Metabolism of Oxamyl in Plants

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Radiolabeled oxamyl, methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate-*1*-^{14}C, was synthesized, and its metabolic fate determined in tobacco, alfalfa, peanuts, potatoes, apples, oranges, and tomatoes. The major route of degradation involved hydrolysis to the corresponding oximino compound, which in turn became conjugated with glucose. Further metabolsim resulted in the loss of one of the *N'*-methyl groups and/or addition of other glucose units to the sugar moiety of the original conjugate. Total breakdown of the pesticide molecule with incorporation of the carbon-14 into normal natural products has been demonstrated. In tobacco small amounts of *N,N*-dimethyloxamic acid were detected, while fruits of apple, orange, and tomato contained small quantities of *N,N*-dimethyl-1-cyanoformamide.

Oxamyl is the approved common name for methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate. This compound is the active material in Du Pont's Vydate oxamyl insecticide/nematicide and was formerly known as DPX-1410.

A method for the determination of oxamyl residues using flame photometric gas chromatography has been published by Holt and Pease (1976). The information reported herein establishes the metabolic fate of oxamyl in a variety of crop plants, and the nature of the residues found in these crops.

EXPERIMENTAL SECTION

Chemicals. Radiolabeled oxamyl was synthesized in a five-step reaction sequence from ethyl acetoacetate-2-^{14}C (Scheme I) purchased from New England Nuclear Corporation.

Authentic samples of the compounds in Table I were prepared for comparison with oxamyl metabolites, as were also the *S*-oxide and *S,S*-dioxide of oxamyl and of I. Details of these syntheses are described in the supplementary material.

Equipment and Methods. Oxamyl and its metabolites were extracted from plant tissue by repeated maceration in a Waring Blendor with methanol followed by filtration. Three extractions were usually sufficient to remove all extractable radioactivity. Radioactivity in extracts, and subsequent liquid fractions, was determined by liquid scintillation counting (LSC) in premixed scintillation cocktail using an Isocap 300 Scintillation Counter (Searle Analytic). The tissue after extraction was air-dried and analyzed for insoluble radioactivity by combustion analysis (CA) in a Model 305 Oxidizer (Packard Instrument Co.).

The methanolic extracts were combined and evaporated under reduced pressure in a rotary evaporator with water bath at <40 °C to a final volume such that the liquid concentrate was actually aqueous from the water found originally in the plant tissue. At this point the evaporation flask typically contained in addition to a fairly clear brownish solution, a considerable quantity of greenish-black gum. Hexane, equal in volume to the aqueous phase, was added and the mixture shaken until all residues dissolved and a clear two-phase system was obtained. In all studies with [^{14}C]oxamyl in foliage, all radioactivity was recovered in the aqueous phase.

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Scheme I

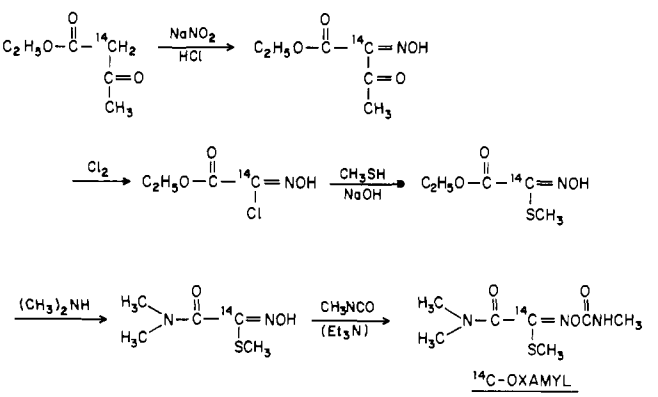
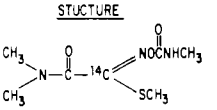
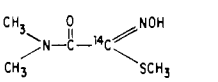
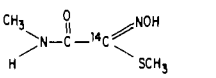
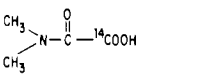
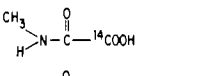
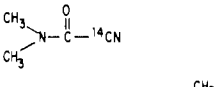
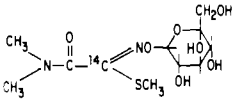
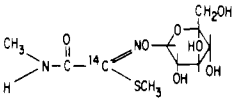


Table I. Names and Structures of Compounds Related to Oxamyl

COMPOUND	NAME	STUCTURE
OXAMYL	METHYL N',N'-DIMETHYL-N [(METHYLCARBAMOYL) OXY] - 1-THIOOXAMIMIDATE	
(I)	OXIMINO METABOLITE METHYL N-HYDROXY-N', N'-DIMETHYL-1-THIOOXAMIMIDATE	
(II)	METHYL N-HYDROXY-N'-METHYL-1-THIOOXAMIMIDATE	
(III)	N, N-DIMETHYLOXAMIC ACID	
(IV)	N-METHYLOXAMIC ACID	
(V)	DMCF N, N-DIMETHYL-1-CYANOFORMAMIDE	
METABOLITE A	GLUCOSE CONJUGATE OF I	
METABOLITE A'	GLUCOSE CONJUGATE OF II	

Oxamyl and other organosoluble metabolites may be extracted from the aqueous phase with ethyl acetate at this point or determined directly in the aqueous phase without prior extraction. Either way these analyses were carried out by thin-layer chromatography (TLC) on silica gel GF chromatoplates (Analtech, 250 μm) developed for 15 cm

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Table II. HPLC of Polar Metabolite Fraction

System	Packing	Dimensions	Solvent	Flow Rate
1	Bio-Gel P-2 (BioRad Laboratories)	13 mm × 1000 mm	Water	6.0 mL min^{-1}
	Evaporation of solvent, followed by transfer of radioactivity to methanol			
2	Sephadex LH 20 (Pharmacia Fine Chemicals)	6 mm × 1000 mm	Methanol	1.6 mL min^{-1}
3	Porasil A (Waters Associates)	2.8 mm × 1000 mm	Methanol	0.5 mL min^{-1}
	Evaporation of solvent, followed by transfer of radioactivity to tetrahydrofuran (THF)			
4	Porasil A (Waters Associates)	2.8 mm × 1000 mm	THF (0.5% H_2O)	0.5 mL min^{-1}

with ethyl acetate. Nonradioactive reference compounds were located by fluorescence quenching under ultraviolet light. Radioactive bands were located with a Berthold Model 6000-2 Automatic TLC Radioscanner (Varian-Aerograph). The amount of radioactivity in each fraction was determined by scraping the silica gel off the plate, eluting the radioactivity with methanol, and counting of aliquots. Metabolites which remained in the aqueous phase after extraction or at the origin of the TLC plates were designated as the polar fraction.

This polar fraction from oxamyl-treated plants was cleaned up and the individual metabolites isolated for identification by high-performance liquid chromatography (HPLC) in the sequential procedure summarized in Table II. HPLC was carried out in glass columns (Chromatronix) with eluting solvent pumped at constant flow rate by a suitable chromatographic pump, e.g., Mini-Pump (Milton-Roy Co.). Radioactive fractions were detected with a Model 3021 TriCarb Scintillation Flow Monitor (Packard Instrument Co.), and quantitation was obtained by LSC of aliquots of fractions collected. A Model 410 ultraviolet absorbance detector (Du Pont) was used where appropriate. In general, HPLC steps 1, 2, and 3 were necessary to clean up and separate the radioactive derivatives of oxamyl from the bulk of the nonradioactive natural products in the extracts. Resolution of individual metabolites from each other did not occur until the attempted transfer to THF and the subsequent absorption chromatography on column no. 4.

Special techniques for the identification of special radioactive products will be discussed in connection with the crop where they occurred.

Mass spectra of metabolites were obtained with a Model 21-492 Mass Spectrometer (Du Pont) coupled with an all-glass GC/MS system using a Perkin-Elmer Model 990 Gas Chromatograph. An all-glass system was essential for analysis of these metabolites as they decomposed when exposed to metals. The GC column was 2 ft × 2 mm glass tubing packed with 10% OV-1 on 100/120 mesh Gas-Chrom Q. Special conditions will be indicated where appropriate.

Treatment of Tobacco (Growth Chamber). The foliage of each of two tobacco plants, var. *Xanthi*, grown in soil in 6-in. pots was treated by pipet with droplets of an aqueous solution of [^{14}C]oxamyl (10 mg, 37.2 μCi) in 0.2% Tween 20. The treated plants were maintained in healthy normal growth in a plant growth chamber (Scherer-Gillett Model CEL 25-7). One of them was enclosed in a glass metabolism apparatus (Harvey and Reiser, 1973) so that all volatile metabolites could be trapped. The first plant was harvested 7 days after treatment; the second (metabolism jar), 15 days after treatment. Foliage was washed first with water to remove surface residues. Aerial and root tissues were extracted and analyzed separately. All fractions from the metabolism apparatus experiment were analyzed for radioactivity and a material balance obtained (95% recovery). Results are summarized in Table III.

Table III. Percent Applied ^{14}C Present in Fractions of Tobacco

	Days after treatment	
	7	15
Volatile components		
Prefurnace traps	*[a]	1.3%
Postfurnace traps	*	0.0%
Condensate	*	3.8%
Plant Tissues		
New growth		
Surface	[b]	0.0%
Extract	[b]	1.3%
Nonextractable	[b]	<0.1%
Treated portion		
Surface	57.3%[b]	50.0%
Extract	32.5%[b]	37.1%
Nonextractable	0.3%[b]	0.5%
Root		
Extract	0.1%	0.05%
Nonextractable	0.1%	0.05%
Soil	*	0.7%
Total recov.		94.8%

[a] (*) not determined. [b] New growth combined with treated portion.

Treatment of Alfalfa (Field Conditions). A small field plot was established in an alfalfa field near Newark, Delaware which was beginning to regrow after mowing 1 month previously. This plot was sprayed three times at 2-week intervals beginning mid-July with a solution of [^{14}C]oxamyl equivalent to 0.5 lb/acre in 0.2% Tween 20. Two and one-half weeks after the last treatment, the aerial portions of the treated plants were harvested, cut up, and frozen for analysis.

Treatment of Peanuts (Field Conditions). Peanut plants for this experiment were grown and treated under typical peanut belt conditions near Clayton, N.C. A small test plot was rototilled and seeded somewhat more densely than normal to peanuts in a single row. When the plants developed the first true leaves and were 3–4 in. wide, a foliar spray of [^{14}C]oxamyl equivalent to 2 lb/acre was applied over the center of the row, 6 in. wide. All emerging plants received this first treatment. Four weeks later at first flower stage, the plants were "thinned" to normal commercial spacing and the *young plants* that were removed were immediately frozen for investigation of short-term metabolites. The plants that remained in the field were immediately given a second treatment of [^{14}C]oxamyl at 2 lb/acre over the center of the row, now 12 in. wide. Additional normal fungicide treatments were applied as needed to maintain healthy growth. Weeds that developed were cut off at ground level and left to decompose on the treated soil surface. When the treated

plants were mature, they were harvested, separated into *mature hay* and *mature nut* fractions, and frozen prior to analysis.

Although the analysis of the *young plant* fraction was carried out as described earlier, difficulties were encountered with the *mature hay* and *mature nut* fractions. The polar metabolite fraction from *mature hay* was purified by HPLC up to the point where methanol was evaporated and the transfer of radioactivity to THF attempted. Repeated attempts to transfer the radioactivity to THF were unsuccessful. Incubation of this THF insoluble polar metabolite fraction was carried out for 48 h at 37 °C with β-glucosidase (Sigma Chemical Co.) at pH 6.8. The radioactivity after the enzyme treatment was extracted into THF and the analysis on Porasil/THF completed in the usual way.

The extraction procedure for the *mature nuts* was modified because of the oily nature of this material. Clean shelled peanut meats were macerated first with hexane (three times), then with ethyl acetate (three times), and finally with methanol (four times). An attempt was made to separate the radioactivity from the peanut oil in the hexane extract by passing a benzene solution of this fraction through a 9 mm × 1000 mm column of BioBeads SX-12 (Bio-Rad Laboratories) in benzene. All radioactivity passed through the column unretained with the peanut oil. The peanut oil with the associated radioactivity was saponified by refluxing with 5% potassium hydroxide in ethanol. The fatty acid fraction which contained 70% of the radioactivity was converted to the methyl esters with diazomethane and analyzed by gas chromatography on two different 4 mm × 6 ft columns, 3% OV-101 on Chromosorb 80–100 mesh, and 15% Carbowax 20M on Diatoport "S". The gas chromatograph was equipped with a splitter so that the effluent gas fractions were trapped by bubbling portions of each fraction through scintillation counting solution, and the amount of radioactivity determined by LSC. The radioactivity in the ethyl acetate and methanol extracts from the mature nuts was characterized by TLC and HPLC.

Both peanut foliage and meats at maturity showed fairly high amounts of radioactivity that could not be removed by exhaustive but nondestructive extraction (i.e., approximately 40% unextractable carbon-14). Even Soxhlet extraction with boiling methanol removed only further traces of radioactivity. These "bound" residues were subjected to attack with a mixture of cellulase enzyme, types I, II, and III (Sigma Chemical Company) at pH 4.7 and 47 °C for 48 h. The radioactivity solubilized into water or ethanol by this treatment was determined by LSC, as well as the amount extractable from aqueous solution by ethyl acetate.

Treatment of Potatoes (Field Conditions). Shortly after the plants had emerged, a section of row of potatoes (var. *Kennebec*) was selected which contained four uniform plants to receive foliar treatments with [^{14}C]oxamyl. Five foliar treatments were applied at approximately 10-day intervals between the time when the plants emerged and up to 14 days before harvest. The first treatment was applied at a rate equivalent to 0.5 lb of oxamyl/acre and the remaining treatments were at 1.0 lb/acre. At harvest the four plants yielded 1190 g of potato tubers. An aqueous wash of the surface of the potato tubers removed little or no radioactivity (<2%). The potato tubers were divided into peel and interior fractions, each of which was freeze-dried separately and analyzed by CA. The interior fraction of the foliar treated potato tubers, containing approximately 90% of total radioactivity, was singled out for residue characterization. The freeze-dried potato flour was extracted exhaustively with several solvents: water, methanol, ethyl acetate, and THF. Less than 3% of the radioactivity was extracted into any of these solvents.

Twenty grams of lyophilized potato tuber (less peel) was hydrolyzed by incubation with stirring for 24 h at 37 °C with 100 mL of anhydrous methanolic hydrochloric acid (Applied Science Laboratory). The mixture was centrifuged and the supernatant was removed from the centrifuge tube. The residue was washed three times with 50-mL portions of methanol which were added to the supernatant. The residue in the centrifuge tube was air-dried and analyzed for ^{14}C content by CA.

The combined supernatant and methanol washings containing 78% of the original ^{14}C activity were evaporated to dryness under nitrogen and taken up with ethyl acetate (3 × 25 mL). The ethyl acetate solution, containing 64% of the original radioactivity, was taken to dryness under nitrogen and the residue dissolved in 2 mL of methanol. Aliquots of the methanol solution were analyzed by TLC together with standards I and II.

As a qualitative cross-check, 5 g of freeze-dried potato flour was treated with β-glucosidase at 37 °C overnight. After purification procedures two ^{14}C compounds soluble in THF were found. These compounds were identified by LC retention times as metabolites A and A′ using the chromatographic systems previously described.

In order to account for the remaining radioactivity in the potato tubers, the possibility of reincorporation of the ^{14}C-labeled carbon into starch itself was investigated by hydrolyzing the starch into its component glucose fraction and providing that ^{14}C was present in the glucose. Ten grams of lyophilized potato tuber (less peel) was suspended in 150 mL of 2% sulfuric acid solution and carefully heated in a flask. After heating for 2 h at 90 °C, the contents were refluxed for 5 h. The mixture was cooled to about 60 °C, neutralized with calcium hydroxide to pH 5, and filtered to remove calcium sulfate and insoluble residue. The filtrate was then heated to 60 °C and 1 g of activated carbon was added. After stirring for 30 min the mixture was filtered with suction through a Buchner funnel. After removal of the solvent, a heavy syrup was obtained which was mixed with 5 g of silica gel and transferred onto a silica gel column (2.5 cm diameter, 35 cm long) with methanol. The column was successively eluted with 200 mL of methanol, 200 mL of 5:1 methanol–H_2O, and 300 mL of 1:1 methanol–H_2O. The latter solvent was found to elute glucose. This fraction was purified by crystallization. The solvent was removed and the resulting syrup was poured into a petri dish and allowed to stand for 3 days until the crystallization was complete. The mother liquor was removed, and the crystalline mass was broken up and washed with ethanol and THF to remove the mother liquor from the crystals. The weight of the isolated, air-dried crystals was 438 mg.

A sample of the dried crystalline material was dissolved in distilled water and counted by liquid scintillation. For gas chromatographic analysis, a 100-mg sample of the material was silylated with 2 mL of Tri-Sil Z (Pierce Chemical Co.) at 60 °C for 30 min. A sample of α-D-glucose was similarly treated with Tri-Sil Z to serve as standard TMS-α-D-glucose. A sample of β-D-glucose was not available. The derivatized samples were analyzed on a F&M Model 810 gas chromatograph equipped with a flame ionization detector, using a glass column (6 ft × 0.25 in.) containing 3% OV-101 on 80–100 mesh Chromosorb W. Column temperature was programmed from 140 to 210 °C at a rate of 10 °C/min. The gas chromatograph was

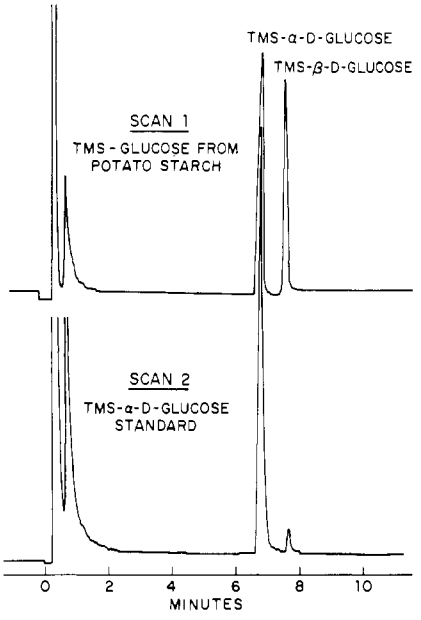

Figure 1. GC of [^{14}C]glucose from hydrolyzed potato starch.

equipped with a 10 to 1 splitter, which permitted trapping of the effluent gas in scintillation vials for radioassay. The trapping efficiency of the effluent was determined to be 86% with standard ^{14}C-labeled hexadecane. Typical chromatographic scans of standard TMS-α-D-glucose and TMS-α-D-glucose and TMS-β-D-glucose from potato starch are shown in Figure 1.

A total of 80 mg of glucose (as the TMS derivatives) from the potato starch was injected into the GC. The TMS-α-D-glucose and the TMS-β-D-glucose peaks were trapped in the same scintillation vial and the radioactivity counted. The GC trapping-counting results were in excellent agreement with direct radioassay of the crystallized glucose. By the GC trapping technique 34% of the original radioactivity was found to be [^{14}C]glucose whereas by direct radioassay of the glucose we found 35% [^{14}C]glucose.

Mass spectra of the TMS-α-D-glucose standard and the TMS-α-D-glucose and the TMS-β-D-glucose obtained by acid hydrolysis of the potato tubers were in excellent agreement. The following characteristic fragment ions were found: *m/e* 525, 435, 393, 361, 345, 332, 319, 305, 291, 243, 231, 217, 204, 191, 147, 129, 117, 103, and 73.

Treatment of Apple Fruit. Four young apples on a Jonathan tree in a Delaware orchard were treated once by brushing with [^{14}C]oxamyl at 1 lb/100 gal on July 25. The ripe apples were harvested Sept 10. An aqueous wash of the surface of the apples removed at most a trace of total radioactivity (0.0–0.3%). Each of the four apples were divided into peel and interior fractions, each of which was extracted separately with methanol in the usual manner. Pulp after extraction was dried and analyzed for ^{14}C by combustion. Levels of total radioactivity ranged from 0.8–2.0 ppm (calculated as oxamyl) and tended to be similar in peel and interior fractions. Most of the activity (98%) was extractable with methanol.

After removal of the methanol from the combined extracts in a rotary evaporator, 77% of the radioactivity partitioned from the resulting aqueous concentrate into ethyl acetate and was analyzed by TLC.

An unknown appeared as a band 9–12 cm above the origin on silica gel TLC plates developed for 15 cm with ethyl acetate. It had never been seen before in any of the numerous oxamyl metabolite analyses which we have run with this TLC system. A sample was purified by TLC and identified by mass spectrum analysis as *N,N*-dimethyl-1-cyanoformamide. Comparison of retention times of the ^{14}C metabolite with an authentic synthetic sample by LC, TLC, and GC confirmed the identification.

The entire polar metabolite fraction, 23% of total ^{14}C in the apples (i.e., that fraction not partitioned into ethyl acetate), was purified by passage through the usual series of chromatographic columns. Its behavior was like that of the polar fraction from mature peanut foliage. Like the peanut fraction, the apple polar metabolite fraction failed to transfer radioactive materials to THF, indicating the absence of significant quantities of metabolites A and A′, as such. The purified polar metabolite fraction from apple fruit was submitted to β-glucosidase attack. The enzymolysis did not appear to be complete, about 65% remained unchanged. However, 35% of the radioactivity was released and identified in a LC effluent fraction with the retention time of metabolite A.

Treatment of Oranges. Immature Hamlin oranges were treated by brushing on Dec 12 in Florida with [^{14}C]oxamyl solution equivalent to 1.2 lb/100 gal (0.2% Tween 20). Six weeks later the oranges were harvested at maturity and frozen until analyzed. Four oranges were partially thawed, sliced in half, and "juiced" with a home-type rotary juicer.

The rind fraction (300 g) was macerated and extracted exhaustively with methanol. The tissue after extraction was air-dried, and analyzed for residual ^{14}C content by combustion. The methanol extract of the rind contained 96% of the radioactivity. After evaporation of these extracts to an aqueous concentrate, 34% of the extracted radioactivity partitioned into ethyl acetate, leaving the remainder as a polar fraction in the water. The ethyl acetate part of the rind metabolites was analyzed by TLC. The positive identification of the DMCF was carried out as described under apple metabolism.

The orange juice fraction was lyophilized and the total ^{14}C content determined by combustion analysis. A portion of the orange juice residue was dissolved in water and extracted with ethyl acetate. The ethyl acetate soluble portion (41%) was analyzed by TLC. The radioactivity remaining in the aqueous phase (59%) was a polar metabolite fraction.

The polar metabolite fractions from both orange rind and orange juice were purified as previously described by HPLC (system 1 and 2). At this point it became evident that the presence of unusually large quantities of natural products remaining with the rather small amount of radioactivity necessitated the introduction of an additional purification step. Accordingly, the partially purified polar fractions were purified by HPLC on a 6 mm × 500 mm column of Aminex A-6 (Bio-Rad Laboratories) in the Ca^{2+} form using water as eluent. Purification was good, and the polar radioactivity was resolved into major (75–80%) and minor (25–20%) components. After in vacuo evaporation of the water, the residues of the major component were triturated with tetrahydrofuran and the resulting extracts chromatographed with HPLC system no. 4. Too little radioactivity was found in the minor components for further identification.

Treatment of Tomatoes (Greenhouse). Each of two small green fruit on a Bonny Best tomato plant were spotted by micropipet as evenly as possible over the entire surface with 300 μL of an aqueous solution of [^{14}C]oxamyl (0.37 mg; 2.40 μCi) containing 0.2% Tween 20. The plant was grown for 14 days more in the greenhouse, and the red fruit harvested. The surface residues were washed off with distilled water and analyzed. The tomatoes were extracted with methanol, and the extract and insoluble pulp were

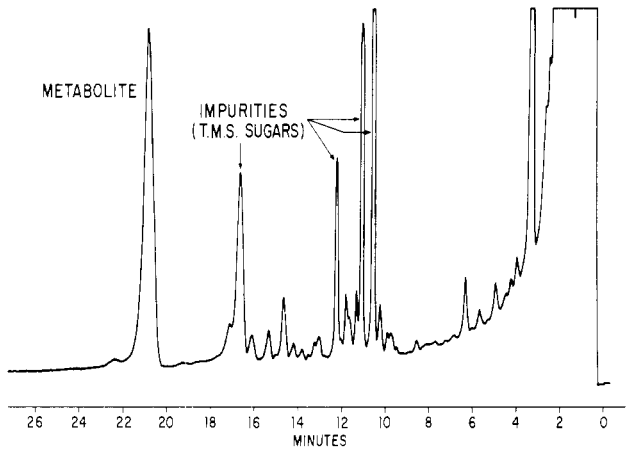

Figure 2. GC of polar metabolite from tobacco (TMS derivative).

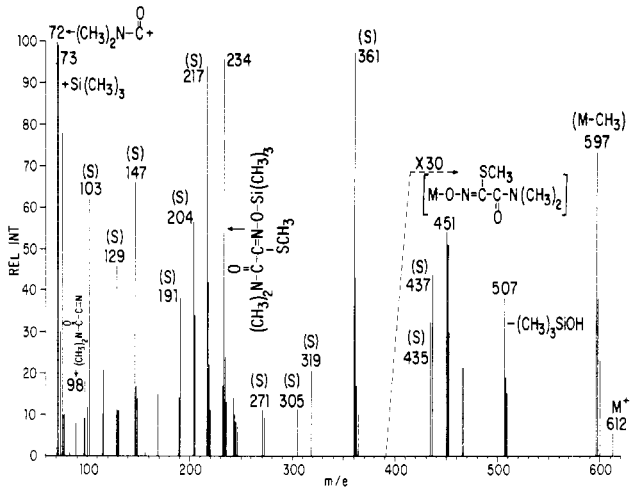

Figure 3. Mass spectrum of tobacco metabolite A (TMS derivative).

analyzed as previously described. Final purification of the polar metabolites was carried out as for oranges.

RESULTS AND DISCUSSION

Tobacco. A tobacco plant treated on the foliage with [^{14}C]oxamyl and grown for 15 days in a metabolism apparatus in a plant growth chamber was completely fractionated and analyzed as shown in Table III. The overall recovery was 95% of the radioactivity in the original treatment (O.T.). A small amount of radioactivity (1.3% O.T.) was trapped as $^{14}CO_2$. There was no evidence of any other volatile metabolites. Fifteen days after treatment 50% of the radioactive treatment could be washed off the surface of the treated leaves. Analysis of this aqueous wash showed 96% oxamyl and 3% compound I.

Radioactivity was distributed within the tissues of the plant with 37.6% O.T. in the treated leaves, 1.3% O.T. in the new growth, and 0.1% O.T. in the root. A similar distribution was found in the tissues of an identical plant only 7 days after treatment except that 57% was still a surface residue and 33% had entered the plant tissues.

Qualitative and quantitative analysis of the extract of the treated leaves (after removal of surface residue by washing) showed that the principal residue 15 days after treatment was [^{14}C]oxamyl (56%). Some of the oximino compound (I) (5%) was found, plus a polar unknown fraction (39%). In the 7-day plant, 73% of the radioactivity in the extract was still oxamyl while 8% had been converted to I and only 19% to the polar unknowns. Clearly oxamyl was degrading via hydrolysis to the oximino compound with buildup of a polar unknown fraction.

The polar unknown fraction was separated into two radiolabeled components and purified by gel permeation and adsorption chromatography. The major component (93%) was a single compound which was designated metabolite A. In HPLC system no. 4, it exhibited a characteristic retention time of 26–32 min. The mass spectrum of this compound as run by direct probe varied with temperature. At a relatively high probe temperature, pyrolysis was obtained and ions indicative of I and others indicative of sugar fragments were obtained, suggesting that the metabolite is a sugar conjugate of I. However, no molecular ion of the conjugate was observed.

When the sample was derivatized with bis(trimethylsilyl)trifluoroacetamide plus an equal volume of DMF, which acts as a solvent and catalyst, and analyzed by GC–MS, the chromatogram in Figure 2 was obtained. Mass spectra showed that the 21-min peak is the metabolite and the other peaks in the chromatogram were TMS sugars, i.e., natural products from the tobacco. Figure 3 shows the mass spectrum of the metabolite peak.

The molecular ion at mass 612 is extremely weak, but a significant M – CH_3 fragment is obtained, which is characteristic of TMS sugars. The intensity of the ions above mass 400 have been increased by a factor of 30 in this figure. The actual relative intensity of the 597 ion is about 3%. The fragment ions marked with an "S" are characteristic of TMS glucose, for example, m/e 191, 204, and 217 (Radford and DeJongh, 1972). The base peak at m/e 72 is due to the dimethylcarbamoyl fragment. An unusual fragment ion was obtained at m/e 234 due to a rearrangement of a TMS group back onto the =NOH. The identity of the 234 and 72 ions were confirmed by high-resolution mass measurements. The 72 ion was checked because of the possibility that this could have been a formyl NH group which has the same nominal mass as the dimethylamino group. These data indicate that the metabolite A is a glucose conjugate of the oximino compound (I) and the structure is shown in Table I. When this metabolite was synthesized, the HPLC and GC retention times and mass spectrum of the TMS derivative of the synthetic compound were identical with those of the isolated metabolite.

A minor component (7%) of the polar fraction from the 15-day tobacco plant was shown by chromatographic behavior to be identical with *N,N*-dimethyloxamic acid (III), a major degradation product of oxamyl in river water (Harvey and Han, 1978a) and as a conjugate in the urine of rats (Harvey and Han, 1978b).

Alfalfa. When alfalfa hay, which had been treated three times with [^{14}C]oxamyl at 0.5 lb/acre and harvested 17 days after the last treatment, was extracted with methanol 75% of the ^{14}C residue was extractable. TLC analysis of the crude extract indicated that 98.4% of the radioactivity was in the form of polar metabolites. Only traces of oxamyl (0.8%) and of I (0.8%) were found. The principal polar metabolite (>90%) was identified by HPLC as metabolite A, and the structure was confirmed by GC–MS study.

Peanuts. The *young plants* from this experiment were examined not because this fraction is significant in terms of final residues but because these plants give us a picture of the intermediate stages of oxamyl metabolism 4 weeks after a single foliar treatment under field conditions. No trace was found of either oxamyl or I in the extract of these *young plants*. Instead, >99% of the radioactivity in the extract was highly polar. When this polar fraction was purified by HPLC the radioactivity was soluble in THF and resolved into two ^{14}C compounds on the Porasil

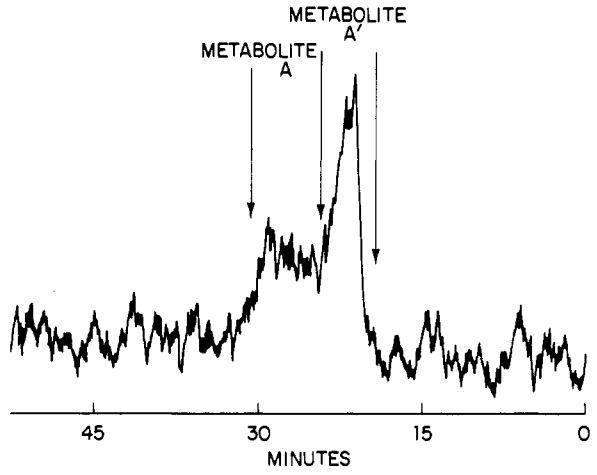

Figure 4. Resolution of ^{14}C metabolites A and A' from young peanut plants on porasil A/THF.

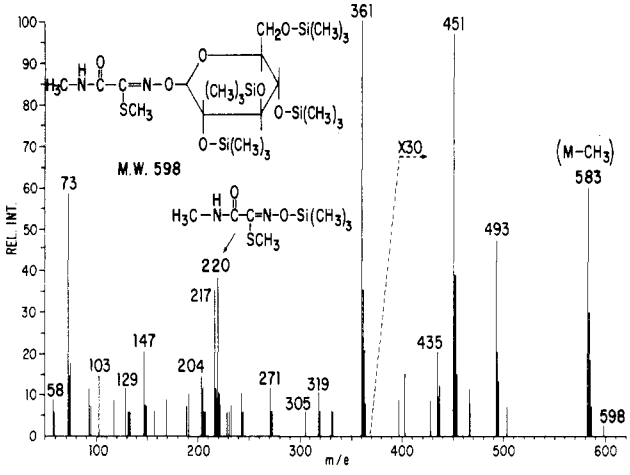

Figure 5. Mass spectrum of peanut metabolite A' (TMS derivative).

A/THF column in an approximate ratio of 2:1 (Figure 4). The metabolite with retention time 25–32 min was isolated as previously described and confirmed as metabolite A by GC–MS. The metabolite with the shorter retention time (20–25 min) was designated metabolite A' and was converted to the trimethylsilyl derivative and purified by GC as described for metabolite A. The derivative of metabolite A' eluted from the GC at about 18 min and Figure 5 shows the mass spectrum obtained. The structure of metabolite A' was assigned as the N'-demethylated structure shown in the figure and in Table I. The M – CH_3 ion is 14 amu lower than that obtained on the previous metabolite, indicating metabolic demethylation. Also the rearrangement ion at 220 is 14 amu lower, and the 72 ion is replaced with a 58 ion due to $CH_3NHC{=}O$. (Note that the NH is not silylated.) The 451 ion is due to the TMS glucose part of the molecule. This identification was also confirmed by synthesis and HPLC, GC, and MS comparisons of the ^{14}C metabolite and synthetic materials.

In *mature hay*, which was harvested about 7 weeks after the second [^{14}C]oxamyl treatment, or about 11 weeks after the first treatment, no oxamyl was present (<0.5%) in the extract, although a trace of compound I was observed (1%). Most of the radioactivity was in the form of very polar conjugates (99% of the extractable radioactivity). This polar metabolite fraction was purified by HPLC up to the point where methanol was evaporated and the transfer of radioactivity to THF attempted. Repeated attempts to transfer the radioactivity to THF were unsuccessful, indicating that neither metabolites A or A' were

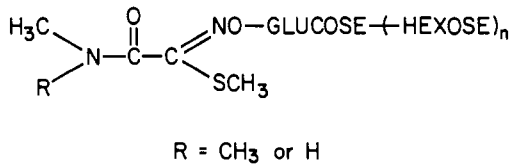

Figure 6. Polar residue at harvest.

present in significant quantity.

Although the enzyme β-glucosidase had been shown earlier to be without effect on metabolites A or A', isolated or synthetic, treatment with β-glucosidase converted the polar at-harvest metabolite fraction into two ^{14}C compounds which were soluble in THF. These compounds were positively identified as metabolites A and A' by the same techniques described previously. Since there are no sites on the oxamyl-related moiety of these compounds available for glycoside formation, we conclude that the difference between metabolites A and A' and the residues present at harvest in mature peanut foliage lies in conjugation of the glucose moiety with additional hexose units (as shown in Figure 6). In other words, the oximino moiety is now attached to a polysaccharide type of compound.

Turning now to our final crop fraction, the *mature nuts*, we found that a modification of the extraction procedure was necessary because of the oily nature of this material. Extraction with hexane removed 30% of the total radioactivity in the peanut meats; ethyl acetate, 15%; methanol, 18%; while 37% remained unextracted.

It became obvious on evaporation of the hexane extract that the major component as expected was peanut oil. We tried to separate the radioactivity from the oil by passing a benzene solution of the fraction through Bio-Beads SX-12 in benzene. This gel permeation medium has a molecular weight exclusion limit of 400; which means that peanut oil passes unretained through the column while all known metabolites of oxamyl are retained. In our case, no separation was evident and all radioactivity passed through the column with the unretained peanut oil.

The peanut oil was then saponified, after which approximately 70% of the radioactivity was recovered in the fatty acid fraction, which was converted to the corresponding methyl esters. Gas chromatography confirmed the literature (Robinson, 1967) that the major fatty acids in peanut oil are palmitic (9%), oleic (59%), and linoleic (21%). The latter two were unresolved on these columns. In this experiment, all radioactivity was recovered in the methyl palmitate and the methyl oleate plus linoleate peaks. Furthermore, the proportion of palmitate to the total in all three esters was repeatedly found to be 11%, nearly the same as the reported natural proportion of 10%. This experiment showed radioactive carbon from [^{14}C]-oxamyl had become reincorporated into the normal naturally occurring lipids of peanut oil.

The same amounts of radioactive metabolites recovered in the methanol were carried through the standard purification scheme, where they behaved like the polar at-harvest foliage metabolites. The intermediate ethyl acetate extract, containing even less radioactivity, was even more difficult to work with. It definitely contained peanut oil and therefore some of the carbon-14 as lipids. It probably also contained traces of the glucose conjugates. No oxamyl or corresponding oximino compound could be detected in any of the extracts of mature peanut meats.

One further point needs to be made. Both peanut foliage at maturity and the peanut meats at maturity showed fairly high amounts of radioactivity that could not be removed by exhaustive, but nondestructive extraction (i.e.,

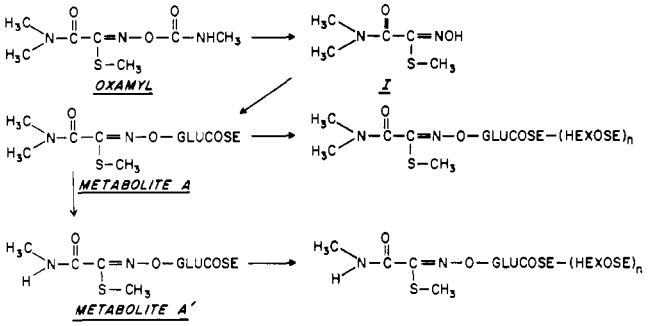

Figure 7. Metabolic pathway of oxamyl in foliage.

approximately 40% unextractable carbon-14). Even Soxhlet extraction with boiling methanol removed only further traces of radioactivity. When we subjected this "bound" residue in the macerated extracted peanut tissue to attack with a mixture of Cellulase enzymes, this treatment liberated 60% of the "bound" residue leaving only 12% of the original ^{14}C still unextracted. This liberated ^{14}C is soluble in water and ethanol, but insoluble in ethyl acetate. This characteristic eliminates the oximino compounds as products of the reaction but is characteristic of metabolites A and A'. We speculate that the process by which the plant converts metabolites A and A' into the extractable at-harvest residues, i.e., by adding additional hexose units to the sugar moiety, may well continue until the resulting hexose polymers become starch or cellulose-like and therefore unextractable. Cellulase thus would act to reverse this process like β-glucosidase does with the soluble forms.

Potatoes. Mature potato tubers from plants which had received five foliar applications at 0.5 to 1 lb/acre of [^{14}C]oxamyl contained a radioactive residue equivalent to 0.7 ppm, calculated as oxamyl. Because the peels contained less than 10% of the radioactivity, the residue of the interior portion of the tuber was characterized. Extraction of the freeze-dried potato flour with several solvents failed to remove significant amounts of radioactivity, indicating very little or no free oxamyl or oximino compound (I) (<3%).

A mild acid hydrolysis using anhydrous methanolic hydrochloric acid liberated 39% of the radioactivity as the oximino compounds I and II. Enzymatic hydrolysis of a portion of the potato flour with β-glucosidase yielded two ^{14}C compounds soluble in THF, which were purified and identified by HPLC as metabolites A and A'. The lack of extractability, even with methanol and water, combined with enzymolysis to metabolites A and A' indicates that the original form of these materials was as insoluble polysaccharide conjugates as discussed under *mature peanut hay*.

A strong acid hydrolysis of potato flour followed by crystallization, GC, trapping, and counting demonstrated that at least 35% of the ^{14}C had been reincorporated as glucose into the tuber starch. Total radioactivity accounted for in the tubers was at least 74%. Since it is difficult to drive hydrolysis reactions of the type employed herein all the way to completion, and since some radioactivity was lost in the lengthy isolation and purification procedures after all three types of hydrolyses, it is likely that the remainder of the radioactivity in the tubers is the same as these compounds already identified.

Apples. Apple fruits 6 weeks after direct application of [^{14}C]oxamyl at 1 lb/100 gal contained ^{14}C residues (0.8–2.0 ppm) evenly distributed throughout the fruit which were readily extracted with methanol (98%). Most of the metabolites (77%) were organosoluble, when partitioned between water and ethyl acetate. This fraction consisted of oxamyl, the corresponding oxime I, and a metabolite positively identified as *N,N*-dimethyl-1-cyanoformamide (DMCF), which had not been encountered previously. These compounds accounted for 16, 42, and 17%, respectively, of the total ^{14}C residue in the fruit at harvest. The remainder of the apple metabolites appeared as a water-soluble, polar fraction (23% of residue in fruit) which does not appear to contain significant quantities of metabolites A or A' in free form. However, β-glucosidase treatment of the partially purified polar fraction liberated some metabolite A, indicating the presence of the polysaccharide-type conjugates discussed earlier.

Oranges. Oranges harvested 6 weeks after direct application of a solution of [^{14}C]oxamyl at 1.2 lb/100 gal contained a total ^{14}C residue equivalent to 2.5 ppm in the whole fruit expressed as oxamyl. Of this, 82% of the radioactivity was in the rind and 18% was in the juice. Characterization of the ^{14}C residue gave the following results expressed on a whole fruit basis: oxamyl (9% of the total ^{14}C), the corresponding oximino compound (6%), *N,N*-dimethyl-1-cyanoformamide (20%), metabolite A (35%), metabolite A' (22%), and unidentified polar metabolites (8%).

Tomatoes. In and on the surface of greenhouse ripened tomatoes, 14 days after treatment with [^{14}C]oxamyl, the principal residue was oxamyl itself (59%). Lesser amounts were recovered as the oximino compound I (13%) and its glucose conjugate, metabolite A (5%). A small amount of DMCF (4%) was detected. The remainder of the residue was present as a mixture of polar metabolites or natural products (19%), that were not further characterized.

Metabolic Pathway. Figure 7 summarizes the metabolic pathway of oxamyl in plant tissues. The first step is the hydrolysis of the methylcarbamoyl group of oxamyl to yield the noninsecticidal oximino compound, followed by conjugate formation through the oximino group with glucose. The resulting glucoside (metabolite A), which is the predominant polar species in short-term studies (2–6 weeks), is gradually demethylated. The monomethyl oximino glucoside (metabolite A') has been identified in moderate length studies (1–2 months). At harvest, the radioactivity from [^{14}C]oxamyl is extracted as very polar conjugates but significant quantities of metabolites A and A' could not be detected. Instead, further conjugates of these metabolites with additional hexose units were found. An extension of this process would lead to insoluble starch-like products which accounts for the rather large amounts of unextractable residues in some mature tissues. Finally, complete breakdown of the [^{14}C]oxamyl molecule with reincorporation of carbon-14 into normal plant lipids in the peanut and into the normal glucose of potato tuber starch has been demonstrated. In several fruits, the conversion of some radioactivity into *N,N*-dimethyl-1-cyanoformamide in small quantities has been observed. Although the *S*-oxide and *S,S*-dioxide of oxamyl and of I were available for comparison with metabolites, none of these compounds was observed as a metabolite. In this respect oxamyl resembles the closely related methomyl rather than the more distantly related aldicarb (Harvey, 1975).

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Supplementary Material Available: Synthesis of radiolabeled oxamyl and the mass spectrum of metabolites A and A' (10 pages). Ordering information is given on any current masthead page.

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Decomposition of Oxamyl in Soil and Water

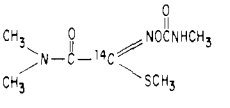
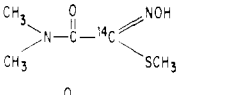
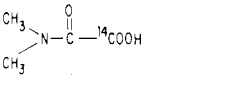
John Harvey, Jr.* and Jerry C-Y. Han

Oxamyl was stable in water at pH 5 or lower, but hydrolyzed rapidly to the oximino compound (I) at pH 9. Ultraviolet light accelerated hydrolysis and caused the formation of the syn–anti isomer of I. In river water exposed to sunlight, oxamyl hydrolyzed immediately to I, which gradually was converted to its isomer and to *N,N*-dimethyloxamic acid (III) and CO_2. In soil [^{14}C]oxamyl degraded rapidly to $^{14}CO_2$. Traces of I and a polar fraction were extractable, and radioactivity became incorporated into normal soil organic matter. Under anaerobic conditions, oxidation to CO_2 and incorporation into organic matter were delayed. These results were confirmed in field studies where the half-life of oxamyl was about 1 week.

We have already described in a previous paper (Harvey et al., 1978) the synthesis of [^{14}C]oxamyl and the metabolic fate of Vydate oxamyl insecticide–nematicide in crop plants. Because the application of oxamyl to crops unavoidably brings the compound into contact with soils and water, its fate in these media is of considerable importance. Bromilow (1973) has reported a half-life of about 2 weeks for oxamyl (Du Pont 1410) in fallow soil in pots kept outdoors in late summer.

This paper describes investigations of the decomposition of oxamyl in water and its decomposition and movement in soils under laboratory and field conditions. The structures and designations of compounds which were encountered in this work but described in our previous paper are shown in Scheme I.

Scheme I

COMPOUND	NAME	STUCTURE
OXAMYL	METHYL N',N'-DIMETHYL-N[(METHYLCARBAMOYL)OXY]-1-THIOOXAMIMIDATE	
(I)	OXIMINO METABOLITE METHYL N-HYDROXY-N',N'-DIMETHYL-1-THIOOXAMIMIDATE	
(III)	N,N-DIMETHYLOXAMIC ACID	

EXPERIMENTAL SECTION

Equipment and Methods. Liquid scintillation counting (LSC), combustion analysis (CA) of solid samples, thin-layer chromatography (TLC) on silica gel developed with ethyl acetate, and high-performance liquid chromatography (HPLC) were all carried out as described in Harvey et al. (1978).

A Bendix Time-of-Flight Model 12-107 mass spectrometer (MS) was used to characterize metabolites after purification.

Effect of pH on Aqueous Solutions of Oxamyl. The stability of [^{14}C]oxamyl was evaluated in 0.01 M solutions of sodium acetate (pH 4.7), sodium chloride (pH 6.9), and sodium bicarbonate (pH 9.1). [^{14}C]oxamyl was introduced at an initial concentration equivalent to 16 oz/100 gal (~1200 ppm). The solutions were kept in stoppered glass flasks at room temperature in the laboratory. Aliquots were withdrawn at appropriate time intervals and analyzed by thin-layer chromatography. The results are shown in Figure 1.

Decomposition in Water Exposed to UV Light. The Brandywine River is a pastoral stream in northern Delaware from which the City of Wilmington draws its water supply. Water drawn from the Brandywine in February, pH 6.5, and distilled water, pH 6.2, was used in these experiments. Th pH of the samples remained unchanged throughout. [^{14}C]Oxamyl solutions were prepared at 1 ppm and 1000 ppm in both distilled and river water. Control samples were transferred into glass bottles and placed in the dark at 24 °C for 10 days. The remaining samples were stirred in beakers exposed to a Blak-Ray XX-15 long wavelength lamp (A. H. Thomas Co.) continuously for 7 days. This lamp emits wavelengths between 300 and 400 nm with an intensity of 1200 μW cm^{-2} when placed 7 in. above the surface of the test solutions. This is equivalent to about half the intensity of summer sun-

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