Metabolism of Carboxin (Vitavax) by Barley and Wheat Plants

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Carboxin (5,6-dihydro-2-methyl-1,4-carboxanilide) used as a seed treatment on barley and wheat is absorbed by the seedlings. It is oxidized in the plants, mainly to the sulfoxide (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide 4-oxide), but small amounts of sulfone (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide 4,4-dioxide) are also found. As plants approach maturity, the extractable oxathiin residues are converted to insoluble anilide complexes, probably with lignin. In no case has hydrolysis of carboxin been detected in plants.

Carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) is a highly effective systemic fungicide as shown by von Schmeling and Kulka (1966), von Schmeling et al. (1966), and Kulka et al. (1968). Its activity has also been studied by Edgington and coworkers (1966, 1967), Hardison (1966), Rowell (1967), and Mathre (1968). Since it is useful as a seed treatment for food crops, a clear understanding of its fate in the crops is necessary. In a previous paper, the degradation of carboxin in water and soil was reported (Chin et al., 1970). This investigation covers the metabolism of carboxin in barley and wheat plants.

Consideration was given to possible products resulting from hydrolysis, oxidation, and complex formation, as well as to the parent compound. For convenience of eventual identification of these metabolites, a preliminary separation of these residues with common organic solvents, such as benzene and acetone, into extractable and nonextractable portions is helpful. The formulas of carboxin and its oxidation analogs are shown in Figure 1.

EXPERIMENTAL

Analytical Procedures. THIN-LAYER CHROMATOGRAPHY (TLC). For qualitative detection of extractable residues, tlc was used as described in a previous paper (Chin et al., 1970).

MICROCOULOMETRIC GAS CHROMATOGRAPHY (MCGC). For the specific quantitative determination of carboxin a Dohrman Model-100 MCGC (Lane, 1967) equipped with a Vycor quartz tube in the injection port was used. The fractionating column was 8 in. long, 0.25 in. o.d. glass tube packed with 15% silicon grease on Anakrom ABS. The titrating cell was Model-T-200-S. The inlet, column, and furnace temperatures were 220°, 220°, and 600° C, respectively. Helium was used as the carrier gas at the rate of 4 on the flow meter steel balls (20 lb of pressure, 150 cc per min). The oxygen rate was 3 on the oxygen flow meter glass ball (20 lb pressure, 35 cc per min). All the analyses were conducted at the range ohm setting 128.

Plant extracts were concentrated with an air jet and aliquots were taken for determination.

COLORIMETRY. This procedure was used for the determination of total residues of carboxin, extractable, and nonextractable (Lane, 1970).

The basic principle of this procedure involves caustic hydrolysis of carboxin and its degradation products (extractable and nonextractable) to give aniline, which is then distilled and determined colorimetrically. This method is sensitive to about 1 ppm. This procedure was considered suitable for

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determining the total residue of carboxin, including metabolites present in the samples. Our previous experiments indicated that carboxin was subject to oxidation rather than hydrolysis in both water and soils, and the oxidation products are also anilides. If free aniline were present due to hydrolysis, it too would be measured.

Radioautography. Barley and wheat seeds treated with ^{14}C labelled carboxin (in both aniline and hetero moieties) were planted in the greenhouse. Two weeks after emergence, plants were extracted by acetone and concentrated for tlc and radioautography.

Preparation of Samples and Residue Analysis. PLANT TREATMENT. Barley and wheat seeds were treated with 84 g of carboxin (112 g formulated 75% wettable powder) per 45.4 kg. (4 oz per 100 lb). The treated seed were planted in several flats to be grown in the greenhouse for periodic harvest. The plants to be harvested at 1 and 3 weeks were grown in 30 × 60 × 10 cm flats, which were densely planted (to get reasonable amounts of tissue even though the individual plants were small at harvest). The plants to be harvested at 5 and 7 weeks were grown in deeper flats (30 × 60 × 20 cm) and fewer seeds were planted per flat. This, of course, means less carboxin was used per flat for the longer time periods. Plants were cut for analysis weekly after emergence. The overall scheme of residue analysis is diagramed in Figure 2. Results of total, extractable, and nonextractable residues were calculated as carboxin and shown in Table I.

TOTAL RESIDUE ANALYSIS. Plants were cut 2.5 cm above the soil surface at different time intervals. One flat of plants was always processed as one sample. The intact plant materials were washed twice with water and twice with acetone, then cut into pieces about 1 cm long. Ten grams of plant material was taken as a single sample for the total residue determination by colorimetry.

EXTRACTABLE RESIDUES. Plant materials were put in thick plastic bags and frozen on dry ice. The fresh plant materials (5 weeks or younger) could be pounded to a fine powder form in the deep frozen condition. The older plant materials (6 weeks or older) could be ground in a blender. The plant materials were ground with acetone (5 ml per 1 g) for 3 min. The slurry was wrung in a piece of cheesecloth to collect the acetone. This solution was dried over Na_2SO_4, filtered through filter paper, and stored in bottles in a refrigerator. Fifty milliliters of acetone extract was evaporated in a 250-ml beaker containing 10 g of Florisil at room temperature. The Florisil containing the extract from 10 g of plant material was analyzed colorimetrically for the extractable residue by the aniline method.

NONEXTRACTABLE RESIDUES. The plant materials extracted by acetone were further cleaned by Soxhlet extraction for 7 hr with a solvent mixture (benzene:methanol:acetone =

Table I. Carboxin Residues in Barley and Wheat Plants

	Total Residue			Acetone Extractable Residue			Nonextractable Residue		
Interval	ppm	μg	%	ppm	μg	% of Total Residue	ppm	μg	% of Total Residue

In Barley Plants

Interval	ppm	μg	%	ppm	μg	% of Total Residue	ppm	μg	% of Total Residue
1st week	20.0	1400	100	18.0	1360	98	52.0	140	10.0
3rd week	13.0	2145	100	11.0	1815	85	13.0	163	7.3
5th week	7.9	1288	100	6.0	978	76	3.8	106	8.3
7th week	2.9	452	100	2.1	328	73	3.1	105	23.0

In Wheat Plants

Interval	ppm	μg	%	ppm	μg	% of Total Residue	ppm	μg	% of Total Residue
1st week	12.0	720	100	14.0	840	110	23.8	68	9.5
3rd week	16.0	2080	100	14.0	1820	90	8.0	108	5.2
5th week	7.7	1540	100	5.5	1100	71	3.4	88	5.7
7th week	2.8	476	100	1.2	204	43	4.4	191	40.0

1:1:1:v/v). Samples were taken from the cleaned plant materials for the nonextractable residue determination by colorimetry.

Identification of Extractable Residues. A series of preliminary studies showed that the sulfoxide was the dominant extractable residue in both barley and wheat plants grown from carboxin-treated seeds. This can be demonstrated easily by applying concentrated plant extract (1 ml = 5 g of plant) to thin-layer sheets and developing with $CHCl_3$. After Fluo-
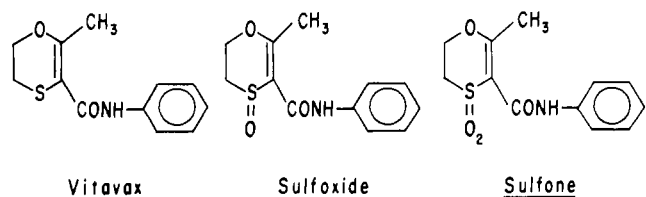

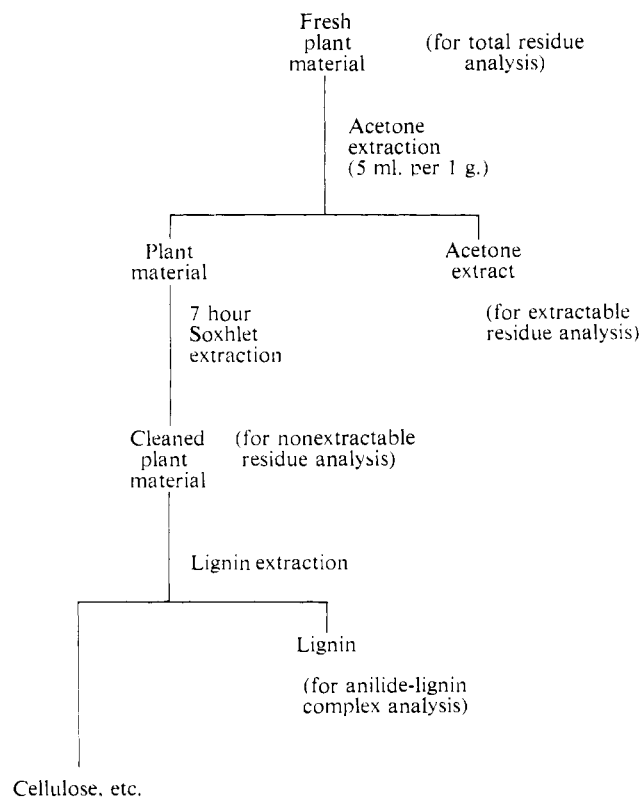Figure 1. Carboxin and its oxidation analogs

Figure 2. Scheme of residue analysis

Table II. Extractable Residues in Carboxin-Treated Barley and Wheat Plants

In Barley Plants

Residues Determined	Procedure Used	Residues Determined ppm				
		1st Week	2nd Week	4th Week	6th Week	Mature[a]
Total ext.	Color	28.3	17.3	6.3	1.3	0.0
Carboxin	MCGC	3.4	1.2	0.3	0.0	0.0
Sulfoxide	Tlc and color	19.0	15.0	6.6	0.5	0.0

In Wheat Plants

Residues Determined	Procedure Used	1st Week	2nd Week	4th Week	6th Week	Mature[a]
Total ext.	Color	27.9	28.1	6.0	1.4	0.0
Carboxin	MCGC	5.0	3.3	0.4	0.0	0.0
Sulfoxide	Tlc and color	22.6	26.1	6.8	2.1	0.0

[a] Dried 15 weeks old mature plant tissue with seeds.

rescein was sprayed, a clear spot corresponding to the sulfoxide could be seen under UV light, but the area corresponding to sulfone was obscured by interference from plant materials. The spot corresponding to the sulfoxide was eluted with benzene and was identical to the standard sulfoxide, as shown by tlc with several other solvents. The quantity of sulfoxide was determined colorimetrically after tlc separation as shown in Table II.

From the radioautogram of ^{14}C-carboxin-treated wheat plants, sulfone was also detected. By counting the relative radioactivity of these spots, as shown in Figure 3, it was found that the total extractable residue was composed of 90–92% sulfoxide and 8–10% sulfone. Only a trace of carboxin could be detected, even when the radioautogram was exposed for longer periods of time.

IDENTIFICATION OF ANILIDE-LIGNIN COMPLEX. Previous work (Chin et al., 1964) showed that anilide-lignin complexes were formed when Swep, Solan, or 3,4-dichloroaniline were applied to wheat, rice, corn, oat, and barnyardgrass plants. Based on this information, the possibility of lignin complex formation with carboxin-type chemical(s) in barley and wheat plants was investigated. Colorimetric determination of anilide residues in the lignin fraction are shown in Table III. A separate experiment with higher rates of carboxin was conducted and results are shown in Table IV.

DISCUSSION

In all cases, the amounts of residues determined in terms of ppm decreased significantly, starting from the first week.

Table III. Residues in Anilide-Lignin Fraction of Barley and Wheat Plants

Interval	Residues Determined ppm	μg	Total Residue in μg (Table I)	% of Total Found in Lignin Fraction
Barley				
1st week	4.0[a]	0.1[a]	1400[a]	0.0[a]
3rd week	75.0[a]	29.3[a]	2145[a]	1.4[a]
5th week	3.7	3.2	1288	0.3
7th week	9.1	30.9	452	6.8
Wheat				
1st week	0.0[a]	0.0[a]	720[a]	0.0[a]
3rd week	5.0[a]	1.8[a]	2080[a]	0.9[a]
5th week	0.6	1.0	1540	0.7
7th week	8.6	36.0	476	7.6

[a] Sample size inadequate for reliable analysis.

Figure 3. Counting of relative radioactivity

Table IV. Comparison of Anilide-Lignin Residue at Three Different Rates

Anilide-lignin residue ppm

Plant	Normal rate[a]	High rate[b]	Soil treatment[c]
Barley	9.1	37.6	731
Wheat	8.6	15.6	503

[a] 85 g active carboxin/45 kg (3 oz/100 lb) seed; 7th week data of Table III. [b] 170 g active carboxin/45 kg (6 oz /100 lb) seed; harvested 7 weeks after emergence; grown in the greenhouse; injured. [c] 2000 ppm (calculated on soil weight) soil treatment; harvested 2 weeks after emergence; grown in the greenhouse; seriously injured.

This was considered mainly due to dilution as the plants grew bigger.

The highest total absolute weights of residues in terms of μg per flat occurred at the third week for both barley and wheat. From then on, the amounts decreased rapidly. The low residue at 1 week may be due to inadequate time for root absorption and translocation upward. The smaller amounts found per flat at 5 and 7 weeks is because of the decrease in number of seeds planted per flat for the longer time studies, which also decreases the amount of carboxin available.

Repeated preliminary experiments revealed that it would take only about 10 weeks for both spring barley and spring wheat to grow from emergence to maturity. This is about 4 weeks shorter than the time required under field conditions. Barley and wheat grown to maturity in the field contained much less total residue than similarly treated crops grown in the greenhouse. These exaggerated residues in greenhouse treatment make it possible to see the degradation pathway more clearly.

Since amounts of carboxin applied were different for different time intervals, inference could not be drawn by comparing the absolute μg at different time intervals. However, if taking the total absolute μg of residue per flat as 100% and comparing the percentage change of extractable and nonextractable residues as shown in Table I, the following phenomena were seen. As plants approached maturity, the total percentage of the extractable residues decreased and the percentage of nonextractable residues increased. At maturity, no extractable residue could be detected.

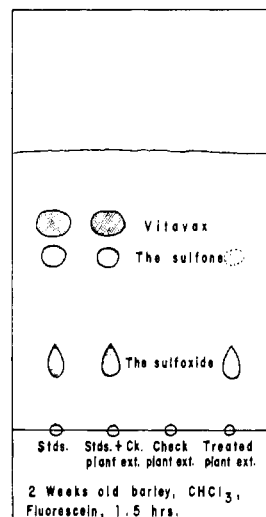Quantitative analysis of the extractable residues shown in

Table II indicated that carboxin itself represented only minor quantities of the total extractable residues in both cases. It was found that no carboxin could be detected 6 weeks after emergence. The disappearance of carboxin could be due to its conversion to the sulfoxide, sulfone, or to the nonextractable form.

The dominant extractable residue was the sulfoxide. Since carboxin can be oxidized to the sulfoxide readily in the soil (Chin et al., 1970), the sulfoxide found in the plants may be derived from two sources: oxidation of carboxin in the soil and absorption of the sulfoxide; and absorption of carboxin from soil and oxidation within the plants.

The possibility of direct absorption of the sulfoxide from soil by barley and wheat plants was confirmed. Barley and wheat seeds were treated with 84 g of the sulfoxide (112 g formulated 75% wettable powder) per 45.4 kg seeds. Plants grown from these seeds and harvested one week after emergence showed 26 ppm extractable residue (colorimetric text) which was identified as the sulfoxide by tlc.

The possibility of oxidation of carboxin within plants to form the sulfoxide was also established. Barley and wheat seeds were water cultured for 1 week. To these seedlings, a water culture containing 200 ppm of carboxin was introduced on the eighth day. Plants were harvested 9 days later and analyzed for total anilide residue (color), carboxin (MCGC), and the sulfoxide (tlc-color) analyses.

Analyses of the carboxin-water cultured wheat and barley plants showed that 60 ppm of the sulfoxide and only 3.6 ppm of carboxin were present. Since during the culturing period no significant oxidation of carboxin to the sulfoxide was observed by tlc in the water solution, carboxin was believed to be the only anilide absorbed by plants. The great amounts of the sulfoxide determined in the plants indicated that rapid oxidation of carboxin within the plants did take place.

Examining the quantities of insoluble anilide-complex in terms of percentage of the total residue, in both barley and wheat plants the percentage tended to increase as plants approached maturity. Previous experience indicated that anilide-lignin complex formation would be more significant for higher rates of application. This was confirmed by results shown in Table IV. All this information suggests that in the carboxin-treated barley and wheat plants, the anilide-lignin complexes do form as the plant grows. Considering the rapid oxidation of carboxin to the sulfoxide within the plants, the

anilide moiety of the lignin complex may be mainly the sulf-oxide rather than carboxin itself. As mentioned in a previous paper (Chin et al., 1964), the mechanism of the anilide-lignin complex formation is not known but was considered to be a possible detoxication mechanism by plants through immobilization.

ACKNOWLEDGMENT

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