Metabolism of 2-Methyl-2-(methylthio)propionaldehyde *O*-(Methylcarbamoyl)oxime (Temik Aldicarb Pesticide) in Potato Plants

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The metabolism of ^{14}C-Temik aldicarb pesticide [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] was investigated in greenhouse- and field-grown potato plants. Aldicarb was metabolized through oxidation to 2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)-oxime and 2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime. The former was the major metabolite in the foliage during early stages of plant growth and the latter became the predominant one during maturation of the plant. Subsequent degradation of these two carbamate metabolites yielded 2-methyl-2-(methylsufinyl)propionaldehyde oxime and 2-methyl-2-(methylsulfonyl)propionaldehyde oxime. The relative concentrations of these nontoxic oximes were higher in the tuber than in the foliage. Treatment of tuber buds with ^{14}C-aldicarb showed that conjugates of 2-methyl-2-(methylsulfinyl)propanol and 2-methyl-2-(methylsulfonyl)propanol constituted the major portion of the water-soluble metabolites in the tuber.

The compound 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime (Temik aldicarb pesticide) is a systemic pesticide exhibiting insecticidal, acaricidal, and nematocidal activity (Weiden *et al.*, 1965). In laboratory and field tests, aldicarb has shown excellent activity against several pests attacking potato plants (Gerhardt, 1966; Hague and Pain, 1970; Hofmaster, 1966; Hofmaster *et al.*, 1967; McClure, 1967; Thomas and Campbell, 1968).

The many potential uses of aldicarb on field crops and its prolonged pesticidal activity have stimulated detailed research on its metabolic fate in a variety of biological systems. Bartley *et al.* (1970), Bull (1968), Coppedge *et al.* (1967), and Metcalf *et al.* (1966) reported the metabolism of aldicarb as well as certain of its degradation products in cotton plants. This report describes a laboratory and field investigation of the metabolism of aldicarb in potato plants. Special emphasis is placed on the qualitative and quantitative aspects of the terminal residues in various plant parts under field conditions.

MATERIALS AND METHODS

Chemicals and Apparatus. Three radiolabeled preparations of aldicarb were synthesized according to described procedures of Bartley *et al.* (1966). These were 2-methyl-2-(methyl - ^{14}C - thio)propionaldehyde *O* - (methylcarbamoyl)-oxime (specific activity 0.286 mCi/mmol, designated as *S*-methyl - ^{14}C - aldicarb), 2 - methyl - 2 - (methylthio)propionaldehyde-2-^{14}C-*O*-(methylcarbamoyl)oxime (specific activity 0.278 mCi/mmol, designated as *tert*-^{14}C-aldicarb), and 2 - methyl - 2 - (methylthio)propionaldehyde *O* - (methyl-^{14}C - carbamoyl)oxime (specific activity 0.328 mCi/mmol, designated as *N*-methyl-^{14}C-aldicarb). The radiochemical purity of these samples was in excess of 98.5% as determined by thin-layer chromatography, radioautography, and liquid scintillation counting. Nonlabeled aldicarb and its derivatives reported as metabolites in plants were also synthesized and their identity established by methods of Bartley *et al.* (1966) and Durden *et al.* (1970). These included 2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)-oxime (aldicarb sulfoxide), 2-methyl-2-(methylsulfonyl)-propionaldehyde *O*-(methylcarbamoyl)oxime (aldicarb sulfone), 2-methyl-2-(methylsulfinyl)propionaldehyde oxime (oxime sulfoxide), 2-methyl-2-(methylsulfonyl)propionaldehyde oxime (oxime sulfone), 2-methyl-2-(methylsulfinyl)propionitrile (nitrile sulfoxide), 2-methyl-2-(methylsulfonyl)propionitrile (nitrile sulfone), 2-methyl-2-(methylsulfinyl)propanol (alcohol sulfoxide), 2-methyl-2-(methylsulfonyl)propanol (alcohol sulfone), 2-methyl-2-(methylsulfinyl)propionamide (amide sulfoxide), 2-methyl-2-(methylsulfinyl)propionic acid (acid sulfoxide), and 2-methyl-2-(methylsulfonyl)propionic acid (acid sulfone).

Radioactivity was determined with a Model 722 Nuclear Chicago or Beckman LS-150 liquid scintillation counter. Quench corrections were made according to the method described by the manufacturer of each counter. Organic extracts were counted in toluene containing 4 g of 2,5-diphenyloxazole (PPO) and 50 mg of *p*-bis-2-(5-phenyloxazolyl)benzene (POPOP) per liter of solution. Aqueous extracts were counted in a solution consisting of one part xylene, three parts dioxane, and three parts ethylene glycol monoethyl ether, with 1% PPO, 0.5% POPOP, and 8% naphthalene (Bruno and Christian, 1961). Ten milliliters of the liquid scintillation mixture were used for counting of each sample.

Laboratory Studies. Irish potatoes (Cobbler variety) were cut into seed pieces and planted in 20-cm pots containing an artificial soil composed of peat moss and sand (1:1 ratio). Nutrients were supplied as a complete liquid fertilizer. The plants were held in a greenhouse programed for 16 hr of light at 27° C and 8 hr of dark at 21° C. Insects were controlled by dusting with malathion as necessary. At the time of treatment, the foliage was 30 to 45 cm tall and the plants were flowering. Foliage weight ranged from 150 to 400 g per plant and the tubers weighed from 10 to 100 g.

A known concentration of radiolabeled aldicarb (dissolved in 50% acetone) was injected into the stem of each plant using the method described by Dorough and Casida (1964). The actual amount of aldicarb (applied dose) injected into each plant was determined by subtracting the radioactivity remaining in the injection tube and the amount remaining on the stem surface at harvest time from the amount placed in the tube initially. Approximately 220 μg of aldicarb was introduced into each plant in this manner. Ten plants were treated with one of the three different labeled prepara-

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tions of aldicarb, resulting in a total of 30 treated plants. Immediately after the solutions were absorbed by the plants, two plants were removed from each treatment for analysis. This sampling time was considered as 0 time after treatment. Other samples, two plants from each treatment, were harvested for analysis at 1, 7, 14, and 28 days following treatment. Untreated control plants were grown and sampled in the same manner as the treated ones. At each harvest date the plants were divided into tuber and foliage (the latter included any remaining stem and fibrous root tissue). Each part was weighed. The stem at the point of injection was washed with acetone (20 ml) to remove any surface radioactivity which might have oozed through the wound made by the capillary tube. In no case was this greater than 0.2% of the initial dose.

Field Studies. Irish potatoes (Cobbler variety) were grown in the field during the 1967 growing season for detailed residue and metabolism studies. A selected area of the Union Carbide Agricultural Research Station, Clayton, N.C., was used for this investigation. A trench, 1.5 m wide, 12.2 m long, and 1.0 m deep, was prepared and was then filled with Norfolk sandy loam soil (pH 6.0). Fertilizer (10-10-10) was broadcast 8 cm to the side and 5 cm below the seed furrow.

A stock solution of *S*-methyl-^{14}C aldicarb fortified with cold technical material (specific activity 29.3 μCi/mmol) was prepared to contain 10.9 mg of aldicarb per milliliter of 80% acetone in water. A furrow, approximately 10 cm wide and 10 cm deep, was opened in the prepared area and marks were placed at 30-cm intervals. Ten milliliters of the stock solution were pipetted into each 30-cm section, spreading the solution over a 5-cm width. This treatment would represent in-furrow application of 3.4 kg of technical aldicarb per hectare (equivalent to the commercial recommended rate of 3 lb per acre). The seed pieces were placed in the treated sections, 30 cm apart, and then covered with Norfolk sandy loam soil. The untreated control area was planted in the same manner. Preparation of soil and planting of potatoes were completed during the day of April 25, 1967. A summary of weather data on the farm during the growing season is shown in Table I. The land was irrigated as needed during the month of June by an overhead sprinkler.

Plants were harvested at 30, 60, and 90 days following treatment. At each harvest two plants were carefully removed from the soil and each plant sectioned into foliage, roots, and tubers. Samples were weighed and then stored at −20° C until analyzed.

Extraction. Among several solvents examined, a mixture of ethanol–water (1:1 v/v) proved to be the most efficient solvent in extracting aldicarb and its metabolites from plant tissues. Frozen samples were allowed to thaw under room temperature and soil particles clinging to tubers and roots removed by washing with water. Each sample was homogenized in a Waring blender in the ethanol–water mixture, 6 ml for each gram of plant material. Insoluble fragments were removed from the homogenate by filtration through Whatman No. 1 filter paper with the aid of suction. After washing the residue twice with the ethanol–water mixture, the volume of the filtrate was reduced under vacuum at 40° C to contain 1 g equivalent of plant material per milliliter of liquid. Two different aliquots, 0.1 and 0.5 ml, were removed from each extract for radioassay. Analysis of 12 treated plants using this extraction procedure showed an average recovery of 101 ± 10%.

The concentrated aqueous plant extract was processed for extraction studies and separation of various aldicarb metabolites. In preliminary studies chloroform and benzene were tested for their efficiency in the extraction of organosoluble metabolites from the aqueous extracts. Benzene was selected because it effectively extracted aldicarb (97.3%) from fortified tissue homogenates while leaving a high percentage of aldicarb sulfoxide in the aqueous layer. Chloroform was selected because it extracted aldicarb sulfoxide reasonably well (84.9%) and gave essentially complete extraction (97.8%) following the addition of a volume of acetonitrile equal to that of the aqueous phase. To determine the extraction values, the aqueous plant extract was partitioned three times with equal volumes of chloroform or benzene and the radioactivity in each fraction determined by liquid scintillation counting.

Table I. Summary of Daily Temperature and Rainfall Records Taken during the Growing Season of Field-Grown Potato Plants

Periods[a]	Temperature, °C High	Temperature, °C Low	Rainfall, cm
April 25–May 24	21.6	8.7	12.07
May 25–June 23	27.8	15.1	12.57
June 24–July 23	29.2	18.8	7.49

[a] Correspond to first, second, and third harvests.

Since addition of acetonitrile improved the efficiency of extraction, the final procedure used in fractionating aqueous plant extracts was based on the addition of an equal volume of acetonitrile to the aqueous phase, followed by chloroform extraction (four times). Acetonitrile–chloroform extracts were combined, dried over anhydrous sodium sulfate, and filtered. Total radioactivity in the aqueous and organic layers was determined and the percent of residues in each fraction calculated. Untreated control samples were carried through the entire extraction procedure for the determination of background during radioassay.

Chromatography and Detection of Aldicarb and its Metabolites. Ascending one- and two-dimensional thin-layer chromatography (tlc) was used to resolve various metabolic components in organic extracts of plant samples. Thin-layer glass plates (20 × 20 cm) coated 0.2 mm thick with silica gel G (Brinkmann Instruments, Inc., Westbury, N.Y.) were used throughout this investigation. An aliquot from the concentrated organic fraction was spotted evenly along a straight line (10 cm long) 3 cm from the bottom. Known standards of aldicarb metabolites were spotted next to the band at each end. Two chromatograms were prepared in this manner for each sample. One chromatogram was developed in ethyl acetate–acetone (25:3 v/v; designated as EA solvent) and the other in dioxane–benzene (3:1 v/v; designated as DB solvent). Nonradioactive standards were located on the plate by spraying with 1% solution of potassium permanganate or 1% iodine in hexane, and were compared with radioactive zones, detected by radioautography using Kodak no-screen X-ray film. Each radioactive zone was scraped from the plate and extracted with chloroform–acetone (1:1 v/v). This solution was filtered through a Buchner funnel and each filtrate made up to 10 ml. Two-milliliter samples were transferred into liquid scintillation vials, solvent reduced to approximately 0.2 ml before adding scintillator solution, and radioactivity determined by liquid scintillation counter. Results obtained from the two chromatograms were averaged to determine the percentage of each component in the organic fraction.

The identity of the metabolites in the zones obtained from one-dimensional tlc was verified by subsequent cochromatog-

Table II. Distribution of Radiocarbon from Differentially Labeled Aldicarb after Injection into Potato Plants[a]

^{14}C Label	Fraction	% of the Applied Radioactivity at Indicated Days After Treatment[b]				
		0	1	7	14	28
N-methyl	Foliage	83.7	99.6	78.7	61.5	61.9(1.76)
	Tuber	0	0	1 (0.01)[c]	1.4 (0.05)	0.8 (0.03)
S-methyl	Foliage	101.8	104.7	80.2	70.3	64.4 (1.03)
	Tuber	0	0	1.5 (0.02)	0.2 (0.02)	0.4 (0.02)
tert-C	Foliage	101.7	117.5	77.7	76.1	45.8 (1.52)
	Tuber	0	0	0.3 (0.01)	0.2 (0.01)	0.3 (0.02)

[a] Approximately 220 μg of radiolabeled aldicarb were applied to each plant. [b] Average values of two replicates for each harvest. [c] Numbers in parentheses are parts per million of total ^{14}C-aldicarb equivalents.

Table III. Partition Distribution of Radioactivity Recovered from Potato Plants Treated with Aldicarb by Stem Injection

Days After Treatment	^{14}C Label	Partition Distribution of Recovered Radioactivity Between[a,b]			
		Foliage		Tuber	
		$H_2O/HCCl_3$	H_2O/Benzene	$H_2O/HCCl_3$	H_2O/Benzene
0	*N*-methyl	0.04	0.13	...[c]	...
	S-methyl	0.03	0.13	...	...
	tert-C	0.04	0.22	...	...
1	*N*-methyl	0.10	1.15	...	...
	S-methyl	0.14	3.61	...	...
	tert-C	0.11	2.67	...	...
7	*N*-methyl	0.29	18.13	7.00	12.50
	S-methyl	0.54	21.91	6.75	31.00
	tert-C	0.27	...	4.50	...
14	*N*-methyl	0.33	19.50	10.90	39.00
	S-methyl	0.40	26.00	7.83	12.00
	tert-C	0.42	18.39	4.20	...
28	*N*-methyl	0.55	15.57	27.00	3.00
	S-methyl	0.83	24.07	3.15	2.86
	tert-C	0.76	28.18	3.83	...

[a] Based on three times partitioning of the aqueous plant extracts with equal volume of chloroform or benzene. [b] Average results of two replicates. [c] Insufficient radioactivity for partition distribution determination.

raphy with nonradioactive standards in two dimensions. Solvents used in this technique were essentially the same as those described by Andrawes *et al.* (1967), Bartley *et al.* (1970), and Coppedge *et al.* (1967). Radioactive spots were located on the chromatograms by radioautography and then scraped from the plates into scintillation vials for direct radioassay.

Mass Isolation of Aldicarb Metabolites from Potato Tubers. Five-week-old field-grown Irish potato plants (Cobbler variety) were transplanted into 30-cm pots containing a fertilized 1:1 mixture of peat moss–sand. The plants were maintained under greenhouse conditions for 2 weeks before treatment. Each plant was then removed from the pots and the tuber buds (5 mm in diameter, 5 to 10 buds per plant) were treated with *S*-methyl-^{14}C-aldicarb (specific activity 5.5 mCi/mmol); 2 μg were administered in 50% acetone in water to the surface of the bud. The acetone was allowed to evaporate for 10 min and then the plants were returned to the original pots. After 6 weeks in the greenhouse, the plants were carefully removed from the pots and the tubers (3 to 5 cm in diameter) separated for analysis. The foliage and roots were analyzed collectively for total radioactivity. Techniques used for characterization and determination of various metabolic components of aldicarb in the tubers were previously published (Bartley *et al.*, 1970). The test included two replicates with five plants in each replicate.

RESULTS AND DISCUSSION

Distribution and Metabolism of Aldicarb in Greenhouse-Grown Potato Plants. Preliminary experiments on the fate of aldicarb in potato plants were conducted using aldicarb labeled with ^{14}C at the *N*-methyl, *S*-methyl, and *tert-C* positions. Distribution of radioactive residues extracted by ethanol–water between the foliage and tuber following stem injection of each of the three labeled aldicarb samples is shown in Table II. No residues were detected in tubers harvested immediately and 1 day after treatment. This was based on radioassay of the crude ethanol–water extract and after fractionation into organic and aqueous fractions. Determination limit of the radiometric method was 0.01 ppm in the crude extracts and 0.003 ppm in the organic fractions. After 7 days, small amounts of radioactivity appeared in the tubers. The amount increased from the 7- to the 14-day harvest but remained level at the end of 28 days. This lack of accumulation from the 14 to the 28 days indicated that movement into the tuber was a passive process. The amount of the radioactivity in the tuber was a relatively small percentage (2%) of that available in the foliage. In the case of *N*-methyl-^{14}C aldicarb, the radioactivity in the tubers was higher than that found after treatment with *S*-methyl and *tert-C* labels. This suggested that half of the tuber radioactivity in the case of the *N*-methyl label was derived by hydrolysis of the carbamic acid ester linkage.

Over the 28-day test period there was a gradual decrease in the percent of the total radioactivity recovered by ethanol–water extraction. Approximately 40–50% of the applied aldicarb was lost at the end of 28 days. No attempt was made to determine if this loss resulted from incorporation into insoluble materials, by root or leaf exudation, or by complete metabolism to CO_2.

Table IV. Metabolites of Aldicarb Present in Potato Plants Treated by Stem Injection

Metabolic Products	% of the Applied Radioactivity[a]				
	1-Day Foliage		28-Day Foliage		28-Day Tuber
	N-Methyl-^{14}C	*S*-Methyl-^{14}C	*N*-Methyl-^{14}C	*S*-Methyl-^{14}C	*S*-Methyl-^{14}C
Water-solubles[b]	8.94	13.17	22.08	29.22	0.30
Aldicarb sulfoxide	78.43	80.45	35.29	19.71	0.03
Aldicarb sulfone and oxime sulfoxide	4.35	5.21	0.88	3.66	0.01
Aldicarb and oxime sulfone	1.09	0.64	0.56	2.18	0.02
Origin of tlc	6.80	5.21	3.11	9.64	0.05

[a] Average results of two replicates in each harvest. [b] Remaining in the aqueous phase after chloroform extraction.

The radioactivity recovered in the aqueous ethanol was partitioned between chloroform and water or benzene and water, and the results are shown in Table III. Extraction values are given as a ratio between the amount of radioactivity remaining in the water layer to that produced by extracting three times with the organic phase. Immediately after treatment the extraction values of the residues recovered from the foliage were slightly higher than those observed when aldicarb was extracted from fortified tissue homogenates ($H_2O/CHCl_3$ = 0.01; H_2O/benzene = 0.03). This difference reflects the partial metabolism which had occurred during the 3-hr uptake period. The shift of radioactivity to the aqueous phase following chloroform or benzene extraction was more evident after 24 hr, and gradually increased with time. This suggested conversion of aldicarb to aldicarb sulfoxide, since the latter compound has a higher extraction value ($H_2O/CHCl_3$ = 0.18; H_2O/benzene = 7.20) than aldicarb.

Partition studies on tuber extracts from the three labeled aldicarb treatments indicated higher concentrations of chloroform-insoluble materials than those found in foliage (Table III). This pattern of distribution was not clear when benzene was used for extraction. Extraction values of tuber extracts increased significantly with time in the case of *N*-methyl-^{14}C aldicarb while remaining level for *S*-methyl-^{14}C and *tert*-^{14}C aldicarb. This indicated hydrolysis of the carbamate linkage which results in the formation of methylamine and its metabolites (water-soluble) and oximes (chloroform-solubles). Methylamine and its metabolites are detected only when the label is in the *N*-methyl group, thus giving a high partition distribution value, while the oximes are detected in the chloroform when the label is in the oxime moiety.

As shown in Table III, chloroform was a more efficient solvent in extracting organosoluble radioactivity from the aqueous plant extracts than benzene. Therefore, chloroform-extractable radioactivity was analyzed by tlc for the determination of the metabolic components. In this preliminary experiment only organic extracts of 1- and 28-day foliage of *N*-methyl-^{14}C and *S*-methyl-^{14}C aldicarb and 28-day tubers of *S*-methyl-^{14}C aldicarb treatments were studied. Thin-layer chromatograms were developed in the DB solvent system. After development, each chromatogram was divided into zones corresponding to the R_f values of 0 to 0.2 (origin), 0.2 to 0.5 (aldicarb sulfoxide), 0.5 to 0.8 (aldicarb sulfone and oxime sulfoxide), and 0.8 to 1 (aldicarb and oxime sulfone). After 1 day only a trace amount of radioactivity was detected in the aldicarb zone (Table IV). This would indicate that aldicarb had a half-life of considerably less than 24 hr in potato plants. Of the residues remaining after 28 days, the maximum concentration of apparent aldicarb present in foliage was 1.2 μg or 0.007 ppm. This was estimated from the amount of radioactivity chromatographed in aldicarb zone in the case of the *N*-methyl^{14}C aldicarb treatment. Any metabolic products containing this group and having R_f value similar to that of aldicarb would be a part of this fraction. The larger percentage present in this zone at 28 days with *S*-methyl-^{14}C aldicarb indicated the presence of hydrolytic products, most probably oxime sulfone, which would not be detectable in the case of *N*-methyl-^{14}C aldicarb.

The major portion of the radioactivity recovered from the foliage 1 day following treatment was identified as aldicarb sulfoxide. Seventy-eight and 80% of the applied radioactivity was detected in this fraction for *N*-methyl-^{14}C and *S*-methyl-^{14}C aldicarb, respectively (Table IV). This metabolite remained as a major chloroform-extractable material in the foliage at the 28-day harvest and represented 35.29 and 19.71% of the original *N*-methyl-^{14}C and *S*-methyl-^{14}C aldicarb, respectively. This would give an estimated half-life of aldicarb sulfoxide in potato plants at slightly under 28 days.

The thin-layer chromatographic system used in this study was not capable of resolving aldicarb sulfone from oxime sulfoxide and aldicarb from oxime sulfone. Therefore, the data reported in Table IV represent the amount of each mixture as recovered from the chromatograms. It is possible, however, to calculate values for the respective carbamates and oximes by comparing the value of each mixture in *N*-methyl-^{14}C and *S*-methyl-^{14}C aldicarb treatments. In the case of *N*-methyl-^{14}C only carbamate products were determined, while in the case of *S*-methyl-^{14}C both carbamate and hydrolytic products were detected. On this basis the data suggest 4.4% of the applied radioactivity was present as aldicarb sulfone in the foliage at 1 day after treatment and declined to 0.9% after 28 days. By difference, the calculated amount of oxime sulfoxide would be 0.9% at 1 day and 2.8% at 28 days. Similar calculations suggest oxime sulfone to be 1.6% of the applied materials at the end of 28 days.

Analysis of tubers from *S*-methyl-^{14}C aldicarb treatment at the end of the 28-day test period indicated the largest percentage of recovered radioactivity (76%) was present as water-soluble materials (Table IV). Of the radioactivity partitioned into chloroform (24%), 12% remained at the origin of thin-layer chromatograms in DB solvent system. The identity of this material and the water-solubles was investigated in a separate experiment. Only 6.12% of the total radioactivity recovered from the tubers chromatographed as aldicarb sulfoxide which represented a final concentration of 0.001 ppm.

Distribution of Aldicarb Residues in Field-Grown Potato Plants. *S*-methyl-^{14}C aldicarb applied at the rate of 3.4 kg/ha in-furrow at planting time resulted in an initial residue of 13.1 ppm in the soil sampled in the treated band (Andrawes *et al.*, 1971). Total residue in various plant parts, expressed as ppm of ^{14}C-aldicarb equivalents in fresh weight and as percent of the applied radioactivity, is shown in Table V.

Table V. Distribution of *S*-Methyl-^{14}C Aldicarb in Field-Grown Potato Plants after In-Furrow Application at Planting[a]

Plant Part	ppm of ^{14}C-Aldicarb Equivalents Found at			% of the Applied Radioactivity at		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Foliage	4.66	6.66	4.38	0.56	3.29	2.04
Tubers	...	1.39	0.79	...	0.48	0.95
Roots	3.25	2.51	2.31	0.12	0.16	0.04
Seed piece	36.90	10.60	...	3.17	0.13	...

[a] Aldicarb applied at the rate of 3.4 kg/ha.

Over the 90-day growing season there was an effective uptake of radioactivity from the soil and systemic movement throughout the plant. Thirty days after treatment the highest level of total ^{14}C-aldicarb equivalents was found in the seed potato which contained 36.9 ppm of ^{14}C-aldicarb equivalents. This high level might be expected since seed pieces were placed directly in the treated furrow. Radioactivity in the seed diminished to 10.6 ppm at 60 days. This would suggest that the seed piece acted as a reservoir in which the compound was absorbed from the treated soil and released slowly to other parts of the growing plant. Roots did not accumulate aldicarb and its metabolites; 3.25 ppm was detected in the roots at 30 days, 2.51 ppm at 60 days, and 2.31 ppm at 90 days. The foliage contained 4.66 ppm of ^{14}C-aldicarb equivalents at 30 days and continued to accumulate residues up to 60 days, at which time residues reached 6.66 ppm. This buildup of radioactivity in the foliage was followed by slow decrease to 4.38 ppm at the end of 90-day test period. A part of this decline was probably due to the loss of the older leaves, which may have contained a higher concentration of total ^{14}C-residues than the remainder of the plant. Analysis of tubers indicated lower levels of ^{14}C-aldicarb equivalents than those found in the foliage or in the roots. As shown in Table V, only 1.39 ppm of ^{14}C-aldicarb equivalents was found in tubers at 60 days (the harvest at which tubers were present) and 0.79 ppm at 90 days. Undoubtedly, however, the decline in residue levels observed in the foliage and tubers is attributable in part to dilution as a result of plant growth.

Metabolism of Aldicarb in Field-Grown Potato Plants. Radiocarbon analysis of foliage and tubers exposed to 3.4 kg/ha in-furrow was accomplished by partitioning of the aqueous plant extracts with acetonitrile–chloroform and by subsequent tlc to determine the metabolic fate of aldicarb during growth and maturity of potato plants. Based on one- and two-dimensional tlc in several solvents, no aldicarb *per se* could be detected in the foliage at any sampling time. Aldicarb sulfoxide constituted the major metabolite in the 30-day foliage. Approximately 45% of the total radioactivity chromatographed as this product (Table VI). Its relative concentration diminished to 6.6% (or 0.29 ppm) at 90 days. A quantitative increase in the relative concentration of aldicarb sulfone was coincident with the decrease in that of aldicarb sulfoxide. Concentration of aldicarb sulfone increased from 25.8% (or 1.20 ppm) at 30 days to 55.9% (or 2.45 ppm) at 90 days. Only trace amounts (1% of total residues) of oxime sulfoxide were detected in the foliage up to 90 days. Similarly, oxime sulfone was a minor metabolite up to 60 days and only 4% of the terminal residues chromatographed as this product at 90 days.

Radiochemical analysis of the residues recovered from the tubers indicated similar metabolites to those found in the foliage. However, the relative concentration of these products at individual harvest dates varied considerably between the two parts of the plants. Fractionation of tuber residues into the various metabolic components indicated the major portion of radioactivity at 60 days was equally distributed among water-solubles, aldicarb sulfoxide, and aldicarb sulfone (Table VI). It appears that the formation of water-soluble metabolites of aldicarb was an effective mechanism during maturation of the tubers. This accumulation of water-solubles was coincident with a decline in the relative concentration of both aldicarb sulfoxide and aldicarb sulfone. However, the dissipation of these two carbamates also contributed to an increase in the relative concentrations of hydrolytic metabolites, oxime sulfoxide, and oxime sulfone. These data and those obtained for the foliage would suggest that hydrolytic mechanisms in the developing tubers were more active in degrading carbamate metabolites of aldicarb than those in the foliage.

Identification of the Water-Soluble Metabolites of Aldicarb in Potato Tubers. Water-soluble metabolites of aldicarb constituted a large percentage of the recovered residues in the tuber, especially in the final harvests (at 28 days in the preliminary study and 90 days in the field tests; Tables III, IV, and VI). However, the identification of the metabolic products in this fraction was difficult as a result of the low level of activity. To generate sufficient radioactive metab-

Table VI. Radiolabeled Components Present in the Foliage and Tubers of Field-Grown Potatoes after In-Furrow Application of *S*-Methyl-^{14}C Aldicarb[a]

Metabolic Products	% of the Recovered Radioactivity Present at			ppm ^{14}C-Aldicarb Equivalents		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Foliage						
Water-solubles[b]	20.5	27.2	29.8	0.96	1.81	1.30
Aldicarb sulfoxide	45.3	22.9	6.6	2.11	1.53	0.29
Aldicarb sulfone	25.8	43.9	55.9	1.20	2.92	2.45
Oxime sulfoxide	1.1	0.9	1.1	0.05	0.06	0.05
Oxime sulfone	1.4	1.6	4.0	0.07	0.10	0.18
Origin of tlc	5.7	3.6	2.6	0.27	0.24	0.11
Tubers						
Water-solubles[b]	...	30.7	65.7	...	0.42	0.52
Aldicarb sulfoxide	...	33.4	4.6	...	0.46	0.03
Aldicarb sulfone	...	30.0	10.1	...	0.42	0.08
Oxime sulfoxide	...	1.6	11.3	...	0.02	0.09
Oxime sulfone	...	4.0	8.0	...	0.06	0.06
Origin of tlc	...	0.3	0.3	...	0.01	0.01

[a] Aldicarb applied at the rate of 3.4 kg/ha at planting time. [b] Remaining in the aqueous phase after chloroform–acetonitrile extraction.

Table VII. Metabolism of *S*-Methyl-^{14}C Aldicarb by Potato Tuber

Metabolic Products	% of Recovered Radioactivity: Organo-solubles	Water-Solubles
Aldicarb	...	...
Aldicarb sulfoxide	51.58	...[a]
Aldicarb sulfone	2.75	...[a]
Oxime sulfoxide	8.77	0.24[b]
Oxime sulfone	0.77	0.24[b]
Nitrile sulfoxide	7.66	1.09
Nitrile sulfone	1.26	...
Alcohol sulfoxide	0.84	5.22[b]
Alcohol sulfone	ND	5.63[b]
Amide sulfoxide	2.57	0.49
Acid sulfoxide / Acid sulfone	ND[c]	0.27
Unknown 1	...	0.27
Unknown 2	...	0.27
Unknown 3[d]	...	0.68
Unknown 4[d]	...	1.19
Unhydrolyzed conjugate	0.43	7.78

[a] Minor amount remaining after extraction; value combined with organosolubles. [b] As glycoside conjugate. [c] ND—none detected. [d] Unknowns 3 and 4 chromatographed as peak 3 on ion exchange column.

olites, the developing tuber buds were treated with undiluted *S*-methyl-^{14}C aldicarb. As a result, the determination limit of the radiochemical method was increased approximately ten times. Radioassay of the foliage plus roots and tubers showed 93% of the recovered radioactivity was present in the tubers. Details of the analysis of the tuber residues are shown in Table VII. Although the method of application was artificial in nature, the same biotransformation products reported in the tubers in the previous tests were also detected in tubers treated directly with aldicarb. This indicated that aldicarb metabolism proceeded through the normal pathway.

Aldicarb was not detected in the tubers after 6 weeks of treatment, thus confirming previous findings of the high sensitivity of the compound to biological degradation. Its oxidation resulted in the formation of aldicarb sulfoxide as a major product and aldicarb sulfone. The hydrolysis of these two carbamates in the tubers was evident from the recovery of the oxime sulfoxide and oxime sulfone and their subsequent biotransformation products listed in Table VII. The high ratio of aldicarb sulfoxide to aldicarb sulfone (18.8:1) may indicate a slow rate of oxidation of the sulfoxide and/or a faster rate of degradation of the sulfone. The former explanation is derived from the high percentage (51.58%) of aldicarb sulfoxide as compared to that of aldicarb sulfone (2.75%) and the latter based on the finding of 5.63% as alcohol sulfone conjugate. Bartley *et al.* (1970) reported only trace amounts of the alcohol sulfone in cotton foliage. They showed that only 1.6% of the alcohol sulfoxide fed to cotton plants was converted to the alcohol sulfone glycoside. Therefore, it is highly unlikely that the large quantities of the alcohol sulfone conjugate could have originated from the free or conjugated alcohol sulfoxide. The data in Table VII show that conjugates of the alcohol sulfoxide and alcohol sulfone constituted the major portion of the water-soluble metabolites in the tuber. Based on hydrolysis by the enzyme β-glucosidase (emulsin from almonds, Sigma Chemical Co., St. Louis, Mo.), it was concluded these conjugates were glycosidic in nature. Trace amounts of the amide sulfoxide, acid sulfoxide, and acid sulfone and four unknown metabolites (represented 2.41% of the recovered residues) were found in the water-soluble fraction. Although the identities of the latter products are still uncertain, they cochromatographed on tlc and ion exchange column with the corresponding unknowns generated by cotton foliage (Bartley *et al.*, 1970).

The organic fraction of tuber residues was subjected to tlc systems described for the preliminary and field tests in an attempt to characterize the components remaining at the origin. Radioactivity isolated from several chromatograms was combined and then analyzed by the methods used for the water-soluble metabolites. It was found that the origin of tlc consisted of trace amounts of the alcohol sulfoxide, amide sulfoxide, and conjugates of the alcohol sulfoxide and alcohol sulfone. Therefore it was concluded that the origin radioactivity reported in the earlier potato tests consisted of a small fraction of the known water-soluble metabolites, which was carried over into organic solvent during the partitioning procedure.

CONCLUSIONS

Aldicarb was found to be a biologically unstable compound with a half-life of much less than 24 hr in potato plants. When injected into the plants, aldicarb was rapidly oxidized to yield aldicarb sulfoxide as a major product. This metabolite was more stable in the plants than the parent compound, with a half-life of slightly less than 28 days. Further oxidation of aldicarb sulfoxide to the corresponding sulfone was a minor mechanism. These results are consistent with metabolic picture found in cotton plants after leaf and stem application (Coppedge *et al.*, 1967; Metcalf *et al.*, 1966).

When aldicarb was applied in the field at planting time, the residue picture quantitatively varied from that found in greenhouse potato injection experiments and those reported for cotton. At 30 days from planting potatoes in the treated soil, larger relative concentrations of aldicarb sulfone were encountered in the foliage than in the studies in which aldicarb was applied directly to the plant. Aldicarb sulfone progressively increased in its relative concentration with time and became the major organosoluble metabolite at 90 days after planting. In the meantime a quantitative decrease in aldicarb sulfoxide was noted. It is possible to demonstrate the relationship of aldicarb sulfoxide to aldicarb sulfone by calculating the ratio of these two metabolites and its change with time. These calculations are shown in Figure 1. It is evident that the ratio of aldicarb sulfoxide to aldicarb sulfone is progressively decreasing with time. Similar calculations are also made on the concentrations of these two carbamate metabolites found in cotton plants grown in soil treated with aldicarb-S^{35} as reported by Coppedge *et al.* (1967). Although the conditions in the latter experiment and that in field-grown potato plants are not strictly comparable, a striking similarity between cotton and potato plants is found in the change in the ratio of aldicarb sulfoxide to aldicarb sulfone with time (Figure 1). Possible explanations suggested by Coppedge *et al.* (1967) for this phenomena were the plant uptake of aldicarb sulfone from the soil and also the faster rate of metabolism of the toxicant by newly formed cotton leaves. At any rate, these findings demonstrate the contribution of aldicarb sulfone to the terminal residues when aldicarb is applied as a soil systemic pesticide.

A xylem movement of aldicarb residues was shown to occur when the compound, labeled at different sites, was injected through the stem into the plant. Similarly, soil treatment under field conditions indicated preferential accumulation of residues in the foliage. In both studies the

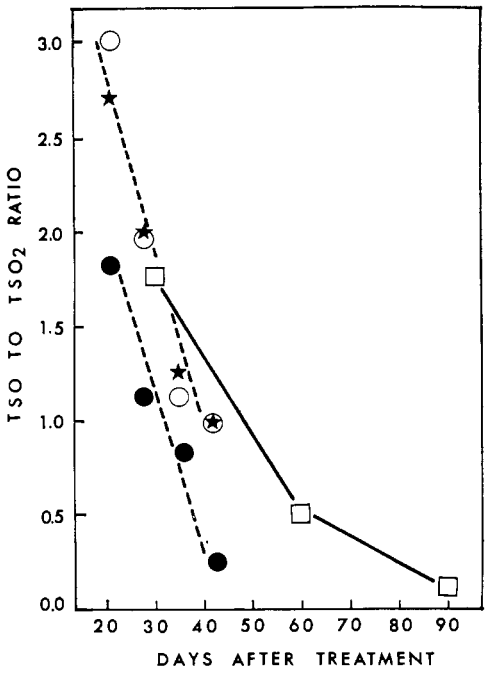

Figure 1. Ratio of aldicarb sulfoxide (TSO) to aldicarb sulfone (TSO_2) and its change with time as found in field-grown potato plants (□—□) in the present study as compared with that reported by Coppedge *et al.* (1967) for cotton plants grown in aldicarb-treated soil (new leaves ●- - - -●, young leaves ○- - -○, and mature leaves ★- - - -★)

tubers contained lower radiolabeled materials than the foliage; however, residues in tubers from soil treatment were higher than those from stem injection. Radiochemical analysis of foliage and tuber residues indicated a difference in the biotransformation activities between the two parts of the plant. In the foliage, oxidative mechanisms appeared more active than hydrolytic ones and the reverse was true for the tubers. This was more evident during maturation of the plant. In the foliage the absorbed radioactivity was metabolized mainly to aldicarb sulfoxide and to the sulfone. In the tubers the two carbamates were degraded more efficiently by hydrolysis to the corresponding oximes, which resulted in the formation of the water-soluble metabolites. This would suggest oxidative reactions were more active under photosynthetic conditions.

In conclusion, the general pathway of aldicarb metabolism in potatoes was found to be qualitatively similar to that previously established for cotton plants (Bartley *et al.*, 1970; Coppedge *et al.*, 1967; Metcalf *et al.*, 1966). The formation of the carbamate metabolites, aldicarb sulfoxide, and aldicarb sulfone and their subsequent degradation to the corresponding oximes appear to be involved in the fate of aldicarb in both plants. Further metabolism of the two oximes resulted in the formation of the water-soluble metabolites previously reported in cotton plants (Bartley *et al.*, 1970).

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