can be established governing the persistence and apparent biological resistance of phenoxyalkyl carboxylic acid herbicides and related chlorophenols or of their respective decomposition products. Certain structural characteristics apparently govern the persistence of aromatic molecules, and these may serve as a means of predicting the relative persistence of such phytotoxic compounds in soil. The present study suggests three hypotheses: (1) The aromatic nucleus of halogenated phenoxyalkyl carboxylic acids and phenols remains intact for long periods in compounds containing the halogen in a position meta to the phenolic hydroxyl. Should such compounds undergo polymerization, it is possible that this change may mask the decomposition. (2) With the exception of 2-CPA, ω-substituted phenoxyalkyl carboxylic acids are readily attacked and degraded by the soil microflora, provided condition 1 does not apply. (3) The decomposition of α-substituted phenoxyalkyl carboxylic acids to the stage of rupture of the aromatic nucleus is dependent upon the meta halogen as well as the length of the aliphatic acid, cleavage being rapid for acetate and caproate but not for propionate and valerate.

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Literature Cited

(1) Bell, G. R., *Can. J. Microbiol.* **3,** 821 (1957).
(2) Dorschner, K. P., Buchholtz, K. P., *Weeds* **5,** 102 (1957).
(3) Steenson, T. I., Walker, N., *J. Gen. Microbiol.* **16,** 146 (1957).
(4) Walker, N., *Plant and Soil* **5,** 194 (1954).
(5) Walker, R. L., Newman, A. S., *Appl. Microbiol.* **4,** 201 (1956).
(6) Webley, D. M., Duff, R. B., Farmer, V. C., *J. Gen. Microbiol.* **18,** 733 (1958).
(7) Whiteside, J. S., Alexander, M., *Weeds,* **8,** 204 (1960).

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HERBICIDE RESIDUES

Decline and Residue Studies on 4-Chloro-2-butynyl *N*-(3-Chlorophenyl)carbamate

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Interest in the use of 4-chloro-2-butynyl *N*-(3-chlorophenyl) carbamate (barban) as a post-emergence herbicide for the control of wild oats has created a need for decline and residue studies. This chemical declines rapidly on wheat, barley, flax, peas, and sugar beets; final barban residues are below 0.01 p.p.m. A method has been provided for determining barban residues on these crops.

A NEW SELECTIVE POST-EMERGENCE HERBICIDE, 4 - chloro - 2 - butynyl *N* - (3 - chlorophenyl)carbamate (barban), has been evaluated in the United States, Canada, and Europe during the past two years (*9, 10*). This chemical has shown promise for the control of wild oats (*Avena fatua*) in the presence of wheat, barley, peas, flax, safflower, rapeseed, sunflower, and sugar beets (*7, 8*). Decline and residue studies have been run on wheat, barley, flax, peas, and sugar beets, using an analytical method based on the colorimetric determination of 3-chloroaniline. This is a modification of the method of Bratton *et al.* (*4*) and Werner (*12*), and is similar to those methods used for the determination of monuron (*3*), acetanilide (*5*), parathion (*1*), and certain sulfa drugs (*6*).

Treatment of Crops

The crops were treated with an emulsifiable concentrate containing 1 pound of barban per gallon in a heavy aromatic oil with an emulsifier and a corrosion inhibitor. The recommended method for wild oat control calls for dilution of 1 gallon of concentrate with 5 to 10 gallons of water and the application of this emulsion at rates of 0.5 pound per acre on wheat, barley, flax, and peas, and at 1 pound per acre on sugar beets. The decline studies on peas, flax, and sugar beets were run at these recommended rates, while wheat and barley were treated at twice their recommended dosage. Preliminary decline studies had revealed that the latter crops could easily tolerate the 1-pound-per-acre dosage; hence more stringent rates were assigned to them in the present studies.

The crops were treated when the majority of the wild oats were in the two-leaf stage. At this date wheat and barley will be only slightly more advanced in growth, flax will be at the eight- to ten-leaf stage, peas at the four- to six-leaf stage, and sugar beets at the two-leaf stage. Peas and flax may respond to this treatment with a very slight leaf burn, while the other crops are not affected. In one series of decline studies, the herbicide was applied at too late a date to obtain control of wild oats, thus simulating a possible error on the potential user's part. In this treatment the wheat and barley were near the "boot" stage, flax and peas were flowering, and the sugar beets had reached the six- to eight-leaf stage. All residue studies were run in triplicated experiments using various rates and spray volumes. Three emulsifiable concentrates, differing only in the solvent and emulsifier brands, were evaluated. Rates as high as 2 pounds of barban per acre were used on barley and wheat, 1 pound per acre on flax and peas, and 4 pounds per acre on sugar beets, a very tolerant crop.

Samples for the decline studies were taken by cutting the grain crops, flax, and peas at ground level, and harvesting the entire sugar beet plant. These samples were placed immediately in polyethylene bags, frozen with dry ice, and shipped frozen to a cold locker for safekeeping until analyzed. The mature food portion of the crop was used in residue studies. The ripe grain crops were harvested at ground level and threshed, and the seed was cleaned and stored. Peas were harvested in their pods, bagged, and held frozen until analyzed. Sugar beets were collected, "crowned" to remove their tops, bagged, and stored.

Analytical Procedure

Samples of the green tissue are chopped into small pieces, weighed, and ground in a Waring Blendor for 3 to 5 minutes with sufficient ethylene dichloride to form a thin paste. The homogenate is filtered through a glass wool plug in a funnel; the tissue is reprocessed in the Blendor with fresh ethylene dichloride, filtered, and washed twice with fresh

ethylene dichloride, with the extracts and washes kept separate. When this procedure is used with mature sugar beets and green peas, an emulsion is formed with ethylene dichloride, necessitating a change in the solvent system to isopropyl alcohol or acetonitrile. The slurry is then filtered through cheesecloth and processed as described above.

Samples of grain are homogenized with ethylene dichloride in the Waring Blendor, the solvent is removed in a basket-head centrifuge at 2000 to 2500 r.p.m., and the solids are washed twice with fresh ethylene dichloride.

Extraction studies of barban added to plant tissues just prior to homogenization show recoveries of 68 to 102%. These studies were run using green tissue from 3- to 4-week-old field-grown plants and the final harvest samples. These results are presented in Table I.

The extracts are freed from interfering substances by treatment on a column consisting of a mixture of 1 part of Celite, 2 parts of anhydrous sodium sulfate, and 2 parts of Attaclay, by weight (*11*). The extracts, followed by the first wash, second wash, and fresh ethylene dichloride, are passed through the columns containing 20 grams of the mixture per 100 ml. of extract. A 30 × 500 mm. column is used for large samples (20 to 200 grams) and a 20 × 300 mm. column for smaller samples. The mixture is packed in the column with firm tamping and is supported by a 1-inch layer of sea sand on glass wool. From 3 to 5 p.s.i. of air pressure may be used, but care must be taken to prevent the column from running dry, as shrinkage will occur, ruining subsequent column washing.

The column effluents are combined and evaporated in a 300-ml. flask at 30° to 50° C. with the aid of a gentle stream of filtered air. To all flasks, except those containing flax residues, is added 100 ml. of 10% aqueous sodium hydroxide and the flask's contents are refluxed for 1 hour under a condenser. Flax residues, being oily, require 20% sodium hydroxide and 2 hours of reflux time. The condenser is then rinsed thoroughly into the flask and a one-piece distillation head–condenser is placed on the flask. The 3-chloroaniline resulting from the hydrolysis of barban is then steam-distilled into a 50-ml. volumetric flask containing 5 ml. of 1*N* hydrochloric acid until a total volume of about 40 ml. is obtained.

One milliliter of a 1% potassium nitrite solution is added to the volumetric flask, mixed well, and allowed to stand for 10 minutes at ambient temperature. One milliliter of 2% ammonium sulfamate is then added to destroy the excess nitrous acid. After 3 minutes, 1 ml. of a 0.1% solution of *N* - (1 - naphthyl) - ethylenediamine dihydrochloride (recrystallized twice from 6*N* hydrochloric acid) is added. After 20 minutes, 5 ml. of 6*N* hydrochloric acid is added, the flasks are brought to volume, and the absorbance at 550 mμ is measured.

The curve of concentration *vs.* absorbance (read on a Beckman Model DU spectrophotometer using a 1-cm. cell) for barban is presented in Figure 1.

When extracts of older plants are analyzed, interfering dyes may result from *o*-aminoacetophenone formed by the alkaline hydrolysis of tryptophan (*2*). These dyes may be removed by chromatography on a cellulose column using a 1 to 15 (v./v.) mixture of glacial acetic acid and 1*N* hydrochloric acid as the developer. This solvent mixture was more suitable than 1*N* hydrochloric acid alone.

A water slurry of Whatman standard cellulose powder is used to prepare a 10 × 360 mm. chromatographic column. The dyes to be separated are placed on this column in 1*N* hydrochloric acid. The addition of 100 to 125 ml. of a 1 to 15 (v./v.) mixture of glacial acetic acid and 1*N* hydrochloric acid elutes the dye formed by the *o*-aminoacetophenone, while the dye from 3-chloroaniline moves only about 1/8 the length of the column. A 1 to 1 (v./v.) mixture of glacial acetic acid and 1*N* hydrochloric acid quantitatively removes the 3-chloroaniline dye. The dye is collected in a 50-ml. volumetric flask, and brought to volume with glacial acetic acid, and the absorbance is measured

Table I. Barban Recovery Studies

	Green Plants[a]				Mature Food Portion[b]			
	Barban Added		Barban found,	Recovery,	Barban Added		Barban found,	Recovery,
Crop	μg.	P.p.m.	μg.	%	μg.	P.p.m.	μg.	%
Barley	0	0.00	0	...	0	0.00	0	...
	10	0.25	10	100	20	0.10	15.5	78
	20	0.50	17	85	40	0.20	31	78
	40	1.0	35	88	60	0.30	47	78
	80	2.0	75	94	80	0.40	73.5	92
					100	0.50	89	89
Flax	0	0.00	0	...	0	0.00	1	...
	10	0.25	10	100	20	0.20	18.5	88
	20	0.50	20	100	40	0.40	32	78
	40	1.0	27.5	69	60	0.60	53	87
	80	2.0	62	78	80	0.80	67	83
Peas	0	0.00	0	...	0	0.00	0	...
	10	0.25	10	100	20	0.10	20	100
	40	1.0	41	102	40	0.20	38	95
	80	2.0	69	87	60	0.30	46	77
					80	0.40	60	75
Sugar beets	0	0.00	0	...	0	0.00	0	...
	10	0.25	8	80	20	0.10	18.5	93
	20	0.50	17	85	40	0.20	34	85
	40	1.0	34.5	86	60	0.30	43.5	89
	80	2.0	74	93	80	0.40	63	79
Wheat	0	0.00	0	...	0	0.00	0	...
	10	0.25	8	80	20	0.10	18.5	93
	20	0.50	20	100	40	0.20	34	85
	40	1.0	31	78	60	0.30	55	92
	80	2.0	54	68	80	0.40	71	89
					100	0.50	74	74

[a] All crops, 40-gram samples.
[b] Barley, peas, sugar beets, and wheat, 200-gram samples; flax, 100-gram samples.

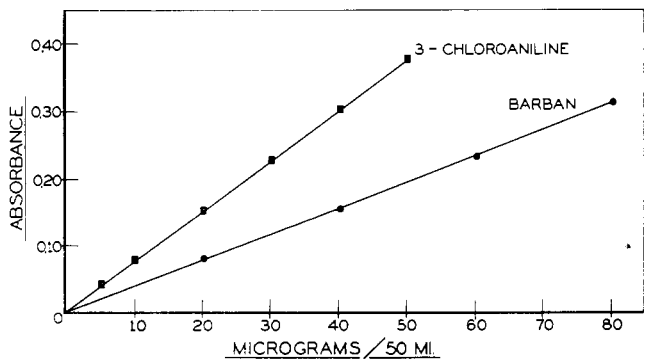

Figure 1. Standard curves

Table II. Barban Decline Studies

Days after Spraying	Barban, P.P.M. Study A Barley	Flax	Peas	Sugar beets	Wheat	Study B Barley	Flax	Peas	Sugar beets	Wheat
0	138.0	22.4	52.3	32.3	75.0	51.0	8.1	32.0	31.0	59.0
1	31.5	1.80	24.0	12.8	40.0	13.5	4.1	8.0	3.5	18.0
2	15.0	1.30	12.0	4.3	13.0	8.7	2.3	9.7	2.4	8.0
3	10.0	1.20	11.0	3.0	12.0	1.8	2.7	4.5	2.1	7.8
4	6.0	0.85	6.4	0.80	4.0	4.3	2.6	5.8	0.9	3.5
6	2.4	0.45	2.8	1.1	3.0	1.7	2.4	1.9	1.0	1.8
9	1.2	0.25	1.2	0.30	0.50	1.3	1.0	1.9	0.40	0.30
13	0.20	0.09	0.20	0.10	0.20	0.40	0.51	0.60	0.50	0.40
16	0.05	0.06	0.20	0.03	0.07	0.30	0.55	0.08	0.45	0.26
20	0.02	0.04	0.05	0.00	0.01	0.14	0.21	0.20	0.31	0.40
28	0.00	0.01	0.01	0.00	0.00	0.14	0.20	0.02	0.14	0.30
34	0.00	0.00	0.02	0.05	0.00	0.10	0.10	0.04	0.04	0.00
48	0.00	0.00	0.04	0.00	0.00	...	0.00	...	0.01	...
55	0.00	0.00	0.01	0.01	0.00	...	...	...	0.00	...
62	0.00	0.00	0.00	0.00	0.00	...	...	...	0.00	...
Harvest	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00

at 550 mμ. The acetic acid lends stability to the system, the colors being unchanged for at least 3 days.

The sensitivity of the analytical method is dependent on the background encountered in the procedure. It was found that the absorbance readings of the color-developing reagents were identical to values obtained with distilled water. When the untreated green plants used as controls were analyzed, absorbances of 0.004 to 0.010 were obtained. Harvest samples of grain from untreated plants gave somewhat lower readings of 0.000 to 0.008. The quantities of tissue used, from 10 to 200 grams, had no effect on this background. These readings represent an apparent background of 0 to 2 μg. of barban. A sensitivity of twice the background appears to be valid and would, therefore, be 0.01 to 0.02 p.p.m. of barban for a 100- to 200-gram sample of plant material.

Results and Discussions

The decline studies with barban on barley, flax, peas, sugar beets, and wheat show a rapid disappearance of the active ingredient even under the adverse conditions of overdosage and late application. The results of the decline studies are shown in Table II. In study A, the crops were sprayed at the recommended time and within 2 weeks had lost over 99% of the applied material. Four weeks after application the barban residues in these crops were 0.01 p.p.m. or less. In study B, the crops were sprayed about 4 weeks after the recommended time. The initial decline of barban was rapid; however, 4 weeks after spraying the barban residues were slightly higher than those encountered in crops sprayed at the recommended times.

In the late spraying of the plants, more of the barban was deposited on older plant tissues whose nature could prevent its penetration into the plant. Thus, a higher level of barban would appear in the studies; however, this barban would not be able to move into the food portion of the plant. The final barban values of studies A and B were 0.01 p.p.m. or less.

A 200-gram sample of the mature food crop was used in determining barban residues in barley, peas, sugar beets, and wheat, and a 100-gram sample for flax. Variations in the formulation used or in the spray volume applied as described in the section headed Treatment of Crops had no effect upon the barban residues. In all cases the barban residues found in these crops were below 0.01 p.p.m. of barban, which equals the sensitivity of the analytical method. This was true even when rates fourfold greater than the recommended ones were used.

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Literature Cited

(1) Averell, P. R., Norris, M. V., *Anal. Chem.* **20,** 753 (1948).
(2) Bleidner, W. E., J. Agr. Food Chem. **2,** 682 (1954).
(3) Bleidner, W. E., Baker, H. M., Levitsky, M., Lowen, W. K., *Ibid.*, **2,** 476 (1954).
(4) Bratton, A. C., Marshall, E. K., Babbitt, D., Hendrickson, A. R., *J. Biol. Chem.* **128,** 537 (1939).
(5) Brodie, B. B., Axelrod, J., *J. Pharmacol. Exptl. Therap.* **94,** 22 (1948).
(6) Eckert, H. W., *J. Biol. Chem.* **148,** 197 (1943).
(7) Hoffmann, O. L., Hopkins, T. R., Pullen, J. W., "Wild Oat Control with 4-Chloro-2-butynyl *N*-(3-Chlorophenyl)-Carbamate," Proceedings of 12th Annual Meeting, Western Section, National Weed Committee (Canada), pp. 3–6, 1959; North Central Weed Control Conference, Cincinnati, Dec. 9, 1958.
(8) Hopkins, T. R., Hoffmann, O. L., Pullen, J. W., "Wild Oats, A New Chemical Control," Western Weed Conference, Salt Lake City, Utah, March 19, 1959.
(9) Hopkins, T. R., Pullen, J. W., U. S. Patent **2,906,614** (Sept. 29, 1959).
(10) Hopkins, T. R., Strickler, P. D., Neighbors, R. P., Phillips, L. V., *J. Org. Chem.* **24,** 2040 (1959).
(11) Lane, J. R., J. Agr. Food Chem. **6,** 668 (1958).
(12) Werner, A. E. A., *Lancet* **1,** 18 (1939).

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