thion standard represents 97.7% of the total shoot radioactivity. The remaining two unidentified bands (R_f's 0.05 and 0.31) represent 0.7 and 1.6% of the total shoot radioactivity.

Injection of a sample of the major tlc band into the gas chromatograph described earlier produced a single peak having the same retention time (3 min) as that of a Guthion standard injected under the same conditions. Further chromatographic analyses involving the use of a gc/ms produced a mass spectrum of unit resolution similar to that reported by Damico (1966) for Guthion. Final proof of structure was apparent after comparing the infrared spectrum of the major labeled tlc band to the infrared spectrum of [^{14}C]Guthion standard (Figure 8). Guthion, therefore, may be classified as a systemic since it is readily absorbed through roots and foliage of the plants and is translocated intact without harm throughout the plant system, thus rendering untreated areas insecticidal.

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Metabolism and Residues of Temik Aldicarb Pesticide in Cotton Foliage and Seed Under Field Conditions

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The fate of *S*-methyl-^{14}*C*-aldicarb pesticide [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] was studied in field-grown cotton. Aldicarb sulfoxide [2-methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)-oxime], aldicarb sulfone [2-methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)-oxime] and water-soluble noncarbamate metabolites constituted the major portion of the residual ^{14}C materials in the foliage and seed. Pesticide residue analysis of samples from commercially treated fields qualitatively agreed with the carbamate residues found in the metabolism studies with [^{14}C]aldicarb. Residue methods are described for characterizing the aldicarb carbamate residues in cotton foliage and for determining the total toxic residue in cottonseed.

The metabolism of Temik aldicarb pesticide [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] in the cotton plant is well documented. In a series of greenhouse experiments, the pathways of biotransformation of aldicarb in cotton plants were studied following uptake by excised leaves (Coppedge *et al.*, 1967; Metcalf *et al.*, 1966), injection into the petiole of intact leaves (Coppedge *et al.*, 1967), root uptake into young seedlings (Bartley *et al.*, 1970), stem application to large plants (Metcalf *et al.*, 1966; Ridgway *et al.*, 1968), and soil application (Bartley *et al.*, 1970; Coppedge *et al.*, 1967; Ridgway *et al.*, 1968). Under field conditions, the metabolic behavior was studied by treating individual leaves and stems of cotton plants (Bull, 1968). These reports have dealt with the individual aspects of the fate of aldicarb in cotton plants. The present study provides detailed analyses of the metabolites and residues resulting from soil application of the formulated compound under field conditions.

METHODS AND MATERIALS

Chemicals and Apparatus. Radiolabeled aldicarb (*S*-methyl-^{14}*C*, specific activity 5.85 mCi/mmol) as well as nonlabeled standards of aldicarb degradation products were prepared and authenticated according to previously described procedures (Bartley *et al.*, 1966; Durden *et al.*, 1970). The radiochemical purity of the [^{14}C]aldicarb sample was in excess of 98.5%, as determined by thin-layer chromatography, radioautography, and liquid scintillation counting. The impurities consisted largely of ^{14}C material(s) remaining at the origin of the thin-layer chromatogram. Nonlabeled standards and the abbreviations used in the present study are as follows: 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 Beckman LS-150 liquid scintillation spectrometer with its carbon-14 channel adjusted to count a nonquenched [^{14}C]hexadecane standard (Beckman Instruments, Fullerton, Calif.) at 90%

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efficiency. The scintillator solution consisted of 100 ml of xylene, 300 ml of dioxane, 300 ml of methyl cellosolve, 7.0 g of 2,5-diphenyloxazole (PPO), 350 mg of 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP), and 56 g of naphthalene (Bruno and Christian, 1961). Ten milliliters of the mixture was used for counting each sample. All samples were counted until enough counts accumulated to give a statistical error of 3.0% or less. Quench corrections were made using the external standard channels ratio method. The quench correction curve was established using the above described scintillator solution (10 ml per sample), [^{14}C]hexadecane as a standard, and CCl_4 as a quenching agent. Counting efficiency of 50% was considered as the minimum acceptable level for quench corrections. Background samples were prepared by processing samples from untreated cotton plants grown in adjacent plots using identical procedures to those used for samples from treated plants. All samples and backgrounds were prepared in duplicate and the average was taken as the activity for the individual sample. The determination limit of carbon-14 counting was established at 10 dpm above background.

A Micro-Tek MT-220 gas chromatograph equipped with a Melpar flame photometric detector incorporating a 394-mμ filter specific for sulfur compounds was used for residue analyses (Maitlen *et al.*, 1968). The column was 10 ft of $\frac{3}{16}$ in. aluminum, packed with 5% Carbowax 20M on 60/80 mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.). Sections of $\frac{1}{4}$ in. diameter aluminum tubing packed with glass wool were attached to each end of the column. These were inserted into and attached to both the oven injection port and oven detector port, and about 2 in. was allowed to protrude into the oven. These sections served as heat insulation for the column packing and with reducing fittings afforded a convenient way to attach the column. Columns were conditioned 24 hr at 200° before use. Several hundred injections could normally be made before the properly conditioned column became unusable. The following instrument conditions were used: oven, 160°; injector temperature, 300°; detector temperature, 165°; nitrogen carrier gas, 60 cm^3/min. For the flame: oxygen (25 cm^3/min), air (25 cm^3/min), and hydrogen (200 cm^3/min) were used. Under these conditions the retention time of the pesticide was about 7 min. Samples were diluted with acetone and 8-μl quantities were injected. Quantitation was by referring peak height to a calibration curve prepared by injection of aldicarb sulfone standard solutions.

Metabolism of [^{14}C]Aldicarb in Cotton Foliage and Seed. An acetone stock solution of *S*-methyl-^{14}*C*-aldicarb fortified with "cold" technical aldicarb (specific activity of the mixture 0.101 mCi/mmol) was prepared to contain 26.38 mg of aldicarb and 5 mg of Carbowax 400/ml. The latter material was added to minimize losses of aldicarb during solvent evaporation. Fifty milliliters of this solution was added to 10.790 g of corncob granules (15–30 mesh) and the mixture was stirred with a glass rod during solvent evaporation under a stream of nitrogen. The sides of the beaker containing the mixture were washed several times with acetone while being stirred continuously. After the final wash, a stream of nitrogen was passed over the corncob formulation to remove all traces of acetone. The formulation was then transferred to a jar and rolled on a ball mill for 3 hr. Several samples of the corncob formulation were removed, weighed, and radioassayed by direct liquid scintillation counting to determine the actual concentration of the chemical in the formulation. Two subsamples, each containing 363.3 mg of [^{14}C]aldicarb, were removed for field applications. One subsample was used for the 1 lb of active ingredient (ai)/acre in-furrow treatment (10 ft of row treated) and the other was used for the 2 lb of ai/acre sidedress treatment (5 ft of row treated).

The area used in this study was located within a cotton field at the Union Carbide Agricultural Research Station, Clayton, N. C. The soil was Norfolk sandy loam with a pH of 6.0, the cotton variety was Carolina Queen, and the row spacing was 42 in. Fertilizer (5:10:10, 500 lb/acre) was applied in row during field preparation. The tops of three rows were leveled with a garden rake and trenches (2 in. wide, 120 in. long, and 0.5 in. deep) were formed on the top of each row. The calculated quantity of granular formulation of [^{14}C]aldicarb was applied in-furrow to the center row, leaving the outer two as guard rows. Based on 42-in. row spacing, this treatment would represent an in-furrow application of 1 lb of ai/acre. Acid delinted cottonseed, previously treated with a fungicide (DuPont Demosan, 1,4-dichloro-2,5-dimethoxybenzene), was then spaced uniformly in the prepared trenches, covered with soil, and then watered. A normal and uniform stand of cotton plants started to appear after approximately 1 week. At 58 days after planting, one-half of the plants were treated with 2 lb of ai/acre of aldicarb granular formulation (containing 363.3 mg of technical [^{14}C]aldicarb) as a sidedress application about 3 in. on each side of the row. The second half of the plants received no further pesticide treatment. Immediately thereafter, 250 lb/acre of 20% nitrogen fertilizer was sidedressed to the entire plot.

At various intervals, three representative samples of cotton foliage were harvested at random from the treated and untreated plots. The plant material was weighed and then stored at −20° until analyzed. During the first 37 days of the season, each sample consisted of the entire aerial portion of individual plants chosen to simulate, as closely as possible, the usual thinning procedure during the early part of the growing season. Samples collected during the latter part of the season (from 58 to 146 days after planting) consisted of randomly picked leaves of different ages and sizes. At approximately 90 days after planting, the fresh weight of the leaves of the treated and control plants began to decrease. The seeds were harvested for analysis at the 146-day sampling, at which time the plants had reached maturity, the leaves had desiccated, and the bolls were fully opened.

The nature of aldicarb metabolites which were found in the cottonseed in the test described above was studied further in a separate experiment. Developing bolls on plants growing under practical field conditions were treated with *S*-methyl-^{14}*C*-aldicarb (specific activity 0.43 mCi/mmol). One to 2 days after the corolla of the cotton flowers had turned pink, the petals were removed to expose the forming boll. Fifty microliters of an acetone–water solution (4:1, v/v) containing 2 mg of [^{14}C]aldicarb was deposited between the forming boll and the surrounding sepals. Two separate replicates were made in this manner using 200–300 bolls in each replicate. The cotton was harvested at 65–70 days after treatment and ginned to separate the seeds. In each replicate the seeds were thoroughly mixed, subsampled, and then stored at −20° until analyzed.

Field Residue Studies with Temik 10G Formulation. Foliage and seed samples were collected from fields which had been treated with Temik 10G formulation at rates recommended on the currently registered label. Aldicarb and its cholinesterase-inhibiting metabolites, aldicarb sulfoxide and aldicarb sulfone, are the toxicologically significant residues of aldicarb and constitute the total toxic residue, which was determined using the glc methods described below. Green foliage samples were taken at intervals during the 1968 growing season in California cotton treated with 1 lb of ai/acre in-furrow at planting and an additional 3 lb of ai/acre sidedress at 72 days thereafter. Additional cotton foliage samples were taken at intervals during the 1970 growing season from a South Carolina field which had been treated hill-dropped with the seed at the recommended rate for this cultural practice of 0.54 lb of ai/acre. Seed samples were collected at normal harvest

from the United States cotton belt in 1969 after application of Temik 10G at planting (0.5, 1, and 2 lb of ai/acre), at planting plus sidedress (1 + 3, and 1 + 2 + 2 lb of ai/acre) and as sidedress only (2, 4, and 8 lb of ai/acre). All the samples were analyzed in duplicate.

Extraction, Cleanup, and Chromatography. *Radiometric Analysis of Foliage and Seed Samples.* Frozen samples were thawed at room temperature and then blended to a fine powder with crushed Dry Ice in a Waring blender. After the Dry Ice had completely sublimed, ethanol–water (1:1, v/v; 6 ml for each gram of plant material) was added and the mixture was homogenized at high speed for 5 min. Insoluble materials were removed from the homogenate by vacuum filtration through Whatman no. 1 filter paper. The filter cake was transferred to the blender and again homogenized with fresh ethanol–water solvent and filtered. The extracts were combined and the volume was reduced under vacuum at 40° to contain 1 g-equiv of plant material per ml of solution. Aliquots were removed from each extract for radioassay.

The resulting aqueous extract of plant material was partitioned four times with an equal volume of chloroform–acetonitrile mixture (1:1, v/v). The organic fractions were combined, dried over anhydrous sodium sulfate, and then filtered. Aliquots were removed from each organic and aqueous fraction for determination of ppm of [^{14}C]aldicarb equivalents present as organo- and water-solubles, respectively. The partition distribution values were subsequently calculated as ratios of organo- to water-soluble radioactivity.

Two-dimensional thin-layer chromatography (tlc) was used to resolve various metabolic components present in the organic extracts of plant samples. Thin-layer glass plates (20 × 20 cm) coated with a 0.2-mm thick layer of Silica Gel G (Brinkman Instruments, Inc., Westbury, N. Y.) were used throughout this investigation. The plates were first developed in a 2:1 ether–hexane mixture containing 20% acetone and then, after drying at room temperature, were developed in the second dimension with a 3:2 chloroform–acetonitrile mixture. Radioactive materials were located on the chromatograms by radioautography using a no-screen type medical X-ray film (Eastman Kodak Company, New York, N. Y.). The radioactive regions were scraped from the plates into scintillation vials for direct radioactivity determination. Duplicate chromatograms were prepared in this manner for each sample and the results were averaged to obtain the relative percentage of the metabolic components. The individual components of the water-soluble metabolites were determined by methods described by Bartley *et al.* (1970).

Foliage Residue Method. A 50-g sample of finely chopped cotton foliage and 25 g of Hyflo Super-Cel (Johns-Manville) were weighed into a 1-qt Waring blender jar and the foliage was extracted by blending for 10 min with 300 ml of a 1:1 mixture of acetone–chloroform. The solvent was vacuum filtered into a 500-ml flask and the filter cake and paper were returned to the blender jar for an additional 5 min extraction with 175 ml of fresh solvent. The second extract was filtered into the same flask as before and the combined extracts were dried by pouring through a bed of anhydrous granular sodium sulfate and collecting in a 500-ml Erlenmeyer flask. The extracts were evaporated under reduced pressure to about 5 ml, while immersing the flask in a warm water bath. The remaining solvent was evaporated by purging the flask with a gentle stream of air and the residue was dissolved in 100 ml of 1:1 benzene–ethyl ether.

The benzene–ethyl ether solution was transferred to a 6-in. column of PR Grade Florisil in a 13-mm i.d. glass chromatograph tube, the Florisil being prewet with 25 ml of the same solvent. This solution was allowed to elute dropwise, after which the column was washed with 25 ml of ethyl ether. All eluate was discarded. Aldicarb, aldicarb sulfone, and aldicarb sulfoxide residues were then eluted from the column into separate flasks with progressively more polar mixtures of acetone–ethyl ether. Aldicarb was eluted with 150 ml of 2% acetone in ethyl ether, aldicarb sulfone was eluted with 150 ml of 20% acetone in ethyl ether, and finally aldicarb sulfoxide was eluted with 100 ml of acetone.

Each fraction was evaporated just to dryness with a gentle stream of air and the residue was redissolved in 10 ml of acetonitrile. One milliliter of 40% peracetic acid (FMC Corporation, Becco Inorganic Chemicals Division, New York, N. Y.) was added to each flask and allowed to react for 30 min with occasional swirling. The peracetic acid oxidized aldicarb and aldicarb sulfoxide to aldicarb sulfone and also served a cleanup function in oxidizing extraneous materials extracted from the samples. Excess acid was neutralized by pouring the solution into 50 ml of 10% aqueous sodium bicarbonate containing 2 g of Hyflo Super-Cel to aid the subsequent filtration. After 5 min of stirring, each mixture was vacuum filtered and the cake was washed with 50 ml of water. The filtrate was extracted four times with 25-ml quantities of chloroform, each time draining the extracts through a sodium sulfate bed, as previously described. The extracts were evaporated just to dryness using vacuum and then air purged as before.

Each residue was dissolved in 100 ml of chloroform and transferred to a 4-in. column of Florisil which was prewet with 25 ml of chloroform. The column was next eluted with 100 ml of 4% acetone in ethyl ether to remove the oxime sulfone and nitrile sulfone metabolites which are not part of the total toxic residue and which would interfere in the glc determinative step. The aldicarb sulfone was then eluted from the column in 100 ml of 1:1 acetone–ethyl ether and the solvent was evaporated with an air purge.

The residue was dissolved in an appropriate volume of acetone and reserved for glc determination of aldicarb sulfone.

Seed Residue Methods. Fifty grams of ground cottonseed was extracted for 10 min in a Waring blender with 250 ml of a 3:1 acetone–water mixture containing 3 ml of 40% peracetic acid. The contents of the jar were then vacuum filtered through a ¼-in. pad of Hyflo filter aid. The filter cake and paper were returned to the blender jar and extracted for 5 min with 300 ml of fresh solvent mixture containing 3 ml of peracetic acid. The contents of the jar were vacuum filtered into the same flask as before without using any additional filter aid. The filter cake was washed with 50 ml of clean mixed solvent. The combined extracts were filtered through a cotton plug into two 500-ml Erlenmeyer flasks. Ten milliliters of chloroform was added to each portion to alleviate bumping, and the extracts were reduced in volume under vacuum with the flasks in a warm water bath. The two portions were then combined in one flask and evaporation was continued to a final volume of about 150 ml. Two grams of Hyflo was then added and, while being stirred on a magnetic stirrer, excess acid was neutralized with careful addition of 100 ml of 10% aqueous sodium bicarbonate. Stirring was continued for 5 min and the aqueous solution was then vacuum filtered through a Hyflo pad. The filter pad was washed with 10 ml of water. The filtrate was then transferred to a 500-ml separatory funnel and the residue was extracted by shaking four times with 50-ml quantities of chloroform, each time draining the chloroform layer through a bed of anhydrous granular sodium sulfate into the same 250-ml Erlenmeyer flask. The combined extracts were concentrated to about 5 ml under vacuum with the flask immersed in a warm water bath. The remaining solvent was removed by a gentle purge of air.

The residue was dissolved in 100 ml of 1:1 benzene–chloroform and poured onto a 4-in. column of Florisil contained in a 13-mm i.d. glass chromatograph tube, the Flo-

risil being prewet with 25 ml of the benzene–chloroform mixture. The column was allowed to elute in rapid drops and this eluate was discarded, as well as subsequent elutions of 25 ml of ethyl ether, 125 ml of 2% acetone in ethyl ether, and 20 ml of 10% acetone in ethyl ether. These discard elutions were necessary to remove cottonseed oil and the nitrile and oxime metabolites of aldicarb which would interfere in the carbamate determination. Finally, the carbamate residues, now oxidized to aldicarb sulfone, were eluted from the column in 100 ml of 20% acetone in ethyl ether. The solvent was evaporated with an air purge, the residue was dissolved in an appropriate volume of acetone, and aldicarb sulfone was determined by glc analysis.

RESULTS AND DISCUSSION

Nature of Foliage ^{14}C Metabolites. Monitoring of the concentration of total [^{14}C]aldicarb equivalents in the foliage during the early stages of plant growth indicated aldicarb was effectively absorbed from the soil by cotton seedlings. A total of 209.0 ppm was detected in the first samples harvested 9 days after planting (Table I). At this time the cotyledons had just opened. Further uptake was evidenced by the fact that while fresh weight of the seedlings increased from 0.57 g at 9 days to 0.98 g at 14 days, the concentration of total ^{14}C residues increased to 241.9 ppm in the 14-day samples. Accumulation of residues was less evident after the first 14 days, since the concentration of ^{14}C materials dropped to 126.8 ppm at the 22-day sampling time. Such a decline could be attributed to dilution of residues due to plant growth and more limited uptake due to the reduced ^{14}C residues remaining in the soil. Average weights of the plants were 3.10 g at 22 days and 6.35 g at 37 days.

Samples analyzed at 58 days immediately before sidedress application contained 20.0 ppm of [^{14}C]aldicarb equivalents. While the residues in plants from in-furrow application declined to 2.4 ppm at 86 days, plants receiving the sidedress treatment showed the anticipated second buildup in aldicarb equivalents, which reached a peak concentration of 70.3 ppm at 1 month after treatment.

Due to normal plant senescence, the foliage fresh weight began to decrease at approximately 90 days after planting. As a result, the concentration of total [^{14}C]aldicarb equivalents was three times higher in plants receiving 1 lb/acre in-furrow and harvested at 100 days than those harvested at 86 days. Plants receiving the 2 lb/acre sidedress treatment apparently desiccated at a much slower rate. Thus, the sharp increase in the total ^{14}C residues occurred between the 100-day (42.8 ppm) and the 146-day (111.8 ppm) sampling periods.

The results presented in Table I show the concentration of individual metabolic components in the foliage resulting from the in-furrow and sidedress application of Temik 10G through a period of 146 days after treatment. It should be noted that, after the application of the 2 lb of ai/acre sidedress, the concentration of each component was a cumulative amount resulting from the first and second applications.

Metabolic products found in cotton plants after both the first and second aldicarb applications were qualitatively similar to those previously reported in cotton plants (Bartley *et al.*, 1970; Bull, 1968; Coppedge *et al.*, 1967; Metcalf *et al.*, 1966) and potato plants (Andrawes *et al.*, 1971a). However, confirmation of the metabolic pathways elucidated in these earlier studies was hampered by the dynamic nature of the soil–plant system involved in the present study. Aldicarb, when applied to the soil, is known to undergo extensive transformation to compounds similar to those generated metabolically by the plants from the parent compound (Andrawes *et al.*, 1971b; Bull, 1968; Bull *et al.*, 1970; Coppedge *et al.*, 1967). At least some of these soil transformation products are also absorbed (Ridgway *et al.*, 1968) and translocated by the plants, and therefore contribute added amounts to those

Table I. Radiolabeled Components Present in the Foliage of Field-Grown Cotton after In-Furrow and Sidedress Applications of *S*-Methyl-^{14}C-aldicarb[a]

Residues	ppm at indicated days after treatment									
	9	14	22	37	58	65	72	86	100[b]	146[b]
	1 lb of ai/acre in-furrow									
Aldicarb	2.2	1.1	1.0	0.4	T[c]	T	T	T	T	T
Aldicarb sulfoxide	147.6	146.8	45.3	13.0	2.5	2.1	0.7	0.2	0.7	0.4
Aldicarb sulfone	14.8	37.7	39.2	12.9	7.3	7.5	2.5	1.1	2.0	0.6
Oxime sulfoxide	0.4	1.1	1.1	1.1	1.2	1.3	0.7	0.2	0.6	0.5
Oxime sulfone	ND[c]	1.0	1.4	0.7	0.3	0.4	0.2	T	T	T
Nitrile sulfoxide	ND	4.8	4.7	2.0	0.1	0.2	0.1	T	T	T
Nitrile sulfone	ND	ND	ND	T	T	T	T	T	T	T
Alcohol sulfone	ND	2.7	1.1	0.4	ND	ND	ND	ND	ND	ND
Origin of tlc	2.2	3.1	0.9	1.0	0.3	T	T	T	T	0.2
Water-solubles	41.8	43.6	32.1	22.0	8.3	8.7	5.0	0.9	3.8	7.2
Total	209.0	241.9	126.8	53.5	20.0	20.2	9.2	2.4	7.1	8.9
	1 lb of ai in-furrow plus 2 lb of ai sidedress/acre									
Aldicarb						0.1	0.2	T	T	T
Aldicarb sulfoxide						12.5	17.9	25.5	8.9	10.8
Aldicarb sulfone						7.2	5.7	16.2	11.7	13.1
Oxime sulfoxide						0.5	0.6	1.1	1.2	2.5
Oxime sulfone						0.1	0.2	0.7	0.5	0.5
Nitrile sulfoxide						T	0.6	2.9	ND	1.1
Nitrile sulfone						T	T	0.7	0.5	1.4
Alcohol sulfone						0.6	0.7	0.5	0.3	0.1
Origin of tlc						0.2	0.3	0.2	0.4	1.5
Water-solubles						12.1	12.9	22.5	19.3	80.8
Total						33.3	39.1	70.3	42.8	111.8

[a] Aldicarb (10% granular formulation) applied at the rate of 1 lb of ai/acre at planting plus 2 lb of ai/acre sidedress 58 days after planting. [b] Desiccation of foliage began at approximately 90 days. [c] T, traces of less than 0.1 ppm; ND, none detected.

formed metabolically in the plant. In addition, the plants continually dilute these products by their growth and by loss of some of the absorbed materials through volatility of some of the metabolites from the leaves (Coppedge *et al.*, 1967).

Aldicarb *per se* was found in the foliage in small concentrations shortly after treatment. Its concentration was at a maximum of 2.2 ppm in the newly emerged cotton seedlings at 9 days after planting and continued to decline thereafter to less than 0.1 ppm at 58 days. All of the aldicarb detected in the sidedressed plants was derived from the second application. Its concentration reached a maximum of 0.2 ppm at the end of 14 days after the sidedress treatment, and then declined to less than 0.1 ppm at the last sampling date. It is, therefore, conclusive that neither in-furrow application nor sidedress treatment resulted in a major accumulation of aldicarb *per se* in the plants. These data suggested that aldicarb was rapidly metabolized as it was absorbed by the cotton plants. Its rapid metabolism resulted in an early appearance of the insecticidally active aldicarb sulfoxide. This is in agreement with previously published reports on the high degree of susceptibility of aldicarb to biological oxidation (Andrawes *et al.*, 1971a; Bull, 1968; Coppedge *et al.*, 1967; Metcalf *et al.*, 1966). The major portion of the total isolated ^{14}C materials during the first 22 days after in-furrow application of aldicarb at planting time consisted of the carbamate metabolite aldicarb sulfoxide (Table I). This metabolite was at its highest concentration (147 ppm) during the first 2 weeks of plant growth. While the total ^{14}C residues increased from the 9 to 14-day harvest, no increase was noted in the concentration of aldicarb sulfoxide during this period. A sharp decline in the concentration of aldicarb sulfoxide occurred after the second week, which resulted in a residue approximately one-third of that found in the 9-day harvest. This rapid decrease in the concentration of aldicarb sulfoxide during the early part of the season was due primarily to dilution by plant growth. The initial rapid decrease in the concentration of aldicarb sulfoxide was followed by a somewhat slower rate with a minimum concentration of 0.2 ppm at the end of 86 days. The concentration of the less toxic aldicarb sulfone increased at a slower rate during the seedling stage of plant growth. Undoubtedly, a part of the aldicarb sulfone was derived through uptake from the soil (Ridgway, 1968) in which this compound is known to be formed (Andrawes *et al.*, 1971b; Coppedge *et al.*, 1967). Aldicarb sulfone concentration began to decline slowly after 22 days and only 0.6 ppm was detected at 146-day harvest.

Sidedress of aldicarb resulted in a buildup in the amounts of aldicarb sulfoxide and aldicarb sulfone, as shown in the samples taken 1 week after application (65 days after planting, Table I). It is possible to calculate the contribution of the sidedressed aldicarb to the accumulation of toxic carbamates by comparing the concentration of these materials in plants receiving this treatment with those in the plants receiving the in-furrow treatment only. Thus, it can be concluded that aldicarb sulfoxide accumulated more rapidly than aldicarb sulfone. During the first 2 weeks after the sidedress treatment, cotton plants accumulated approximately 17 ppm of additional aldicarb sulfoxide. The corresponding figure for aldicarb sulfone was 3 ppm. The two carbamates continued to increase in concentration and reached a peak of 25.3 ppm of aldicarb sulfoxide and 15.1 ppm of aldicarb sulfone 1 month after sidedressing (86 days from planting). After this time their concentration decreased and at the end of 146 days their total was 23.9 ppm in the desiccated foliage.

The calculations in Table II show the change with time in the ratio of aldicarb sulfoxide to aldicarb sulfone (T_1/T_2). The T_1/T_2 ratio progressively decreased with time up to 86 days after the original 1 lb of ai/acre in-furrow application and between the 72-day to the 100-day harvests in the 1 lb in-furrow plus 2 lb of sidedress/acre. A similar relationship was reported in cotton plants (Coppedge *et al.*, 1967) and in potato foliage (Andrawes *et al.*, 1971a). During the latter part of the growing season (100 to 146 days after planting), the T_1/T_2 ratio did not follow the decline pattern just discussed. This could have resulted from the lack of soil aldicarb sulfone residues and/or a faster rate of metabolism of aldicarb sulfone than the rate of its formation from aldicarb sulfoxide.

As discussed above, the conversion of aldicarb sulfoxide to aldicarb sulfone alone did not account for the rate of dissipation of this metabolite; *i.e.*, the increase in the concentration of aldicarb sulfone did not equal the reduction in concentration of aldicarb sulfoxide. This would support earlier studies (Coppedge *et al.*, 1967; Metcalf *et al.*, 1966) which concluded that other metabolic activities such as hydrolytic and elimination reactions play an important role in the detoxification of aldicarb sulfoxide. Evidence for this was found in the large amounts of conjugated alcohol sulfoxide in the water-solubles (Table III). The isolation of oxime sulfoxide and nitrile sulfoxide from the treated plants (Table I) is further evidence for the cleavage of the carbamate metabolite. In addition, Metcalf *et al.* (1966) and Coppedge *et al.* (1967) demonstrated that aldicarb sulfoxide was metabolized mainly through hydrolysis of the carbamate ester linkage to yield oxime sulfoxide. It appears that aldicarb sulfone also undergoes

Table II. Aldicarb Sulfoxide to Aldicarb Sulfone Ratios in Foliage of Field-Grown Cotton after Treatment with *S*-Methyl-^{14}C-aldicarb

Days after planting	1 lb of ai/acre in-furrow	1 lb of ai/acre in-furrow plus 2 lb of ai/acre sidedress
9	9.97	
14	3.89	
22	1.16	
37	1.01	
58	0.34	
65	0.28	1.74
72	0.28	3.14
86	0.18	1.57
100	0.35	0.76
146	0.67	0.82

Table III. Metabolic Products of *S*-Methyl-^{14}C-aldicarb Isolated from Cotton Foliage at 22 Days after 1 lb of ai/acre In-Furrow Application at Planting Time

	Percent of recovered radioactivity	
Metabolic products	Organosolubles	Water-solubles
Aldicarb	0.8	
Aldicarb sulfoxide	35.7	[a]
Aldicarb sulfone	30.9	
Oxime sulfoxide	0.9	3.5[b]
Oxime sulfone	1.1	
Nitrile sulfoxide	3.7	[a]
Nitrile sulfone	ND	
Alcohol sulfoxide		7.8
Alcohol sulfone	0.9	
Origin of tlc	0.7	
Amide sulfoxide		1.6
Acid sulfoxide		0.6
Acid sulfone		1.1
Unknown[c]		2.2
Unhydrolyzed conjugate		8.5

[a] Value combined with organic. [b] As glycoside conjugate. [c] Peak no. 3, ion exchange, two components, 2:1 ratio.

Table IV. Organic/Aqueous Partition Distribution Values of Radioactivity Present in the Foliage of Cotton Plants Receiving S-Methyl-^{14}C-aldicarb Treatments

Days after planting	1 lb of ai/acre in-furrow	1 lb of ai/acre in-furrow plus 2 lb of ai/acre sidedress
9	4.00	
14	4.55	
22	2.95	
37	1.44	
58	1.41	
65	1.33	1.76
72	0.90	2.02
86	1.72	2.12
100	0.89	1.22
146	0.27	0.38

similar degradation reactions to yield oxime sulfone, nitrile sulfone, and alcohol sulfone (Tables I and III).

The water-soluble metabolites constituted a major portion of the ^{14}C residues recovered from plant foliage. This is readily evident when one considers their relationship to the organosolubles at various times during the growing season, as shown by the partition distribution values (organosolubles/water-solubles) (Table IV).

These partition distribution values progressively decreased with time, indicating a positive trend toward the formation of highly polar ^{14}C metabolites remaining in the aqueous fraction after chloroform–acetonitrile partitioning. Bartley *et al.* (1970) have identified the various water-soluble metabolites of aldicarb and described the pathways leading to their formation. In the present study, only the 22-day water-soluble sample was analyzed and the data are presented in Table III. Metabolic products found in this sample were similar to those previously reported by Bartley *et al.* (1970) in cotton plants under greenhouse conditions. In addition, these data substantiate the conclusion presented above concerning the hydrolytic degradation of the carbamate metabolites and show that water-solubles are formed through further metabolism and conjugation of the intermediates in the plant.

Table V. Nature of ^{14}C Residues Found in Cottonseeds after Application of S-Methyl-^{14}C-aldicarb to the Soil and as Surface Treatment to Developing Cotton Bolls

Metabolic products	Percent of the recovered radioactivity as: Soil application[a]	Surface-treated bolls[b]
Aldicarb	ND[c]	ND
Aldicarb sulfoxide	6.97	24.12
Aldicarb sulfone	7.57	3.96
Oxime sulfoxide	0.10	1.25
Oxime sulfone	1.12	0.94
Nitrile sulfoxide	1.01	8.41
Alcohol sulfone	1.02	1.26
Solvent front	2.19	13.98
Origin of tlc	10.25	1.66
Water-solubles	69.77	44.42
Total ppm of [^{14}C]aldicarb equivalent	2.81	7.49

[a] Radiolabeled aldicarb applied at the rate of 1 lb of ai/acre in-furrow at planting plus 2 lb of ai/acre sidedress at 58 days thereafter. Seeds were harvested 88 days after last treatment. [b] Developing cotton bolls were treated on the surface with 2 mg of [^{14}C]aldicarb per boll. Seeds were harvested 65 to 70 days after treatment. [c] ND, none detected.

Table VI. Residues of Aldicarb and Its Carbamate Metabolites as Determined by glc in Cotton Foliage after Application of Temik 10G According to the Commercially Recommended Methods

Days after last application	Residues in ppm[a]: Aldicarb	Aldicarb sulfoxide	Aldicarb sulfone	Aldicarb sulfoxide to aldicarb sulfone, avg ratio
	California samples[b]			
15	0.16	32.0	4.7	6.8
31	0.10	7.7	2.7	2.9
60	<0.01	2.8	1.5	1.9
89	<0.01	0.6	0.6	1.0
	South Carolina samples[c]			
36	0.02	3.3	2.2	1.5
46	0.01	3.9	3.4	1.1
52	0.01	2.5	2.7	0.9
60	<0.01	0.4	0.6	0.7

[a] Residues are calculated as aldicarb sulfone equivalents. Determination limit of the method is 0.01 ppm. [b] Temik 10G applied at the rate of 1 lb of ai/acre in-furrow at planting and 3 lb of ai/acre sidedress at 72 days thereafter. [c] Temik 10G hill-dropped with the seed at the rate of 0.54 lb of ai/acre.

Nature of Seed ^{14}C Metabolites. Shown in Table V are the radiometric analyses of cotton seed harvested from plants receiving [^{14}C]aldicarb at a normal soil application rate of 1 lb/acre in-furrow plus 2 lb/acre sidedress and those treated artificially by placing the chemical directly on the surface of the developing bolls. Translocation of ^{14}C metabolites from cotton leaves into the seed in the soil application of [^{14}C]aldicarb was minimal. At the final harvest (146 days after planting), the concentration of total ^{14}C residues found in the seed was 2.81 ppm, which represents 2.5% of that available in the leaves. The metabolic products found in the seeds were qualitatively similar to those previously identified in the leaves. With minor exceptions, the relative distribution of the seed metabolic components in the soil application closely resembled that of the 146-day harvested leaves. For example, the water-soluble metabolites constituted approximately 70% of the recovered radioactivity in the seeds, as compared to 72% for the leaves. Although the relative, as well as the absolute concentration of aldicarb sulfoxide and aldicarb sulfone metabolites, was much lower in the seed than in the leaves, the aldicarb sulfoxide–aldicarb sulfone ratio was 0.92 in the seed, as compared to 0.82 for the leaves. Other organosoluble metabolites occurred in quantities of less than 2% of the total recovered from both the seeds and the leaves. Major differences between the seed and leaf residues were observed in the quantities at the origin of the tlc and a component designated as "solvent front." The former was most likely a carryover of water-soluble radioactivity into the organic fraction during the partitioning step (Andrawes *et al.*, 1971a; Bartley *et al.*, 1970). Radioactivity present at the solvent front of the thin-layer chromatogram was associated with an oily material traveling with the two solvents used in the two-dimensional tlc. None of the known aldicarb metabolites moved to this region. The low level of activity in this fraction precluded its identification by conventional analytical techniques.

The higher concentration of total radioactivity (Table V) obtained by direct treatment of developing cotton bolls with [^{14}C]aldicarb allowed confirmation of the identities of various metabolites by cochromatography with authentic standards. The qualitative similarity of these metabolites to those formed after soil application indicated that cotton bolls are capable of metabolizing aldicarb through a metabolic pathway similar to that of the soil–plant system. Quantitative differences between the normal residues in the seeds from soil application and those from the

Table VII. Total Carbamate Residues in Cottonseed Resulting from Temik 10G Applications as Determined by glc in Samples Collected at Normal Harvest from the United States Cotton Belt in 1969

	Type treatment and rate, lb of ai/acre							
	At planting			At planting + sidedress[a]		Sidedress only		
Location	0.5	1	2	1 + 3	1 + 2 + 2	2	4	8
				Residues, ppm[b]				
North Carolina		0.12						
South Carolina			0.11		0.10			
Mississippi	<0.01	0.02						
		0.01						
Arkansas		0.03						
Texas			<0.01	0.04				
				0.08				
Arizona						0.03	0.05	0.08
						0.03	0.06	0.07

[a] 1 + 2 + 2 indicates 1 lb of ai/acre at planting and two 2 lb of ai/acre sidedress treatments. [b] Residues are calculated as aldicarb sulfone equivalents. Determination limit of the method is 0.01 ppm.

artificial method of treatment are shown in Table V. A higher relative concentration of aldicarb sulfoxide and a lower percentage of the water-solubles occurred in the seed of plants to which aldicarb was applied to the boll rather than in the seed of plants grown in aldicarb-treated soil. These results suggest that the metabolism in the latter was at a more advanced stage than the former treatment.

Residues in Cotton Foliage and Seed. The qualitative aspects of the carbamate residues and metabolites found in the above experiments and their pertinence to describing the transformation and degradation of aldicarb in cotton are substantiated by analyses of commercially grown cotton by residue methodology. Shown in Table VI are the details of aldicarb residue determinations on cotton foliage collected from California and South Carolina tests. Verification of the lack of appreciable transfer of aldicarb or its carbamate metabolites to cottonseed is found in the low level of residue in seed from fields which had been commercially treated with Temik 10G. Table VII shows results of analysis for total carbamate residues of spot samples of seed collected at normal harvest from the United States cotton belt in 1969. Maximum rates of 1 lb of ai/acre at planting and 3 lb of ai/acre at sidedress are recommended for cotton in the directions for application portion of the Temik 10G product label. Seed residue does not exceed 0.1 ppm total carbamate, even in cotton treated at rates in excess of those recommended. Due to the low level residue in commercial cottonseed, residue methodology has not been developed to characterize the seed residue.

The validity of the analytical method for seed was determined both by analysis of laboratory fortified samples

Table VIII. Recovery of Individual Components of the Total Toxic Residue from Fortified Cottonseed

ppm of component added[a]	Aldicarb recovered		Aldicarb sulfoxide recovered		Aldicarb sulfone recovered	
	ppm	%	ppm	%	ppm	%
0.011	0.011	100	0.011	100	0.012	109
0.022	0.022	100	0.021	105	0.024	109
0.022	0.022	100	0.019	86	0.021	95
0.022	0.021	96	0.018	82	0.020	91
0.044	0.036	82	0.044	100	0.041	93
0.044	0.041	93	0.043	98	0.045	102
0.044	0.045	102	0.045	102	0.045	102
0.044			0.043	98		
0.044			0.041	93		
0.088	0.108	121	0.098	111	0.106	120
0.088	0.081	92	0.081	92	0.089	101
0.088	0.072	82	0.070	80	0.086	98
0.176	0.172	98	0.154	88	0.174	99
0.488	0.363	75	0.410	84	0.440	90
Average		95		94		101

[a] Three separate samples, one for each compound, were fortified at this level. All ppm are in aldicarb sulfone equivalents.

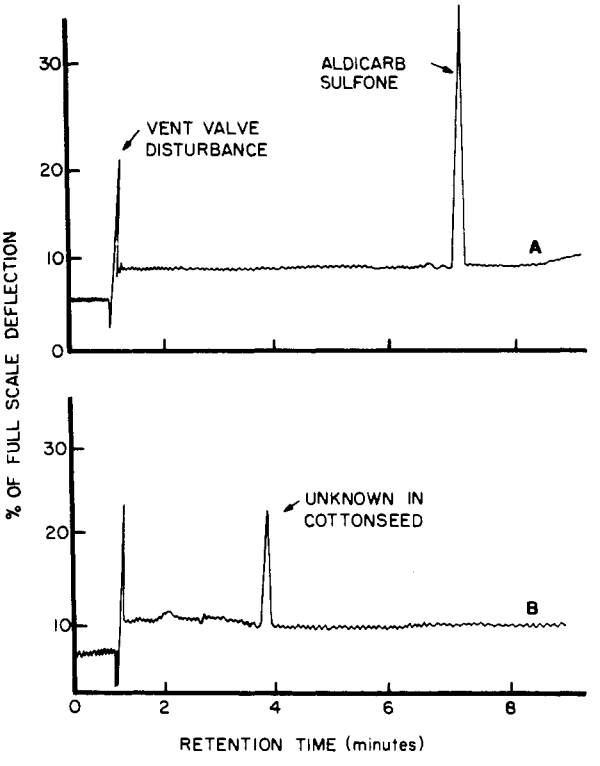

Figure 1. Typical chromatograms obtained with the flame photometric detector (FPD) from injection of: A, 8 μl of a standard solution containing 2.2 μg/ml of aldicarb sulfone, a total injection of 17.6 ng; B, 8 μl of a 1-ml final dilution of an untreated control sample of cottonseed showing no aldicarb sulfone residue and no interfering peaks eluting near the 7-min retention time of aldicarb sulfone.

Table IX. Comparison of the Aldicarb Carbamate Residues Found in Cottonseed and Foliage by the Radiometric and Residue Analysis Procedures

Residue	Cottonseed		Cotton foliage			
			Radiometric method		Residue method	
	Radiometric method	Residue method	Sample 1	Sample 2	Sample 1	Sample 2
Aldicarb	ND[a]	—[b]	Trace	Trace	<0.01	0.06
Aldicarb sulfoxide	0.08	—	0.4	8.7	0.27	8.7
Aldicarb sulfone	0.21	—	0.6	13.3	0.69	14.1
Total toxic residue	0.29	0.23	1.0	22.0	0.96	22.9

[a] ND, none detected. [b] A dash signifies the particular residue was not separately determined.

(Table VIII) and by analysis of seed from plants treated with radioactive aldicarb and thus containing grown-in radioactive residues. Comparative results from radiometric and residue analyses of these seeds, as well as foliage from the plants, are seen in Table IX. These data prove the applicability of the residue methods to cotton seed and foliage, as have corollary exhaustive extraction tests on cottonseed, which show 98% of the seed residue is extracted in two extractions as stipulated in the residue method.

The determination limit of the residue method is about 0.01 ppm. Typically, untreated control samples show little variation in the base line near the retention time of the pesticide (Figure 1). One peak, eluting with a retention time of about 4 min, is seen in varying quantities in cottonseed as well as other agricultural and environmental substrates. No serious attempt has been made to identify this peak, since it does not interfere in the determinations.

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Photodecomposition of the Herbicide Methazole

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Exposure of methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] to ultraviolet light in methanol or to sunlight in water or as surface deposits resulted in loss of carbon dioxide from the oxadiazolidine ring with subsequent generation of several derivatives. Photoproducts identified include 3,4-dichloronitrobenzene, 1-(3,4-dichlorophenyl)-3-methylurea, 1-(3,4-dichlorophenyl)urea, and two isomeric dichloro-1-methyl-2-benzimidazolinones. The methylurea and urea compounds were not produced in methanol, but 1-(3,4-dichlorophenyl)-3-methoxymethylurea was generated in rather large quantities. This compound degraded to the urea during workup of the photolysis mixture. The two isomeric dichloro-1-methyl-2-benzimidazolinone photoproducts were not phytotoxic to bean or tomato foliage, but were more toxic to mice than methazole when administered ip.

Methazole [Probe, bioxone, VCS-438, 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] (I) is an experimental herbicide currently under development by Velsicol Chemical Corp. This compound has shown excellent potential for control of selected weed species in several crops, particularly cotton.

In studies of methazole metabolism by cotton plants, 1-(3,4-dichlorophenyl)-3-methylurea (II) and 1-(3,4-dichlorophenyl)urea (III) were identified as the major metabolites, while several other unidentified products also were observed (Jones, 1971; Jones and Foy, 1972). Metabolites

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