Fifteen grams of the above ester was hydrolyzed in 25% aqueous ethanol with 3.0 g of sodium hydroxide for 3 hr. The ethanol was removed *in vacuo*. The residual solution was extracted with diethyl ether to remove unhydrolyzed material. The aqueous phase was acidified with 10% sulfuric acid and extracted with diethyl ether. The ether extract was dried with anhydrous magnesium sulfate. The ether was removed *in vacuo* to give 10.0 g of crude light yellow oil which crystallized on standing in the refrigerator. Recrystallization from ethanol-water gave 7.7 g of white crystals, mp 82–84°. Fractional crystallization from ethanol-water gave white needles: mp 117–118°; nmr δ_{TMS}^{CDCl3} 1.22 (3 H, s), 1.32 (3 H, s), 1.63 (1 H, d), 10.36 (1 H, s). *Anal.* for $C_8H_{10}O_2Br_2$, C, H.

(5-Benzyl-3-furyl)methyl 2-(2,2-Dibromovinyl)-3,3-dimethylcyclopropanecarboxylate. 2-(2,2-Dibromovinyl)-3,3-dimethylcyclopropanecarboxylic acid, 2.5 g, was added to 500 ml of dry benzene. 5-Benzyl-3-furyl methyl alcohol, 4.0 g, was dissolved in 50 ml of dry benzene and added to the acid solution in the dark. Cyclohexylcarbodiimide, 3.0 g, was then added to the solution, which then stood for 48 hr in the dark. A white precipitate formed, which was filtered from the solution. The benzene was removed from the filtrate *in vacuo* in the dark to give a light yellow oil. Purification of the final product was accomplished employing silica gel chromatography. Two fractions, obtained by elution with hexane, gave, upon concentration *in vacuo*, 1.9 g of a clear oil. Recrystallization from hexane gave the pure *dl-trans* isomer, 0.5 g: mp 65°; nmr δ_{TMS}^{CDCl3} 1.12 (3 H, s), 1.20 (3 H, s), 1.56 (1 H, d), 2.10 (1 H, m), 3.84 (2 H, s), 4.80 (2 H, s), 5.88 (1 H, s), 6.00 (1 H, d), 7.08 (6 H, m). *Anal.* for $C_{20}H_{20}O_3Br_2$, C, H; ir (carbonyl) 1730 cm^{-1}.

ACKNOWLEDGMENT

The authors thank S. B. Penick and Co., Orange, N. J., for their assistance and contribution of material.

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Received for review March 26, 1973. Accepted June 11, 1973. This paper reflects the results of research only. Mention of a pesticide or of a commercial or proprietary product in this paper does not constitute a recommendation by the U. S. Department of Agriculture. This study was supported in part by a grant from North Texas State University Faculty Research Fund, The Robert A. Welch Foundation of Texas, B-133, and by the Environmental Protection Agency/Water Supply and Pollution Control.

Metabolism of Methomyl in the Rat

John Harvey, Jr.,* Arthur G. Jelinek, and Henry Sherman

Radiolabeled methomyl (*S*-methyl [1-^{14}C]*N*-[(methylcarbamoyl)oxy]thioacetimidate) was administered orally to rats. Essentially all of the radioactivity was rapidly eliminated from the animal body within 24 hr in the ratio of 1 part [^{14}C]carbon dioxide, 2 parts [1-^{14}C]acetonitrile, and 1 part urinary metabolites. Although the chemical identity of the polar radiolabeled material excreted in the urine has not been established, the absence of methomyl, *S*-methyl *N*-hydroxythioacetimidate, the *S*-oxide of methomyl, and the *S,S*-dioxide of methomyl and conjugates thereof has been demonstrated. The synthesis of these compounds is described.

Methomyl (*S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate) is the active ingredient in Lannate methomyl insecticide (formerly Du Pont Insecticide 1179, E. I. du Pont de Nemours & Co., Inc.). It is effective against pests such as beetles, aphids, thrips, leaf hoppers, and caterpillars, and particularly loopers, beet armyworm, and corn earworm. At the present time, it has been registered by the Environmental Protection Agency for insect control on 13 crops, including tobacco, sweet corn, tomatoes, cabbage, cauliflower, broccoli, and head lettuce.

A method for the determination of methomyl residues using microcoulometric gas chromatography has been published by Pease and Kirkland (1968). Metabolism information furnished in support of registration for methomyl in the United States has been summarized by the IUPAC Commission on Terminal Residues (1970) and by Baron (1971). The original information contained in this and the two papers immediately following presents the experimental details and complete data.

Biochemicals Department and Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours & Co., Inc., Wilmington, Delaware 19898.

EQUIPMENT AND METHODS

Countercurrent fractionations were carried out in a Model EC-520 countercurrent fractionator (100 tubes, 10 ml/phase/tube) manufactured by the E-C Apparatus Company, Philadelphia, Pa.

Gas chromatography was carried out using a F&M model 720 dual-column programmed temperature gas chromatograph equipped with a thermal conductivity detector.

Silica gel tlc plates were prepared by the following procedure: 3.6 g of Du Pont luminescence chemical No. 609 and 152 ml of water were mixed in a Waring blender at low speed. To this was added 1.8 g of Baymal and 54 g of Kieselgel and the mixture was blended for 3 min. Thin-layer chromatographic apparatus from Colab (Shandon Unoplan Leveller) was used to spread the films with a thickness of 250 μ. The plates were allowed to air-dry for 24 hr, and then were placed in an oven at room temperature. The oven was slowly heated to 110° and held at this temperature for 2 hr. Finished plates were stored in a desiccator until used.

Instruments used in the characterization of the methomyl metabolites were the Bendix Time-of-Flight Model

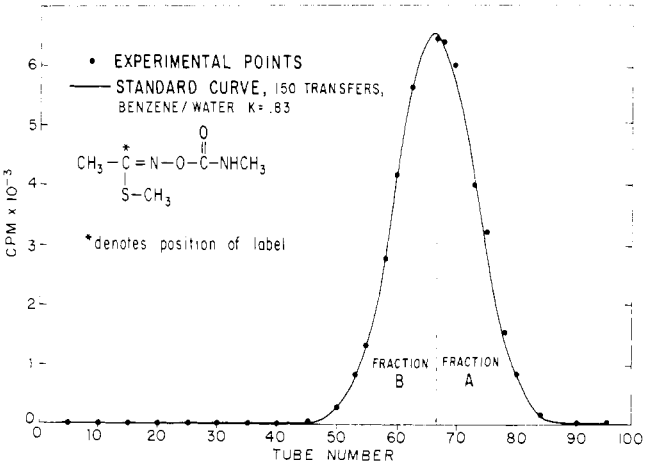


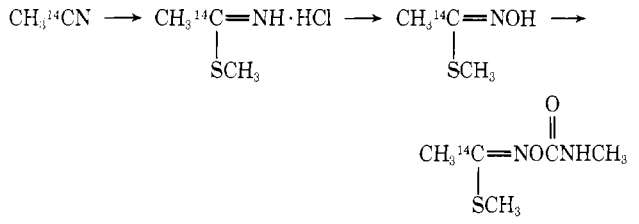
Figure 1. Countercurrent distribution of [^{14}C]methomyl preparation.

12-107 mass spectrometer and the Beckman infrared spectrophotometer IR-12.

Radioactive counting procedures are described below.

SYNTHESIS OF RADIOLABELED METHOMYL

Radiolabeled methomyl was synthesized in a three-step reaction sequence, beginning with [1-^{14}C]acetonitrile from New England Nuclear Corp.



S-Methyl [1-^{14}C]Thiolacetimidate Hydrochloride. A 268-mg sample of [1-^{14}C]acetonitrile (specific activity 2 mCi/mmol) was transferred to a 25-ml three-necked reaction flask with enough nonradiolabeled acetonitrile to bring the total to 620 mg (0.015 mol). The center neck of the flask was equipped with a vacuum distilling receiver topped with a Dry Ice condenser. At once, 8.5 ml of a prepared solution of approximately 9 g of anhydrous hydrogen chloride solution dissolved in 75 ml of a 50:50 mixture of ether–pentane was added. While maintaining the reaction flask at 5 ± 3° with a Dry Ice–acetone bath and stirring with a small magnetic stirrer, 2 ml of methyl mercaptan (0.03 mol) was introduced into the distilling receiver through the side arm while keeping the Dry Ice condenser filled with acetone and Dry Ice. The mercaptan was then added dropwise through the stopcock. Shortly after the addition of the mercaptan, the solid product began forming in the reaction flask. The flask was maintained at 5 ± 3° for 4 hr, while keeping the condenser supplied with Dry Ice.

The flask was then cooled by a water bath at approximately 8° for 3 days. For this the Dry Ice trap and distilling head were replaced with a water-cooled, coil-type condenser, fitted with a drying tube. Stirring was discontinued. After the 3-day period, the reaction flask temperature was allowed to slowly rise to 13° and was maintained at this temperature another day. After this holding period, most of the supernatant liquid in the reaction flask was drawn out with a small capillary pipette and replaced with an equal volume of anhydrous ether in order to wash the solid product free of unreacted materials and impurities. This was repeated once. A slow stream of dry nitrogen was then blown through the flask in order to dry the product (1.5 g, mp 83–88°, 80% yield). The entire product was retained in the flask for the next reaction step.

S-Methyl [1-^{14}C]*N*-Hydroxythioacetimidate. While being stirred with the magnetic stirrer, 0.9 g of hydroxylamine hydrochloride (0.013 m) was added to the flask with the above crude product. Addition of 4.7 ml of absolute alcohol was made. The flask was heated to 31 ± 1° and 0.62 g (0.0115 m) of sodium methoxide was added in small portions over a half-hour period. Between additions, the temperature of the reaction flask was kept in the stated range by cooling occasionally with a small dish of ice water. As the reaction became less exothermic, the flask was insulated with aluminum foil to hold the heat as long as possible. About 2 hr after the addition had been completed, the temperature had dropped to 28°.

Approximately 5 ml of water was added and the resulting solution was extracted four times with 5-ml portions of chloroform. After being dried over magnesium sulfate, the combined organic solution was filtered into a tared 25-ml round-bottomed flask. Evaporation of the solvents with a stream of N_2 gave a residue of 0.7 g of product, mp 84–87° (55% yield). The product was not purified but was retained in this flask for the final reaction step.

S-Methyl [1-^{14}C]*N*-[(Methylcarbamoyl)oxy]thioacetimidate ([^{14}C]Methomyl). After the addition of 1 ml each of benzene and cyclohexane to the above flask of material, 0.42 ml of methyl isocyanate (0.007 m) was introduced along with a trace of Dabco catalyst. The flask, which was fitted with a thermometer and water-cooled condenser, was stirred by magnetic stirrer and heated carefully to 60°. The temperature was maintained for 2 hr. The solution was allowed to cool and was evaporated with a stream of nitrogen in the flask. There was obtained 0.9 g of crude solid product (90% yield). The product was used directly for purification by countercurrent fractionation. Overall yield was 40% (based on acetonitrile).

Purification. The entire sample (0.9 g) of crude [^{14}C]methomyl was dissolved in 20 ml of lower phase from the benzene–water solvent system. The resulting solution was used as the first two lower phases in a 150 transfer countercurrent distribution. Since the instrument has only 100 tubes, the first 50 upper phases to emerge from the extraction train were collected as a combined forerun.

Aliquots (0.10 ml) of representative upper phases were diluted to 10.0 ml with toluene, and 50 μl of the resulting solutions were mixed with 15 ml of solutions for liquid scintillation counting.

Figure 1 is a plot of the observed net activity of the upper phases compared with a standard curve for analytical grade methomyl determined experimentally by uv absorption. No significant quantities of impurities were observed except in the forerun, which contained 28.2 μCi of unidentified impurities which were not resolved further. The contents of tubes 0–45 and no. 88–100, as well as the forerun effluent, were discarded.

The contents of tubes no. 68–87 were combined and the phases separated. The aqueous phase was extracted twice with 100-ml portions of chloroform. The chloroform layers were evaporated in succession to a small volume, after which they were combined with the original benzene phase and evaporated to a volume of 20 ml under reduced pressure on a rotary evaporator. The remaining solvent was evaporated in a gentle stream of nitrogen. The crystalline residue was triturated with pentane and the crystals were collected on a filter, dried, and called Fraction A: wt, 230 mg; mp 78.5–80°; sp act., 0.864 mCi/mmol.

The contents of tubes no. 46–67 were combined and treated in the same manner and called fraction B: wt, 260 mg, mp 79–80°; sp act., 0.878 mCi/mmol; overall yield (A + B), 20% based on acetonitrile and 18% based on ^{14}C.

Chemical Purity. A solution was prepared of 8 mg of Fraction A in 1.0 ml of reagent chloroform, and another of

4 mg of Fraction B in 1.0 ml of reagent chloroform. Portions of the two solutions were used to spot a 20 × 20 cm tlc silica gel plate at 1-, 5,- 10-, 25-, and 50-μl levels. The plate was developed in ethyl acetate, with the solvent front advancing 15 cm.

The developed plate, after drying, was examined under ultraviolet light. Compounds were visible as dark spots on the greenish fluorescent background.

Fractions A and B at all levels gave a single prominent spot (R_f 0.29). Because the 1-μl spot from Fraction B was clearly visible, and since no secondary spot from Fraction A or B was visible at 50 μl, it was estimated that chemical purity was greater than 99% based on ultraviolet absorbing contaminants.

Radiochemical Purity. After examination of the above described tlc plate under ultraviolet light, the plate was placed in direct contact with Ansco Non-Screen X-ray film for 17 hr. After development of the film, Fractions A and B at all levels gave only a single prominent spot (R_f 0.29). At the two highest levels of Fraction A and the highest level of Fraction B, there was a slight shadow at the origin. By comparison of the intensity of this shadow with the spot for the lowest level of Fraction B, it was concluded that Fractions A and B had a radiochemical purity of better than 99.9%.

The tlc plate was reexamined under ultraviolet light and the material containing the major spots from all five levels of Fraction A was scraped off the plate and mixed with 15 ml of scintillation solution. The following fractions were also scraped off: the major spot region of Fraction B; the origin region of Fraction A; the origin region of Fraction B; and a similar quantity of silica gel from in front of the solvent front. The samples were counted and the radiochemical purity was computed to be, for Fraction A, 99.92% and, for Fraction B, 99.95%.

Countercurrent Distribution of Fractions A and B. The solution of Fraction A remaining after spotting the tlc plate was evaporated to dryness in a current of nitrogen. The solution of Fraction B was evaporated similarly. Each residue was then taken up in 10 ml of lower phase from the benzene-water system. The resulting solution was used as the first lower phase in a 150 transfer countercurrent distribution (benzene-water).

Aliquots of selected upper phases were diluted with benzene, and 50-μl samples were counted. The results were plotted and, as in the tlc work, Fractions A and B gave identical results.

SYNTHESIS OF METHOMYL *S*-OXIDE

To a solution of 16.2 g of methomyl in 150 ml of chloroform was added 19 g of 40% peracetic acid over 35 min at 3° to 5°.

The reaction mixture was stirred at 3° to 5° for an additional 30 min and for 5 hr at ambient temperature. The upper aqueous phase was separated and discarded. The chloroform was removed by vacuum distillation (down to 0.1 mm) until 18.5 g of residual oil was obtained. Three grams of this oil was dissolved in 150 ml of chloroform and applied to a chromatographic column (150 g of Mallinckrodt no. 2847 silicic acid, 45 × 95 mm). The column was developed using 1% ethanol in chloroform, causing the appearance of an opaque band which slowly moved down the translucent column of silicic acid. After about 2000 ml of 1% ethanol in chloroform had been passed through the column, the lower edge of this band reached the bottom of the column. The band was eluted with the succeeding 1250 ml of eluate. Removal of the solvent yielded 1.0 g of a clear oil, which slowly crystallized; mp 72–73.5°. A mixture with methomyl melted at 35–60°. The infrared spectrum (Figure 2B) of the crystals showed a very strong band at 1050 cm^{-1}, characteristic of sulfoxides, indicating

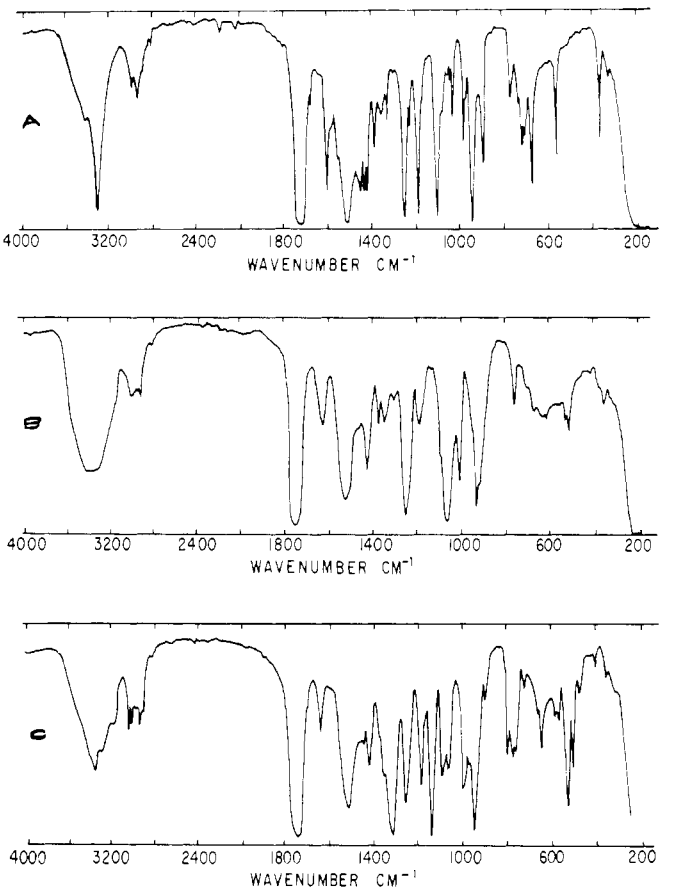


Figure 2. Infrared spectra: A, upper, methomyl; B, middle, methomyl *S*-oxide; and C, lower, methomyl *S,S*-dioxide.

that this material is methomyl *S*-oxide: partition coefficient (benzene–water), 0.00.

Anal. Calcd for $C_5H_{10}N_2O_3S$: C, 33.70; H, 5.66; N, 15.72; S, 17.99. Found: C, 33.59; H, 5.60; N, 16.16; S, 18.47.

SYNTHESIS OF METHOMYL *S,S*-DIOXIDE

To a solution of 48.6 g of methomyl in 500 ml of chloroform was added 120 g of 40% peracetic acid over 1 hr at 0 to 5°. The reaction mixture was stirred for an additional hour at 0–5° and for 20 hr at ambient temperature. The upper aqueous phase was separated and discarded. Most of the chloroform was removed by distillation. The residue (72 g) was slurried with ethyl ether (200 ml), at which point crystals appeared. Ethanol (50 ml) was added, the mixture was chilled and filtered, and the crystals were washed with a chilled mixture (4:1) of ether and ethanol. The wet cake was crystallized twice from ethanol, yielding 17.8 g of methomyl *S,S*-dioxide: mp 107.5–109°. The infrared spectrum (Figure 2C) showed strong bands, characteristic of sulfones, at 1310 and 1135 cm^{-1}: partition coefficient (benzene–water), 0.22.

Anal. Calcd for $C_5H_{10}N_2O_4$: C, 30.92; H, 5.19; N, 14.43; S, 16.51. Found: C, 30.82; H, 5.18; N, 14.67; S, 16.34.

TREATMENT OF ANIMALS

A male Charles River-CD rat, no. 88758, weighing 169 g, was given a diet composed of ground Purina Laboratory Chow to which had been added 1% corn oil and 200 ppm of nonradiolabeled methomyl. Eight days later, when the animal weighed 237 g, he was given by intragastric intubation 2 ml of peanut oil containing 1.2 mg (6.5 μCi) of [^{14}C]methomyl and immediately placed in a glass metabolism unit (Stanford Glassblowing Labs., Inc.) through which 500 ml/min of dried and carbon dioxide-free air was drawn. The effluent air from the chamber was scrubbed

with 4 *N* sodium hydroxide to remove respiratory carbon dioxide. Urine and feces were collected at 24-hr intervals. Each of the 24-hr intervals for carbon dioxide collection contained two samples: 0–7 and 7–24 hr. After 72 hr, the animal (wt, 237 g) was taken from the metabolism chamber and lightly anesthetized with chloroform; blood was removed by syringe and needle from the pumping heart. The animal was killed with an overdose of chloroform and the following organs and tissues were surgically removed: brain, lungs, heart, liver, spleen, kidneys, testes, gastrointestinal tract, and portions of muscle and fat. In addition, the carcass was placed in a plastic bag and was frozen along with the rest of the tissues, urine, and feces.

A second male Charles River-CD rat, No. 88860, weighing 172 g, was preconditioned and treated exactly like the first, except that this test was terminated after 24 hr. The animal weighed 215 g when sacrificed.

The experiment was repeated a third time with the following modification. The effluent gases from the metabolism unit and trapping system previously described were conducted through a 3-ft long × 1-in. diameter quartz tube, the 6-in. middle section of which had been packed with cupric oxide and heated to 700° by an electric furnace. Its purpose was to oxidize to carbon dioxide any volatile organic compounds not trapped in the first sodium hydroxide trap. Any carbon dioxide so generated was collected in 150 ml of 4 *N* sodium hydroxide in a 250-ml fritted disk gas washing bottle. A third male Charles River-CD rat, No. 91510, weighing 198 g, was preconditioned as previously described. Nineteen days later when the animal weighed 312 g, he was given by intragastric intubation 2 ml of peanut oil containing 1.1 mg (6.08 μCi) of [^{14}C]methomyl and immediately placed in the modified metabolism unit. The same procedure was followed for 3 days, as described for the first animal. The animal at sacrifice weighed 308 g.

DETERMINATION OF RADIOACTIVITY

Two scintillation counting solutions were used. Aliquots (1.0 ml) of sodium hydroxide solutions were added to a scintillation vial filled with Cab-O-Sil to which had been added 15 ml of scintillator solution, consisting of 3.4 g of PPO (2,5-diphenyloxazole), 9.0 mg of dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene), 600 ml of toluene, and 378 ml of absolute ethanol. Aliquots (50 μl) of urine, tissue extracts, and other solutions were counted in 15-ml portions of a scintillator solution consisting of 12 vol of spectrograde *p*-dioxane, 2 vol of ethyleneglycol dimethylether, anhydrous, and 1 vol of anisole to which had been added 0.32 g of PPO and 2.1 mg of POPOP/100 ml of solvent, followed by 8 g of naphthalene/100 ml of solvent. Samples were counted on a Nuclear-Chicago liquid scintillation system 6801. Counting efficiency was determined by internal standardization with labeled toluene (New England Nuclear, NES 006).

Small organs were submitted *in toto* for wet combustion analysis for radioactivity by the method of Smith *et al.* (1964). Larger organs were homogenized with ethanol in a glass and Teflon tissue homogenizer. The resulting suspension was centrifuged. The clear supernatant extract was counted by liquid scintillation counting, and the precipitate analyzed by the wet combustion procedure. Recoveries of radioactivity for the three animals are shown in Table I.

Table I. Recovery of Radioactivity after Treatment of Rats with Radiolabeled Methomyl

	Rat no. 88758, 3 days		Rat no. 88860, 1 day		Rat no. 91510, 3 days	
	μCi	% OT[a]	μCi	% OT[a]	μCi	% OT[a]
External fractions						
First sodium hydroxide trap	1.50	23	0.94	15	1.03	17
Second sodium hydroxide trap	*[b]		*[b]		2.00	33
Urine	1.59	24	1.75	27	0.95	16
Feces	0.13	2	0.02	<1	*[b]	
Body fractions						
Blood	0.084		0.034		*	
Brain	0.003		0.003		*	
Fat	0.000		0.002		*	
GI tract	0.122		0.167		*	
Heart	0.005		0.003		*	
Hide	0.169		0.105		*	
Kidneys	0.007		0.010		*	
Liver	0.035		0.042		*	
Lungs	0.004		0.005		*	
Muscle	0.003		0.004		*	
Spleen	0.003		0.003		*	
Testes	0.005		0.006		*	
Carcass	0.234		0.227		*	
Body total	0.674	10	0.611	9		
Recovery		60		52		

[a] OT, original treatment. [b] *, not determined.

CHARACTERIZATION OF METABOLITES

Identification of $^{14}CO_2$ as a Metabolite. When 10-ml aliquots of the most radioactive sodium hydroxide solutions from the first trap were treated with equal volumes of 20% barium chloride solution and centrifuged, it was found that 93% of the radioactivity was precipitated. It was concluded that the radioactivity in the first trap was essentially all the result of [^{14}C]carbon dioxide.

Identification of the Neutral Volatile Metabolite. Four male Charles River-CD rats were conditioned and treated with radiolabeled methomyl essentially as described previously. They were placed two at a time in the glass metabolism chamber for 1-day periods. Neither of the sodium hydroxide traps were used. Instead, laboratory air was drawn directly into the chamber, and the effluent air was drawn immediately through two cold traps. The first cold trap was cooled in Dry Ice; the second was cooled in liquid nitrogen. Urine was collected as previously described.

Aliquots of the liquid nitrogen trap contents and of the Dry Ice trap contents were injected on a 4-ft × 1/4-in. diameter Porapak Q gas chromatographic column. The separation was carried out using a flow of 50 cm^3/min of helium with column temperature held at 70° for 2 min, then programmed at 5°/min to 220°. The radioactivity of both samples was recovered quantitatively in a single peak, with a retention time of 16 min. When the radioactive peak from a 50-μl aliquot of the liquid nitrogen trap contents was trapped on carbon, and a mass spectrum obtained, the mass spectrum was found to be identical with that of acetonitrile (Figure 3). The retention time of acetonitrile on the column described was found to be 16 min.

Countercurrent Distribution of Rat Urine. A 10-ml aliquot of the 0–24-hr sample of urine from the first rat was used as the first lower phase in a 100 transfer countercurrent distribution using the benzene–water system. Standard distributions, run under the same conditions for [^{14}C]methomyl and for *S*-methyl [1-^{14}C]*N*-hydroxythioacetimidate, are shown in Table II.

The radioactivity in representative phases was determined by liquid scintillation counting in the dioxane-based scintillator solution. Virtually all the radioactivity was found as a very polar material in the first few lower phases (Figure 4).

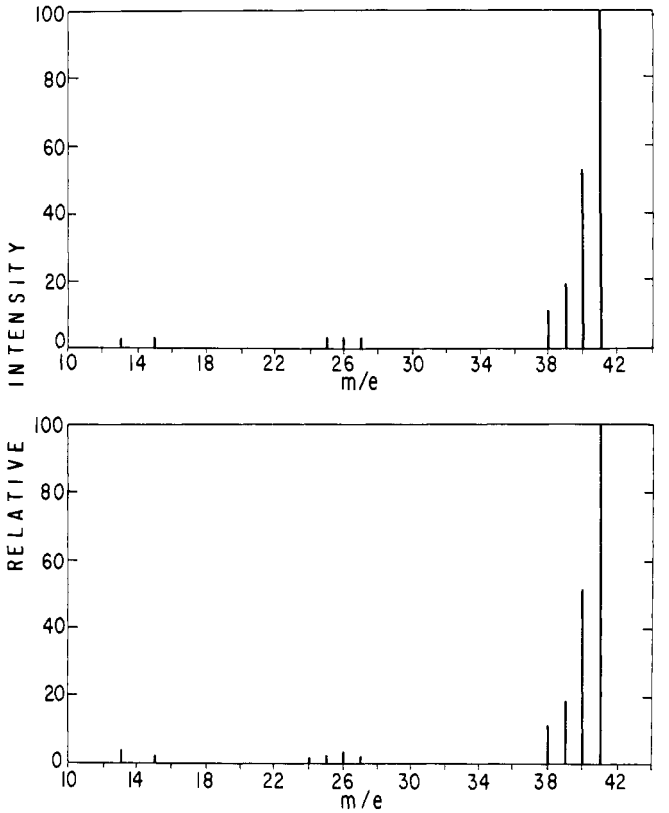


Figure 3. Mass spectra: upper, neutral volatile metabolite; and lower, acetonitrile (API Spectrum =234).

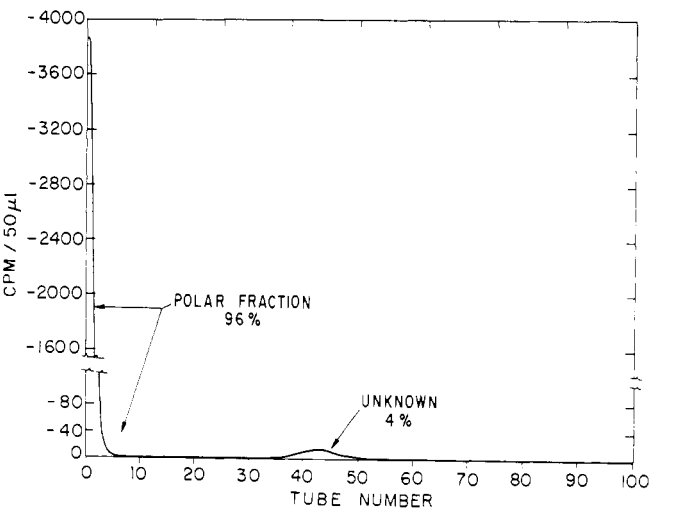


Figure 4. Countercurrent distribution of radioactivity in urine of [^{14}C]methomyl-treated rat.

Table II. Standard Countercurrent Distribution for Methomyl and *S*-Methyl *N*-Hydroxythioacetimidate

Solvent, benzene-water; 100 transfers		
	Fraction of total	
Tube no.	Methomyl[a]	*N*-hydroxy compound[b]
Maximum	0.0680	0.106
Max ± 1	0.0670	0.100
± 2	0.0636	0.090
± 3	0.0599	0.078
± 4	0.0550	0.060
± 5	0.0488	0.043
± 6	0.0411	0.030
± 7	0.0334	0.020
± 8	0.0266	0.014
± 9	0.0207	0.007
± 10	0.0161	0.003
± 11	0.0120	0.002
± 12	0.0090	
± 13	0.0059	
± 14	0.0037	
± 15	0.0019	

[a] Methomyl maximum in tube no. 44, $k = 0.78$. [b] *N*-Hydroxy compound maximum in tube no. 19, $k = 0.23$.

Not more than 0.03 μCi of radioactivity (4% of the total distributed) was found in tubes No. 35–49, where [^{14}C]methomyl would be found, if present. This material was extracted from the aqueous phases into chloroform, but when the benzene phases and the chloroform extracts were concentrated to a small volume, virtually all of the radioactivity was lost. This behavior excludes methomyl, which is not volatile under these conditions. However, acetonitrile has a partition value (k = 0.72) in the benzene–water system which would place it in these tubes if it were present in the original urine.

From the tubes (No. 5–30) which would contain *S*-methyl [1-^{14}C]*N*-hydroxythioacetimidate (k = 0.23) and [^{14}C]methomyl *S,S*-dioxide (k = 0.22), if these compounds were present in the urine, no more than 0.001 μCi of radioactivity (<0.1% of the total distributed) was detected.

It was concluded that the urine does not contain significant quantities of methomyl, *S*-methyl *N*-hydroxythioacetimidate, or methomyl *S,S*-dioxide.

Enzymatic Treatment of Polar Fraction. The aqueous phases No. 0–4 from the countercurrent distribution were combined and concentrated under reduced pressure in a rotary evaporator to a volume of 10 ml. A portion of the concentrated polar fraction was adjusted to pH 5.0 by the addition of dilute acetic acid; 50 μl of β-glucuronidase–aryl sulfatase (C. F. Boehringer & Soehne) was added and mixed thoroughly. The mixture was incubated at 35–37° for 24 hr. A 10 × 20 cm thin-layer chromatographic plate was spotted on the left with 70 μl of the concentrated polar fraction prior to enzyme treatment and on the right with an equivalent quantity of the mixture after enzyme treatment. The plate was developed for 15 cm with water and exposed to Ansco Non-Screen X-Ray Film for 10 days. The enzyme did not appear to affect the polar fraction appreciably, and no spots corresponding to methomyl or its *N*-hydroxy derivative appeared. This indicates that the polar fraction is not a glucuronide or sulfate conjugate of methomyl or *S*-methyl *N*-hydroxythioacetimidate.

Tlc of Methomyl *S*-Oxide with the Urine Metabolites. A solution of urinary metabolites was prepared in the following manner. The urine collected from rats treated with [^{14}C]methomyl was combined and dried by lyophilization, without loss of radioactivity. The residue was triturated with reagent grade methanol and filtered to obtain >97% of the radioactivity in the clear solution. The solution was adjusted to pH 9 by addition of 1 *N* potassium hydroxide, and the alkaline solution concentrated to a small volume under reduced pressure in a rotary evaporator without loss of radioactivity.

A composite sample (1.0 l.) of urine from rats in a 2-year feeding study with 400 ppm of nonradiolabeled methomyl in the diet was lyophilized, extracted, and partially concentrated as described above. The resulting solution was mixed thoroughly with the similar solution from the radiolabeled feeding study. This concentrated mixture of urinary metabolites (0.067 μCi/ml) was used in the following experiment.

A silica gel tlc plate was spotted from left to right as follows: 0.002 μCi of urinary metabolites, 10 μg of meth-

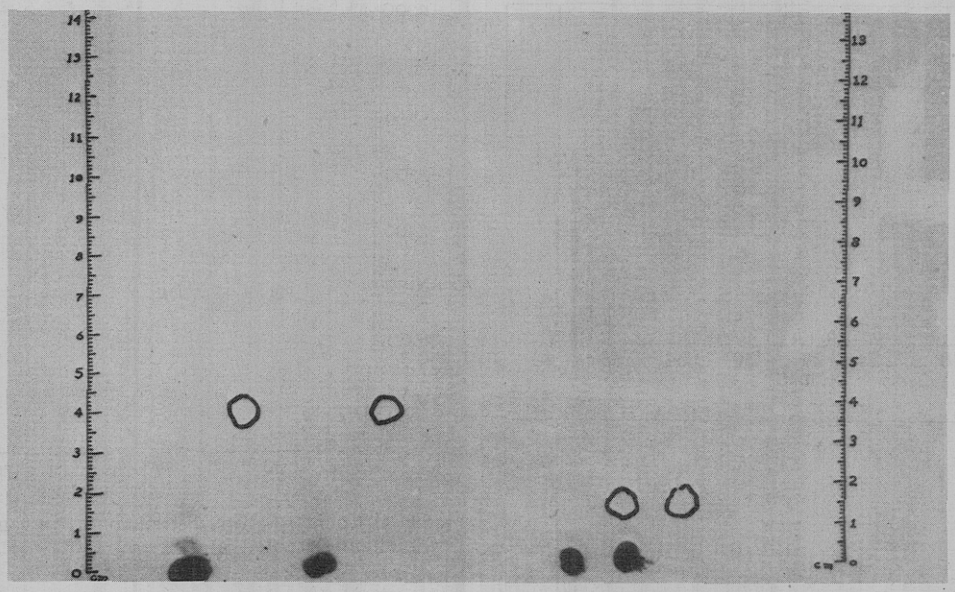


Figure 5. Tlc comparison of radioactive polar metabolites (dark areas) with methomyl *S*-oxide (dotted circles). Left plate (developed in acetonitrile): channels from left to right; 0.002 μCi urinary metabolites; *S*-oxide; 0.001 μCi urinary metabolites; *S*-oxide. Right plate (developed in ethyl acetate): channels from left to right; 0.001 μCi urinary metabolites; 0.001 μCi urinary metabolites + *S*-oxide; *S*-oxide.

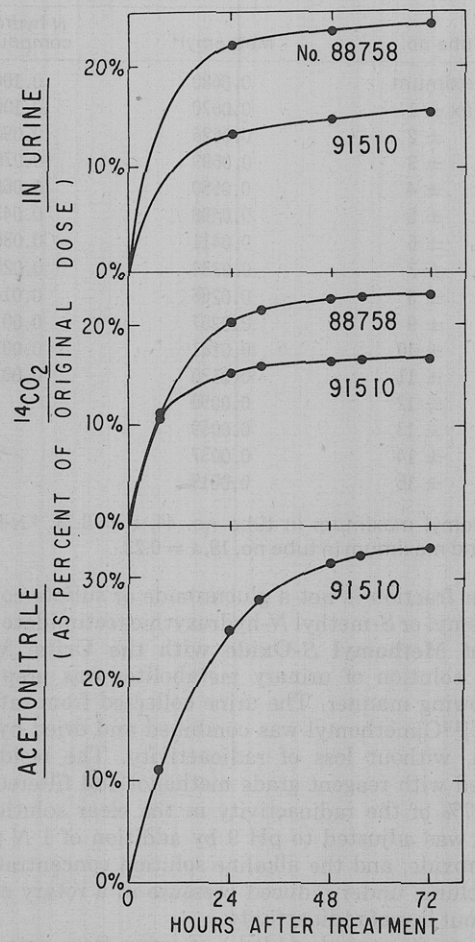


Figure 6. Elimination of radioactivity from the rat after treatment with [^{14}C]methomyl.

omyl *S*-oxide, 0.001 μCi of urinary metabolites, and 10 μg of methomyl *S*-oxide. The plate was developed for 15 cm in acetonitrile. Another tlc plate was spotted from left to right as follows: 0.001 μCi of urinary metabolites, a mixture of 0.001 μCi of urinary metabolites plus 10 μg of methomyl *S*-oxide, and 10 μg of methomyl *S*-oxide. This plate was developed for 15 cm with ethyl acetate. The plates were marked to indicate the position of methomyl *S*-oxide as located by fluorescence quenching under ultraviolet light. The plates were exposed together to a single sheet of X-ray film for 4 weeks. The X-ray film after development was compared to the tlc plates and marked to show the position of the methomyl *S*-oxide. The result is shown in Figure 5.

RESULTS AND DISCUSSION

When two male rats which had been preconditioned on 200 ppm of methomyl in the diet were treated by intragastric intubation with 1.2 mg of radiolabeled methomyl, essentially all of the radiolabeled material was rapidly eliminated from the animal body within 24 hr (Figure 6). Ten percent or less of the original radiolabel was found in the whole body and organs 1 day after treatment. Urine and respiratory carbon dioxide accounted for most of the recovered radioactivity. Urinary contamination probably accounts for the trace of radioactivity recovered in the feces fraction. However, in both experiments, using only a simple NaOH trapping system, almost half of the radioactivity was not recovered at all.

When the experiment was rerun using an oxidizing tube in the train of traps so that any nonacidic organic material in the effluent gases would be oxidized to carbon dioxide and trapped in the final sodium hydroxide solution, a missing respiratory metabolite was discovered. This material was trapped in a final experiment and identified by gas chromatography and mass spectrum as acetonitrile.

The variability in the absolute quantities of metabolites recovered is attributed to uncontrolled variations in the dosage actually administered. However, the data support a conclusion that the radioactivity is rapidly eliminated in the ratio of 1 part [^{14}C]carbon dioxide, 2 parts [1-^{14}C]acetonitrile, and 1 part urinary metabolites.

The chemical identity of the radiolabeled material excreted in the urine has not been established. However, no methomyl or *S*-methyl *N*-hydroxythioacetimidate was detected. β-Glucuronidase–aryl sulfatase treatment of the urinary metabolite fraction was without effect, indicating that there are none of the usual urinary type conjugates of these compounds present either. Finally, the urinary metabolite fraction was shown to be devoid of either the *S*-oxide or *S*,*S*-dioxide of methomyl.

ACKNOWLEDGMENT

The *S*-oxides of methomyl were prepared by J. B. Buchanan. Mass spectra were obtained by R. W. Reiser and infrared spectra by G. Walser.

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Received for review February 5, 1973. Accepted May 24, 1973.

Metabolism of Methomyl in Tobacco, Corn, and Cabbage

John Harvey, Jr.,* and Robert W. Reiser

The metabolic fate of methomyl was investigated in tobacco, corn, and cabbage in the laboratory and greenhouse. Plants treated with radiolabeled methomyl (*S*-methyl [1-^{14}C]*N*-[(methylcarbamoyl)oxy]thioacetimidate) rapidly degraded the compound to [^{14}C]carbon dioxide and [1-^{14}C]acetonitrile, which volatilized from the plant tissues. The half-life for methomyl was on the order of 3 to 6 days. The remainder of the ^{14}C activity had been reincorporated into natural plant components after total decomposition of the methomyl molecule. Radiolabeled lipids, Krebs cycle acids, sugars, and a multiplicity of ^{14}C products have been identified or detected. No evidence was found for the presence of either the *S*-oxide or *S,S*-dioxide of methomyl. The only terminal residue found was methomyl itself. The laboratory studies were extended to season-long treatment of corn and cabbage in the field with radiolabeled methomyl with similar results.

This paper is the second in a series of three. The first paper describes the synthesis of radiolabeled methomyl and its fate in rats and also summarizes pertinent literature (Harvey *et al.*, 1973). The third paper (Harvey and Pease, 1973) covers the decomposition of methomyl in soil. The information reported herein establishes the metabolic fate of methomyl in tobacco, corn, and cabbage and the nature of the residue found in these crops.

EQUIPMENT AND METHODS

The preparations of *S*-methyl [1-^{14}C]*N*-[(methylcarbamoyl)oxy]thioacetimidate (radiolabeled methomyl), *S*-methyl [1-^{14}C]*N*-hydroxythioacetimidate, and the *S*-oxide and the *S,S*-dioxide of methomyl are described by Harvey *et al.* (1973). Procedures for countercurrent fractionation, determination of radioactivity, and the preparation of thin-layer chromatographic plates are also described in the same paper.

Gas chromatography was carried out using a F&M Model 720 dual column programmed temperature gas chromatograph.

ROOT TREATMENT OF TOBACCO

Several weeks before the start of the ^{14}C experiment, three young tobacco seedlings, var. Xanthi, were transplanted to white quartz sand in 4-l. capacity stainless steel beakers with 0.5-in. diameter holes in the bottoms for drainage, and were maintained by watering with nutrient solution (2 g of "Nutrileaf" per gallon). The plants were considered ready for treatment when they had reached a height of 7 in.

Each plant was placed in a glass metabolism apparatus designed to isolate the atmospheres surrounding the aerial and root portions of the plant from each other and connected to a series of traps, as described by Gardiner *et al.* (1969). The trapping system for each portion features two sodium hydroxide traps separated by an oxidizing furnace, the purpose of which is the oxidation to $^{14}CO_2$ of any organic compounds which are not trapped in the first caustic traps. Air flow through the apparatus averaged 1.0 ft^3/hr. A volume equivalent to the volume of the battery jar passed through the upper or foliar section every 90 min and through the lower or root section every 40 min.

Into the lower compartment of each growth chamber was placed 1000 ml of nutrient solution containing 10 mg (54.2 μCi) of radiolabeled methomyl. This solution was recirculated through the sand every second day.

FOLIAGE TREATMENT OF CABBAGE

A 42-day-old cabbage plant, var. Burpee's Surehead, grown in a 6-in. diameter pot of soil was selected for the experiment.

In order to get the droplets of treatment solution to adhere to the cabbage leaves, the upper surface of four leaves was first wiped gently with a cleansing tissue moistened with 0.2% Tween 20 in water. Small droplets of methomyl solution were then distributed on the upper surfaces of the four leaves by use of a Hamilton microsyringe. The plant was treated with 325 μl of an aqueous solution of radiolabeled methomyl (0.85 mg, 4.42 μCi) and 0.2% Tween 20.

Immediately after treatment, the plant was placed inside a glass metabolism apparatus consisting of two large bell jars modified so that single gas inlet and outlet tubes could be attached. The ground flanged rims of the bell jars were sealed with stopcock grease and held firmly together by a circular metal clamp to which legs were attached to support the apparatus. A small stainless steel stand supported the pot above the bottom of the chamber. The plant was completely isolated so that the atmosphere in which it was growing could be monitored. The trapping system employed was essentially the same as that described for the tobacco experiment, except that a cold trap, immersed in Dry Ice–triclene, was inserted between the glass metabolism apparatus and the first sodium hydroxide trap. Its purpose was to collect a portion of the volatile metabolites for qualitative analysis. The volume

Biochemicals Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, Delaware 19898.