Registry No. Efrotomycin A_1, 56592-32-6; aluminum, 7429-90-5; pyridine, 110-86-1; efrotomycin B, 96956-38-6.

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Fate of Dicrotophos in the Soil Environment

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The fate of dicrotophos (Bidrin insecticide), labeled with carbon-14 at the crotonamide moiety, in the aqueous and soil environment was examined. Hydrolysis rates are pH-dependent and follow first-order kinetics. The half-lives of dicrotophos in pH 5, 7, and 9 buffer solutions at 25 °C are 117, 72, and 28 days, respectively. *N,N*-Dimethylacetoacetamide and *O*-desmethyldicrotophos were the major hydrolytic degradation products detected. Aquatic and soil surface photolysis study showed the degradation of dicrotophos was not induced by light exposure. Soil metabolism studies conducted under aerobic and anaerobic conditions indicated the rapid and extensive decomposition of dicrotophos and its soil metabolite(s) to $^{14}CO_2$ and unextractable residues. The soil half-life of dicrotophos in a sandy loam soil was 3 days. *N,N*-Dimethylacetoacetamide and 3-hydroxy-*N,N*-dimethylbutyramide were detected as the major soil degradation products. Soil TLC data showed that dicrotophos has intermediate soil mobility. In view of the rapid and extensive degradation in the soil, results from this study indicated dicrotophos and its degradation products do not persist in the environment.

Dicrotophos (1, 3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide, dimethyl phosphate) is the active ingredient for Bidrin insecticide. Dicrotophos is a contact organophosphorus insecticide, active against a wide spectrum of phytophagous insects (Corey, 1965). Studies of the metabolic fate of dicrotophos have been conducted in plants (Menzer and Casida, 1965; Bull and Lindquist, 1964), insects, and mammals (Menzer and Casida, 1965; Bull and Lindquist, 1964; Hall and Sun, 1965). However, there have been no published reports on the fate of dicrotophos in the environment. This report presents the studies of the hydrolytic, photolytic, and soil degradation of dicrotophos. The soil leaching potentials of dicrotophos and its soil degradation products are also discussed.

EXPERIMENTAL SECTION

Radiosynthesis. Dicrotophos (1), labeled with carbon-14 at the C3 position of crotonamide, was synthesized from *N,N*-dimethyl[3-^{14}C]acetoacetamide (2).

To a stirred solution of **2** [296 mg, 2.3 mmol, 31 mCi (Amersham Corp., Arlington Heights, IL)] in dry methylene chloride (4.6 mL) was added sulfuryl chloride (247 mg, 1.8 mmol; Aldrich Chemical Co.) dissolved in dry methylene chloride (3.7 mL) dropwise at room temperature. After the addition was complete, the reaction mixture was heated to reflux for 1 h. An additional amount of sulfuryl chloride (25 mg, 0.18 mmol) was added, and the solution was refluxed for an additional 15 min. The reaction mixture was cooled and was washed with 10% aqueous sodium bicarbonate followed by water. The organic phase was dried and concentrated to provide 2-chloro-*N,N*-dimethyl[3-^{14}C]acetoacetamide (3; 283 mg, 24 mCi). Trimethyl phosphite (455 mg, 3.7 mmol) was added dropwise at room temperature to **3** (283 mg, 1.7 mmol, 24 mCi) in a 10-mL round-bottom flask. Acetic acid (16 μL), used as an isomer enhancer, was added, and the mixture was heated to 95 °C, while stirring. The reaction was maintained between 95 and 110 °C for 4 h. At the end of this period, the reaction solution was cooled and the excess trimethyl phosphite was removed under vacuum. The residue was diluted with nonradioactive dicrotophos (60 mg) and was purified by preparative thin-layer chromatography (TLC; silica gel F-254, 0.5 mm, E. Merck) using hexane–acetone (1:3, v/v) as the developing solvent. The radiochemical purity and the specific activity of [^{14}C]dicrotophos were 98.3% and 52 μCi/mg (12.4 mCi/mmol), respectively. Mass spectral and infrared data of [^{14}C]dicrotophos were consistent with those of the unlabeled reference standard.

Other reference standards were synthesized at the Biological Sciences Research Center (BSRC), Shell Agricultural Chemical Co. These standards included unlabeled *N,N*-dimethylacetoacetamide (2), *O*-desmethyldicrotophos (4, 3-hydroxy-*N,N*-dimethylcrotonamide, methyl hydrogen phosphate), and 3-hydroxy-*N,N*-dimethylbutyramide (5). Chemical structures of these compounds are presented in Figure 1.

Chromatography and Radioassay. Radioactivity was quantitated in 15 mL of Aquasol-2 scintillation solution (New England Nuclear) on a Packard Model Tri-Carb 300 liquid

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Table I. TLC R_f, R-HPLC R_t Values, and Mass Spectral Data of Dicrotophos and Model Metabolites

	TLC R_f		HPLC	
	system 1[a]	system 2[b]	R_t, min	EI MS data, m/z
dicrotophos (1)	0.63	0.69	8.9	237 (M^+), 193, 164, 127, 109, 95, 79, 77, 67, 44
N,N-dimethylactoacetamide (2)	0.68	0.70	2.4	129 (M^+), 112, 101, 85, 72, 69, 58, 56, 44
O-desmethyldicrotophos (4)[c]	0.01	0.25	1.7	
3-hydroxy-*N*-*N*-dimethylbutyramide (5)	0.59	0.65	2.4	131 (M^+), 116, 98, 87, 72, 69, 58, 45

[a] Dichloromethane–acetone–acetic acid (60:40:5). [b] Acetonitrile–water–ammonium hydroxide (40:9:1). [c] Analyzed as dicrotophos by GC–MS after methylation with diazomethane.

Table II. Characteristics of Test Soils[a]

	Tifton, GA	Hanford (Modesto, CA)	Catlin (Cerro Gordo, IL)	Sharkey (Glen Alen, MS)
cation-exch capacity, mequiv/100 g	2.0	7.1	13.0	23.5
field moisture at $^1/_3$ bar, %	2.3	10.8	27.7	31.7
bulk density, g/cm^3	1.6	1.3	1.0	1.2
hydrogen, mequiv/100 g	0.0	0.6	2.7	3.9
organic matter, %	1.1	1.3	4.0	3.3
pH (soil)	6.7	6.4	5.7	5.9
sand, %	88	60	24	22
silt, %	8	28	52	34
clay, %	4	12	24	44
texture	sand	sandy loam	silty loam	clay loam

[a] Analyzed by A&L Midwest Agricultural Laboratories, Omaha, NE.

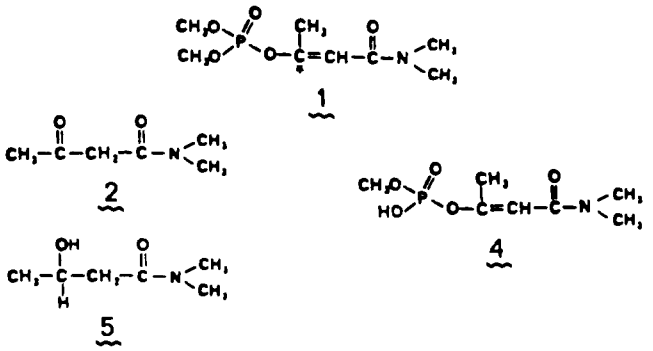

Figure 1. Chemical structures of dicrotophos and related degradation products (*denotes carbon-14).

scintillation system. The radioactive area of the TLC plate, after solvent development and autoradiography, was removed by scraping and analyzed in an Aquasol-2/water (11:4 mL) gel system. ^{14}C residues in the soil sample were analyzed by combusting subsamples (100 mg) in a Packard 306B sample oxidizer. Combustion efficiency was determined on untreated soil and calibrated [^{14}C]dicrotophos solution as the internal standard. The oxidizer counting solution included a Carbo-sorb and Permafluor V (10:12 mL) mixture.

^{14}C residues recovered from the soil and aqueous extracts were analyzed by two-dimensional TLC (silica gel F-254, 0.25 mm, E. Merck). The TLC and HPLC retention times of dicrotophos and reference standards are presented in Table I. The distribution patterns of ^{14}C residues on the TLC plates were visualized by autoradiography on Kodak SB-5 single-coated X-ray film. Extractable radioactivity was also analyzed by high-performance liquid radiochromatography (r-HPLC) (Varian Model 5000 liquid chromatograph) using RP-1 column (5 μm; 5 mm × 10 cm). The mobile phase was composed of methanol–water (1:4, v/v) at the flow rate of 1 mL/min. Radioactivity was monitored by the FLO-ONE Model HP radioactive flow detector (Radiomatic Instruments and Chemical Co.).

Mass spectral analysis was carried out on the HP-5970A GA–mass spectrometer (equipped with a mass-selective detector) on a 25 m × 0.2 mm fused silica, wall-coated cross-linked 5% phenylmethylsilicone column. A summary of the mass spectral data of dicrotophos and model metabolites is also included in Table I.

Test Soils. Four different test soils were utilized in this study: Hanford sandy loam from Modesto, CA; a sandy soil from Tifton, GA; Catlin silty loam from Cerro Gordo, IL; and Sharkey clay loam from Glen Allen, MS. Test soils were collected from 0 to 6 in. depth from each location. Soils were passed through a $^1/_4$-in. mesh screen to remove rock and other large debris. Samples of each test soil were air-dried at room temperature for 24 h and screened to remove any particles larger than 2 mm prior to the analysis for soil characteristics (Table II).

Hydrolysis Rate Determination. The hydrolysis rate was determined by monitoring the rate of disappearance of [^{14}C]dicrotophos in the aqueous buffer solutions. These buffer solutions included pH 5 (0.01 M phosphate buffer), pH 7 (0.01 M phosphate buffer), and pH 9 (0.02 M borate buffer). To ensure that the observed hydrolytic degradation was not caused by microorganisms, buffer solutions and all glass apparatus were sterilized by autoclaving prior to the initiation of the hydrolysis experiment.

Hydrolysis studies were carried out at 25 °C in darkness. Duplicate solutions containing 50 ppm [^{14}C]dicrotophos were prepared in each type of sterilized buffer solutions. A 10-mL aliquot of the buffer solution was transferred to 25-mL test tubes that were sealed with a standard tapered ground-glass stopper and placed in Haake Model E-52 constant-temperature water bath equipped with a circulating pump. At zero time and various time intervals thereafter, a 0.5-mL aliquot from each type of buffer solution was removed from the water bath and quantitated directly by liquid scintillation counting (LSC). In addition, the nature and distribution of dicrotophos and its hydrolysis products were determined. A 5-μL aliquot of the aqueous solution, without solvent extraction, was analyzed directly by TLC and r-HPLC.

For the isolation and identification of the hydrolysis products, aqueous samples (from the day 30 sampling interval) were extracted three times with an equal volume of chloroform. The combined chloroform extract was dried over anhydrous sodium sulfate, concentrated, and analyzed by TLC, r-HPLC, and mass spectrometry (MS). To analyze the water-soluble products, the aqueous phase after chloroform extraction was freeze-dried and the residual material was resuspended in methanol, filtered, concentrated, reacted with diazomethane, and analyzed by TLC, r-HPLC, and MS.

Aqueous Photolysis Rate Determination. The rate of photolysis was determined by monitoring the disappearance of [^{14}C]dicrotophos in the pH 7 buffer solution at various time intervals after exposure to simulated sunlight in the laboratory.

A single concentration of approximate 50 ppm [^{14}C]dicrotophos in pH 7 buffer solution was prepared. An 8-mL portion of the treatment solution was transferred to an 11-mm-i.d. quartz glass tube. Test solutions were prepared in duplicate. Additional duplicate control sample tubes were wrapped in aluminum foil. Samples were placed under the OPTI-Beam 1000 laboratory solar simulator (Optical Radiation Corp., Azusa, CA). The instrument was operated at the 2 V, 45-A setting and maintained in a 12-h light/dark cycle. Tubes were inclined at approximately 30° from

vertical. Both the exposed and dark control samples were maintained under the identical conditions during the entire test period. The temperatures at the test tube surface during the light irradiation and dark periods were 29 and 25 °C, respectively. The spectra of the high-pressure xenon lamp and natural sunlight are consistent.

At zero time and at each time interval thereafter, 10-μL aliquots of the test solutions were quantitatively radioassayed by LSC for material balance and qualitatively analyzed by TLC and r-HPLC as described above.

Soil Surface Photolysis. The photolytic degradation of [^{14}C]dicrotophos on the soil surface was examined. A concentration of 25 ppm [^{14}C]dicrotophos was established in the sterilized Hanford sandy loam soil (25 g) by applying the appropriate amount of ^{14}C test material in acetone directly onto the soil surface. Treated soil was placed in a glass Petri dish (150 × 25 mm) as a thin layer (<1 mm). Corresponding control samples were covered with aluminum foil. Test (exposed) and control (dark) samples (a total of 26 individual samples) were placed together 18 in. below the high-intensity UV light chamber (GE P40BL and BLB fluorescent lamps) during the entire test period. The light chamber was operated in a 12-h light/dark cycle. The temperatures at the soil surface during the irradiation and dark periods were 35 and 25 °C, respectively.

To determine the chemical nature of the radioactivity recovered from the treated soil after light exposure, duplicate soil samples (both exposed and control) were extracted three times with 50 mL of acetone–methanol (1:1, v/v) solvent mixture. The combined solvent extract was concentrated to approximately 10 mL, and the final volume was readjusted to 50 mL with saturated sodium chloride solution. This mixture was partitioned three times with 50 mL of chloroform. The combined chloroform extract was dried over anhydrous sodium sulfate, concentrated, and analyzed by TLC and r-HPLC. Radioactivity remaining with the extracted soil was considered as unextractable residues, which were quantitated by oxygen combustion and LSC.

Aerobic and Anaerobic Soil Metabolism. Freshly collected Hanford sandy loam was used in this study. A concentration of 10 ppm [^{14}C]dicrotophos was established in air-dried (24-h) test soil by applying the appropriate amount of the test material in water (7 mL/100 g) directly onto the soil surface. Treated soil samples (100 g each) were maintained individually in a 500-mL Erlenmeyer flask. The soil moisture level was equivalent to approximately 75% of the field moisture capacity at $^1/_3$ bar. Each soil sample was connected to an incubation system consisting of (1) an Ascaritte gas filter (to remove any CO_2 and gas impurities), (2) a 500-mL gas washing bottle containing water (air humidifier), (3) manifold, (4) treated soil container, (5) a 250-mL gas washing bottle containing 200 mL of ethylene glycol (volatile trap), and (6) a 250-mL gas washing bottle containing 200 mL of ethanolamine ($^{14}CO_2$ trap). The soil metabolism study was conducted in darkness at 23 ± 1 °C.

In the aerobic soil metabolism study, [^{14}C]dicrotophos-treated soils were purged continuously with humidified air (20–30 mL/min). In the anaerobic soil metabolism study, soil samples were initially incubated under aerobic conditions for 3 days (1 half-life) prior to establishing anaerobic conditions by water-logging the soil container with 200 mL of tap water (2–3 cm above soil surface) followed by the continuous purging of the soil container with humidified nitrogen.

Aliquots of the ethylene glycol and ethanolamine solution were sampled at each time interval, and triplicate subsamples (0.5 mL) were assayed directly by LSC.

To examine the chemical nature of the soil residues, two soil metabolism units were removed at each time interval: aerobic study, immediately after application and 1, 2, 3, 5, 8, 14, 24, and 36 days after treatment; anaerobic study, 11, 21, and 33 days after maintenance under anaerobic conditions. Soil samples were extracted three times with 100 mL of 0.01 N calcium sulfate followed by a single extraction with 75 mL of acetone for 10 min each on a wrist-action shaker. The acetone extract was concentrated to approximately 10 mL, and the residual material was transferred to the aqueous soil extract. This aqueous mixture, after being acidified to pH 3 with 1 N HCl, was saturated with sodium chloride and partitioned three times with equal volumes of chloroform. The combined chloroform extract was dried over anhydrous sodium sulfate, concentrated, and analyzed by TLC and r-HPLC. Unextractable soil residues, after the aqueous and acetone extractions, were quantitated by LSC after combustion.

In the anaerobic metabolism study, flood water was separated from the soil sample by centrifugation. The water sample was freeze-dried, and the solid residual material was resuspended in methanol. Radioactivity associated with the methanol extract was analyzed directly by TLC and r-HPLC. Radioactivity remaining in the anaerobically aged soil, after the removal of the water-logging fraction, was analyzed by the same soil extraction procedures as described above.

Soil Leaching Studies. The soil leaching potential of [^{14}C]dicrotophos and its soil degradation products was evaluated by soil TLC and adsorption/desorption studies.

[^{14}C]Dicrotophos in four different concentrations (0.1, 1, 3, 10 ppm) in aqueous 0.01 N calcium sulfate were prepared. Adsorption was determined by mixing a solution of [^{14}C]dicrotophos (10 mL) with soil (2 g) in a 5:1 volume to weight ratio. Each sample was duplicated. Screw-cap Pyrex tubes (16 × 125 mm) with Teflon-lined plastic caps were used as containers. Immediately after the addition or the solution, test tubes were vigorously agitated for 2 min on a Vortex mixer. Subsequent shaking was continued at a sufficient rate on a Labquake mixer to keep the soils in suspension. After equilibration (24 h in darkness at 25 ± 1 °C), the tubes were centrifuged at 2000 rpm for 15 min. Triplicate 0.25-mL aliquots of the supernatant were transferred from each tube into scintillation vials for direct radioassay.

Desorption was determined on the same samples used for the adsorption study, and the method was essentially the same. After 24 h, equilibration of 2 g of soil sample with 10 mL of aqueous calciuim sulfate–[^{14}C]dicrotophos solution, the suspension was centrifuged. A 5-mL portion of the aqueous solution was replaced with 5 mL of 0.01 N calcium sulfate solution, and the contents were shaken again on a Labquake mixer for 2 h followed by centrifugation. A 5-mL portion of the supernatant was again replaced with 5 mL of 0.01 N calcium sulfate. After each centrifugation, radioactivity associated with the supernatant was radioassayed. Equilibrium adsorption and desorption isotherms for dicrotophos were described by the Freundlich equation.

The mobility of [^{14}C]dicrotophos was also examined by soil TLC. Soil TLC plates were prepared by using a variable-thickness TLC spreader on a 20 × 20 cm glass plate. Distilled water was added to the sieved soil until a smooth moderately fluid slurry was attained. The thickness of the soil layer was 0.75 mm for all test soils. Plates were air-dried for a minimum of 24 h after slurry application and were stored in a dark location prior to use.

A horizontal line was scribed through the soil layer 11.5 cm above the base. [^{14}C]Dicrotophos and reference standard compounds [dichlorodi[U-^{14}C]phenyltrichloroethane (DDT), 2,4-dichlorophenoxy[2-^{14}C]acetic acid (2,4-D), trichloro[2-^{14}C]acetic acid (TCA), [^{14}C]triazine ring labeled atrazine, and 3-(3,4-dichloro-[U-^{14}C]phenyl)-1,1-dimethylurea (diuron)] were spotted 1.5 cm from the bottom edge and 2 cm apart. The day 3 aged soil extract was also included. Approximately the same quantities of test materials (1–5 μg, 0.05–0.30 μCi) were used for each duplicate plate.

Soil TLC plates were immersed vertically in a closed TLC solvent tank containing 0.5 cm of water and were removed when the solvent front had migrated to the 11.5-cm line. Soil TLC plates, after development, were air-dried for 24 h, and the movement of the test chemical was determined from the autoradiogram. The soil TLC mobility was reported as the average frontal R_f of the spot or streak.

RESULTS AND DISCUSSION

Hydrolytic and Aqueous Photolytic Degradation. The hydrolysis rates of dicrotophos are pH dependent. Reaction rates follow first-order kinetics and are catalyzed in alkaline solution. The calculated half-lives for dicrotophos at pH 5, 7, and 9 at 25 °C are 117, 72, and 28 days, respectively. *N,N*-Dimethylacetoacetamide (**2**) and *O*-desmethyldicrotophos (**4**) were identified as primary hydrolysis products by GC–MS. **4** was identified as dicrotophos (**1**) after methylation with diazomethane. The quantitative distribution of these hydrolysis products

Table III. Distribution of the Applied Radioactivity as Dicrotophos, *N,N*-Dimethylacetoacetamide, and *O*-Desmethyldicrotophos in Various Buffer Solutions

	% appl radioact recovered as[a]								
	dicrotophos			*N,N*-dimethylacetoacetamide			*O*-desmethyldicrotophos		
	pH 5	pH 7	pH 9	pH 5	pH 7	pH 9	pH 5	pH 7	pH 9
zero time	98.0	98.1	97.8						
day 7	94.7	92.6	88.5	1.2	1.2	4.8	3.5	5.2	5.5
day 14	90.8	85.6	72.4	1.5	3.5	17.6	7.0	10.0	9.0
day 21	87.8	82.4	62.4	0.8	1.6	21.2	10.5	14.8	13.5
day 28	83.7	74.3	49.9	1.3	4.8	31.2	13.6	18.8	16.5

[a] Average of two replicates.

Table IV. Distribution of Dicrotophos (1) and Degradation Products [*N,N*-Dimethylacetoactamide (2) and *O*-Desmethyldicrotophos (4)] in the pH 7 Buffer Solution at Various Time Intervals under Dark and Light Exposed Conditions

	% appl radioact[a]					
	light exposed			dark control		
day	1	2	4	1	2	4
7	88.6	1.6	7.9	89.7	1.5	7.6
14	78.8	4.1	14.3	79.4	4.8	14.9
21	74.1	1.6	19.9	75.4	1.3	20.9
28	64.3	5.5	25.3	66.3	5.8	25.5

[a] Average of two replicates.

differed for the various buffer solutions tested (Table III). Quantitative differences in the amount of the 4 between the pH 5, 7, and 9 buffer solutions were not observed. However, the cleavage of the phosphorus–crotonamide linkage to yield 2 was clearly predominant in alkaline solution.

Hydrolysis and aqueous photolysis studies showed the degradation rate and the chemical nature of the degradation products were not affected by exposure of the test solution (pH 7 buffer) to UV light (Table IV).

Soil Surface Photolysis. The photolysis of [^{14}C]dicrotophos on sterilized Hanford sandy loam soil surface was examined under high-intensity UV light source. After 30 days, approximately 80% of the applied [^{14}C]dicrotophos was recovered from both the light exposed and dark control soil samples (Table V). Photolytic induced degradation of dicrotophos on sterilized soil surface was not observed. Volatile materials, $^{14}CO_2$, and degradation products accounting for greater than 1% of the applied radioactivity were not observed. Additional soil residue, accounting for approximately 20% of the applied radioactivity, could be recovered by an aqueous solvent extraction and was characterized as the undegraded parent molecule. The formation of this "aged" residue was not light induced, since it occurred under both light and dark conditions.

Photolysis studies concluded that the degradation of dicrotophos was not significantly affected by light exposure. Its decomposition in the aqueous environment is primarily by hydrolytic reaction, and its degradation on soil surfaces is due mainly to microbial actions.

Soil Metabolism. Rapid degradation of dicrotophos in the Handord sandy loam soils under laboratory aerobic and anaerobic conditions was observed. Less than 3% of the applied [^{14}C]dicrotophos could be recovered after 14 days of aerobic incubation.

N,N-Dimethylacetoacetamide was detected as the major soil degradation product and was found mainly in the earlier sampling time intervals (up to 20% at day 5, Table VI). It dissipated rapidly and was not detected in the later sampling intervals. In the aerobically aged soil samples, several minor degradation products were also observed; however, each accounted for less than 1% of the applied radioactivity. The majority of the applied radioactivity was recovered as $^{14}CO_2$ (accounted to 75%). Other volatile metabolites were not detected. Unextractable residues, accounting for up to 27%, could not be readily recovered by exhaustive extraction procedures with organic solvents (chloroform, ethyl acetate, acetone, methanol, and acetonitrile) and water. Further fractionation showed the majority of these unextractable ^{14}C residues were associated within the fulvic acid fraction. Since dicrotophos and its degradation products decomposed readily under alkaline conditions, the chemical nature of the released radioactivity in the fulvic acid fraction was not further characterized.

The initial degradation pathway for dicrotophos in the soil environment probably involved hydrolytic reactions to generate *N,N*-dimethylacetoacetamide, which subsequently underwent rapid decomposition to yield $^{14}CO_2$ and intermediate metabolites that bound to soil macromolecules as unextractable residues. In addition, the generated $^{14}CO_2$ during the decomposition of dicrotophos could be utilized by the soil microorganisms as its carbon source to form natural constituents not readily recovered by solvent extraction.

The metabolism of [^{14}C]dicrotophos in the Handford sandy loam under laboratory anaerobic conditions was also examined. Treated soil was incubated under aerobic conditions for 1 half-life (3 days) prior to changing to anaerobic conditions. The dissipation rate of dicrotophos appeared to be reduced under anaerobic conditions (Figure

Table V. Material Balance and Distribution of the Applied Ratioactivity in the [^{14}C]Dicrotophos-Treated Soil (Exposed and Dark Control) at Different Sampling Intervals

		% appl radioact[a]							
		dark control				light exposed			
	zero time	5	14	21	30	5	14	21	30
extractable	104.5	92.1	85.8	81.0	81.4	90.7	84.8	81.7	82.7
dicrotophos	100.3	87.1	83.4	78.9	79.6	86.5	81.5	79.3	80.6
other	4.2	5.0	2.4	2.1	1.8	4.2	3.3	2.4	2.1
unextractable	3.4	11.5	13.9	18.6	17.3	10.5	16.8	21.0	18.0
total	107.9	103.6	99.7	99.6	98.7	101.2	101.6	101.8	100.7

[a] Results are expressed as an average of two replications.

Table VI. Distribution and Recovery of the Applied [^{14}C]Dicrotophos in the Hanford Sandy Loam Soil under Laboratory Aerobic and Anaerobic Incubation Conditions

	% appl radioact										
	zero time	aerobic								anaerobic[a]	
		1	2	3	5	8	14	24	36	14	24	36
$^{14}CO_2$		<0.5	2.5	5.8	29.4	36.8	57.7	66.5	73.5	17.8	18.0	18.0
water-logging phase										52.3	48.6	59.7
1										12.2	4.3	2.0
2										30.3	28.5	35.5
5										4.3	6.0	12.7
others[b]										5.5	9.8	9.5
total extractable	98.4	93.3	89.8	76.8	50.1	28.0	6.9	3.4[c]	2.3[c]	20.7	23.1	23.3
organic extractable	98.3	90.6	83.0	68.8	43.7	23.7	5.1			16.9	17.3	16.5
1	95.3	80.9	66.2	52.1	26.4	13.9	2.8			5.5	2.0	0.6
2	1.5	4.0	13.7	13.9	20.0	8.4	1.0			9.7	10.7	12.4
others[b]	1.5	5.5	3.1	2.8	2.3	1.4	1.3			1.7	4.6	3.5
water-soluble	<0.1	2.7	6.8	8.0	6.4	4.3	1.8			3.8	5.8	6.8
unextractable	2.9	3.5	6.8	13.0	20.6	27.9	26.5	21.4	28.5	7.6	7.1	6.2
total recovery	101.3	97.8	99.6	96.1	100.6	93.2	91.6	91.8	104.8	98.9	97.3	107.7

[a] Anaerobic soil samples were incubated under aerobic conditions for 3 days prior to changing to anaerobic condition. [b] To include minor products. [c] Because of the low level of radioactivity, chromatographic analysis was not conducted.

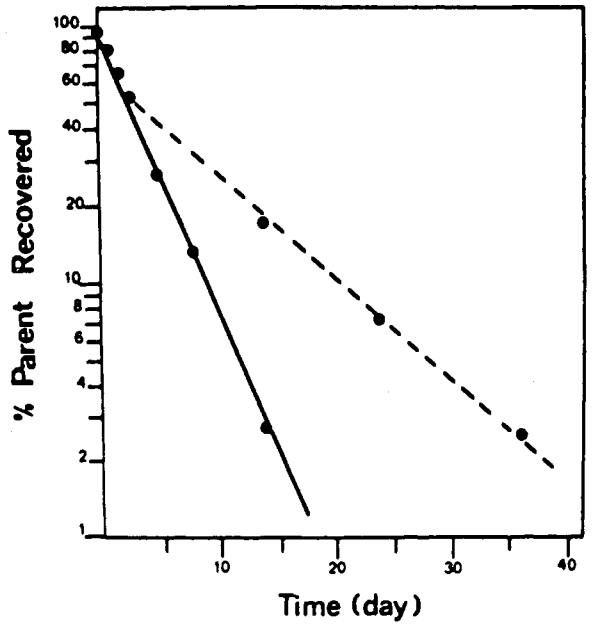

Figure 2. Soil degradation rate of dicrotophos under aerobic (—) and anaerobic (- - -) conditions.

2); less than 3 and 18% of the applied [^{14}C]dicrotophos were recovered after 14 days of aerobic and anaerobic incubations, respectively. The half-lives of dicrotophos under aerobic and anaerobic conditions were approximately 3 and 7 days, respectively. In addition to *N,N*-dimethylacetoacetamide, 3-hydroxy-*N,N*-dimethylbutyramide (**5**) was recovered in the water-logging fraction under anaerobic conditions.

Soil Mobility Potentials. The soil mobility of dicrotophos was evaluated by soil TLC and adsorption/desorption studies. Adsorption reached equilibrium within 24 h. Dicrotophos was adsorbed in the various test soil ranging from 2 (sandy loam) to 40% (clay loam). The Freundlich adsorption constants ranged from 0.07 to 3.58 (Table VII). There is a direct correlation between adsorption and soil organic matter and clay contents. Adsorption was reversible, and 10–55% of the adsorbed materials was released by the first desorption cycle.

On the basis of mobility classification of Helling (1971), DDT (immobile), diuron (low), atrazine (intermediate), 2,4-D (mobile), and TCA (very mobile) soil TLC autoradioagrams showed dicrotophos has an intermediate soil mobility.

Table VII. Freundlich Equation Parameters[a] for the Soil Adsorption/Desorption of Dicrotophos

	K	$1/n$	% adsorption	% desorption[b]
Georgia sandy soil	0.07	0.77	1–3	ND[c]
Handford sandy loam	0.40	0.96	7–8	10–55
Catlin silty loam	0.92	0.95	14–18	24–52
Sharkey clay loam	3.58	0.98	40–43	25–29

[a] Freundlich equation: $\log (X/M) = \log K + 1/n \log Ce$. [b] Percent of the adsorbed materials recovered after the first desorption cycle (at four concentrations). [c] Not determined.

CONCLUSION

The fate of dicrotophos in the aqueous and soil environment was investigated. Under hydrolytic conditions, dicrotophos degraded via O-dealkylation and ester cleavage to yield *N,N*-dimethylacetoacetamide and *O*-desmethyldicrotophos. Aqueous and soil surface photolysis studies showed the degradation of dicrotophos did not accelerate by light exposure. In the soil environment, the crotonamide moiety of dicrotophos degraded rapidly and the majority of the applied radioactivity was recovered as $^{14}CO_2$ and unextractable materials not be readily recovered by exhaustive solvent extraction. Dicrotophos has intermediate soil mobility as indicated by soil TLC and adsorption/desorption studies. Because of its rapid degradation to CO_2 and unextractable materials, only a very limited amount of the applied dicrotophos residues could be environment relevant. Dicrotophos degraded in the aqueous and soil environments to yield common degradation products that were also observed in the plant and animal metabolism studies. Results from this study indicated that dicrotophos and its degradation products do not persist in the environment.

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Recovery of Protein-Rich Byproducts from Sugar Beet Stillage after Alcohol Distillation

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Ground sugar beets and sugar beet juice were fermented to ethanol. After ethanol was distilled, residual stillage was separated by screening and centrifugation into filter cake, centrifuged solids, and stillage solubles. Pectinase was partially effective in reducing the viscosity of sugar beet slurries before fermentation. Centrifuged solids and stillage solubles had crude protein contents (nitrogen × 6.25, dry basis) of 47 and 24%, respectively, and contained 20 and 79% of the total nitrogen of sugar beet juice. Of the nitrogen in sugar beet juice stillage solubles, 91% passed through a 10-kDa molecular weight cutoff membrane. Permeate from sugar beet juice stillage solubles processed by combined ultrafiltration and reverse osmosis had much lower nitrogen, solids, and ash contents than those of stillage solubles. This practical method to ferment sugar beet juice for ethanol and to recover valuable protein-rich byproducts may have commercial potential.

Although most fuel ethanol produced by fermentation in the United States comes from corn, other fermentable substrates are of interest. Parrish et al. (1985) examined production of readily fermentable carbohydrates and biomass from grain sorghum, Jerusalem artichoke, corn, sugar beet, sweet potato, and sweet sorghum at three temperate locations and concluded that sugar beets are superior to corn in producing fermentable substrates.

Gibbons et al. (1984) reported a continuous, farm-scale, solid-phase fermentation process to produce fuel ethanol and protein feed from fodder beets, which could compete with corn as an ethanol feedstock. Boudarel and Ramirez (1984) studied fermentation of beet juice to ethanol; productivity of an industrial reactor was 30% higher than on a laboratory scale. Larsen et al. (1981) investigated various methods of processing sugar beets for fermentation in relation to alcohol yield. Little is known, however, of yield and composition of fermentation residues from sugar beets or sugar beet juice after ethanol distillation.

Optimum use and efficient processing of fermentation residues are important for commercial success of all ethanol processes. This paper reports on fermentation of ground sugar beets and sugar beet juice to ethanol, effects of commercial pectinases on viscosities of sugar beet slurries before fermentation, yield and composition of stillage fractions, and use of ultrafiltration (UF) and high-pressure reverse osmosis (RO) to concentrate sugar beet juice stillage solubles and produce a permeate suitable for reuse or safe disposal.

MATERIALS AND METHODS

Sugar Beets. Z8 sugar beets (1984 crop) were obtained from Michigan Sugar Co., Carrollton, MI. Z8 is a high-yielding breeding line; its sugar content ranges from 15 to 17% at harvest. Beets were stored at 5 °C for 9 months before arrival and then at 1 °C for 2 weeks before use.

B1230 sugar beets were obtained from D. Cole, U.S. Department of Agriculture, Agricultural Research Service, Fargo, ND. These beets contained 14.8% sugar upon harvest in 1983 and had been stored at 5 °C for 4 months before arrival; beets were then stored at 1 °C for 16 months before use.

Sugar beets were ground in a Cuisinart food processor, and juice was obtained by pressing ground beets at 520 lb/in.^{2} (3540 kPa) in a Model B Carver Laboratory Press (Summit, NJ).

Pectinases. Clarex L, Spark-L HPG, Pectinol 80SB, and Klerzyme Liquid 200 pectinases were described by Wu and Bagby (1987). Pectinex 3XL (Novo Laboratories, Wilton, CT) is a purified pectolytic enzyme preparation from a selected strain of *Aspergillus niger*. Ultrazyme 100 is a pectolytic enzyme from Swiss Ferment Co. (Basel, Switzerland). Optimum pH values for Clarex L, Spark-L HPG, Pectinol 80SB, Pectinex 3XL, and Klerzyme Liquid 200 are 3.5, 3.5, 4.5, 3.5, and 3.4 and optimum temperatures are 50, 50, 55, 50, and 60 °C, respectively. Ground sugar beets (100 g) were incubated with 0.3 and 1.5 mL of pectinase at optimal temperatures and pH values, stirred periodically for 6 h with a spatula (since suspensions were too thick to stir magnetically), and centrifuged at 6000*g* for 20 min.

Fermentation. Viscosities of sugar beet slurries without pectinase treatment were too high for proper stirring without significant aqueous dilution. Ground sugar beets (4900 g) were put in a 20-L stainless-steel, temperature-controlled, jacketed fermentor equipped with stirrers. The pH was adjusted to 3.5, and the mixture was maintained at 90 °C for 30 min with stirring. The temperature was then reduced to 50 °C, 74 mL of Pectinex 3XL and 500 mL of hot water were added, and the slurry was maintained at 50 °C for 3 h with agitation. The slurry was then adjusted to pH 4.0, and 18 mL of Diazyme L-100 glucoamylase (Miles Laboratories, Elkhart, IN) was added. The mixture was maintained at 50 °C for 2 h with agitation and cooled to 30 °C. The slurry was then inoculated with 500 mL of yeast (*Saccharomyces cerevisiae*) containing 500 million cells/mL and fermented at pH 4.5. Samples were withdrawn at 0, 24, 48, and 66 h, at which time fermentation was stopped. Figure 1 is a schematic diagram of sugar beet fermentation, fractionation of stillage, and UF and RO recovery.

For fermentation of sugar beet juice (8758 g), pH was adjusted to 6.2, 6 mL of Taka-therm α-amylase (Miles Laboratories) was

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