for the estimation of Bayer 22,408 residues in milk, butterfat, or plant samples, especially when all the material that may cause interference can be removed by the repeated solvent extractions, evaporation, and chromatography. However, to avoid excessively high reagent blanks, certain general precautions should be followed. First, all glassware should be cleaned with cleaning solution, thoroughly rinsed with water, and dried in an oven at 100° C. Second, contact of the analytical solution with stopcock grease, rubber, cork, soap powder, or soap film must be avoided, as these materials can introduce additional fluorescence and cause high readings.

With the use of a Fisher Nefluoro-Photometer and the filters specified, the range of the method is limited to 5 to 100 γ of Bayer 22,408 in the samples analyzed. However, with an Aminco-Bowman spectrophotofluorometer, the activation and fluorescence maxima under the conditions of the method were 372 and 480 mμ, respectively. As little as 0.01 γ in 1 ml. can be detected with this instrument. Therefore, the sensitivity and range of the method can be greatly increased if a spectrophotofluorometer is used or if more accurate filters of proper wave lengths are used.

Under the experimental conditions, Bayer 22,408 fluoresces strongly in the alcoholic sodium hydroxide solution. The intensity of the fluorescence is diminished when a small amount of acid is added to the reaction solution, and is completely quenched when the solution is made strongly acidic, but it does not change if an excess of alcoholic sodium hydroxide is added. Acetone and water also have a quenching effect on the fluorescence intensity.

Increasing the temperature up to 50° C. increases the intensity of fluorescence, but if the reaction mixture is kept between 20° and 30° C., the intensity will not change.

Experience shows that it is important to include hydrogen peroxide in the dioxane. Chemically purified dioxane was first tried, and the intensity of the fluorescence was very low; however, when 0.1 ml. of 30% hydrogen peroxide was added to the same solution, the intensity became extremely high. The intensity could not be further increased by the addition of larger amounts of hydrogen peroxide.

Although practically no interference has thus far been encountered in runs on milk, butterfat, and plant materials not treated with Bayer 22,408, it is always important to run such control analyses. According to reports (*2*), extracts of many plants fluoresce when exposed to ultraviolet light; therefore, in the analysis of plant samples the inclusion of untreated control samples is especially important.

Most insecticides do not interfere; however, some, such as Co-Ral and Potasan [*O*-(4-methylumbelliferone) *O,O*-diethyl phosphorothioate], give some fluorescence in this method, but will interfere only if present in amounts exceeding 10 mg.

Acknowledgment

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HERBICIDE STRUCTURE AND STABILITY

Effect of Chemical Structure on Microbial Decomposition of Aromatic Herbicides

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The effect of molecular structure on the persistence and microbial decomposition of phenoxyalkyl carboxylic acid herbicides, chlorophenols, and some related compounds is presented. The resistance of some aromatic herbicides or their derivatives to microbial degradation is governed by the position of the halogen on the aromatic nucleus and by the linkage and type of aliphatic side chain.

THE USE of herbicides to control and eradicate weeds has presented several problems in addition to the initial plant response to the applied chemical. An aspect of these studies which becomes more important with the greater utilization of herbicides in agriculture is that dealing with the persistence and decomposition of the added material. The duration of activity of the herbicide has a distinct bearing upon agronomic practice, because phytotoxicity may be observed not only in the season of application but in succeeding years as well.

In many instances where herbicide breakdown occurs, evidence is available to show that the active agents in the process are members of the soil microflora. With the exception of a small group of compounds, however, little is known of the conditions favoring the decomposition or of the effects of molecular structure upon microbial decomposition. A study has been made to ascertain the rate of breakdown of a number of halogenated phenols and phenoxyalkyl carboxylic acids by certain soil populations and to determine which of these substances are transformed microbiologically.

Methods

The ultraviolet absorption characteristics of each of the chemicals investigated was determined with the Beckman spectrophotometer using aqueous solutions containing 10 to 100 p.p.m. of the compound. From each absorption spectrum, an absorbance was chosen for the analytical determination either at the point of maximal absorption or at a wave length close to it, with the latter choice adopted to minimize the number of individual spectrophotometric manipulations. The wave lengths used are included in Tables I and II. Of the materials tested, the absorbances were proportional to their concentration up to levels of more than 50 p.p.m. This is in agreement with the results of Dorschner and Buchholtz (*2*), who investigated the optical properties of several of these herbicides.

The following medium was used to determine the pattern of decomposition of the compounds through the activities of the soil population: 0.5 gram of ammonium nitrate, 0.2 gram of monobasic and 0.8 gram of dibasic potassium phosphate, 0.2 gram of magnesium sulfate, 0.03 gram of ferrous sulfate, and 0.1 gram of calcium chloride in 1000 ml. of distilled water. After sterilization of the medium, which had a final pH of 7.2 to 7.3, the aromatic substrate was added to a final concentration of 50 or 80 γ per ml., and a 4.0-gram aliquot of freshly sampled soil was added to 100 ml. of medium as source of the microbial inoculum. The flasks containing the soil suspensions were aerated by shaking on a rotary shaker at a temperature of 30° C. Three soil types were included: Honeoye silt loam, Mardin silt loam, and Dunkirk silt loam. Rates of herbicide were in terms of the acid equivalents.

At regular intervals, aliquots removed from each of the flasks were centrifuged at 1000 × G for 20 minutes to remove the soil particles, and the absorbance of the resulting supernatant was measured against distilled water at the selected wave length. At each sampling time, a reading was made upon the check samples at the same wave length, these controls containing the soil–inorganic medium mixture with no herbicide. The adsorption values were corrected by subtracting the absorbance of the soil-medium constituents. At the time of initiation, the differences in absorbance between experimental and check treatments were 0.3 to 0.7, depending upon the chemical. The technique and some of its applications have been described more fully by Whiteside and Alexander (*7*).

Results

The ultraviolet absorption of 2,4,5-T and 4-(2,4,5-TB) did not disappear when these chemicals were incubated with soil suspensions under conditions in which the corresponding dichloro-substituted compounds were degraded (*7*). To determine which pesticides of this group are suscepitble to decomposition, a variety of ω-substituted phenoxyalkyl carboxylic acids were tested for breakdown when incubated with several New York soils for periods ranging up to more than 4 months. The persistence data presented in Table I demonstrate distinct structural effects. Disappearance was noted only with 4-CPA, 3-(4-CPP), 4-(4-CPB), 2,4-D, 3-(2,4-DP), 4-(2,4-DB), MCPA, 3-(MCPP), and 4-(MCPB). The results of decomposition of these compounds in different soils generally agree, even with results obtained with Honeoye silt loam in which the process was slow. Only with MCPA and 4-(MCPB) did two soil samples react differently in terms of decomposition. In no instance was there evidence of loss of aromatic absorption of any of the 3,4-dichloro- or 2,4,5-trichlorophenoxyalkyl carboxylic acids in the test period. Failure to observe microbial oxidation of phenoxyacetate and 2-chlorophenoxyacetate is surprising because, in culture at least, organisms have been found to utilize these compounds (*1*, *5*). The transformation in soil, however, may proceed very slowly and not be detected by the technique used.

Examination of the compounds still persisting reveals certain areas of similarity. No compound having on its aromatic nucleus a chlorine in the meta position was transformed to an extent sufficient to cause a significant loss in ultraviolet absorption. This included the 3,4-dichloro- and 2,4,5-trichlorophenoxyalkyl carboxylic acids regardless of the nature of the aliphatic side chain. On

Table I. Decomposition of Phenoxyalkyl Carboxylic Acids in Three Soils

Compound	Abbreviation	Wave Length, Mμ	Days for Complete Disappearance		
			Mardin[a]	Honeoye[a]	Dunkirk[b]
ω-Substituted Phenoxyalkyl Carboxylic Acids					
Phenoxyacetate	...	269	...	124+	205+
2-Chlorophenoxyacetate	2-CPA	274	47+	124+	205+
4-Chlorophenoxyacetate	4-CPA	279	...	94	11
3-(4-Chlorophenoxy)propionate	3-(4-CPP)	279	...	...	11
4-(4-Chlorophenoxy)butyrate	4-(4-CPB)	279	...	...	53
2,4-Dichlorophenoxyacetate	2,4-D	283	23	94	26
3-(2,4-Dichlorophenoxy)propionate	3-(2,4-DP)	283	...	...	4
4-(2,4-Dichlorophenoxy)butyrate	4-(2,4-DB)	283	10	94	11
3,4-Dichlorophenoxyacetate	3,4-DA	283	47+	124+	205+
3-(3,4-Dichlorophenoxy)propionate	3-(3,4-DP)	283	...	...	81+
4-(3,4-Dichlorophenoxy)butyrate	4-(3,4-DB)	283	47+	124+	205+
2-Methyl-4-chlorophenoxyacetate	MCPA	279	47+	124+	70
3-(2-Methyl-4-chlorophenoxy)-propionate	3-(MCPP)	279	...	...	17
4-(2-Methyl-4-chlorophenoxy)-butyrate	4-(MCPB)	279	47+	124+	39
2,4,5-Trichlorophenoxyacetate	2,4,5-T	288	47+	124+	205+
3-(2,4,5-Trichlorophenoxy)propionate	3-(2,4,5-TP)	288	...	...	81+
4-(2,4,5-Trichlorophenoxy)butyrate	4-(2,4,5-TB)	288	47+	124+	205+
α-Substituted Phenoxyalkyl Carboxylic Acids					
2-(4-Chlorophenoxy)propionate	2-(4-CPP)	279	...	...	205+
2-(4-Chlorophenoxy)valerate	2-(4-CPV)	279	...	...	81+
2-(4-Chlorophenoxy)caproate	2-(4-CPC)	279	...	...	11
2-(2,4-Dichlorophenoxy)propionate	2-(2,4-DP)	283	47+	124+	205+
2-(2,4-Dichlorophenoxy)valerate	2-(2,4-DV)	283	...	...	81+
2-(2,4-Dichlorophenoxy)caproate	2-(2,4-DC)	283	...	...	9
2-(3,4-Dichlorophenoxy)propionate	2-(3,4-DP)	283	47+	124+	205+
2-(3,4-Dichlorophenoxy)valerate	2-(3,4-DV)	283	...	...	81+
2-(3,4-Dichlorophenoxy)caproate	2-(3,4-DC)	283	...	...	81+
2-(2-Methyl-4-chlorophenoxy)-propionate	2-(MCPP)	279	47+	124+	205+
2-(2-Methyl-4-chlorophenoxy)-valerate	2-(MCPV)	279	...	...	81+
2-(2-Methyl-4-chlorophenoxy)caproate	2-(MCPC)	279	...	...	81+
2-(2,4,5-Trichlorophenoxy)propionate	2-(2,4,5-TP)	288	47+	124+	205+
2-(2,4,5-Trichlorophenoxy)valerate	2-(2,4,5-TV)	288	...	...	81+
2-(2,4,5-Trichlorophenoxy)-caproate	2-(2,4,5-TC)	288	...	...	81+

+ after a figure indicates ultraviolet absorption was still present at end of incubation period.

[a] Substrate added, 50 γ per ml.
[b] Substrate added, 80 γ per ml.

Table II. Microbial Decomposition of Chlorophenols in Soil Suspensions

Compound	Wave Length, Mμ	Days for Complete Disappearance	
		Dunkirk[a]	Mardin[a]
Phenol	269	2	1
2-Chlorophenol	274	14	47
3-Chlorophenol	274	72+	47+
4-Chlorophenol	279	9	3
2-Bromophenol	274	14	...
3-Bromophenol	274	72+	...
4-Bromophenol	279	16	...
2,4-Dichlorophenol	283	9	5
2,5-Dichlorophenol	279	72+	...
2,4,5-Trichlorophenol	288	72+	47+
2,4,6-Trichlorophenol	288	5	13
2,3,4,6-Tetrachlorophenol	300	72+	...
Pentachlorophenol	320	72+	...

[a] Substrate added, 50 γ per ml.

the other hand, the ω-linkage of acetate, propionate, and butyrate did not prevent cleavage of the aromatic nucleus, although the length of the aliphatic moiety did affect the rate of cleavege.

Application of the spectrophotometric technique to α-substituted phenoxyalkyl carboxylic acids (Table I) revealed the absence of ring rupture in the 3,4-dichloro- and 2,4,5-trichlorophenoxyalkyl carboxylic acids. A definite influence of aliphatic substitution did appear, however, for the nuclei of none of the 2-valerate or propionate derivatives were degraded to a detectable extent, regardless of the position or number of halogens. Only certain of the 2-caproates and 2-acetates were decomposed to the extent that the ultraviolet absorption was lost. Although no 2-butyrate was available for study, the results strongly suggest that a two-carbon mechanism is important in the persistence and metabolism of such herbicides.

Of the three chlorophenoxyacetates that showed appreciable ring cleavage during the incubation period, the persistence of MCPA was greatest and that of 4-CPA and 2,4-D least; on the other hand, the aromatic ring of 2-CPA apparently remained intact for the entire period of investigation, suggesting either that the 2-chlorine confers greater resistance or that the other substitutions make the molecule more susceptible to breakdown. In the 3-propionate herbicides that had disappeared, 3-(MCPP) was slower than 3-(2,4-DP) and 3-(4-CPP), a situation analogous to the resistance of acetates. The 4-(2,4-dichlorophenoxy)-butyrate was the only one of the butyrates to undergo ring cleavage in short periods. Thus, the data clearly suggest the greater suitability of the ω-substituted 2,4-dichlorophenoxyalkyl carboxylic acids than the 2-methyl-4-chlorophenoxy series for microbial attack. A similar conclusion is suggested by the results with the caproate group. Study of the influence of chain length on rate of disappearance of ω-substituted compounds (Table I) shows that the aromatic nucleus of the 2,4-dichloro-, 2-methyl-4-chloro-, and 4-chloro- series generally is metabolized more readily for the propionic than for the corresponding acetic and butyric acids.

To test further the apparent effect of the position of the chlorine in the aromatic nucleus, the decomposition patterns of a number of halogenated phenols were examined by the same spectrophotometric method (Table II). Phenol, 4-chlorophenol, 2,4-dichloro-, and 2,4,6-trichlorophenol disappeared rapidly, whereas the 2-chloro- and 2- and 4-bromophenol were lost more slowly. The phenoxy nuclei of 3-chloro-, 3-bromo-, 2,5-dichloro-, 2,4,5-trichloro-, 2,3,4,6-tetrachloro-, and pentachlorophenol persisted for the full duration of the incubation. Other absorption experiments have shown that the absorption of 2,3-, 3,4-, and 3,5-dichlorophenol remained under conditions where 4-chlorophenol and 2,6-dichlorophenol has been metabolized, the latter two requiring approximately 2 weeks for disappearance. In agreement with the phenoxyalkyl carboxylic acids, the only features that the resistant compounds have in common is the halogen in the meta position of the ring. Phenoxyacetate and 2-CPA, however, have no meta halogen, but their decomposition was not detected.

With several of the chemicals, second applications of the compounds were made to the initial soil enrichments. These subsequent increments consistently disappeared at rates greater than the first additions, suggesting the build-up of an active population.

The results obtained have no bearing upon possible alterations in the side chains, as only activities leading to a ring cleavage can be observed by the technique used, but the data do demonstrate the greater duration of the aromatic nucleus of certain substances—that is, the phenoxy moiety is not cleaved to an appreciable extent. Lack of apparent breakdown is not indicative of a complete resistance to biological attack; rather, it signifies only that the aromatic nucleus is not cleaved. The absorbance of the treated reaction vessel rarely falls to zero—i.e., to the same value as the soil-medium blank. Hence, complete disappearance is assessed by the time to attain a 75 to 90% decrease in absorbance.

The possibility that the observed disappearance was not biological was investigated by adding the chemicals to two sets of flasks containing the medium and 4.0 grams of soil, a Dunkirk silt loam. One set was treated with sodium azide to a final concentration of 0.01% and the parallel series had none of the inhibitor added. Azide serves as an inhibitor of microbiological transformations. Breakdown was determined by the procedures outlined. With azide in the reaction mixture, no disappearance of ultraviolet absorption of the following compounds was detectable even after all the absorbance had disappeared in the flasks not treated with azide: 4-CPA; 4-(4-CPB); 2,4-D; 3-(2,4-DP); 4-(2,4-DB); 4-(MCPB); 2-(2,4-DC); phenol; 2-chlorophenol; 4-chlorophenol; 2,4-dichlorophenol; and 2,4,6-trichlorophenol. The remaining compounds which had been metabolized were not tested.

Discussion

It has been shown (*7*) that 2,4-D and 4-(2,4-DB) were readily decomposed by the microbial population of the soil whereas 2-(2,4-DP), 2,4,5-T, 2-(2,4,5-TP), and 4-(2,4,5-TB) were not degraded appreciably. Such results indicated that the ring substitutions and nature of the aliphatic side chain determine the susceptibility of the aromatic compounds to microbiological decomposition. These preliminary observations led to the observations reported herein, work that confirms and extends the effect of structure upon availability to biological attack.

It is possible that the microbiologically catalyzed disappearance of certain of these compounds may proceed at a rate too slow to be detected by the method used herein, but nonetheless be sufficiently rapid so that none of the pesticide is present in the following growing season. Further, alterations of the aliphatic moiety of the molecule may be of consequence in herbicide treatments in agricultural practice, because such modifications would undoubtedly alter phytotoxicity. Webley, Duff, and Farmer (*6*) have studied a *Nocardia* species which attacks the side chain of certain phenoxyalkyl carboxylic acids but does not rupture the aromatic nucleus, in one instance converting 4-phenoxybutyrate to phenoxy acetate but being inactive on the latter compound. The present investigations, on the other hand, deal only with systems leading to loss of ultraviolet absorption and would fail to detect modifications in the aliphatic moiety.

A comparison of investigations using pure bacterial cultures with the soil incubation technique reveals considerable similarity. Oxidation of phenol, 4-chlorophenol, 2,4-dichlorophenol, 2,4-dibromophenol, phenoxyacetate, 2-CPA, 4-CPA, 2,4-D, and MCPA by *Achromobacter*, *Flavobacterium*, and *Mycoplana* strains has been demonstrated (*1*, *3*, *5*). Walker (*4*) has also shown the disappearance of phenol and 2- and 4-chlorophenol when these materials were percolated through a soil column. The *Mycoplana* species that grew on 4-chlorophenol, however, failed to utilize the 2- and 3-chlorophenol as carbon sources for growth (*5*). Similarly, resting cells of 2,4-D grown *Achromobacter* sp. and *Flavobacterium peregrinum* metabolized 2-CPA and 4-CPA, but not 3-chlorophenoxyacetate (*3*), an observation which may explain the greater persistence of the latter in soil. These same bacteria would not oxidize the 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-dichlorophenoxyacetates even when adapted to 2,4-D decomposition. These observations are in agreement with the hypothesis that meta chlorine substitution confers resistance upon phenolic and phenoxy compounds. Bell (*1*) has reported a low rate of oxygen consumption by an *Achromobacter* sp. with 2,4,5-T, the oxidation being appreciably less than that on 2,4-D. This, together with the well known greater persistence in field soil of 2,4,5-T when compared with 2,4-D, is further support for the specific effect of halogen substitution.

The results suggest that basic principles

can be established governing the persistence and apparent biological resistance of phenoxyalkyl carboxylic acid herbicides and related chlorophenols or of their respective decomposition products. Certain structural characteristics apparently govern the persistence of aromatic molecules, and these may serve as a means of predicting the relative persistence of such phytotoxic compounds in soil. The present study suggests three hypotheses: (1) The aromatic nucleus of halogenated phenoxyalkyl carboxylic acids and phenols remains intact for long periods in compounds containing the halogen in a position meta to the phenolic hydroxyl. Should such compounds undergo polymerization, it is possible that this change may mask the decomposition. (2) With the exception of 2-CPA, ω-substituted phenoxyalkyl carboxylic acids are readily attacked and degraded by the soil microflora, provided condition 1 does not apply. (3) The decomposition of α-substituted phenoxyalkyl carboxylic acids to the stage of rupture of the aromatic nucleus is dependent upon the meta halogen as well as the length of the aliphatic acid, cleavage being rapid for acetate and caproate but not for propionate and valerate.

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HERBICIDE RESIDUES

Decline and Residue Studies on 4-Chloro-2-butynyl *N*-(3-Chlorophenyl)carbamate

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Interest in the use of 4-chloro-2-butynyl *N*-(3-chlorophenyl) carbamate (barban) as a post-emergence herbicide for the control of wild oats has created a need for decline and residue studies. This chemical declines rapidly on wheat, barley, flax, peas, and sugar beets; final barban residues are below 0.01 p.p.m. A method has been provided for determining barban residues on these crops.

A NEW SELECTIVE POST-EMERGENCE HERBICIDE, 4 - chloro - 2 - butynyl *N* - (3 - chlorophenyl)carbamate (barban), has been evaluated in the United States, Canada, and Europe during the past two years (*9, 10*). This chemical has shown promise for the control of wild oats (*Avena fatua*) in the presence of wheat, barley, peas, flax, safflower, rapeseed, sunflower, and sugar beets (*7, 8*). Decline and residue studies have been run on wheat, barley, flax, peas, and sugar beets, using an analytical method based on the colorimetric determination of 3-chloroaniline. This is a modification of the method of Bratton *et al.* (*4*) and Werner (*12*), and is similar to those methods used for the determination of monuron (*3*), acetanilide (*5*), parathion (*1*), and certain sulfa drugs (*6*).

Treatment of Crops

The crops were treated with an emulsifiable concentrate containing 1 pound of barban per gallon in a heavy aromatic oil with an emulsifier and a corrosion inhibitor. The recommended method for wild oat control calls for dilution of 1 gallon of concentrate with 5 to 10 gallons of water and the application of this emulsion at rates of 0.5 pound per acre on wheat, barley, flax, and peas, and at 1 pound per acre on sugar beets. The decline studies on peas, flax, and sugar beets were run at these recommended rates, while wheat and barley were treated at twice their recommended dosage. Preliminary decline studies had revealed that the latter crops could easily tolerate the 1-pound-per-acre dosage; hence more stringent rates were assigned to them in the present studies.

The crops were treated when the majority of the wild oats were in the two-leaf stage. At this date wheat and barley will be only slightly more advanced in growth, flax will be at the eight- to ten-leaf stage, peas at the four- to six-leaf stage, and sugar beets at the two-leaf stage. Peas and flax may respond to this treatment with a very slight leaf burn, while the other crops are not affected. In one series of decline studies, the herbicide was applied at too late a date to obtain control of wild oats, thus simulating a possible error on the potential user's part. In this treatment the wheat and barley were near the "boot" stage, flax and peas were flowering, and the sugar beets had reached the six- to eight-leaf stage. All residue studies were run in triplicated experiments using various rates and spray volumes. Three emulsifiable concentrates, differing only in the solvent and emulsifier brands, were evaluated. Rates as high as 2 pounds of barban per acre were used on barley and wheat, 1 pound per acre on flax and peas, and 4 pounds per acre on sugar beets, a very tolerant crop.

Samples for the decline studies were taken by cutting the grain crops, flax, and peas at ground level, and harvesting the entire sugar beet plant. These samples were placed immediately in polyethylene bags, frozen with dry ice, and shipped frozen to a cold locker for safekeeping until analyzed. The mature food portion of the crop was used in residue studies. The ripe grain crops were harvested at ground level and threshed, and the seed was cleaned and stored. Peas were harvested in their pods, bagged, and held frozen until analyzed. Sugar beets were collected, "crowned" to remove their tops, bagged, and stored.

Analytical Procedure

Samples of the green tissue are chopped into small pieces, weighed, and ground in a Waring Blendor for 3 to 5 minutes with sufficient ethylene dichloride to form a thin paste. The homogenate is filtered through a glass wool plug in a funnel; the tissue is reprocessed in the Blendor with fresh ethylene dichloride, filtered, and washed twice with fresh