Degradation of Carbamate Herbicides in Soil

D. D. KAUFMAN

Certain *N*-phenyl-, thio-, dithio-, and methylcarbamates are finding wide application as pre- and/or postemergent herbicides for use in agronomic and horiticultural crops. Volatilization of some carbamate herbicides from moist soil surfaces constitutes a major source of dissipation. Persistence of these carbamates is increased by application to dry soil surfaces or by soil incorporation. Environmental factors increasing microbial activity in soil generally decrease the persistence of carbamate herbicides. Soil microorganisms that metabolize certain carbamate herbicides have been isolated and identified. Degradation pathways have been proposed. In most of these degradation reactions, the initial cleavage of the molecule occurs at the ester linkage. Properties of hydrolytic enzymes isolated from adapted soil microorganisms have been determined. Enzymatic hydrolysis of some carbamates can be correlated to substrate acidity, and rate differences explained by consideration of certain steric and electronic properties of the carbamate.

Friesen (1929) reported that certain carbamate compounds retarded the germination of wheat and oats and resulted in abnormal seedling development. Since then numerous workers have investigated the effects of various carbamates upon plants (Haubein and Hansen, 1965; Herrett and Berthold, 1965; LeFevre ,1939; Shaw and Swanson, 1953; Simonet and Guinochet, 1939; Swingle and Mayer, 1944). Today, several carbamates (Table I shows common names) are used at rates of 1 to 9 pounds per acre as selective pre- and/or postemergent herbicides for control of weeds in several vegetable, forage, and field crops under a wide range of edaphic and climatic conditions. Carbamate herbicides, in general, are becoming increasingly important owing to their low mammalian toxicity, relatively short persistence in soils, ease of degradation by nontarget organisms, and comparatively innocuous degradation products.

Several processes are known to determine the fate of herbicides in soil. These include leaching, adsorption, volatilization, photodecomposition, chemical reaction, and absorption and metabolism by plants and soil microorganisms. Physical and/or chemical properties of herbicides, soils, and environmental variables act directly and interact in many combinations to influence herbicidal activity. One or two of these processes often predominate in effecting loss of a particular herbicide or class of herbicides from soil.

Although carbamate herbicides generally are not particularly persistent in soil, relatively little research has been done to determine their possible degradation products in soils. Volatilization has been an important factor in dissipation of several carbamate herbicides (Freed and Montgomery, 1963; Gray and Weierich, 1965; Parochetti and Warren, 1966; Taylorson, 1966), particularly from moist surfaces. Immediate incorporation or application to dry surfaces has enhanced both their residual life in soil and herbicidal activity (Hauser, 1965; Havis *et al.*, 1959; Klingman *et al.*, 1961). Under conditions where volatility was a minor factor, extensive losses indicate that microbiological decomposition was an important factor (Danielson *et al.*, 1961; Kaufman and Kearney, 1965; MacRae and Alexander, 1965; Sheets, 1958). Environmental factors conducive to increased microbial activity—e.g., high organic matter levels, increased aeration, and increased soil moisture content and temperatures—tend to reduce persistence of carbamate herbicides in soil (DeRose, 1946; Dubrovin, 1962; Freed, 1951; Kaufman and Kearney, 1965; Kaufman and Kearney, 1966; Newman *et al.*, 1948; Ogle and Warren, 1954; Stevens and Carlson, 1952). With few exceptions, however, the products resulting from microbial degradation are not known. The purpose of this paper is to review the present knowledge of carbamate herbicide degradation in soil and to discuss possible degradation mechanisms.

Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

Phenylcarbamate Herbicides

The *N*-phenylcarbamates, acylanilides, and phenylureas are members of a large class of compounds known as phenylamides. Several of these compounds have found wide application as herbicides in agronomic crops. All have the general formula C_6H_5—NH—CO—R. In the acylanilides, R is an alkyl group; in the phenylureas, R represents an alkylamino group; whereas in the phenylcarbamates, R is an alkoxy group. Although structurally similar, their activity and degradation is quite different.

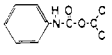
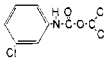
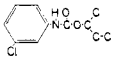
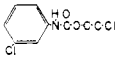
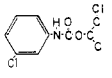
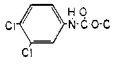
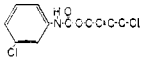
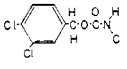
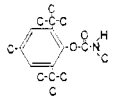
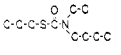
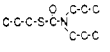
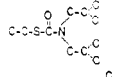
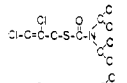
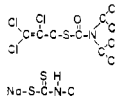
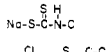
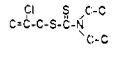
More is known concerning the fate of phenylcarbamate herbicides in soil than that of any other group of carbamate herbicides. Evidence obtained by many workers (DeRose, 1946; Dubrovin, 1962; Freed, 1951; Kaufman and Kearney, 1965; Newman *et al.*, 1948; Ogle and Warren, 1954; Stevens and Carlson, 1952) has indicated that soil microorganisms are responsible for degradation of phenylcarbamate herbicides. Dubrovin (1962) observed that 4-chloro-2-butynyl-*N*-3-chlorophenylcarbamate (barban) disappeared more rapidly in nonautoclaved soil than in autoclaved soil. Freed (1951) and Newman *et al.* (1948) obtained similar results with isopropyl *N*-phenylcarbamate (IPC). Environmental factors favoring microbiological activity reduce

the persistence of both IPC and isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) in soil (Dubrovin, 1962; Freed, 1951; Newman *et al.*, 1948; Ogle and Warren, 1954; Stevens and Carlson, 1952). Differences in soil persistence also may be attributable to structural variations. DeRose (1946) found CIPC to be more persistent in soil than IPC. Moore *et al.* (1953) compared the residual life of isopropyl *N*-(3-methylphenyl)carbamate, isopropyl *N*-(2-methoxy-5-chlorophenyl)carbamate, and BCPC with that of CIPC. All three compounds showed greater residual life than CIPC. Of the three chemicals compared with CIPC, isopropyl *N*-(3-methylphenyl)carbamate was the least persistent.

Kaufman and Kearney (1965) isolated and identified several soil microorganisms capable of degrading CIPC and CEPC. Soil bacteria effective in degrading CIPC included *Pseudomonas striata* Chester, *Flavobacterium* sp., *Agrobacterium* sp., and *Achromobacter* sp. Isolates effective in degrading CEPC included an *Achromobacter* sp. and an *Arthrobacter* sp. Although isolated and effective on different carbamate substrates, the two *Achromobacter* isolates appeared identical. All isolates were tested for their ability to degrade CIPC and CEPC analogs. CEPC isolates readily metabolized CIPC, and, conversely, CIPC isolates metabolized CEPC, although much more slowly than the former. All isolates degraded IPC more readily than either CIPC or CEPC. Low concentrations of 3-chloroaniline were also readily metabolized by all isolates.

Some elucidation of products of phenylcarbamate

Table I. Chemical Structure, Chemical Name, and Common Name or Designations of Carbamate Herbicides

Chemical Structure	Chemical Name	Common Name or Designation
	Isopropyl *N*-phenylcarbamate	IPC
	Isopropyl *N*-(3-chlorophenyl)carbamate	CIPC
	*sec*-Butyl *N*-(3-chlorophenyl)carbamate	BCPC
	2-Chloroethyl *N*-(3-chlorophenyl)carbamate	CEPC
	1-Chloro-2-propyl *N*-(3-chlorophenyl)carbamate	CPPC
	Methyl 3,4-dichlorocarbanilate	Swep
	4-Chloro-2-butynyl *m*-chlorocarbanilate	Barban
	3,4-Dichlorobenzyl *N*-methylcarbamate	UC-22463
	2,6-Di-*tert*-butyl-*p*-tolyl *N*-methylcarbamate	Terbutol
	*S*-Propyl butylethylthiocarbamate	Pebulate
	*S*-Propyl dipropylthiocarbamate	Vernolate
	Ethyl *N*,*N*-dipropylthiolcarbamate	EPTC
	Ethyl *N*,*N*-diisobutylthiolcarbamate	R-1910
	*S*-2,3-Dichloroallyl *N*,*N*-diisopropylthiolcarbamate	Diallate
	*S*-2,3,3-Trichloroallyl *N*,*N*-diisopropylthiolcarbamate	Triallate
	Sodium *N*-methyldithiocarbamate	SMDC
	2-Chloroallyl diethyldithiocarbamate	CDEC

metabolism in soil has been made. Microbial degradation of CIPC and CEPC in perfused muck soil was measured both by microbial production of 3-chloroaniline, and by liberation of chloride ion (Kaufman and Kearney, 1965). Production of 3-chloroaniline and chloride ion was detected in both CIPC- and CEPC-treated soils. In both instances, 3-chloroaniline disappeared as chloride ion content of the perfusate increased. Metabolism of both isopropyl-C^{14} and uniformly ring-C^{14}–labeled CIPC by soil microorganisms has been studied (Kearney and Kaufman, 1966; Kearney and Kaufman, 1965). $C^{14}O_2$ was evolved from both labeled forms of the molecule in perfused muck soil. $C^{14}O_2$ evolution from isopropyl-C^{14}–labeled CIPC preceded $C^{14}O_2$ evolution from ring-C^{14}–labeled CIPC. This, and the detection of 3-chloroaniline (Kaufman and Kearney, 1965) in the early stages of microbial degradation would indicate an initial attack on either the amide or ester linkage or alkyl group of the molecule, followed by metabolism of the isopropyl and 3-chloroaniline moieties. Under pure culture conditions, however, only the ring portion of the molecule gave rise to $C^{14}O_2$ when incubated with *Pseudomonas striata* Chester (Kearney and Kaufman, 1966). The isopropyl moiety presumably was lost from the culture solutions as some volatile component and was not recoverable as $C^{14}O_2$.

Enzymatic hydrolysis of CIPC by cell free extracts of *P. striata* has also been reported (Kearney, 1965; Kearney and Kaufman, 1965). The isolated enzyme catalyzed the hydrolysis of CIPC to 3-chloroaniline, CO_2, and isopropyl alcohol. The enzyme also catalyzes the decomposition of a large variety of other alkyl *N*-phenylcabamate compounds (Kearney, 1965; Kearney, 1967). Although the enzyme also hydrolyzed two acylanilides to the corresponding anilines, it was inactive on the methylcarbamate insecticide carbaryl, 1-naphthyl *N*-methylcarbamate, and phenylurea herbicide monuron, 3-(*p*-chlorophenyl)-1,1-dimethylurea. Other properties of this enzyme such as substrate specificity, inhibition, metal ion requirement, and pH optimum have been reported (Kearney, 1965).

Whether enzymatic cleavage proceeds by hydrolysis of the amide bond or by hydrolysis of the ester linkage is not clear. Preliminary evidence (Kearney, 1965; Kearney and Kaufman, 1965), however, indicates that the ester linkage is the primary site of attack. Regardless of which bond is broken first, the products resulting from hydrolysis of CIPC would be the same—i.e., 3-chloroaniline, CO_2, and isopropyl alcohol. On the basis of the information available, the pathway illustrated in Figure 1 has been proposed (Kearney, 1965; Kearney *et al.*, 1965; Kearney and Kaufman, 1965).

Available evidence indicates that degradation of other phenylcarbamate herbicides proceeds by a similar mechanism. Chin *et al.* (1964) observed that trace amounts of Swep were hydrolyzed in soil to 3,4-dichloroaniline. In plants, however, most of the herbicide was immobilized and bound as a Swep-lignin complex. Such a complex also might occur in soil where lignin or ligninlike substances occur as a portion of the soil organic matter. Kaufman (1967) has observed that the corresponding anilines were produced during

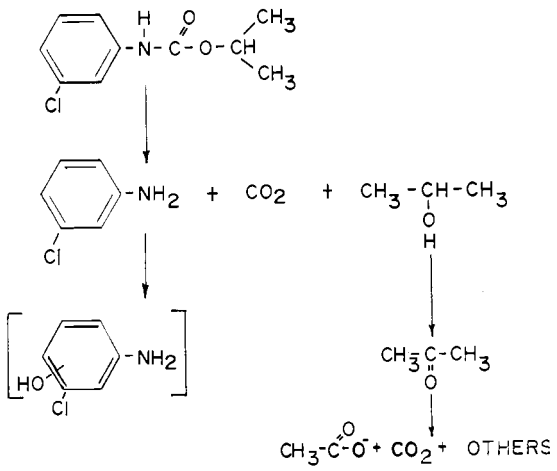


Figure 1. Proposed pathway of microbial degradation of CIPC (Kearney and Kaufman, 1966)

microbial degradation of a large number of alkyl *N*-phenylcarbamate herbicides in perfused soils, including IPC, CIPC, BCPC, CEPC, and CPPC.

This mechanism appears to be in contrast to that proposed in plants. Baskakov and Zemskaya (1959) suggested herbicidal carbamates are metabolized to their *N*-hydroxy derivatives and thereby activated. Stefange and DeRose (1959), however, in comparative tests between isopropyl carbamates and their *N*-substituted derivatives on oat plants, observed that a halogen atom is not necessary on the nitrogen to preclude strong phytotoxic effects. Substitution with other groups altered, but did not destroy, the phytotoxicity.

Products resulting from the degradation of aniline in soil have not been identified or reported. Aniline degradation by soil microorganisms (Chambers *et al.*, 1963; Kaufman, 1967; Kaufman and Kearney, 1965) and sewage microflora (Heukelekian, 1955) has been observed, however. Presumably a hydroxylation, resulting in the formation of a phenol, would occur prior to ring cleavage. 2-Chloro-4-aminophenol has been identified as a metabolite of barban in plants (Riden and Hopkins, 1962). Whether or not conversion of anilines to their corresponding phenol occurs in soil is not known. Considerable information has been gathered concerning the oxidative breakdown of phenols by microorganisms (Stanier, 1955; Stanier, 1950). Typically, microorganisms convert phenols to the corresponding catechols, followed by muconic acid, lactone, and ketoadipic acid, respectively. The possible formation of glucuronides, and *N*-sulfates of sulfamate conjugates cannot be excluded, however (Williams, 1959).

Certain anilines may be quite persistent in soil. Alexander and Lustigman (1966) observed that *o*-, *m*-, and *p*-chloroanilines resisted microbial attack for periods of more than 64 days. Kearney and Kaufman (1965), however, observed that in studies with CIPC-adapted microorganisms, *m*-chloroaniline did not accumulate, about half of the C^{14} from uniformly ring-labeled CIPC having been recovered as $C^{14}O_2$ after a 3-hour incubation period. Kaufman (1967) also ob-

served that at low concentrations, aniline and *m*-chloroaniline were readily degraded by soil microorganisms, whereas *p*- and *o*-chloroanilines were more resistant to microbial attack. Dichloroanilines were generally resistant to microbial degradation. The corresponding chloroaniline has also been shown to be a residual product of the phenylurea herbicides monuron, 3-(*p*-chlorophenyl)-1,1-dimethylurea and diuron, 3-(3,4-dichlorophenyl)-1,-1-dimethylurea (Dalton *et al.*, 1965). Inasmuch as chloroanilines have shown some phytotoxicity (Shaw, 1966), accumulation of these materials in soil should be avoided. Degradation of chloroanilines in soil warrants further investigation.

Although phenylcarbamate degradation appears to occur principally by hydrolysis of the ester linkage, other sites such as the ring, the amide linkage, or the alkyl group could be primary sites of attack. Riden and Hopkins (1962) considered four sites where initial attack on barban could occur: the aromatic ring, the carbamate linkage, the triple bond, and the chloromethyl group. No evidence has been presented as yet, however, to support the occurrence of these degradation mechanisms in soil. Phenylcarbamates with unsubstituted rings may be degraded by different pathways than those with substituted rings. According to Williams (1959), the initial reaction in degradation of ethyl *N*-phenylcarbamate is hydroxylation of the phenyl ring, resulting in the formation of ethyl *N*-4-hydroxyphenylcarbamate. Crick and Jackson (1952) obtained evidence indicating that ethyl *N*-phenylcarbamate was not converted to aniline, whereas ethyl *N*-4-iodophenylcarbamate yielded 4-iodoaniline. Halogenation of the para position evidently blocked hydroxylation, permitting attack at the ester linkage as the alternative reaction, the amide linkage being somewhat more stable.

The effect of structure on degradation of numerous phenylcarbamates has been studied (Kaufman, 1967; Kearney, 1965). In cell-free systems, enzymatic hydrolysis rates of several phenylcarbamates was positively correlated with relative acidities of the substrates, and rate differences could be explained by considering certain steric and electronic properties of the phenylcarbamates (Kearney, 1965; Kearney, 1967). Inductive effects with the molecule that tended to lower electron density of the carbonyl carbon would facilitate enzymic hydrolysis of the compound, owing to the enhanced susceptibility of the carbonyl carbon to attack (Kearney, 1965). A good correlation was established between rates of hydrolysis and relative acidity for seven of the nine phenylcarbamates examined. Hydrolytic rates increased with increased relative acidity. Exceptions occurred with the 1-chloroisopropyl and 1,3-dichloroisopropyl *N*-phenylcarbamates where hydrolysis rates decreased with increased relative acidities, respectively. Steric inhibition of the enzymatic attack was cited as the probable cause of this effect.

More recently, Kearney (1967) examined the effect of various meta substituents on enzymic hydrolytic rates of several additional phenylcarbamates. Again the inductive effects of the meta substituents correlated positively with HNP or relative acidity. The meta substituted nitrophenylcarbamate was the most acidic of the meta series, and consequently the most readily hydrolyzed, followed by aceto-, chloro-, methoxy-, and hydrogen-substituted phenylcarbamates. Isopropyl *N*-phenylthionocarbamate was the most acidic carbamate examined (Kearney, 1965; Kearney, 1967) and the most rapidly hydrolyzed. This observation was attributed to the fact that the sulfur exerts a strong influence on the amide hydrogen, thus increasing the protons' leaving ability.

Positional effects of ring substituents were also important in governing hydrolytic rates. Although the isopropyl ester of 2-chlorophenylcarbamate was more acidic than CIPC, its rate of hydrolysis was somewhat less than CIPC. In this case, the close proximity of the ortho-substituted chlorine to the carbonyl carbon which is attacked indicates that steric hindrance may decrease enzymic cleavage. The ability of the para-substituted nitro group to pull electrons away from the reactive site of the molecule also influenced the relative acidity of the compound, and hence the ease with which it is hydrolyzed. The *p*-nitro–substituted phenylcarbamate was hydrolyzed faster than the *m*-nitro–substituted compound. Here, the strong electron-withdrawing power of the para-situated nitro group translated through the system of alternate single and double bonds reduces the charge density in the vicinity of the carbonyl group, thus influencing the relative ease with which it is hydrolyzed (Kearney, 1967).

The size of the molecule also affects its hydrolytic rate (Kearney, 1967). The bulky naphthyl group of isopropyl *N*-naphthylcarbamate had a decreasing effect on the hydrolytic rate, when compared to IPC. As the size of the alkyl group increases from ethyl to *N*-propyl to isopropyl to the benzyl group, the hydrolytic reaction rate decreased. The steric effects, or unique spatial relationships imposed by the various sized alkyl groups, indicate that enzyme-substrate fit also may be important in determining the hydrolytic rates.

These data show that at least in relatively pure chemical systems, enzymatic hydrolysis of structurally related phenylcarbamates follows relative rates which may be predictable through consideration of classical organic mechanisms. Although such studies may be of value as an indicator of relative biodegradability by a given soil microorganism, final consideration and evaluation of structural effects must involve the intact microorganism.

Structural effects on degradation of certain phenylcarbamates by intact microbial cells have been determined (Kaufman, 1967). The number, type, and position of substituents on the ring affected not only the decomposition of the basic ring structure, but the removal of the side chain as well. Variations such as size, halogenation, and type of linkage of the alkyl group to the ring also affected the rate of microbial degradation. At present, little is known concerning influence of chemical structure on adsorption and absorption of pesticides and their subsequent metabolism by soil microorganisms.

Methylcarbamate Herbicides

Methylcarbamates are newcomers to the field of carbamate herbicides. Two of the most promising compounds are Terbutol (Haubein and Hansen, 1965) and

UC-22463 (Herrett and Berthold, 1965). Terbutol has shown preemergence crabgrass activity, whereas UC-22463 is a selective preemergence herbicide active against both grass and broadleaf weeds. The activity of Terbutol or its analog is specific to the type of substituents in the 2-, 4-, and 6-positions of the phenyl group. It has remained active in moist soil in greenhouse for more than 2-month periods and has proved relatively resistant to leaching (Haubein and Hansen, 1965). Loss by volatility was considered negligible. The degree and type of activity of UC-22463 or its analogs vary markedly with variation of the *N*-substituent (Herrett and Berthold, 1965). Owing to its relatively recent appearance in the herbicide field, little or nothing is known about its persistence in soil under field or greenhouse conditions.

Although little is known about the degradative mechanisms of methylcarbamate herbicides per se, certain analogies can be made with degradative mechanisms observed with certain methylcarbamate insecticides. Spencer (1965) suggested that methylcarbamates were subject to several possible points of metabolic attack, including hydrolysis of the ester linkage and oxidation of the *N*-methyl group, a ring substituent, or the ring itself. Casida (1963) reported that degradation generally involved an initial esterase attack followed by degradation of the hydrolyzed fragments. Earlier observations by Casida and Augustinsson (1959) indicated the site of attack on *N*-methylcarbamates was at the ester rather than the amide bond, since both I-naphthyl and *p*-nitrophenyl *N*-methylcarbamate and the acetate of 1-naphthol or *p*-nitrophenol were cleaved by albumin. Products resulting from an initial attack at the ester linkage would probably be the enol, carbonate ion, and alkylamine, with methylisocyanate as a possible intermediate according to Aeschlimann and Reinert (1931) and Casida *et al.* (1959, 1960). Methylcarbamic acid also might be formed as an intermediate, rather than the methylisocyanate. In either case, the subsequent products would be methylamine and CO_2 (Figure 2).

Whether or not the degradation of Terbutol would occur by this mechanism is questionable, because of possible steric inhibition by the presence of the *tert*-butyl groups in close proximity to the ester linkage. Evidence of such a steric effect has been obtained by Kaufman and Kearney (1966). They observed a competitive inhibition between numerous methylcarbamate compounds and CIPC for a CIPC-hydrolyzing enzyme. Failure of Terbutol to act in a competitive fashion was attributed to steric inhibition. Because of this apparent steric inhibition of hydrolysis of the ester linkage, Terbutol probably will be more persistent in soil than most other carbamate herbicides.

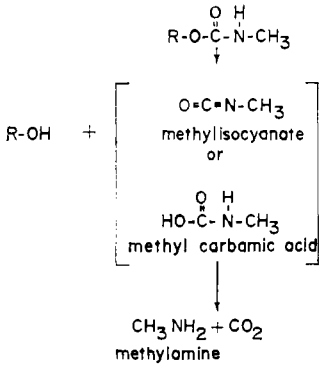


Figure 2. Probable degradation pathway of methylcarbamate herbicides

Thio- and Thiolcarbamate Herbicides

Several thiocarbamate compounds have found use as pre- and/or postemergence herbicides on certain field and vegetable crops, and forage legumes. Comparatively little research has been done with the thiocarbamates to determine their degradation products in soil. Volatility is a major factor in the behavior of many thiocarbamates when applied to soil surfaces (Ashton and Dunster, 1961; Ashton and Sheets, 1959; Fang *et al.*, 1961; Gray and Weierich, 1965; Hauser, 1965; Horowitz, 1966; Sheets, 1959). Retention of EPTC (Fang *et al.*, 1961) and pebulate (Horowitz, 1966) was greater on dry soils than wet soils, and weed control was frequently poor when applications were made to moist soils. Under conditions where pebulate was applied on dry soil without incorporation, it persisted for 1 to 2 months in soil. Adsorption to dry soil probably prevents loss of EPTC vapors (Ashton and Sheets, 1959). Water in films surrounding soil particles may prevent adsorption of the relatively insoluble EPTC by competition or a shielding of the adsorption sites (Sheets *et al.*, 1964). Loss of EPTC (Ashton and Dunster, 1961; Hauser, 1965), pebulate, and vernolate (Hauser, 1965) by volatilization is apparently reduced by soil incorporation since herbicidal activity was increased by this practice.

Available data indicate that soil microorganisms contribute significantly to the disappearance of thiocarbamate herbicides when incorporated in soil (Danielson *et al.*, 1961; Kaufman, 1966; MacRae and Alexander, 1965; Sheets, 1958). Danielson *et al.* (1961) compared the persistence of EPTC and CDEC with several phenylcarbamate herbicides. Although EPTC was less persistent in environmental conditions conducive to increased microbial activity, persistence was noticably affected by incorporation, solvent carrier system, and surfactants. EPTC persisted much longer in autoclaved soil than nonautoclaved soil (Sheets, 1959), EPTC being inactivated about $^1/_3$ as rapidly in autoclaved as in nonautoclaved soil. Therefore, microbial degradation was a major factor of EPTC loss from soils when incorporated, and its activity might be greatly prolonged under field conditions unsuitable for microbial growth. Burschel and Freed (1959) also concluded that microbiological decomposition was an important factor in persistence of EPTC under conditions where volatility was a minor factor.

Kaufman (1966) (Figure 3) noted that although bioassay determinations revealed a rapid disappearance of EPTC, the rate of $C^{14}O_2$ evolution from ethyl-C^{14}–labeled EPTC applied to soil occurred more slowly. The difference between the slow release of $C^{14}O_2$ from labeled EPTC and the more rapid microbial inactivation of the herbicide, as revealed by bioassay, indicates detoxication without appreciable mineralization of the

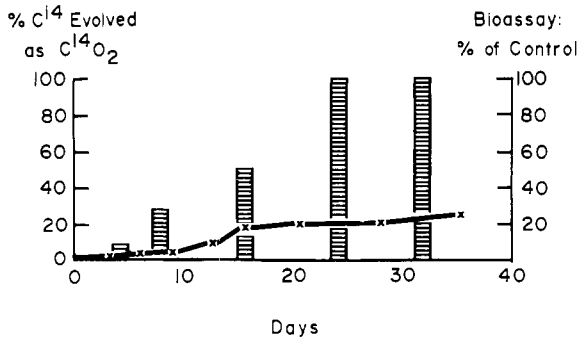


Figure 3. Persistence and degradation of EPTC in a Hagerstown silty clay loam soil

Bioassay (bar graph) determined with oat seedling measurements after a 3-week growing period

ethyl moiety of the molecule. Similar results were obtained earlier by MacRae and Alexander (1965). Since numerous compounds structurally related to EPTC have significant effect on microbial activity, they also investigated the effect of several thiocarbamate herbicides on the growth of a *Bacillus* sp. They observed that comparatively low levels of thiocarbamate compounds were toxic to *Bacillus* sp. in vitro. The *n*-propyl diethyl, *tert*-butyl ethyl *n*-butyl, and *n*-butyl di-*n*-butyl thiolcarbamates were toxic at concentrations of 5 p.p.m., whereas EPTC, *n*-amyl diethyl, ethyl di-*n*-butyl, ethyl ethyl *n*-butyl, propyl ethyl *n*-butyl (pebulate), *n*-propyl di-*n*-propyl (vernolate), ethyl diallyl, ethyl *n*-propyl allyl, allyl di-*n*-propyl, *tert*-butyl di-*n*-propyl, and 2-chloroallyl di-*n*-propyl thiocarbamates were toxic at 10 p.p.m. Although other data indicate that somewhat higher concentrations are needed for toxic effects to be observed in vivo on specific groups of soil microorganisms, it is quite conceivable that 5.0- and 10.0-p.p.m. concentrations may be attained in soil under certain conditions, and that these compounds may, at least temporarily, retard the development of populations of microorganisms effective in their degradation.

Isolation or identification of soil microorganisms capable of degrading thiocarbamate herbicides has not been reported, nor has the mechanism of microbial degradation been established. Several sites of attack are conceivable—e.g., the alkyl groups, the amide linkage, or the ester linkage. The initial site of attack is probably determined, to some extent, by the nature of the alkyl groups attached to the carbamate linkage In the presence of relatively small alkyl groups, the thiocarbamate molecule is probably hydrolyzed at the ester linkage, resulting in the formation of the corresponding mercaptan, alkylamine, and CO_2. The mercaptan could then be converted to an alcohol by a transthiolation and further oxidized to an acid form prior to entering the metabolic pool. Such a mechanism has been proposed in plants and/or animals with both EPTC (Nalewaja *et al.*, 1964) and pebulate (Fang and George, 1961; Fang *et al.*, 1964) (Figure 4). In studies with ethyl-C^{14}–labeled EPTC, in which the label was on the carbon atom adjacent to the sulfur atom, direct incorporation into the sulfur-containing amino acids cystine and cysteine was

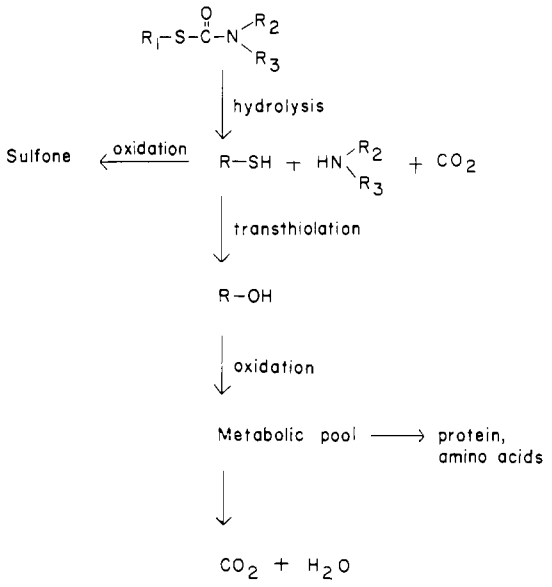


Figure 4. Probable degradation pathway of thiocarbamate herbicides

expected but not found (Nalewaja *et al.*, 1964). This, along with liberation of $C^{14}O_2$, indicated that there was a cleavage between the sulfur atom and the ethyl group. Whether or not this cleavage occurred subsequent to the formation of a mercaptan, or during the initial attack on the intact carbamate molecule, was not determined. Nalewaja *et al.* (1964) concluded, however, that the lack of radioactivity in sulfur amino acids ruled out direct incorporation of ethyl mercaptan. This supports the conclusion of Fang and Yu (1959) who stated that the sulfur was oxidized to sulfate and then remetabolized, after finding S^{35} from EPTC-S^{35} distributed in all sulfur-containing compounds.

Certain mercaptans and sulfides, besides those related to cysteine and methionine, occur in nature, but little is known concerning their formation or degradation. Mercaptan formation from sulfur-containing amino acids is common among soil microorganisms (Alexander, 1961; Fruton and Simmonds, 1959). Mercaptans and H_2S are frequently formed under anaerobic conditions as a result of microbial degradation of sulfur-containing compounds (Alexander, 1961). Metabolism of methyl mercaptan is similar to that of methyl alcohol which could arise from the mercaptan by transthiolation (Williams, 1959). Carbon-14 from labeled methyl mercaptan has appeared as the beta carbon of serine and in the methyl group of methionine, choline, and creatine (Canellakis and Tarves, 1953). The metabolism of ethyl mercaptan per se has not been reported, but its fate has been deduced from that of compounds such as diethylsulfide, which are converted to it (Williams, 1959). The ethyl mercaptan formed from the disulfide is presumably methylated in vivo to ethyl methyl sulfide which is subsequently oxidized to the sulfone via a sulfoxide. The alternate possibility is conversion of the ethyl mercaptan to ethanol by a transthiolation, with the sulfur ultimately appearing as sulfate, and the ethanol entering the two-carbon metabolic pool as acetic acid. Under aerobic conditions, microbial metabolism of organic

sulfur compounds frequently terminates in the formation of inorganic sulfate (Alexander, 1961).

The fate of the aliphatic amines formed in the degradation of thio- and dithiocarbamates in soil is also unknown. Short chain aliphatic amines are mainly degraded to the corresponding carboxylic acid and urea (Williams, 1959), the intermediate compounds being the corresponding aldehyde and ammonia. Alcohol formation by reduction of the aldehyde is theoretically possible but has not been observed. Secondary amines are more resistant to metabolic change (Williams, 1959). Dealkylation to the corresponding aldehyde and primary amine is probably involved. Microbial degradation of aliphatic amines probably occurs slowly, since they do have some antimicrobial activity (Eckert and Kolbezen, 1963; Fuller, 1942; Tilley and Schaffer, 1928).

The occurrence of such a mechanism (Figure 4)—i.e., hydrolysis, followed by transthiolation—could explain results observed in persistence and degradation studies with diallate in the author's laboratory. In two separate experiments, a bioassay of diallate-treated soils indicated a gradual partial loss of phytotoxicity followed by a temporarily increased phytotoxicity, with a subsequent complete disappearance of phytotoxicity (Figure 5). Hydrolysis of the ester linkage and transthiolation of the allylic group would result in the formation of 2,3-dichloroallyl alcohol. Although the phytotoxicity of 2,3-dichloroallyl alcohol is not known, allyl alcohol is highly phytotoxic and is frequently used as an herbicide. The conversion of allyl alcohol to lactic acid and subsequent metabolism by soil microorganisms has been reported previously (Jensen, 1961).

Preliminary results indicate some correlation between chemical structure and soil persistence of thiocarbamate herbicides. Thiocarbamates having branched-chain alkyl groups were more persistent in soil than those having similar straight chain alkyl groups. Similar results have been obtained with various alkyl *N*-phenylcarbamate herbicides (Kaufman, 1966)—i.e., compounds having branched alkyl groups were more persistent than those having the corresponding straight chain alkyl groups (Figure 6). Evidence obtained with thiocarbamates, however, is too limited to correlate accurately structural features with soil persistence.

Dithiocarbamate Herbicides

The dithiocarbamate herbicide CDEC is used largely as a pre-emergent herbicide for control of foxtail, bromegrass, cheatgrass, crabgrass, and certain broadleaf weeds in vegetable crops, particularly in lighter soils where leaching can be a problem (Hamm *et al.*, 1955). Several reports have indicated that soil microorganisms are involved in the loss of CDEC activity from soils. High temperature and moisture content which also stimulate microbial activity are most conducive to a rapid loss of phytotoxicity from soil (Gantz and Slife, 1960; Otten *et al.*, 1957).

The mechanism whereby soil microorganisms degrade dithiocarbamate herbicides has not been elucidated. As in the case of the thiocarbamates, several points of attack are possible. These include attack at either the allyl or ethyl moieties of the molecule, or attack at some portion

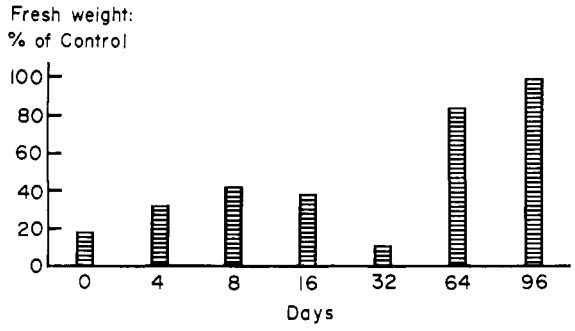


Figure 5. Persistence of diallate in a Hagerstown silty clay loam soil

Bioassay determinations were made with oat seedlings after a 3-week growing period

of the carbamate linkage. Direct attack of the ester linkage would result in the formation of 2-chloroallyl alcohol and diethyldithiocarbamic acid which would subsequently decompose to CS_2 and diethylamine. Conversion of 2-chloroallyl alcohol to lactic acid and subsequent incorporation into amino acids could follow. Such a mechanism has been observed (Jaworski, 1964) in higher plants (Figure 7). Conversion of allyl alcohol to lactic acid prior to incorporation has been demonstrated with soil microorganisms (Jensen, 1961).

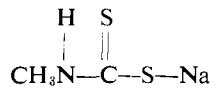
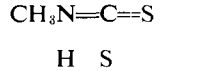
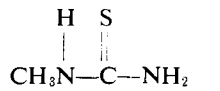
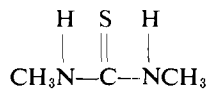
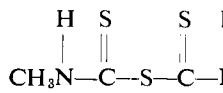
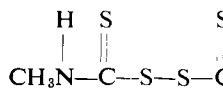
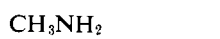
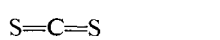
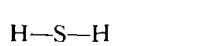
An alternate mechanism, listed by Menzies (1966), involves an initial attack on the 2-chloroallyl moiety. Formic acid, free chloride, and 2-(diethylthiocarbamyl)-acetic acid are the first products, the 2-(diethylthiocarbamyl)acetic acid subsequently being degraded to glycolic acid, CS_2, and diethylamine. This mechanism has not been observed in soil, however.

Sodium *N*-dimethyldithiocarbamate (SMDC or Vapam) has been used extensively for control of numerous soil-borne plant pathogenic microorganisms. More recently, however, it has also been used as a herbicide, particularly for killing weed seeds in soil. Owing to its relatively high degree of volatility, its most frequent application is as a fumigant.

SMDC decomposes in soil to methylisocyanate (MIT) (Gray, 1962; Hughes, 1960; Lloyd, 1962; Munnecke *et al.*, 1962; Turner, 1962; Turner and Corden, 1963; Turner *et al.*, 1962), and it is this form which is the primary toxicant. Turner and Corden (1963) examined some of the chemical reactions of SMDC and its decomposition products (Table II) under varying conditions of soil environment. SMDC decomposed to MIT and elemental sulfur in dilute aqueous solution at pH 9.5. Carbon disulfide (CS_2), hydrogen sulfide (H_2S), methylamine, MIT, and *N,N*-dimethylthiuram disulfide (DMTD) were formed in acid solutions. Methylamine and CS_2 reacted, forming MIT which in turn could react with SMDC, yielding DMTD, and with methylamine or H_2S, forming DMTU. Methylamine, MIT, and *N,N*-dimethylthiourea (DMTU) (Gray and Streim, 1962; Turner and Corden, 1963) have been identified in commercial samples of SMDC.

The occurrence of DMTU in commercial preparations of SMDC has been associated with phytotoxicity symptoms following SMDC treatments (Gray and Streim, 1962). Phytotoxicity is most likely a problem in sandy

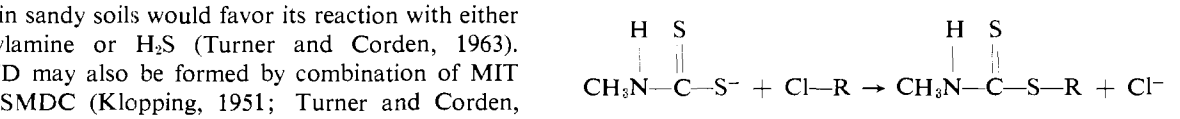
Table II. Chemical Decomposition Products of SMDC (Turner and Corden, 1963)

Structure	Chemical Name	Common Name or Designation
	Sodium *N*-methyldithiocarbamate	SMDC
	Methyl isothiocyanate	MIT
	Methyl thiourea	
	*N,N'*-Dimethylthiourea	DMTU
	*N,N'*-Dimethylthiuram monosulfide	DMTM
	*N,N'*-Dimethylthiuram disulfide	DMTD
	Methylamine	
	Carbon disulfide	CS_2
	Hydrogen sulfide	H_2S

soils, since MIT persists in these soils longer than in heavier soils (Von Kotter *et al.*, 1961). Methylamine and SMDC combine, forming DMTU (Moore and Crossley, 1955). Hydrogen sulfide and MIT undergo a similar reaction (Challenger, 1959). Persistence of MIT in sandy soils would favor its reaction with either methylamine or H_2S (Turner and Corden, 1963). DMTD may also be formed by combination of MIT with SMDC (Klopping, 1951; Turner and Corden, 1963). Since the effectiveness of SMDC as a soil fumigant is dependent on its conversion to MIT (Turner and Corden, 1963), production of DMTU and DMTD reduces its effectiveness. These relatively nonvolatile decomposition products may represent a potential hazard for contamination of soil, water, and plants with undesirable residues. Although other potential products of SMDC decomposition—e.g., CS_2 and H_2S—are also toxic compounds, their high degree of volatility would tend to preclude their accumulation in soils.

Low moisture and high temperature increase the rate of SMDC decomposition in soil (Turner and Corden, 1963). Decreases in soil moisture content from 20 to 6% decreased the time required for maximum SMDC decomposition from 7 to 2.5 hours, whereas increasing soil temperature from 10° to 40° C. decreased decomposition time from 7 to 1.5 hours. Production of MIT from SMDC was inhibited in a nitrogen atmosphere, since it is primarily an oxidative process. The conversion of SMDC to MIT was increased under conditions which favored soil aeration—e.g., low soil moisture and an increased liquid-air interface associated with the greater surface area of smaller soil particles.

According to recent reports by Miller and Lukens (1966), SMDC may be detoxified through esterification of the carbamic acid by halogenated hydrocarbon nematocides. Apparently esterification occurs by direct addition of a halogenated hydrocarbon when the two are combined.



R may be D—D (ethylene dibromide), Nemagon (1,2-dibromo-3-chloropropene), 1,3-dichloropropene, or related C_3 chlorinated hydrocarbons.

Several SMDC transformations are known to occur as a result of microbial activity. Cell suspensions of *Saccharomyces cerevisiae*, however, produced a compound identified as γ-(dimethylthiocarbamoylthio)-α-aminobutyric acid (Sijpesteijn *et al.*, 1962) (Figure 8). Another compound was tentatively identified as the corresponding keto acid, γ-(dimethylthiocarbamoylthio)-α-ketobutyric acid. Similar results were obtained with other soil microorganisms. Plant tissues have been shown to transform SMDC into two other products, namely the β-glucoside (Kaslander *et al.*, 1961) and the alanine derivative (Kaslander *et al.*, 1962). The conversion of foreign —SH compounds into aminobutyric acid derivatives by microorganisms was also observed with diethyl, dipropyl, and dibutyldithiocarbamates (Sijpesteijn *et al.*, 1962). Although it has not been reported elsewhere, this degradation mechanism may occur in the degradation of mercaptans formed during decomposition of thiocarbamate compounds. The fate of these compounds in microbial systems is not known.

Decomposition of dithiocarbamate herbicides might

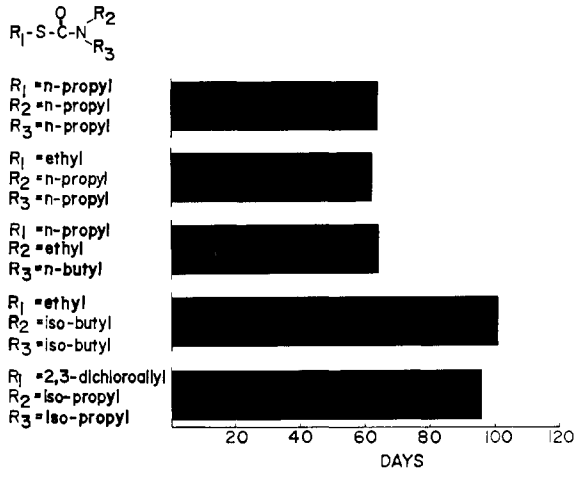


Figure 6. Effect of structure on persistence of several thiocarbamate herbicides in a Hagerstown silty clay loam

Bioassay determinations were made with oat seedlings after a 3-week growing period

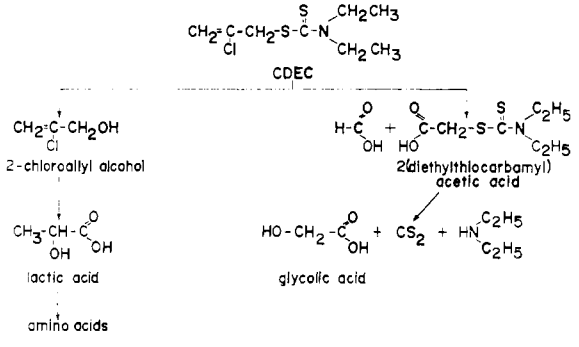


Figure 7. Probable degradation pathway of CDEC (Jaworski, 1964; Menzies, 1966)

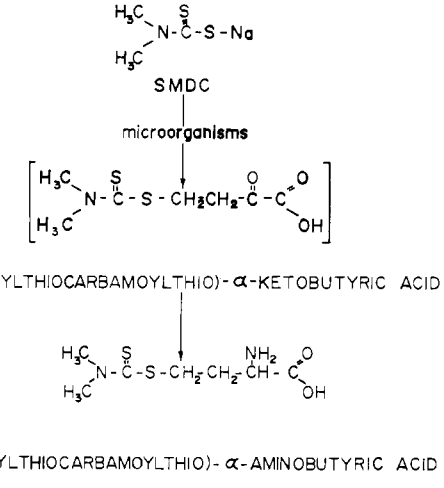
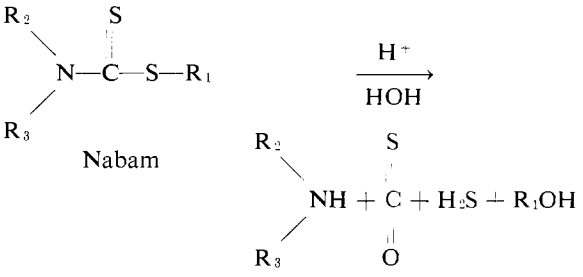


Figure 8. Microbial degradation of SMDC (Sijpesteijn *et al.*, 1962)

also proceed by a mechanism similar to that reported for the decomposition of the fungicide Nabam (disodium ethylene-bisdithiocarbamate) (Moje *et al.*, 1964). Products resulting from this mechanism of degradation would be the corresponding alkylamine and alcohol, hydrogen sulfide, and carbonyl sulfide.



This mechanism has not been observed, however, with dithiocarbamate herbicides.

Microbial degradation of dithiocarbamates may occur more slowly than other carbamates owing to their inherent microbial toxicity. Dithiocarbamic acid and its derivatives are well known as microbial inhibitors (Carter *et al.*, 1963; Rich and Horsfall, 1961). *N,N*-dialkyldithiocarbamic acids in particular have received considerable attention. In general, however, replacement of *N*-methyl substituents by higher alkyl groups or by hydrogen, or esterification, leads to lower microbial activity (Carter *et al.*, 1963).

Summary

Several carbamate compounds are used as selective pre- and/or postemergent herbicides in a variety of crops. Volatilization and microbial degradation are important factors in the dissipation of carbamate herbicides from soil. Loss by volatilization is a minor factor when the herbicides are incorporated or applied to dry surfaces. Environmental factors conducive to increased microbial activity—i.e., increased moisture content and temperature and organic matter level—tend to reduce the persistence of carbamate herbicide residues in soil, under conditions where volatilization is not an important factor.

The actual mechanism of microbial degradation of most carbamate herbicides has not been determined. Although several sites of attack are possible, available data indicate that the ester linkage constitutes the most probable site of attack. Subsequent degradation to relatively innocuous compounds and incorporation of the carbon into cellular substituents appears likely.

Rates of herbicide inactivation vary tremendously in response to difference in soil types and climate. However, carbamate herbicides or their degradation products are not likely to constitute a residue problem when areas receive rainfall sufficient for crop production.

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