LITERATURE CITED

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Metabolism of Methomyl in Tobacco, Corn, and Cabbage

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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,

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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

Table I. Percent Applied ^{14}C Present in Various Fractions

Plant	Tobacco no. 1	Tobacco no. 2	Cabbage	Corn
Site of application	Root	Root	Foliage	Foliage
Volatile components				
Pre-furnace traps	8.6*[a]	6.7*	7.8	6.9
Post-furnace trap	10.5*	7.3*	7.6	26.5
Condensate	0.2	0.5	4.4	13.5
Plant tissues				
Aerial portion, extract	4.4	3.5	51.8	28.3
Aerial portion, not extractable	1.6	1.6	21.1	20.6
Root	0.1	0.1	0.8	0.4
Growth medium residue				
Nutrient solution	70.0	77.0		
Sand	0.6	0.7		
Soil			2.0	12.1
Total recovery	96.0	97.4	95.5	108.3

[a] * indicates sum of activity from root and foliar sections; about $^2/_3$ of the volatile radioactivity was eliminated from the foliar sections and $^1/_3$ from the root sections.

of air drawn through the apparatus was sufficient to provide a complete change inside the bell jars once an hour.

During the course of the experiment, the plant, inside the metabolism apparatus, was grown in a Sherer-Gillett Co. Model CEL-25-7 plant growth chamber programmed to maintain the plant in healthy active growth.

FOLIAGE TREATMENT OF CORN

Eleven days before the start of the experiment, field corn, var. Eastern States 8-30, was planted in soil in 6-in. diameter plastic pots. After germination the plants were thinned to four plants per pot and grown in the greenhouse. At time of treatment, the growing points of the plants were approximately 11 in. above the soil in the pot.

The plants were treated by pipetting 100 μl of a solution of radiolabeled methomyl (0.26 mg, 1.36 μCi) and 0.2% Tween 20 into the whorl at the growing point of each plant. This made a total treatment of 5.44 μCi/pot of four plants. The treated plants were placed immediately inside the glass metabolism apparatus and growth chamber described in the cabbage experiment, and the experiment was begun.

QUANTITATIVE RECOVERY STUDIES

Traps. At suitable intervals the contents of the sodium hydroxide traps were replaced with fresh sodium hydroxide solution, and the cold traps were emptied. The amount of radioactivity in each trap was determined by liquid scintillation counting in appropriate cocktails as described by Harvey et al. (1973).

Tobacco Plants. After 4 weeks of treatment, the chambers containing plant no. 1 and plant no. 2 were disassembled and the plants were fractionated. The leaves were cut off the plants at the stem. The leaves from a single plant were combined in a polyethylene bag and immediately frozen in Dry Ice. Each stem was cut off at ground level, sectioned into 0.5-in. long pieces, placed in a polyethylene bag, and immediately frozen in Dry Ice. The root of each plant was removed as carefully as possible from the sand and washed with water which was allowed to drain back on the sand. Each root was stored and frozen as above.

The frozen leaf tissue from each plant was pulverized and extracted with 100 ml of ethyl acetate in a Waring Blendor and reextracted in the blender with an additional 50-ml portion of solvent. Root and stem tissues were each pulverized and extracted three times with 50-ml portions of ethyl acetate. After filtration, aliquots of the extracts were counted in dioxane scintillator solution. The insoluble residues from the extractions were dried in air and

analyzed by the wet combustion technique of Smith et al. (1964).

Cabbage Plant. Seven days after treatment, the plant was removed from the glass metabolism apparatus. Plant growth was normal and no chemical injury was evident. The plant was cut off at ground level. The aerial portion of the plant was macerated immediately with methanol in a Waring Blendor. The extraction was repeated three additional times. After filtration, aliquots of each extract were counted. Essentially all of the soluble radioactivity was removed in the first three extractions, with only traces appearing in the fourth extract. The insoluble residue from the extractions was dried in air and analyzed for radioactivity by wet combustion. The root, washed free of soil, was dried in air and analyzed by wet combustion.

Corn Plant. This experiment was terminated 10 days after treatment. The plants were cut off and extracted as described for cabbage.

Sand and Nutrient Solution or Soil. After the tobacco root had been removed from the sand and washed, at least 2 l. more water was allowed to drain through the sand into the nutrient solution. When the sand had drained reasonably well, it was transferred to a large Buchner funnel, where it was washed thoroughly with approximately 4 l. more water. After it had been sucked dry, the sand was spread out in a shallow stainless steel tray to dry. The liquid samples were analyzed by liquid scintillation counting and the dry sand was analyzed by wet combustion.

In the corn and cabbage experiments, the soil and the water used to wash the soil from the plant roots were mixed in a ball mill for 2 hr. The resulting slurry was spread in a shallow stainless tray to air dry. The dry solids were forced through a no. 10 sieve and analyzed by wet combustion.

Condensate. When the growth chambers were first opened, care was taken to wash out the condensate which had collected on the inside of the glass and to save it, together with an aqueous rinse of the inside walls of the glass chamber. Radioactivity was determined by counting.

The results of the quantitative recovery studies are shown in Table I. Recoveries were essentially quantitative in each experiment.

IDENTIFICATION OF VOLATILE ^{14}C COMPONENTS

Of the ^{14}C activity collected in the sodium hydroxide traps for the cabbage experiment, approximately equal amounts were collected in the trap before the oxidizing furnace and in the trap after the oxidizing furnace. For corn, almost four times as much was collected in the trap after oxidation to [^{14}C]carbon dioxide as was collected

from the air stream before oxidation. In the tobacco experiments, where air streams from the aerial and root chambers were monitored separately, less than one-quarter of the volatile ^{14}C activity from the root zone appeared in the trap after oxidation. From the aerial portions, twice as much volatile ^{14}C was trapped after the furnace as was trapped before it. Clearly, the treated plants were metabolizing [^{14}C]methomyl to varying proportions of an acidic and a nonacidic ^{14}C-containing volatile compound.

Carbon Dioxide. Aliquots of the contents of the six sodium hydroxide traps connected with tobacco plant no. 1 during the second week of the experiment were mixed with excess 10% barium chloride solution. Each mixture was filtered. The radioactivity remaining in the clear filtrate was determined by liquid scintillation counting.

All of the radioactivity in traps no. 1 and 2 connected to the root chamber was precipitated by barium chloride, indicating that the only significant source of radioactivity in these traps was [^{14}C]carbon dioxide. For the traps connected to the aerial portions of the plant, 82% of the radioactivity in trap no. 1 and 50% of the radioactivity in trap no. 2 was precipitated by barium chloride, indicating that only these proportions of the trap contents were attributable to carbon dioxide. As expected, all of the contents of the two traps no. 3 (after the oxidizing furnace) were precipitated by barium chloride.

When the contents of trap no. 1 (aerial) were extracted with ethyl acetate, 18% of the radioactivity was extracted into ethyl acetate from the alkaline solution. When this solution was partially evaporated under reduced pressure in a rotary evaporator, no concentration of the radioactivity occurred. The specific activity of the distillate and of the residue remained essentially the same as that of the starting solution. Since this behavior is characteristic of the neutral volatile component, it was concluded that the radioactivity in trap no. 1 (aerial) and trap no. 2 (aerial) which was not due to carbon dioxide was attributable to the neutral volatile component discussed in the following section.

Similar treatment of the contents of the first sodium hydroxide traps from the cabbage and corn experiments with excess barium chloride solutions showed that 90% or more of the radioactivity was precipitated, demonstrating carbon dioxide as the volatile acidic metabolite.

Acetonitrile. Four weeks after the start of the tobacco experiment, the trapping system on the upper compartment of plant no. 3 was modified so that the effluent air flowed through one sodium hydroxide trap, one dilute sulfuric acid trap, two cold traps immersed in Dry Ice–triclene baths, and, finally, the furnace and trap no. 3 in sequence. Essentially no radioactivity was collected in the acidic trap, demonstrating that the second volatile compound was not a base. The neutral volatile metabolite was collected in the cold traps over a 2-week period as a frozen aqueous solution containing 0.06 μCi in slightly more than 100 ml of water.

Samples of the crude aqueous solution of neutral volatile metabolite were equilibrated against equal volumes of selected solvents. Aliquots of the resulting upper and lower phases were counted by liquid scintillation counting techniques. The partition coefficient was determined by dividing the net counts per minute of the upper phase by the net counts per minute of the lower phase in equilibrium with it. Partition coefficients of the neutral volatile metabolite were: benzene–water, 0.73; ethyl acetate–water, 1.6; and ether–water, 0.65. Partition coefficients for [1-^{14}C]acetonitrile (NEC-157, New England Nuclear Corp.) in the same systems were 0.72, 1.5, and 0.62, respectively.

A 50-μl aliquot of the crude solution of the neutral volatile metabolite was injected into a 6-ft long gas chromatographic column of 20% Carbowax 20M on Teflon. The

separation was carried out using a flow of 50 cm^3/min of helium with column temperature held at 70° for 2 min, and then programmed at 5°/min to 220°. All the radioactivity was found to elute with the water peak at 14 min. Since acetonitrile is not resolved from water on this column under these conditions, this behavior is consistent with the identification suggested by the partition data described above.

It was found that water (retention time 3.2 min) and acetonitrile (retention time 16 min) were well separated on a 4 ft × ¼-in. diameter Porapak Q column using the same conditions described previously. A 1.0-ml sample of the neutral volatile fraction was spiked with 0.06 μl of nonradiolabeled acetonitrile. An aliquot of this mixture was injected on this column. The acetonitrile peak was trapped in scintillation counting solution and, after counting, was found to contain all the radioactivity.

On the basis of identical partition ratios in three solvent systems and identical retention times on two chromatographic columns, it was concluded that the neutral volatile metabolite from tobacco was acetonitrile.

Similar partition and gas chromatographic experiments with the cold trap contents from the cabbage and corn experiments yielded similar results. When the aqueous solution from the cold trap was extracted three times with equal volumes of ethyl acetate, essentially all the ^{14}C activity passed into the ethyl acetate. Concentration of the extract in a stream of nitrogen to a final volume of 1 ml resulted in the loss of more than 90% of the radioactivity by volatilization. Thin-layer chromatography of the less volatile residue in comparison with authentic nonradiolabeled methomyl showed that a very small proportion of the radioactivity in the cold trap was due to [^{14}C]methomyl.

The experiments with cabbage and corn in which the cold trap was located between the metabolism chamber and the first chemical trap clearly establish that the neutral volatile metabolite of [^{14}C]methomyl in these species is [^{14}C]acetonitrile. However, in the case of tobacco, a sodium hydroxide trap intervened between the chamber and the cold trap. Accordingly, the possibility existed that the acetonitrile detected in that experiment might be an artifact of the trapping system.

In order to clarify this point, a 10-in. tall Xanthi tobacco plant growing in quartz sand watered with Nutrileaf solution was treated at the root with 11.5 μCi of [^{14}C]methomyl in 10 ml of water. The plant was placed in a metabolism chamber with a trapping system like that used in the cabbage and corn experiments. After 5 days, the radioactivity in the various traps was determined. The prefurnace NaOH trap had accumulated 0.53 μCi and the postfurnace trap had 0.43 μCi, indicating a ratio between the two volatile metabolites essentially the same as that obtained previously. The cold trap contained 0.01 μCi, which was investigated as previously described. The partition behavior, gas chromatographic analysis, and volatility on concentration clearly established that more than 90% of the radioactivity in the cold trap was acetonitrile. Thin-layer chromatography of the small amount of less volatile material established its identify as [^{14}C]methomyl.

CONDENSATE FRACTION

Extraction of the aqueous condensate fractions with ethyl acetate, followed by drying, and concentration of the extracts under reduced pressure on a rotary evaporator resulted in essentially quantitative recovery of the radioactivity as a single component which cochromatographed with authentic nonradiolabeled methomyl on silica gel tlc plates developed in ethyl acetate. All condensate fractions consisted of the water that condensed on the sides of the metabolism jars plus the water that was in the bottom of

Table II. Percent ¹⁴C Activity in Fractions of Plant Extracts

Fraction	Tube no.	Tobacco[a]	Cabbage[b]	Corn[b]
Polar	0–6 (lower)	7.5	62.4	81.8
	10–25	0.1	1.2	1.4
Methomyl	30–60	82.2	6.7	5.6
Nonpolar	80–94 (upper)[c]	10.2	29.7	11.2

[a] Root application. [b] Foliar application. [c] Because of emulsion difficulties, the passage of upper phase through the apparatus was impeded, and tubes no. 95–100 contained no upper phase and no radioactivity.

the jars. It is believed that most of the observed material had simply dripped off the plants, particularly in the corn experiment, where the leaves of the plant were touching the walls of the jar.

COMPOSITION OF THE NUTRIENT SOLUTION AFTER THE TOBACCO EXPERIMENT

One-half of the nutrient solution fraction (1900 ml) from plant no. 2 was extracted eight times with 500-ml portions of ethyl acetate. Aliquots of the extracts were counted by liquid scintillation counting. Extraction recovery was 96%. The eight extracts were combined and evaporated to 50 ml. An equal volume of water was added and the mixture was concentrated to a single aqueous phase.

A 10-ml aliquot of the concentrate was analyzed by countercurrent fractionation. A comparison of the curve obtained for the radioactivity in tubes no. 30–60 with a standard curve for methomyl indicated that 96% of the total radioactivity in solution was unchanged methomyl. S-Methyl N-hydroxythioacetimidate was present to the extent of 1%, while 3% was obtained as a polar fraction found in the first few aqueous phases.

FRACTIONATION OF PLANT EXTRACTS

The extract of the aerial portions of a treated plant was mixed with 20 ml of water and concentrated in a rotary evaporator under reduced pressure until a single aqueous phase (volume 10 ml) was obtained. The green gummy deposit on the walls of the flask was dissolved in small portions of benzene until a total volume of 10 ml of benzene solution was obtained. The resulting water and benzene solutions were used as the first lower and upper phases, respectively, in a 100 transfer countercurrent distribution using the benzene–water system. The amount of radioactivity in representative tubes following the fractionation was determined by liquid scintillation counting. The distribution of methomyl and S-methyl N-hydroxythioacetimidate under these conditions has been described elsewhere in connection with the analysis of rat urine (Harvey et al., 1973). The results of these analyses are summarized in Table II. The countercurrent distribution of the extract of cabbage leaves is shown in Figure 1.

Although in each case the distribution of ¹⁴C activity in tubes no. 30–60 was in excellent agreement with a calculated curve for pure [¹⁴C]methomyl, confirmation of the identity and purity of this fraction was obtained by cochromatography on silica gel tlc plates developed in ethyl acetate. In each case, a single radioactive spot was obtained which cochromatographed with authentic nonradiolabeled methomyl. In the tobacco experiment where methomyl itself appeared to be the major component, this identification was confirmed by mass spectroscopy on the isolated material.

CHARACTERIZATION OF THE NONPOLAR FRACTION FROM CABBAGE EXTRACT BY SAPONIFICATION

The material used in these studies was obtained by combining the benzene phases from tubes no. 80–93 inclusive of the countercurrent fractionation of the material ex-
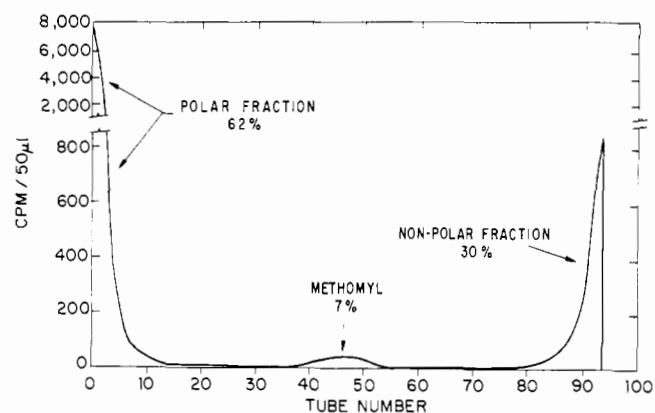

Figure 1. Countercurrent distribution of cabbage leaf extract.

tracted from the treated cabbage plant. The radioactive components of the nonpolar fraction, which had been shown by the countercurrent fractionation to have no appreciable water solubility, were also found to have no solubility in aqueous potassium hydroxide or aqueous hydrochloric acid by shaking aliquots of the benzene solution with these aqueous solutions and counting aliquots of the resulting phases.

A portion of the benzene solution of this neutral, nonpolar metabolite fraction from cabbage (95 ml, 0.403 µCi) was heated under reflux for 20 hr with an equal volume of 10% potassium hydroxide in 95% ethanol. To the reaction mixture 30 ml of water was added, and the mixture was concentrated to 25 ml under reduced pressure in a rotary evaporator. The volume of the aqueous residue was adjusted to exactly 30 ml and equilibrated by shaking with an equal volume of ethyl ether. The neutral compounds in the ether solution amounted to 24.3% of the original radioactivity in the nonpolar fraction. When the ether phase was extracted in turn with equal volumes of 0.1 N potassium hydroxide and 1 N hydrochloric acid, no significant amounts of radioactivity were extracted.

The original alkaline aqueous phase was acidified by the addition of concentrated hydrochloric acid with external cooling. The acidic solution was extracted twice with equal volumes of ethyl ether. Acidic compounds passed into the ether extracts essentially quantitatively and accounted for 73.5% of the original radioactivity in the nonpolar fraction.

The ether solution of acids that resulted from the saponification was dried over anhydrous magnesium sulfate and concentrated to one-tenth its original volume under reduced pressure in a rotary evaporator without loss of radioactivity. The resulting concentrate was esterified quantitatively by treatment with an excess of diazomethane in ether solution.

A portion of the ether solution of the methyl esters was analyzed by gas chromatography using a 2-ft column packed with 5% cyclohexane dimethanol succinate on 60-80 mesh Diatoport S at 180°, 50 cm³/min He flow rate. Four peaks were visible to the thermal conductivity detector with retention times of 3, 4.5, 6.8, and 9.3 min. These peaks correspond to palmitate (C_{16}), palmitoleic, stearate and/or oleate (C_{18}), and arachidate (C_{20}), the most common fatty acids in plants. The material in each peak was trapped in scintillation counting solution and counted. The results showed that 95% of the radioactivity in the methyl ester solution was recovered in these four peaks in the ratio of 5:2:3:3, respectively.

CHARACTERIZATION OF THE NONPOLAR FRACTION FROM CORN BY SAPONIFICATION

The material obtained by combining the benzene phases from tubes no. 80–94 inclusive of the countercurrent

fractionation was subjected to the same saponification procedure.

The alkaline aqueous phase was found to contain 17% of the radioactivity in the original sample of nonpolar fraction. The remainder of the radioactivity was found in the ether phase. The material in the ether phase was neutral and partially water soluble. It did not partition into 10% potassium hydroxide or 1 N hydrochloric acid. It would partially partition into 0.1 N potassium hydroxide, but could be quantitatively returned to the ether phase by saturating the dilute alkaline solution with sodium chloride. This characterization is similar to that in cabbage, but more of the ^{14}C appears to be incorporated in the alcohol portion of the lipid.

CHARACTERIZATION OF POLAR FRACTION

Assay for AChE Inhibition. As a check on the possibility of toxicity, the polar radioactive fractions found in the extracts of treated cabbage and corn leaves were first tested for inhibitory activity against purified bovine red blood cell acetylcholinesterase by an adaptation of the procedure of Robbins et al. (1958). The lower phase most concentrated in polar fraction from the countercurrent distribution (ca. 1×10^{-4} M based on specific activity for both corn and cabbage) was used for these studies. An identical polar fraction of normal plant constituents was prepared by the same extraction, concentration, and countercurrent distribution procedure using an untreated control corn or cabbage plant of the same variety and age.

The polar fractions from both treated and untreated corn plants showed no AChE inhibitory activity. The polar fractions from both treated and untreated cabbage plants showed a very low level of AChE inhibitory activity due to the presence of a naturally occurring inhibitor, but the fraction from the treated plant did not exhibit any increase in activity over that found for the untreated plant. In fact, the extract from the particular untreated cabbage plant analyzed was more of an inhibitor than that obtained from the treated plant.

Absence of Methomyl S-Oxide as a Metabolite in Polar Fraction. The preparation of methomyl S-oxide and its physical properties are described by Harvey et al. (1973). If methomyl S-oxide were present as a metabolite in a leaf extract, its partition coefficient ($k = 0$) is such that it would have to be in the polar metabolite fraction after countercurrent distribution. A 5 cm × 20 cm tlc plate (Analtech, silica gel GF, not activated) was spotted in one channel with a mixture of 0.002 μCi of polar metabolite fraction from cabbage plus 50 μg of methomyl S-oxide, and was spotted in the other channel with 50 μg of methomyl S-oxide alone. Similar plates were prepared using the polar metabolite fraction from corn and tobacco.

All three plates were developed for 15 cm in acetonitrile. The spot of methomyl S-oxide was located in each channel by fluorescence quenching under ultraviolet light and marked. The plates were then exposed to X-ray film for 4 weeks. The X-ray film after development was compared to the tlc plates and marked to show the areas occupied by methomyl S-oxide. The results for cabbage and corn are shown in Figure 2. Identical results were obtained with the fraction from tobacco. Since no ^{14}C activity coincided with methomyl S-oxide, it was concluded that this material was not present as a metabolite in the polar fraction.

Treatment of Polar Metabolites with β-Glucosidase. The reaction was carried out according to the procedure of Bergmeyer (1963). The enzyme solution was prepared by dissolving 8 mg of β-glucosidase (Calbiochem No. 346801) in 4 ml of pH 6.2 buffer. The buffer was prepared by mixing Na_2HPO_4 (358 g of $Na_2HPO_4 \cdot 12H_2O/l$. in distilled
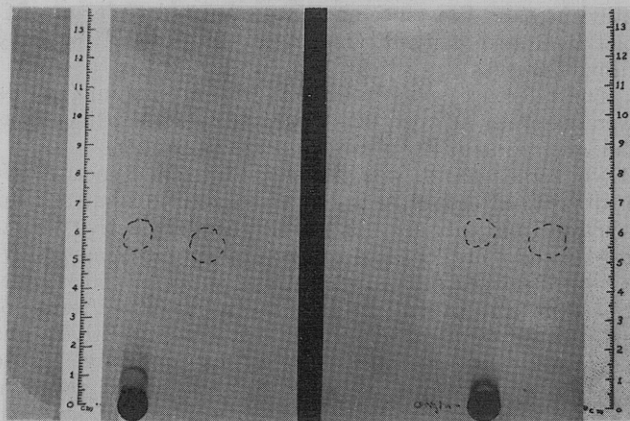

Figure 2. Tlc comparison of radioactive polar compounds (dark areas) with methomyl S-oxide (dotted circles). Left plate: left channel, polar fraction from corn plus S-oxide; right channel, methomyl S-oxide. Right plate: left channel, polar fraction from cabbage plus S-oxide; right channel, methomyl S-oxide.

water) with dilute acetic acid (60 ml of acetic acid/l. in distilled water) until pH 6.2 was attained.

Polar metabolites from corn (2 ml) were mixed with enzyme solution (1 ml) and diluted to 5 ml with buffer. Polar metabolites from cabbage (2 ml) were mixed with enzyme solution (1 ml) and diluted to 5 ml with buffer. Dilute aqueous S-methyl [1-^{14}C]N-hydroxythioacetimidate solution (2 ml) was mixed with enzyme solution (1 ml) and diluted to 5 ml with buffer. Aliquots of each solution were counted by liquid scintillation counting before incubation. Each solution was incubated at 37° for 44 hr.

A nonincubated control of S-methyl [1-^{14}C]N-hydroxythioacetimidate was prepared after the incubations were complete. All four solutions were then adjusted to pH 8–9 with 20% NaOH and extracted three times with 2-ml portions of ethyl acetate. The ethyl acetate extracts from a single solution were combined, dried over magnesium sulfate, and concentrated in a current of dry nitrogen to 1 ml. Aliquots of each concentrated extract were counted by liquid scintillation counting. Sixty and 71%, respectively, of the ^{14}C was extracted from the incubated and nonincubated controls. However, only 3.8 and 5.4% of the ^{14}C was extractable from the incubated solutions of the corn and cabbage metabolites. The latter values indicated little, if any, effect by the enzyme treatment.

The identity of the ^{14}C in the control extracts was examined by spotting a 5 cm × 20 cm × 250 μ silica gel GF tlc plate (Analtech) with 10 μl of the extract from the incubated control mixed with 40 μg of S-methyl N-hydroxythioacetimidate and with 10 μl of extract from the nonincubated control mixed with 40 μg of S-methyl N-hydroxythioacetimidate. The plate was developed for 15 cm with ethyl acetate. The S-methyl N-hydroxythioacetimidate spots were located and marked by examining the fluorescent plates under ultraviolet light. That spot was then scraped carefully from each channel, extracted with reagent methanol, and counted by liquid scintillation counting. From the nonincubated control, 85% of the radioactivity was recovered in the reference spot. This is consistent with repeated observations that a portion of this compound always volatilizes from tlc plates. From the incubated control, 56% of the radioactivity was recovered in the reference spot. This indicates slight decomposition of the reference compound under incubation conditions.

Four similar tlc plates were spotted from left to right with 50 μl of extract from incubated corn polar fraction mixed with 40 μg of S-methyl N-hydroxythioacetimidate reference, 50 μl of extract from incubated cabbage polar fraction mixed with 40 μg of reference, and 40 μg of reference compound. All plates were developed for 15 cm in

ethyl acetate. Reference spots were located under ultraviolet light and marked. One plate was exposed to X-ray film for 4 weeks.

The reference spots from each of the remaining plates were carefully scraped off, separated, eluted with methanol, and counted. The results were as follows.

No radioactivity was detected in the S-methyl N-hydroxythioacetimidate spots mixed with incubated polar fraction from cabbage. Of the original radioactivity spotted on the plate, 6% was recovered with the S-methyl N-hydroxythioacetimidate spot from incubated polar fraction from corn. Examination of the X-ray film after exposure to the tlc plate confirmed that no radioactivity was associated with the spot of reference compound in the case of cabbage. In the case of corn, the X-ray film showed that the small amount of radioactivity counted with the spot of reference compound is due to contamination by a small amount of radioactivity from a much larger spot adjacent to but slightly slower moving than the reference spot.

Thin-Layer Chromatography of the Polar Fraction. One 20 cm × 20 cm × 250 μ tlc plate (Analtech, silica gel GF, not activated) and one 20 cm × 20 cm × 250 μ tlc plate (Analtech, normal cellulose) were each spotted in four channels with 60-μg reference spots of the normal plant acids glycolic acid, glyoxylic acid, oxalic acid, and tartaric acid. In four adjacent channels the same acids were spotted after being mixed individually with 642 dpm portions of the polar metabolite fraction from cabbage. Two similar plates were spotted in the same manner with the same reference acids and the same mixtures, except that 450 dpm of polar fraction from corn was used.

The plates were developed for 15 cm in a mixture of 1-propanol–2 N aqueous ammonia (70:30). After drying in air at room temperature, each plate was exposed to X-ray film for 4 weeks. After the film had been developed, the spots were located on the tlc plates by spraying with a mixture of Universal pH Indicator (90 ml) to which 1 N sodium hydroxide solution (1 ml) had been added. The reference acid spots were marked and the corresponding spot in each mixture was marked. When the X-ray film was superimposed on the proper tlc plate, the location of the marked spots was transferred to the film. Both of the tlc plates spotted with the extract from cabbage showed shadows from radioactivity that coincided with the marked spots for glycolic acid and tartaric acid. Significant quantities of these acids do not appear to have been labeled in corn. All the plates show a multiplicity of radioactive spots and shadings throughout each channel of the polar fraction.

One 10 cm × 20 cm × 250 μ silica gel GF tlc plate (Analtech) and one 10 cm × 20 cm × 250 μ normal cellulose tlc plate (Analtech) were each spotted from left to right as follows: mixture of 642 dpm polar fraction from cabbage with 30 μg of glycine; 30 μg of glycine; 642 dpm polar fraction from cabbage with 30 μg of serine; and 30 μg of serine. Two similar plates were prepared using 450 dpm of polar fraction from corn instead of the polar fraction from cabbage. These plates were developed, dried, and exposed to X-ray film as previously described. The color spots were developed by spraying the plates with ninhydrin solution (Fisher) and heating in an oven at 100° for 4 min. Spots were marked as previously described. When the X-ray film was superimposed on the corresponding plate, the marked spots were transferred to the film. Although some radioactivity shadow appeared in the areas occupied by glycine and serine, it was not uniformly distributed throughout the spot and hence did not appear to be either of these compounds. On the other hand, both extracts showed two ninhydrin positive spots with higher R_f values that were presumed to be other amino acids. A large number of weak spots, as shown on all plates, would be predicted once the radiolabeled carbon has gotten into the metabolic pool.

One 5 cm × 20 cm tlc plate (Analtech PEI/cellulose) was spotted from left to right with 60 μg of D-glucose, 3 μl of polar fraction from corn, and 60 μg of sucrose. Another plate was spotted with 3 μl of polar fraction from cabbage and the two reference spots. Each plate was developed twice with a mixture of formic acid, methyl ethyl ketone, tert-butanol, and water (15:30:40:15, v/v). The plates were exposed to X-ray film for 4 weeks.

The plates were sprayed with 2-aminodiphenyloxalic acid dissolved in 85% ethanol and heated for 5 min at 110° in an oven. The glucose spots were located 5.7 cm from the origin and the sucrose was 3.6 cm from the origin, in excellent agreement with the literature. There was evidence from the X-ray film of radioactivity in the region occupied by those and other sugars.

MOVEMENT OF [¹⁴C]METHOMYL FROM TOBACCO LEAF

In order to see if methomyl would move from treated foliage to untreated parts of the plant, two tobacco plants, var. Xanthi, growing in soil in 6-in. plastic pots were selected when each was approximately 12 in. tall and had ten fully expanded leaves. The upper and lower portions of each plant were carefully wrapped in Saran Wrap, leaving only the fifth leaf from the ground exposed. The exposed leaf was sprayed with 0.5 ml of a solution containing 0.5 mg (2.70 μCi) of [¹⁴C]methomyl. The solvent was a mixture of water (95.8%), glycerine (4.0%), and Tween 20 (0.2%). After treatment the plants were placed in the greenhouse. The plants were watered as required at the base.

Three days after treatment, one of the plants was fractionated, as follows: growing tips, stem, and leaves not fully expanded; mature leaves and stem above treated leaf; treated leaf; mature leaves and stem below treated leaf; and roots. Each fraction was separately macerated in a Waring Blendor with an appropriate volume of ethyl acetate. Each solution was decanted through a sintered glass funnel and the residue was reextracted with ethyl acetate. The extracts of each fraction were combined and counted by liquid scintillation counting technique. The residues were analyzed by the wet combustion technique.

Seven days after treatment, the other plant was fractionated and analyzed exactly as described with one exception. The treated leaf, before being macerated with ethyl acetate, was washed thoroughly in 1 l. of distilled water. The washings were counted by liquid scintillation technique. The results of this study are shown in Table III. No methomyl was detected in extracts of any part of the plant other than the originally treated leaf. On the other hand, methomyl is absorbed into the treated foliage, and only 1% of the radioactive residue after 7 days could be removed by washing.

STUDY OF AChE INHIBITORY ACTIVITY IN TREATED TOBACCO AS A FUNCTION OF TIME

We have already shown that methomyl degrades rapidly in plant tissue and that the polar radioactive fraction derived from [¹⁴C]methomyl does not possess AChE inhibitory activity. The following experiment was conducted to determine if loss of total AChE inhibitory activity from the plant does indeed parallel loss of methomyl itself.

Xanthi tobacco plants with four to seven leaves were sprayed almost to runoff with an aqueous solution of methomyl at a concentration of 0.4 lb/100 gal of spray. The aqueous solvent contained 12.5% by weight of acetone and about 0.03% by weight of Duponol L144-WDG, a fatty alcohol sodium sulfate, as a wetting agent. Control plants were sprayed as described above except that methomyl was omitted from the spray solution. The growing tips of each plant were pinched to prevent new growth. Plants

Table III. Movement of Radioactivity in the Tobacco Plant after Foliar Application

Portion of plant	Radioactivity found	
	3 days after treatment, μCi	7 days after treatment, μCi
Growing tip	0.00	0.01
Leaves and stem above treated leaf	0.00	0.02
Treated leaf[a]	1.57	0.88[a]
Leaves and stem below treated leaf	0.00	0.02
Roots	0.00	0.02

[a] Only 0.01 μCi of radioactivity was obtained by washing the surface of the leaf 7 days after treatment.

were fertilized with Peters Water Soluble Fertilizer as required to keep the leaves green, on the 14th, 21st, and 28th day. Two plants of each treatment were harvested immediately as the 0-day harvest. Two additional plants were harvested 3, 7, and 14 days, while one plant was harvested 21 and 28 days after treatment. The plants were frozen immediately after harvest, and maintained at -5 to $0°F$ until analyzed.

After determination of the fresh weight of the plant leaves and stems, that tissue was transferred to a semi-micro Waring Blendor and macerated with sufficient reagent grade methanol to obtain a good homogenate. The mixture was then transferred to a sintered-glass Buchner funnel and filtered. The plant tissue was then returned to the blender and the extraction and filtration were repeated twice. The three filtered extracts were combined, mixed with 10 ml of water, and evaporated under reduced pressure in a rotary evaporator to slightly less than 10 ml of liquid phase. After dilution to exactly 10 ml with distilled water, the aqueous concentrate was analyzed for acetylcholinesterase inhibitory activity by the method of Robbins et al. (1958).

The inhibition constant, K_I, for each plant extract was first calculated in terms of the milligrams of original plant tissue required to produce 50% inhibition in the 1 ml of final hydrolysis mixture employed. Therefore, the more active the residues in the plant, the lower the K_I value. The anticholinesterase activities (or toxicities) of the plant tissue after 3, 7, 14, 21, and 28 days may also be expressed relative to that of the treated plant on day 0. Thus, the activity immediately on application is taken as unity, and all later values for treated plants are expressed relative to this common starting point. These data are shown in Table IV, which indicates that the half-life of total toxic residues from methomyl treatment on tobacco was about 7 days under greenhouse conditions.

CABBAGE

Treatment under Field Conditions. Cabbage was sown in flats on Sept 12 and transplanted into the field on a test farm near Bradenton, Fla., on Oct 12. Plants were spaced 1 ft apart in rows 3 ft apart. The soil type was a Leon Immokalee fine sand. Two sections of row, each containing three plants, were selected for treatment. The radiolabeled methomyl used in the laboratory studies was diluted with nonradiolabeled methomyl to obtain material with a specific activity of 0.428 μCi/mg. The plants were sprayed with this methomyl at the rate of 0.5 lb/acre in water at 50 gal/acre containing Du Pont Spreader-Sticker at 4 oz/100 gal. Eight treatments were applied on Oct 31, Nov 7, 14, 21, and 27, and Dec 5, 17, and 26. The plants also received: EPN-300 at 2 lb of active ingredient/acre on Sept 24 and Oct 10; Fermate at 2 lb/100 gal on Oct 22; and parathion 4E at 0.5 lb of active ingredient/acre on Oct 28. The remainder of the treated row, as well as one

Table IV. AChE Inhibitory Activity in Extracts of Treated Tobacco

Time after treatment	Day					
	0	3	7	14	21	28
$K_I = \dfrac{(\text{mg plant top fresh weight})}{(\text{ml reaction mixture})}$	0.78	1.11	1.41	4.13	11.2	18.8
Inhibiting ability relative to day zero	1.00	0.70	0.56	0.19	0.07	0.04

row on each side of it, was treated on the same schedule with Lannate 90WD at 0.5 lb of active ingredient/acre. The ^{14}C treatment was applied with a hand-held carbon dioxide pressure sprayer with a commercial nozzle in as close to the commercial method of application as possible. All sprays with nonradiolabeled pesticides were applied with conventional farm equipment.

The cabbage was harvested Jan 3 at maturity. Each head was cut and packaged separately in a polyethylene bag. The waste fraction, consisting of outer leaves and root, was packaged separately in the same manner. All fractions were frozen immediately and kept frozen until analyzed.

ANALYSIS OF FIELD-TREATED CABBAGE

One head of cabbage with its corresponding waste fraction was selected at random from each of the two treated plots. The fresh weight of each fraction was determined. All of each fraction was macerated separately with methanol in a Waring Blendor. Each slurry was decanted onto a sintered glass filter and the insoluble residue was sucked free of excess liquid. The residue was then reextracted twice more with methanol using the same procedure. The three extracts were combined and concentrated to 100 ml under reduced pressure in a rotary evaporator. The concentrate consisted of an aqueous solution mixed with a green gummy material. The gummy material was dissolved completely by shaking the entire concentrate with 100 ml of benzene. Aliquots of each of the resulting phases were radioassayed by liquid scintillation counting in Fluoralloy DXA (Beckman Instrument Co.). The composition of the extracts was determined by countercurrent fractionation as previously described. The insoluble plant material after extraction was dried in air and analyzed by wet combustion. The results of these analyses are shown in Table V.

CORN

Treatment under Field Conditions. Sweet corn was sown in the field adjacent to the cabbage on Oct 1. A single 6-ft segment of row was selected for treatment with radiolabeled methomyl, diluted to a specific activity of 0.222 μCi/mg. This material was applied at the rate of 0.5 lb/acre in water at 50 gal/acre containing Du Pont Spreader-Sticker at 4 oz/100 gal. Seven treatments were applied on Oct 31, Nov 7, 14, 21, and 27, and Dec 5 and 9. Two treatments with nonradioactive methomyl at 0.5 lb/acre were applied earlier on Oct 10 and 28. The remainder of the treated row, as well as one row on each side of it, was treated on the same schedule with Lannate 90WD at 0.5 lb of active ingredient/acre. Applications were made as described for the field treatment of cabbage.

The corn was killed at the early mature stage by a freeze on Dec 17. Before the plants had thawed, they were harvested, divided into ears, chopped fodder, and roots, and stored in a freezer in polyethylene bags.

ANALYSIS OF FIELD-TREATED CORN

Two packages of chopped fodder from radiolabeled methomyl-treated plants were selected at random and the fresh weight of each was determined. One bag of 12 ears

Table V. ¹⁴C Residues in Field-Treated Crops

Sample	Fresh weight, g	Total ¹⁴C, μCi	Distribution as percentage of total			
			Methomyl	Polar	Nonpolar	Unextractable
Cabbage						
Head "A"	935	1.16	3 (0.09 ppm)	49	4	44
Head "F"	1659	1.17	2 (0.03 ppm)	51	2	45
Waste "A"	607	7.29	3 (0.8 ppm)	31	12	54
Waste "F"	782	7.32	4 (0.9 ppm)	37	13	46
Corn						
Fodder "A"	514	1.57	13 (1.8 ppm)	35	6	46
Fodder "B"	578	2.44	14 (2.6 ppm)	31	6	49
Waste "A"	737	0.73	30 (1.4 ppm)	26	3	41
Waste "B"	757	0.74	36 (1.6 ppm)	26	3	35
Kernels	490	0.08	0 (0 ppm)	63	0	37

from the treated plot was also selected at random and divided into two portions of six ears each. The ears were husked and the kernels cut off the cobs to form a kernel fraction. The husks were kept with the corresponding cobs to give two cannery waste fractions. The fresh weights of the kernels and of the two cannery waste fractions were determined. Each fraction was extracted, concentrated, and analyzed by countercurrent fractionation as previously described. The results of these analyses also shown in Table V.

RESULTS AND DISCUSSION

Tobacco plants growing continuously in a nutrient solution containing 10 ppm of radioactive methomyl absorbed 20-25% of the available radioactivity over a 4-week period (Table I). Of the amount actually taken up, one-fourth was retained in the plant tissues, principally in the leaves, when the experiment was terminated, and three-fourths had already been lost to the atmosphere as volatile compounds. When an extract of the plant leaves was submitted to countercurrent fractionation, radioactivity was found in three main areas (Table II). The principal component, accounting for 14% of the absorbed dose, was identified as methomyl itself. In addition, small amounts of a polar radiolabeled fraction and of a nonpolar radiolabeled fraction, representing a total of about 3% of the absorbed dose, were also observed in the leaf extract. Of the radioactivity taken up by the plant and evolved as volatile components, approximately one-half was shown to be [¹⁴C]carbon dioxide and one-half as [¹⁴C]acetonitrile. The material remaining in the nutrient solution at the end of the experiment was nearly all unchanged methomyl.

When radiolabeled methomyl was applied to the surface of a single tobacco leaf on an intact plant, the material was absorbed into the treated leaf but was not translocated to other parts of the plant (Table III). The half-life of the methomyl in this experiment was between 3 and 7 days.

One week after treatment of the leaves of a young cabbage plant with radiolabeled methomyl, 20% of the radioactivity had been lost from the plant as volatile metabolites (Table I). These volatiles were identified as [¹⁴C]carbon dioxide and [¹⁴C]acetonitrile in approximately equal amounts. A total of 74% remained in or on the plant, and 2% had washed onto the soil. An extract of the radioactivity in or on the aerial portion of the plant after analysis by countercurrent fractionation was resolved into the same three components as the tobacco extract (Figure 1). In this species, the highly polar fraction accounted for most of the radioactivity in the extract. A nonpolar fraction contained about half as much, and there was a small residue of methomyl (Table II).

When the growing shoots of young corn plants were treated with radiolabeled methomyl, 47% of the original radioactivity was lost from the plants as volatile components within 10 days (Table I). A total of 49% remained

on the plant and 12% had washed onto the soil. Approximately four times as much [¹⁴C]acetonitrile as [¹⁴C]carbon dioxide was produced by the corn plant. The extract of the plants after countercurrent fractionation contained, as its principal component, the highly polar fraction with small amounts of nonpolar material and methomyl (Table II).

The extracts of the treated plants were all investigated specifically for the presence of three suspected metabolites of methomyl. One was S-methyl N-hydroxythioacetimidate, the hydrolysis product of methomyl. The other two were the S-oxide and S,S-dioxide derivatives of methomyl because biological oxidation of sulfur-containing compounds has been reported frequently in the literature. The partition coefficients of the hydrolysis product and of the S,S-dioxide derivative, as described previously, are such that both compounds, if present, would occur in tubes no. 10-25 of the countercurrent fractionations of the leaf extracts. Accordingly, the contents of these tubes were combined and the total amount of radioactivity was determined. As shown in Table II, the upper limit for the presence of these compounds as determined by radioactivity present was 0.1% of the applied dose for tobacco and approximately 1% of the applied dose for cabbage and corn. In view of the relatively large amounts of polar fraction in tubes no. 0-6 for cabbage and corn, even this low level of activity in tubes no. 10-25 is probably due to mechanical carryover of droplets of aqueous phase from the first few tubes.

The S-oxide of methomyl, on the other hand, would, by virtue of its partition coefficient, appear in the polar fraction if present in the original plant extracts. This possibility was examined by thin-layer chromatography of methomyl S-oxide alone and in mixture with the various polar fractions. As shown in Figure 2, no evidence was obtained for the presence of any methomyl S-oxide in any of the plant extracts.

The polar metabolite fractions from cabbage and corn were subjected to enzymolysis with β-glucosidase. The reaction failed to liberate any S-methyl N-hydroxythioacetimidate, confirming the absence of this compound even as a sugar conjugate in the polar fraction.

Methomyl is a potent acetylcholinesterase (AChE) inhibitor. When the polar metabolite fraction from cabbage was assayed in an AChE inhibition test, the polar fraction from the methomyl-treated plant did not exhibit any increase in activity over that found for the untreated plant. In fact, the extract from the particular untreated cabbage plant analyzed was more of an inhibitor than that obtained from the treated plant. This variability is undoubtedly due to the presence of a natural inhibitor in cabbage. In corn, neither the polar fraction from the methomyl-treated plant nor the polar fraction from the untreated plant showed any AChE inhibitory activity.

A kinetic study of the loss of AChE inhibitory activity in methomyl-treated tobacco plants as a function of time

indicated that the half-life of total toxic residues from methomyl treatment in tobacco was about 7 days, in general agreement with the rate of disappearance of [^{14}C]methomyl from a treated leaf in this species (Table IV).

The radioactivity in the nonpolar metabolite fraction is found in neutral compounds which are saponified in base to yield fatty acids and other neutral compounds. The fatty acids are those normally found in plants, *i.e.*, C_{16}, C_{18}, and C_{20}. The original neutral compounds that show this behavior must therefore be esters, *i.e.*, lipids, and the neutral compounds remaining after saponification must be the natural alcohols which originally formed the esters.

With regard to the polar fraction, [^{14}C]glycolic acid has been shown to be present by thin-layer chromatography. It is interesting to note here that glycolic acid is the substrate for photorespiration which results in the oxidation of glycolic acid to carbon dioxide. Corn is reported by Downton and Tregunna (1968) to lack this metabolic pathway, and hence does not produce carbon dioxide from glycolic acid in the light. In our experiments, tobacco and cabbage both produced acetonitrile and carbon dioxide in approximately equal amounts, whereas corn produced four times as much acetonitrile as carbon dioxide.

Evidence for the presence of radiolabeled tartaric acid as a metabolite has been obtained on the thin-layer chromatographic plates as well as an indication of labeling in the plant sugars.

The thin-layer plates do not show conclusive evidence for labeling of glycine or serine. However, two of the spots obtained on the autoradiograms of the plates definitely coincide with materials that give a violet color with the ninhydrin reagent and, hence, probably are amino acids.

Finally, it is concluded from the very multiplicity of spots and the shadings of radioactivity throughout each channel of radioactive polar fraction on the thin-layer plates that large numbers of natural products have become radiolabeled through reincorporation of $^{14}CO_2$ or other small fragments formed during the rapid and complete metabolic breakdown of methomyl.

Repetitive season-long treatment of cabbage and corn under field conditions has confirmed the results obtained with single treatments on young plants in the laboratory (Table V). The outer leaves or "waste fraction" of cabbage contain most of the radioactive residue. An extract of the radioactivity in these leaves is composed of methomyl, a polar fraction, and a nonpolar fraction. The amount of methomyl in the waste fraction 8 days after the last application was equivalent to 0.8–0.9 ppm. Very little radioactivity moved into the head of the cabbage and, again, the extract was composed of the same three types of material—a large polar fraction, a small nonpolar fraction, and a trace of methomyl (0.06 ppm).

In corn, the outer portions of the plant, as expected, contain most of the radioactivity. Very little radioactivity was found in the kernels. Eight days after the last application, the amount of methomyl in the various fractions was equivalent to 2 ppm in fodder, 1.5 ppm in cannery waste, and 0.00 ppm in the kernels. Reincorporation of ^{14}C has resulted in the formation of a polar and a nonpolar fraction of normal plant constituents. The relatively larger amounts of nonextractable radioactivity in the field studies probably result from reincorporation of ^{14}C into the basic structural material of the plant itself. In the field study the plants had a whole season to utilize the relatively soluble radiolabeled natural products produced by reincorporation, whereas the plants were restricted to 1 week reincorporation periods in the laboratory studies.

In conclusion, we find that in all crops studied and by either foliar or root application, methomyl itself is the only terminal residue resulting from methomyl application. Therefore, the analysis of crops for intact methomyl using the published selective gas chromatographic method is a valid measure of the total toxic residues.

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