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ARMSTRONG LABORATORY

ACUTE TOXICITY OF QUADRICYCLANE

E. R. Kinkead
R. E. Wolfe
S. A. Salins

MANTECH ENVIRONMENTAL TECHNOLOGY, INC.
P.O. BOX 31009
DAYTON, OH 45437-0009

J. H. Grabau

OCCUPATIONAL AND ENVIRONMENTAL HEALTH DIRECTORATE
TOXICOLOGY DIVISION

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TECHNICAL REVIEW AND APPROVAL

AL-TR-1993-0046

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Maj, USAF, BSC
Deputy Director, Toxic Hazards Division
Armstrong Laboratory

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E.R. Kinkead, R.E. Wolfe, S.A. Salins, and J.H. Grabau

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The Air Force is currently developing high energy density matter (HEDM) for use as advanced rocket propellants. One group of compounds considered are the strained-ring hydrocarbons. These compounds will be mixed with kerosene to increase the propellants' performance. A compound of immediate interest is quadricyclane (tetracycloheptane). Quadricyclane (C_7H_{10}) is a colorless, flammable liquid with a boiling point of 180 °C. Although quadricyclane is available commercially, no acute toxicity information is available in the literature. The Air Force is in the process of screening various propellant condidates in order to select the most promising for further development. Toxicological hazard will be an important criterion for screening candidate compounds. Quadricyclane produced 100% mortality in male Fischer 344 rats within 24 h following gavage at 3.5 g/kg. Gavage treatment with a quadricyclane/kerosene mixture (70% quadricyclane, 30% kerosene), similar to the proposed rocket fuel mixture, produced toxic effects at a dose level below the EPA limit test. No treatment-related deaths occurred in rabbits following a 24-h dermal exposure to the EPA limit dose of 2 g test material/kg body weight.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc , located at Wright-Patterson Air Force Base (WPAFB), OH This document serves as a final report on the acute toxicity of quadricyclane The research described in this report began in May 1992 and was completed in November 1992 under Department of the Air Force Contract No F33615-90-C-0532 (Study No F18) Lt Col James N McDougal served as Contract Technical Monitor for the U S Air Force, Armstrong Laboratory, Toxicology Division

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, Department of Health and Human Resources, National Institutes of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended

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ABBREVIATIONS

bw	Body weight
CNS	Central nervous system
°C	Degrees Celsius
EPA	Environmental Protection Agency
F 344	Fischer 344 (rats)
g	Gram
h	Hour
HEDM	High energy density matter
kg	Kilogram
mL	Milliliter
NZW	New Zealand white (rabbits)
p	Probability
RP	Rocket Propellant
SD	Standard Deviation
WPAFB	Wright-Patterson Air Force Base

SECTION 1

INTRODUCTION

The Air Force is currently developing high energy density matter (HEDM) for use as advanced rocket propellants (RP) The most near-term development effort is that of the strained-ring hydrocarbons These compounds will be added to rocket propellant RP-1 (kerosene) to improve performance An HEDM of immediate interest is quadricyclane Present plans are for producing a rocket fuel mixture of 70% quadricyclane and 30% RP-1

Although quadricyclane (tetracycloheptane) is commercially available, a toxicologic evaluation of the compound has not been performed Quadricyclane will undergo metal ion catalyzed rearrangement to norbornadiene with the release of heat One of the components of the jet fuel JP-9 is RJ-5, a mixture of dimers of norbornadiene The acute oral toxicity of RJ-5 has been evaluated in this laboratory (Haun et al , 1978) A peroral dose of 4 g RJ-5/kg body weight (bw) administered in corn oil to three rats was not lethal, however, two of three mice died following a 0 25 g/kg dose Dermal toxicity of RJ-5 was not investigated

Vernot et al (1990) reported acute toxicity data on a straight-run kerosene (without additives) that has a slightly higher boiling range than RP-1 Oral and dermal limit tests performed on the neat kerosene produced no mortality; however, the material was severely irritating to rabbit skin following 24-h contact Evaluation of eye irritation tests determined that kerosene was "practically nonirritating " Minimal irritation was noted at 1-h posttreatment; washed eyes had a slightly higher Draize score than unwashed eyes All appeared normal by 24-h posttreatment Dermal sensitization studies were negative

The Air Force is in the process of screening various propellant candidates in order to select the most promising for further development Toxicological hazard will be one of the major screening criteria Promising candidates will be transferred to a Systems Program Office for engineering development At that point, it is necessary to have initial data on chemical and physical properties, explosive hazards, and toxicology

The objective of this study is to provide acute hazard information on quadricyclane to the industrial hygienists at Edwards Air Force Base who are responsible for the safe handling of this material Acute oral and dermal studies will be performed to provide information on accidental exposure by these routes

SECTION 2

MATERIAL

ANIMALS

Fischer 344 (F-344) male rats, 101 to 125 g, were purchased from Charles River Breeding Labs, Kingston, NY. Male New Zealand white (NZW) rabbits weighing between 2 and 3 kg were purchased from Myrtle Rabbitry, Thompson Station, TN. All animals were identified by tattoo and were subjected to a 2-week acclimatization period. Rats were group housed (three per cage) in clear plastic cages with wood-chip bedding. The rabbits were housed individually in suspended, wire-bottom, stainless steel cages. Water and feed (Purina Rabbit Chow #5320 and Purina Formulab #5008) were available ad libitum, except for 16 h prior to oral dosing. Animal room temperatures were maintained at 21 to 25 °C, and the light/dark cycle was set at 12-h intervals.

TEST AGENT

The quadricyclane test compound was purchased from Aldrich Chemical Co., Inc. Pertinent physical and chemical properties are listed below.

CAS number:	278-06-8
Appearance:	Colorless liquid
Empirical formula:	C_7H_{10}
Formula weight:	92.14
Boiling point:	108 °C
Specific gravity:	0.919 g/mL
Purity:	99%

RP-1 (kerosene) will make up approximately 30% of HEDM fuel mixture. The RP-1 sample was provided by the Air Force and consisted of approximately 20% solvent-refined heavy naphtha and 80% hydrotreated light petroleum distillates.

Boiling point:	185-221 °C
Vapor pressure:	approx. 4 mmHg @ 25 °C
Specific gravity:	0.800 g/mL

Saline, sodium chloride irrigation, USP, for use as a negative control, was purchased from Abbott Labs, North Chicago, IL.

SECTION 3

EXPERIMENTAL APPROACH

ORAL TOXICITY

Male F-344 rats were fasted approximately 16 h prior to the administration of the oral dose Each rat was weighed prior to dosing and the test substance was administered on a g/kg bw basis Prior to treatment, the animals were randomized using a proprietary modular software system (Path/Tox® System, Cedar Knolls, NJ) that assigns animals to groups The doses were as follows

five rats at 1 7 g/kg rocket fuel mixture*

five rats at 4 3 g/kg RP-1 (kerosene)

five rats at 3 5 g/kg quadricyclane

five rats at 5 0 g/kg saline (control)

* The rocket fuel mixture (prepared in house) contained 30% RP-1 and 70% quadricyclane

The body weights of surviving rats were measured at 1, 7, and 14 days posttreatment On the 14th day posttreatment, the rats were sacrificed and gross pathology was performed Additionally, sections of liver, stomach, small and large intestine, and kidneys were sampled for histopathologic examination

Blood was sampled from all rats prior to gavage treatment for alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase evaluations In addition, red cells, hematocrit, and hemoglobin values were determined These evaluations were again determined following the 14-day posttreatment observation period

A one-factorial repeated measures analysis, and multiple comparisons using Ryan-Einot-Gabriel-Welsh multiple F-test (SAS Institute, Inc , 1985), were used to analyze bw Clinical pathology parameters were analyzed by a one-factorial analysis of covariance (SAS Institute, Inc , 1985) Histopathology results were analyzed using a two-factorial analysis of variance with multivariate comparisons (Barcikowski, 1983)

DERMAL TOXICITY

Dermal toxicity was determined on the rocket fuel mixture only The backs and sides of five male rabbits were clipped prior to dosing A dose of 2 g rocket fuel mixture/kg bw was applied to the backs of the rabbits and spread evenly to both sides The dose was kept in place by applying an eight-ply gauze patch over the test substance A clear plastic wrap was then applied over the entire midsection and was held in place with Vetrap (3M, St Paul, MN) and elastoplast tape The test

material remained in contact with rabbit skin for 24 h, at which time the tape, plastic wrap, and gauze were removed and the residual test material was wiped from the skin. Records were kept of body weights (at time of dosing and on Days 1, 7, and 14 posttreatment), signs of toxicity, and mortality. Gross pathology was performed at the termination of the study (Day 14).

SECTION 4

RESULTS

ORAL TOXICITY

The group of five F-344 rats gavaged with 3.5 g quadricyclane/kg bw all died within 24 h following treatment. Three rats died overnight while the other two died the following morning. All quadricyclane-treated rats were prostrate immediately following dosing and remained so until death. Five male rats gavaged with 1.7 g rocket fuel mixture/kg bw were prostrate through 24 h posttreatment, but returned to normal appearance and activity on Day 2, and all survived the 14 day posttreatment observation period. Body weight loss on posttreatment Day 1 reflects the lack of food or water intake during the 24 h period immediately following treatment. The group of five rats gavaged with 4.3 g RP-1/kg bw showed no signs of toxic stress, but had a slight depression in body weight gain (Table 1). All RP-1-treated rats survived the 14 day posttreatment period.

Blood parameters measured on all rats prior to treatment and on survivors at 14 days posttreatment showed no treatment-related differences when compared to the saline control group. Because all quadricyclane treated animals died, no posttreatment blood determinations were performed.

Results from the histopathologic examination of the tissues removed following death were equivocal. Tissues removed from three of the dead quadricyclane-treated animals were autolytic which precluded evaluation of subtle changes. Tissues from the two remaining animals that died (these were necropsied immediately after death) appeared to be normal. Tissues from the remaining animals sacrificed following the 14 day observation period were all normal.

DERMAL TOXICITY

One of the five rabbits treated dermally with 2 g rocket fuel mixture/kg bw for 24 h died of accidental injury during the treatment period. The four remaining rabbits survived the 14 day observation period and appeared normal upon gross observation. Body weight gains during the 14 day observation period appeared normal (Table 2).

TABLE 1. BODY WEIGHTS (g) OF RATS FOLLOWING ORAL GAVAGE

Group	Animal #	Day -1	0	1	7	14
Saline	17	164 6	162 0	176 9	191 0	213 3
	22	167 0	163 5	176 4	197 4	213 2
	10*	189 4	196 1	197 7	214 1	233 0
	15	159 3	154 9	167 8	182 3	202 1
	04	176 7	174 2	185 5	200 0	216 9
Mean		171 4	170 1	180 9	197 0	215 7
SD		11 9	16 0	11 3	11 8	11 4
RP-1	21	157 7	153 9	163 1	174 8	190 2
	23	179 1	175 9	181 8	193 6	214 2
	09	164 3	158 6	171 2	178 9	199 8
	19	173 4	167 9	179 0	196 3	215 5
	20	171 4	171 6	173 2	192 5	207 5
Mean		169 2	165 6	173 7[a]	187 2[b]	205 4[b]
SD		8 3	9 1	7 3	9 7	10 6
Mixture	03	184 2	179 8	163 3	189 1	216 0
	13	164 9	159 7	142 1	181 5	208 2
	07	163 5	157 0	143 1	183 1	211 4
	16	177 8	172 7	158 8	195 7	234 1
	08	160 0	157 5	141 5	182 7	210 2
Mean		170 1	165 3	149 8[b]	186 4[b]	216 0
SD		10 4	10 3	10 4	6 0	10 5
Quadricyclane	02	184 4	182 4	----[c]		
	01	166 3	160 6	----[c]		
	14	163 4	157 4	----[c]		
	06	175 2	167 4	156 6	----[c]	
	18	153 9	149 3	140 6	----[c]	
Mean		168 6	163 4	148 6		
SD		11 6	12 4	11 3		

* Animal was not fasted prior to treatment
[a] Significantly different from saline control at $p \leq 0.05$
[b] Significantly different from saline control at $p \leq 0.01$
[c] Animal died prior to weighing

TABLE 2. BODY WEIGHT (kg) OF RABBITS FOLLOWING DERMAL APPLICATION OF 2 g ROCKET FUEL MIXTURE/kg bw

Animal #	Day 0	1	7	14
01	2 6	2 4	2 7	2 8
02	2 5	2 3	2 7	2 7
03	2 9	2 7	2 9	3 0
04	2 7	2 5	2 6	2 8
05	2 7	2 4	---[a]	---

[a] Animal died of self-inflicted injury

SECTION 5

DISCUSSION

Death following oral gavage of 3.5 g quadricyclane/kg was rapid and no target organs of toxicity were identified. Rats dosed with the rocket fuel mixture survived the 14-day observation period and no gross or microscopic lesions were observed. Although an oral LD_{50} of quadricyclane cannot be calculated from this limited data, the results of the assay with the rocket fuel mixture containing 0.7 g quadricyclane per g mixture and the neat quadricyclane indicate an LD_{50} between 1.19 and 3.5 g/kg. The maximum toxicity rating that would be assigned to this compound is moderately toxic, which includes compounds with oral LD_{50}s ranging between 0.5 and 5.0 g/kg (Kraassen and Doull, 1980). The clinical signs produced following oral ingestion of quadricyclane are consistent with an effect on the central nervous system (CNS). However, because of the rapid onset of prostration in these animals, behavioral signs typical of CNS toxicity were not observed. Examination of animals treated at the lower dose level indicates that the toxic effects of low doses of this compound are reversible with no indication of tissue damage noted 14 days posttreatment.

Ingestion of these quantities of quadricyclane (1.2 to 3.5 g/kg) could be equated to a 70 kg man drinking 3 to 8 oz of the compound, a quantity not likely to be ingested accidentally. However, ingestion of even small quantities of the compound, especially the rocket fuel mixture which contains kerosene, could be aspirated, resulting in cyanosis, tachycardia, tachypnea and, possibly, chemical pneumonitis. Hydrocarbon- or chemical-induced pneumonias normally develop within 24 h of the ingestion and usually require several weeks for complete resolution.

The rocket fuel mixture is not lethal when in contact with rabbit skin for 24 h at the EPA limit dose of 2 g/kg. In this study, the rabbit skin surface in contact with the compound represented approximately 10%, of the total body surface of the rabbit. If one relates this body exposure to humans, it would be somewhat similar to having both legs (or 13% of total body surface, excluding feet) (Berkow, 1931), exposed to the rocket fuel mixture. Both hands (5%) or both arms (13.5%) would serve as other means of comparative dermal exposure. Bartek et al., (1972) determined that rabbit skin was much more permeable to topically applied compounds than was human skin. Therefore, if percutaneous absorption of the rocket fuel mixture was not a hazard in the rabbit, the possibility of toxic effects by this route in humans is unlikely. In general, prolonged contact of kerosene or related hydrocarbons with skin may result in irritation, drying, and dermatitis.

SECTION 6

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QUALITY ASSURANCE

The study, "Acute Toxicity of Quadricyclane," was conducted by the ManTech Environmental Technology, Inc., Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Standards, 40 CFR 792. No claim will be made that this was a "GLP" study as no attempt was made to adhere to the strict requirements of those standards.

The various phases of this study were inspected by members of the Quality Assurance Unit. Results of the inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION	ITEM INSPECTED
August 5, 1992	Animal QC (Lot# K81)
August 18, 1992	Pre-dosing blood specimens and gavage.
September 1, 1992	Animal sacrifice and 14-day post-dosing blood specimens.
November 17-19, 1992	Data and final report audit.
December 8, 1992	Audit response review.
January 8, 1993	Audit response review.

The Quality Assurance Unit has determined through review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretations presented in this Final Report.

M. G. Schneider
QA Coordinator
Toxic Hazards Research Unit

Date January 8, 1993