosa and mucosa of the airway.

Appendix G (cont.): PATHOLOGY REPORT

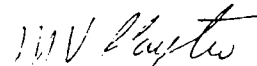
Pathology Report  
GLP Study 85015

Dx: Bronchiolitis, lymphocytic, multifocal, minimal, lung.

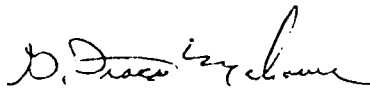
85001074: Lungs - Morphologically, these lungs are the same as 85001033.

Dx: Bronchiolitis, lymphocytic, multifocal, minimal, lung.

Comments: The lung lesions are common in rats, most likely mycoplasmal in origin and unrelated to the test compound.



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