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Acute Oral Toxicity of JA-2 Solid Propellant in ICR Mice

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Acute Oral Toxicity of JA-2 Solid Propellant in ICR Mice (Toxicology Series 177)--
Morgan *et al.*

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William C. Cole 29 December '89

for Donald G. Corby (date)
COL, MC
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The acute oral toxicity of JA-2 Solid Propellant was determined in male and female ICR mice by using an oral gavage, split-dose method. The MLD was 3774.6 ± 150.5 mg/kg for male mice and 3528.8 ± 133.8 mg/kg for female mice. JA-2 produced clinical signs that were attributed to its nitrate ester component, diethyleneglycol dinitrate and nitroglycerin. These signs included tremors, inactivity, depression of reflexes, loss of equilibrium, opisthotonus, and increased respiratory activity. Other clinical signs observed were associated with the general malaise of the animals following dosing and included perianal staining, hunched posture, squinting, and rough coat. Most animals exhibited signs by 2 hours after dosing and either had died or the signs had cleared within 5 days of dosing. According to the classification scheme of Hodge and Sterner, these results place JA-2 in the slightly toxic class.

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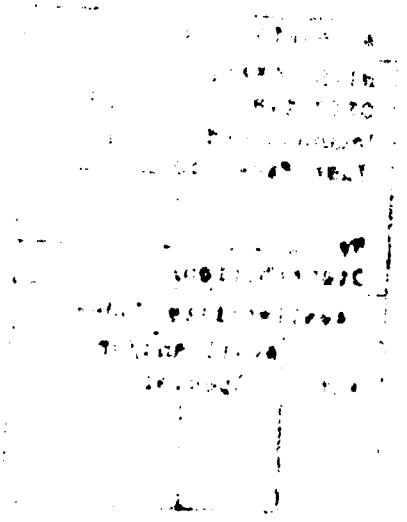
ABSTRACT

The acute oral toxicity of JA-2 Solid Propellant was determined in male and female ICR mice by using an oral gavage, split-dose method. The MLD was 3774.6 ± 150.5 mg/kg for male mice and 3528.8 ± 133.8 mg/kg for female mice. JA-2 produced clinical signs that were attributed to its nitrate ester component, diethyleneglycol dinitrate and nitroglycerin. These signs included tremors, inactivity, depression of reflexes, loss of equilibrium, opisthotonus, and increased respiratory activity. Other clinical signs observed were associated with the general malaise of the animals following dosing and included perianal staining, hunched posture, squinting, and rough coat. Most animals exhibited signs by 2 hours after dosing and either had died or the signs had cleared within 5 days of 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, Mice



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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US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

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

GLP STUDY NUMBER: 85016

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PATHOLOGIST: MAJ Michael V. Slayter, DVM, VC

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: JA-2 Solid Propellant

INCLUSIVE STUDY DATES: 17 Dec 85 - 17 Jan 86

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

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SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85016 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 14 Dec 89
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REPLY TO
ATTENTION OF

SGRD-ULZ-QA

29 December 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85016

1. This is to certify that the protocol for LAIR GLP Study 85016 was reviewed on 10 April 1985.

2. The institute report entitled "Acute Oral Toxicity of JA-2 Solid Propellant in ICR Mice," Toxicology Series 177, was audited on 29 December 1989.

Carolyn M. Lewis

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

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Acute Oral Toxicity of JA-2 Solid Propellant in ICR Mice--Morgan *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, DIGL-RP and JA-2. 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 ICR mice.

MATERIALS

Test Substance

Chemical Name: JA-2 Solid Propellant

LAIR Code No.: TP56

Description: Solid black cylinders (stick configuration)

Lot Number: RAD83K001S153

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 No. 34F0156, Sigma Chemical Company, St. Louis, MO) in sterile water for injection (Lot 65-914-DM-03, Abbott Laboratories, North Chicago, IL). The expiration date was Mar 1995 for the gum tragacanth and Jun 1986 for the sterile water for injection.

Animal Data

Eighty-one male and 81 female ICR mice were obtained from Charles River Laboratories, Inc. (Kingston, NY) for this study. They were identified individually with cervical tags. Twenty-three animals were used as approximate lethal dose (ALD) animals and two males and two females were submitted as necropsy quality controls. One hundred and ten animals were used in the study. Twenty-five animals were not used in this study. The animal weights on receipt ranged from 22 to 33 g. Additional animal data appear in Appendix B.

Husbandry

Mice 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 22.2°C to 26.4°C and the relative humidity was maintained in a range of 38% to 61% with spikes to 76% during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Male and female mice were randomized separately into five dose groups and a vehicle control group with a stratified, weight-biased computer program (Beckman TOXSYS® Animal Allocation Program run on a Beckman TOXSYS® Data Collection Terminal). The animals were quarantined/acclimated for 13-17 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 greater than 2000 mg/kg and less than 5000 mg/kg. Based on these data, test doses were selected (Table 1).

TABLE 1: JA-2 Solid Propellant Doses

Group	Dose Levels (mg/kg)
1	2820
2	3550
5	3970
3	4470
4	5010
6 (control)	Vehicle (10 ml/kg)

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

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. 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 3.5% for any suspension. The JA-2 dosing suspensions used in this study were within 91.2 - 104.8% of target.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP OP-STX-36 (3). 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, all animals, except the controls which only received a single dose, 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. The vehicle control (1% gum tragacanth) group received 0.25 to 0.36 ml. Split dose volumes ranged from 0.30 to 0.40 ml in the males and 0.25 to 0.32 ml in the females. The total volume administered each test 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 (Becton, Dickenson & Co., Rutherford, NJ) fitted with 18-gauge, 3-inch, ball-tipped feeding tubes (Popper & Sons, Inc., New Hyde Park, NY) were used. Dosing took place on three different days 4 hours after food had been removed from the animals' cages. Dosing began no earlier than 1003 hours and was concluded in all cases by 1316 hours (Appendix C).

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages, (b) animals were removed from their cages and given a physical examination, and (c) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Recorded observations were performed approximately 1, 2, and 4 hours after the second 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 LD_{10}, LD_{50}, and LD_{90} 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 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 JA-2 suspensions were administered as a split dose one hour apart because their high viscosity made concentrations greater than 200 mg/ml impossible to administer via the feeding tubes. Dosing was performed in 3 phases instead of two in an attempt to describe more accurately the dose-response curve. Mid-study weighings took place on Days 7, 8, or 9 depending on the dose group. 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

Fifty-two of 86 animals (21/40 males, 31/46 females) dosed with JA-2 died as a result of its toxicity. Two (3.8%) deaths occurred within 24 hrs of dosing. An additional 44 (84.6%) deaths occurred by 48 hrs after dosing, and the remaining 6 (11.5%) deaths occurred within 5 days of 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.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis and the equations for the probit regression line were: $Y = -45.8 + 14.2 \log X$ (males); $Y = -53.7 + 16.5 \log X$ (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 2: Compound-Related Deaths by Group

Dose Level (mg/kg)	Deaths/ Group	Percent Mortality
	Males	
2280	1/8*	12.5
3550	2/9*	22.2
3970	5/10	50.0
4470	6/6*	100.0
5010	7/7*	100.0
Vehicle	0/4*	0.0
	Females	
2820	0/9*	0.0
3550	6/9*	66.7
3970	8/10	80.0
4470	9/10	90.0
5010	8/8*	100.0
Vehicle	0/5	0.0

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

Clinical Observations

Frequently observed categories of clinical signs in animals administered JA-2 were behavioral disturbances (82 of 86 animals dosed), hunched posture (47 of 86), ocular signs (45 of 86), miscellaneous signs (29 of 88), changes in reflex activity (28 of 88), rough coat (20 of 86), opisthotonus (14 of 86), and respiratory changes (10 of 86). Behavioral signs included tremors, inactivity, loss of equilibrium, ataxia, and jumping. Ocular signs included squinting. Miscellaneous

Figure 1

JA-2 Dose Response Curve for Male Mice

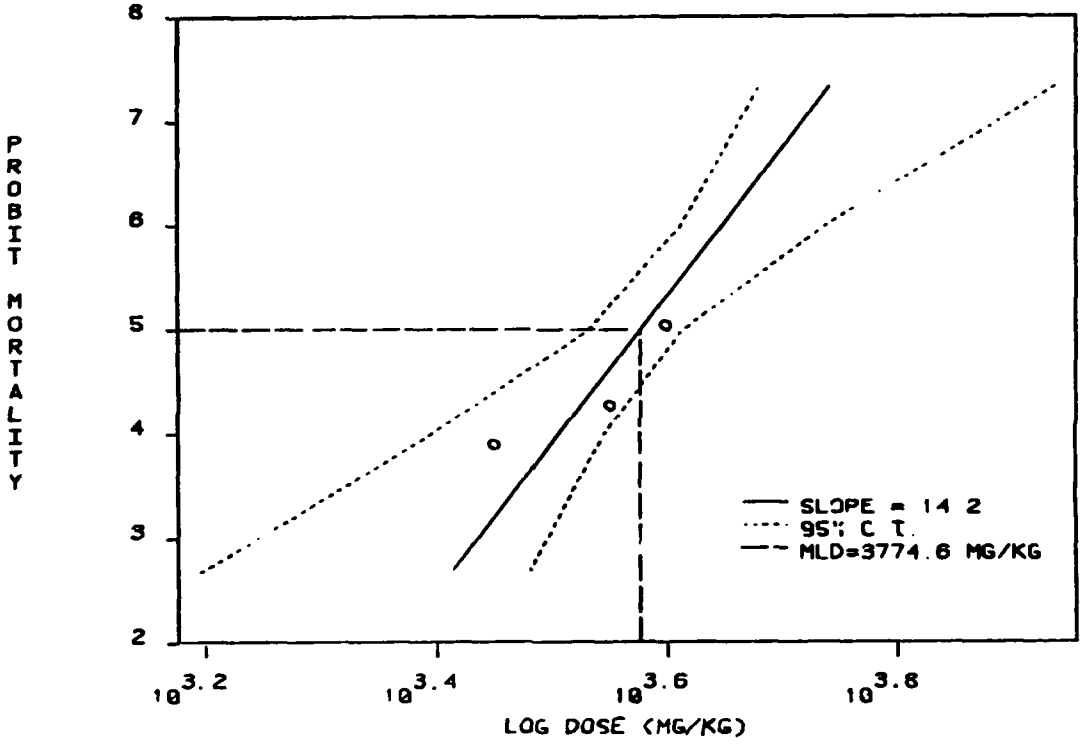

Figure 2

JA-2 Dose Response Curve for Female Mice

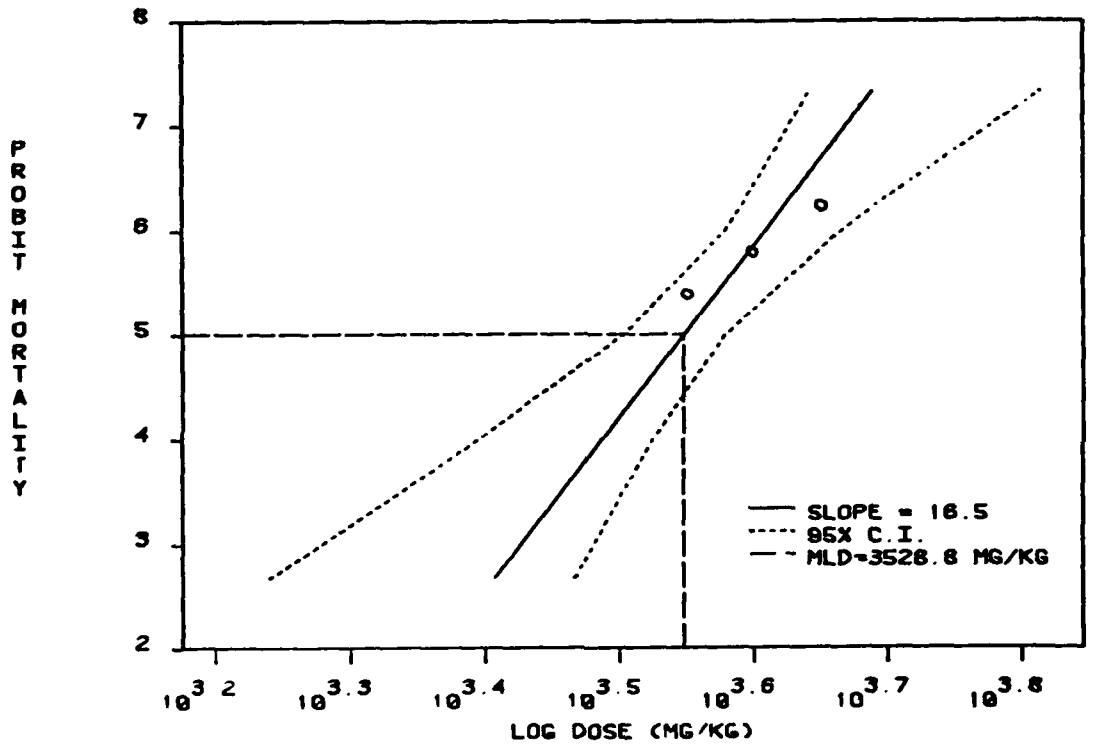

TABLE 3: Calculated Lethal Doses (LD) of JA-2 Solid Propellant in ICR Mice

Level	Calculated Dose* (mg/kg)	95% Confidence Limits (mg/kg)
	Males	
LD_{10}	3066.3 ± 232.0	(2262.9, 3401.8)
LD_{50}	3774.6 ± 150.5	(3402.6, 4107.3)
LD_{90}	4646.6 ± 303.6	(4235.5, 5990.6)
	Females	
LD_{10}	2952.5 ± 201.5	(2305.2, 3250.6)
LD_{50}	3528.8 ± 133.8	(3180.3, 3787.5)
LD_{90}	4217.6 ± 203.9	(3913.4, 4947.7)

* Calculated dose ± standard error.

signs included fecal and urine stains of the abdomen and perineum and a brown stain on the face of one mouse. Changes in reflex activity include depressed grasping and righting reflexes. Respiratory changes included increases in rate and depth. Although clinical signs were observed at each dose level, there was no clear dose-response relationship for severity or duration of the symptoms. All vehicle control animals survived until study termination at 14 days. The only clinical signs observed in the control animals were rough coat and perianal staining.

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

Gross and microscopic changes were noted only in animals that died during the study. Hepatic swelling and pulmonary congestion were the most frequently observed findings. The veterinary pathologist's report appears in Appendix G.

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

MALES	Dose	Vehicle	2820	3550	3970	4470	5010
	(n=)	4	8	9	10	6	7
Respiratory[a]		0	0	0	1	3	5
Behavorial[b]		0	7	8	9	6	7
Convulsions[c]		0	0	0	0	2	2
Gastrointestinal[d]		0	0	0	0	0	1
Rough Coat		1	4	5	0	3	1
Ocular[e]		0	4	6	0	4	7
Hunched posture		0	5	6	6	3	3
Reflex[f]		0	0	2	1	4	5
Prostrate/Moribund		0	0	0	2	1	2
Miscellaneous[g]		2	1	5	6	3	5
Normal throughout		2	0	0	1	0	0
Deaths		0	1	2	5	6	7
FEMALES	Dose	Vehicle	2820	3550	3970	4470	5010
	(n=)	5	9	9	10	10	8
Respiratory[a]		0	0	0	0	1	0
Behavorial[b]		0	9	9	9	10	8
Convulsions[c]		0	0	0	0	5	5
Rough Coat		0	0	2	3	1	1
Ocular[e]		0	6	8	0	5	5
Hunched posture		0	6	6	9	2	1
Reflex[f]		0	0	3	1	5	7
Prostrate/Moribund		0	0	0	2	2	0
Miscellaneous[g]		0	0	1	4	1	3
Normal throughout		5	0	0	0	0	0
Deaths		0	0	6	8	9	8

[a] Includes increases in rate or depth.

[b] Includes tremors, inactivity, ataxia, jumping, and loss of equilibrium.

[c] Includes opisthotonus.

[d] Includes increased salivation.

[e] Includes squinting.

[f] Includes depressed grasping and righting reflexes.

[g] Includes urine and fecal stains on abdomen or perineum and brown stains on the face.

TABLE 5: Mean Body Weights in Grams ± S.E (N)

Dose Groups (mg/kg)	At Receipt	Dosing Day	Midtrial Day	Termination Day
		MALES		
2820	29.1 ±0.8 (8)	34.4 ±0.6 (8)	34.7 ±0.4 (7)	34.7 ±0.4 (7)
3550	30.6 ±0.4 (9)	33.7 ±0.8 (9)	35.4 ±1.0 (7)	35.8 ±1.1 (7)
3970	29.8 ±0.5 (10)	33.8 ±0.8 (10)	35.4 ±1.5 (5)	36.0 ±1.8 (5)
4470	29.3 ±0.7 (6)	33.0 ±0.9 (6)	N/A	N/A
5010	29.7 ±0.7 (7)	32.9 ±0.7 (7)	N/A	N/A
Vehicle	30.0 ±1.1 (4)	33.8 ±1.4 (4)	36.0 ±1.8 (4)	36.5 ±1.6 (4)
		FEMALES		
2820	24.6 ±0.3 (9)	28.3 ±0.6 (9)	30.0 ±0.5 (9)	29.2 ±0.5 (9)
3550	26.0 ±0.4 (9)	28.3 ±0.5 (9)	29.3 ±0.9 (3)	28.0 ±0.6 (3)
3970	25.4 ±0.4 (10)	27.6 ±0.3 (10)	29.5 ±0.5 (2)	31.0 ±1.0 (2)
4470	25.5 ±0.4 (10)	27.1 ±0.3 (10)	29.0 (1)	31.0 (1)
5010	25.1 ±0.5 (8)	26.5 ±0.4 (8)	N/A	N/A
Vehicle	24.6 ±0.9 (5)	26.4 ±0.4 (5)	29.4 ±0.5 (5)	29.2 ±0.5 (5)

DISCUSSION

The calculated median lethal dose (MLD) for JA-2 Solid Propellant was 3774.6 mg/kg in male mice and 3528.8 mg/kg in female mice. These values place JA-2 within the slightly toxic classification (5).

JA-2 has as its major constituents, nitrocellulose and diethyleneglycol dinitrate (DEGDN) (Appendix A). Nitrocellulose is relatively nontoxic (MLD >5000 mg/kg) to mice (6) while a MLD of 1321-1395 mg/kg for DEGDN in mice has been reported (7). The oral MLD for nitroglycerin in mice is 500 mg/kg (8). The spectrum of clinical signs (behavioral, reflex, and convulsions) observed following JA-2 administration supports the assumption that the nitrate esters, nitroglycerin and DEGDN (9), are the toxic components of JA-2.

The relative contribution of nitroglycerin and DEGDN to the MLD of JA-2 can be determined using their percentage compositions by weight (JA-2 is 15.9% nitroglycerin and 24.8% DEGDN). The calculated quantity of nitroglycerin or DEGDN contributing to the oral MLD of JA-2 is 600 mg/kg and 936 mg/kg, respectively. These data suggest there is no additive relationship between the toxicity of DEGDN and nitroglycerin in the JA-2 formulation. 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 female mice contains approximately 2208 mg/kg nitrocellulose, which is approximately 40% of the MLD for nitrocellulose.

CONCLUSION

JA-2 Solid Propellant is a slightly toxic compound that produces behavioral changes, convlusions, and changes in reflex activity. Calculated MLD values were 3774.6 ± 150.5 mg/kg in male and 3528.8 ± 133.8 mg/kg in ICR mice.

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7. Ryabik JRG, Brown LD, Wheeler CR, Korte DW, Jr. Acute oral toxicity of diethyleneglycol dinitrate (DEGDN) in mice. Toxicology Series 137. Presidio of San Francisco, CA: Letterman Army Institute of Research. Institute Report No. 336, May 1989.

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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[1]

Chemical Analysis:

DEGDN was the only major component of JA-2 that could be easily analyzed.[2,3] 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 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. 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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[1] Wheeler CR. Toxicity testing of propellants. Laboratory Notebook #85-12-023, p. 43. 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.

[3] 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 (µg/ml)	Peak Area* ($x10^6$)
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 ($x\ 10^6$)	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:
$=\{[\text{Peak Area}(\div 10^6) - 0.109]/0.57\} \times \text{wgt JA-2(mg)} \times 10$.

The average value for the concentration of DEGDN in JA-2 was 26.8% and this agrees closely with the value of 24.82 ± 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: RAD83KO01S153

Appendix A (cont.): CHEMICAL DATA

Stability: The aqueous stability of the DEGDN component of JA-2 propellant was determined.[4] 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*

Aliquot	0 Hours	24 Hours
1	2.79×10^7	2.83×10^7
2	2.94×10^7	2.96×10^7
3	3.02×10^7	3.05×10^7
Mean ($\times 10^7$) $\pm$ S.D.	2.91 ± 0.12	2.95 ± 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[5]: 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.

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[4] 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.

[5] 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 (µg/ml)	Peak Area* (x 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 x 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 + 2.27×10^6)/5.8×10^4] x D.F. x 3.73/1000 µg/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[6] and 85016[7]. 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	1.883×10^7	122.3	103.2
	158.0	3 Dec 85	100	2.561×10^7	168.0	106.3
	211.0	3 Dec 85	100	3.350×10^7	221.2	104.8
	137.0	5 Dec 85	100	2.290×10^7	149.7	109.3
	244.0	5 Dec 85	250	1.607×10^7	259.2	106.2
	281.0	5 Dec 85	250	1.889×10^7	306.7	109.1
85016†	223.0	30 Dec 85	250	1.357×10^7	219.1	98.3
	250.0	30 Dec 85	250	1.476×10^7	238.9	95.6
	141.0	2 Jan 86	125	1.586×10^7	128.6	91.2
	177.5	2 Jan 86	125	2.278×10^7	186.0	104.8
	199.0	2 Jan 86	125	2.477×10^7	202.6	101.8

* Equation for the standard curve (Study #85015):[6]
Y (peak area) = 5.531×10^4 X (µg/ml) + 7.028×10^5, $R^2 = 0.999$.

† Equation for the standard curve (Study #85016):[7]
Y (peak area) = 5.617×10^4 X (µg/ml) + 3.74×10^5, $R^2 = 0.999$.

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

[7] Ibid. p. 51-63.

Appendix A (cont.): CHEMICAL DATA

CHEMICAL ANALYSIS FOR JA-2
(Information from the Manufacturer's Data Sheet)

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

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

Appendix B: ANIMAL DATA

Species: Mus musculus

Strain: Albino ICR (Institute of Cancer Research)

Source: Charles River Laboratories, Inc.
Kingston, NY

Date of Birth: Males: 1 November 1985
Females: 15 October 1985

Sex: Male and female

Method of Randomization: TOXSYS animal allocation
(SOP OP-ISG-24)

Initial Animal Allocation: 10/group, male or female, except vehicle control groups had 5 each

Animal Condition at Study Initiation: Normal

Body Weight Range at Dosing: 25 - 40 g

Identification Procedures: Cervical tag.

Conditioning: Quarantine/acclimation 18 Dec 85 - 2 Jan 86

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

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

Date	Event
17 Dec 85	Received ICR mice. Animals were checked for physical condition, sexed, and individually caged.
18 Dec 85	Mice were weighed and tagged. Four mice (2 males and 2 females) were submitted for necropsy quality control.
18 Dec 85-2 Jan 86	Animals were observed daily while under quarantine/acclimation.
23 Dec 85	Animals were weighed and randomized into dose groups.
30 Dec 85	Phase I animals (4470 mg/kg, 5010 mg/kg, and controls) were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
31 Dec 85-12 Jan 86	Phase I animals were observed daily in the am and pm.
2 Jan 86	Phase II animals (2820 mg/kg, 3550 mg/kg) were fasted 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
3-15 Jan 86	Phase II animals were observed daily in the am and pm.
3 Jan 86	Phase III animals (3970 mg/kg) were fasted for 4 hours, weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
4-17 Jan 86	Phase III animals were observed daily in the am and pm.
8 Jan 86	Phase I animals were weighed.

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

Date	Event
10 Jan 86	Phase II and III animals were weighed.
13 Jan 86	Phase I animals were fasted, weighed, observed, and submitted to necropsy.
16 Jan 86	Phase II animals were fasted, weighed, observed, and submitted to necropsy.
17 Jan 86	Phase III animals were fasted, weighed, observed, and submitted to necropsy.

Appendix D: CUMULATIVE MORTALITY DATA (deaths/group)

Dose mg/kg	Animals/ Group	Time After Dosing: Hours 2	Hours 4	Days 1	Days 2	Days 3	Days 4	Days 5	Days 6	Days 7	Days 8-14
				MALES							
2820	8	0	0	0	0	0	0	1	1	1	1
3550	9	0	0	0	2	2	2	2	2	2	2
3970	10	0	0	0	4	4	4	5	5	5	5
4470	6	0	0	0	6	6	6	6	6	6	6
5010	7	0	0	0	7	7	7	7	7	7	7
Vehicle	4	0	0	0	0	0	0	0	0	0	0
TOTAL*	40	0	0	0	19	19	19	21	21	21	21
				FEMALES							
2820	9	0	0	0	0	0	0	0	0	0	0
3550	9	0	0	0	4	6	6	6	6	6	6
3970	10	0	0	0	6	6	7	8	8	8	8
4470	10	0	0	0	9	9	9	9	9	9	9
5010	8	0	0	2	8	8	8	8	8	8	8
Vehicle	5	0	0	0	0	0	0	0	0	0	0
TOTAL*	46	0	0	2	27	29	30	31	31	31	31

* TOTAL reflects only animals receiving JA-2.

Appendix E: INDIVIDUAL ANIMAL HISTORIES

MALE: 2820 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00682	Hunched Posture	Jan 2	Slight
	Rough Coat	Jan 2	Slight
	Squinting	Jan 2	Slight
85C00687	Hunched Posture	Jan 2	Slight
	Inactive	Jan 2	Slight
	Rough Coat	Jan 2	Slight
85C00691	Inactive	Jan 2	Slight
85C00700	Hunched Posture	Jan 2	Slight
	Inactive	Jan 2	Slight
	Squinting	Jan 2	Slight
85C00703	Squinting	Jan 2	Slight
	Inactive	Jan 2	Slight
	Hunched Posture	Jan 5,6	Moderate
	Rough Coat	Jan 5,6	Slight
	Death	Jan 7	4.9 days
85C00715	Removed From Study	N/A	N/A
85C00736	Irritable	Jan 2	Slight
	Stain, Yellow, Perianal	Jan 10-13,15,16	Slight
85C00738	Misdosed	N/A	N/A
85C00743	Inactive	Jan 2-4	Moderate
	Loss of Equilibrium	Jan 2	Present
	Ataxia	Jan 3,4	Slight
	Squinting	Jan 3,4	Slight
	Rough Coat	Jan 5	Slight
85C00744	Hunched Posture	Jan 2	Slight
	Inactive	Jan 2	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3550 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00675	Hunched Posture	Jan 2,3	Moderate
	Inactive	Jan 2,3	Slight
	Tremors	Jan 2	Slight
	Squinting	Jan 2,3	Moderate
85C00676	Inactive	Jan 2	Moderate
	Squinting	Jan 2	Slight
	Rough Coat	Jan 4	Slight
85C00677	Hunched Posture	Jan 2,3	Marked
	Inactive	Jan 2,3	Marked
	Squinting	Jan 2	Marked
	Rough Coat	Jan 2,5,6	Moderate
	Tremors	Jan 2	Slight
	Stain, Yellow, Perianal	Jan 4,15,16	Slight
85C00678	Hunched Posture	Jan 2	Marked
	Squinting	Jan 2	Marked
	Inactive	Jan 2	Moderate
	Rough Coat	Jan 4	Slight
85C00680	Inactive	Jan 2,3	Slight
	Tremors	Jan 2,3	Slight
	Squinting	Jan 2,3	Slight
	Stain, Yellow, Perianal	Jan 4,7,10-16	Slight
85C00694	Misdosed	N/A	N/A
85C00709	Inactive	Jan 2	Slight
	Rough Coat	Jan 2,16	Slight
	Hunched Posture	Jan 2	Slight
	Stain, Yellow, Perianal	Jan 5-13,15,16	Slight
	Stain, Yellow, Abdominal	Jan 3,4	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3550 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00721	Hunched Posture	Jan 2,3	Slight
	Inactive	Jan 2,3	Moderate
	Squinting	Jan 2,3	Moderate
	Tremors	Jan 3	Slight
	Depr. Grasping Reflex	Jan 3	Slight
	Death	Jan 4	1.9 days
85C00725	Hunched Posture	Jan 2,3	Moderate
	Inactive	Jan 2,3	Marked
	Tremors	Jan 2,3	Slight
	Depr. Grasping Reflex	Jan 3	Slight
	Stain, Yellow, Perianal	Jan 3	Slight
	Death	Jan 4	1.9 days
85C00726	Stain, Yellow, Perianal	Jan 3,16	Slight
	Rough Coat	Jan 16	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3970 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00685	Tremors	Jan 3	Slight
	Inactive	Jan 3	Slight
	Hunched Posture	Jan 4	Slight
85C00692	Inactive	Jan 3	Slight
	Tremors	Jan 3,4	Marked
	Hunched Posture	Jan 3	Slight
	Stain, Yellow, Abdomen	Jan 4	Moderate
	Depr. Grasping Reflex	Jan 4	Moderate
	Death	Jan 5	2.0 days
85C00695	Normal	N/A	N/A
85C00696	Inactive	Jan 3-5	Slight
	Tremors	Jan 3-5	Slight
	Stain, Yellow, Abdomen	Jan 5-9,15-17	Marked
	Stain, Yellow, Perianal	Jan 14	Slight
85C00708	Inactive	Jan 3	Moderate
	Tremors	Jan 3	Moderate
	Prostrate	Jan 3,4	Present
	Stain, Yellow, Abdomen	Jan 3,4	Marked
	Incr. Respiration Rate	Jan 3,4	Present
	Death	Jan 5	2.0 days
85C00714	Hunched Posture	Jan 3,4	Slight
	Inactive	Jan 3,4	Moderate
	Tremors	Jan 3,4	Slight
	Stain, Yellow, Perianal	Jan 4	Slight
	Death	Jan 5	2.0 days
85C00720	Hunched Posture	Jan 3-6	Slight
	Tremors	Jan 3-6	Moderate
	Inactive	Jan 5,6	Moderate
	Stain, Yellow, Abdomen	Jan 5-7	Moderate
	Prostrate	Jan 7	Present
	Death	Jan 7	4.2 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 3970 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00723	Tremors	Jan 3	Slight
	Inactive	Jan 3	Slight
85C00735	Stain, Yellow, Perianal	Jan 3	Slight
	Hunched Posture	Jan 3	Slight
	Tremors	Jan 3	Slight
85C00741	Tremors	Jan 3,4	Moderate
	Inactive	Jan 3,4	Moderate
	Hunched Posture	Jan 3,4	Slight
	Stain, Yellow, Abdomen	Jan 4	Moderate
	Death	Jan 5	2.0 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4470 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00679	Misdosed	N/A	N/A
85C00688	Misdosed	N/A	N/A
85C00697	Inactive	Dec 30,31	Marked
	Tachypnea	Dec 30	Moderate
	Squinting	Dec 30,31	Marked
	Loss of Equilibrium	Dec 30	Present
	Depr. Righting Reflex	Dec 30	Slight
	Depr. Grasping Reflex	Dec 30,31	Slight
	Tremors	Dec 31	Moderate
	Opisthotonus	Dec 31	Present
	Rough Coat	Dec 31	Moderate
	Death	Dec 31	28.7 h
85C00702	Inactive	Dec 30,31	Marked
	Squinting	Dec 30,31	Marked
	Rough Coat	Dec 30,31	Marked
	Stain, Yellow, Abdomen	Dec 30,31	Marked
	Depr. Grasping Reflex	Dec 31	Moderate
	Death	Jan 1	1.9 days
85C00705	Misdosed	N/A	N/A
85C00712	Misdosed	N/A	N/A
85C00727	Inactive	Dec 30,31	Marked
	Tremors	Dec 30,31	Marked
	Tachypnea	Dec 30	Moderate
	Squinting	Dec 30,31	Marked
	Stain, Yellow, Abdomen	Dec 30,31	Moderate
	Depr. Grasping Reflex	Dec 30,31	Marked
	Opisthotonus	Dec 31	Present
	Death	Dec 31	24.9 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 4470 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00734	Hunched Posture	Dec 30	Moderate
	Inactive	Dec 30	Moderate
	Tachypnea	Dec 30	Moderate
	Tremors	Dec 30,31	Moderate
	Depr. Grasping Reflex	Dec 30	Moderate
	Prostrate	Dec 31	Present
	Death	Dec 31	28.6 h
85C00737	Hunched Posture	Dec 30,31	Slight
	Squinting	Dec 30,31	Moderate
	Inactive	Dec 30,31	Moderate
	Rough Coat	Dec 30,31	Slight
	Stain, Yellow, Abdomen	Dec 30,31	Moderate
	Death	Jan 1	1.9 days
85C00742	Hunched Posture	Dec 30,31	Moderate
	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Slight
	Death	Jan 1	1.9 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 5010 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00674	Hunched Posture	Dec 30	Marked
	Inactive	Dec 30,31	Marked
	Hyperactive	Dec 30	Moderate
	Tachypnea	Dec 30	Moderate
	Squinting	Dec 30,31	Marked
	Tremors	Dec 30,31	Marked
	Stain, Yellow, Perianal	Dec 30,31	Moderate
	Depr. Grasping Reflex	Dec 31	Slight
	Death	Jan 1	1.9 days
85C00681	Misdosed	N/A	N/A
85C00690	Inactive	Dec 30,31	Moderate
	Tachypnea	Dec 30	Moderate
	Squinting	Dec 30,31	Moderate
	Tremors	Dec 30,31	Moderate
	Opisthotonus	Dec 30,31	Present
	Depr. Grasping Reflex	Dec 30,31	Slight
	Stain, Yellow, Perianal	Dec 30,31	Slight
	Death	Dec 31	28.2 h
85C00698	Misdosed	N/A	N/A
85C00701	Inactive	Dec 30	Marked
	Tremors	Dec 30,31	Slight
	Incr. Respiration Depth	Dec 30	Present
	Tachypnea	Dec 30	Moderate
	Squinting	Dec 30,31	Marked
	Prostrate	Dec 31	Present
	Death	Dec 31	24.5 h
85C00710	Inactive	Dec 30,31	Marked
	Squinting	Dec 30,31	Marked
	Tachypnea	Dec 30	Moderate
	Hunched Posture	Dec 30,31	Moderate
	Rough Coat	Dec 30,31	Slight
	Tremors	Dec 30,31	Slight
	Depr. Grasping Reflex	Dec 30,31	Slight
	Stain, Yellow, Perianal	Dec 30,31	Slight
	Jumping	Dec 31	Slight
	Death	Jan 1	1.9 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 5010 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00718	Inactive	Dec 30,31	Moderate
	Squinting	Dec 30,31	Moderate
	Tachypnea	Dec 30	Moderate
	Tremors	Dec 30,31	Moderate
	Stain, Yellow, Abdomen	Dec 30,31	Marked
	Death	Dec 31	28.1 h
85C00722	Misdosed	N/A	N/A
85C00739	Inactive	Dec 30	Marked
	Tremors	Dec 30,31	Marked
	Squinting	Dec 30,31	Marked
	Hunched Posture	Dec 30	Moderate
	Depr. Righting Reflex	Dec 30	Moderate
	Stain, Yellow, Abdomen	Dec 30,31	Marked
	Incr. Salivation, Red	Dec 30,31	Present
	Prostrate	Dec 31	Present
	Death	Dec 31	24.4 h
85C00740	Inactive	Dec 30,31	Moderate
	Squinting	Dec 30,31	Moderate
	Tremors	Dec 30,31	Moderate
	Opisthotonus	Dec 31	Present
	Depr. Grasping Reflex	Dec 31	Slight
	Death	Jan 1	1.9 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: *Vehicle Control*

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00683	Normal	N/A	N/A
85C00693	Ear Tag Missing	Dec 30	N/A
	Stain, Yellow, Perianal	Jan 4-11	Slight
	Rough Coat	Dec 31-Jan 2,5	Slight
85C00704	Misdosed	N/A	N/A
85C00706	Normal	N/A	N/A
85C00707	Stain, Yellow, Perianal	Jan 4-11	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2820 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00751	Removed From Study	N/A	N/A
85C00762	Hunched Posture	Jan 2	Marked
	Inactive	Jan 2	Slight
	Squinting	Jan 2	Moderate
	Loss of Equilibrium	Jan 2	Present
85C00764	Inactive	Jan 2	Slight
	Squinting	Jan 2	Slight
85C00767	Inactive	Jan 2	Slight
85C00782	Inactive	Jan 2	Moderate
	Squinting	Jan 2	Slight
	Hunched Posture	Jan 2	Slight
85C00794	Inactive	Jan 2	Slight
85C00798	Hunched Posture	Jan 2	Slight
	Inactive	Jan 2	Slight
	Squinting	Jan 2	Slight
85C00800	Hunched Posture	Jan 2	Slight
	Squinting	Jan 2	Slight
	Tremors	Jan 2	Slight
85C00805	Hunched Posture	Jan 2	Slight
	Inactive	Jan 2	Slight
	Tremors	Jan 4	Slight
85C00813	Hunched Posture	Jan 2	Slight
	Inactive	Jan 2	Slight
	Squinting	Jan 2	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3550 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00753	Inactive	Jan 2-4	Marked
	Squinting	Jan 2-4	Marked
	Tremors	Jan 2-4	Moderate
	Loss of Equilibrium	Jan 2	Present
	Stain, Yellow, Abdomen	Jan 3,4	Moderate
	Death	Jan 5	3.0 days
85C00763	Inactive	Jan 2	Slight
	Squinting	Jan 2	Slight
85C00769	Inactive	Jan 2-4	Marked
	Hunched Posture	Jan 2-4	Slight
	Tremors	Jan 2-4	Slight
	Squinting	Jan 2-4	Moderate
	Death	Jan 5	3.0 days
85C00781	Hunched Posture	Jan 2	Moderate
	Inactive	Jan 2,3	Moderate
	Depr. Grasping Reflex	Jan 2,3	Moderate
	Rough Coat	Jan 2,3	Slight
85C00787	Hunched Posture	Jan 2,3	Moderate
	Tremors	Jan 2,3	Moderate
	Squinting	Jan 2,3	Slight
	Inactive	Jan 3	Moderate
	Depr. Grasping Reflex	Jan 3	Moderate
	Death	Jan 4	1.9 days
85C00790	Hunched Posture	Jan 2,3	Moderate
	Tremors	Jan 2,3	Slight
	Squinting	Jan 2,3	Moderate
	Inactive	Jan 2,3	Moderate
	Depr. Grasping Reflex	Jan 3	Moderate
	Death	Jan 4	1.9 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3550 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00792	Hunched Posture	Jan 2,3	Moderate
	Inactive	Jan 2,3	Moderate
	Tremors	Jan 2,3	Slight
	Squinting	Jan 2,3	Slight
	Death	Jan 3	27.6 h
85C00809	Misdosed	N/A	N/A
85C00816	Tremors	Jan 2,3	Moderate
	Inactive	Jan 2,3	Moderate
	Rough Coat	Jan 3	Slight
	Squinting	Jan 2,3	Marked
	Death	Jan 4	2.0 days
85C00817	Inactive	Jan 2	Slight
	Squinting	Jan 2	Moderate
	Hunched Posture	Jan 2	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3970 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00757	Tremors	Jan 3,4	Slight
	Hunched Posture	Jan 3,4	Slight
	Inactive	Jan 3,4	Moderate
	Death	Jan 5	2.0 days
85C00772	Hunched Posture	Jan 3-5	Slight
	Rough Coat	Jan 5	Slight
85C00780	Inactive	Jan 3	Slight
	Tremors	Jan 3,4	Slight
	Hunched Posture	Jan 3	Slight
	Stain, Red, Face	Jan 4	Present
	Prostrate	Jan 4	Present
	Death	Jan 4	26.9 h
85C00786	Hunched Posture	Jan 3-5	Slight
	Inactive	Jan 3-6	Marked
	Tremors	Jan 3-6	Slight
	Stain, Yellow, Abdomen	Jan 4-6	Moderate
	Depr. Grasping Reflex	Jan 6	Moderate
	Death	Jan 6	3.2 days
85C00788	Hunched Posture	Jan 3-7	Slight
	Tremors	Jan 3-7	Slight
	Stain, Yellow, Perianal	Jan 3-8	Marked
	Inactive	Jan 5-7	Moderate
	Moribund	Jan 8	Present
	Death	Jan 8	4.9 days
85C00793	Hunched Posture	Jan 3,4	Slight
	Tremors	Jan 3,4	Slight
	Inactive	Jan 4	Moderate
	Death	Jan 5	2.0 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 3970 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1986)	Severity
85C00804	Rough Coat	Jan 3,4	Slight
	Inactive	Jan 4	Moderate
	Tremors	Jan 4	Slight
	Death	Jan 5	2.0 days
85C00812	Hunched Posture	Jan 3,4	Slight
	Inactive	Jan 3,4	Slight
85C00815	Hunched Posture	Jan 3	Slight
	Inactive	Jan 3,4	Marked
	Tremors	Jan 3,4	Moderate
	Stain, Yellow, Abdomen	Jan 4	Marked
	Stain, Brown, Mouth	Jan 4	Marked
	Death	Jan 5	2.0 days
85C00821	Rough Coat	Jan 3,4	Moderate
	Hunched Posture	Jan 3,4	Slight
	Inactive	Jan 3,4	Moderate
	Tremors	Jan 4	Moderate
	Death	Jan 5	2.0 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4470 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00748	Inactive	Dec 30	Moderate
	Tremors	Dec 30,31	Moderate
	Squinting	Dec 30,31	Marked
	Depr. Grasping Reflex	Dec 30	Slight
	Prostrate	Dec 31	Present
	Death	Dec 31	28.5 h
85C00760	Inactive	Dec 30,31	Moderate
	Tachypnea	Dec 30	Slight
	Loss of Equilibrium	Dec 30	Present
	Tremors	Dec 30,31	Moderate
	Hunched Posture	Dec 30,31	Slight
	Death	Jan 1	1.9 days
85C00768	Inactive	Dec 30,31	Slight
	Tremors	Dec 30,31	Slight
	Hunched Posture	Dec 30	Marked
	Squinting	Dec 30,31	Moderate
	Depr. Grasping Reflex	Dec 31	Slight
	Death	Jan 1	1.9 days
85C00775	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Slight
	Opisthotonus	Dec 31	Present
	Death	Jan 1	1.9 days
85C00776	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Slight
	Squinting	Dec 30,31	Marked
	Opisthotonus	Dec 30,31	Present
	Depr. Grasping Reflex	Dec 30,31	Slight
	Death	Jan 1	1.9 days
85C00779	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Moderate
	Opisthotonus	Dec 30,31	Present
	Death	Jan 1	1.9 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4470 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00797	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Moderate
	Loss of Equilibrium	Dec 30	Present
	Depr. Grasping Reflex	Dec 30,31	Slight
	Depr. Righting Reflex	Dec 30,31	Moderate
	Opisthotonus	Dec 31	Present
	Death	Jan 1	1.9 days
85C00808	Inactive	Dec 30	Marked
	Tremors	Dec 30,31	Marked
	Squinting	Dec 30	Marked
	Depr. Righting Reflex	Dec 30	Moderate
	Rough Coat	Dec 30	Moderate
	Stain, Yellow, Abdomen	Dec 30	Moderate
	Prostrate	Dec 31	Present
	Death	Dec 31	28.3 h
85C00819	Inactive	Dec 30	Slight
85C00820	Inactive	Dec 30,31	Marked
	Squinting	Dec 30,31	Marked
	Tremors	Dec 30,31	Slight
	Loss of Equilibrium	Dec 30	Present
	Jumping	Dec 30	Slight
	Opisthotonus	Dec 31	Present
	Death	Jan 1	1.9 days

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 5010 mg/kg JA-2

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00755	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Moderate
	Opisthotonus	Dec 30,31	Present
	Stain, Yellow, Perianal	Dec 30,31	Slight
	Depr. Righting Reflex	Dec 30,31	Slight
	Death	Jan 1	1.9 days
85C00756	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Marked
	Squinting	Dec 30,31	Marked
	Depr. Grasping Reflex	Dec 30,31	Slight
	Hunched Posture	Dec 30,31	Moderate
	Opisthotonus	Dec 31	Present
	Death	Dec 31	28.0 h
85C00759	Misdosed	N/A	N/A
85C00761	Inactive	Dec 30	Marked
	Tremors	Dec 30	Marked
	Depr. Righting Reflex	Dec 30	Moderate
	Squinting	Dec 30	Marked
	Stain, Yellow, Perianal	Dec 30	Slight
	Death	Dec 30	5.0 h
85C00773	Inactive	Dec 30,31	Moderate
	Squinting	Dec 30,31	Moderate
	Tremors	Dec 30,31	Slight
	Opisthotonus	Dec 31	Present
	Death	Jan 1	1.9 days
85C00783	Inactive	Dec 30,31	Marked
	Tremors	Dec 30,31	Moderate
	Squinting	Dec 30,31	Marked
	Opisthotonus	Dec 31	Present
	Depr. Grasping Reflex	Dec 30,31	Moderate
	Death	Dec 31	27.9 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 5010 mg/kg JA-2 (cont.)

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00784	Inactive	Dec 30,31	Moderate
	Tremors	Dec 30,31	Moderate
	Stain, Yellow, Abdomen	Dec 30,31	Moderate
	Depr. Grasping Reflex	Dec 31	Slight
	Death	Jan 1	1.9 days
85C00802	Misdosed	N/A	N/A
85C00810	Inactive	Dec 30,31	Marked
	Tremors	Dec 30,31	Moderate
	Rough Coat	Dec 30,31	Slight
	Depr. Grasping Reflex	Dec 31	Slight
	Opisthotonus	Dec 31	Present
	Death	Jan 1	1.9 days
85C00818	Inactive	Dec 30	Marked
	Tremors	Dec 30	Marked
	Squinting	Dec 30	Marked
	Depr. Grasping Reflex	Dec 30	Marked
	Depr. Righting Reflex	Dec 30	Slight
	Death	Dec 31	19.7 h

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1985/1986)	Severity
85C00752	Normal	N/A	N/A
85C00770	Normal	N/A	N/A
85C00774	Normal	N/A	N/A
85C00795	Normal	N/A	N/A
85C00799	Normal	N/A	N/A

Appendix F: INDIVIDUAL BODY WEIGHTS IN GRAMS

Males: 2820 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 8	Termination Day 14
85C00682	30	36	36	36
85C00687	30	33	34	34
85C00691	30	35	35	35
85C00700	31	32	36	35
85C00703	24	37	Dead	---
85C00736	29	35	35	36
85C00743	30	34	34	34
85C00744	29	33	33	33
Mean	29.1	34.4	34.7	34.7
Standard Deviation	2.2	1.7	1.1	1.1
Std. Error of Mean	0.8	0.6	0.4	0.4

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

Males: 3550 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 8	Termination Day 14
85C00675	30	34	35	35
85C00676	31	33	36	39
85C00677	32	35	37	39
85C00678	31	30	32	33
85C00680	29	32	32	33
85C00709	33	37	39	39
85C00721	29	31	Dead	---
85C00725	30	35	Dead	---
85C00726	30	36	37	33
Mean	30.6	33.7	35.4	35.8
Standard Deviation	1.3	2.3	2.6	3.0
Std. Error of Mean	0.4	0.8	1.0	1.1

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

Males: 3970 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85C00685	31	34	34	34
85C00692	30	34	Dead	---
85C00695	31	34	36	35
85C00696	26	40	41	43
85C00708	31	34	Dead	---
85C00714	30	33	Dead	---
85C00720	29	31	Dead	---
85C00723	30	33	34	35
85C00735	29	33	32	33
85C00741	31	32	Dead	---
Mean	29.8	33.8	35.4	36.0
Standard Deviation	1.5	2.4	3.4	4.0
Std. Error of Mean	0.5	0.8	1.5	1.8

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

Males: 4470 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85C00697	31	35	Dead	---
85C00702	31	35	Dead	---
85C00727	30	33	Dead	---
85C00734	27	31	Dead	---
85C00737	29	30	Dead	---
85C00742	28	34	Dead	---
Mean	29.3	33.0	N/A	N/A
Standard Deviation	1.6	2.1		
Std. Error of Mean	0.7	0.9		

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

Males: 5010 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85C00674	32	35	Dead	---
85C00690	32	34	Dead	---
85C00701	30	34	Dead	---
85C00710	27	33	Dead	---
85C00718	29	33	Dead	---
85C00739	29	31	Dead	---
85C00740	29	30	Dead	---
Mean	29.7	32.9	N/A	N/A
Standard Deviation	1.8	1.8		
Std. Error of Mean	0.7	0.7		

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

Males: Vehicle Control

Animal No.	Receipt	Dosing	Day 9	Termination Day 14
85C00683	28	30	31	32
85C00693	33	36	39	39
85C00706	30	36	38	38
85C00707	29	33	36	37
Mean	30.0	33.8	36.0	36.5
Standard Deviation	2.2	2.9	3.6	3.1
Std. Error of Mean	1.1	1.4	1.8	1.6

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

Females: 2820 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 8	Termination Day 14
85C00762	25	28	30	31
85C00764	23	26	28	28
85C00767	24	28	30	27
85C00782	25	28	29	29
85C00794	25	32	33	32
85C00798	25	30	30	29
85C00800	23	26	29	28
85C00805	26	28	31	30
85C00813	25	29	30	29
Mean	24.6	28.3	30.0	29.2
Standard Deviation	1.0	1.9	1.4	1.6
Std. Error of Mean	0.3	0.6	0.5	0.5

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

Females: 3550 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 8	Termination Day 14
85C00753	28	31	Dead	---
85C00763	26	28	29	28
85C00769	27	28	Dead	---
85C00781	25	29	31	29
85C00787	27	29	Dead	---
85C00790	26	29	Dead	---
85C00792	25	27	Dead	---
85C00816	25	28	Dead	---
85C00817	25	26	28	27
Mean	26.0	28.3	29.3	28.0
Standard Deviation	1.1	1.4	1.5	1.0
Std. Error of Mean	0.4	0.5	0.9	0.6

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

Females: 3970 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85C00757	26	27	Dead	---
85C00772	28	28	29	30
85C00780	26	26	Dead	---
85C00786	25	28	Dead	---
85C00788	24	27	Dead	---
85C00793	26	29	Dead	---
85C00804	25	28	Dead	---
85C00812	24	29	30	32
85C00815	25	27	Dead	---
85C00821	25	27	Dead	---
Mean	25.4	27.6	29.5	31.0
Standard Deviation	1.2	1.0	0.7	1.4
Std. Error of Mean	0.4	0.3	0.5	1.0

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

Females: 4470 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85C00748	27	28	Dead	---
85C00760	23	26	Dead	---
85C00768	26	26	Dead	---
85C00775	26	28	Dead	---
85C00776	27	27	Dead	---
85C00779	24	26	Dead	---
85C00797	26	26	Dead	---
85C00808	25	28	Dead	---
85C00819	26	28	29	31
85C00820	25	28	Dead	---
Mean	25.5	27.1	29	31
Standard Deviation	1.3	1.0	N/A	N/A
Std. Error of Mean	0.4	0.3	N/A	N/A

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

Females: 5010 mg/kg JA-2

Animal No.	Receipt	Dosing	Day 7	Termination Day 14
85C00755	26	27	Dead	---
85C00756	25	28	Dead	---
85C00761	24	25	Dead	---
85C00773	24	25	Dead	---
85C00783	28	27	Dead	---
85C00784	25	26	Dead	---
85C00810	24	27	Dead	---
85C00818	25	27	Dead	---
Mean	25.1	26.5	N/a	N/A
Standard Deviation	1.4	1.1	---	---
Std. Error of Mean	0.5	0.4	---	---

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

Females: Vehicle Control

Animal No.	Receipt	Dosing	Day 9	Termination Day 14
85C00752	27	27	31	31
85C00770	26	27	30	29
85C00774	24	27	29	28
85C00795	22	26	29	29
85C00799	24	25	28	29
Mean	24.6	26.4	29.4	29.2
Standard Deviation	2.0	0.9	1.1	1.1
Std. Error of Mean	0.9	0.4	0.5	0.5

Appendix G: PATHOLOGY REPORT

GLP Study #85016
Principal Investigator: CPT Morgan

I. INTRODUCTION

Study: Oral LD50/JA-2 Solid Propellant
Animal: Mouse (ICR)/albino
Reference: SOP OP-STX-36
Procedure: Euthanized with sodium pentobarbital

II. GROSS FINDINGS

Group 1 Males - 2820 mg/kg JA-2
(Live animals indicated by '*')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 38908	85C00682	14 Days	Not Remarkable (NR)
* 38909	85C00687	14 Days	NR
* 38910	85C00691	14 Days	NR
* 38911	85C00700	14 Days	NR
38842	85C00703	5 Days	NR
* 38914	85C00736	14 Days	NR
* 38915	85C00743	14 Days	NR
* 38916	85C00744	14 Days	NR

Group 2 Males - 3550 mg/kg JA-2
(Live animals indicated by '*')

* 38903	85C00675	14 Days	NR
* 38904	85C00676	14 Days	NR
* 38905	85C00677	14 Days	NR
* 38906	85C00678	14 Days	NR
* 38907	85C00680	14 Days	NR
* 38912	85C00709	14 Days	NR
38823	85C00721	2 Days	Pulmonary congestion
38824	85C00725	2 Days	NR
* 38913	85C00726	14 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

Group 3 Males - 4470 mg/kg JA-2
(Live animals indicated by '*')

	ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
	38787	85C00697	1 Day	NR
	38798	85C00702	2 Days	NR
	38790	85C00727	1 Day	Serosanguinous discharge around mouth and eyes
	38791	85C00734	1 Day	NR
	38800	85C00737	2 Days	NR
	38802	85C00742	2 Days	NR

Group 4 Males - 5010 mg/kg JA-2
(Live animals indicated by '*')

	38797	85C00674	2 Days	NR
	38786	85C00690	1 Day	Mild hepatic swelling
	38788	85C00701	1 Day	Mild hepatic and renal swelling
	38799	85C00710	2 Days	NR
	38789	85C00718	1 Day	Diffuse pulmonary congestion
	38792	85C00739	1 Day	Diffuse pulmonary congestion
	38801	85C00740	2 Days	NR

Group 5 Males - 3970 mg/kg JA-2
(Live animals indicated by '*')

*	38940	85C00685	14 Days	NR
	38829	85C00692	2 Days	NR
*	38941	85C00695	14 Days	NR
*	38942	85C00696	14 Days	NR
	38830	85C00708	2 Days	Diffuse pulmonary congestion
	38831	85C00714	2 Days	NR
	38843	85C00720	4 Days	Diffuse pulmonary congestion Diffuse hepatic pallor
*	38943	85C00723	14 Days	NR
*	38944	85C00635	14 Days	NR
	38832	85C00741	2 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

Group 6 Males - Vehicle Control
(Live animals indicated by '*')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 38874	85C00683	14 Days	NR
* 38875	85C00693	14 Days	NR
* 38876	85C00706	14 Days	NR
* 38877	85C00707	14 Days	NR

Group 1 Females - 2820 mg/kg JA-2
(Live animals indicated by '*')

* 38917	85C00762	14 Days	NR
* 38919	85C00764	14 Days	NR
* 38920	85C00767	14 Days	NR
* 38922	85C00782	14 Days	NR
* 38923	85C00794	14 Days	NR
* 38924	85C00798	14 Days	NR
* 38925	85C00800	14 Days	NR
* 38926	85C00805	14 Days	NR
* 38927	85C00813	14 Days	NR

Group 2 Females - 3550 mg/kg JA-2
(Live animals indicated by '*')

38833	85C00753	3 Days	Diffuse pulmonary congestion
* 38918	85C00763	14 Days	NR
38835	85C00769	3 Days	NR
* 38921	85C00781	14 Days	NR
38826	85C00787	2 Days	Diffuse pulmonary congestion and brown discoloration of blood
38827	85C00790	2 Days	Diffuse pulmonary congestion
38822	85C00792	1 Day	Red discharge around mouth Diffuse pulmonary congestion
38828	85C00816	2 Days	Diffuse pulmonary congestion Bilateral renal swelling Brown discoloration of blood
* 38928	85C00817	14 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

Group 3 Females - 4470 mg/kg JA-2
(Live animals indicated by '*')

	ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
	38793	85C00748	1 Day	Mild hepatic swelling
	38804	85C00760	2 Days	NR
	38805	85C00768	2 Days	NR
	38807	85C00775	2 Days	NR
	38810	85C00776	2 Days	NR
	38811	85C00779	2 Days	NR
	38813	85C00797	2 Days	NR
	38796	85C00808	1 Day	Mild hepatic swelling
*	38883	85C00819	14 Days	NR
	38809	85C00820	2 Days	NR

Group 4 Females - 5010 mg/kg JA-2
(Live animals indicated by '*')

	38803	85C00755	2 Days	NR
	38794	85C00756	1 Day	NR
	38779	85C00761	1 Day	NR
	38806	85C00773	2 Days	NR
	38795	85C00783	1 Day	NR
	38812	85C00784	2 Days	NR
	38808	85C00810	2 Days	NR
	38780	85C00818	1 Day	Diffuse pulmonary congestion

Group 5 Females - 3970 mg/kg JA-2
(Live animals indicated by '*')

	38834	85C00757	2 Days	NR
*	38945	85C00772	14 Days	NR
	38825	85C00780	1 Day	Diffuse pulmonary congestion
	38840	85C00786	3 Days	NR
	38844	85C00788	5 Days	Minimal pulmonary congestion Marked hepatic pallor
	38836	85C00793	2 Days	Mild hepatic swelling
	38837	85C00804	2 Days	Mild hepatic swelling
*	38946	85C00812	14 Days	NR
	38838	85C00815	2 Days	Diffuse pulmonary congestion Bilateral renal swelling
	38839	85C00821	2 Days	NR

Appendix G (cont.): PATHOLOGY REPORT

Group 6 Females - Vehicle Control
(Live animals indicated by '*')

ACCESSION NUMBER	ANIMAL ID NUMBER	DOSE-DEATH INTERVAL	OBSERVATIONS
* 38878	85C00752	14 Days	NR
* 38879	85C00770	14 Days	NR
* 38880	85C00774	14 Days	NR
* 38881	85C00795	14 Days	NR
* 38882	85C00799	14 Days	NR

TABLE 1: Incidence of Prominent Gross Findings

Group	Lesions*				
	HS	RS	HP	PC	BB
2-Male				1/9(11%)	
4-Male	2/7(29%)	1/7(14%)		2/7(29%)	
5-Male			1/10(10%)	2/10(20%)	
2-Female		1/9(11%)		5/9(56%)	2/9(22%)
3-Female	2/10(20%)				
4-Female				1/8(12%)	
5-Female	2/10(20%)	1/10(10%)	1/10(10%)	3/10(30%)	

* HS=hepatic swelling, RS=renal swelling, HP=hepatic pallor, PC=pulmonary congestion, BB=brown discoloration of blood

TABLE 2: Numbers of Animals with Various Gross Findings Grouped by Survival Time.

Lesions*	Survival Time				
	1 Day	2 Days	3 Days	4 Days	5 Days
HS	4	2			
RS	1	2			
HP				1	1
PC	5	6	1	1	1
BB		2			

* HS=hepatic swelling, RS=renal swelling, HP=hepatic pallor, PC=pulmonary congestion, BB=brown discoloration of blood

Appendix G (cont.): PATHOLOGY REPORT

Comments: Out of 52 non-survivors, 21 (40%) presented remarkable gross findings at necropsy (Table 1). Occasional (rare) animals had combinations of more than one finding. Most animals with lesions died by Day 3. Survival time did not seem to correlate with the presence or absence of lesions in general or any lesion in particular. Although hepatic swelling and pulmonary congestion seemed to stand out in the first two days (Table 2), the number of animals involved were small compared to the number in each group. In conclusion, highly consistent gross lesions were not the case, and there was no evidence of an extraneous cause of death.

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