Metabolism in Rats and Mice of the Soil Fumigants Metham, Methyl Isothiocyanate, and Dazomet†

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Isotopic labeling of metham, methyl isothiocyanate (MITC), and dazomet with $^{13}CH_3$ and $^{14}CH_3$ and of metham and MITC with $^{13}C{=}S$ provided the materials for metabolite identification by ^{13}C NMR and quantitation by HPLC analysis and radiocarbon counting. Rats and mice were treated intraperitoneally and the metabolites studied at 48 h. Most of the $^{14}CH_3$ label for each compound in mice appears in urine (58–80%) or is retained in the body (8–12%), particularly the liver and kidney. The major metabolites in each case from rats are *S*-(*N*-methylthiocarbamoyl)glutathione in the bile and *S*-(*N*-methylthiocarbamoyl)mercapturic acid in the urine, whereas from mice the mercapturate is a minor metabolite. Methylamine is a major urinary component following treatment with methylamine or dazomet (rats and mice) or metham (mice) but not with MITC. Detoxification by conjugation with glutathione (GSH) appears to involve direct reaction for MITC, GSH *S*-transferase-catalyzed reaction for metham, and the intermediacy of either MITC or metham for dazomet.

INTRODUCTION

Metham (metam, sodium *N*-methyldithiocarbamate), methyl isothiocyanate (MITC), and dazomet (tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiadiazine-2-thione) are soil fumigants used to control weeds, soil fungi, insects, and nematodes (Worthing and Hance, 1991). Metham is a major agricultural pesticide, particularly in California where 6 million pounds was used in 1990 alone. The environmental fate of metham is of special interest and concern following a July 1991 spill of ~60 000 lb of the active ingredient into the Sacramento River, an event called "California's worst environmental disaster inland" (California Environmental Protection Agency, 1992).

Metham and dazomet are probably propesticides which break down in soil to MITC as the ultimate toxicant (Figure 1) (Worthing and Hance, 1991). MITC is metabolized in rats to the mercapturate *N*-acetyl-*S*-(*N*-methylthiocarbamoyl)cysteine [$CH_3NHC(S)SMA$] (Mennicke et al., 1983, 1987) via the glutathione (GSH) conjugate *S*-(*N*-methylcarbamoyl)-GSH [$CH_3NHC(S)SG$] (Figure 1) which serves as a potential carrier for later release of the MITC moiety (Baillie and Slatter, 1992). The metabolic fate of metham and dazomet in mammals has not been reported. MITC is reactive with biological thiols and amines, and its toxicity is assumed to be associated with such derivatization reactions. MITC and dazomet are more toxic than metham, with mouse intraperitoneal (ip) LD_{50} values of 100, 100, and 750 mg/kg, respectively [determined for this study using methyltriglycol, dimethyl sulfoxide (DMSO), and water as carrier vehicles, respectively, and similar to the report for dazomet of BASF Japan Ltd. (1992)]. These relative toxicity values indicate that not all of the metham is converted immediately to MITC but rather that metham may have alternative detoxification mechanisms for a portion of the dose.

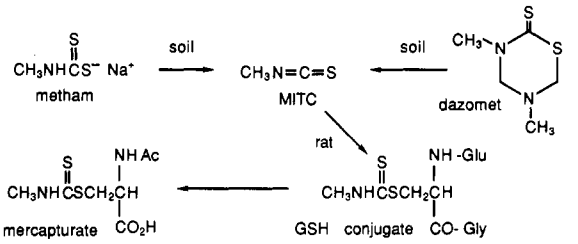

Figure 1. Structures of the soil fumigants metham, MITC, and dazomet showing conversion of metham and dazomet to MITC in soil and of MITC to the urinary mercapturate via the GSH conjugate in rats. See text for references.

This study compares the metabolism of metham, MITC, dazomet, and methylamine in rats and mice using various ^{13}C and ^{14}C preparations to facilitate identification and quantitation of the metabolites. It evaluates the importance of MITC as a metabolic intermediate or activation product of these soil fumigants.

MATERIALS AND METHODS

Chemicals. Sources for the chemicals used were as follows: MITC and *N*-acetylcysteine, Aldrich Chemical Co. (Milwaukee, WI); unlabeled metham and dazomet, Chem Service (West Chester, PA); reduced GSH, Calbiochem (La Jolla, CA); rat liver GSH *S*-transferase (GST), Sigma Chemical Co (St. Louis, MO); $^{13}CS_2$ and $^{13}CH_3NH_2{\cdot}HCl$, each 99 atom % ^{13}C, Cambridge Isotope Laboratories (Woburn, MA) and Sigma, respectively; $^{14}CH_3NH_2{\cdot}HCl$ (55 mCi/mmol), Amersham (Arlington Heights, IL).

Spectroscopy. ^1H and ^{13}C NMR spectra, the latter with nuclear Overhauser enhancement, were acquired at 300 and 75 MHz, respectively, on a Bruker AM-300 spectrometer with an ASPECT 3000 data system. For water-soluble compounds, D_2O was used as the lock solvent and the ^{13}C signals were recorded downfield from 3-(trimethylsilyl)propanesulfonic acid sodium salt (0.00 ppm). Chemical shift values in DMSO-d_6 and $CDCl_3$ are relative to tetramethylsilane.

Chromatography. TLC used 0.25-mm silica gel GF plates (Analtech, Newark, DE) developed as indicated later with detection of unlabeled compounds under UV light and labeled compounds by radioautography. Silica gel (Merck, Darmstadt, Germany) was also used for column chromatography. Reversed-phase high-performance liquid chromatography (HPLC) for analysis of all samples was performed as follows: Waters 600E solvent delivery system; Waters 994 photodiode array detector

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† This work was supported in part by NIH Grants ES PO1 00049 and ES RO1 04863 and the University of California Toxic Substances Research and Training grant.

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0021-8561/93/1441-1497$04.00/0

Table I. ^{13}C NMR Chemical Shifts (δ) for Metham, MITC, Dazomet, and Some of Their Candidate Metabolites

compound	solvent	CH_3	C(S)S	SCH_2	other
$CH_3NHC(S)SNa$	D_2O	37.39	213.60		
$CH_3N{=}C{=}S$	DMSO-d_6	30.19			125.88 (NCS)
$CH_3N{=}C{=}O$	$CDCl_3$	28.35			121.96 (NCO)
dazomet	DMSO-d_6	39.83	189.80		72.15 (CH_2)
		38.14			59.59 (CH_2)
$CH_3NHC(S)SG$	D_2O	37.01	199.85	38.64	179.03, 177.69, 176.82, 174.18, 57.15, 56.01, 46.49, 29.21, 34.45
$CH_3NHC(S)SMA$	D_2O	36.97	200.34	39.66	179.13 (NHCO), 176.27 (COOH), 57.54 (CH), 25.15 ($COCH_3$)
$CH_3NHC(O)SMA$	D_2O	30.62		34.68	172.32 (COS), 179.26 (NHCO), 176.34 (COOH), 58.28 (CH), 25.13 ($COCH_3$)

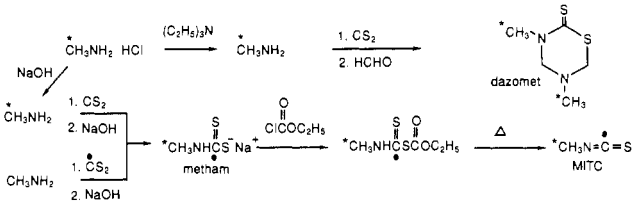

Figure 2. Synthesis of [^{13}C]- and [^{14}C]metham, -MITC, and -dazomet labeled in the CH_3 (*) and C=S (●) positions.

at 200 and 260 nm; Merck 100RP-18 column, 5 μm, 0.4 × 12.5 cm; linear gradient of 0–50% methanol in water with constant 0.1% trifluoroacetic acid over 20 min at 1.5 mL/min. Fractions were collected and radioactivity quantified by liquid scintillation counting.

Synthesis of ^{13}C- and ^{14}C-Labeled Compounds (Figure 2). The syntheses were based on reported procedures for metham and MITC (Moore and Crossley, 1955, and references cited therein) and dazomet (Delépine, 1896; Yeder, 1958). ^{13}C NMR established that the ^{13}C preparations of metham and dazomet were >99% pure, while that of MITC was >95% pure (Table I).

[$^{13}CH_3$]Metham. $^{13}CH_3NH_2$·HCl (200 mg, 2.9 mmol) in water (250 μL) was treated with NaOH as a 40% aqueous solution (320 μL), and the CH_3NH_2 solution was distilled. CS_2 (253 mg, 3.3 mmol) was added to the distillate with stirring for 30 min before aqueous NaOH (3.6 mmol) was added. The reaction mixture was stirred for 7 h followed by evaporation of the solvent to give an off-white solid (448 mg, 93%).

[$^{13}C{=}S$]Metham. $^{13}CS_2$ (303 mg, 3.9 mmol) was reacted with CH_3NH_2 as above to give metham in 91% yield.

[$^{13}CH_3$]MITC. [$^{13}CH_3$]Metham (201 mg, 1.2 mmol) in water (1 mL) was treated dropwise with ethyl chloroformate (147 mg, 1.3 mmol). After stirring for an additional 30 min, the reaction mixture was extracted with ether which was dried (Na_2SO_4) and evaporated to give $^{13}CH_3NHCS_2CO_2C_2H_5$ as a pale yellow liquid (197 mg, 0.41 mmol, 91%) from which an aliquot (34 mg, 0.19 mmol) was heated at 85 °C for 2.5 h in a sealed vial to give $^{13}CH_3NCS$ (13.9 mg).

[$^{13}C{=}S$]MITC. [$^{13}C{=}S$]Metham (170 mg, 1.02 mmol) in water (700 μL) was reacted with ethyl chloroformate (136 mg, 1.21 mmol) with workup as before to give $CH_3NH^{13}CS_2CO_2C_2H_5$ (140 mg, 76%). After further purification by column chromatography (gradient of 0–100% CH_2Cl_2 in hexane), a portion of the $CH_3NH^{13}CS_2CO_2C_2H_5$ (26 mg) was converted to $CH_3N^{13}CS$ (11 mg) as above.

[$^{13}CH_3$]Dazomet. A solution of $^{13}CH_3NH_2$·HCl (250 mg, 3.65 mmol) in water (300 μL) and triethylamine (431 mg, 4.3 mmol) was stirred for 10 min, and then CS_2 (142 mg, 1.9 mmol) was added dropwise at room temperature with stirring for 20 min. Formaldehyde (115 mg, 3.8 mmol, 37%) was added dropwise to the above reaction mixture, which was stirred for 30 min until a white precipitate formed. [$^{13}CH_3$]Dazomet (211 mg) was obtained as a white solid by filtration. Additional product (46 mg) was recovered by extraction of the aqueous phase with chloroform and column chromatography (hexane/ethyl acetate; total 257 mg, 86%).

$^{14}CH_3NH_2$. An aqueous solution of $^{14}CH_3NH_2$ (310 mg, 10 mmol, 0.10 mCi/mmol) was prepared by adding a 20% aqueous solution of CH_3NH_2 (310 mg, 10 mmol) to $^{14}CH_3NH_2$·HCl (0.018 mmol) with stirring.

[$^{14}CH_3$]Metham. CS_2 (7.5 mmol, 570 mg) was added dropwise to a 20% solution of $^{14}CH_3NH_2$ (217 mg, 7 mmol), and stirring was continued for 30 min. Addition of aqueous NaOH (6.9 mmol), stirring overnight, and solvent evaporation gave the desired product as a yellow solid (1.08 g, 94%, 0.10 mCi/mmol) (95% radiopurity analyzed as the ethyl chloroformate derivative described below).

[$^{14}CH_3$]MITC. [$^{14}CH_3$]Metham (78.8 mg, 0.48 mmol) in water (350 μL) was reacted with ethyl chloroformate (68.1 mg, 0.62 mmol) in the same manner as for [$^{13}CH_3$]MITC to give $^{14}CH_3NHCS_2CO_2C_2H_5$ as a yellow liquid (68.4 mg, 80%, 0.10 mCi/mmol) (95% radiopurity of TLC, silica gel, hexane/ether 2:1). An aliquot (44.8 mg) was heated in a sealed vial at 85 °C for 2.5 h to give [$^{14}CH_3$]MITC in almost quantitative yield and 92% radiopurity by TLC (ethyl acetate/hexane 1:1) after conversion to the corresponding thiourea with *n*-butylamine (Mennicke et al., 1988).

[$^{14}CH_3$]Dazomet. $^{14}CH_3NH_2$ (62 mg, 2 mmol, 0.1 mCi/mmol, 20% aqueous solution) was added to CS_2 (82 mg, 1.07 mmol) dropwise, and stirring was continued for 20 min. Formaldehyde (63 mg, 1.05 mmol, 37% aqueous solution) was added dropwise with stirring for 30 min, prior to evaporation of the water, to give dazomet (133.3 mg, 82%, 0.20 mCi/mmol) of 99% radiopurity based on TLC (hexane/ethyl acetate/methanol 2:2:1).

Synthesis and Conformational Isomers of Glutathione and Mercapturic Acid Conjugates. Reaction of the thiol and isothiocyanate or isocyanate (1–3 equiv) gave the desired products which were characterized by NMR (Table I) directly on the reaction mixtures (^{13}C) and following lyophilization to dryness (^1H and ^{13}C).

$CH_3NHC(S)SG$. GSH (1.5 g, 4.9 mmol) was dissolved in water (2.0 mL) by addition of 20% aqueous NaOH to pH 7.0. MITC (0.402 g, 15.5 mmol) was then added, and the cloudy mixture was stirred vigorously and held at 37 °C for 17 h.

$CH_3NHC(S)SMA$. *N*-Acetylcysteine (4.45 g, 27 mmol) in water (2.0 mL) was treated with NaOH and MITC (2.05 g, 28 mmol) as above and incubated at 37 °C overnight.

N-Acetyl-S-(N-methylcarbamoyl)cysteine [$CH_3NHC(O)SMA$]. In a similar manner, *N*-acetylcysteine (1.65 g, 10.1 mmol) in water (1.0 mL) was treated with methyl isocyanate (MIC) (0.59 g, 10.3 mmol), and the two-layer mixture was stirred vigorously for 30 min.

Conformational Isomers of Dithiocarbamates. Two $^{13}CH_3$ signals at about δ 36.8 (major) and 35.3 (minor) are evident for each of $^{13}CH_3NHC(S)SG$ and $^{13}CH_3NHC(S)SMA$. These resonances are due to conformers resulting from the barrier to free rotation about the C–N bond based on analogy with reports on *S*-alkyl-*N*-methyl- and *N*,*N*-dimethyl dithiocarbamates (Gayathri Devi et al., 1981; Holloway and Gitzlitz, 1967) and on variable-temperature ^1H NMR experiments using presaturation conditions. Thus, the ^1H signals at δ 3.16 and 3.06 show one-bond C–H correlations with ^{13}C signals at δ 36.8 and 35.3, respectively. Accordingly, the ^1H signals of $CH_3NHC(S)SMA$ in D_2O were examined at 23, 30, 59, 67, and 29 °C (final) with irradiation of the water signal at δ 4.80. The two sets of AB doublets from the methylene protons (δ 4.00 and 3.84; δ 3.64 and 3.50) and two singlets (δ 3.16 and 3.06) from the methylcarbamoyl groups shifted toward each other as the temperature was raised from 23 to 30 °; a coalescence temperature was reached at 59 °C at which the signals became three different plateaus, and at 67 °C, they became three sets of signals. Finally, when the temperature was lowered to 29 °C, the spectrum was identical to that at 30 °C, confirming the chemical stability.

Metabolism Investigations. *Studies with Mice.* Male albino Swiss-Webster mice (20–25 g) were treated ip with the ^{14}C compounds at 0.05 mmol/kg of body weight equivalent to 7.0, 4.0, 8.8, and 1.7 mg/kg for metham, MITC, dazomet, and CH_3-

Table II. Radiocarbon Recovery for Mice 48 h after Intraperitoneal Administration of $^{14}CH_3$-Labeled Metham, Methyl Isothiocyanate, Dazomet, and Methylamine at 0.05 mmol/kg

excreta and retention	radiocarbon recovery, %			
	metham	MITC	dazomet	methylamine
urine	58.0 ± 18.7[a]	80.2 ± 9.7[a]	64.7 ± 6.1[b]	35.4 ± 9.2[a]
feces	5.9 ± 1.6[c]	4.8 ± 1.4[c]	3.4 ± 1.4[c]	1.6 ± 0.15[c]
$^{14}CO_2$	4.1 ± 1.1[c]	3.8 ± 1.3[c]	9.0 ± 2.0[c]	5.0 ± 1.8[c]
carcass	7.5[d]	5.9[d]	11.7[d]	21.0[d]
total	75.5	94.7	88.8	63.0

[a] $n = 5$. [b] $n = 7$. [c] $n = 3$. [d] $n = 1$.

NH_2, respectively. The carrier solvents (10–50 μL) were water for metham and CH_3NH_2 and DMSO for MITC and dazomet. In radiocarbon-balance studies the treated animals were held in glass metabolism cages for collection of urine, feces, and $^{14}CO_2$. Mice were also sacrificed at 6, 24, and 48 h for tissue analyses.

Studies with Rats. Male Sprague-Dawley rats (140–300 g) were treated ip with the ^{14}C-labeled compounds using the same rates and carriers as used for mice. Rats for ^{13}C NMR studies were treated orally, ip, and ip with cannulation (ipc) according to the following protocols (mg/kg for oral, ip, and ipc, respectively; carrier vehicle): metham (167, 167, 167; water), MITC (33, 33, 33; corn oil), and dazomet (53, 66, 80; corn oil). Rats were anesthetized with ether for oral dosing by stomach tube but not for ip administration. Larger rats (300–400 g) were anesthetized with ketamine/xylazine/acepromazine cocktail for bile duct cannulation. When bile was flowing, these anesthetized rats were injected ip with the ^{13}C-labeled compound with additional injectable anesthesia as required. Treated animals were placed in metabolism cages for urine and/or bile collection.

Tissue Residues. Radiocarbon in mouse tissues on sacrifice was analyzed by combustion to $^{14}CO_2$ (Harvey oxidizer, OX 500). Certain carcasses were extracted with methanol using a Waring blender.

^{13}C NMR Analysis of Urine and Bile of Rats Treated with the ^{13}C-Labeled Compounds. Urine and bile were analyzed directly or after lyophilization. Urine but not bile was filtered using a Millex unit. The ^{13}C resonances varied somewhat with the solution environment, but verification of structural assignments was obtained by spiking with standards.

HPLC Analysis of Urine of Rats and Mice Treated with the ^{14}C-Labeled Compounds. Urine (50–100 μL) was analyzed directly by HPLC. Alternatively, certain urine samples (50–100 μL) plus 1 M NaOH (400 μL) were treated with benzoyl chloride (1 drop) for 15 min at 0–4 °C to convert CH_3NH_2 to its *N*-methylbenzamide derivative, which was extracted into ether prior to HPLC analysis monitoring the UV absorbance at 220 nm. Under the reaction conditions authentic $^{14}CH_3NH_2$ is converted quantitatively to *N*-[^{14}C]methylbenzamide, while $^{14}CH_3NHC(S)SMA$ is stable to hydrolysis.

Studies with GST. Metham (500 μg, 3.0 μmol) and GSH (1800 μg, 6.0 μmol) were dissolved in phosphate buffer (500 μL, 50 mM, pH 7.4). Commercial rat liver GST (0.3 mg) was added to certain samples, and all mixtures were incubated at 37 °C with periodic removal of aliquots over 3 h for product analysis by HPLC using UV absorbance at 260 nm to monitor $CH_3NHC(S)SG$ formation. Metham plus GSH without GST was used as an experimental control.

RESULTS

Radiocarbon Balance for Mice. *Excretion and Retention (Table II).* Mice injected with $^{14}CH_3$-labeled metham, MITC, and dazomet excrete 58–80% of the radiocarbon in the urine within 48 h, whereas the comparable value for $^{14}CH_3NH_2$ is only 35%. Feces are a minor route of excretion, accounting for only 2–6% of each dose. Recovery of $^{14}CO_2$ was 4–9% of the administered radiocarbon, but the low overall recovery of the administered dose for [^{14}C]metham and $^{14}CH_3NH_2$ indicates that possibly not all of the $^{14}CO_2$ was trapped. Radiocarbon in whole carcasses 48 h after dosing ranged from 6% for MITC to 21% for CH_3NH_2, and 85–90% of these residues were refractory to extraction by methanol with MITC, dazomet, and CH_3NH_2.

Tissue Residues (Table III). Radiocarbon from $^{14}CH_3$-labeled metham, MITC, dazomet, and CH_3NH_2 is widely distributed in the tissues at 6, 24, and 48 h posttreatment. The radiocarbon tissue distribution pattern is not unique for any one of the four compounds, but liver and kidney generally retained higher ^{14}C residues. When the average residues in the 16 tissues examined are expressed as a percentage of the administered level (ppm tissue/ppm administered), the values at 6, 24, and 48 h, respectively, are 44, 23, and 20% for metham; 25, 13, and 10% for MITC; 44, 22, and 18% for dazomet; and 41, 35, and 29% for CH_3NH_2.

Urinary and Biliary Metabolites from Rats Identified by ^{13}C NMR. *Metabolites Evident with Unlabeled (Natural Abundance) Metham.* Several ^{13}C NMR signals are evident in the spectrum of the 6–8-h urine and 8-h bile of cannulated rats treated ip with unlabeled metham at 250 mg/kg that are absent in the untreated controls. In the lyophilized bile, the additional signals at δ 57.27, 55.90, 38.60, 36.75, and 34.57 are very similar or identical to those of synthetic $CH_3NHC(S)SG$ (although some signals are obscured). The DEPT spectrum indicated that the δ 36.75

Table III. Radiocarbon in the Tissues (Parts per Million Equivalents) of Mice (n = 3–4) at 6, 24, and 48 h after Intraperitoneal Administration of $^{14}CH_3$-Labeled Metham, Methyl Isothiocyanate, Dazomet, and Methylamine at 0.05 mmol/kg

sample analyzed	metham (7.0 mg/kg)			MITC (4.0 mg/kg)			dazomet (8.8 mg/kg)			methylamine (1.7 mg/kg)		
	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
blood	2.4[c]	0.74[b]	0.9[a]	1.2[b]	0.29[b]	0.24[b]	10.5[c]	1.2[b]	0.53[b]	0.19[b]	0.12[b]	0.23[b]
bone	1.6[b]	0.76[b]	1.5[a]	0.45[b]	0.34[c]	0.32[b]	2.1[a]	1.8[b]	1.2[a]	0.71[b]	0.63[a]	0.45[a]
brain	0.91[b]	0.41[b]	0.43[b]	0.48[b]	0.19[c]	0.12[b]	1.3[b]	0.52[b]	0.42[a]	0.17[c]	0.15[b]	0.14[a]
fat	2.0[b]	0.88[b]	0.80[a]	0.27[c]	0.25[c]	0.19[c]	2.9[b]	1.0[b]	0.70[b]	0.30[c]	0.26[b]	0.32[b]
hair	4.9[a]	1.8[b]	3.1[a]	0.46[b]	1.5[b]	0.82[b]	4.7[b]	0.99[d]	2.7[d]	0.21[a]	0.29[b]	0.95[b]
heart	2.1[b]	1.1[b]	0.89[a]	0.57[a]	0.32[b]	0.33[b]	2.2[a]	1.9[a]	1.4[b]	0.39[b]	0.52[b]	0.52[b]
intestine												
large	3.7[a]	1.6[b]	1.6[b]	1.9[b]	0.70[b]	0.36[b]	4.0[b]	2.9[b]	2.4[b]	1.8[a]	1.3[b]	0.95[b]
small	2.3[b]	1.3[b]	1.4[b]	1.4[b]	0.49[b]	0.34[b]	4.0[a]	2.7[a]	2.7[b]	0.82[b]	1.2[b]	0.96[b]
kidney	4.5[b]	3.1[b]	1.8[b]	1.6[b]	0.66[b]	0.50[b]	4.1[a]	3.0[b]	2.5[a]	0.67[b]	0.95[b]	0.66[a]
liver	10.0[b]	4.7[a]	3.2[b]	2.2[a]	0.87[c]	0.76[b]	6.1[b]	3.5[b]	2.4[a]	0.44[b]	0.58[b]	0.47[a]
lung	5.7[b]	2.7[b]	1.4[b]	1.3[b]	0.51[b]	0.51[b]	3.5[a]	2.1[b]	1.8[a]	0.49[b]	0.43[a]	0.43[a]
muscle	0.91[a]	0.74[b]	1.3[a]	0.34[b]	0.26[c]	0.32[b]	1.7[a]	1.1[b]	0.85[a]	0.44[b]	0.40[b]	0.31[b]
skin and hair	2.3[b]	1.3[b]	1.6[b]	0.88[c]	0.69[b]	1.1[a]	3.3[a]	2.1[b]	1.8[b]	0.43[b]	0.84[b]	0.62[b]
spleen	2.4[a]	1.8[b]	1.2[b]	0.92[b]	0.41[a]	0.53[b]	5.9[b]	2.5[b]	2.0[b]	1.1[b]	0.89[a]	0.57[b]
stomach	2.9[c]	1.5[b]	1.4[b]	0.99[b]	0.48[b]	0.34[b]	4.0[b]	2.0[b]	1.9[b]	2.5[a]	0.34[c]	0.60[a]
testes	1.0[a]	1.1[b]	0.60[b]	0.32[b]	0.28[b]	0.24[a]	1.6[b]	0.90[a]	0.89[a]	0.38[c]	0.34[b]	0.28[a]

[a] Standard error (SE) ≤ 10%. [b] SE 11–25%. [c] SEE 26–50%. [d] SE 51–75%.

Table IV. ^{13}C NMR Signals of Metabolites in Urine and Bile of Rats after Administration of $^{13}CH_3$-Labeled Metham, Methyl Isothiocyanate, and Dazomet

compound[a]	metabolite	^{13}C NMR of urine oral[b]	ip[b]	ipc[b]	^{13}C NMR of bile ipc
[$^{13}CH_3$]metham	$^{13}CH_3NHC(S)SR$[c]	36.81[d]	36.74	36.81	36.78
		35.39[d]	35.26	35.72	35.29
	$^{13}CH_3NH_2$	27.60[d]			
[$^{13}CH_3$]MITC	$^{13}CH_3NHC(S)SR$[c]	36.75	36.77	36.81	36.84
		35.34	35.36	35.40	35.35
[$^{13}CH_3$]dazomet	$^{13}CH_3NHC(S)SR$[c]	36.82	36.81	36.82	36.77
		35.40	35.38	35.40	35.28
	$^{13}CH_3NH_2$	27.70	27.68	27.70	

[a] Doses are given under Materials and Methods. [b] Large amount of glucose in rat urine evident by ^{13}C NMR signals at δ 98.79, 94.98, 78.78, 78.69, 77.10, 75.71, 74.41, 74.28, 73.78, 72.75, 72.53, 72.20, 63.65, and 63.50. [c] R = $-CH_2CH(NHCOCH_3)COOH$ in the case of urine; R = G from GSH in the case of bile; two signals from conformational isomers. [d] Identification confirmed by spiking with authentic standard.

Table V. Urinary Metabolites of Rats and Mice after Intraperitoneal Administration of $^{14}CH_3$-Labeled Metham, Methyl Isothiocyanate, Dazomet, and Methylamine at 0.05 mmol/kg

metabolite	radiocarbon in urine,[a] % metham 0–6 h[b]	metham 0–48 h[c]	MITC 0–48 h[c]	dazomet 0–6 h[b]	dazomet 0–48 h[c]	methylamine 0–48 h[c]
			Rat			
mercapturate	58.0	34.0	26.0	37.0	38.0	
methylamine	3.0	4.9	1.8	22.0	9.1	13.0
unidentified polar[d]		26.7	61.2		36.9	
other unidentified		34.4	11.0		16.0	87.0
			Mouse			
mercapturate	4.0	3.3	2.1	4.0	0.92	
methylamine	14.0	14.2	2.2	13.0	15.5	25.0
unidentified polar[d]		76.8	84.6		79.2	
other unidentified		5.7	11.1		4.4	75.0

[a] See Table II for recovery of administered radioactivity. [b] $n = 1$. [c] $n = 2$. [d] HPLC solvent front.

signal was due to a methyl group, and on addition of authentic $CH_3NHC(S)SG$ the diagnostic signals overlapped. Metham itself was not detected; *i.e.*, addition of authentic metham gave additional signals at δ 213.12 and 37.39. Interestingly, the $HSCH_2$ signal at δ 29.21, presumably from GSH, was missing in treated rat bile, suggesting a compound-related GSH depletion. On a comparable basis, the NMR spectrum of unlyophilized urine from a rat treated orally with metham (315 mg) revealed $CH_3NHC(S)SMA$ with all signals clearly evident except carbonyl carbons (*i.e.*, δ 57.30, 39.55, 36.90, and 24.95) plus a metham signal at δ 37.54.

Metabolites Identified with [$^{13}CH_3$]- and [^{13}C=S]-Metham. When $^{13}CH_3NHC(S)SNa$ was administered at 167 mg/kg in water, $^{13}CH_3NHC(S)SG$ was clearly evident in bile following ipc treatment and $^{13}CH_3NHC(S)SMA$ and $^{13}CH_3NH_2$ were found in urine following oral treatment (Table IV; Figure 3). Similar ^{13}C signals for urine were observed with ip treatment of metham except δ 27.55 (methylamine) was absent. The ^{13}C signals in urine were similar with and without bile duct cannulation. Oral administration of [^{13}C=S]metham resulted in ^{13}C signals at δ 203.24 and 200.33 (major) in the urine associated with $CH_3NH^{13}C(S)SMA$.

Metabolites Identified with [$^{13}CH_3$]MITC and [$^{13}CH_3$]Dazomet. $^{13}CH_3NCS$ administered at 33 mg/kg gave ^{13}C NMR spectra of urine and bile appropriate for

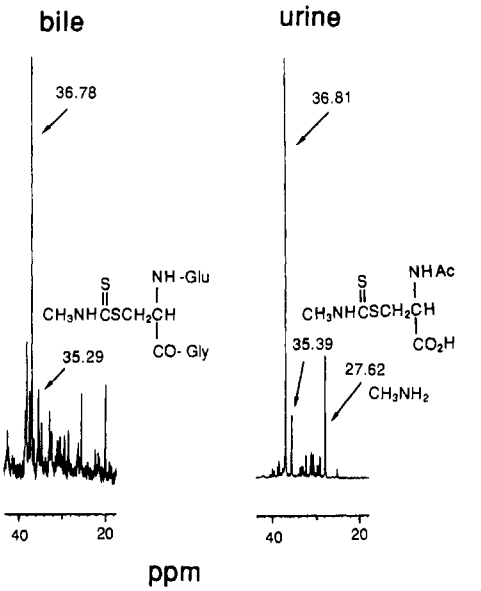

Figure 3. ^{13}C NMR spectra of bile and urine of rats dosed intraperitoneally (with cannulation) or orally, respectively, with [$^{13}CH_3$]metham (167 mg/kg administered in water) showing signals of GSH conjugate in bile and mercapturate and methylamine in urine. Samples were lyophilized and reconstituted in D_2O.

the mercapturate (δ 36.8) in urine and the GSH conjugate (δ 36.8) in bile (Table IV). Similar results were obtained with dazomet-treated rats, but methylamine was also present in the urine.

Glucose in Urine. Metham, MITC, and dazomet, administered either orally or ip, each gave strong ^{13}C NMR signals in the urine identical to those of glucose (Table IV) and not present in control urine, suggesting that these compounds may cause hyperglycemia at high doses.

Urinary Metabolites in Rats and Mice Identified by HPLC Cochromatography and Quantified by ^{14}C Content. HPLC analysis of the metabolite profile in urine allowed qualitative and quantitative comparisons of individual compounds and of the fate in rats with that in mice. Metham, MITC, and dazomet each gave the mercapturate as the major metabolite from rats (26–38% of the ^{14}C in urine after 48 h) (Table V; Figure 4). Mice metabolize these compounds somewhat differently since the mercapturate contributed only 1–4% of the ^{14}C in their urine. $^{14}CH_3NH_2$ was also identified, representing up to 22% of the ^{14}C in 0–6-h urine from dazomet-treated rats. There are substantially reduced amounts of $^{14}CH_3NH_2$ in urine of both rats and mice dosed with [^{14}C]MITC (only 2% of urinary ^{14}C) compared to the amounts from metham and dazomet. Metabolic differences between rats and mice are demonstrated also by the lower amounts of $^{14}CH_3NH_2$ in rat urine compared to mouse urine (5–9% *vs* 14–16% for both metham and dazomet at 48 h).

Enzymatic Metabolism. While a low rate of GSH conjugate formation occurred with metham and GSH alone in buffer, the rate accelerated 5-fold with GST present (Figure 5). The low rate in the absence of enzyme may arise from slow decomposition of metham to MITC and subsequent reaction of MITC with GSH. The nonenzymatic reaction of MITC with GSH was much faster than that of metham. Metham alone in buffer gave no detectable MITC after 3 h.

DISCUSSION

Metham, MITC, and dazomet have several metabolites in common in rats and mice (Figure 6). A principal

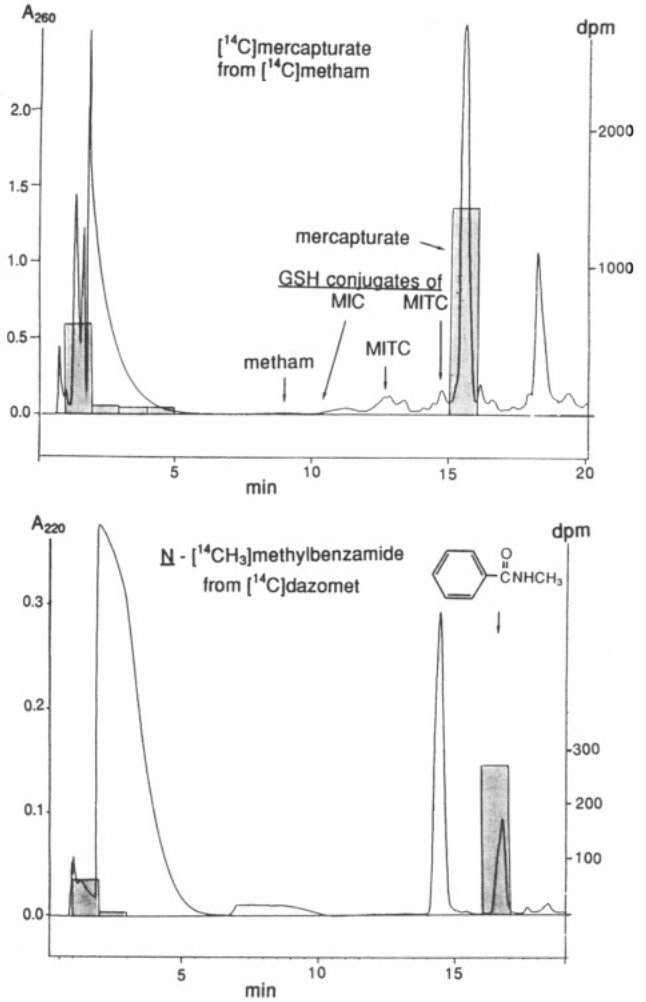

Figure 4. HPLC chromatograms of urinary metabolites of rats dosed orally with [$^{14}CH_3$]metham (7.0 mg/kg administered in water) and [$^{14}CH_3$]dazomet (8.8 mg/kg administered in DMSO) showing [$^{14}CH_3$]mercapturate (neat urine) and *N*-[$^{14}CH_3$]-methylbenzamide (ether extract following treatment with benzoyl chloride), respectively. Solid lines are UV absorbance, and shaded bands are histograms of eluted radioactivity. See text for methods.

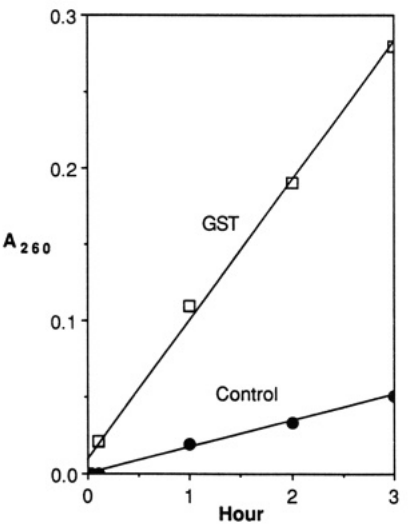

Figure 5. Conjugation of metham with GSH catalyzed by GSH *S*-transferase (HPLC analysis conditions in Figure 4).

detoxification step for metham and MITC is their conversion to the GSH conjugate. MITC reacts readily with GSH without GST, whereas the metham reaction is catalyzed by GST without the requirement of MITC as

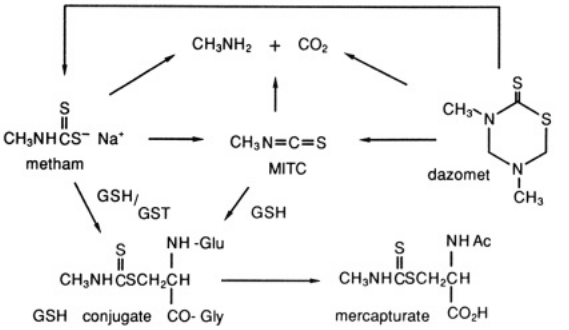

Figure 6. Metabolic fate of metham, MITC, and dazomet in rats and mice showing proposed pathways from the current study for conversion to GSH conjugate and mercapturate.

an intermediate. Each compound results ultimately in a large amount of the mercapturate in urine with CH_3NH_2 as an additional product from metham and dazomet but less so for MITC. Thus, the metabolism of metham involves GSH conjugation for a major part of the dose, but there are also other pathways leading to CH_3NH_2 liberation. It is not clear if all of the CH_3NH_2 from dazomet originates from the tertiary amine methyl group or if some is also generated from the thiocarbamate methyl substituent.

There are large amounts of unidentified metabolites particularly in mice. The vast majority of the unknown ^{14}C is very polar (eluting in water from the standard reversed-phase C_{18} HPLC column), so a cyclic mercaptopyruvate conjugate as found from benzyl isothiocyanate in guinea pigs and rabbits (Görler et al., 1982) is unlikely to be a major metabolite. Substantial conversion of any of these ^{14}C substrates to MIC is contraindicated by the absence of $CH_3NHC(O)SMA$ in urine, a known major metabolite of MIC (Slatter et al., 1991). In contrast to 2-naphthyl isothiocyanate, which is converted to an isocyanate *in vitro* by rat liver microsomes and NADPH (Lee, 1992), the corresponding transformation of MITC to MIC with mouse liver microsomes, NADPH, and cytosol could not be demonstrated by trapping with GSH [see Han et al. (1990) for synthesis of GSH conjugate] and HPLC analysis (data not shown). Since metabolism of $^{14}CH_3NH_2$ also produced considerable polar unknown ^{14}C, much of the unidentified radiocarbon from metham, MITC, and dazomet may arise from secondary metabolism of CH_3NH_2. Methylurea accounts for 2–5% of the urinary ^{14}C in rats and rabbits administered $^{14}CH_3NH_2$ (Dar and Bowman, 1985) and may represent some of the unidentified ^{14}C in the present study. More importantly, CH_3NH_2 is metabolized to formaldehyde, which produces a plethora of products. Much of the ^{14}C residue in tissues may be derived from extensive degradation to formaldehyde and formic acid and redistribution via the one-carbon pool. The fairly general ^{14}C residue profile in tissues supports this contention. The ^{14}C retained in carcasses probably was from covalently bound metabolites since 85–90% was unextractable with methanol.

MITC occurs in the plant *Cleome spinosa* as the glucosinolate precursor (Mennicke et al., 1983) for later release upon injury or ingestion. Another potential MITC progenitor is *S*-(*N*-methylthiocarbamoyl)-L-cysteine, which acts as a suicide substrate for L-methionine γ-lyase from *Pseudomonas putida* (Esaki et al., 1984). Although related GSH and cysteine conjugates of MIC can carbamoylate peptides and proteins (Pearson et al., 1990, 1991; Baillie and Slatter, 1991), there is no evidence for MIC or MITC as an intermediate in metabolism of GSH-related conjugates in this work. The biological target(s) for poisoning

by metham, MITC, and dazomet remain(s) to be defined.

This study illustrates both the advantages and limitations of ^{13}C NMR at 75 MHz in pesticide metabolism research. The ^{13}C-enriched compounds were administered at 10–50 mg per rat, which was consistent with the acute toxicity and adequate for direct ^{13}C NMR analysis of urine and bile even without concentration. Chemical shifts of the $^{13}CH_3$ moieties allowed tentative identification of the GSH conjugate in bile and mercapturate in urine but were not sufficiently different to distinguish these metabolites from each other. Metabolites with dissimilar chemical shifts (*e.g.*, $^{13}CH_3NH_2$) could be identified with confidence. These high-yield metabolites were also evident from diagnostic signals by natural abundance ^{13}C NMR, but for enhanced sensitivity lyophilization was preferred. In the present investigation ^{13}C NMR is used in conjunction with ^{14}C analysis to verify the identification and provide quantitation.

ABBREVIATIONS USED

DEPT, distortionless enhancement polarization transfer; DMSO, dimethyl sulfoxide; GSH, glutathione; GST, glutathione *S*-transferase; HPLC, high-performance liquid chromatography; ip, intraperitoneal; ipc, intraperitoneal with cannulation; MIC, methyl isocyanate; MITC, methyl isothiocyanate; $CH_3NHC(S)SG$, *S*-(*N*-methylthiocarbamoyl)-L-glutathione; $CH_3NHC(O)SMA$, *N*-acetyl-*S*-(*N*-methylcarbamoyl)-L-cysteine; $CH_3NHC(S)SMA$, *N*-acetyl-*S*-(*N*-methylthiocarbamoyl)-L-cysteine.

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Received for review March 22, 1993. Accepted May 25, 1993.