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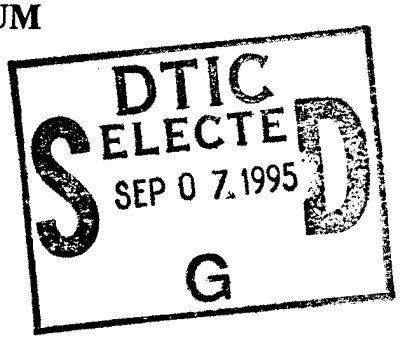
ARMSTRONG LABORATORY

ACUTE AND SUBACUTE TOXICITY EVALUATION OF AMMONIUM DINITRAMIDE



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TECHNICAL REVIEW AND APPROVAL

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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

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
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The Department of Defense is considering replacing ammonium perchlorate with ammonium dinitramide (ADN). Ammonium dinitramide, a Class 1.1 explosive oxidizer, would be used in solid rocket engine propellant mixtures as well as used as a high explosive. The first objective of this study was to provide acute hazard information on ADN to industrial hygienists responsible for the safe handling of this material. Because the most likely form of accidental exposure would be by the dermal or oral routes, acute oral and dermal toxicity tests were performed. Oral gavage of ADN solutions greater than 1 g/kg resulted in mortality preceded by convulsions. An oral LD_{50} of 823 mg/kg was determined for male Fischer 344 rats. Rats receiving nonlethal doses of ADN showed no treatment-related effects when necropsied following a 14-day observation period. Dermal toxicity in New Zealand white rabbits, performed at the Environmental Protection Agency's limit dose level of 2 g/kg body weight, resulted in no mortality, no clinical signs or differences in clinical pathology following a 14-day post-treatment observation period.

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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. This document serves as a final report on the acute and subacute toxicity evaluation of ammonium dinitramide in New Zealand white rabbits, Fischer 344 rats, and Sprague-Dawley rats. The research described in this report began in April 1993 and was completed in January 1994 under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F22). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory. This study was sponsored by the U.S. Air Force under the direction of Maj Donald Tocco.

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 the Care and Uses of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #85-23, 1986, and the Animal Welfare Act of 1966, as amended.

The authors would like to acknowledge Richard Godfrey, Harold Leahy, and Jerry Nicholson for their excellent technical assistance.

TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS

ADN	Ammonium dinitramide
DOD	Department of Defense
F-344	Fischer 344 (rats)
g	gram
h	Hour
kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliter
NZW	New Zealand white (rabbits)
SD	Sprague-Dawley (rats)
SIDS	Screening Information Data Set

SECTION I
INTRODUCTION

The Department of Defense (DOD) currently is considering replacing ammonium perchlorate with ammonium dinitramide (ADN). Ammonium dinitramide, a Class 1.1 explosive oxidizer, would be used in solid rocket engine propellent mixtures and as a high explosive. No acute or chronic toxicity information is currently available for ADN; however, field reports from exposed personnel indicate that the compound is readily absorbed by the skin, resulting in numbness of the fingers (Koppes, 1993).

Preliminary toxicology information was required for this compound to determine potential acute toxicity hazards of ADN. The most significant exposure routes expected would be dermal and possible accidental ingestion. This study addresses these potential routes of exposure as well as providing data necessary in selecting dose levels for a 90-day modified Screening Information Data Set (SIDS) study.

In addition, increased interest in ADN by the DOD indicated that a SIDS test would be conducted on this compound. A SIDS test provides preliminary information on general toxicity, reproductive toxicity, and developmental toxicity following repeated administration of the test material. The ADN, which is highly water soluble, would be administered to rats via drinking water. To determine palatability of ADN in drinking water and to possibly identify target organs, a three-week range-finding study was included in this project.

SECTION II
MATERIALS

Test Compound

The ammonium dinitramide [$NH_4N(NO_2)_2$] was supplied by SRI International, Menlo Park, CA. The test compound, a water-soluble powder, is light sensitive and was maintained in an enclosed cabinet. The test compound is known to be contaminated with 1 to 2% ammonium nitrate (Koppes, 1993).

Animals and Animal Husbandry

Male Fischer 344 (F-344) rats, six weeks of age, and male and female Sprague-Dawley (SD) rats, nine weeks of age, were purchased from Charles River Breeding Labs, Raleigh, NC. Male New Zealand white (NZW) rabbits weighing between 2 and 3 kg were purchased from Myrtle's Rabbitry, Inc., Thompsons Station, TN. All animals were identified by tattoo and subjected to a two-week acclimation period. Rats used in the oral gavage toxicity study were group housed (two per cage) in clear plastic cages with wood-chip bedding (Betta-Chip, Northeastern Products Corp., Warrensburg, NY). Rats used in the 3-week palatability drinking-water study were single housed under the same conditions. The rabbits were housed individually in suspended, wire-bottom, stainless steel cages. Water and feed (Purina Formulab #5008 for rats, Purina Rabbit Chow #5320) were available *ad libitum*, except for 12 h prior to oral gavage dosing. Animal room temperatures were maintained at 21 to 28 °C and the light/dark cycle was set at 12-h intervals.

SECTION III
METHODS

Oral Toxicity

Five male F-344 rats per dose level were fasted 12 h prior to administration of the oral dose. The ADN was diluted in saline at a concentration that provided a constant dose volume of 1 mL per 100 grams of body weight. The rats were individually weighed prior to dosing to determine the proper dose volume. A group of five rats were initially dosed at the Environmental Protection Agency's limit test dose of 5 g ADN/kg body weight. Following that, geometrically spaced dose levels were used which allowed for the calculation of an LD_{50} using the moving average method of Weil (1952). A group of five rats were gavaged with an equivalent volume of saline. Surviving rats were weighed on days 1, 7, and 14 posttreatment. At necropsy, sections of stomach, small and large intestine, liver, kidneys, and gross lesions were sampled from selected dead animals and all surviving animals for histopathologic examination.

Dermal Toxicity

The backs and sides of five male NZW rabbits were clipped 5 h prior to dosing. A neat dose of 2 g ADN/kg body weight 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 the 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). Sections of skin (treated and untreated), liver, and kidneys were removed for histopathologic examination. Blood samples (via the vena cava) were taken at necropsy for AST, ALT, LDH, GGT, and alkaline phosphatase measurements. A complete hematology evaluation also was made. Erythrocytes were enumerated on a Coulter counter (Coulter Electronics, Hialeah, FL) and sera for clinical chemistry evaluation were assayed on an Automated Chemistry Analyzer (DuPont Company, Wilmington, DE). Selected hematological parameters and absolute leukocyte differentials were determined according to established procedures.

Three-Week Palatability Study

Three SD rats, per sex, received ADN in drinking water for a three-week

period (21 days). Treatment levels were 1.0, 0.5, 0.25, or 0.12 g ADN/L drinking water. A control group of three rats per sex was maintained on water obtained from the animal drinking water system of Building 838. The same water source was used for the four ADN-treated drinking-water solutions, which were prepared as needed. Water consumption was measured in individual rats for a two-week period prior to treatment and during the three-week treatment period. All rats were observed daily and weighed weekly. At necropsy blood samples were analyzed for methemoglobin concentration using a cooximeter (Model IL282, Instrumentation Laboratory, Lexington, MA). Liver, heart, and spleen, as well as testes in male rats, were weighed. No tissues were prepared for histopathologic evaluations.

SECTION IV
RESULTS

Oral Toxicity

All rats orally gavaged at 5, 2, and 1 g ADN/kg body weight died within an hour of dosing (Table 1). In each case, death was preceded by convulsions (both tonic and clonic spasms). Rats gavaged with 0.5 g ADN/kg body weight displayed mild tremors that persisted for several hours. Surviving rats gained weight similar to the control group during the 14-day posttreatment period. Peroral administration of ADN to fasted male rats produced an LD_{50} value of 823 mg/kg.

TABLE 1. ACUTE ORAL TOXICITY OF ADN

Dose Level (g/kg)	Mortality Ratio	Time to Death
5.0	5/5	<0.5 h
2.0	5/5	1.0 h
1.0	5/5	1.0 h
0.5	0/5	-----
0.0 (control)	0/5	-----
	LD_{50} = 823 mg/kg	

Gross observations in rats that died following treatment included of reticulated livers, red liquid covering the brain, and multiple discolorations on the glandular portion of the stomach. Congestion, either as a postmortem change or an agonal event at or near the time of death, was observed in most tissues examined histopathologically. The red fluid observed on the meninges was most likely blood-tinged serum that seeped from congested blood vessels after the rats died. Similarly, a dark reticulated liver is a sign of congestion. The purple discoloration of the glandular stomach is attributed to hemorrhage and thought to be a treatment-related effect. Tissues examined from rats that survived the 14-day observation period were found to be essentially normal.

Dermal Toxicity

The rabbits were treated with 2 g ADN/kg body weight and maintained 14-days posttreatment. No mortality occurred and all rabbits appeared unaffected by treatment. Blood evaluations, measured 14 days following treatment, were

all within normal limits. All tissues examined histopathologically were essentially normal. No evidence of dermal irritation was noted in the skin sections sampled.

Three-Week Palatability Study

No clinical signs of toxic stress were observed and no mortality occurred in any of the rats during the three-week treatment period. No differences were noted in mean water consumption or mean body weights of treated rats when compared to their respective control groups. During the study, male rats consumed approximately 46 mL/day and the females approximately 28 mL/day resulting in doses of approximately 98, 49, 24, and 6 mg ADN/kg/day for male rats and approximately 120, 60, 28, and 12 mg ADN/kg/day for female rats.

No treatment-related increases in methemoglobin concentrations were noted at necropsy. Mean absolute and relative (to body weight) organ weights of the treated rats did not differ significantly from the weights of their respective control groups.

SECTION V
DISCUSSION

Ammonium dinitramide proved to be more toxic than ammonium nitrate which has an oral LD_{50} of 4.8 g/kg. When ADN was administered to rats by gavage at doses between 1.0 and 5.0 g/kg, death occurred rapidly, preceded by convulsions. Acute toxicity produced by nitrites and nitrates results from the vasodilating properties of the compounds and from methemoglobinenemia-induced hypoxia. These compounds can produce peak vasodilating effects rapidly, often within 30 seconds (Ellenhorn and Barceloux, 1988). Signs and symptoms of nitrate-induced vasodilation include syncope, tachycardia, decreased peripheral vascular resistance, and cardiovascular collapse. Gross examination of the animals that died following dosing displayed discoloration in many vascular organs as well as seepage of serum on the meninges, indicative of the severe vasodilating effects of the treatment. The rapid onset of death precluded the observation of discolored blood indicative of methemoglobinenemia; however, the related hypoxia was probably the cause of the convulsions that preceded death. Gastric hemorrhage in animals following ingestion of excessive amounts of nitrates has been reported by Valli (1993). An oral LD_{50} of 823 mg/kg was established for this compound, which would place it in the moderately toxic classification of compounds having oral LD_{50}s ranging between 0.5 and 5.0 g/kg (Klaassen and Doull, 1980). Ingestion of this quantity of ADN (823 mg/kg) could be equated to a 70 kg man ingesting approximately 58 g of the compound, a quantity not likely to be ingested accidentally.

The rabbit dermal exposure test does not provide the type of information that would define neurologic effect to the extremities such as numbness of fingers. However, it has been determined that a dose of 2 g/kg was not lethal and no persistent blood or tissue abnormalities resulted. In this study, the rabbit 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) in contact with the compound. 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 ADN was not a toxic hazard in the rabbit, the possibility of toxic effects by this route in humans is questionable.

Oral ingestion of low levels of ADN in drinking water likewise showed no signs of toxic effects in rats for the parameters studied in this project. Information derived from this project was used for setting drinking-water ADN concentrations for a modified SIDS study. Results from the SIDS study will provide more definitive information on the effects of long-term administration of ADN.

SECTION VI
REFERENCES

Bartek, M.J., J.A. LaBudde, and H.I. Maibach. 1972. Skin permeability, *in vivo*: Comparison in rat, rabbit, pig, and man. *J. Invest. Dermatol.* **58**:114-123.

Berkow, S.G. 1931. Value of Surface Area Proportions in the Prognosis of Cutaneous Burns and Scalds. *Am. J. Surg.* **11**:315.

Ellenhorn, M.J. and D.G. Barceloux. 1988. Medical Toxicology, Diagnosis and Treatment of Human Poisoning. Elsevir, New York. pp. 848-849.

Klaassen, C.D. and J. Doull. 1980. Evaluation of Safety: Toxicologic Evaluation. In: J.D. Doull, C.D. Klaassen, and M.D. Amdur, eds. *Casarett and Doull's Toxicology, The Basic Science of Poisons,* 2nd Edition, New York: MacMillan, p. 12.

Koppes, William. 1993. Personal communication.

Valli, V.E.O. 1993. The hematopoietic system. In: K.V.F. Jubb, P.C. Kennedy, and N. Palmer, eds. *Pathology of Domestic Animals,* 4th edition, New York: Academic Press. pp. 208-209.

Weil, C.S. 1952. Tables for Convenient Calculation of Median-Effective Dose (LD_{50} or ED_{50}) and Instructions for Their Use. *Biometrics* **8**,3:249-263.

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