RESIDUES OF METHIOCARB IN BURLEY AND FLUE-CURED TOBACCO[1]

By T. J. SHEETS, R. B. LEIDY, and W. J. MISTRIC, JR.[2]

Studies conducted in 1978 with burley tobacco showed levels of total methiocarb (methiocarb, the sulfoxide, and the sulfone) of 0.35 and 0.48 ppm, respectively, for the 1.0 and 2.0 kg/ha rates (season totals of 4.0 and 8.0 kg/ha) on bottom stalk samples. Residues were less in leaf samples from middle and upper stalk positions. For a 1981 experiment, total residues in flue-cured tobacco for the 1.0 and 2.0 kg/ha rates were 0.62 and 1.05 ppm in first-harvest samples and declined in samples taken from progressively higher stalk positions. The residue was present as the sulfoxide and sulfone. The sulfoxide was present in greater amounts than the sulfone in most samples. Methiocarb treatment had no effect on reducing sugar and total alkaloid of tobacco.

Additional key words for indexing: methiocarb sulfoxide, methiocarb sulfone, reducing sugars, alkaloids, slugs.

INTRODUCTION

Methiocarb (3,5-dimethyl-4-methylthiophenyl methylcarbamate) is an insecticide and acaricide with good residual activity. It is effective also as a molluscicide and bird repellent.

Specific data on residues of methiocarb and its sulfoxide and sulfone metabolites in plants were not located in the literature; however a method of analysis for methiocarb, including the sulfoxide and sulfone metabolites, in plants was published by Thornton and Drager (5). They provided recovery values for apple and pear peel and pulp, corn grain and fodder, and sugar beet tops and roots. In their method Thornton and Drager (5) oxidized methiocarb and the sulfoxide to the sulfone and measured the sulfone with flame-photometric gas chromatography.

Much is known about the metabolism of carbamates in plants (2), and the metabolism of methiocarb in beans and apples has been studied (1)[3]. The sulfoxide and sulfone are the principal metabolites in plants. In soils, metabolic changes produce both the sulfoxide and water soluble products including the phenol derivative of methiocarb.[4]

A 2% bait form of methiocarb applied broadcast at 1 kg/ha ai one to three times provided good control of slugs (*Arion fasciatus* Nilsson in burley tobacco (4). These organisms often damage burley tobacco on small farms where pesticides are applied by hand or with inferior power equipment. Technical methiocarb is moderately toxic to the rat with an oral LD_{50} of 100 mg/kg (3). Formulation as 2% pellets provides sufficient dilution to allow application safely by gloved hand; and that formulation is, therefore, a desirable treatment for the small tobacco grower where slugs are a problem.

The research reported here was undertaken as an Interregional-4 project to obtain residue data to support registration of methiocarb for this minor use. Although these pests do not usually damage flue-cured tobacco, the U.S. Environmental Protection Agency (US-EPA) required a residue study with two tobacco types in order to extend the label to cover all tobaccos grown in the United States. Thus, two residue studies were conducted with burley and one with flue-cured tobacco.

MATERIALS AND METHODS

Burley Tobacco. Burley tobacco Variety KY 14 was

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[2] *Professor and Senior Researcher, Pesticide Residue Research Laboratory and Professor, retired, Department of Entomology, N.C. State University, Raleigh, North Carolina 27695-7613.*

[3] *Church, D.D. 1969. Metabolism of 4(methylthio)-3,5-xylylmethylcarbamate (BAY 37344) on apples and beans. Chemagro Report No. 25160.*

[4] *Church, D.D. and D.R. Flint. 1971. The fate of Mesurol [4-(methylthio)-3,5-xylylmethylcarbamte] in soil. Chemagro Report No. 29591.*

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Table 1. Recoveries of known amounts of methiocarb added to 25 g of untreated burley tobacco before extraction.

Number of samples	Amount added (ppm)	Amount recovered Range (%)	Amount recovered Avg (%)
2	0.15	80-93	87
4	0.20	90-90	90
2	0.30	77-97	87
2	0.60	92-98	95
2	1.0	83-92	88
2	3.0	87-89	88
2	6.0	84-86	85
All samples	0.15-6.0	77-97	89

transplanted on clay loam soil May 29, 1978 at the Upper Mountain Research Station, Laurel Springs, N.C., and May 31 at the Mountain Research Station, Waynesville, N.C. Experimental plots four rows wide by 18 m long were established at each location. Recommended fertilization and cultural practices were followed. Methiocarb as the 2% bait was broadcast by hand (with gloves) over plants and soil at rates of 0, 1.0, and 2.0 kg/ha ai on June 12, 19, and 26 and July 3, 1978 at the Upper Mountain Research Station and June 14, 21, and 28 and July 5, 1978 at the Mountain Research Station. Thus, the totals applied per season were 4.0 and 8.0 kg/ha. The 1.0 kg/ha is the recommended rate for slug control with a maximum of four applications per year. The 2.0 kg/ha rate applied four times is twice the maximum recommended rate as required by the US-EPA to be included in residue studies of this kind.

The three pesticide rates were arranged in a randomized complete block design with four replications. Superimposing stalk positions on the design created a randomized split plot with rates of methiocarb as whole plots and a systematic arrangement of subplots (stalk positions) over the whole plots.

The tobacco was harvested September 12, 1978 at Waynesville and September 14 at Laurel Springs. The tobacco was air cured by conventional methods, and 48 leaves each were selected from bottom, middle, and upper stalk positions. Leaves from each sample were ground in a Wiley Mill to pass a 2-mm screen. After mixing, a representative subsample from each sample was stored at -20 C until analyzed.

Our method of analysis differed substantially from that of Thornton and Drager (5) because, by their method, several constituents of the tobacco extracts cochromatographed with methiocarb making quantitation impossible. Twenty-five grams of tobacco and 200 mL of acetone were added to 970-mL jars and blended for 10 min. The extract was decanted into a funnel containing 50 g of anhydrous sodium sulfate; an additional 200 mL of acetone were added to the jar and contents were again blended for 10 min. The combined extracts were evaporated to dryness under vacuum at 40 C. Ten milliliters of benzene were added to the flask.

This fraction was transferred onto a 20 by 2.5-cm glass column containing, from bottom to top, 2.5 cm of sodium sulfate, 5 g of silica gel (Woelm Activity Grade I), and 2.5 cm of sodium sulfate. The column had been prerinsed with 50 mL of 97.5:2.5 v/v of benzene:acetone. Methiocarb and its metabolites were eluted with 100 mL of 97.5:2.5 v/v of benzene:acetone. The eluate was evaporated to 2 to 3 mL under vacuum at 40 C and diluted with methanol for high performance liquid chromatographic analysis (HPLC).

The liquid chromatograph was a Varian Model 8500 dual pumping system with a 25 by 0.46-cm column containing Partisil-10 PAC (5 to 10 μm). The solvent system was 5% 2-propanol in hexane at a flow rate of 0.5 mL/min. The detector was a Schoeffel Model SF 770 variable UV/vis spectrophotometer operated at 225 nm and 0.04 AUFS. A 10 mV recorder with a drive speed of 0.17 cm/min was used to record peaks. The retention time of methiocarb and each of the two metabolites was 8.0 min. The response per unit of each compound injected was the same, thus allowing calculation of total methiocarb residue (methiocarb plus the sulfoxide and sulfone). Data were calculated by the peak height method.

Flue-Cured Tobacco. Flue-cured tobacco variety 'McNair 944' was transplanted on sandy loam soil May 4, 1981 at the Central Crops Research Station, Clayton, N.C. Recommended fertilization and cultural practices were followed. Plots were four rows wide and 12 m long.

The 2% commercial bait formulation of methiocarb was applied by hand, using disposable polyethylene gloves, over-the-top broadcast on May 14, May 22, June 5, and June 11, 1981 at rates of 0, 1.0, and 2.0 kg/ha. Thus, the totals applied were 2.0 and 4.0 kg/ha. The treatments were replicated four times in a randomized plot design. The addition of harvests (equivalent to stalk position) into the design gave a randomized split block with rates of application of methiocarb as whole plots and harvests as subplots.

The tobacco was harvested five times (July 15, July 28, August 4, August 18, and September 1, 1981) and cured in bulk-curing barns by standard procedures. After curing was complete, samples consisting of 50 randomly selected leaves from each plot and each harvest were air-dried and ground in a Wiley mill to pass a 0.5 mm screen. Representative subsamples were stored at -20 C until analyzed.

The method of analysis was modified for the 1981 samples. Twenty-five grams of tobacco were blended twice for 10 min with 200 mL of acetone. The extracts were combined and filtered by a vacuum through a 0.45 μm nylon filter (48 mm diam) containing 10 g of sodium sulfate above and 2.5 cm of Celite below. The filtrate was evaporated just to dryness at 40 C under reduced pressure, and the residue was taken up in 10 mL of 97.5:2.5 v/v of benzene:acetone.

A 2.5 by 20-cm glass column containing 2.5 cm of sodium sulfate, 10 g of Silica Gel (Woelm Activity Grade I), and 2.5 cm of sodium sulfate was rinsed with 50 mL of benzene, and the acetone-benzene solution containing the methiocarb and two

Table 2. Recoveries of known amounts of methiocarb and its sulfoxide and sulfone added to 25 g of untreated flue-cured tobacco 30 min before adding the extraction solvent.

Compound	Number of samples	Amount added (ppm)	Amount recovered Range (%)	Amount recovered Avg (%)
Methiocarb	2	0.15	77-83	80
	6	0.50	85-89	87
	2	1.00	86-90	88
	2	5.00	86-91	90
	All samples	0.15-5.0	77-91	86
Sulfoxide	5	0.10	81-86	83
	3	0.25	82-87	84
	4	0.50	82-88	85
	All samples	0.1-0.5	81-88	84
Sulfone	5	0.10	80-89	86
	3	0.25	82-85	84
	4	0.50	84-89	87
	All samples	0.1-0.5	80-89	86

Table 3. Residues of methiocarb in burley tobacco treated with 1 and 2 kg/ha.

Rate of application[a] (kg/ha)	Stalk position	Total methiocarb residues: Laurel Springs (ppm)	Waynesville (ppm)	Avg (ppm)
0.0	Bottom	<0.10	<0.10	<0.10
	Middle	<0.10	<0.10	<0.10
	Top	<0.10	<0.10	<0.10
1.0	Bottom	0.42	0.29	0.35
	Middle	0.15	0.17	0.16
	Top	0.18	<0.10	0.11
2.0	Bottom	0.44	0.53	0.48
	Middle	0.34	0.28	0.31
	Top	0.16	<0.10	<0.10
Significance level by F test:[b]				
Rate of application		N.S.	0.01	0.05
Stalk position		0.01	0.01	0.01
Rate x stalk position		N.S.	0.01	N.S.

[a]Methiocarb was applied four times at the rates shown to give season rates of 4.0 and 8.0 kg/ha.

[b]Values for the untreated control were omitted from the analysis of variance. Other values that were less than the lowest detectable limit (0.10 ppm) were assigned a value of 0.05 for the statistical analysis.

Table 4. Residues of methiocarb in flue-cured tobacco after application of 1 and 2 kg/ha, Clayton, N.C.

Rate of application[a] (kg/ha)	Harvest	Methiocarb (ppm)	Sulfoxide (ppm)	Sulfone (ppm)	Total residue (ppm)
0.0	First	<0.10	<0.10	<0.10	<0.30
	Second	<0.10	<0.10	<0.10	<0.30
	Third	<0.10	<0.10	<0.10	<0.30
	Fourth	<0.10	<0.10	<0.10	<0.30
	Fifth	<0.10	<0.10	<0.10	<0.30
1.0	First	<0.10	0.42	0.15	0.62
	Second	<0.10	0.24	0.16	0.45
	Third	<0.10	0.14	<0.10	<0.30
	Fourth	<0.10	<0.10	<0.10	<0.30
	Fifth	<0.10	<0.10	<0.10	<0.30
2.0	First	<0.10	0.64	0.36	1.05
	Second	<0.10	0.33	0.26	0.64
	Third	<0.10	0.20	0.19	0.44
	Fourth	<0.10	0.22	0.18	0.45
	Fifth	<0.10	0.14	0.18	0.37
Significance level by F test:[b]					
Rate of application			N.S.	N.S.	0.05
Stalk position			0.01	0.05	0.01
Rate x stalk position			N.S.	N.S.	N.S.

[a]Methiocarb was applied four times at the rates shown to give season rates of 4.0 and 8.0 kg/ha.

[b]Values for the untreated control were omitted from the analysis of variance. Other values that were less than the lowest detectable limit (0.10 ppm) were assigned a value of 0.05 for the statistical analysis.

metabolites was tranferred to the column. Fifty milliliters of 97.5:2.5 v/v of benzene:acetone were passed through the column and discarded, and the methiocarb and metabolites were eluted with 5% acetone in benezene. The eluate was evaporated to dryness at 40 C under reduced pressure. Ten milliliters of acetonitrile were added and the resulting solution was transferred to a 125-mL separatory funnel. The acetonitrile solution was partitioned three times with *n*-hexane and the *n*-hexane was discarded. The volume of acetonitrile was adjusted to 10 mL for analysis by HPLC.

The liquid chromatograph used for analysis of the 1981 samples was a Waters Model 6000A equipped with a Waters Model 710B Autosampler. The column was a Waters RCSS-C_{18} (10 by 0.8 cm ID). The solvent system was water:acetonitrile (8:2 v/v) with flow rate of 0.8 mL/min. The detector was identical to that used on the 1978 samples operated at 225 nm and 0.1 AUFS. A 10-mV recorder was operated at 0.25 cm/min. With this system the retention times for methiocarb, the sulfoxide, and the sulfone were 14.8, 3.8, and 5.6 min, respectively.

Known amounts of methiocarb and the sulfoxide and sulfone were added to untreated tobacco 30 min before the extraction solvent was added, and the fortified samples were carried through the procedure to determine the efficiency of the method. Two such recoveries were analyzed with each set of 10 experimental samples.

RESULTS AND DISCUSSION

The average recovery for 1978 samples fortified with methiocarb at levels of 0.15 to 6.0 ppm before extraction was 89% (**Table 1**). With the method of analysis used for 1978 samples the parent compound could not be separated from the sulfoxide and sulfone metabolites. Only total residues (methiocarb + methiocarb sulfoxide + methiocarb sulfone) are reported.

The method of analysis was modified between the time the samples for 1978 and those for 1981 were analyzed. The methiocarb, the sulfoxide, and the sulfone were separated and each measured separately by the procedure used for the 1981 samples. Recoveries averaged 86, 84, and 86%, respectively, for methiocarb, the sulfoxide, and the sulfone (**Table 2**). The lowest recovery was 77%; all others ranged from 80 to 91%.

Residues of total methiocarb were greatest in burley leaf from the bottom stalk position (**Table 3**). Levels for the 1.0 and 2.0 kg/ha rates (season totals of 4 and 8 kg/ha respectively) in bottom stalk samples averaged 0.35 and 0.48 ppm, respectively, and were progressively lower for leaf from the middle and upper stalk positions. The upper most leaves were small or only forming at the time of the last application in early July; the tobacco was harvested about 9 weeks later. The decreasing direct exposure during application may account to a large extent for the low residues in middle and upper stalk tobacco. There was no significant difference between residues for the two locations.

Total residues of 0.62 and 1.05 ppm were found in first harvest samples (bottom stalk) of flue-cured tobacco about 5 weeks after the last application of 1 and 2 kg/ha (season total of 4 and 8 kg/ha), respectively (**Table 4**). Residues declined in samples taken from progressively higher stalk positions. At the low rate of application, residues of all three components were below the detectable limit in fourth and fifth harvest samples.

The parent compound, methiocarb, was not detected in any flue-cured samples (**Table 4**). All values were below the detectable limit even at the first harvest. All measurable residues were in the sulfoxide and sulfone forms, and in most samples the sulfoxide was present in greater amounts than the sulfone. Thus, conversion of methiocarb to the metabolites occurred either within the plant or in the soil before absorption.

From the data, it was impossible to determine whether the

methiocarb and (or) the metabolites present in cured leaf of both burley and flue-cured tobacco were absorbed by the roots and transported to the leaves, absorbed directly by leaf tissue from pellets retained by the leaves, or caused by the forces of wind and rain or contamination from hand harvesting. Because top leaves were not present at the time of application, some transport within the plant may have accounted for residues found in samples from the upper stalk positions of both burley and flue-cured tests.

Methiocarb at rates of application employed in these experiments caused no measurable effect on yield of cured leaf, percent reducing sugar, or percent total alkaloids (**Table 5**). In flue-cured samples the sugar to nicotine ratio ranged from 7.2 to 7.7. Sugar and nicotine are of major importance in determining smoke flavor, and good tobacco produced in the same part of North Carolina as the site of our experiment with flue-cured tobacco should show a sugar/nicotine ratio of about 7 (6). From a series of experiments to control slugs in burley tobacco, Mistric et al. (4) reported that one to three applications of methiocarb increased yields and consequently value but did not alter quality. Thus, methiocarb does not appear to affect tobacco except indirectly by reducing loss in weight caused by slugs.

Table 5. Yield, reducing sugar, and total alkaloids of burley and flue-cured tobacco after application of methiocarb.

Location	Rate of application[a] (kg/ha)	Yield[b] (kg/ha)	Reducing sugar[b] (%)	Total alkaloids[b] (%)
Waynesville (burley)	0.0	3304	1.00	2.99
	1.0	3237	0.68	3.24
	2.0	3458	0.73	3.07
Laurel Springs (burley)	0.0	2851	0.85	2.61
	1.0	2733	1.23	2.84
	2.0	2703	0.98	2.50
Clayton (flue-cured)	0.0	3732	23.6	3.08
	1.0	3710	22.5	3.12
	2.0	3701	22.9	3.12

[a] Methiocarb was applied four times at the rates shown to give season rates of 4.0 and 8.0 kg/ha.

[b] Statistical analysis showed no significant difference at the 5% level among treatments for yield, reducing sugar, and total alkaloids in flue-cured samples. Statistical analyses are unavailable for burley samples.

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