Bull. Environm. Contam. Toxicol. 26, 393-400 (1981)

Residue Analysis of Methiocarb Applied to Ripening Sorghum as a Bird Repellent in Senegal

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Pest birds, and the Red-billed Quelea (Quelea quelea) in particular, are responsible for annual losses of sorghum exceeding 6 tons or about 4% of the total sorghum production in Senegal (BRUGGERS & RUELLE in press). Using chemical repellents on ripening cereal crops for reducing these losses is gaining increasing acceptability in Africa. Methiocarb is highly repellent to Q. quelea and has shown promise in West Africa (BRUGGERS 1979) in protecting ripening cereal crops. The increased use of the chemical on food crops requires an understanding of its degradation dynamics under African conditions.

Methiocarb is an insecticide/molluscicide with a low dermal, and moderately high oral toxicity to rats (Table 1). Methiocarb breakdown produces several toxic metabolites (KUHR & DOROUGH 1976) and since it is being increasingly used as a crop protection method by applying it directly to ripening grain, it is necessary to assure permissible residue levels at the time of harvest.

Table 1. Comparative oral toxicity of methiocarb and its metabolites to rats.

Chemical	Oral LD_{50} (mg/kg)	Reference
Methiocarb--oral	100-135	EDSON et al. 1966
--dermal	2000	KENAGA & CHERYL 1974
Methiocarb sulfoxide	42	HOFFMAN pers. comm.
Methiocarb sulfone	>1000	" "
4-(methylthio)-3,5-dimethylphenol	>1000	" "
4-(methylsulfinyl) 3,5-dimethylphenol	>1000	" "
4-(methylsulfonyl) 3,5-dimethylphenol	>1000	" "

Carbamate residues can be analyzed using several methods, but many of the methods, and particularly the spectrophotometric ones, are inappropriate for methiocarb (NIESSEN & FREHSE 1963; BENSON & FINOCCHIARO 1965; VONECH & DE RIVEROS 1971). Flame photometric gas chromatography can be used to specifically analyze for the sulfur

0007-4861/81/0026-0393 $01.60

in methiocarb (BOWMAN & BEROZA 1969), however, since methiocarb and its metabolites decompose when heated (STROTHER 1968; WHEELER & STROTHER 1969), they must be transformed into more stable volatile derivates (JOHNSON & STANSBURY 1965; LAU & MARXMILLER 1970; COHEN et al. 1979; BUTLER & MCDONOUGH 1971; SEIBER 1973), such as the silyl derivative (THORNTON & DRAGER 1973).

Despite the sensitivity and specificity of these methods, they often are impractical to use in Africa. Pure hydrogen and oxygen gases are not regularly available, nor is maintenance of the equipment reliable or dependable. Therefore, we developed a rapid, simple, practical, and sensitive method to mark the hydrolysis products of methiocarb and its metabolites with dansyl chloride, a fluorescent compound, and to separate the dansylated de ivatives using thin-layer chromatography (TLC). The technique of quantifying carbamates by dansylation was proposed by LAWRENCE & FREI (1972, 1974) and LAWRENCE et al. (1972). The dansylation reaction for methiocarb is indicated in Fig. 1. The fluorescent intensity of the spot is measured with a densitometer. The results were compared to those obtained by gas chromatography (THORNTON & DRAGER 1973).

MATERIALS AND METHODS

Application and Sampling. Methiocarb was applied with a motorized backpack sprayer at the rate of 2 kg/80 L of water/ha to milk-stage sorghum (variety CK 60) in a 46-m^2 area on 30 Jan 1978. Adhesive/spreader Triton AE was added at 60 mL/100 L of water. Sorghum was irrigated overhead 20-25 mm twice weekly for a total of 120-150 mm during the maturation period. Average maximum and minimum temperatures during this period were 35.7° and 16.3°C, respectively; no rainfall occurred.

Stalks of heads in four rows were marked prior to applying the methiocarb for subsequent residue analysis collection. One row was left untreated (Sample A). Heads were collected from the treated rows (Sample B) immediately after spraying, at 12 h, 24 h, and 5, 10, 15, 20, 25, and 30 days and frozen until analyzed. Residues were determined for the entire head (grain and glume) and for the grain only.

Apparatus, Chemicals, and Procedures. Methiocarb and its metabolites were qualitatively analyzed using two-dimensional TLC on F 254 silica gel plates, with the solvents petroleum ether:diethyl ether (1:1 v/v) and diethyl ether:hexane (4:1 v/v). The plates were visualized at 254 nm (Table 2).

Methiocarb, its sulfone and its sulfoxide, were quantitatively analyzed by placing between 10 and 100 µL of the dansylated methylamine hydrolysis products (extracted with cyclohexane) on silica gel G chromatographic plates. The plates were developed using a benzene:acetone system (98:2 v/v) followed by a 20% solution of triethanolamine in 2-propanol and measured by densitometer after 48 h at emission and excitation wavelengths of 525 and 366 nm, respectively. The spot intensity is linear from 0 to 250 ng of methiocarb.

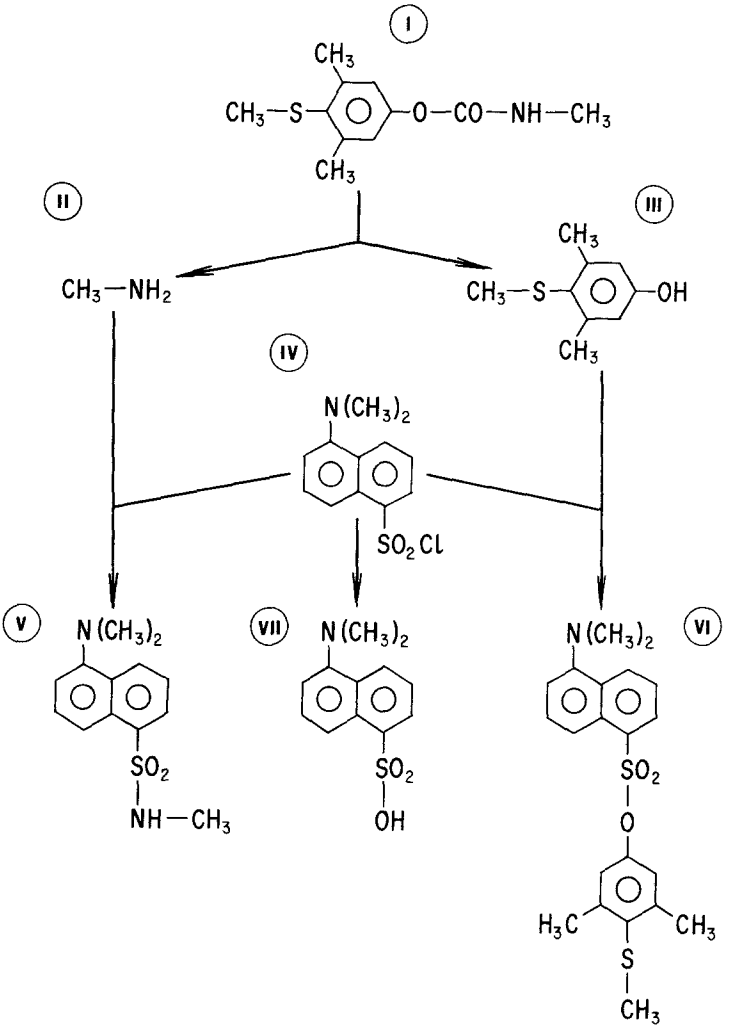

Figure 1: Dansylation reaction of methiocarb.

I: Methiocarb
II: Methylamine
III: 4-(methylthio)-3,5-dimethylphenol
IV: Dansyl chloride
V: Dansylated derivative of methylamine
VI: Dansylated derivative of 4-(methylthio)-3,5-dimethylphenol
VII: Hydrolyzed dansyl chloride

Table 2. Results of thin-layer chromatography of methiocarb and its principal metabolites from sorghum treated with 2 kg (ai)/ha.

Chemical products	Sensitivity limits	R_f (on F 254 thin-layer plate) Ether-hexane (4:1)	Ether-pet. ether (1:1)
Methiocarb	0.5 - 1 ug	0.58	0.38
Methiocarb sulfoxide	" "	0	0
Methiocarb sulfone	" "	0.37	0.13
4-(methylthio)-3,5-dimethylphenol	" "	0.72	0.56

This extraction method is a slight modification of THORNTON & DRAGER's (1973) technique in that 25-g samples were used instead of 100-g samples, and the volume of reactants was reduced accordingly. The extract was hydrolyzed for 30 min and dansylated. We also quantitatively extracted the dansylated products with three successive extractions using 1 mL of cyclohexane instead of one extraction of 0.5 mL of cyclohexane. Once the extract was obtained, it was evaporated and recovered with 0.5 mL of cyclohexane.

The results obtained by TLC were confirmed using a gas chromatograph equipped with an FPD detector and a 1.8 m x 2 mm x 2 mm glass column packed with 5% DC-200 on 80-100 mesh Gas Chrom Q. The silyated derivatives from the different extracts were injected into the column in quantities of 1-5 ug from each sample. The corresponding quantity of methiocarb was then calculated using a standard curve. Analytic references for methiocarb, methiocarb sulfoxide, methiocarb sulfone, and 4-(methylthio)-3,5-dimethylphenol of 99% purity were furnished by Bayer AG, West Germany.

RESULTS AND DISCUSSION

Analysis by TLC. Residue analysis was conducted on untreated (Sample A) and treated (Sample B) heads, both on grains alone and grains with their protective glumes. Analysis of the seeds of the untreated heads confirmed the absence of methiocarb or other chemicals which might possibly interfere with analysis under the treatment conditions. In the treated sample, methiocarb alone was identified at 5 days; methiocarb sulfone was evident in the samples after that time period. Neither methiocarb sulfoxide nor 4-(methylthio)-3,5-dimethylphenol were observed. The absence of methiocarb sulfoxide is particularly notable since it constitutes the most toxic metabolite.

The procedural recovery of methiocarb and its sulfone and its sulfoxide metabolites was determined using fortified samples. The results yielded 99, 96, and 100%, respectively, for methiocarb, its sulfoxide, and its sulfone. When samples are quantitatively

analyzed only by TLC, the method can be considerably simplified without losing the reliability and quality of the results. The chloroform extract can be dried on sodium sulfate, evaporated, and reconstituted with 5 mL of 95% alcohol. Recoveries of this method conform closely to those previously obtained; 99% for methiocarb, 95% for methiocarb sulfoxide, and 98% for methiocarb sulfone.

Degradation Curves and Residues. The methiocarb degradation curves of Fig. 2 are based on the data in Table 3. The logarithmic curves theoretically are composed of two different slopes corresponding to two particular kinetics of chemical disappearance. The steep slope corresponds to the chemical breakdown under the influence of external climatic factors such as the sun, humidity, and wind. The penetration by the chemical through the cell wall into the tissues is a more slow, enzymatic metabolism process which is exemplified by a more gradual slope. These results show that initial degradation is very rapid, and that metabolism effects begin to appear the second day. The mathematical interpretation of the curve is explained by the equation

$$\text{Log } Y = K_1 t + \text{Log } Y_0$$

with Y_0 being the quantity of chemical at t = 0, "Y" the quantity at any time, "K_1" the slope of the regression line, and "t" the time in days. The half-life of methiocarb can be determined from this equation (Table 4), and was found to be about 7 days, which corresponds to the normal values for carbamates.

The residues obtained by TLC were slightly lower, but compared favorably with those obtained by gas chromatography (Table 3). The half-life of methiocarb was 7 days by TLC and 6-7 days by gas chromatography. For Q. quelea, the methiocarb R_{50} = 0.015% (SHUMAKE et al. 1976) or 150 ppm which is equivalent to the amount remaining on grain during the first 3 days after spraying.

The daily acceptable dose (DAD) for human consumption for carbamates in general, as provisionally established by a joint FAO/WHO committee is 0.025 mg/kg (ROIG 1973). The maximum admissible concentration (MAC) can be calculated as

$$\text{MAC} = \frac{\text{DAD (mg/kg) x P x 1000}}{Q}$$

where "P" represents the average weight of an individual and "Q" the daily intake of potentially contaminated food. Assuming an average daily consumption of 500 g of sorghum by a 70-kg individual in Senegal, the MAC would be

$$\text{MAC} = \frac{0.025 \times 70 \times 1000}{500} = 3.5 \text{ mg/kg or } 3.5 \text{ ppm.}$$

It also is possible to calculate the time to treatment inhibition (the time delay between the last treatment and harvest). Since the methiocarb residues should not exceed the MAC (Yn = 3.5 ppm), the time to treatment inhibition can be expressed as

$$t_{Yn} = \frac{\text{Log } n}{K_1}$$

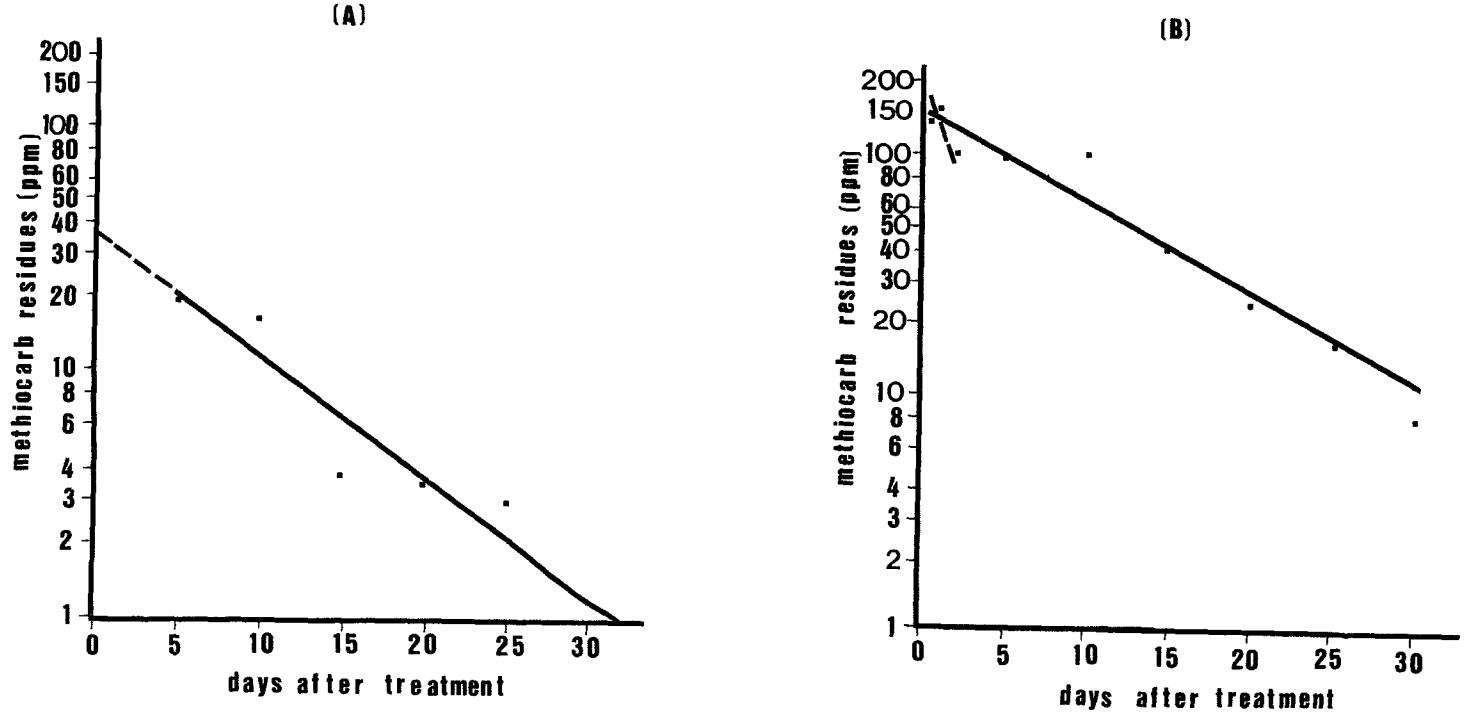

Figure 2: Degradation of methiocarb on the grain (A) and on grain and glumes (B) of sorghum following a 2-kg/ha application in Senegal during 1978.

Table 3. Persistence of methiocarb on sorghum (seeds only and seeds with glumes) after spraying with 2 kg/ha in Bambey, Senegal, 1978.

Sample no.	Time in days after application	Amount of methiocarb (ppm)	
		Thin-layer chromatography	Gas chromatography
Seed only			
1	5	20	36
2	10	17	13
3	15	3.8	8.8
4	20	3.5	4.3
5	25	3.0	4.0
6	30	0.9	2.2
Seed and glumes			
7	0	180	200
8	0.5	140	150
9	1	150	170
10	2	100	140
11	5	92	140
12	10	100	140
13	15	11	76
14	20	24	56
15	25	17	24
16	30	8	11

Table 4. Regression curve parameters for half-life determination of methiocarb.

Treated heads	K_1	Y_0 in ppm	Correlation	Half-life in days
Grain only	0.052	39	0.97	6
Grain and glumes	0.040	150	0.98	7.5

with "K_1" being the slope of the regression line and "n" the relationship $\frac{Y_0}{Yn}$ (Y_0 is the amount of methiocarb in the sample at t = o). From these results, the time to treatment inhibition is 20 days (Fig. 2) by TLC densitometry and 23 days by gas chromatography. This time period corresponds well with actual field application methods. Second applications usually are applied within 7-10 days of the first, leaving approximately 3 weeks until harvest in a normal 4- to 5-week maturation period. It also should be noted that in Senegal sorghum always is cooked prior to eating and that methiocarb is heat unstable; any remaining residues most likely would be partially or completely degraded during preparation. These results, in conjunction with methiocarb's low R_{50}, indicate that

it can be safely applied to ripening sorghum at normal repellent use levels in Senegal against pest birds.

Acknowledgments. Research was financed by UNDP/FAO Regional Project (RAF 73/055) 'Research into the Biology and Control of Grain-eating Birds.' H. Hoffman and G. Drager, Bayer AG, furnished methiocarb and its metabolites and contributed helpful suggestions. Sorghum was planted at the Centre National de Recherches Agronomiques, Bambey, Senegal in collaboration with C. Ndao and J. Denis. Additional support was provided by U.S. Agency of International Development under PASA ID/TAB 473-1-67. A. Medbery and E. Schafer, Jr., Denver Wildlife Research Center, reviewed the manuscript.

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