Biochemical Pharmacology, 1966, Vol. 15, pp. 1325–1332. Pergamon Press Ltd., Printed in Great Britain.

THE METABOLIC FATE OF MONOMETHYLHYDRAZINE AND UNSYMMETRICAL DIMETHYLHYDRAZINE

F. N. Dost, D. J. Reed and C. H. Wang

Science Research Institute and Radiation Center, Oregon State University, Corvallis, Ore., U.S.A.

(*Received* 28 *December* 1965; *accepted* 2 *March* 1966)

Abstract—The respiratory and urinary excretion of intraperitoneally administered unsymmetrical dimethylhydrazine (UDMH) and monomethylhydrazine (MMH) and their metabolites by rats has been studied by means of radiotracer techniques. Animals given a very low dose of UDMH-^{14}C metabolized almost 30 per cent of the compound to respiratory $^{14}CO_2$ in 10 hr. The conversion of a convulsive dose of UDMH-^{14}C to $^{14}CO_2$ amounted to slightly more than 13 per cent at the end of 20 hr. At the various doses of UDMH-^{14}C used, at least 50 per cent of the administered radioactivity appeared in the urine in a 2-day period.

Rats given 0·12 m-mole MMH-^{14}C/kg i.p. respired approximately 45% of the ^{14}C during the following 24 hr. Of the respired radioactivity, 20% to 25% was $^{14}CO_2$; the remainder was $^{14}CH_4$. At the subconvulsive doses, 40 per cent of the administered radioactivity in MMH-^{14}C was excreted in urine. The percentage of urinary excretion of ^{14}C from higher doses of MMH-^{14}C was less, but the net amount excreted was slightly higher.

Toxicity of hydrazines and their derivatives is of importance in applications ranging from rocket propulsion to their use in drugs having effects on the central nervous system. Several investigators have reported on the toxicologic and pharmacologic properties of simple alkylhydrazines,[1–6] but only limited information is available concerning their metabolic fate in intact animals. Back *et al.*[7] investigated the absorption, distribution, and excretion of unsymmetrical dimethylhydrazine (UDMH) in rats, rabbits, cats, dogs, and monkeys, using ^{14}C tracer and colorimetric techniques. Their work suggested that a UDMH metabolite was present in the blood of treated animals and the UDMH was not preferentially concentrated in any organ or tissue. An earlier report from our laboratory confirms this observation.[5] The only reported study of the metabolism of MMH of which we are aware has originated in this laboratory.[8]

The present report describes findings on the metabolic fate of the two compounds, followed by radiotracer means. MMH was found to be converted by rats to respiratory carbon dioxide and methane, whereas UDMH was converted to carbon dioxide, but not to methane. Unidentified urinary ^{14}C compounds derived from UDMH-^{14}C and MMH-^{14}C were also observed.

MATERIALS AND METHODS

UDMH-^{14}C (spec. act. 1·2 mc/m-mole) and MMH-^{14}C (spec. act. 0·8 mc/m-mole) were synthesized by New England Nuclear Corp., Boston, Mass. ^{14}C-Labeled UDMH and

MMH were supplied in glass ampules, under vacuum, in lots of 200 μc and 100 μc respectively. The purity and identity of MMH-^{14}C was established by paper chromatography and by preparation and characterization of a derivative, 2-isonicotinyl, 1-methylhydrazide.[9] Unlabeled UDMH and MMH were obtained from Matheson Coleman and Bell Co., East Rutherford, N.J., and used without further purification.

The rats in these studies were Sprague-Dawley males, each 250 $\pm$ 5 g in weight, and were obtained from Pacord Research, Inc., Portland, Ore. The rats were maintained on Purina laboratory chow and water *ad libitum*.

Administration of the hydrazines to rats

The hydrazine compounds were injected intraperitoneally as aqueous solutions, in volumes less than 1 ml. Dosage varied according to the objective of the experiment and in no case exceeded 75 per cent of a median lethal dose. The respective median lethal doses for rats, as established in this laboratory, are 1·8 m-moles UDMH and 0·6 m-mole MMH per kg body weight. When each original container of unlabeled UDMH or MMH was unsealed, 2- to 3-ml quantities were removed and stored in ampules, under a nitrogen atmosphere, for use in individual experiments. UDMH-^{14}C was dissolved in distilled water and stored similarly in 0·1-ml (approximately 18·7 μc) quantities. MMH-^{14}C was dissolved in 0·5 N HCl to minimize decomposition, and 0·1-ml lots (approximately 9 μc) were kept in separate ampules. Preliminary studies indicated that the presence of HCl does not alter the lethality, toxic symptoms, or metabolic fate of MMH. The amount of radioactivity in each ampule was determined at the time of use by the liquid scintillation counting procedure described below for measurement of radioactivity in urine.

Metabolic studies

Liquid scintillation counting techniques were utilized to assay urinary radioactivity derived from intraperitoneally administered UDMH-^{14}C or MMH-^{14}C. In a typical procedure, 10 μl urine was transferred into a glass counting vial containing 6 ml of an ethanol : ethanolamine solution (1 : 2 v/v) plus 10 ml toluene containing 0·3% (w/v) terphenyl and 0·003% (w/v) POPOP [1,4-bis-2-(5-phenyloxazole)-1 benzene]. The samples so prepared were counted in a liquid scintillation spectrometer (model 314-EX-2, Packard Instrument Co., La Grange, Ill.). A sufficient number of counts was collected to ensure that the relative standard deviation of the counting data was no greater than 2 per cent. Counting efficiency was established by internal standardization.

The radioactivity in the respiratory gases was monitored continuously by a radiorespirometer constructed for this purpose, the design of which has been described previously.[10] Each of the four parallel radiorespirometric systems consists of an animal chamber, ion chamber, vibrating reed electrometer, voltage to frequency converter, and digital scaler. A common air supply system, electronic timer, programmer, and digital data printer complete the system.

In the experiments with MMH-^{14}C, the radiorespirometer was modified to determine separately the radioactivity in the respiratory $^{14}CO_2$ and that appearing in any other volatile ^{14}C-labeled compound(s). The respired gases were first passed through 2 N HCl to remove any free MMH-^{14}C. They were then dried and assayed for total ^{14}C radioactivity in a flow ionization chamber. The gas mixture was finally passed

through a soda-lime column to remove CO_2 and through a second flow ion chamber to determine the radioactivity in neutral ^{14}-labeled volatile compounds.

A Beckman IR-5A infrared spectrophotometer equipped with a gas cell having a 10-m light path and a 4-l volume was used in the identification of methane as a metabolite of MMH. In these experiments, individual rats were injected with unlabeled MMH and placed in a small metabolism chamber. The gas cell was evacuated to a pressure of 1 mm Hg and air was then bled into the cell by way of the animal chamber and a column for drying and CO_2 absorption. At a flow of 100 ml/min, respiratory gases were collected in the cell for measurement of methane over a period of about 40 min; a major portion of the total respired methane derived from MMH was evolved during this time. For the verification of the identity of methane, spectra of collected respiratory gases from MMH-treated rats were compared with spectra of respiratory gases from rats given methane gas intraperitoneally, and with the spectrum of methane gas admitted directly to the infrared gas cell.

RESULTS AND DISCUSSION

Metabolism of UDMH-^{14}C

Figure 1 shows the hourly and cumulative molar conversion of UDMH to CO_2, as established from yields of $^{14}CO_2$ from doses of 0·013, 0·33, 1·0, and 1·33 m-moles UDMH-^{14}C/kg. The cumulative percentage of conversion of the various UDMH-^{14}C doses (Table 1) shows that at the lowest dose the rate of UDMH metabolism was comparatively high, suggesting that the capacity of the rats to metabolize UDMH was not reached.

TABLE 1. CUMULATIVE PERCENTAGE YIELDS OF $^{14}CO_2$ FROM UDMH-^{14}C ADMINISTERED TO RATS

Duration of expt. (hr)	m-moles UDMH-^{14}C/kg body weight 0·013 (%)	0·33 (%)	1·0 (%)	1·33 (%)
3	20·5	10·8	6·3	4
6	25·2	25·2	9·5	5·8
9	27·0	15·2	10·8	7·0
12		15·8	11·2	8·7
20		16·9	11·4	13·4
53		21·1	12·0	19·0

Cumulative percentage conversion to $^{14}CO_2$ of UDMH-^{14}C administered to rats.

The lower percentage of recovery and similar maximal molar conversion rates at the two higher doses presumably reflect the limiting concentration of the enzyme system(s) responsible for metabolism of the toxic agent. At each of the definitely subconvulsive doses, the rate of conversion decreases in a few hours to a very low level, possibly because the agent has been exhausted from the active sites of UDMH metabolism. The curve representing output of $^{14}CO_2$ after an acutely toxic dose of 1·33 m-moles/kg appears to be biphasic. The continuing conversion of high doses of UDMH-^{14}C to $^{14}CO_2$ during the latter part of the experiments is difficult to relate to the fact that death following UDMH administration usually occurs within 2 hr of

administration. The comparatively high urinary ^{14}C excretion at this dose (Table 2) may be related to the mechanism of intoxication, but a precise time relationship between appearance of urinary ^{14}C and toxic manifestations has not been established.

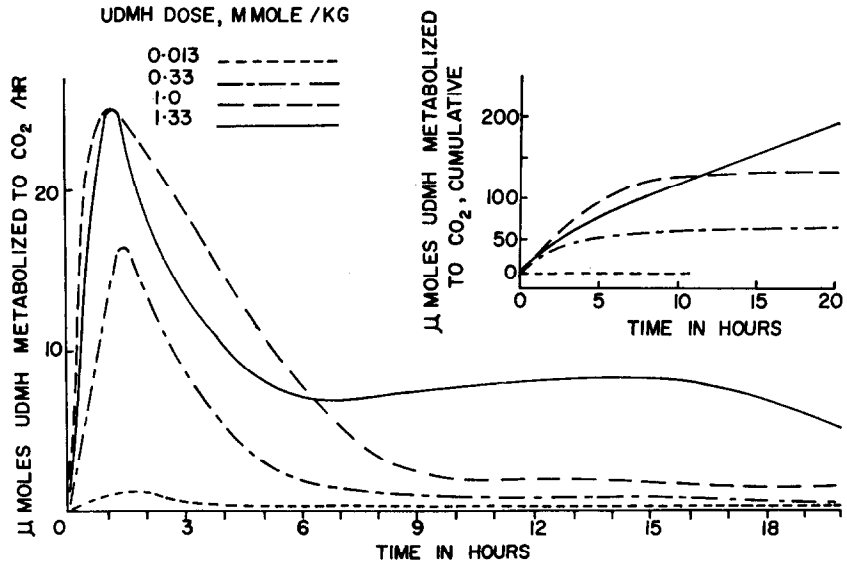

Fig. 1. Hourly and cumulative yield of respiratory $^{14}CO_2$ expressed as μmoles of intraperitoneally administered UDMH-^{14}C converted to respiratory $^{14}CO_2$.

Table 2. Distribution of ^{14}C in respiratory CO_2, urine, and tissue 53 hr after administration of UDMH-^{14}C to rats*

	20 mg (0·33 m-mole)/kg		60 mg (1·00 m-mole)/kg		80 mg (1·33 m-moles)/kg	
	^{14}C yield (%)	UDMH equivalent (mg/kg)	^{14}C yield (%)	UDMH equivalent (mg/kg)	^{14}C yield (%)	UDMH equivalent (mg/kg)
In urine	56	11·2	48·2	28·9	64·0	51·0
	55	11·0	57·5	34·5	76·0	61·0
As $^{14}CO_2$	22	4·5	13·1	7·3	21·1	17·0
	19·4	3·9	10·8	6·5	16·9	13·5
Difference retained in tissue	22	4·3	38·7	23·8	14·9	12·0
	25·6	5·1	31·7	19·0	7·1	5·5

* Observations of two animals at each dose.

Metabolism of MMH-C^{14}

Two catabolic products of MMH-^{14}C appeared in the respiratory gases: methane-^{14}C and carbon dioxide-^{14}C. Cumulative recovery of these metabolites in m-moles of ^{14}C is shown for three dose levels in Fig. 2. The total respiratory radioactivity at the lowest dose used was about 45 per cent of the administered amount; as the dose was increased this percentage decreased.

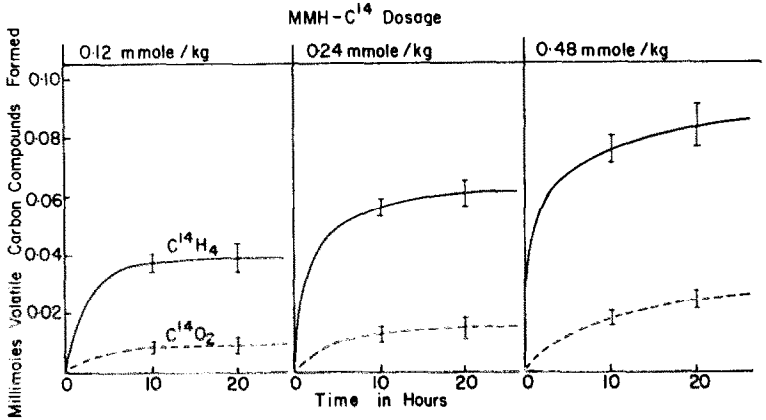

FIG. 2. Cumulative production of respiratory $^{14}CO_2$ by rats metabolizing varying doses of monomethylhydrazine-^{14}C administered intraperitonaelly.

Of the respiratory radioactivity, only 20 to 25 per cent was found in $^{14}CO_2$. The remainder was identified as methane by the three infrared spectra shown in Fig. 3. The first spectrum is an analysis of the respiratory gases, less CO_2 and water, of a rat given unlabeled MMH; the second represents respired gases from a rat given methane gas intraperitoneally. The third is the spectrum of methane gas admitted directly to the infrared cell.

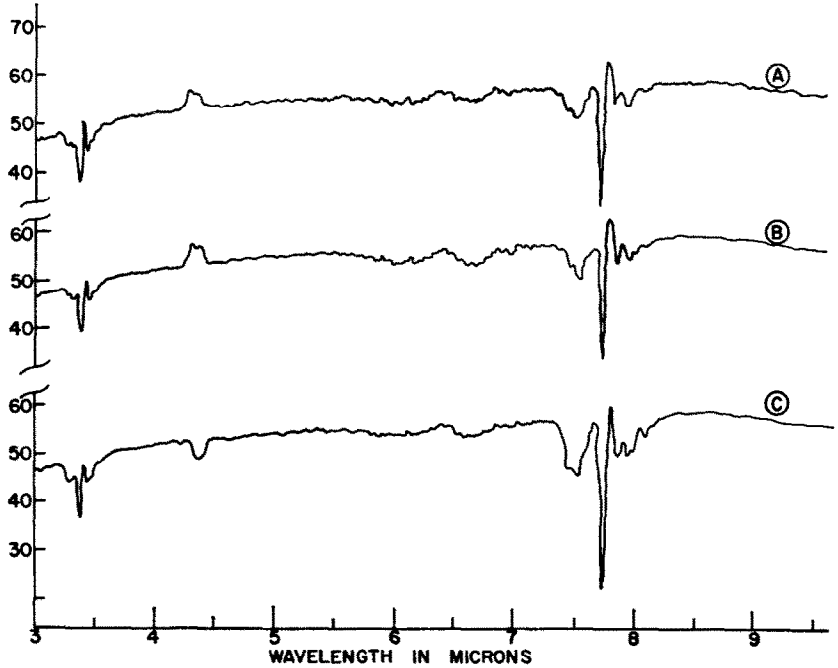

FIG. 3. A: Respiratory gases of rat given monomethylhydrazine, 0·48 m-mole/kg i.p. (water and carbon dioxide removed). B: Respiratory gases of rat given methane gas, 0·1 m-mole/kg i.p. (water and carbon dioxide removed). C: Methane gas, (0·044 m-mole, admitted directly to infrared cell).

TABLE 3. DISTRIBUTION OF ^{14}C IN RESPIRATORY GASES, URINE, AND TISSUE 27 HR AFTER ADMINISTRATION OF MMH-^{14}C TO RATS*

	5·5 mg (0·12 m-mole)/kg		11 mg (0·24 m-mole)/kg		22 mg (0·48 m-mole)/kg	
	^{14}C yield (%)	MMH equivalent (mg/kg)	^{14}C yield (%)	MMH equivalent (mg/kg)	^{14}C yield (%)	MMH equivalent (mg/kg)
In urine	36	2·0	44	4·8	19·6	4·3
	45·5	2·5	35	3·8	24·5	5·4
As $^{14}CO_2$	10·8	0·6	7·9	0·9	6·2	1·4
	5·8	0·3	4·6	0·5	5·0	1·1
As $^{14}CH_4$	33	1·8	27·5	3·1	19·6	4·3
	25	1·4	22·5	2·5	16·2	3·5
Difference retained in tissue	20·4	1·1	20·6	2·2	54·6	12
	24	1·3	37·9	4·2	54·3	12

* Observations of two animals at each dose.

The mechanism involved in the conversion of MMH to methane is not yet ascertained. Eberson and Persson[11] have studied oxidation of β-phenylisopropylhydrazine when it was catalyzed by cupric ion under conditions resembling those in biological systems. Their data provide evidence for a free radical mechanism leading to oxidation products which include isopropyl benzene, propenyl benzene, phenyl acetone, 1-phenyl-2-propanol, and β-phenyl-2-propanol. Neuman and Nadeau[12] have reported the formation of methane, nitrogen, and small quantities of carbon monoxide from a dilute aqueous solution of MMH upon oxidation by dilute sodium hypochlorite. At least one biological system has previously been shown to produce hydrocarbon from a substituted hydrazine. Beaven and White[13] have shown that oxyhemoglobin can produce benzene and molecular nitrogen from phenylhydrazine in the presence of oxygen.* From these studies it appears that mono-alkyl- or aryl-hydrazines are attacked by relatively weak oxidizing systems. If a free radical mechanism is involved, methane should be expected as a product of MMH.

The kinetics of the production of CH_4 from MMH are shown in Fig. 4. The data are plotted as hourly recovery of the respective volatile compounds, expressed as micromoles of MMH converted. The great preponderance of CH_4 over CO_2 early in the experiment can be appreciated by the tenfold difference in scale between the two graphs represented here. Output of carbon dioxide originating from MMH peaked at approximately the same time as methane production and then remained elevated for a longer time, particularly at higher dose levels. Methane production followed the same time course at all doses of MMH; rapid rise, an abrupt fall, and equilibrium occurred at the same time in each case. The ratio of yields: CH_4/CO_2, early in these experiments, was approximately 10, but because of sustained CO_2 production the ratio eventually fell to 3 or 4.† The contrast suggests that independent mechanisms

* Preliminary observations in this laboratory of the metabolism of 1-methyl-H^3-2-*p*-(isopropylcarbamoyl) benzylhydrazine (Ro 4-6467/8) indicate that a substantial amount of its methyl carbon is also catabolized to methane.

† Preliminary observations of metabolism of very low doses of MMH-^{14}C indicate that total conversion to respiratory radioactive products increases with decreasing dose. At 10 μmoles MMH/kg, conversion may exceed 80% and the ratio CH_4/CO_2 appears relatively constant.

may be involved in the production of respiratory CO_2 and CH_4 from the administered MMH.

An inventory of ^{14}C in tissue was estimated by difference (Table 3). At lower dose levels the renal excretion of radioactivity of MMH-^{14}C or its derivatives was proportional to the administered doses. However, when the dosage reached 0·48 m-mole/kg the absolute amount of radioactivity excreted in urine was essentially the same as that observed at 0·24 m-mole/kg. This implies that there is a limit to the excretion rate of MMH or its metabolic derivative(s). At the highest dose, a greater amount of MMH-^{14}C radioactivity was retained in the tissues, which suggests that the excretion capabilities of the subject may have been impaired by intoxication. On the other hand, the absolute amounts of CO_2 and CH_4 produced from MMH increased with the dose, although not proportionally (Fig. 2). MMH, therefore, probably does not seriously impair its own metabolism by the intoxicated animal. It is also probable that the metabolism of this hydrazine is not related to the amine oxidases, which are known to be inhibited by MMH.[11, 14, 15]

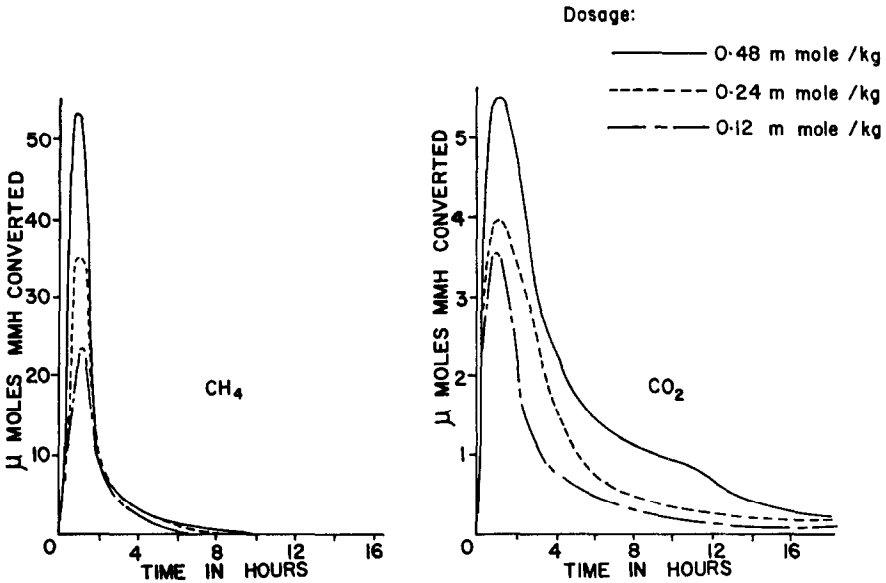

FIG. 4. Hourly production of respiratory $^{14}CH_4$ and $^{14}CO_2$ by rats which had received varying doses of monomethylhydrazine-^{14}C intraperitoneally.

Acknowledgements—This research was sponsored by the Aerospace Medical Research Laboratories, Air Force Systems Command, under Contract AF 33(657)-11757. We thank Dr. James W. Groshong, English Department, Oregon State University, for critically reviewing this manuscript.

REFERENCES

1. K. H. JACOBSEN, J. H. CLEM, H. J. WHEELWRIGHT, W. E. RINEHART and N. MAYES, *A.M.A. Archs. industr. Hlth* **12**, 609 (1955).
2. L. B. WITKIN, *A.M.A. Archs. industr. Hlth* **13**, 34 (1956).
3. M. H. WEEKS, G. C. MAXEY, M. E. SICKS and E. A. GREENE, *Am. industr. Hyg. Ass. J.* **24**, 137 (1963).

4. K. C. BACK and A. A. THOMAS, *Am. industr. Hyg. Ass. J.* **24**, 23 (1963).
5. D. J. REED, F. N. DOST, R. S. MCCUTCHEON, R. D. BARBOUR and C. H. WANG, *Aerospace Medical Research Laboratories Report*, No. AMRL-TDR-63-127 (1963).
6. A. L. GREEN, *Biochem. Pharmac.* **13**, 249 (1964).
7. K. C. BACK, M. K. PINKERTON, A. B. COOPER and A. A. THOMAS, *Toxicol. appl. Pharmac.* **5**, 401 (1963).
8. F. N. DOST, D. J. REED and C. H. WANG, *Fedn. Proc.* **24**, 547 (1965).
9. H. FOX and J. T. GIBAS, *J. org. Chem.* **18**, 994 (1953).
10. F. N. DOST, D. J. REED and C. H. WANG, *Aerospace Medical Research Laboratories Report*, No. AMRL-TR-64-111 (1964).
11. L. E. EBERSON and K. PERSSON, *J. med. pharmac. Chem.* **5**, 738 (1962).
12. E. W. NEUMAN and H. G. NADEAU, *Analyt. Chem.* **36**, 640 (1964).
13. G. H. BEAVEN and J. C. WHITE, *Nature, Lond.* **173**, 389 (1954).
14. D. J. REED, F. N. DOST and C. H. WANG, *Aerospace Medical Research Laboratories Report*, No. AMRL-TR-64-113 (1964).
15. G. WERNER and N. SEILER, *Biochem. Z.* **337**, 383 (1963).