

# Metabolism of Propanil in Soils

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The metabolism of unlabeled and <sup>14</sup>C-labeled 3',4'-dichloropropionanilide (propanil) was studied in five different soil types at two concentrations. Soil type influenced propanil disappearance and 3,3',4,4'-tetrachloroazobenzene (TCAB) formation. <sup>14</sup>CO<sub>2</sub> evolution from carbonyl-<sup>14</sup>C-propanil was rapid and varied between 60 and 80% of the original

<sup>14</sup>C applied, depending on soil type and concentration. Ring <sup>14</sup>CO<sub>2</sub> evolution was slower and amounted to less than 3% of the original <sup>14</sup>C. A major unknown (*m/e* = 333) was isolated from one soil receiving 850 ppm. This unknown appears to be related to TCAB by the addition of an —NH group to the azo compound.

Propanil (3',4'-dichloropropionanilide) is one of the major herbicides used in rice (*Oryza sativa* L.) due to its selective herbicidal action between rice and certain weeds, especially barnyardgrass (*Echinochloa* spp.). Propanil is cleaved enzymatically to form 3,4-dichloroaniline (DCA) in plants (Adachi *et al.*, 1966; Frear and Still, 1968; Still, 1968), mammalian liver (Williams and Jacobson, 1966), and soils (Bartha and Pramer, 1967; Bartha, 1968). Free DCA converted from propanil in rice plants forms a DCA metabolic complex with sugars identified as N-(3,4-dichlorophenyl)glucosylamine (Still, 1968) and with lignin (Yih *et al.*, 1968).

In soil, two molecules of DCA derived microbiologically from propanil condense and form 3,3',4,4'-tetrachloroazobenzene (TCAB) (Bartha and Pramer, 1967). TCAB has not been found in plant tissues treated with propanil (Lieb and Still, 1969; Still, 1969). It has been suggested that microbiological action (Bartha and Pramer, 1967) and more specially peroxidase activity (Bartha and Bordeleau, 1969; Bartha *et al.*, 1968; Daniels and Saunders, 1953) are involved in the condensation of DCA to TCAB in soils.

In laboratory studies, TCAB-<sup>14</sup>C supplied to rice roots in nutrient solution was absorbed and translocated to the shoots, though the amount absorbed was fairly low (Still, 1969). In field surveys conducted on rice-producing soils in Stuttgart, Ark., TCAB was detected only at low concentrations (<0.1 ppm) (Kearney *et al.*, 1970).

A variety of azobenzenes, anilinoazobenzenes, and azoxybenzene have recently been detected in photosensitized reactions. Plimmer and Kearney (1969) photolyzed 3,4-dichloroaniline to TCAB and 3,3',4,4'-tetrachloroazoxybenzene in benzene solution with benzophenone as the photosensitizer. Rosen *et al.* (1969) exposed 3,4-dichloroaniline in aqueous solution with riboflavin-5'-phosphate (FMN) to sunlight and obtained TCAB and 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene. Linke (1970) isolated the latter compound in soil incubated for 4 weeks with 500 ppm of propanil and from the action of peroxidase on 3,4-dichloroaniline.

The formation of azo compounds and related metabolites should be investigated in greater detail, since anilines are produced in soil from many herbicides, such as phenylcarbamates (Kaufman and Kearney, 1965), phenylureas (Dalton

*et al.*, 1966), and acylanilides (Bartha, 1969). Rats fed TCAB at a level of 4 mg per week for an initial 3 week period and at 10 mg per week for an additional 37 weeks produced no tumors when the animals were sacrificed at the 60th week (Bartha and Pramer, 1970).

The present investigation examined the rate of breakdown and formation of propanil metabolites at several application rates in five Japanese rice producing soils.

## METHODS AND MATERIALS

**Synthesis and Purification of Compounds.** Technical propanil was purified by column chromatography on alumina. Fractions collected from the column were recrystallized several times from petroleum ether (60° to 110° C). DCA was purified by charcoal treatment and several recrystallizations from petroleum ether. TCAB was prepared by the method of Corbett and Holt (1963). The purity of these products was established by thin-layer and gas chromatography, melting point determination, and mass spectral analysis.

Ring-<sup>14</sup>C propanil was synthesized and supplied by Rohm and Haas Co., Research Laboratories, Spring House, Pa. Carboxy-<sup>14</sup>C-propanil was prepared by Gerald Still of the Metabolism and Radiation Laboratory, ARS, USDA, Fargo, N.D. Ring-<sup>14</sup>C DCA was prepared from <sup>14</sup>C-ring labeled diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] by basic hydrolysis in 2-methoxyethanol. Radioactive compounds were purified by thin-layer chromatography on silica gel by using a solvent system of hexane, benzene, acetone (7:3:1 v/v), and detected on no-screen x-ray film. Purity was verified by comparison with authentic compounds on thin-layer chromatograms. The specific activity of ring-<sup>14</sup>C propanil, carbonyl-<sup>14</sup>C propanil, and <sup>14</sup>C DCA were 0.26, 0.02, and 1.00 mc per mmole, respectively.

**Soil Properties.** Five soil types were selected from typical paddy rice fields in Japan. These soils consist of two light clays, two clay loams, and one coarse sandy loam. Their properties are shown in Table I. Soil types were classified according to the international system (Buckman and Brady, 1960). These soils represent a wide range of physical and chemical properties.

Soil C, originating from volcanic ash, contains a high percentage of organic matter and has a high cation exchange capacity. The other four are alluvial soils. Soils were kept moist during storage and activated microbiologically by growing rice and returning the shoots to the soils before starting the experiments.

**TCAB Formation in Five Soils.** Experiments were conducted to determine the rates of propanil disappearance and DCA and TCAB formation among soils. Purified propanil

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was dissolved in ethanol and added to duplicate samples (500 g oven-dry basis) of each soil at the rate of 85 and 850 ppm on a weight basis (ppmw). Soils were thoroughly mixed after evaporation of the ethanol, and incubated in beakers at 30° C. Soil moisture content was maintained at 80 to 90% of field capacity, and soils were mixed at frequent intervals during incubation.

At 15, 30, 45, 60, and 105 days after propanil treatment, 100 g (oven-dry basis) of each soil was extracted with 200 ml of acetone in a blender for 3 min. The extract was filtered, the acetone was evaporated in a rotary flask evaporator, and the water layer was washed three times with benzene. The water layer was discarded. The benzene was reduced to a suitable volume in the evaporator, and then subjected to gas chromatographic analysis for propanil, DCA, and TCAB.

All analyses were performed by flame-ionization gas chromatography with a 6-ft column packed with 10% methylvinyl silicon gum rubber on diatoport S 80-100 mesh. The carrier gas (N<sub>2</sub>) flow rate was 40 ml per min. Injection port and detector temperatures were 270° and 310° C, respectively. Column temperatures were 200°, 200°, and 245° C for propanil, DCA, and TCAB, respectively. Under these conditions, the retention times of propanil, DCA, and TCAB were 4 min 35 sec, 1 min 20 sec, and 5 min 57 sec, respectively. Products were quantitated by comparing peak heights with appropriate calibration curves. The limits of quantitative analysis were 0.5, 0.2, and 0.3 ppmw for propanil, DCA, and TCAB, respectively. Recoveries from soils were more than 90% for propanil and TCAB, and roughly 60% for DCA. The results of analyses were used without correction for recoveries.

**Metabolism of Propanil- and DCA-<sup>14</sup>C in Soils.** The rate of <sup>14</sup>CO<sub>2</sub> evolution and the distribution of <sup>14</sup>C in metabolites from radioactive propanil and DCA in two different soils was determined. <sup>14</sup>CO<sub>2</sub> production from soils treated with carbonyl-<sup>14</sup>C propanil, ring-<sup>14</sup>C propanil, and <sup>14</sup>C DCA were measured in the metabolic flasks described by Bartha and Pramer (1965). Soils sterilized by steam and KN<sub>3</sub> (500 ppmw) were maintained as controls.

Soils C and E were used in this experiment. Selection of these soils was based on the differences in TCAB formation determined by gas chromatographic analysis. Acetone solutions of the three radioactive compounds were added to the soils at two concentrations, 8.5 and 850 ppmw for the two labeled forms of propanil, and 6.3 and 630 ppmw for DCA. DCA at 6.3 ppm is equivalent to 8.5 ppm of propanil in the aromatic moiety. When the acetone had evaporated, the soils were thoroughly mixed and moisture adjusted to 80% of field capacity. A 50-g sample (oven-dry basis) of each treated soil was transferred to duplicate flasks and incubated at 30° C for 25 days.

Ten ml of 0.1N KOH was used as the CO<sub>2</sub> trapping solution in the side arm. The solution was removed and replaced every day for the first week, and every other day for the following period of incubation. All determinations of radioactivity were carried out in a Nuclear Chicago Mark I liquid scintillation counter. All measurements were corrected for background and quenching. It was verified by titrating at each sampling time that the CO<sub>2</sub> trapping solution removed from the side arm was not saturated with CO<sub>2</sub>.

Duplicate 1 ml samples of CO<sub>2</sub> trapping solution were added to 15 ml of scintillation solution (PPO 12 g, POPOP 0.6 g, naphthalene 60 g, and 2-ethoxyethanol 200 ml in scintillation grade 1,4-dioxane 1000 ml); and the radioactivity was measured by liquid scintillation.

Table I. Properties of Five Soil Types from Typical Paddy Rice Fields in Japan

SOILS		SAND SILT CLAY			ORGANIC MATTER %	P H	C.E.C. m.e.	
		%						
A	KONOSU (SAITAMA)	LiC	31	29	40	5.8	5.2	18.7
B	AZUMIDAIRA (NAGANO)	CoSl	74	16	10	3.3	5.3	6.6
C	UTSUNOMIYA (TOCHIGI)	CL (VOLCAN.)	44	35	21	12.8	6.1	30.2
D	FUKUYAMA (HIROSHIMA)	CL	52	29	19	1.5	6.3	10.0
E	CHIKUGO (FUKUOKA)	LiC	40	32	28	4.1	5.6	20.3

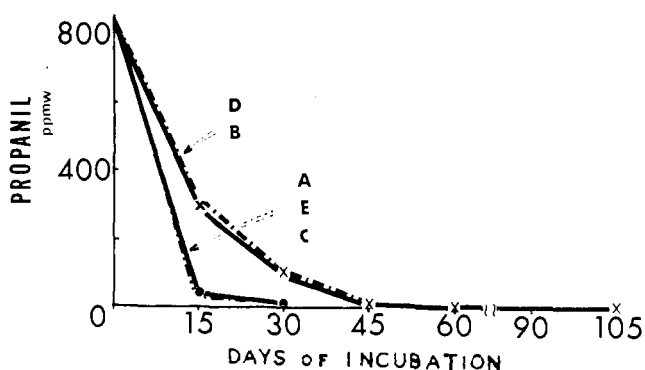


Figure 1. Concentration of propanil in five soils after 15, 30, 45, 60, and 105 days. (Initial propanil concentration was 850 ppm)

At the completion of the incubation period, the soil from each metabolic flask was extracted with acetone and the extract transferred to benzene by the procedures outlined previously. Radioactivity in the acetone extract and the water washed with benzene were measured by adding 15 ml of scintillation solution to 1 ml of each sample.

The benzene was reduced to a suitable volume after removal of trace amounts of water by MgSO<sub>4</sub>, and then thin-layer chromatography was performed on plates 20 × 20 cm coated with silica gel G. Chromatograms were developed first with benzene and second with hexane, benzene, and acetone (7:3:1 v/v). Chromatograms were exposed to no-screen x-ray film for 10 days and then developed. Activity in each spot was determined by scraping the silica gel into a vial containing 15 ml of CO<sub>2</sub> cocktail (PPO, 5 g and POPOP, 0.15 g in toluene, 1000 ml) and counting by liquid scintillation.

Approximately 100 mg of each soil sample was combusted in duplicate to determine the amount of <sup>14</sup>C in soils before and after extraction. <sup>14</sup>CO<sub>2</sub> from combusted soil was trapped in 10 ml of 2-methoxyethanol and monoethanolamine (7 to 1 v/v), and 5 ml aliquot of this CO<sub>2</sub> trapping solution was added to 10 ml scintillation solution for measuring radioactivity. Mass spectral analysis of the products was performed with a Perkin-Elmer Model 270 combination gas chromatograph-mass spectrometer.

## RESULTS AND DISCUSSION

Most of the propanil disappeared within 15 days from soils receiving 85 ppmw. Differences among soils could not be determined, since the amount of propanil was less than 1 ppm after 15 days in all soils. At 850 ppmw, propanil decomposed more slowly in soils B and D (Figure 1).

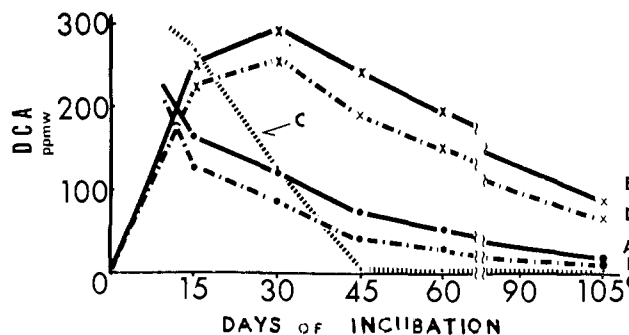


Figure 2. Concentration of DCA in five soils after 15, 30, 45, 60, and 105 days. (Initial propanil concentration was 850 ppmw)

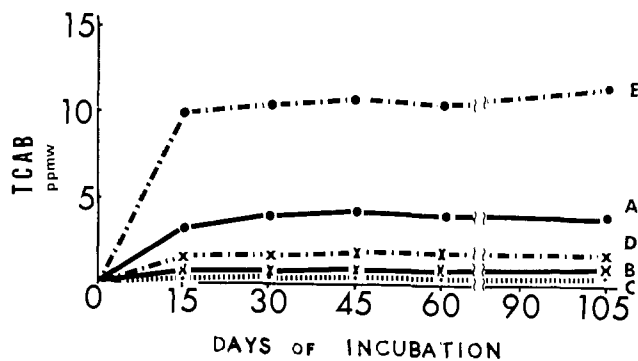


Figure 3. Concentration of TCAB in five soils after 15, 30, 45, 60, and 105 days. (Initial propanil concentration was 85 ppmw)

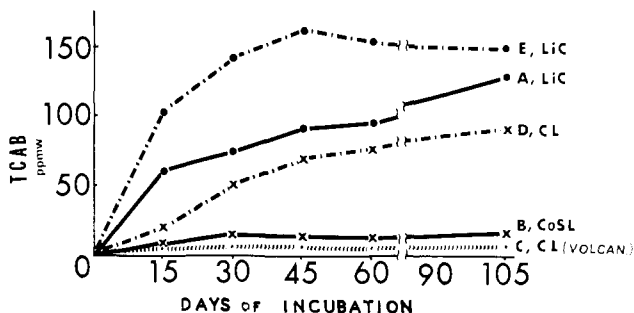


Figure 4. Concentration of TCAB in five soils after 15, 30, 45, 60 and 105 days. (Initial propanil concentration was 850 ppmw)

The effect of soil type on DCA appearance and loss is shown in Figure 2 for soils receiving 850 ppmw of propanil. In recovery studies, known quantities of DCA were added to soils, and extracted with acetone. Recovery was a function of soil type and initial DCA concentration. The range varied from 80% in soil D to 60% in soil C at an initial concentration of 200 ppmw. At 20 ppmw recoveries from all soils were less than 50%. Low recoveries may be caused by substantial amounts of aniline and/or alteration products binding to soil particles. Vapor loss of DCA also could have occurred during evaporation of solvents. Consequently, differences among soils in Figure 2 may be related to adsorption or volatilization of DCA. In soils B and D, which have a high sand content and low organic matter, DCA formed slowly and persisted longer.

TCAB was detected in every soil (Figures 3 and 4). At 85 and 850 ppmw of propanil, the order of TCAB concentration was E > A > D > B > C after 15 days. Formation of TCAB decreased at the lower rate of propanil application. Kearney

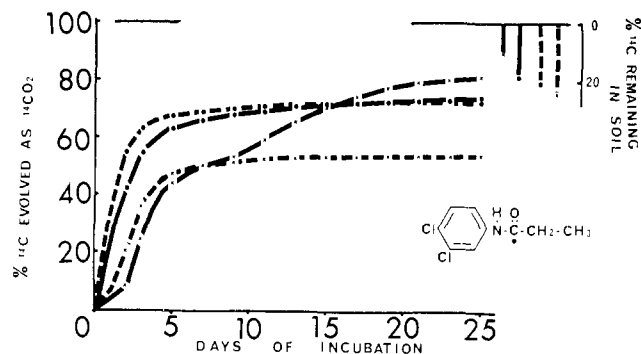


Figure 5.  $^{14}\text{CO}_2$  evolution from carbonyl- $^{14}\text{C}$  propanil in two soils receiving 8.5 and 850 ppmw (--- soil C, 8.5 ppmw propanil; X---soil C, 850 ppmw propanil; — soil E, 8.5 ppmw propanil; X— soil E, 850 ppmw propanil)

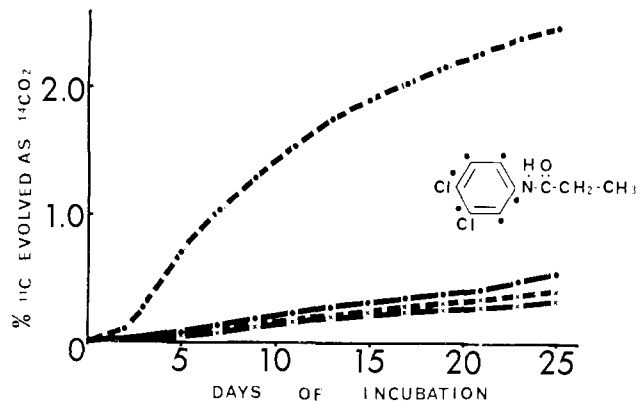


Figure 6.  $^{14}\text{CO}_2$  evolution from ring- $^{14}\text{C}$  propanil in two soils receiving 8.5 and 850 ppmw (--- soil C, 8.5 ppmw propanil; X--- soil C, 850 ppmw propanil; — soil E, 8.5 ppmw propanil; X— soil E, 850 ppmw propanil)

*et al.* (1969) found that condensation of DCA to TCAB increased as the concentration of DCA was increased logarithmically in soil. Since there was no apparent decomposition of TCAB in the present experiment after 105 days, it is presumed that TCAB is persistent in most soils. The percent conversion of propanil to TCAB for the five soil types receiving 850 ppmw was: A, 21.0%; B, 2.5%; C, 1.3%; D, 14.6%; and E, 26.2%. At 85 ppmw, the percent conversion was: A, 6.7%; B, 1.6%; C, 1.0%; D, 3.1%; and E, 18.6%.

**Metabolism of Propanil- and DCA- $^{14}\text{C}$  in Soils.** The production of  $^{14}\text{CO}_2$  from carbonyl- $^{14}\text{C}$  propanil is shown in Figure 5. More than 60% of the initial  $^{14}\text{C}$  added was evolved as  $^{14}\text{CO}_2$  within 5 days in soils C and E when the initial concentration of propanil was 8.5 ppmw. Thereafter, the evolution of  $^{14}\text{CO}_2$  continued slowly and reached 70% of the initial  $^{14}\text{C}$  added after 25 days except in soil at 850 ppm propanil. Approximately 20% of the  $^{14}\text{C}$  was recovered in soils by combustion and 10% was unaccounted for in soils receiving 8.5 ppm propanil. Total  $^{14}\text{CO}_2$  evolution was greater in soil E at 850 ppm propanil and occurred in two phases. This may indicate that metabolism of the carbonyl labeled propionyl moiety occurs in two separate steps.  $^{14}\text{CO}_2$  evolution from ring labeled propanil was very slow, and amounted to less than 3% after 25 days (Figure 6). Therefore, differences in rates of ring cleavage among soil types could not be a major reason for differences in TCAB formation observed in Figures 3 or 4 at either concentration of propanil in soils.

Table II. Balance Sheet of  $^{14}\text{C}$

Soil	Treatment	Rate, ppm	Initial activity of $^{14}\text{C}$ 1000 cpm	$^{14}\text{C}$ % of initial activity			Recovery <sup>a</sup>
				As $^{14}\text{CO}_2$	in soils after extraction	extracted	
C	nonsterile						
	Ring- $^{14}\text{C}$ Propanil	8.5	1350	2.5	80.5	14.3	14.9
	Propanil	850	1350	0.4	50.4	48.1	49.6
	$^{14}\text{C}$ DCA	6.3	1260	0.3	81.9	16.3	16.8
C	sterile						
	Ring- $^{14}\text{C}$ Propanil	8.5	1506	0.0	3.4	95.9	98.0
	Propanil	850	1550	0.0	2.5	94.5	97.5
	$^{14}\text{C}$ DCA	6.3	1260	0.2	78.2	19.9	19.8
E	nonsterile						
	Ring- $^{14}\text{C}$ Propanil	8.5	1350	0.5	63.0	30.1	31.6
	Propanil	850	1350	0.3	48.7	42.5	44.5
	$^{14}\text{C}$ DCA	6.3	1260	0.2	74.0	18.0	18.7
E	sterile						
	Ring- $^{14}\text{C}$ Propanil	6.3	1295	0.4	47.9	39.2	43.3
	Propanil	850	1350	0.3	48.7	42.5	44.5
	$^{14}\text{C}$ DCA	6.3	1260	0.3	73.3	23.0	23.1
		630	1295	1.1	39.4	50.7	51.3

<sup>a</sup> $^{14}\text{C}$  extracted/ $^{14}\text{C}$  in soil before extraction.

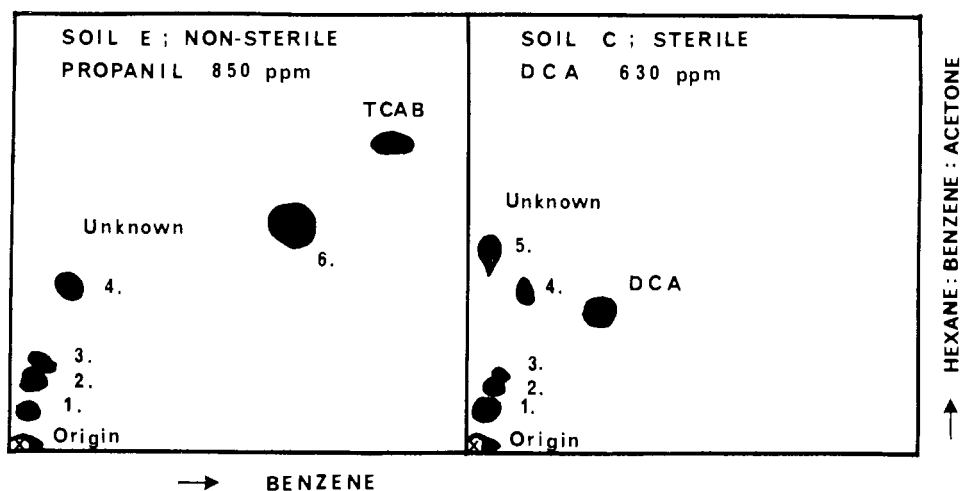


Figure 7. Distribution of radioactive spots on thin-layer chromatograms from ring- $^{14}\text{C}$  propanil in soil E (nonsterile) and soil C (sterile) developed first with benzene and second with hexane:benzene:acetone (7:3:1 v/v)

Carbon balance studies for soils treated with ring-labeled propanil and DCA are shown in Table II and for products from thin-layer chromatograms, as shown in Figure 7. Values for  $^{14}\text{C}$  contained in the water-layer of the extracts are omitted, since these were well below 0.5% of the initial activity. Propanil recovery was almost 100% from sterile soils after 25 days incubation, whereas DCA and several unidentified labeled products were found in sterile DCA treated soils (Table III). Percent recoveries at 630 ppm of DCA were 87% and 37% in soils C and E, respectively.

A major metabolite (unknown 6 in Table III and Figure 7) similar in structure to TCAB was formed in soil E treated with propanil or DCA at the higher rate. This compound contained more than 60% of the total radioactivity in the extract or approximately 25% of the initial activity. This bright yellow compound was not detected in any samples of soil C or soil E treated with propanil at the lower rate, or in sterilized soil E. Mass spectral analyses showed a molecular ion at  $m/e$  333 indicating three nitrogen atoms, peaks at higher mass indicating four chlorine atoms, and suggests the empirical formula  $\text{C}_{12}\text{H}_7\text{N}_3\text{Cl}_4$ . No peaks were detected in the

region of TCAB by gas chromatography on the 10% methyl-vinyl silicon gum rubber column. Control beakers of soil E maintained under similar conditions with the metabolic flasks also produced the unknown. Consequently, production of the unknown was not caused by the metabolic flask method.

Based on the pathways shown in Figure 8, the following differences can be shown between soil types and their capacity to form TCAB or related products. In soil E, a clay soil forming the largest amounts of TCAB by GC analysis, and soil C, a volcanic ash forming little TCAB by comparison, reaction A, or the formation of DCA and propionate takes place rapidly. Enzymes responsible for this reaction, acyl acylamidase (aryl acylamine amidohydrase EC 3.5.1.a) have been isolated and characterized extensively. Microbial oxidation of propionate (reaction B) to  $\text{CO}_2$  occurs within 4 days and amounts to about 70% after 25 days. Little ring oxidation (reaction C <3%) occurs in this same time period. Up to this point there appears to be little difference between soil types and propanil decomposition. The subsequent transformations of DCA determine the significance of condensation products in soils. Reaction E or binding of DCA

Table III. <sup>14</sup>C Distribution on TLC, Percent of Initial Activity

Products	Soil C								Soil E							
	Sterile				Nonsterile				Sterile				Nonsterile			
	DCA	Propanil			DCA	Propanil			DCA	Propanil			DCA	Propanil		
	6.3	630	8.5	850	6.3	630	8.5	850	6.3	630	8.5	850	6.3	630	8.5	850
Propanil	...	...	95.9	94.5	...	...	<sup>a</sup>	<sup>a</sup>	...	...	<sup>a</sup>	<sup>a</sup>	...	...	<sup>a</sup>	<sup>a</sup>
DCA	0	6.4	0	0	0	1.1	0	3.6	0	31.8	0	0	0	0.2	0	0.2
TCAB	0	0	0	0	0	0.5	0.1	0.4	0	0	0.5	1.8	1.1	1.1		
Unknown (Origin)	3.8	7.6	0	0	3.7	2.5	2.0	3.2	4.7	4.6	2.8	2.2	2.3	1.8		
1	4.8	17.2	0	0	2.9	6.5	1.5	10.6	6.4	6.6	5.7	4.4	4.3	3.4		
2	4.3	3.2	0	0	4.6	10.4	3.1	10.3	4.6	2.1	3.0	3.1	3.5	3.3		
3	1.7	3.2	0	0	2.4	10.4	3.3	10.3	3.1	2.1	3.0	3.1	15.8	1.8		
4	3.7	10.7	0	0	1.6	6.8	2.1	11.7	1.2	2.1	0.9	1.8	1.3	1.7		
5	0	4.2	0	0	0	2.4	0	7.1	0	0	0	0	0	0		
6	0	0	0	0	0	2.5	0	0	0	0	0.3	24.3	0	27.5		
Others	1.6	0.2	0	0	1.1	0.2	2.2	1.1	3.0	3.5	1.8	1.4	1.9	1.6		
Total	19.9	49.6	95.9	94.5	16.3	32.9	14.3	48.1	23.0	50.7	18.0	39.2	30.1	42.5		

<sup>a</sup>Propanil was not separated well from a metabolite, unknown 3. Consequently, activity of unknown 3 contains possibly some propanil.

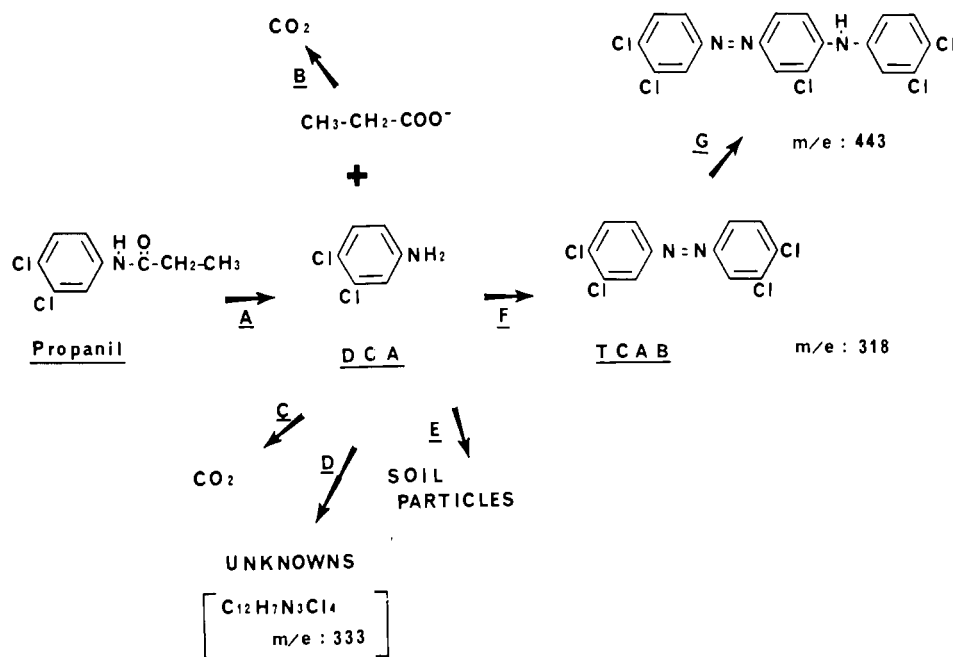


Figure 8. Proposed pathways for propanil metabolism in soils

and/or a product to soil particles is a major pathway for both soils. Binding of DCA to cellular components is also a major reaction in plants. DCA also forms a number of unknown products, some of which probably arise by chemical reactions, as demonstrated in sterile DCA-<sup>14</sup>C controls by reaction D. The major difference between soil E and C is the amount of DCA that gives rise to TCAB and related products. These are not major pathways in soil C. A further reaction product 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene (m/e = 443), has been described by Rosen *et al.* (1969) and may arise by further reaction of TCAB with DCA, as indicated by reaction G.

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