Determination of Tricyclazole and Alcohol Metabolite in Rice Grain and Straw

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An analytical method for determining residues of the fungicide tricyclazole (5-methyl-1,2,4-triazolo-[3,4-*b*]benzothiazole) and the major metabolite (1,2,4-triazolo[3,4-*b*]benzothiazole-5-methanol) in rough rice, grain components, and straw has been developed with flame photometric–gas chromatographic detection. Rice plants were foliar sprayed with tricyclazole at booting growth stage with a single 0.5 lb/A application or dual applications of 0.25 lb/A at booting and early heading growth stages. Maximum tricyclazole residues found in rough rice and straw were 3.9 and 3.5 ppm, respectively. Maximum metabolite residues found in rough rice and straw were 1.1 and 10.2 ppm, respectively. The limit of detection for tricyclazole or the alcohol metabolite in rough rice was 0.02 ppm based on a 25.0-g sample. Recoveries at the 0.10 ppm fortification level were approximately 77% for tricyclazole and 57% for the metabolite. The alcohol metabolite was derivatized and chromatographed as the trimethylsilyl (Me_3Si) ether.

INTRODUCTION

Tricyclazole is a systemic fungicide for control of the rice blast disease, *Piricularia oryzae*. The fungicidal properties of tricyclazole were first reported by Froyd (1973). Its potential for controlling rice blast disease was reported by Froyd et al. (1976) and methods of applying the material to rice plants were described by Froyd et al. (1978).

This paper describes a sensitive residue procedure for the determination of tricyclazole and the trimethylsilyl (Me_3Si) ether derivative of the alcohol metabolite in rough rice, brown rice, white rice, bran, hulls, and straw.

EXPERIMENTAL CONDITIONS

Field Applications. Field experiments were conducted in the typical rice growing regions of Arkansas, Louisiana, Mississippi, and Texas. Rice plants in the booting growth stage were sprayed with either 0.25 or 0.5 lb/A of tricyclazole. Those plants which received the 0.25 lb/A treatment were given a second application of 0.25 lb/A at early heading. All rice plants received a total tricyclazole treatment of 0.5 lb/A. Residue data were obtained from aerial and hand sprayed application methods. Experiments were designed to compare efficacy of one large application of tricyclazole vs. two smaller applications and to provide residue data from two typical methods of application.

Sampling. At maturity grain and straw samples from control and treated rice paddies were each placed in special residue shipping bags, identified, and sent to Lilly Agricultural Analytical Chemistry, Greenfield, IN. Ample amounts of rough rice from aerial applications were processed through a typical commercial milling operation to provide all of the rice fractions for analysis: brown rice, white rice, bran, and hulls.

Sample Preparation. The rough rice grain (unhulled rice), referred to as the raw agricultural commodity, was finely ground in a Burr Mill. Brown and white rice were also ground in this manner. Rice straw and hulls were frozen in liquid nitrogen and then finely ground in a Homoloid Machine using a fine screen. All samples were stored at −20 °C until analysis.

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Chemicals. All solvents were pesticide grade or reagent grade redistilled in glass. The analytical grade tricyclazole and alcohol metabolite were prepared in the Lilly Research Laboratories. All steps involving Regisil (*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (Me_3SiCl), Regis Chemical Co.) were performed in a well ventilated fume hood.

Sample Extraction. Analysis weights of the tissue components were rough rice, brown or white rice 25.0 g, hulls 12.5 g, bran or straw 10.0 g. The correct amount of finely ground sample was weighed into a 250-mL boiling flask and refluxed for 1 h in 125 mL of 4 N H_2SO_4. The reflux condenser was washed with 25 mL of distilled water and added to the sample. The hot mixture was filtered with Schleicher and Schuell No. 558 folded filter paper and the filtrate was collected in a 250-mL beaker. The spent tissue was washed twice using 30 mL of distilled water per wash. The combined filtrates were cooled in an ice water bath, neutralized with 50% NaOH and adjusted to pH 8–9 with 2 N NaOH.

Partitioning. Sample solutions were transferred to 500-mL separatory funnels and extracted twice with 100-mL portions of dichloromethane. Emulsions, which formed occasionally, were broken by centrifuging the mixtures for approximately 10 min. The dichloromethane extracts were dried by passage through a bed of anhydrous sodium sulfate and combined in a 250-mL boiling flask. Extracts were evaporated to dryness with a rotary vacuum evaporator and a 45–50 °C water bath to aid evaporation.

Column Chromatography. Basic Woelm Alumina Super I (Universal Scientific, Inc.) was deactivated with 10% water and used for column chromatographic cleanup and separation of tricyclazole and the alcohol metabolite. The chromatographic column (Kimax or Pyrex, 250 mm × 14 mm i.d. with 250-mL reservoir) was prepared by placing 50 mL of dichloromethane in the column prior to adding 14 g of the deactivated basic alumina. A 2-cm band of anhydrous sodium sulfate was placed at the top of the column and the dichloromethane was drained to the top of the sodium sulfate. The residue in the evaporation flask was dissolved in 10 mL of dichloromethane and transferred to the column. Two additional 10-mL dichloromethane rinses of the flask were added to the column. The column was washed with an additional 70 mL of dichloromethane and all washings were discarded. Tricyclazole was eluted with 100 mL of dichloromethane–methanol (99:1 v/v),

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Table I. Tricyclazole Residues after Aerial Application[a]

expt	rough rice		brown rice		white rice		bran		hulls		straw	
	Tr[b]	Al[c]	Tr[b]	Al[c]	Tr[b]	Al[c]	Tr[b]	Al[c]	Tr[b]	Al[c]	Tr[b]	Al[c]
RWW 80-19												
0.5 lb/A	3.9	1.1	0.16	0.05	0.04	NDR[d]	0.79	0.18	11.5	3.0	0.70	3.9
0.25 + 0.25 lb/A Katy, TX	1.7	0.87	0.09	0.04	0.05	NDR	0.64	0.14	8.6	2.2	0.48	3.6
KRB 80-19												
0.5 lb/A	0.21	0.05	NDR	NDR	NDR	NDR	NDR	0.02	0.88	0.21	0.43	2.1
0.25 + 0.25 lb/A Crowley, LA	1.1	0.11	0.03	NDR	NDR	NDR	0.14	0.10	4.5	0.91	0.33	3.2
HLW 79-24												
0.5 lb/A	0.13	0.03	0.03	0.07	NDR	NDR	0.13	0.10	0.34	0.15	0.23	2.7
0.25 + 0.25 lb/A Cherry Valley, AR	0.33	0.03	0.05	0.10	NDR	NDR	0.26	0.14	0.88	0.18	0.18	1.9
DLG 79-13												
0.5 lb/A	0.06	NDR	NDR	NDR	NDR	NDR	insufficient		0.06	NDR	0.21	1.1
0.25 + 0.25 lb/A Robinsonville, MS	0.11	0.03	0.03	0.02	NDR	NDR	sample		0.32	0.11	0.18	1.5
RWW 79-9												
0.5 lb/A	0.15	0.04	0.03	NDR	no		no		0.43	0.17	0.06	0.40
0.25 + 0.25 lb/A Katy, TX	1.7	0.31	0.08	0.04	sample		sample		5.9	0.86	0.23	1.3

[a] Values given in ppm. [b] Tr = tricyclazole. [c] Al = alcohol metabolite. [d] NDR = no detectable residue at detection limit of 0.02 ppm.

Table II. Tricyclazole Residues 0.25 + 0.25 lb/A Hand Application[a]

expt	rough rice		brown rice		hulls		straw	
	Tr	Al	Tr	Al	Tr	Al	Tr	Al
JLP 8,9,0-18								
1978	*b*		0.23	0.07	8.0	1.2	0.54	0.84
1979	0.75	0.03	0.31	0.03	0.72	0.37	0.63	1.1
1980	1.6	0.25	0.68	0.07	7.0	0.15	1.2	1.1
Beaumont, TX								
JLP 8,9,0-19								
1978	*b*		0.31	0.13	4.8	0.84	0.48	1.6
1979	0.73	0.19	0.15	0.08	2.0	0.73	0.12	1.6
1980	0.03	0.03	NDR	NDR	0.08	0.11	0.11	0.77
Iota, LA								
JLP 8,9,0-20								
1978	*b*		0.29	0.08	9.3	3.5	3.5	10.2
1979	*c*							
1980	2.5	0.05	0.13	0.06	5.9	0.76	1.9	1.4
Eagle Lake, TX								
HLW 8,9,0-19								
1978	*b*		0.28	0.11	5.9	1.6	0.69	2.8
1979	0.61	0.17	0.02	NDR	2.4	0.12	0.22	1.8
1980	2.6	0.48	0.28	0.08	5.2	1.4	1.4	4.8
Colt, AR								

[a] See abbreviations in Table I. [b] The complete sample of rough rice was dehulled. [c] Samples were taken in the green immature, stage and were not assayed.

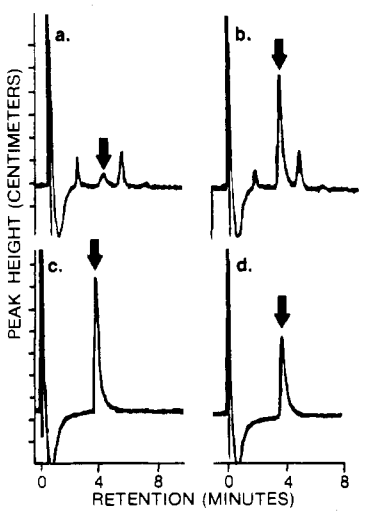

Figure 1. Tricyclazole in rough rice: (a) control 25.0 g → 2.0 mL; (b) recovery 0.10 ppm, 25.0 g → 2.0 mL; (c) treated 0.5 lb/A, 25.0 g → 10.0 mL; (d) standard 1.0 μg/mL.

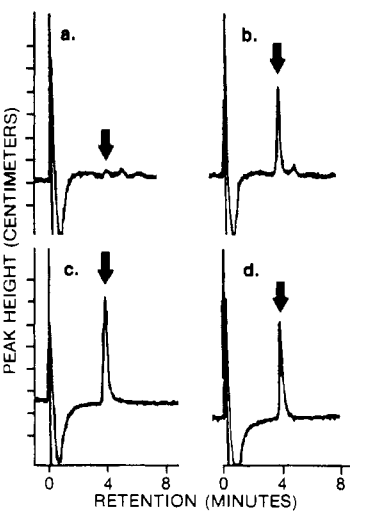

Figure 2. Me_3Si alcohol metabolite in rough rice: (a) control 25.0 g → 1.0 mL; (b) recovery 0.10 ppm, 25.0 g → 1.0 mL; (c) treated 0.5 lb/A, 25.0 g → 10.0 mL; (d) standard 1.0 μg/mL.

Table III. Tricyclazole Residues 0.5 lb/A Hand Application[a]

	rough rice		brown rice		hulls		straw	
expt	Tr	Al	Tr	Al	Tr	Al	Tr	Al
JLP 8,9,0-18								
1978	[b]		NDR	NDR	0.35	0.19	0.64	1.2
1979	0.03	NDR	0.02	NDR	0.05	0.03	0.11	0.16
1980	0.12	0.06	NDR	NDR	0.55	0.58	0.70	1.2
Beaumont, TX								
JLP 8,9,10-19								
1978	[b]		0.04	0.03	0.83	0.16	0.22	1.07
1979	0.17	0.06	0.05	NDR	0.70	0.22	0.19	2.0
1980	0.03	0.02	NDR	NDR	0.07	0.09	0.18	0.80
Iota, LA								
JLP 8,9,0-20								
1978	[b]		0.07	0.04	2.4	1.1	0.76	4.7
1979	[c]							
1980	0.11	0.03	NDR	NDR	0.34	0.07	0.34	0.74
Eagle Lake, TX								
HLW 8,9,0-19								
1978	[b]		0.08	0.02	1.7	0.33	0.11	1.4
1979	0.02	0.02	NDR	NDR	NDR	0.08	0.12	1.0
1980	0.08	NDR	NDR	NDR	0.19	0.06	0.50	4.2
Colt, AR								

[a] See abbreviations in Table I. [b] The complete sample of rough rice was dehulled. [c] Samples were taken in the green immature stage and were not assayed.

Table IV. Growth Stage of Rice Plants at Application Time

	growth stage	
expt	0.5 lb/A	2nd 0.25 lb/A
RWW 80-19	30% heading	90% heading
KRB 80-19	1% heading	50–70% heading
HLW 79-24	late booting	50–70% heading
DLG 79-13	2–4 in. panicle	50–75% heading
RWW 79-9	2% heading	90% heading
JLP 8-18	booting	heading
JLP 8,9-18	booting	very late heading
JLP 8,9,0-18	early booting	early heading
JLP 8-19	1% heading	full heading
JLP 8,9-19	booting	late heading
JLP 8,9,0-19	booting	no information
JLP 8-20	10% heading	full heading
JLP 8,9-20	booting	no information
JLP 8,9,0-20	early booting	no information
HLW 8-19	early heading	full heading
HLW 8,9-19	2–4 in. particle	full heading
HLW 8,9,0-19	late booting	50–70% heading

followed by elution of the alcohol metabolite with 175 mL of dichloromethane–methanol (95:5 v/v). The eluates were collected in separate 250-mL boiling flasks and evaporated to dryness on a rotary evaporator.

Derivatization. The alcohol metabolite was derivatized by the addition of 0.5 mL of acetonitrile and 0.5 mL of Regisil to the evaporation flask. After rotating the flask to insure solution of the residual material, a 0.5-mL aliquot was transferred to a 25-mL glass screw-cap vial and capped tightly with a cap containing an aluminum insert (note: avoid any type of wax insert). The reaction was allowed to proceed for 1 h at 100 °C and then the vials were cooled in the hood to room temperature. The contents in the vials were evaporated to dryness in a well-ventilated hood using a stream of dry nitrogen and heat of about 50 °C. The sample vials were capped immediately upon removal from the nitrogen and cooled for approximately 2 min. Exactly 0.5 mL of acetonitrile was added to each vial and the vial was again securely capped. The Me_3Si metabolite samples were now ready for GC analysis.

Fortification. Recoveries of tricyclazole and the alcohol metabolite were determined by extraction of a control rice fraction fortified with 2.5 μg of each compound, i.e., 0.10 ppm in grain, 0.20 ppm in hulls, and 0.25 ppm in bran or straw. The fortified samples remained at room temperature overnight before extraction and analysis.

Gas Chromatography. A Tracor Model 560 Gas Chromatograph equipped with a flame photometric detector (FPD), operated in the sulfur mode, was used with an Omni Scribe 1.0 mV recorder. The 1.8 m × 2 mm i.d. coiled glass column was packed with 1.5% Carbowax 4000M on Chromosorb W – HP 80/100 mesh. Retention times for tricyclazole and derivatized metabolite were approximately 4–5 min. Operating conditions were column 220 °C, injector 250 °C, detector 220 °C, helium (carrier gas) 30 mL/min, hydrogen and air (detector gases) 100 and 200 mL/min, and electrometer 3.2×10^{-9} AFS. Under these conditions a 10.0-ng injection of tricyclazole (5.0 μL of 2.0 μg/mL) or the Me_3Si alcohol metabolite gave 50% recorder deflection. The flame photometric detector is not a linear response detector (Fredriksson and Cedergran, 1981), therefore a standard curve was established with each set of samples and concentrations in experimental samples were determined from the standad curves.

RESULTS AND DISCUSSION

Many polar compounds such as the alcohol metabolite of tricyclazole do not chromatograph, per se, but after derivatization with BSTFA the Me_3Si ether derivative chromatographs excellently (Poole and Zlatkis, 1979). The derivatization procedure for the alcohol metabolite was relatively simple. A drying oven set at 100 °C provided a constant heat for the 1-h reaction. An aluminum block, 6 in. square with sixteen 1.125-in. diameter holes, was used to hold the glass vials during the derivatization and evaporation steps. During sample evaporation the block was heated by placing on a hot plate. Caution should be observed during the evaporation of the BSTFA by using a well ventilated hood. After removing a sample from the nitrogen, immediately cap the vial. Allow 2–3 min for the vial to cool, then add the acetonitrile, and secure the cap on the vial. Extra precautions should be observed when working with Me_3Si derivatives because these compounds are easily hydrolyzed.

The optical filter used with the FPD was the broad band sulfur filter, 396 ± 20 nm. This filter allowed for maximum sensitivity without increasing background noise. Validation of the acid hydrolysis extraction was accomplished

Table V. Summary of Recovery Data[a]

recovery equiv expt	percent							
	0.10 ppm rough rice		0.10 ppm brown rice		0.20 ppm hulls		0.25 ppm straw	
	Tr	Al	Tr	Al	Tr	Al	Tr	Al
RWW 80-19	80	36	85	58	76	38	68	72
KRB 80-19	60	82	85	58	81	79	100	51
HLW 79-24	71	61	66	32	82	36	58	45
RWW 79-9	60	49	56	54	38	42	76	43
DLG 79-13	58	51	77	60	80	50	78	40
JLP 8-18	*b*		87	40	76	46	74	50
JLP 8,9-18	100	51	70	54	100	54	90	50
JLP 8,9,0-18	80	69	94	84	77	58	86	74
JLP 8-19	*b*		89	52	82	80	96	52
JLP 8,9-19	82	43	66	36	64	27	77	35
JLP 8,9,0-19	84	63	68	58	65	44	86	70
JLP 8-20	*b*		88	50	74	67	81	48
JLP 8,9-20	*c*							
JLP 8,9,0-20	80	52	72	90	66	82	89	94
HLW 8-19	*b*		84	41	70	34	88	52
HLW 8,9-19	100	37	94	52	68	72	86	68
HLW 8,9,0-19	72	92	72	100	88	62	100	84
$\bar{X}$	77.3	57.2	78.3	57.4	74.2	54.8	83.3	58.0
σ	14.0	17.2	11.4	19.0	13.4	17.9	11.3	16.9

[a] See abbreviations in Table I. [b] Complete samples of rough rice were dehulled. [c] Rice samples were green, immature, and not assayed.

utilizing the straw from [^{14}C]tricyclazole-treated rice plants. Comparison of results from the two assay procedures: FPD-GC values, 0.82 ppm tricyclazole and 1.83 ppm alcohol metabolite; radiochemical values, 1.05 ppm tricyclazole and 2.24 ppm alcohol metabolite. The results compare favorably with the FPD-GC analysis equaling 78% and 81% of the radiochemical values.

Extracts from tricyclazole-treated rough rice were prepared according to the analytical procedure and injected into a Hewlett-Packard Model 5985 GC–MS System. The instrument was operated in the select ion mode to monitor tricyclazole at 189 and 162 (molecular weights of tricyclazole and primary fragment with loss of HCN), and the Me_3Si alcohol derivative was monitored at 277 and 262 (molecular weights of the derivative and primary fragment with loss of CH_3). The presence of both tricyclazole and metabolite was clearly identified in the treated rough rice.

Residues of tricyclazole and the alcohol metabolite were determined in rough rice, rice grain fractions, and straw from nine field experiments involving aerial and hand sprayed application (Tables I, II, and III). The quantity of tricyclazole residue found in rough rice and resulting fractions was directly affected by timing of application relative to the growth stage of the rice plants. The field experiment at Katy, TX, produced samples giving the largest residue values because the dual 0.25 lb/A applications were made when the plant growth stage was at 30% heading and again at 90–100% heading (Table IV). This delay of application was caused by adverse weather conditions and represented the latest spraying schedule which would prove beneficial for the control of rice blast.

A summary of recovery data is listed in Table V. The average recovery value for tricyclazole in rough rice was 77.3% and the alcohol metabolite was 57.2%. The detection limit for tricyclazole or the alcohol metabolite in rough rice, brown rice, or white rice was 0.02 ppm based on a 25.0-g sample.

Figures 1 and 2 illustrate typical chromatograms of control and treated rough rice extracts. These samples were from field experiment RWW 80-19, Katy, TX. Background interference with tricyclazole was observed with some control rough rice samples. This interference of 2–3% chart scale was insignificant considering that the treated rough rice sample was diluted 5-fold compared to the control. Fortified recoveries were corrected for the control response in calculating percent recovery, but control corrections were not applied in calculations of residues found in treated samples.

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Registry No. Tricyclazole, 41814-78-2; 1,2,4-triazolo[3,4-*b*]-benzothiazole-5-methanol, 69243-49-8.

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