

Fate of Herbicide-Derived Chloroanilines in Soil

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Uniformly ^{14}C -ring-labeled 3',4'-dichloropropionanilide (propanil) and 4-chloroaniline were utilized for tracing the fate and persistence of herbicide-derived aromatic moieties in soil. Only low percentages of ^{14}C were lost due to mineralization and volatilization of chloroanilines. At a 5 ppm application rate, 73% of propanil ring-carbon became bound to the soil. At 500 ppm only 54% were immobilized, and a corresponding increase in solvent-extractable

metabolites was observed. Evidence is presented to support the contention that the bulk of the immobilized residues consists of intact chloroanilines that are chemically bonded to humic substances. Projection of mineralization rates observed in short term experiments indicate that complexing may extend the residual life of chloroanilines in soil to as much as 10 years.

As recently reviewed, the fate of some chloroaniline-based herbicides received much attention (Bartha and Pramer, 1970). These herbicides are biodegradable in the sense that their aliphatic moiety is readily cleaved and metabolized. Their aromatic portion, however, was shown to give rise to relatively persistent complex residues, such as chloroazobenzenes and other polymerized compounds. The yields of polyaromatic products accounted for only up to 40% of the decomposed herbicides. It was of prime interest to know whether the rest of the aromatic moieties was mineralized or whether it persisted in a yet unidentified form. The balance studies with labeled 3',4'-dichloropropionanilide (propanil) and 4-chloroaniline (CA) described here were performed to answer this question.

MATERIALS AND METHODS

Uniformly ^{14}C -ring-labeled propanil was a gift of the Rohm and Haas Co. (Philadelphia, Pa.). Uniformly ^{14}C -ring-labeled CA was purchased from Mallinckrodt/Nuclear (St. Louis, Mo.). Unlabeled propanil and CA were recrystallized from technical material and assayed for purity by melting point determination, thin-layer, and gas chromatography (Bartha, 1968). 4-(3,4-Dichloroanilino)-3,3',4'-trichloroazobenzene (Rosen *et al.*, 1970) was donated by J. D. Rosen.

Freshly collected, sieved Nixon sandy loam (pH 5.5) was treated with propanil (7.5 μCi) and CA (4.5 μCi) at 5 to 500 ppm levels. The labeled compounds were diluted with sufficient unlabeled material to reach the desired concentrations. Both propanil and CA were applied in 0.5 ml ethanol to an amount of fresh soil that was equivalent to 50 g by dry weight. After thorough mixing, the soil was moistened to 60% of its water holding capacity, and was incubated with periodic aerations at 27° C either in polyethylene film-covered beakers, or in special flasks described earlier (Bartha and Pramer, 1965). For these experiments, the flasks were fitted with a second side arm. One of the side arms was charged with 1.0 M KOH for absorption of CO_2 , the other with 0.5 M H_2SO_4 for trapping volatilized chloroanilines. The contents of the side arms were withdrawn, analyzed, and replaced at appropriate time intervals.

At the end of each experiment, the soil samples were extracted while stirring three times with a total of 250 ml acetone, and three times with a total of 250 ml benzene for removal of all solvent-soluble residues and metabolites.

The extract was analyzed by gas and thin-layer chromatography (Bartha, 1968). For quantitation of 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene, cold carrier was added. After drying with Na_2SO_4 , the soil extracts were concentrated on steam 25-fold, and 10 μl amounts were applied to the thin-layer plates. Developed by benzene for a 10 cm distance, both 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene and TCAB moved with R_f 0.91. After drying and 90° rotation, the plates were developed in the second direction by *n*-hexane for an identical distance. TCAB moved with R_f 0.52; 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene moved with R_f 0.09. The latter spot which was completely separated from all other uv-absorbing material was quantitatively transferred to counting vials.

Aliquots of the extracted soil were subjected to wet combustion (Allison *et al.*, 1965), and the $^{14}\text{CO}_2$ evolved was trapped in 0.1 M KOH. Other aliquots were subjected to caustic digestion followed by steam distillation and ether extraction of the distillate (Still and Mansager, 1969). The ether extract was concentrated on steam and analyzed for 3,4-dichloroaniline (DCA) by gas chromatography (Bartha, 1968).

Soil devoid of organic matter was prepared by H_2O_2 -treatment, as described by Bourget and Tanner (1953). Solubilization of the soil organic matter by 0.1 M neutral $\text{Na}_4\text{P}_2\text{O}_4$ solution was carried out as described by Bremner and Lees (1949). The time period of the extraction was limited to 1 hr. Radioactivity of all samples was measured with internal standardization using a Nuclear-Chicago Model 723 liquid scintillation counter. The scintillation solution (10 ml per vial) consisted of 70.0 g naphthalene, 7.0 g PPO, and 0.05 g POPOP in 1000 ml *p*-dioxane. Each of the described experiments was replicated at least once.

RESULTS

At the end of a 20-day incubation period, no remaining propanil or DCA was detected by gas chromatography in extracts of propanil-treated (5100 and 500 ppm) soil. $^{14}\text{CO}_2$ and radioactive DCA released from the 5 ppm sample are shown in Figure 1. The patterns of release from the 100 and 500 ppm samples were similar. The nature of the trapped products was substantiated by additional tests. The radioactivity of the alkaline trap could be removed by BaCl_2 precipitation. The contents of the acid trap gave positive Bratton-Marshall reactions (Sharabi and Bordeleau, 1969) that decreased in intensity and finally became undetectable toward the end of the experiment. Only 1.3–1.5% of the ring

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Table I. Metabolites of Uniformly ^{14}C -Ring-Labeled Propanil after 20 Days Incubation in Soil

Propanil ppm	Percent Ring Label Recovered				
	Volatile DCA	Carbon Dioxide	Soluble Metabolites	Soil-bound	Total Recovery
5	0.6	1.5	19.7	73.3	95.1
100	0.5	1.0	28.4	69.0	98.9
500	0.4	0.7	42.2	54.0	97.3

Table II. Metabolites of Uniformly ^{14}C -Ring-Labeled 4-Chloroaniline (CA) after 13 Days Incubation in Soil

CA ppm	Percent Ring Label Recovered				
	Volatile CA	Carbon Dioxide	Soluble Metabolites	Soil-bound	Total Recovery
5	0.1	2.1	10.8	77.4	90.4
500	0.2	0.6	40.3	53.2	94.3

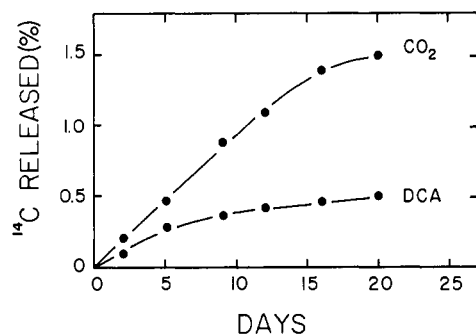


Figure 1. ^{14}C -ring-label release from propanil in soil. A 50 g by dry weight soil sample was treated with 0.25 mg (5 ppm) uniformly ring-labeled propanil. The ^{14}C evolved as CO_2 and as DCA during incubation was trapped and recorded as percent of the total radioactivity applied to the soil

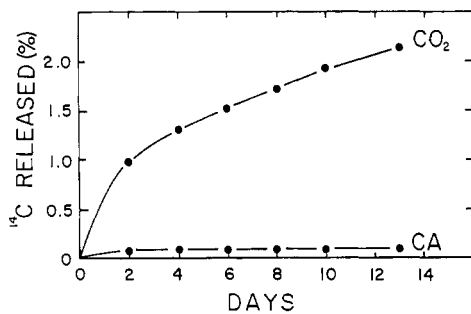


Figure 2. ^{14}C -ring-label release from 4-chloroaniline in soil. A 50 g by dry weight soil sample was treated with 0.25 mg (5 ppm) uniformly ring-labeled 4-chloroaniline (CA). The ^{14}C evolved as CO_2 and as CA was trapped and recorded as percent of the total radioactivity applied to the soil

carbon was mineralized and 0.4–0.6% was volatilized as DCA in 20 days. The solvent-extractable and soil-bound portions of the radioactivity are shown in Table I. High herbicide concentrations favored the formation of solvent-extractable products; lower ones favored immobilization of the label.

From the 500 ppm treatment, 40% of the soil-bound aromatic moiety could be recovered as unchanged DCA following caustic digestion of the previously extracted soil. After steam distillation, no significant radioactivity was retained by the solubilized soil organic matter or in the insoluble residue.

As the digestion, distillation, extraction, and concentration procedures resulted in 50–70% loss of added DCA, the 40% recovery is clearly a minimal value. It appears that most of the immobilized residue consists of 3,4-dichloroaniline moieties that are bound to unidentified sites of the soil.

Caustic digestion cleaves the amide bond of propanil. To ascertain that the observed DCA was, in fact, not derived from soil-bound propanil, the previous experiment was repeated using a free chloroaniline. Since uniformly labeled DCA was not obtainable, CA was utilized for the same purpose. The metabolism of this compound proceeded in a pattern similar to that of propanil-derived DCA. Figure 2 shows that volatile CA was lost only in the first 2 days of the experiment. Presumably within this time most CA was converted to less volatile compounds. Mineralization of CA-carbon proceeded somewhat faster than that of DCA. Again, high CA concentrations favored the formation of solvent-extractable metabolites; low ones favored the formation of soil-bound residues (Table II). The described similarities suggest that in the previous experiment not propanil but its degradation product DCA was the soil-bound compound.

An attempt was made to elucidate the mechanism of chloroaniline immobilization by soil. The failure of organic solvents, H_2O , 10% NaCl solution, and 0.1 M HCl to elute any substantial portion of the bound chloroanilines counterindicated binding by physical adsorption alone. In contrast, alkaline hydrolysis that is known to solubilize soil organic matter succeeded in releasing the bound radiocarbon. The amounts released correlated with the severity of the treatment and with the amount of organic matter solubilized. Pretreatment of soil by H_2O_2 strongly reduced its capacity to immobilize CA, although this treatment can be expected to free new sites for physical adsorption. All this suggested that the immobilized chloroanilines were chemically bound to humic substances, and physical adsorption was, at most, a secondary mechanism. This view was further substantiated by the fact that treatment with neutral pyrophosphate solution solubilized 23% of the radioactivity remaining in the previously solvent extracted soil. This figure agrees well with the reported average 25% efficiency of this treatment in solubilization of soil organic matter (Mortensen, 1965).

Linke (1970) identified in extracts of propanil-treated soil 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene, a compound previously reported by Rosen *et al.* (1970) to be a photochemical transformation product of DCA. For lack of a suitable gas chromatographic technique, no quantitative data were available on production of 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene in soil. The soil extracts containing the labeled propanil metabolites offered an opportunity for the quantitative determination of this product after its separation by two-dimensional thin-layer chromatography from other components of the extract. In the soil samples treated with 0.25 mg (5 ppm) and 25 mg (500 ppm) propanil, the amount of 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene formed was calculated to be 7.5 and 146.0 μg , respectively. At both propanil concentrations, this metabolite was responsible for 2% of the total solvent-extractable radioactivity.

DISCUSSION

While this article was in preparation, Chisaka and Kearney (1970) published an extensive study on the metabolism of ^{14}C -labeled propanil and DCA in several soils. The results on ring carbon mineralization and on proportions of solvent-extractable and soil-bound residues reported here confirm and

extend their findings. In addition, this study evaluated losses of ring-carbon due to volatility of free DCA, and found them to have little significance in closed laboratory systems. However, under field conditions such losses are expected to be substantial.

Results of Chisaka and Kearney (1970) and those reported here both show a pronounced concentration dependence of solvent-extractable residue formation from propanil. Since formation of solvent-extractable residues requires the coupling of two or more free chloroaniline moieties, their concentration in soil at any one time determines the proportions of residues formed. At high concentrations, polymerization reactions predominate, but at low concentrations binding sites on the soil organic matter compete successfully for the chloroaniline moieties. In consequence, the proportion of solvent-extractable metabolites decreases, and most chloroaniline becomes soil-bound. This phenomenon may explain the paradox that although degradation of phenylurea herbicides involves the release of chloroanilines (Dalton *et al.*, 1966), chloroazobenzene residues could not be detected (Bartha and Pramer, 1969; Belasco and Pease, 1969). In comparison to many acylanilides, chloroaniline release from phenylureas is very slow, and it is likely that in consequence the bulk of their chloroaniline moieties becomes soil-bound.

4-(3,4-Dichloroanilino)-3,3',4'-trichloroazobenzene is not a major transformation product of propanil in soil, but like TCAB it is quite recalcitrant. Rosen and Siewierski (1971) recovered it from soil quantitatively at the end of a 60-day incubation period.

Chisaka and Kearney (1970) focused their attention on solvent-extractable metabolites and succeeded in identifying new products, but they made no attempt to characterize the nature of the soil-bound residue. Since at herbicide concentrations approaching the recommended field application rates, up to 70–80% of the aromatic herbicide moiety were shown to be rendered nonextractable, the chemical nature, biological activity, and the persistence of this material have become questions of major importance. The evidence presented here strongly suggests that the bulk of the immobilized aromatic propanil moiety is a humus–3,4-dichloroaniline complex. The undefined nature of humic compounds renders the chemical characterization of this complex extremely difficult. The

biological activity of DCA bound in this manner is, at this time, unknown. The binding of chloroanilines to soil organic matter and the resulting increase in their persistence has an interesting counterpart in plant herbicide metabolism. Still and Mansager (1969) detected bound DCA residues in most commercial rice (*Oryza sativa*) grain samples they have examined.

Degradation of humic DCA complexes can be expected to depend on the turnover rate of the humus which, in most soils, is a rather slow process. A rough estimate of persistence can be projected from the ^{14}C mineralization rates observed here and in the experiments of Chisaka and Kearney (1970). Assuming that in the later stages of the experiments all trapped $^{14}\text{CO}_2$ arose from degradation of such humic DCA complexes, and assuming further that the mineralization rate will continue to be linear, in the various soils tested 4–10 years would be required for their complete elimination. Considering the degradation of the parent herbicide within 2–3 weeks, such recalcitrance of the complexed DCA moieties was not expected.

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