FOREWORD

The investigations described in this report were carried out during the period from November 1960 to February 1962. The research was conducted in the Toxic Hazards Section, Physiology Branch, Biomedical Laboratory of the 6570th Aerospace Medical Research Laboratories, under Project No. 6302, "Toxic Hazards of Propellants and Materials," and Task No. 630202, "Pharmacology and Biochemistry."

The invaluable assistance rendered by Capt D. F. Dixon, Jr., USAF, VC, Capt G. Fischer, USAF, VC, Mr. K. Atkinson, Mrs. Mildred K. Pinkerton, Miss Barbara Reynolds, and Maj J. R. Prine, USAF, VC, of the 6570th Aerospace Medical Research Laboratories is gratefully acknowledged.

Animal experimentation reported herein was conducted in accordance with "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

ABSTRACT

The acute LD_{50} of UDMH in mice, rats, dogs, and monkeys by intraperitoneal administration was found to be 125 mg/kg, 104 mg/kg, 60-100 mg/kg, and 60-100 mg/kg, respectively. All animals exhibited clonic-tonic convulsions as the predominant symptom, and death was via respiratory arrest. Dogs and monkeys routinely showed emesis within 15-60 minutes following dose of UDMH, regardless of route. In the anesthetized dog UDMH did not alter the effects of epinephrine, norepinephrine, acetylcholine, histamine, or reserpine on blood pressure. It did not significantly affect autonomic ganglia or postganglionic nerve endings, nor did it markedly alter the electrocardiogram or blood pressure in 1-2 hours. In the unanesthetized dog, however, blood pressure was significantly increased until convulsions and respiratory arrest occurred.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

JOS. M. QUASHNOCK
Colonel, USAF, MC
Chief, Biomedical Laboratory

PHARMACOLOGY AND TOXICOLOGY OF 1, 1-DIMETHYLHYDRAZINE (UDMH)

INTRODUCTION

1, 1-dimethylhydrazine (UDMH) has become increasingly important, from a medical viewpoint, because of its large scale use as a missile propellant. Its reputation as a potentially toxic agent is well documented. Examination of the literature has revealed a great deal of data regarding the inhalation and parenteral toxicology of UDMH but little information on its pharmacodynamic properties (refs. 1, 2, 3, and 4). This report concerns the pharmacologic and toxicologic activity of UDMH.

MATERIALS AND METHODS

All experiments were performed using Practical Grade UDMH obtained from Eastman Organic Chemicals, Rochester, N. Y.

The acute (single dose, 24-hour) intraperitoneal (i. p.) toxicity of UDMH was determined in male albino Swiss mice (19-25 g). The statistical method for computation of the LD_{50} was that of Litchfield and Wilcoxon (ref. 5). Limited acute toxicity data were obtained from mongrel dogs and cynomolgus monkeys following intravenous (i. v.) and intraperitoneal administration of UDMH.

Subacute toxicity data were obtained using 30 male Sprague-Dawley rats weighing 182-210 g. The animals received 10 mg/kg/day UDMH i. p. for a period of 20 days. Body weights were determined and recorded five times during the experiment. A control group of 30 rats receiving saline i. p. was programmed simultaneously. On the day following the last injection, an LD_{50} for UDMH was determined for both the UDMH-treated and control groups. All animals were killed at the end of the experiment, and gross and microscopic pathology was performed on all major organ systems.

The effect of UDMH on pentobarbital sleep time was determined in male Swiss mice (24-46 g). A dose of either 100 mg/kg UDMH i. p. or saline 0. 2 ml was administered to controls 30 minutes prior to a dose of pentobarbital sodium 65 mg/kg i. p. The elapsed time until a mouse attained an erect sitting position was taken as the end-point of the experiment.

The effect of UDMH on the time of onset and severity of pentylenetetrazol (Metrazol)^{R} induced convulsion was determined in male albino Swiss mice (21-23 g). Fifteen mice were given 100 mg/kg UDMH i.p. 30 minutes prior to the i.p. administration of 85 mg/kg Metrazol, and the times to the appearance of convulsions and death were recorded. Control mice were given Metrazol for comparison.

When exposed to light for a period of time, UDMH changes to a yellow-orange solution. To determine if there was a concomitant change in toxicity during the aging process, a solution containing 10 mg UDMH per ml distilled water was exposed to light in a tightly-stoppered glass volumetric flask for 27 days. An LD_{50} in mice was determined using a fresh solution, and toxicity tests were run at 8, 12, and 27 days thereafter, for comparison.

Dogs of either sex were used to study the effects of UDMH on the cardiovascular system, autonomic and somatic-motor nervous systems, and smooth muscle (duodenal) motility. The animals were anesthetized with pentobarbital sodium 30 mg/kg. Recordings were made on a Sanborn 4-channel recorder. Carotid arterial pressures were measured with a Statham P 23 AA pressure transducer. Duodenal motility was recorded by means of a water-filled balloon attached to a Statham P 23 BB pressure transducer. The effects of UDMH on autonomic nerves and ganglia were studied by stimulation of the peripheral and central cut ends of the right vagus nerve by a 4-volt, square-wave pulse, 1 millisecond in duration at a frequency of 40 cps for 5 seconds. The response of the nictitating membrane to stimulation of the central end of the vagus nerve was noted. All test drugs and agents were given through an indwelling femoral vein catheter. Electrocardiographic tracings were recorded using standard leads.

Unanesthetized dogs were also used to determine the effects of UDMH on ECG and blood pressure. Blood pressure was recorded by attaching a pressure transducer to an indwelling catheter inserted into a femoral artery under local anesthesia.

RESULTS

Acute Toxicity

The acute 24-hour LD_{50} of UDMH is 125 mg/kg in the mouse and 104 mg/kg in the rat (table I). These results are not consistent with those of Witkin (ref. 2) who reported LD_{50}'s of 290 mg/kg i.p. for the mouse and 131 mg/kg i.p. for the rat. The mice displayed clonic-tonic convulsions occurring between 50 and 120 minutes following the dose, and death was caused by respiratory arrest. Mice also showed tonic-extension of the hind limbs immediately before death, a phenomenon not exhibited by rats.

Seven dogs were given 100 mg/kg UDMH either by the i.v. or i.p. route. All animals vomited in 30-60 minutes and showed clonic-tonic convulsions in 30-120 minutes. Death by respiratory arrest occurred in 1-4 hours. Symptoms occurred in the same time sequence regardless of route of administration. Dogs given doses of 50 mg/kg either i.p. or i.v. convulsed in 2-4 hours but survived.

Macaca iris (cynomolgus) monkeys were given i.p. doses of 1-40 mg/kg UDMH in an effort to define the emetic and convulsive threshold in this species. The threshold for emesis is approximately 30 mg/kg and the convulsive threshold is approximately 40 mg/kg. The monkeys receiving 40 mg/kg displayed clonic-tonic convulsions in 2-4 hours after UDMH was administered and the convulsions lasted 0.5-1.5 minutes. No animals had more than two convulsive episodes. The UDMH did not hamper the appetite of the animals, and some of the animals ate within 5 minutes after a convulsion.

TABLE I

THE ACUTE TOXICITY OF UDMH

Species	Route	LD_{50} (mg/kg)	Slope Function
Mouse	i.p.	125 (117-134)*	1.19 (1.09-1.31)
Rat	i.p.	104 (95-114)	1.12 (0.89-1.40)
Dog	i.p., i.v.	60-100†	---
Monkey	i.p.	60-100 †	---

* 95 percent confidence limits
† approximate

The acute toxicity of UDMH in mice shifted as a water solution of the compound was allowed to deteriorate (table II). An 8-day-old solution of UDMH produced an LD_{50} of 100 mg/kg while the same solution 12 days old gave an LD_{50} of 78 mg/kg; this in contrast to 125 mg/kg for a fresh solution. After 27 days the solution was a very dark yellow; however, it was less toxic than the 12-day-old material.

TABLE II

THE ACUTE TOXICITY OF AGED UDMH IN MICE

Dose mg/kg	No. Dead/No. Used (%)			
i.p.	Fresh	8 Day	12 Day	27 Day
70		1/20 (5)	2/12 (17)	0/12 (0)
85	0/6 (0)	5/32 (16)	9/12 (75)	0/12 (0)
100	5/38 (13)	14/32 (44)		1/12 (8)
115	7/20 (35)			
130	11/20 (55)	20/20 (100)		
150	17/20 (85)			
175	20/20 (100)			

Subacute Toxicity

The i.p. administration of UDMH 10 mg/kg/day for 20 days failed to cause weight loss, convulsions, or other clinical symptoms in 30 experimental rats (table III). This dosage (10 mg/kg) represents approximately 10 percent of the established acute LD_{50} for rats. The acute LD_{50}'s determined on the 21st day of the experiment failed to reveal any significant difference between UDMH-treated and control animals. Gross and microscopic pathological study on the animals showed no significant pathologic changes. The data indicate that UDMH, when given in relatively high doses, does not exhibit accumulative effects. The experiment further demonstrates that neither increment nor decrement in tolerance is experienced in rats subjected to repeated sub-convulsive doses of the agent.

TABLE III

TWENTY-DAY SUBACUTE TOXICITY OF UDMH IN THE RAT

Dose UDMH mg/kg/day i.p.	Number of Rats	Day 1 Average Weight	Day 20 Average Weight	Day 21 LD_{50} mg/kg
0	30	197	313	100
10	30	199	316	105

UDMH and Pentobarbital Sleep Time

Since UDMH was shown to produce convulsions, we wanted to determine whether the compound would decrease pentobarbital sleep time in mice or pentobarbital would decrease UDMH toxicity. Analysis of the data (table IV) shows that UDMH did not affect significantly the pentobarbital sleep time of mice. Further, pentobarbital (65 mg/kg) did not prevent convulsions and death. A dose of 100 mg/kg UDMH in mice usually kills between 5 and 15 percent. In this experiment, 22 percent of the mice receiving both UDMH and pentobarbital convulsed and died.

TABLE IV

THE EFFECT OF UDMH ON PENTOBARBITAL SLEEP TIME IN MICE

Dose UDMH mg/kg i.p.	Dose Pento. mg/kg i.p.	Number of Mice	Av. Sleep Min. (range)	Number Dead
0	65	20	87 (42-136)	0
100	65	18	81 (31-200)	4
100	0	38	----	5

UDMH and Metrazol Convulsions

The onset of seizures and death in Metrazol-treated mice was hastened by UDMH (table V). The difference in the onset of convulsions and time of death between Metrazol- and Metrazol-UDMH treated animals was significant at the $p = 0.05$ level. A 100 mg/kg dose of UDMH caused convulsions in 106-190 minutes in 30 percent of the mice tested. Since UDMH when given alone could not have caused convulsions within 0.5-3 minutes, it is extremely attractive to postulate that UDMH causes a reduction in the convulsive threshold to Metrazol; the reverse could also be true.

TABLE V

THE EFFECT OF UDMH ON METRAZOL SEIZURES IN MICE

Dose UDMH mg/kg i.p.	Dose Metrazol mg/kg i.p.	Number of Mice	Av. Time to Seizure Min. (range)	Av. Time to Death Min. (range)
100	0	20	138 (106-190) 6/20*	115 1/20**
0	85	15	2.6 (1-7) 15/15*	7.0 (3-13) 15/15**
100	85	15	1.2 (0.5-3) 15/15*	4.8 (0.5-9) 15/15**

* Number with seizures
** Number dead

Cardiovascular, Smooth Muscle, and Autonomic Responses

In the pentobarbital-anesthetized dog, doses of UDMH ranging from 1-50 mg/kg i.v. produced no effect or a slight transient fall in blood pressure for 1-3 minutes. A dose of 100 mg/kg i.v. caused an approximate 50 percent decrease in blood pressure lasting for about 2-1/2 minutes (figure 1). There was no change in heart rate or ECG and only a slight transient diminution of respiratory rate. Duodenal motility was enhanced for the duration of the experiment until the animal died.

Doses of UDMH ranging from 1-200 mg/kg i.v. did not alter the blood pressure effects of epinephrine (2 μg/kg i.v.), acetylcholine (2 μg/kg i.v.), norepinephrine (2 μg/kg i.v.), histamine phosphate (2 μg/kg i.v.), or of reserpine (2.5 mg/kg i.v.). Further, the blood pressure effect induced by stimulation of the peripheral cut end of the right vagus nerve was not altered by UDMH administration, nor was the response of the nictitating membrane altered. The lack of an enhancing effect by UDMH on pressor agents and the fact that it did not cause a reversal of the blood pressure effects of reserpine indicates that UDMH does not possess monoamine-oxidase inhibitory properties to any great extent. Finally, UDMH does not cause any acute effects at autonomic ganglia or postganglionic nerve endings.

The dogs routinely convulsed in 2-4 hours, although they were under surgical plane anesthesia. In a number of experiments, d-tubocurarine was administered in an effort to prevent skeletal muscle activity during convulsions. This was done while the animals were supported by a respiration pump. Despite heavy curarization, generalized muscle fasciculations were still evident. This may indicate some peripheral action of UDMH beyond the motor end plate in skeletal muscle. All animals that were given 100 mg/kg or more UDMH finally died in cardiovascular collapse, even though respiration was supported artificially. Fluids, plasma expanders, and pressor agents failed to alter this cardiovascular collapse.

A remarkably different effect on blood pressure was experienced when UDMH (120 mg/kg i.v.) was administered to the unanesthetized dog. In this instance, blood pressure markedly increased and remained elevated until convulsions began (figure 2). In these experiments, the animals were immediately given an anesthetizing dose of pentobarbital sodium at the first sign of convulsions. The magnitude of increase in blood pressure was not attributable to the concomitant increase in heart rate. The exact mechanism of this phenomenon has not been elucidated.

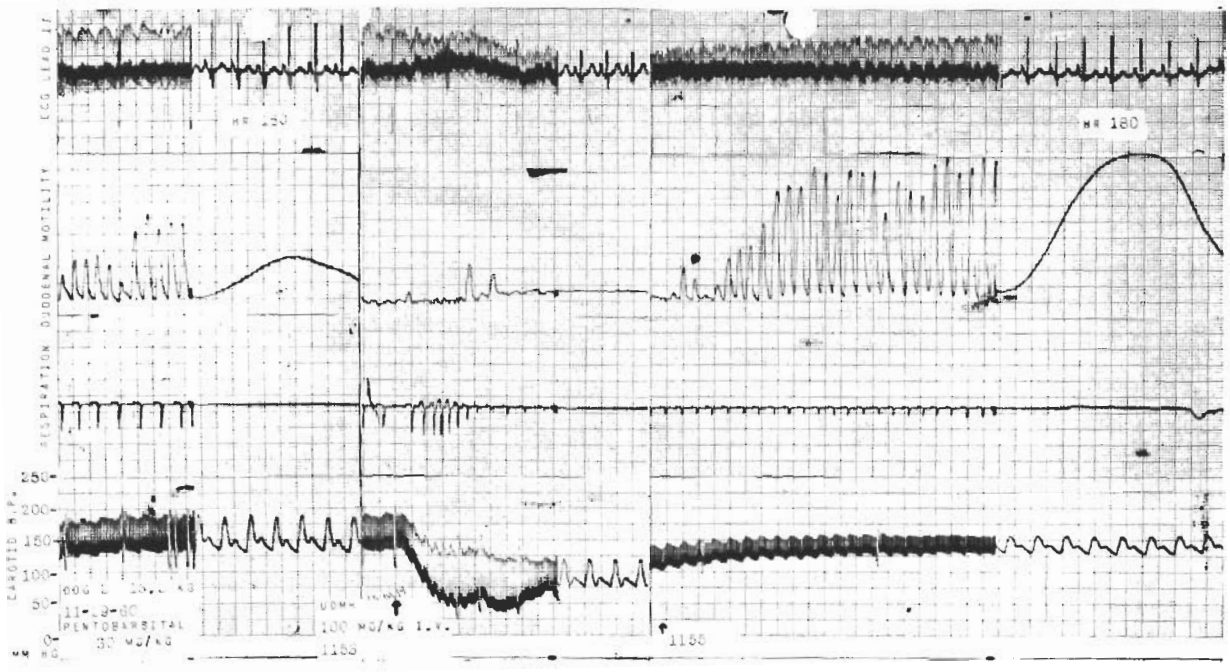

Figure 1. Affect of UDMH on Anesthetized Dog

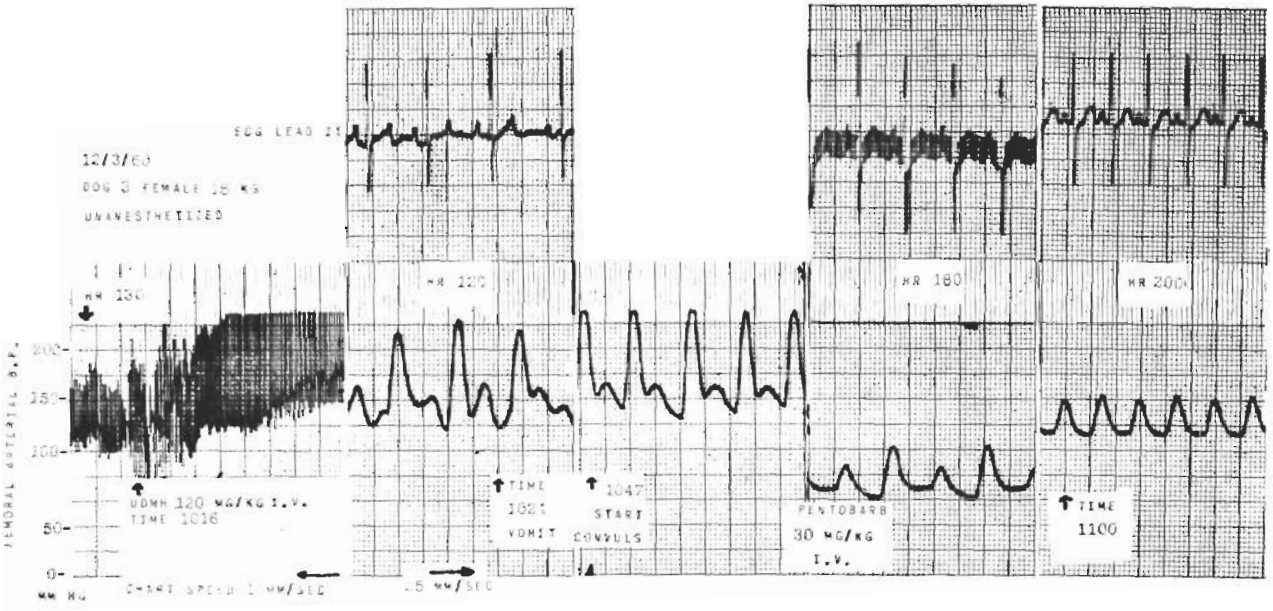

Figure 2. Affect of UDMH on Unanesthetized Dog

DISCUSSION

The information gathered from this research does not, unfortunately, provide the clues to the mechanism of toxicological activity of UDMH. Nothing in these studies has shown the reason for the long latent period between dosage and the onset of central nervous system (CNS) effects. The mechanism of death, however, has been shown to be respiratory arrest with cardiovascular collapse as a secondary cause. Administration of an acute dose of UDMH appears to produce no discernible manifestations until vomiting and convulsions occur. The primary activity of UDMH is apparently centered in the central nervous system. Recent work by Medina et al* has implied that UDMH effects on the CNS are due to an effect on the gamma aminobutyric acid shunt. More specifically, it appears to act as an inhibitor of glutamic acid decarboxylase, which could explain the reason for the long delay in CNS activity even when UDMH is given intravenously. The above-mentioned work, studies by Reeves (ref. 6) and Back et al** have further shown that certain Vitamin B_6 analogues can effectively prevent convulsions and death of laboratory animals exposed to lethal doses of UDMH. The mechanism for this protection is not completely understood, but it undoubtedly is related to the action of pyridoxine as a co-enzyme to decarboxylase in the gamma aminobutyric acid shunt.

Of practical interest is the finding that UDMH dose-response curves are characterized by very steep slopes. A steep slope combined with an apparent species nonspecificity makes it easier for the toxicologist to extrapolate data to possible human toxicity. Additional insight has been provided for use in the transposition of animal data to human tolerance by Reynolds et al (ref. 7) who showed that in monkeys doses of 30 mg/kg UDMH i.p. caused no decrement of performance function in a shock avoidance test program.

The foregoing information coupled with the fact that UDMH is extremely rapidly excreted in the urine (ref. 8) offers strong support for the belief that a human being could be exposed to at least 10 mg/kg UDMH without harmful effects.

---

*Medina, M.A., H.D. Braymer, and J.L. Reeves, In Vitro Reversal of Glutamic Acid Decarboxylase Inhibition Induced by 1,1-Dimethylhydrazine (Unpublished report), School of Aerospace Medicine, Brooks AFB, Texas

**Back, K.C., M.K. Pinkerton, and A.A. Thomas, Effect of Vitamin B_6 Analogues on UDMH Toxicity (Unpublished), 6570th Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

REFERENCES

1. Jacobson, K.H., J.H. Clein, H.J. Wheelwright, and N. Mayes, "The Acute Toxicity of the Vapors of some Methylated Hydrazine Derivatives," AMA Arch. Ind. Health, Vol 12, p 609, 1955.

2. Witkin, L.B., "Acute Toxicity of Hydrazine and Some of Its Methylated Derivatives," AMA Arch. Ind. Health, Vol 13, p 34, 1956.

3. Rinehart, W.E., E. Donati, and E.A. Greene, "The Subacute and Chronic Toxicity of 1,1-Dimethylhydrazine Vapors," Am. Ind. Hyg. Assoc. J., Vol 21, p 207, 1960.

4. Weeks, M.H., G.C. Maxey, M.E. Sicks, and E.A. Greene, Vapor Toxicity of UDMH in Rats and Dogs from Short Exposures, Aeronautical Systems Division Technical Report 61-526, Wright-Patterson Air Force Base, Ohio, October 1961.

5. Litchfield, J.T., and F. Wilcoxon, "A Simplified Method for Evaluating Dose-Effect Experiments," J. Pharmacol. Exptl. Therap., Vol 96, p 99, 1949.

6. Reeves, J.L., Influence of Large Doses of Pyridoxine Hydrochloride on the Convulsigenic Activity of UDMH in Monkeys, School of Aerospace Medicine Technical Report 62-31, Brooks Air Force Base, Texas, December 1961.

7. Reynolds, H.H., F.H. Rohles, Jr., J. Fineg, K.C. Back, and A.A. Thomas, "The Effects of 1,1-Dimethylhydrazine on Learned Behavior in the Java Monkey," presented at the 33rd Annual Meeting Aerospace Medical Association, April 1962.

8. Back, K.C., M.K. Pinkerton, A.B. Cooper, and A.A. Thomas, Absorption, Distribution and Excretion of 1,1-Dimethylhydrazine, 6570th Aerospace Medical Research Laboratories Report No. MRL-TDR-62-30, Wright-Patterson Air Force Base, Ohio, May 1962.