Degradation of Alachlor by a Soil Fungus, *Chaetomium globosum*

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Degradation of alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide] by a common soil fungus, *Chaetomium globosum*, produced chloride and four identifiable organic metabolites: 2-chloro-2′,6′-diethylacetanilide, 2,6-diethyl-*N*-(methoxymethyl)aniline, 2,6-diethylaniline, and 1-chloroacetyl-2,3-dihydro-7-ethylindole. Incubation of *C. globosum* with 2-chloro-2′,6′-diethylacetanilide, 2,6-diethylaniline, and monochloroacetic acid showed further degradation of these products. Six other soil fungi studied were unable to effectively degrade alachlor.

Alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide] is a preemergence herbicide which has become popular for weed control in corn and soybeans. Information from soil incubation studies suggests that alachlor is biodegraded relatively rapidly in soils (Beestman and Deming, 1974; Chou and Tiedje, 1973) but that very little ring-labeled [^{14}C]alachlor is converted to $^{14}CO_2$ (Chou and Tiedje, 1973). The majority of the radioactivity could be recovered from soil only after alkaline hydrolysis, suggesting that herbicide metabolites were bound to soil organic matter.

Studies on alachlor metabolism in barley (Hamill and Penner, 1973), in yellow nutsedge (Armstrong *et al.*, 1973), and by a soil fungus, *Fusarium oxysporum* (Kaufman and Blake, 1973), have shown alachlor to be degraded but in no case have metabolites been identified. Hargrove and Merkle (1971) reported that 2-chloro-2′,6′-diethylacetanilide was formed in alachlor-treated air-dried soils incubated at 46° but showed this product to result from acid-catalyzed hydrolysis of alachlor on mineral surfaces.

This investigation was initiated to identify metabolites of alachlor and to elucidate the pathway of alachlor degradation. The soil fungus, *Chaetomium globosum*, was chosen for the studies since Taylor (1972) had shown that this organism rapidly metabolized ring-labeled [^{14}C]alachlor without producing $^{14}CO_2$. In addition, Kaufman and Blake (1973) found *F. oxysporum* released some chloride from alachlor but did not produce aniline intermediates. This organism quantitatively released chloride from a related herbicide, propachlor (2-chloro-*N*-isopropylacetanilide), and accumulated 2-hydroxy-*N*-isopropylacetanilide as the major metabolite (Kaufman *et al.*, 1971).

In this paper we report on the identification of four alachlor metabolites produced by the soil fungus, *C. globosum*, and suggest pathways of degradation.

APPARATUS AND REAGENTS

Instrumental Analyses. Gas–liquid chromatography (glc) was done on a Perkin-Elmer Model 900 dual-column gas chromatograph equipped with flame ionization and thermal conductivity detectors. Operating temperatures were: detector and inlet, 220°; column, in the range of 160–200°. Carrier gas flow rate and column temperature were adjusted for optimal resolution of all sample components. The columns used were the following: (A) 6 ft × ⅛ in., 5% SE-30 on 60–80 mesh Chromosorb W; (B) 6 ft × ⅛ in., 4% SE-30, 6% QF-1 on 60–80 mesh Chromosorb W; and (C) 6 ft × 0.25 in., 10% SE-30 on 60–80 mesh Chromosorb W. All column supports were acid and base washed and silanized. Triphenylmethane was used as the internal standard for all glc quantitative analyses.

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Mass spectra were obtained on an Hitachi RMU-6 instrument with an ionizing voltage of 70 eV and on an LKB 9000 gas chromatograph–mass spectrometer with an ionizing voltage of 70 eV. The glass chromatography column for the gc–mass spectrometry unit was 4 ft × ⅛ in., 3% SE-30 on 80–100 mesh Chromosorb W, and was programmed at 2°/min from 160 to 210°.

Proton nmr spectra were run on a Varian T60 instrument and obtained using dilute solutions in carbon tetrachloride or deuteriochloroform.

Infrared spectra (ir) were measured on a Perkin-Elmer 137 spectrometer; absorptions are reported as frequencies (in cm^{-1}) and are referenced to the 1602-cm^{-1} peak of polystyrene. Liquid samples were examined as neat films and solids as Nujol mulls.

Melting points were obtained in open capillaries in a Thomas-Hoover melting point apparatus and are uncorrected. Colorimetric analyses for chloride were performed with a Bausch and Lomb Spectronic 20.

All radioactivity was assayed by liquid scintillation spectrometry in a Packard Tri-Carb scintillation spectrometer, Model 3310. Aqueous samples were counted in Bray's solution (Bray, 1960); organic extracts were counted in Spectrafluor (Amersham/Searle Corp.). All counts were corrected for quenching by external standardization.

Reagents. Alachlor was obtained from City Chemical Co. The dark, greasy product was purified as follows. Alachlor, 5 g, was dissolved in 150 ml of methanol and water was added slowly until the solution remained cloudy on swirling. The aqueous methanolic solution was then extracted with three 200-ml portions of hexane. The hexane extract was dried over Na_2SO_4 and evaporated *in vacuo* to approximately 200 ml total volume. The solution was placed in a −10° freezer overnight. The crystalline material was removed by suction filtration, washed with cold hexane, and allowed to dry at room temperature. The colorless plates (mp 42–43°) were 99.9% pure by glc (column A at 150°, column B at 180°). The ir showed tertiary amide absorption at 1694 cm^{-1}; nmr δ (CCl_4) 1.27 (t, 6 H, J = 7 Hz), 2.62 (q, 4 H, J = 7 Hz), 3.50 (s, 1 H), 3.58 (s, 1 H), 4.92 (s, 3 H), 7.27 (m, 3 H); mass spectra m/e (% base peak) M^+, 269 (28), 237 (65), 224 (67), 188 (100), 160 (97).

Uniformly ^{14}C-ring-labeled alachlor (1.02 mCi/mmol) was supplied by Monsanto Chemical Co., St. Louis, Mo. The label purity was determined to be 99.7% by thin-layer chromatography.

2-Chloro-2′,6′-diethylacetanilide (demethoxymethylalachlor) was prepared by hydrolysis of alachlor in 5 *N* HCl according to the procedure of Hargrove and Merkle (1971). The crude product, which precipitated from acetone solution on standing at room temperature, was filtered, washed with water, and allowed to dry at room temperature. Recrystallization in acetone–water produced white crystalline material (mp 134.0–134.5°) in a 60% yield. The product was 99.9% pure by glc (column A at 150°, column B at 180°). The spectral evidence is in ac-

cord with the desired compound. The ir showed secondary amide absorptions at 3246, 1689, and 1639 cm^{-1}; nmr δ ($CDCl_3$) 1.16 (t, 6 H, J = 8 Hz), 2.50 (q, 4 H, J = 8 Hz), 4.10 (s, 2 H), 7.10 (m, 3 H), 7.80 (br s, 1 H); mass spectra m/e (% base peak) M^+, 225 (17), 176 (100), 147 (61), and 132 (36).

2,6-Diethylaniline, provided by Ethyl Corporation, was further purified by distillation through a short Vigreux column (bp 125–129° (~22 mm)). The ir showed an amine absorption at 3448 cm^{-1}; nmr δ (CCl_4) 1.3 (t, 6 H, J = 8 Hz), 2.42 (q, 4 H, J = 8 Hz), 3.37 (br s, 2 H), 6.4–7.0 (m, 3 H); mass spectra m/e (% base peak) M^+, 149 (3), 148 (100). Due to rapid discoloration, the collected material was dissolved in acetone, precipitated as the hydrochloride, filtered, washed with acetone, and air dried.

2′,6′-Diethylacetanilide was prepared from 2,6-diethylaniline hydrochloride by derivatization with acetic anhydride as described in the analysis section of this paper. White plates (mp 130–131°) were crystallized from ethanol–water. Spectral data are as follows: ir absorptions at 3246, 1689, and 1663 cm^{-1}; nmr δ ($CDCl_3$) 1.14 (t, 6 H, J = 3.5 Hz), 1.92 (s, 3 H), 2.46 (q, 4 H, J = 3.5 Hz) 7.08 (m, 3 H), 8.10 (br s, 1 H); mass spectra m/e (% base peak) M^+, 191 (40), 148 (98), 134 (100).

Monochloroacetic acid was obtained from Fisher Scientific Co. and was used without further purification. Freon 113 (1,1,2-trichlorotrifluoroethane) was obtained from Matheson Gas Products. Other reagents and solvents were reagent grade materials and used as such.

PROCEDURES

Cultural. Pure cultures of *Chaetomium globosum, C. bostrychodes, Fusarium roseum,* and species of *Penicillium, Phoma, Alternaria, Paecilomyces,* and *Trichoderma* were obtained from W. G. Fields, Department of Botany and Plant Pathology, Michigan State University. The cultures were maintained by aseptic transfer on potato dextrose agar. The fungi were grown on 300 ml of potato dextrose broth (Difco Laboratories) in erlenmeyer flasks. Conidia, mycelial fragments, or ascospores (in the case of *C. globosum*) were used as inocula. The flasks were incubated on a rotary shaker (to produce mycelial pellets) or stationary (to produce surface mycelial mats) for 5 days at 28°; the biomass yields were greater when grown on the rotary shaker.

The pellets of fungal mycelia were removed from the flasks by aseptic suction filtration, washed with five 50-ml portions of sterile, deionized water to remove inorganic chloride, and transferred to sterile 500-ml erlenmeyer flasks. The mycelium was resuspended in 300 ml of a filter sterilized solution of 0.37 mM ^{14}C-ring-labeled alachlor (1.12 μCi/l.) in 0.02 M KH_2PO_4 buffer, pH 5.0. In one experiment potato dextrose broth (8 g/l.), pH 5.1, was substituted for phosphate buffer. Control flasks contained mycelium and buffer (or broth) without alachlor and alachlor in buffer without mycelium (sterile control). Similarly, demethoxymethylalachlor, 2,6-diethylaniline, and monochloroacetate, each dissolved in 0.02 M phosphate buffer, pH 5.0, were incubated with *C. globosum;* the substrate concentrations were 0.37 mM except for demethoxymethylalachlor which was 0.27 mM because of its more limited solubility. All flasks were incubated on a rotary shaker at 28°. Thirty-milliliter aliquots were aseptically withdrawn from each flask at the indicated time intervals. All data, except those reported in Table I, are averages from duplicate flasks.

Analyses. Each 30-ml aliquot was apportioned and analyzed as follows: 1 ml was analyzed for total ^{14}C content, 6 ml was analyzed for inorganic chloride according to the method of Bergman and Sanik (1957), and 20 ml was analyzed for substrate and metabolites. The organic layers from each extraction were combined and dried over anhydrous Na_2SO_4; 0.4 ml of the organic layer was analyzed for ^{14}C content and the remainder was used for glc analysis.

Table I. Degradation of Alachlor by Several Soil Fungi

Fungus	Alachlor remaining, % of original: 0 time	2 days	4 days	7 days	Final biomass, mg/flask
Chaetomium globosum	101	18	0	0	201
Chaetomium bostrychodes	100	92	79	82	102
Paeciliomyces	100	62	55	38	310
Phoma	102	100	100	89	131
Alternaria	100	97	98	96	276
Fusarium roseum	100	97	95	100	209
Trichoderma	100	102	101	100	390
Penicillium	100	100	101	100	211

For samples in which 2,6-diethylaniline was quantitated, the aniline (and other amines or alcohols present) was derivatized as follows: 1 ml of acetic anhydride was added to 20 ml of aqueous sample containing up to approximately 10 mmol of amine or alcohol. The solutions were swirled until the acetic anhydride had dissolved, then 1 g of $NaOAc \cdot 3H_2O$ in 2 ml of water was added. The solutions were stoppered and allowed to sit for 30 min at room temperature. The conversion of 2,6-diethylaniline to 2′,6′-diethylacetanilide was quantitative.

Alachlor, demethoxymethylalachlor, and 2,6-diethylaniline (as the acetanilide) were quantitated by gas chromatography using column B, 180°, and a flame ionization detector. All of the organic fractions were subsequently combined, concentrated, and the individual components collected by preparative glc using column C, 180°, and a thermal conductivity detector. The aqueous layers remaining after the initial Freon extraction were combined and continuously extracted for 5 days with Freon 113 to remove additional intermediates. The organic layer was removed, dried over anhydrous Na_2SO_4, and concentrated. The individual components from the concentrated continuous extract were also collected by preparative glc using column C, 180°, and a thermal conductivity detector. Spectral data were obtained on the individual components and structures determined where possible.

RESULTS AND DISCUSSION

Mycelial pellets of *C. globosum* from shaken flasks showed a rate of alachlor degradation that was approximately 30% faster than that of mycelial mats grown in stationary flasks. Therefore, mycelial pellets were used for subsequent experiments. When mats or pellets of mycelium were incubated with alachlor in either phosphate buffer or potato dextrose broth, there was no difference in degradation rate between media indicating that an energy source was not stimulatory to alachlor metabolism. Since phosphate buffer, in contrast to potato dextrose broth, contained no substances that interfered with glc and chloride analyses, it was the medium of choice for subsequent experiments.

The data obtained from incubation of several prevalent soil fungi with alachlor (Table I) show that *C. globosum* readily degrades this herbicide but that this capacity is not widely distributed among the soil fungi tested. A member of the same genus, *C. bostrychodes,* showed only minor metabolic activity on alachlor.

A quantitative analysis of products formed during alachlor degradation by *C. globosum* is shown in Table II. None of the ^{14}C label was released as CO_2 since all of the

Table II. Products Formed during Alachlor Degradation by *C. globosum*

Incubation time, hr	Per cent of total possible[a]						
	Alachlor remaining	Demethoxymethylalachlor formed	2,6-Diethylaniline formed	Chloride formed	^{14}C in org fraction	Unident. organochlorine compd(s)[b]	Unident. compd(s) in org fraction[c]
0	100	0	0	0	96	0	4
12	51.0	0	0	7.1	88.5	41.9	37.5
24	33.0	0	6.7	16.2	74.5	40.8	34.8
36	11.0	10	11.5	30.8	72.5	48.2	40.0
60	<5	10	13.7	68.0	52.5	22.0	26.8

[a] 112 μmol of alachlor and 326 mg of fungal mycelium per flask. [b] Determined from 100% − (alachlor + demethoxymethylalachlor + chloride). [c] Determined from (^{14}C in organic fraction) − (alachlor + demethoxymethylalachlor + diethylaniline).

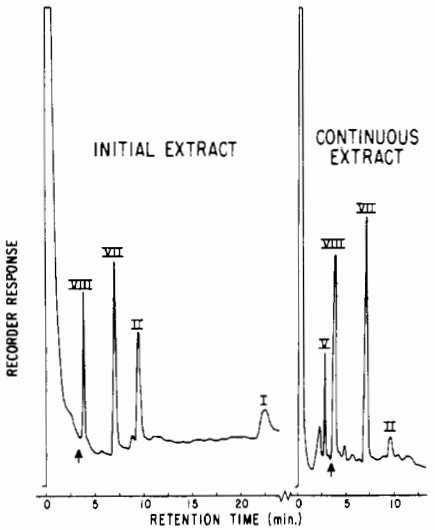

Figure 1. Glc traces of the underivatized initial extract and the continuous extract on column A, 150°. Peaks identified as follows: (I) alachlor; (II) demethoxymethylalachlor; (V) 2′,6′-diethyl-*N*-(methoxymethyl)aniline; (VII) 1-chloroacetyl-2,3-dihydro-7-ethylindole; and (VIII) unidentified. Arrows identify location of 2′,6′-diethylacetanilide peak in derivatized samples.

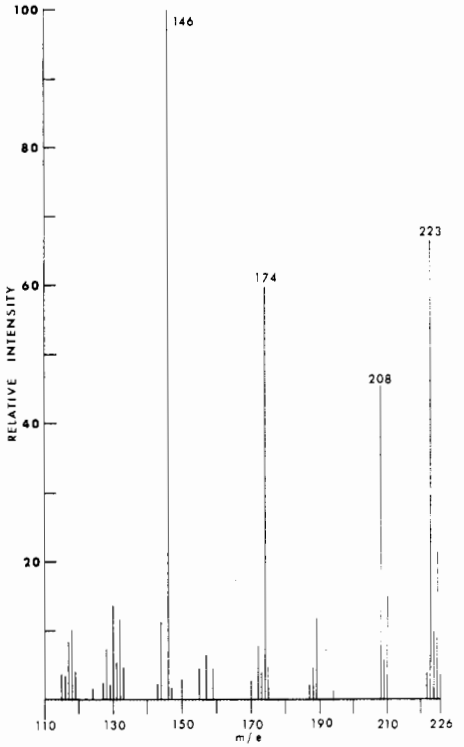

Figure 2. Mass spectrum of product VII, 1-chloroacetyl-2,3-dihydro-7-ethylindole.

label could be recovered. This is consistent with previous results of Taylor (1972) who showed that in a similar incubation system *C. globosum* converted none of the [^{14}C]alachlor to a product that could be trapped in NaOH. After 60 hr essentially no alachlor remained and 68% of the chlorine had been released as chloride. These data suggest that the majority of the alachlor residues were nonchloro aromatic moieties, since dissimulatory reactions subsequent to ring cleavage are common to many microorganisms and would have resulted in $^{14}CO_2$ production.

Glc analysis of the organic extracts showed four components that were probable alachlor products since they did not appear in extracts from the mycelium or from the sterile control. Two of the peaks were identified as demethoxymethylalachlor (Figure 1, peak II) and 2,6-diethylacetanilide (Figure 1, arrow) by coinjection of the sample with authentic material (column A at 150°, column B at 180°). These assignments were confirmed by isolation of the products from preparative glc and comparison of spectral properties of the collected material with authentic material. Since 2,6-diethylacetanilide was not observed in underivatized samples (Figure 1, arrow) 2,6-diethylaniline could be quantitatively correlated with the presence of 2,6-diethylacetanilide. The data in Table II indicate that demethoxymethylalachlor and 2,6-diethylaniline do not appear to be terminal products since they did not accumulate stoichiometrically.

A third organic-soluble metabolite (Figure 1, peak VII) was observed as a major component but it could not be quantitated because of the lack of a standard. This product gave the following ir maxima: (CCl_4) 1666, 1580, and 1510 cm^{-1}, and yielded the mass spectrum shown in Figure 2.

On the basis of the mass spectral data which show the presence of Cl, N, a molecular ion of 223, a single methyl group (208), and a chloroacetyl moiety (146), and the ir data which show aromatic absorptions at 1580 and 1510 cm^{-1}, structures VI and VII (Figure 3) could be drawn. The molecular weight (M^+), in conjunction with the isotope abundance data from the M + 1 and M + 2 peaks, was consistent with the formula $C_{12}H_{14}NOCl$ for VI and VII. The single carbonyl absorption at 1666 cm^{-1}, absence of N–H absorptions in the ir, absence of a C-4 fragment in the mass spectra, and the short retention time on 3% SE-30 (column A) relative to demethoxymethylalachlor (Figure 1) suggest that the metabolite was VII, a tertiary

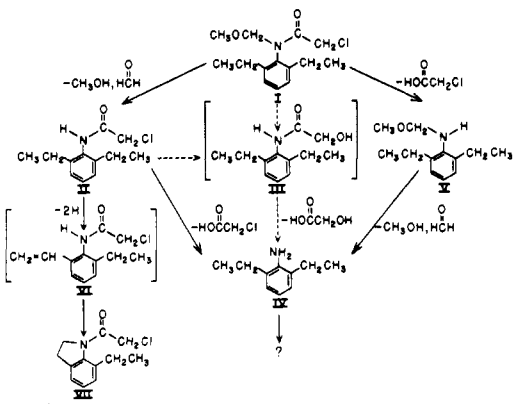

Figure 3. Scheme for alachlor degradation by *C. globosum*.

amide. Other indoline products have been reported for microorganisms (Laskin and Lechevalier, 1973).

Gas chromatographic analysis of the continuous extract showed one additional peak (Figure 1, peak V) which had the following gc–mass spectrometry and ir data: mass spectra *m/e* (% base peak) M^+, 193 (48%), 178 (19%), 148 (77%), 132 (100%), 117 (20%); ir 3226, 1580, and 1550 cm^{-1}. The ir showed an N–H absorption and various aromatic absorptions but no carbonyl absorption. The absence of a strong M − 1 peak, typical of primary aromatic amines, and an important loss of a C_2H_5O unit indicated that the metabolite was an N-substituted compound. Considering the substitution pattern in alachlor, V was assigned as 2,6-diethyl-*N*-(methoxymethyl)aniline.

The remaining peak (Figure 1, peak VIII), found in both extracts, was not identified but mass spectral evidence indicated that it did not contain chlorine.

From Table II one can see that the values for the unidentified components in the last two columns are similar. Since 1-chloro-2,3-dihydro-7-ethylindole contains chlorine and was extracted into the organic fraction, it could account for a significant portion if not all of this unidentified fraction. In any case, the decline in quantity in this fraction after 36 hr suggests further metabolism of this compound(s).

In an effort to elucidate the pathway of alachlor degradation, *C. globosum* was incubated with demethoxymethylalachlor, 2,6-diethylaniline, and chloroacetate in a manner identical with the alachlor incubation. Chloroacetate was included since degradation of alachlor and demethoxymethylalachlor could yield chloroacetate. The rate of chloride release from chloroacetate (Table III) was as fast as the rate of chloride release from alachlor (Table II), which is consistent with its being an intermediate. However, the rates of degradation and chloride release of demethoxymethylalachlor were slower than for alachlor suggesting this product may not be in the major pathway. The indoline intermediate was observed when demethoxymethylalachlor was the substrate; however, 2,6-diethylaniline was the major product.

2,6-Diethylaniline accumulated in relatively small amounts during alachlor degradation (Table II) probably due to its subsequent metabolism. This explanation is supported by data in Table III which show that when 2,6-diethylaniline was used as substrate, it was degraded at a moderate rate. No major products from aniline were detected in organic extracts by glc analysis. These findings are consistent with 2,6-diethylaniline being a major intermediate in the alachlor degradation pathway. The relatively large quantity of 2,6-diethylaniline formed during the rather slow degradation of demethoxymethylalachlor is unexpected. It may have been due to altered metabolism caused by high substrate levels which would not normally be encountered during alachlor metabolism.

Table III. Degradation of Several Possible Alachlor Intermediates and Products Formed by *C. globosum*

Incubation time, hr	Demethoxymethylalachlor substrate, % of total possible[a]: Demethoxymethylalachlor remaining	2,6-Diethylaniline formed	Chloride formed	2,6-Diethylaniline substrate,[b] % remaining	Chloroacetate substrate,[c] % chloride formed
0	100	0	0	100	0
12	92	2.3	0	80.0	7.1
24	92	6.0	0	66.5	24.3
36	77.4	20.7	14.5	61.3	52.6
60	60.5	25.7	31.8	41.7	68.5

[a] 80 μmol of demethoxymethylalachlor and 270 mg of mycelium per flask. [b] 112 μmol of 2,6-diethylaniline and 390 mg of mycelium per flask. [c] 112 μmol of chloroacetate and 349 mg of mycelium per flask.

On the basis of the products isolated, the degradation scheme shown in Figure 3 is proposed. The conversion of II → VII is unlikely as a one-step process. Although VI was not observed, oxidation *via* an intermediate such as VI is more reasonable because bond formation between a secondary amide and a carbon atom β to an aromatic ring is not favorable; it is more reasonable to envision oxidation of the ethyl group prior to cyclization to the indoline.

Although III was not observed, the facile conversion II → III → IV makes this a reasonable sequence. If II were part of the minor pathway, as the data suggest, III could also be generated from 2-hydroxy-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide which could be formed from an initial dechlorination of alachlor. A similar initial dechlorination step has been reported for 2-chloro-*N*-isopropylacetanilide (propachlor) metabolism in several plants (Lamoureux *et al.*, 1971) and by a soil fungus, *F. oxysporum* (Kaufman *et al.*, 1971). In the latter case, 2-hydroxy-*N*-isopropylacetanilide was identified as the major metabolite.

The data are consistent with a major pathway involving compound V (Figure 3) since 2,6-diethyl-*N*-(methoxymethyl)aniline (V) occurred in small quantities and chloroacetate, a possible prior product, was metabolized as rapidly as alachlor. The initial reaction would likely involve an aryl acylamidase similar to the one purified from a bacterium and active on several herbicides (Engelhardt *et al.*, 1973).

ACKNOWLEDGMENT

The authors thank S. F. Chou and Herb Manning for technical assistance with portions of the work. The authors gratefully acknowledge the Department of Chemistry, Charles C. Sweeley, Department of Biochemistry, and Matthew Zabik, Pesticide Research Center, all of Michigan State University, for the use of several instruments.

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Received for review April 29, 1974. Accepted September 13, 1974. Published as Michigan Agricultural Experiment Station Journal Article No 6781. This study was supported in part by Grant No. 8R01EP00801-06 from the Environmental Protection Agency and Regional Research Project NC-96.

Residues in Crops Irrigated with Water Containing Trichloroacetic Acid

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Ten field and vegetable and three fruit crops were irrigated with water that contained about 0.1 and 0.5 ppm of trichloroacetic acid (TCA). Grapes were treated at 0.17 and 1.39 ppm. TCA residues were not detected in peaches, sugar beets, wheat, or tomatoes. Minute amounts of TCA (0.01–0.04 ppm) were detected in alfalfa, corn, garden peas, potatoes, and watermelon treated at 0.5 ppm. Slightly higher residues of TCA, ranging from 0.13 to 0.43 ppm, were detected in field-bean pods and seed, and snapbean herbage treated at 0.5 ppm. Apples treated at 0.1 ppm contained 0.19 ppm of residue, and grapes contained 0.03 and 0.20 ppm when treated at lower and higher rates, respectively. Of the two crops that were sprinkler and furrow irrigated with TCA-treated water, the only difference was in pods of furrow-irrigated field-beans, which contained maximum TCA residues of 0.19 ppm, compared to pods of sprinkler-irrigated beans, which contained 0.03 ppm. At harvest, residues had disappeared from most crops, except apples, field-bean pods and seed, garden peas, and grapes, which contained 0.19, 0.19, 0.13, 0.02, and 0.20 ppm of TCA, respectively.

Under favorable moisture conditions, fall applications of the sodium salt of trichloroacetic acid (TCA) effectively retard the growth and reduce the stand of reed canarygrass (*Phalaris arundinacea* L.) on canal banks (Comes, 1970; Bruns, 1973). This grass may develop from above or from below the waterline; consequently, the swath of a TCA treatment may cover an area from 1 to 2 m above to about 1 m below the normal waterline. Because of minimal absorption of TCA by the mature vegetation and the likelihood of limited leaching and degradation of TCA during the winter, the authors were concerned with any significant contamination of the water, and consequently of irrigated crops, which would occur in the spring when water was introduced into the canals. To determine the significance of possible residues from fall applications of TCA on canal banks, Comes *et al.* (1972) monitored the first water used for priming the canals in the spring. During the first hour of flow, the average levels of TCA in the water of six canals were usually in the range of a few ppb up to 100 ppb. In one instance, the average level of TCA during the first hour of water flow was 142 ppb. Less than 1 ppb of TCA was detected in any of these canals 8 hr after water flow had commenced. While these concentrations of TCA found in the water were quite low, the possible uptake of residues by the crops was of concern. A study was initiated to determine the amount of TCA that might be taken up by crops from irrigation water and to evaluate the potential for damage.

METHODS AND MATERIALS

Field-Scale Treatments. The water in eight small laterals or head ditches was treated with the sodium salt of TCA to provide about 0.1 and 0.5 ppm of TCA. These treatments simulate 1 and 5 times the maximum concentration of this herbicide usually found in water turned into canals in the spring after an autumn ditchbank treatment with TCA at 82 kg/ha. The duration of each treatment was about 2 hr. Duplicate water samples were taken for analysis every 20 min, after the water and TCA had been uniformly mixed, usually at the farm turnout just before the treated water reached the crop land. The treated water was applied to ten field and vegetable crops on several farm units of the Columbia Basin during the 1971 growing season. In 1972, three fruit crops were similarly treated.

Normal irrigation flows preceded and continued after the application of TCA-treated water. Alfalfa was sprinkler irrigated; all other crops were furrow irrigated. Stages of crop maturity at the time of treatment were as follows: potato, blooming; alfalfa, blooming and about ready for cutting; garden peas, fully developed pods; snap beans, blooming stage, with a few immature pods; wheat, early heading; watermelon, vining; sugar beets, 3-cm diameter roots; and corn, about 46 cm high. The treatments were made to apples, grapes, and peaches during the early stage of fruit development.

Triplicate samples of the field and vegetable crops were collected 6–8 days after treatment and at harvest. Fruit crops were sampled only at harvest. An untreated control sample for each crop was collected on dates corresponding to those of early and late harvests. The crops collected are

Cooperative investigations of the Agricultural Research Service, U. S. Department of Agriculture; the Bureau of Reclamation, U. S. Department of the Interior; and the College of Agriculture, Washington State University.

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