Fate of Uniformly Carbon-14 Ring Labeled 2,4,5-Trichlorophenoxyacetic Acid and 2,4-Dichlorophenoxyacetic Acid

Philip J. McCall,* Susan A. Vrona, and Sharon S. Kelley

Laboratory studies were conducted with uniformly ^{14}C ring labeled 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) to determine degradation kinetics in a number of soils and to trace the fate of the ring carbon in the degradation process. Degradation rates were not simple first order but generally increased until ~20% of the chemical remained, after which they declined. Average 50% decomposition times of 4.0 and 14 days were observed for 2,4-D and 2,4,5-T, respectively. Two major metabolites were observed in the degradation of 2,4,5-T: 2,4,5-trichlorophenol and 2,4,5-trichloroanisole. The anisole appears to be formed from the phenol through a microbial methylation process. Analogous metabolites were not observed for 2,4,-D. Soil samples containing measurable levels of 2,4,5-trichloroanisole were subjected to further studies investigating volatility. The compound was found to be quite volatile with a 50% loss time from soil of 1–3 days.

The persistence of the phenoxy herbicides 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) in soils has been extensively studied under field and laboratory conditions during the past 25 years. Effects of various environmental parameters have been investigated in a variety of soil types. However, frequently, only the parent compound has been studied and when metabolites have been detected they have seldom been identified. When ^{14}C tracer studies have been conducted, the tracer has usually been in the side chain so that transformations of the ring itself have not been thoroughly investigated. The degradation pathway of 2,4-D has been elucidated in microbial systems (Loos, 1975; Kearney and Kaufman, 1972); however, 2,4,5-T has not been studied as completely.

The objectives of this study were to determine the fate of the total molecule for 2,4-D and 2,4,5-T in a number of soils and to compare the compounds with respect to their degradation rates and metabolites produced.

MATERIALS AND METHODS

Radiochemical Purity. Uniformly ^{14}C ring labeled 2,4,5-T (sp act. 1.61 mCi/mmol) and 2,4-D (sp act. 9.61 mCi/mmol) were purchased from Mallinckrodt. Radiochemical assay by TLC showed the chemicals to be 98.0% and 97.6% pure, respectively.

Soils. Six soils were collected from various geographical regions throughout the United States. Soils were screened through a 2-mm sieve and stored at 4 °C until used in the study. Classification and properties for each soil are given in Table I.

Soil Treatment. Samples were prepared in a two-compartment incubation flask: one compartment containing soil and the other containing 100 mL of 0.2 N NaOH as a CO_2 trap. The ^{14}C-labeled chemical dissolved in 0.25 mL of acetone was mixed into 50 g of soil to give a concentration of 1 ppm. The soil mixture was adjusted to 75%, $^1/_3$ bar tension, soil–water content, and the flasks were connected to an oxygen manifold and incubated at 25 °C in the dark in a constant temperature cabinet. This constituted a completely closed incubation system which maintains constant temperature, atmosphere, and soil moisture throughout the experiment. For each soil and chemical, a series of eight flasks were prepared which were incubated for various periods of time up to 230 days. At designated times, samples were removed and the NaOH was analyzed for $^{14}CO_2$. The soil was stored frozen until analysis.

Recovery and Extraction of Radioactivity. The recovery of applied radioactivity was determined for each sample by analysis of the NaOH solution for $^{14}CO_2$ and by combustion of the soil for remaining radioactivity. For determination of $^{14}CO_2$, 2 mL of the NaOH solution was mixed with 18 mL of Aquasol scintillation cocktail and counted in Packard 3330 or 3255 scintillation counters by using the automatic external standard method to determine counting efficiency. Soil radioactivity was determined by combustion of 1–2 g of soil in a Harvey Biological Materials Oxidizer. Carbon dioxide from the combusted soil was trapped in 15 mL of a 2:1 mixture of Carbosorb and Permalfluor V. This was diluted with an additional 5 mL of Permafluor V before counting.

After analysis for total radioactivity, the soil samples were extracted to determine the distribution of radioactivity in the soil. Approximately 10-g aliquots of soil were weighed into 35-mL centrifuge tubes fitted with aluminum-lined caps, and then 10 mL of 1.5 M H_3PO_4 and 15 mL of diethyl ether (Mallinckrodt ether absolute-analytical reagent) were added. The samples were shaken overnight and the tubes centrifuged for 10 min at 2000 rpm in a Sorvall GLC-2 centrifuge. After ether removal, samples were extracted 3 additional times with 10-mL aliquots of fresh ether, 30 min each time. The ether was combined in a 50-mL volumetric flask and brought to volume. The aqueous acid phase was decanted and the soil washed twice with 10-mL volumes of water. The total volume was brought to 50 mL. Ten milliliters of 1.0 N NaOH was then added to the soil and the samples were shaken overnight. The NaOH was removed after centrifugation for 20 min at 2000 rpm. Two additional extractions were made with 10-mL aliquots of 1.0 N NaOH with shaking for 4 h each time. After NaOH removal, samples were washed with 10 mL of water, and NaOH and water were combined and brought to 50 mL. The extracted soil was placed on a watch glass and air-dried.

The above overall extraction procedure yielded four fractions for analysis: ether, acid, NaOH, and extracted soil. The ether and acid fractions were assayed for radioactivity content by counting 2-mL aliquots in 18 mL of Aquasol. The amount of radioactivity in the NaOH fraction was determined by combustion of 0.5 mL of NaOH solution applied to 1 g of untreated soil. The radioactivity remaining as unextracted from the soil was

Residue/Environmental/Metabolism Research, Agricultural Products Department, Dow Chemical U.S.A., Midland, Michigan 48640.

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Table I. Soils: Classification and Properties

soil	source	series	textural class	pH	% OC	% sand	% silt	% clay
M-21	Mississippi	Commerce	loam	6.1	0.67	34	50	16
M-32	Illinois	Catlin	silty clay loam	6.1	2.01	10	58	32
M-47	North Dakota	Fargo	clay	7.4	3.77	4	34	62
M-48	Nebraska	Keith	clay loam	6.9	1.20	24	38	28
M-50	Washington	Walla-Walla	silt loam	6.4	0.87	18	62	20
M-51	Georgia	Cecil	sandy loam	6.3	0.65	72	14	14

determined by combustion of 1 g of extracted soil. Percent of applied radioactivity in each fraction was calculated on an oven dry soil weight basis.

The NaOH extract was further examined to determine the distribution of radioactivity in the soil organic carbon (fulvic and humic acid) which was extracted in this fraction. A 25-mL portion of the extract was acidified with 2 mL of concentrated HCl and extracted 3 times with 7 mL of ethyl acetate which were combined and brought to a final volume of 25 mL. After extraction, the radioactivity in ethyl acetate and aqueous phase was determined by liquid scintillation counting. The amount of radioactivity in the humic acid, which precipitated upon acidification, was calculated as the difference between the amount of radioactivity initially in the NaOH extract and that determined to be in the ethyl acetate and aqueous phases.

Gel permeation chromatography was used in the analysis of the fulvic acid fraction obtained upon acidification of the NaOH extract. A 25-mL portion of the fulvic acid aqueous fraction was evaporated to dryness with a rotovap. The residue was redissolved in 4 mL of water and centrifuged, and 3 mL was applied to a 1 m × 2.5 cm i.d. Pharmacia glass column packed with Bio-Gel P-2 polyacrylamide gel. The column was eluted by using ascending chromatography with 1 M NaAc at a flow rate of ~0.5 mL/min. Two-milliliter fractions were collected for liquid scintillation counting. The column was calibrated with methyl red and blue dextran dyes.

2,4-D, 2,4,5-T, and Metabolite Identification. The degradation of 2,4-D and 2,4,5-T and the formation and decline of metabolites were followed by using thin-layer chromatography. Confirmation of metabolite identity was performed by using high-performance liquid chromatography and gas chromatography–mass spectrometry (GC–MS).

TLC was conducted with the ether extracts by application of an aliquot of the extract in a 2.5-cm band 2.5 cm from the bottom of a 5 × 20 cm F-254 silica gel plate (E. Merck). Plates were developed with a solvent system consisting of benzene–ethyl acetate–acetic acid (86:10:4 v/v) and then scraped in 1-cm segments to determine the position of radioactive compounds present. Each segment was counted in a liquid scintillation counter, and a histogram of the radioactivity was constructed by using a Hewlett-Packard 9821A computer. Positions of radioactive peaks were compared to those of standard unlabeled compounds chromatographed in the same manner and detected with ultraviolet light.

For further confirmation of identity of 2,4,5-T metabolites, several ether extracts were analyzed by high-performance LC using a Waters Associates μBondapak C_{18} reverse-phase liquid chromatographic column. The column was eluted with a 40-min linear solvent gradient from 100% H_2O to 100% MeOH (buffered with 0.01 M NH_4Ac) by using two Waters Model 6000A pumps. The radioactivity in the effluent was measured by liquid scintillation counting of collected fractions. Retention times of standard compounds were determined by using a Schoeffel Model SF 770 UV detector in conjunction with a Hewlett-Packard Model 3380A integrator.

The nonpolar 2,4,5-T metabolite (2,4,5-trichloroanisole) was further studied by GC–MS using a Finnigan Model 3100D mass spectrometer operated in the electron impact (EI) mode with a Model 6000 data system. The GC was equipped with a 6 m × 3 mm i.d. glass column packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q. GC conditions were as follows: helium carrier gas, 20 mL/min; column inlet temperature, 235 °C; column temperature, 210 °C.

Volatility Measurements. Volatility measurements were conducted on a few subsamples of soil initially treated with 2,4,5-T that contained higher amounts of the 2,4,5-trichloroanisole metabolite. A 25-g sample of soil was placed in the bottom of a 250-mL Erlenmeyer flask equipped with a sidearm. Humidified air was passed through the flask via the sidearm at a rate of 1000 cm^3/min, and volatile materials were trapped in a polyurethane foam plug located in a tower at the top of the flask. Polyurethane has been shown by Bidleman and Olney (1974) to be an efficient trap for volatile organic chemicals. The experiment was conducted at ambient temperature, ~22 °C, and soil moisture was maintained at 75%, $^1/_3$ bar, by periodic addition of water. The rate of loss of radioactivity from the soil was monitored by periodically changing the polyurethane plug and measuring the amount of radioactivity trapped in the plug. The plug was washed with 250 mL of acetone and a 2-mL aliquot was counted in 18 mL of Aquasol.

RESULTS AND DISCUSSION

Recovery of radioactivity, distribution of radioactivity in the soil extracts, and distribution of radioactivity in the NaOH soil extracts for 2,4-D and 2,4,5-T are summarized in Tables II and III, respectively. In general, recoveries averaged 100%, indicating all the material applied was accounted for. The major metabolic product for both chemicals was CO_2; however, intermediate metabolites were detected for 2,4,5-T in the ether extracts, and a significant amount of radioactivity for both chemicals was associated with the organic fraction of the soil. The organic fraction consists of fulvic and humic acids which are extracted with NaOH and humin which is unextracted with NaOH. Characterization of the radioactivity in these extracts is discussed below.

Metabolite Identification. The radioactivity in the ether extracts was analyzed for 2,4-D, 2,4,5-T, and metabolites in the respective soil studies by thin-layer chromatography. In the 2,4,5-T study two metabolites were found: 2,4,5-trichlorophenol and 2,4,5-trichloroanisole. Analogous metabolites were not detected for 2,4-D; the radioactivity in the ether extracts was identified as mainly 2,4-D. Several minor radioactive compounds were separated by TLC, but levels observed for these compounds never exceeded 1% of applied 2,4-D. The composition of the radioactivity in the ether extracts from the 2,4,5-T study is shown in Table IV and an example of a TLC histogram is shown in Figure 1. R_f values of the radioactive peaks on TLC corresponded to those of analytical standards of 2,4,5-T, the phenol, and the anisole.

Identity of the 2,4,5-T metabolites was further investigated with high-performance LC and GC–MS. A high-

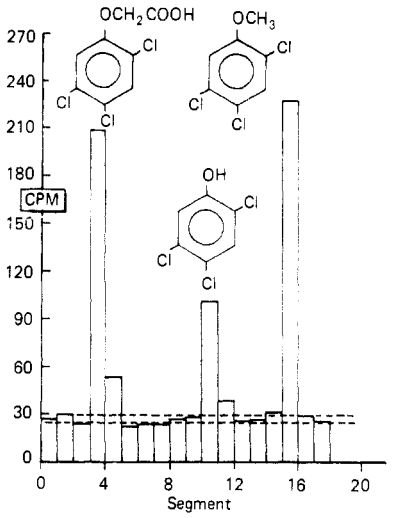

Figure 1. TLC histogram of ether extract of Fargo soil incubated 42 days with 1 ppm of 2,4,5-T.

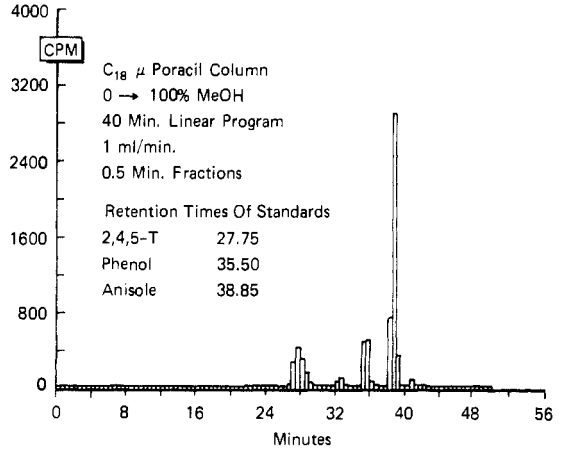

Figure 2. High-performance LC histogram of ether extract of Fargo soil incubated 75 days with 1 ppm of 2,4,5-T.

performance LC histogram showing the position of radioactive peaks is given in Figure 2. Retention times of standards match those of the major radioactive peaks. The anisole, which represents a metabolite that has not been previously reported, was isolated by using TLC. Its identity was confirmed by GC–MS. Ion chromatograms for m/e 210 of this metabolite and the analytical standard of anisole are shown in Figure 3. GC retention times are identical, and the mass spectra for each peak are shown in Figure 4. The material isolated from soil has a spectrum qualitatively identical with that of the analytical standard anisole. The spectra were characterized by the molecular ion at m/e 210 and ion fragments at m/e 195 resulting from the loss of the CH_3 group and at m/e 167 resulting from additional loss of C–O to form a cyclopentadiene structure. Similar rearrangements have been reported for other phenolic compounds (McLafferty, 1973). The presence of three chlorine atoms in the molecule is confirmed by the characteristic 100:97.5:31.7:3.4 isotopic ratio at M, M + 2, M + 4, and M + 6 for each of the three ions.

Characterization of Soil Organic Fraction. The results in Tables II and III show that over the course of the studies with both 2,4-D and 2,4,5-T, the amount of NaOH extractable and unextractable radioactivity increases to combined levels of 20–35% in the different soils. These levels were generally reached after several weeks of incubation and then they declined slowly thereafter. Acidification of the NaOH extract and extraction with

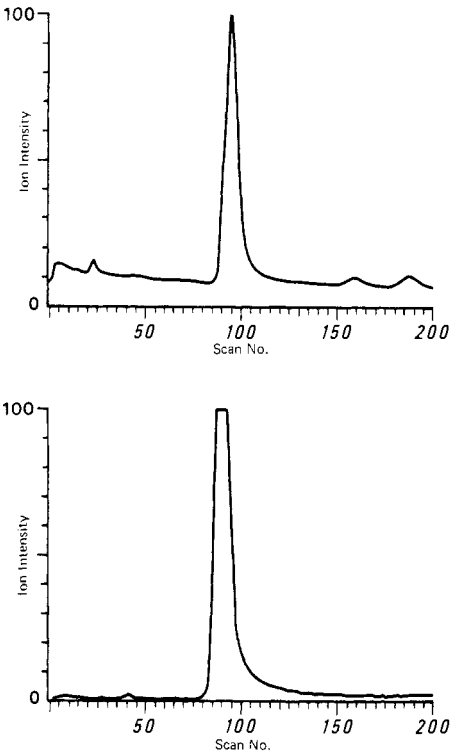

Figure 3. GC–MS m/e 210 ion chromatograms for 2,4,5-trichloroanisole (top) and 2,4,5-T soil metabolites (bottom).

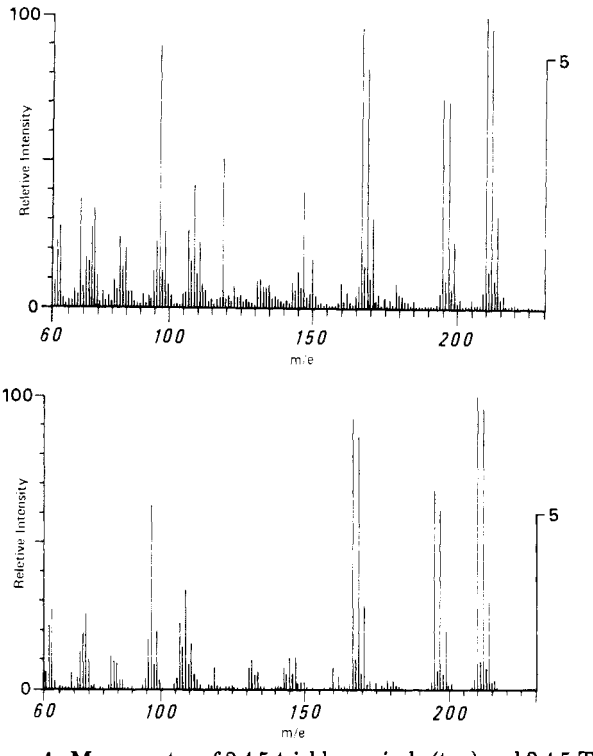

Figure 4. Mass spectra of 2,4,5-trichloroanisole (top) and 2,4,5-T soil metabolite (bottom).

ethyl acetate showed that the major portion of the radioactivity in the NaOH was associated with acid-insoluble humic acid material (which precipitated) and with the acid-soluble fulvic acid fraction (aqueous phase). The radioactivity not extracted with NaOH remained with the alkali-insoluble humin fraction of the soil.

These organic fractions are characterized as being high molecular weight, polydisperse, polymeric molecules. The molecular weight distribution in the fulvic acid fraction was characterized by GPC to determine if any low mo-

Table II. 2,4-D Aerobic Soil Degradation: Recovery and Distribution of Applied Radioactivity (100% = 1 ppm)

	% recovd ^{14}C act.			% soil ^{14}C act.					% NaOH ^{14}C act.		
day	CO_2	soil	recovery	Et_2O	NaOH	H_3PO_4	unextracted	2,4-D in Et_2O	EtAc	aqueous (fulvic acid)	humic acid
					Commerce						
0	0.0	96.9	96.9	89.2	4.0	0.9	2.2	88.9	1.8	1.6	0.6
3	10.9	86.4	97.3	70.6	7.5	2.2	6.7	70.5	2.2	4.0	1.3
7	29.2	69.6	98.9	36.5	17.8	3.1	11.5	36.4	3.6	6.6	7.6
10	41.4	49.4	90.8	7.7	20.3	4.9	17.4	7.3	2.4	9.4	8.5
16	50.8	48.7	99.5	6.0	20.9	4.0	17.6	5.3	2.4	9.8	8.7
21	55.6	44.7	100.3	4.0	20.4	3.3	16.5	3.3	2.2	10.0	8.2
30	57.8	43.1	100.9	3.3	19.9	3.3	14.9	2.7	2.0	9.6	8.3
51	62.3	38.5	100.8	2.2	19.3	2.6	13.2	2.2	2.0	9.6	7.7
					Catlin						
0	0.0	97.9	97.9	94.2	1.8	0.9	2.9	94.0			
3	34.5	63.5	99.0	21.0	21.1	4.9	13.0	20.3	5.4	6.8	8.9
7	49.8	50.6	100.4	8.9	20.2	3.6	15.2	7.5	4.8	6.6	9.4
10	57.0	41.2	98.2	4.7	15.3	3.0	12.3	3.7	3.4	5.8	6.1
16	60.1	39.8	99.9	4.5	15.3	3.1	13.8	3.6	3.4	5.6	6.3
21	60.0	40.0	100.0	3.8	16.9	2.7	15.3	2.8	3.4	5.0	8.5
30	63.3	37.5	100.9	3.4	15.2	2.7	15.8	2.7	2.8	5.6	6.8
51	66.4	33.6	100.0	2.4	13.8	2.8	11.5	1.9	2.4	5.4	6.0
					Fargo						
0	0.0	97.7	97.7	97.9	2.2	0.4	1.6	97.9	1.6	0.4	0.2
2	2.6	94.3	97.0	85.0	5.3	0.8	3.6	85.0	2.2	1.4	1.7
4	5.5	90.2	95.7	85.4	5.5	1.5	6.1	85.4	2.6	1.8	1.1
8	16.3	93.2	109.5	52.1	12.7	3.0	10.9	52.1	4.4	4.8	3.5
21	34.6	68.1	102.7	16.4	15.6	5.0	24.8	15.4	3.2	6.8	5.6
42[a]	3.3	95.2	98.6	90.2	4.3	1.2	4.8	38.0			
75	45.8	53.2	99.0	5.1	14.6	4.2	21.4	3.5	2.4	6.6	5.6
150	49.1	50.1	99.2	3.7	17.3	4.6	22.3	2.5	2.4	7.4	7.5
					Keith						
0	0.0	101.5	101.5	99.3	0.5	0.6	1.6	96.2			
2	6.3	94.5	100.7	78.0	8.4	1.8	3.7	78.0	1.8	4.6	2.0
4	15.5	83.7	99.2	52.0	14.4	3.6	8.4	52.0	2.2	8.8	3.4
8	31.0	67.6	98.5	25.7	20.5	5.0	16.2	25.4	3.2	10.8	6.5
21	56.7	46.6	103.3	3.2	20.8	3.9	16.5	2.5	1.8	11.4	7.6
42	63.9	39.6	103.4	1.8	17.8	3.0	17.6	1.5	1.4	8.8	7.6
75	67.0	34.0	101.0	1.3	17.3	2.3	15.1	0.9	1.4	8.6	7.3
150	70.4	30.1	100.5	1.1	14.4	1.9	9.4	0.7	1.4	7.6	5.4
					Walla-Walla						
0	0.0	101.1	101.1	98.2	3.2	0.6	0.1	97.5	1.2	1.0	1.0
2	11.5	97.8	109.4	65.0	14.8	3.0	5.3	63.7	3.8	4.6	6.4
4	30.2	72.2	102.5	31.4	22.7	4.7	8.6	28.8	4.8	8.2	9.7
8	48.0	52.5	100.5	8.8	26.7	3.9	11.7	5.0	4.6	9.4	12.7
21	60.0	42.0	102.0	5.4	22.9	2.8	10.6	2.8	3.8	7.8	11.3
42	71.6	31.0	102.7	4.2	16.0	3.3	6.6	1.1	2.4	6.6	7.0
77	79.0	21.7	100.7	2.6	11.0	2.5	5.0	1.3	1.6	4.4	5.0
150	82.8	18.5	101.3	1.8	9.3	2.2	3.9	0.9	1.2	4.4	3.7
					Cecil						
0	0.0	99.3	99.3	97.6	3.3	1.2	1.1	97.1	1.2	1.2	0.9
2	13.2	86.9	100.1	64.3	11.6	4.2	2.5	63.6	2.2	4.2	5.2
4	27.3	60.9	88.2	41.2	17.9	5.6	4.9	40.8	2.8	7.4	7.7
8	46.5	53.1	99.6	13.3	26.0	15.5	5.9	8.7	3.0	9.0	14.0
21	56.5	44.1	100.5	6.1	24.4	4.3	7.6	4.0	2.6	7.8	14.0
42	63.6	38.8	102.5	4.3	22.3	4.3	7.3	2.9	2.2	9.0	11.1
77	65.7	33.9	99.6	3.0	18.7	3.9	7.8	2.6	1.6	6.6	10.5
150	55.8	44.8	100.6	9.0	20.9	4.7	7.5	2.1	1.9	9.3	9.7

[a] Inconsistent data; not used in calculations.

lecular weight polar metabolites were present that had not been extracted with ethyl acetate. The GPC radioactivity elution profile for the fulvic acid fraction of 2,4,5-T incubated 62 days in Catlin soil is shown in Figure 5. Two major areas of radioactivity were seen, one at the exculsion volume which represents material greater than 1800 M_r and one at a position corresponding to 1200 M_r. Therefore, this fraction appears to contain primarily polymerized material. Crosby and Tutass (1966) and Crosby and Wong (1973), in conducting photolysis studies, observed a similar tendency of 2,4-D and 2,4,5-T to form polymeric humic substances through addition of hydroxyl groups to the ring. Similar reactions may represent a minor pathway for these molecules in soil.

Degradation Kinetics. Degradation of chemicals in soil is generally treated with first-order kinetics where a plot of the logarithm of concentration remaining vs. time yields a straight line with a slope equal to degradation rate constant. The half-life of the chemical is then calculated from the rate constant. When the degradation data for 2,4-D or 2,4,5-T were plotted in this manner, sigmoidal curves were obtained. The rate of breakdown was not first order but generally increased until ~20% of the applied chemical remained, after which the rate gradually declined.

Table III. 2,4,5-T Aerobic Soil Degradation: Recovery and Distribution of Applied Radioactivity (100% = 1 ppm)

	% recovd ^{14}C act.			% soil ^{14}C act.				% NaOH ^{14}C act.		
day	CO_2	soil	recovery	Et_2O	NaOH	H_3PO_4	unextracted	EtAc	aqueous (fulvic acid)	humic acid
					Commerce					
0	0.0	101.7	101.7	100.9	1.7	0.1	1.5			
7	2.5	98.9	101.4	90.6	5.7	0.6	3.6	2.2	1.8	1.8
16	5.8	91.5	97.3	81.8	8.8	1.0	6.7	3.0	3.0	2.8
28	11.5	97.6	99.1	70.0	13.8	1.5	7.8	3.3	4.6	5.9
43	14.6	81.2	95.8	59.6	13.6	1.6	9.0	3.1	5.3	5.2
62	19.4	79.7	99.1	54.9	13.3	1.8	8.0	2.8	5.4	5.1
153	39.3	59.9	99.1	28.4	15.1	2.4	11.7	3.0	6.3	5.8
230	47.9	46.2	94.1	19.9	14.1	2.1	10.6	2.4	6.1	5.9
					Catlin					
0		101.1	101.1	102.0		0.2	1.5			
7	3.3	97.5	100.7	84.6	12.3	1.0	4.6	4.1	2.8	5.5
16	10.8	91.3	102.1	60.3	20.2	2.0	7.6	5.6	5.5	9.1
28	19.1	81.0	100.1	44.0	27.0	2.4	8.5	6.1	7.8	13.1
43	27.5	71.9	99.4	29.3	26.8	3.1	12.5	6.4	9.2	11.2
62	33.1	68.1	101.1	22.5	27.3	3.6	12.2	5.7	9.8	11.8
153	52.6	47.4	100.1	9.1	22.4	3.0	10.8	4.3	8.9	9.2
230	54.2	45.8	100.0	7.5	22.0	3.2	11.2	4.0	8.8	9.2
					Fargo					
0		95.6	95.6	95.9	0.9	0.1	1.2			
2	0.1	95.7	95.8	98.3	0.7	0.3	1.8			
4	0.5	97.6	98.1	100.7	1.1	0.3	2.0			
8	0.5	95.1	95.5	95.1	1.4	0.4	3.1			
21	1.4	97.2	98.6	93.6	3.0	3.0	3.0	1.3	0.6	1.1
42	3.3	95.2	98.6	90.2	4.3	1.2	4.8	1.4	0.8	2.1
77	6.1	95.1	101.2	85.8	5.8	2.0	6.2	1.9	0.9	3.0
150	14.2	88.6	102.7	69.6	12.7	0.9	0.5	2.0	1.5	9.3
					Keith					
0		98.9	98.9	100.0	1.0	0.1	0.4			
2	0.6	101.6	102.2	97.9	3.9	0.4	1.4			
4	1.3	101.6	102.9	94.0	3.8	0.4	2.0			
8	2.8	95.2	97.9	86.5	7.4	0.9	3.6	2.7	1.5	3.2
21	13.9	86.0	99.9	61.2	11.3	4.1	7.3	2.3	6.1	2.9
42	53.6	48.5	102.1	13.7	14.4	6.5	11.7	2.3	8.1	4.0
77	60.5	44.8	105.3	8.3	12.4	7.6	11.7	2.1	7.7	2.6
150	70.7	32.0	102.7	4.4	13.8	4.5	8.8	1.7	6.4	5.7
					Walla-Walla					
0		103.7	103.7	99.6	0.5	0.1	0.3			
2	0.6	97.6	98.2	96.1	3.1	0.4	1.5			
4	1.4	97.0	98.4	89.6	3.6	0.6	2.1			
8	9.2	89.9	99.1	72.4	11.0	1.4	6.7	4.9	2.9	3.2
21	25.1	75.2	100.3	46.1	21.3	3.3	8.7	5.0	8.3	8.0
42[a]										
77	57.9	43.4	101.3	17.4	16.3	3.9	4.6	3.2	7.0	6.1
150	65.0	35.2	100.3	12.3	15.0	2.9	4.9	2.7	6.8	5.9
					Cecil					
0		101.0	101.0	101.3	0.6	0.2	0.4			
2	0.4	100.4	100.9	96.0	1.9	0.6	0.9			
4	1.4	96.6	98.1	90.7	5.3	0.9	1.7			
8	5.2	92.6	97.8	79.5	8.0	1.7	3.7	1.9	4.2	2.0
21	25.5	73.4	98.8	40.8	22.7	4.4	6.6	5.7	11.6	5.4
42	48.3	56.0	104.3	20.7	18.6	2.7	6.6	3.3	10.4	4.9
77[a]										
150	55.8	44.8	100.6	9.0	20.9	4.7	7.5	3.2	10.5	7.2

[a] Sample lost.

This effect can be seen in Figure 6 for the degradation of 2,4,5-T in Keith soil.

The initial lag in the degradation rate of phenoxy compounds has been observed previously in the soil perfusion experiments of Audus (1960, 1964). A postulated explanation for this behavior is that the enzyme systems necessary for the metabolism of these types of compounds are available in the microbial population; however, they must be stimulated or induced before degradation will take place. On the other hand, mutations may be required to produce the enzymes that can specifically degrade these chemicals. Either mechanism would account for the observed lag in degradation. These hypotheses are discussed in more detail in a review by Loos (1975). The late decline in the rate can be explained by a separate soil phenomenon. As the chemical incubates in soil, it can gradually become adsorbed into interstitial soil spaces. The rate of movement out of these spaces is generally slower than the rate in, such that at later times when little of the chemical remains, the amount of chemical available for the degradation is significantly less than the total amount present. Therefore, the rate appears to decline as observed in these

Table IV. Distribution of 2,4,5-T and Metabolites in Diethyl Ether Extracts: Percent of Applied 2,4,5-T (100% = 1 ppm)

day	% 2,4,5-T	phenol	anisole
	Commerce		
0	100.9	0.0	0.0
7	81.0	1.2	8.4
16	53.8	3.0	24.9
28	30.7	1.5	37.5
43	8.9	2.1	47.4
62	6.3	0.3	48.3
153	1.4	0.3	26.1
230	1.6	0.2	16.7
	Catlin		
0	102.0	0.0	0.0
7	55.7	11.8	17.2
16	16.0	11.1	33.2
28	7.0	5.9	29.0
43	4.0	4.7	19.4
62	3.0	3.6	14.0
153	1.6	1.5	4.2
230	1.4	0.9	3.5
	Fargo		
0	95.9	0.0	0.0
2	97.9	0.4	0.0
4	97.2	2.6	0.8
8	87.3	3.5	3.5
21	69.4	9.7	14.5
42	38.0	14.9	37.3
77	18.6	10.7	56.5
150	3.8	10.8	55.0
	Keith		
0	100.8	0.0	0.0
2	95.9	0.3	1.3
4	92.0	0.0	2.0
8	73.9	0.5	12.1
21	20.8	1.7	38.7
42	5.6	0.8	6.8
77	3.0	0.4	4.6
150	1.7	0.3	2.0
	Walla-Walla		
0	99.6	0.0	0.0
2	82.0	13.4	0.7
4	74.1	13.9	1.6
8	37.0	20.6	14.5
21	7.1	9.7	28.9
42			
77	2.3	2.9	10.7
150	1.2	2.1	7.5
	Cecil		
0	101.3	0.0	0.0
2	92.6	3.4	0.0
4	80.4	7.8	2.5
8	63.9	9.5	6.2
21	12.2	4.2	23.6
42	7.8	2.4	9.9
150	2.1	1.0	4.7

Table V. Time in Days for 50% Decomposition of 2,4,5-T and 2,4-D in Six Soils

soil	time, days 2,4,5-T	2,4-D	2,4,5-T/ 2,4-D
Commerce	17	5.0	3.3
Catlin	7.5	1.5	5.0
Keith	12	3.9	3.1
Cecil	9.9	3.0	3.3
Walla-Walla	6.6	2.5	2.5
Fargo	31	8.5	3.6
av:	14	4.0	3.5

experiments. This hypothesis has been discussed in more detail by Hamaker (1976).

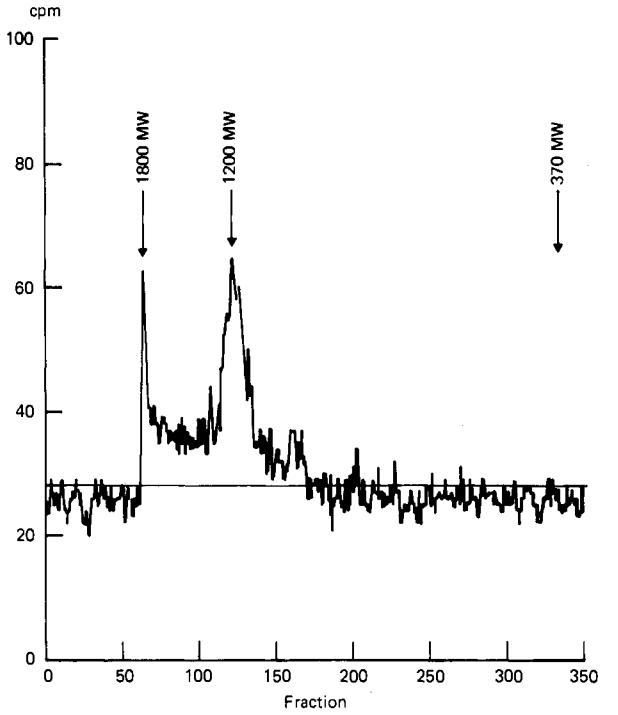

Figure 5. Gel permeation radioactivity chromatograph of fulvic acid extract from Catlin soil incubated 62 days with 1 ppm of 2,4,5-T. Positions of methyl red (M_r 370) and blue dextran (M_r 1800) are shown with an estimated molecular weight of 1200 for the major peak.

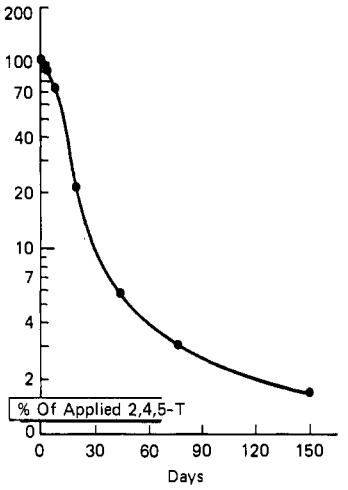

Figure 6. Degradation of 2,4,5-T in Keith soil at 25 °C and an initial concentration of 1 ppm.

Table VI. Time in Days for 90% Decomposition of 2,4,5-T and 2,4-D in Six Soils

soil	time, days 2,4,5-T	2,4-D	2,4,5-T/ 2,4-D
Commerce	41	10	4.1
Catlin	24	5.9	4.1
Keith	33	13	2.6
Cecil	30	7.6	3.9
Walla-Walla	18	6.5	2.8
Fargo	106	25	4.2
av:	42	11	3.6

Since the kinetics are not first order, a half-life as such cannot be expressed for each soil. However, the degradation can be characterized in terms of the time observed experimentally for 50 and 90% of the chemical to be degraded. These times for 2,4-D and 2,4,5-T are given in Tables V and VI along with the ratio of the decomposition

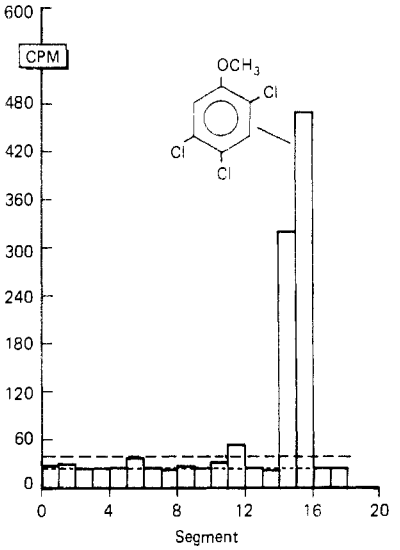

Figure 7. TLC histogram of radioactivity volatilized from Keith soil incubated 21 days with 1 ppm of 2,4,5-T.

times to each other for each soil. The variation in the decomposition times for each chemical among the different soils appears to represent the basic ability of a given soil microbial population to degrade the compounds. No correlation with the soils' properties was found. However, the ratio of the decomposition times is an almost constant value, essentially independent of soil type, such that 2,4-D can be expected to degrade ~3.5 times faster than 2,4,5-T in any soil.

Patterns of formation and decline of 2,4,5-trichlorophenol and 2,4,5-trichloroanisole, as indicated in the data in Table III, suggest the anisole is formed from the phenol via a sequential reaction mechanism. In general, appearance of the anisole and the maximum value it reached in the different soils followed those of the phenol. A similar hydroxylmethlyation has been observed with 3,5,6-trichloro-2-pyridinol to form the analogous 3,5,6-trichloro-2-methoxypyridine compound. This pathway has also been shown to be reversible (Bidlack and Laskowski, 1979).

In addition, studies conducted by Loos et al. (1967) with *Arthrobacter* sp. demonstrated the conversion of 2,4-dichlorophenoxyacetate to 2,4-dichloroanisole via the phenol. Apparently, after formation of the phenol the anisole is produced by an enzmatic O-methylation reaction. Such O-methylations, involving the transfer of a methyl group from *S*-adenosylmethionine in the presence of an *O*-methyltransferase, have also been reported for catecols and other hydroxy compounds (Axelrod, 1965). Therefore, it seems unlikely with 2,4,5-T that the anisole is formed directly from the parent molecule.

Volatility of the Anisole. On the basis of structure of the anisole, it was suspected the compound would be volatile. In the incubation system employed in the degradation study, volatility cannot occur to any appreciable extent because the system is closed. The chemical will tend to equilibrate between the atmosphere, soil, and aqueous caustic contained in the flask; however, the properties of the anisole are such that in this closed system the chemical will tend to remain primarily in the soil. A few samples containing higher amounts of the anisole were studied as described under Material and Methods to gain an estimate of the chemical's tendency to volatilize in an open system. Radioacivity trapped in the polyurethane foam plug was determined to be essentially all anisole (Figure 7). In the example shown (21-day sample from

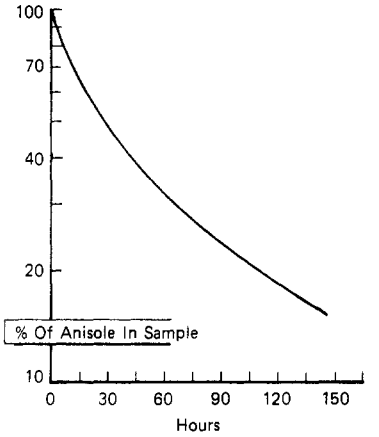

Figure 8. Volatility of 2,4,5-trichloroanisole from Keith soil incubated 21 days with 1 ppm of 2,4,5-T.

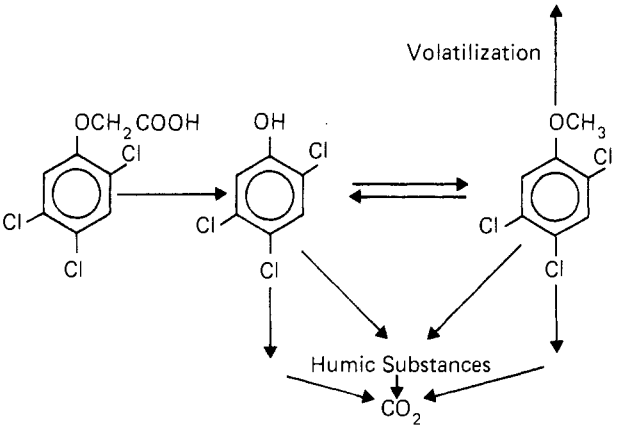

Figure 9. Proposed environmental soil metabolic pathway of 2,4,5-T.

Keith soil), ~30% of the applied radioactivity was anisole. Figure 8 shows the rate at which the anisole was lost from the sample in the experiment with the total anisole initially present normalized to 100%. The anisole was found to be quite volatile with a 50% loss time of ~1–3 days under the conditions of the experiment.

Volatility of chemicals from soil depends on a variety of factors: air speed, soil moisture, temperature, and depth of incorporation in the soil. However, the temperature and moisture conditions of the volatility study were similar to those in the degradation study. Therefore, if the rate of loss through volatility is compared to the rate of decline of this metabolite resulting from soil degradation, it appears that volatility is much faster. Volatility could therefore represent a significant route of dissipation of the anisole under field conditions. One would not expect to find significant amounts of this metabolite in the soil following a field application of 2,4,5-T.

A proposed environmental soil metabolic pathway for 2,4,5-T incorporating the results of this study is shown in Figure 9. The parent 2,4,5-T molecule is decarboxylated to form the phenol, which can then be methylated to form the anisole, which in turn can volatilize in an environmental situation. The phenol and presumably the anisole can also undergo further soil degradation. The degradation takes place predominantly by a series of relatively fast reactions as described by Loos (1975) to yield CO_2. In addition, there is incorporation of some form of the ring carbon into humic substances of the soil. These materials are ultimately degraded slowly to CO_2.

Presumably 2,4-D could follow a similar mechanism of degradation in soil. However, in the absence of the chlo-

rine at the 5 position of the ring, the relative rate of ring opening appears to be much faster than with 2,4,5-T. Thus, if the analogous phenol and anisole metabolites are formed, they are rapidly degraded and do not appear at measurable levels in soil.

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Persistence and Distribution of Fenvalerate Residues in Soil under Field and Laboratory Conditions

Bernard D. Hill

An analytical method is described for determining fenvalerate [(*RS*)-α-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate] residues in soil using hexane–acetone extraction, alumina microcolumn cleanup, and ^{63}Ni electron-capture gas chromatographic detection. Method recoveries ranged from 78.4 to 105% at the 0.005–1.0-ppm fortification levels. Methanol was an unsuitable solvent for residue analysis since fenvalerate exchanged the α proton with methanol, racemization occurred, and the amount of the *RS,SR* enantiomeric pair increased relative to that of the *RR,SS* pair. In a microplot field study, fenvalerate residues had an average half-life of 6 weeks in the 0–2.5-cm soil layer. Residues were not readily leached down to the 2.5–5.0-cm soil layer and lateral surface movement was minimal. Fenvalerate had an average half-life of 5.2 weeks in soil incubated in an environmental chamber. In both soil persistence studies, the *RS,SR* enantiomeric pair degraded slightly faster than the *RR,SS* pair.

Fenvalerate [(*RS*)-α-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate] is one of the "second-generation" (Menn, 1980) synthetic pyrethroid insecticides being evaluated at this laboratory for possible control of cutworm species (McDonald, 1979) and alfalfa insect pests. Although fenvalerate has a relatively low mammalian toxicity (rat, acute oral LD_{50} 451 mg/kg), it is toxic to fish and potentially harmful residues could move from farmlands into water bodies.

Since much of an applied insecticide contacts the soil, this work was conducted to determine the persistence and possible movement of fenvalerate soil residues under southern Alberta conditions. An analytical method for fenvalerate soil residues, the instability of fenvalerate in methanol, a microplot field study, and an environmental chamber incubation study are described. It was of particular interest to determine whether results of an indoor incubation study would approximate the persistence of fenvalerate in field soils.

From an environmental chamber incubation study, Williams and Brown (1979) reported a half-life of 7 weeks for fenvalerate in British Columbia soils. They showed that soil sterilization greatly reduced the rate of degradation of fenvalerate and permethrin and concluded that "microbial activity was the major factor in the loss of these insecticides".

Agriculture Canada Research Station, Lethbridge, Alberta T1J 4B1, Canada.

EXPERIMENTAL SECTION

Soil. The Lethbridge clay loam contained 29.0% clay, 27.0% silt, and 44.0% sand with a cation-exchange capacity of 24.4 mequiv/100 g. It had an organic matter content of 2.1%, a moisture holding capacity of 19.8% at 30-kPa pressure, and a pH of 7.9 as a 1:1 soil–water slurry.

Chemicals. Fenvalerate analytical standard (98.5% purity) and formulated product, 30% emulsifiable concentrate (EC), were supplied by Shell Canada Ltd., Toronto, Ont. Standards of each fenvalerate enantiomeric pair were obtained from Shell Research Ltd., Sittingbourne, U.K. Aluminum oxide (Woelm acidic) was stored as received at 130 °C. Solvents were pesticide-grade or glass-distilled reagent grade.

Field Persistence Study. A microplot technique similar to that reported by Smith (1971, 1972) was used with a randomized block design and four replicates. On June 20, 1978, fenvalerate at 150 g/ha was applied to thirty-two 20 × 20 cm soil microplots situated behind a shelterbelt

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