1980

Carbofuran persistence in soil and efficacy for corn rootworm larval control

Greg William Gorder
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd
Part of the Entomology Commons

Recommended Citation
Gorder, Greg William, "Carbofuran persistence in soil and efficacy for corn rootworm larval control " (1980). Retrospective Theses and Dissertations. 6726.
https://lib.dr.iastate.edu/rtd/6726

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.
GORDER, GREG WILLIAM

CARBOFURAN PERSISTENCE IN SOIL AND EFFICACY FOR CORN ROOTWORM LARVAL CONTROL

Iowa State University

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106
Carbofuran persistence in soil and efficacy for corn rootworm larval control

by

Greg William Gorder

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Entomology
Major: Entomology (Insecticide Toxicology)

Approved: Members of the Committee:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1980
## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>Methods for Studying Carbofuran Residues in Soils</td>
<td>3</td>
</tr>
<tr>
<td>Carbofuran Persistence in Soil</td>
<td>11</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>21</td>
</tr>
<tr>
<td>Analytical Chemicals</td>
<td>21</td>
</tr>
<tr>
<td>Sampling</td>
<td>21</td>
</tr>
<tr>
<td>Soil Analyses</td>
<td>24</td>
</tr>
<tr>
<td>Carbofuran Extraction from Soil and Extract Cleanup</td>
<td>24</td>
</tr>
<tr>
<td>Analytical Techniques</td>
<td>32</td>
</tr>
<tr>
<td>Experimental Series</td>
<td>38</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>53</td>
</tr>
<tr>
<td>Recovery Experiments</td>
<td>53</td>
</tr>
<tr>
<td>Carbofuran Persistence in Soil</td>
<td>60</td>
</tr>
<tr>
<td>Carbofuran Residues and Efficacy</td>
<td>72</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>80</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>88</td>
</tr>
<tr>
<td>APPENDIX A: STRUCTURES OF CARBOFURAN AND METABOLITES</td>
<td>89</td>
</tr>
<tr>
<td>APPENDIX B: CARBOFURAN ANALYSIS METHODS</td>
<td>91</td>
</tr>
<tr>
<td>APPENDIX C: PATHWAYS OF CARBOFURAN BREAKDOWN IN SOIL</td>
<td>96</td>
</tr>
</tbody>
</table>
INTRODUCTION

Larvae of the northern corn rootworm, *Diabrotica longicornis* (Say), and the western corn rootworm, *Diabrotica virgifera* LeConte, are major pests in the corn belt of the United States. Tests have shown that carbofuran applied at planting time is frequently effective in controlling corn rootworm larval feeding damage (Gorder et al., 1980; Mayo, 1980), but it occasionally fails to provide adequate control (Gorder et al., 1980; Ahmad et al., 1979). Very rapid degradation of carbofuran in some soils might be responsible for lack of corn rootworm control. One possibility is that microbial enrichment occurs in soils treated with carbofuran causing rapid degradation after subsequent additions. This theory is widely held, although it has not been substantiated experimentally (Gorder et al., 1980; Ahmad et al., 1979). It has been shown, however, that carbofuran persistence in soil decreases with increased soil pH (Getzin, 1977). Therefore, soil pH might influence carbofuran persistence and efficacy for corn rootworm control. Alternatively, Chio et al. (1978) suggested that corn rootworm resistance to insecticides could account for control failures, but their bioassays did not show appreciable corn rootworm resistance to carbofuran. The reasons for control failures are not known at this time.

This research was done to study carbofuran residues in soil, and to determine if control failures could be explained by an absence of sufficient residues at the time that corn rootworm larvae are present in the soil. Because of time limitations and the large amount of work involved in studying carbofuran residue levels, no further investigation was done.
on the possibility that control failures could be related to insect resistance problems.
LITERATURE REVIEW

Methods for Studying Carbofuran Residues in Soils

Sampling

Sampling errors result when the compound under study is not represented at the same mean level in samples and the entire treated area. Samples are sometimes subsampled to reduce the cost of analysis. In these cases, additional errors are added to the sampling error if the compound under study is not represented at the same mean level in subsamples and the entire sample. Measurement errors result when laboratory techniques show that the compound is present at a mean level different from the actual level (Peterson and Calvin, 1965). Soil sampling procedures usually produce errors that are much larger than measurement errors (Cline, 1944; Rigney and Reed, 1945; Taylor et al., 1971; Caro and Taylor, 1971; Caro et al., 1973a). For that reason, proper soil sampling procedures can greatly increase the accuracy of analysis.

Sampling locations can be selected by judgment, a simple random design, a stratified random design, or a systematic design. A design that provides maximum precision at minimum cost should be used (Peterson and Calvin, 1965). Cline (1944) described a method for determining the number of sampling units that should be taken to provide a reliable estimate of the mean. This method can only be used when preliminary sampling and analysis is possible. When preliminary work is not possible, Cline (1945) suggested starting with 100 sampling units from treatments in cultivated fields treated within the most recent crop year, and 20 sampling units
from treatments in cultivated fields treated in previous crop years. The Federal Working Group on Pest Management (FWGPM, 1974) suggested starting with fifty 5-cm diameter units from a 4-ha field. It is important to specify the diameter of the sampling unit relative to the area being sampled because Taylor et al. (1971) showed that accuracy improved with increasing diameter of sampling units when the number of units collected and the sample area were kept constant. The FWGPM (1974) recommended that all pesticide residue samples be collected to a depth of 5 to 8 cm.

The FWGPM (1974) recommended compositing sampling units into a single sample prior to analysis as a way of reducing analysis costs. Peterson and Calvin (1965) pointed out, however, that the precision of estimation of the mean is dependent on the number of sampling units in the composite. There is no way of determining the precision of the mean from a single composite sample, including analysis of subsamples from the composite. When the significance of the means is needed, it is necessary to collect the sample units according to a randomized design, and analyze the cores separately or as several composites (Cline, 1945). When several composites are analyzed, the resulting standard deviation is actually the standard deviation of means (standard error) because the result from each composite is the mean of the contributing sample units. The standard deviation of composite samples should decrease in proportion to the square root of the number of sampling units in each composite sample, because standard error = σ/√n (Cline, 1944). Results presented by Taylor et al. (1971) suggest that this relationship holds for dieldrin residues in soils.
Extraction

The structures of carbofuran and its metabolites discussed in the following sections are shown in Appendix A. Carbofuran extraction from plant and animal tissues is usually accomplished by acid reflux to release conjugated metabolites (Cook, 1973). This procedure also is effective with soils. It involves refluxing 50 g of soil with 600 ml of 0.25 N HCl for 1 h, cooling in a freezer for 1 h, extracting 3 times with 600 ml of dichloromethane, and concentrating the extract using a Kuderna-Danish apparatus. Caro et al. (1973b) used a similar extraction procedure, but substituted 0.5 M acid ammonium acetate for the 0.25 N HCl.

Apparently due to an absence of conjugated metabolites, direct solvent extraction procedures have been used frequently to extract carbofuran from soil. In one such procedure (Gupta and Dewan, 1974), acetone was used to extract 50 g of air-dried soil for 8 h in a soxhlet apparatus. The acetone extract was subsequently diluted with water and extracted with dichloromethane. These authors suggested, however, that 0.25 N HCl may have extracted more carbofuran metabolites from soil than did acetone. Möllhoff (1975a) extracted carbofuran by shaking 100 g of soil with 300 ml of methanol:water (1:1) for 30 min. The methanol:water was separated from the soil by filtration and extracted 3 times with a total volume of 700 ml of chloroform. Williams et al. (1976a) extracted 20 g of soil by shaking for three 2-h periods with 70-ml portions of methanol:dichloromethane (2:5). The solvent was removed from the pooled extracts, and the remaining water was partitioned with dichloromethane. Venkateswarlu et al. (1977) extracted 20 g of soil plus 25 ml of water by shaking 3 times for
30 min with chloroform:diethyl ether (1:1). The supernatant was separated from the soil by centrifugation, and the solvent was separated from the water in a separatory funnel.

All of the extraction procedures described above involve 2 extractions; first the soil is extracted, then the carbofuran is partitioned from the extract into a less polar solvent. For that reason, all of the procedures are time consuming and costly. It may also be desirable to extract quantities of soil larger than the 20, 50, or 100 g described by these procedures. Procedures described by Cook (1973), Caro et al. (1973b), and Gupta and Dewan (1974) cannot be used on large samples, because extremely large glassware and unmanageable volumes of extractants would be required. Procedures described by Möllhoff (1975a) and Venkateswarlu et al. (1977) are not useful on large samples because of difficulties involved in filtering and centrifuging large quantities of soil.

Several extraction procedures have been described that require only 1 extraction and might be useful on large quantities of soil. Getzin (1973) shook 20 cm$^3$ of soil with 100 ml of acetone:benzene (1:1) 3 times for 30 min. The solvent was decanted without loss of soil. Getzin, however, extracted up to 10% more carbofuran when soil was refluxed with 0.25 N HCl subsequent to solvent extraction. Deuel et al. (1979) shook soil for 2 h with diethyl ether:dichloromethane (1:4) and decanted the solvent through a Büchner funnel to remove traces of soil. Kadoum and Mock (1978) and Klaassen and Kadoum (1979) shook 150-g, water-saturated soil samples with 100 ml of acetone followed by three 100-ml portions of ethyl acetate and
decanted each solvent extract. The variety of methods described shows that there is no universally used carbofuran extraction procedure.

Analysis

Gas-liquid chromatography (GLC) has been the most widely used analysis method. GLC analysis methods are summarized in Appendix B. Carbofuran can be measured by GLC using nitrogen-specific detectors (V, Appendix B), but it is necessary to form derivatives to make carbofuran sensitive to other detectors (VI through XXIII). One metabolite of carbofuran, 3-oxocarbofuran (III, Appendix A, also called 3-ketocarbofuran), can be measured without derivatization with an electron-capture detector (ECD) even though this detector is not nitrogen specific (Ragab, 1977; Ragab et al., 1977).

Direct carbofuran analysis (V, Appendix B) is done with nitrogen-specific detectors that are only available on specific brands of equipment. Cassil et al. (1969) discussed the operating principles of a Dohrmann titration cell (DTC) and a Coulson conductivity detector (CCD), and described their use for carbofuran analysis. Both detectors reduce nitrogen-containing compounds to ammonia gas at 800 to 900°C in the presence of hydrogen gas and a catalyst. The DTC measures nitrogen with coulometric titration of ammonia, and the CCD measures nitrogen through the change in conductivity of water in a flow cell. The DTC is sold by Envirotech Corp., and the CCD is sold by Tracor Co., Inc. (Laski and Watts, 1973). The DTC was used for carbofuran analysis by Cook et al. (1969) and Cook (1973); and the CCD was used by Williams and Brown (1973), Williams et al. (1976a), Laski and Watts (1973), and Klaassen and Kadoum
(1979). An improved version of the CCD called the Hall conductivity detector (Nelson and Cook, 1980) is also sold by Tracor Co., Inc. (Erickson et al., 1979). Nitrogen-specific detectors called thermionic detectors are described by Ives and Giuffrida (1967). These detectors measure the electrical response from excited ions produced by organic nitrogen and phosphorus compounds in the presence of heat, hydrogen, and an alkaline-metal salt. Heat in these detectors may be supplied by modifying commonly used flame-ionization detectors (FID, Ives and Giuffrida, 1967), or from an electrical source. An alkali flame-ionization detector (AFID) was used for carbofuran analysis by Ahmad et al. (1979). Flameless thermionic detectors (TD) manufactured by Hewlett-Packard Corp. were used for carbofuran analysis by Seiber et al. (1978) and Nelson and Cook (1980). Fuhremann and Lichtenstein (1980) used a TD manufactured by Tracor Co., Inc.

Apparently due to wide availability of the ECD, many methods have been described for forming carbofuran derivatives that can be measured with this detector. In some cases, these derivatives also can be measured with other detectors. In general, these indirect analysis methods are more time consuming and less reliable than the direct methods previously described. Holden et al. (1969) hydrolyzed carbamate insecticides and formed dinitroaniline derivatives (VII, Appendix B) from the resulting methylamine or dimethylamine using 1-fluoro-2,4-dinitrobenzene (VI). This method produces different products for methylcarbamate compounds and dimethylcarbamate compounds, but products among either group of compounds are identical. For that reason, Holden (1973) used the same reagent to
form derivatives of the phenolic moieties. The resulting 2,4-dinitrophenyl ether derivatives (IX) are specific for each insecticide, and for metabolites that are altered on the phenolic moiety. This procedure has been used to measure carbofuran residues with an ECD by Caro et al. (1973a,b), Siddaramappa et al. (1978), and Talekar et al. (1977). Cook et al. (1977) used this procedure to analyze carbofuran metabolites with a DTC. Butler and McDonough (1968) used trichloroacetyl chloride (X) to derivatize the phenolic moiety of hydrolyzed carbamate insecticides for analysis with an ECD. This method was used for analysis of carbofuran residues by Butler and McDonough (1971) and Deuel et al. (1979). Coburn et al. (1976) used pentafluorobenzyl bromide (XII) to derivatize the phenolic moiety of hydrolyzed carbamate insecticides for analysis with an ECD. Seiber (1972) described the formation of N-perfluoroacyl derivatives (XV) of intact methylcarbamate insecticides. Each insecticide and metabolite formed a unique product that could be measured with an ECD or an AFID. Lawrence (1976) analyzed these derivatives with a CCD, and Chapman and Robinson (1977) used a mass spectrometer (MS) as a detector. This method also was used for carbofuran residue analysis by Wong and Fisher (1975), Lawrence and Ryan (1977), Archer et al. (1977), Miles and Harris (1979), and Gorder et al. (1980).

Derivatives of carbofuran also have been formed to allow GLC analysis with other detectors. Knaak et al. (1970) derivatized intact carbofuran with acetic anhydride (XVI, Appendix B) for analysis with a FID. Moye (1971) described a procedure that involved transesterification on the GLC column to form methyl N-methylcarbamate (XIX) that was detected by AFID.
This product forms from any methylcarbamate compound, so it is not specific for carbofuran. Nevertheless, the procedure was used for carbofuran analysis by Van Middelem et al. (1971), Möllhoff (1975a, b), and Holland (1977). Bowman and Beroza (1967) hydrolyzed carbofuran and derivatized the phenol with dimethyl chlorothiophosphate (XX) to form a thiophosphoryl ester (XXI) for analysis on a flame photometric detector (FPD). Maitlen and McDonough (1980) described derivatization of the phenolic moiety of carbofuran with methanesulfonyl chloride (XXII) to form a mesylate derivative (XXIII) for analysis by FPD.

Two alternatives to GLC are colorimetric analysis and high-pressure liquid chromatography (HPLC). Gupta and Dewan (1974) developed a colorimetric method that was not described, but another method was described by Mithyantha and Perur (1974). The latter method was used by Venkateswarlu et al. (1977) and Venkateswarlu and Sethunathan (1978, 1979) to measure carbofuran and its metabolites after isolating each compound from extracts by thin-layer chromatography (TLC). Lawrence (1977) and Lawrence and Leduc (1977) described a method for direct measurement of carbofuran and its metabolites by HPLC using an ultra-violet detector (UVD). Lawrence and Leduc (1978) described an alternate HPLC analysis method using fluorescence detection. Nelson and Cook (1979) hydrolyzed carbofuran and analyzed its phenol by HPLC with an UVD. Additional methods of carbofuran analysis include field-cricket bioassay (Harris and Turnbull, 1977), radiocarbon analysis (Getzin, 1973; Williams et al., 1976b), nuclear magnetic resonance analysis of an N-perfluoroacyl derivative of carbofuran (Bose, 1977), TLC with chromogenic spray detection...
(Metcalf et al., 1968; Gupta and Dewan, 1974) or autoradiographic detection (Getzin, 1973), and enzyme inhibition (Gupta and Dewan, 1974).

**Extract cleanup**

The extract may or may not require cleanup prior to analysis, depending on the extraction and analysis procedures used. Those procedures not requiring cleanup include (1) extraction by reflux with 0.5 M acid ammonium acetate prior to formation of the 2,4-dinitrophenyl ether derivative (IX) for analysis by GLC (Caro et al., 1973b), (2) extraction with diethyl ether:dichloromethane (1:4) prior to formation of the trichloroacetyl derivative (XI) for analysis by GLC (Deuel et al., 1979), (3) extraction with methanol:water (1:1) prior to formation of methyl N-methylcarbamate (XIX) for analysis by GLC (Möllhoff, 1975a), and (4) extraction with acetone:benzene (1:1) for radiocarbon analysis (Getzin, 1973). Cleanup methods for soil extracts include liquid column chromatography with various adsorbents including Nuchar-attaclay (Cook, 1973), aluminum oxide (Butler and McDonough, 1971; Williams et al., 1976a), silica gel (Coburn et al., 1976), Florisil (Gorder et al., 1980), and a mixture of Celite 545, MgO, and Norit SG (Klaassen and Kadoum, 1979). Silica gel, preparative TLC plates also have been used for cleanup (Venkateswarlu et al., 1977).

### Carbofuran Persistence in Soil

Hamaker and Goring (1976) theorized that pesticide molecules applied to soil enter compartments in the soil where the molecules are either available or unavailable for degradation. Molecules that are in the soil
solution or adsorbed on rapid-exchange sites are in the available compartment, and molecules that exchange very slowly with the soil solution are in the unavailable compartment. Degradation of molecules in the available compartment follows first-order kinetics as long as the factors responsible for degradation are in excess. This is true for nonbiological and biological degradation. Molecules in the unavailable compartment persist in the soil until they are released into the available compartment. This theory explains why pesticide residues in soil frequently degrade according to first-order kinetics initially, but subsequently degrade at slower rates. These degradation curves appear to follow second-order kinetics.

High Freundlich $k$ values show a high adsorbent (e.g., a soil) capacity for an adsorbate (e.g., a pesticide, Adamson, 1976, p. 389). Adsorption is affected by both the soil and the pesticide, so pesticides are best compared in similar soils. Freundlich $k$ values for carbofuran were 27, 2, 1.6, 0.51, and 0.1 for soils with 75.3, 2.8, 2.5, 1.7, and 0.7% organic matter, respectively (Sharom, 1977; Caro et al., 1974). These values were lower than values obtained with 10 of 11 other insecticides tested in the same soils (Sharom, 1977), and lower than 4 of 5 insecticides tested by Felsot and Dahm (1979). These low values suggest that carbofuran has a low affinity for the unavailable compartment in soil. Conversely, a high affinity for the available compartment is suggested by the high water solubility of carbofuran variously reported as 250 ppm (Caro et al., 1974), 320 ppm (Bowman and Sans, 1979), and 700 ppm (Cook, 1973). Sharom (1977) found that carbofuran had greater mobility in both sand and muck soils than 8 of 9 other insecticides tested, and Felsot and
Wilson (1980) found $R_f$ values between 0.30 and 0.95 that showed substantial carbofuran movement on soil TLC plates. The relative affinity of carbofuran to the available compartment in comparison with other insecticides suggests that degradation of carbofuran in soil should approximately follow first-order kinetics. This was confirmed by Caro et al. (1973a).

**Nonbiological factors affecting persistence**

Nonbiological factors affect the persistence of a soil pesticide by causing movement of the pesticide out of the treated zone or by breaking down the molecule. Movement out of the treated zone can occur when the pesticide is associated with the soil solution, soil particles, or air. Models for pesticide movement in the agricultural environment (Bailey et al., 1974; Frere, 1975) predict that movement of pesticides in the water phase will agree with chromatography theory. Pesticides with the least adsorption to soil solids move farthest in the soil solution. Pesticides with the greatest adsorption to soil move mainly by soil erosion. The previously described $k$ values, water solubility, mobility factor, and $R_f$ values indicate that carbofuran has an affinity for the soil solution. Carbofuran surface runoff from 3 agricultural watersheds ranged between 0.5 and 2.0% of applied when carbofuran was applied as in-furrow or disked-broadcast treatments of Furadan® 10C. Most of the carbofuran was lost in the first 2 runoff events after treatment, regardless of the timing of those events (Caro et al., 1973a). Most other pesticides are lost in a single event, and they are lost only when severe rainfall conditions occur within 2 weeks of application (Wauchope, 1978). Caro et al. (1976) found that more dieldrin than carbofuran was lost with
surface runoff. Carbofuran was found in the runoff water and dieldrin was found on suspended sediments. These results suggest that despite the affinity of carbofuran for the soil solution, the surface runoff losses are no greater than they are for other pesticides. Unfortunately, information on the rate of carbofuran leaching through soil is not available.

Volatilization also affects the persistence of some pesticides in soil, but it probably has little effect on carbofuran persistence. Caro et al. (1976) found that carbofuran volatilized too slowly for accurate measurement, but dieldrin was measured. This suggests that carbofuran volatilization was substantially below 4.5% of the applied dose per year that was found for dieldrin. Deuel et al. (1979) also were unable to detect any volatilized carbofuran, and Harris and Turnbull (1977) determined with bioassays that carbofuran has no fumigant action.

Nonbiological carbofuran breakdown by alkaline and clay-catalyzed hydrolysis may be important in reducing carbofuran persistence in some soils (Appendix C). Getzin (1973) found progressively shorter persistence of carbofuran in pH-adjusted Sultan silt loam between pH 4.3 and 7.8. The time required for 50% breakdown ranged between 21 days at pH 7.8 and 210 days at pH 4.3. Seiber et al. (1978) found a similar pH relationship with rice-paddy water. Getzin (1973) also sterilized samples of 4 soil types by gamma irradiation and compared carbofuran degradation in those samples to unsterilized controls. It was not possible to distinguish completely between biological and nonbiological degradation of carbofuran because the sterilized samples were not kept free from airborne contamination. Gamma irradiation slowed the rate of carbofuran degradation in 3 soils with pH
values of 5.9 to 6.2, but did not slow degradation in a soil with a pH of 7.8. These results indicate that alkaline hydrolysis is the major factor in carbofuran degradation in some high pH soils. Getzin (1973) even suggested that nonbiological reactions might lead to the release of some carbonyl carbon as carbon dioxide. Caro et al. (1973a), Talekar et al. (1977), Siddaramappa et al. (1978), and Seiber et al. (1978) also suggested that increased soil pH might decrease carbofuran persistence in the field. Caro et al. (1973a) found that the half lives of carbofuran in pH 6.35 and 5.20 corn-growing soils were 46 and 117 days, respectively. These half lives, however, were much shorter than the respective 140- and 1600-day half lives in buffer solutions. These results indicate that pH-catalyzed hydrolysis is not the major factor in carbofuran degradation in acidic environments.

Deuel et al. (1979) found that over 50% of applied carbofuran disappeared in 1 day from sucrose-fortified, flooded soils that were either unsterile or autoclaved. They suggested that this was due to surface-catalyzed hydrolysis on montmorillonic surfaces rather than pH hydrolysis; the solution pH of 6.0 to 6.5 was too acidic for pH hydrolysis. The authors only autoclaved the fortified soil solution for 15 min and did not check sterility, however, so they should not have ruled out microbial metabolism. The possibility of clay-catalyzed hydrolysis also was suggested by Caro et al. (1973a) as a possible explanation of why the half life of carbofuran at certain points in a field was 33.5 days and the average half life for the entire field was 94 days.
Deuel et al. (1979) recovered almost 15% more carbofuran from de-ionized-water solutions that sat in laboratory light for 4 days as opposed to sunlight. Seiber et al. (1978) also found that carbofuran in aqueous solution degraded slightly faster in the light than in the dark. Siddaramappa et al. (1978), however, did not find any photodegradation of carbofuran in rice-paddy water.

**Biological factors affecting persistence**

Biological metabolism of carbofuran applied to soil occurs in animals, plants, and microorganisms. No information is available on the total amount of carbofuran that is absorbed by the target pest or other animals. This amount, however, probably represents a very small fraction of the total carbofuran applied to the soil. Uptake by soil insects is probably enhanced by increased soil-moisture levels (Harris and Turnbull, 1977). Carbofuran metabolism in animals has been studied by Metcalf et al. (1968), Dorough (1968), Knaak et al. (1970), Wong and Fisher (1975), Chio and Sanborn (1977), Chio and Metcalf (1979), and Lichtenstein et al. (1979).

Ashworth and Sheets (1970) showed that carbofuran has systemic activity in tobacco plants. The insecticide was absorbed through the roots and rapidly translocated to the leaves without accumulation in the roots. The same insecticide distribution occurred in corn (Turner and Caro, 1973), rice (Siddaramappa and Watanabe, 1979), and oats (Fuhrmann and Lichtenstein, 1980). In corn, over 85% of the carbofuran that was taken up by the plants was translocated to the leaves. In the leaves, over 80% of the carbofuran was converted to 3-hydroxycarbofuran and over
5% was converted to 3-oxocarbofuran (Turner and Caro, 1973). Similar metabolism was found in oats (Fuhremann and Lichtenstein, 1980). The recovery of $^{14}$C-carbofuran dropped 12% during the first 12 days after treatment of tobacco (Ashworth and Sheets, 1970). This indicates that some radiocarbon was volatilized or became unextractable. In rice, 9 to 17% of the radiocarbon taken up by the plants volatilized through the leaves as unmetabolized carbofuran. Slightly lower amounts of radiocarbon also were trapped in alkaline solution suggesting that some of the ring carbons were metabolized to carbon dioxide (Siddaramappa and Watanabe, 1979). Carbofuran and its carbamate metabolites decline 60 to 85% in corn between the silage and harvest stages (Turner and Caro, 1973). At the silage stage, the corn plants contained the equivalent of only 0.14% of the carbofuran applied as in-furrow treatment of Furadan 10G (Caro et al., 1973a). This quantity is small, but it does not include carbofuran lost from the plants due to metabolism or volatilization. The total quantity of carbofuran removed from the soil by corn may be small, but the amount is not known. Fuhremann and Lichtenstein (1980) showed that oat plants grown in the laboratory can remove 55 to 69% of the carbofuran applied to the soil.

Although previously described hydrolysis explained carbofuran breakdown in alkaline soils (Getzin, 1973), it did not explain carbofuran breakdown in acidic soils (Caro et al., 1973a; Getzin, 1973). Microorganisms also are important in carbofuran breakdown (Appendix C). Getzin (1973) found slower breakdown of carbofuran in 3 of 4 gamma-irradiated soils than in the same unsterilized soils. Venkateswarlu et al. (1977)
and Williams et al. (1976b) got the same results by comparing carbofuran degradation in autoclaved and unsterile soil samples. Williams et al. (1976b) found that 8 pure cultures of soil microorganisms metabolized the carbonyl carbon to carbon dioxide. The most active cultures were actinomycetes. Carbofuran phenol (IV, Appendix A) or a similar metabolite is initially bound to the soil, then slowly metabolized to carbon dioxide by microorganisms (Getzin, 1973; Siddaramappa et al., 1978; Venkateswarlu and Sethunathan, 1979). Getzin (1973) showed that the bound material was held covalently rather than by adsorptive forces. The bound material appeared to be carbofuran phenol (IV), because carbofuran phenol (IV) added directly to soil was rapidly bound and slowly metabolized to carbon dioxide. Venkateswarlu and Sethunathan (1979) found that flooded soil samples that were not shaken accumulated extractable carbofuran phenol (IV). When the samples were subsequently shaken, carbofuran phenol (IV) became unextractable and the ring carbons were slowly released as carbon dioxide. This suggested that binding of carbofuran phenol (IV) and microbial metabolism of the ring carbons required aerobic conditions.

The pathway of carbofuran degradation described above appears to be the major pathway for microbial metabolism of carbofuran in soil, but microorganisms also use other pathways (Appendix C). An Aspergillus terreus culture that was isolated from soil produced 8 organic-soluble metabolites of carbofuran in culture medium. Treatment of the extracted medium with acid and subsequent solvent extraction resulted in the release of additional organic-soluble metabolites that might have been conjugated. The possibility of glucoside, glucuronide, sulfate, and phosphate conju-
gates were ruled out (Davis, 1975). Cultures of Aspergillus niger, Trichoderma verde, and Helminthosporium sp. isolated from soil also metabolized carbofuran in culture medium. The last culture was the most active. All of the cultures produced small amounts of 3-hydroxycarbofuran (II, Appendix A), but other metabolites were not identified (Kandasamy et al., 1977). This pathway agrees with reports that small quantities of 3-hydroxycarbofuran and 3-oxocarbofuran (II and III) are found in some soils (Caro et al., 1973a; Talekar et al., 1977; Fuhremann and Lichtenstein, 1980). Several other microorganisms degrade carbofuran by unknown pathways. Venkateswarlu et al. (1977) isolated a bacterium from soil that used carbofuran as a sole carbon and nitrogen source in culture medium, and researchers at the Illinois Natural History Survey isolated a Pseudomonas sp. from soil that degraded carbofuran in culture medium (Allan Felsot, Illinois National History Survey, Urbana, IL, personal communication).

Several factors could potentially affect the rate of carbofuran degradation in soil. The rate is increased in rice soils by reducing the oxygen tension by flooding the soil and adding rice straw to the soil (Venkateswarlu et al., 1977; Venkateswarlu and Sethunathan, 1979). The same authors stated that microbial degradation proceeded after a lag period that was not influenced by repeated treatments of the soil with carbofuran. The lack of influence of repeated treatments on a lag period would be very unusual (Kearney et al., 1969), and the data presented do not clearly agree with the authors' conclusions. So it is possible that
previous treatments of the soil with carbofuran could slightly reduce its persistence in soil by reducing the lag time.

Factors influencing both biological and nonbiological mechanisms

Some factors that might indirectly affect both biological and nonbiological rates of carbofuran degradation are described in this section. Carbofuran degradation rates increased with increased soil temperature (Talekar et al., 1977; Williams et al., 1976b). Increased soil-moisture levels also may increase carbofuran degradation rates (Caro et al., 1973a; Talekar et al., 1977). Perhaps this is due to increased availability of carbofuran to degradative mechanisms through increased amounts of carbofuran in the soil solution (Bailey et al., 1974). Ahmad et al. (1979) found that the formulation of carbofuran affected persistence. Technical carbofuran had a half life of 11 to 13 days, and carbofuran applied as Furadan 10G had a half life of 60 to 75 days in the same soil.
MATERIALS AND METHODS

Analytical Chemicals

A carbofuran granular formulation (Furadan 10G), analytical and technical-grade carbofuran, (carbonyl-$^{14}$C)-carbofuran, and 2 metabolites of carbofuran, 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl-N-methylcarbamate) and 3-oxocarbofuran (2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl-N-methylcarbamate) were obtained courtesy of the FMC Corporation (Middleport, NY). Structures of these chemicals are shown in Appendix A (I, II, and III).

The $^{14}$C-carbofuran was separated from impurities by preparative thin-layer chromatography (TLC). The radiochemical purity of $^{14}$C-carbofuran was 98.4% after purification. The purified $^{14}$C-carbofuran was mixed with unlabeled, analytical-grade carbofuran to give a specific activity of 600 mCi/mole. Bulk-grade acetone; reagent-grade ethyl acetate and methanol; and Nanograde$^R$ dichloromethane, hexane, and benzene were used in these studies. Acetone was redistilled in glass before use.

Sampling

Corn roots were sampled for carbofuran residues and efficacy experiments (1977 and 1979) and soil was sampled for all experiments. The field treatments are described in detail with the carbofuran residues and efficacy experiments.

Corn roots

In 1977 tests, 20 corn roots were removed from carbofuran-treated rows and from untreated rows at each location. In 1979 tests, 5 corn
roots were removed from each row at each location. This resulted in the collection of either 20 or 40 corn roots from the carbofuran-treated rows and untreated rows at each location. The corn roots were always removed from both carbofuran-treated rows and untreated rows on the same day during late July or early August. These dates followed maximum larval feeding and preceded significant root regrowth. The roots were washed and rated for larval feeding on a 1 to 6 scale, with 1 indicating no feeding damage, and 6 indicating severe damage (Hills and Peters, 1971).

Soil

In the 1977 carbofuran residues and efficacy experiment, composite soil samples consisting of 20 to 25 cores, 1.9 by 15 cm, were taken from the carbofuran-treated rows 0, 1, 2, 4, 6, and 10 weeks after planting and insecticide application, with the exception that 0-week samples were not collected at Winterset, Sanborn, and Wellsburg. Sampling was not done within 5 m of the end of a row because of the possibility of terminal uneven insecticide application. The cores were separated into 0 to 7.5-cm (upper) and 7.5 to 15-cm (lower) layers, placed in polyethylene bags, and taken to the laboratory. All soil samples were passed through a 2.38-mm sieve and mixed by rolling on a sheet of paper. Soil samples were stored in glass screw-capped jars at -18°C prior to insecticide extraction.

Portions of the 5 or 6 upper-layer composite samples from each field were composited into a single 150-g sample to study soil properties. Additional upper and lower-layer composite samples were made from twenty to twenty-five 1.9 by 15-cm cores collected 10 weeks after planting from untreated rows. These samples were passed through a 2.38-mm sieve, mixed
by rolling on a sheet of paper, and stored at 0°C prior to use in laboratory recovery experiments.

In the 1979 carbofuran residues and efficacy experiment, 9 soil-sampling locations were selected randomly within the central 20 m of each 30.5-m long carbofuran-treated row or pair of rows. The 10.5 cm in diameter and 7.5 cm in height cores were collected immediately before treatment, immediately after treatment, and 10 weeks after treatment. Carbofuran persistence at Newell and Nashua was studied by collecting 9 soil cores (10.5 by 7.5 cm) from carbofuran-treated rows immediately before treatment, immediately after treatment, and 1, 2, 4, 6, and 10 weeks after treatment; 3 additional cores (10.2 cm in diameter and 37.5 cm in height) were collected 23 weeks after treatment. The latter cores were separated into five 10.2 by 7.5-cm layers. Cores were placed individually in polyethylene bags marked with the collection location and stored at 0°C until insecticide extraction.

Composite samples containing three 10.5 by 7.5-cm cores were collected on the day of planting adjacent to carbofuran-treated rows or between pairs of rows. These samples were passed through a 2.0-mm sieve and mixed by rolling on a sheet of paper prior to testing the soil properties. Some of the samples collected immediately before treatment also were composited, passed through a 2.0-mm sieve, and mixed by rolling on a sheet of paper prior to use in laboratory recovery and persistence experiments.
Soil Analyses

The weight loss that occurred in soil during oven drying for 12 h at 110°C was divided by the dry weight of the soil to determine soil moisture levels. The pH readings were made with a glass electrode (soil/water ratio, 1:2). Oven-dried portions of the composite samples were used for soil particle size analysis by the hydrometer method (Bouyoucos, 1936) after partly destroying organic matter with H₂O₂. Air-dried portions of the composite samples were ground with a mortar and pestle to pass a 50-mesh sieve prior to determination of organic carbon by wet oxidation (Mebius, 1960). The percentage organic matter was determined by multiplying the percentage organic carbon by 1.724 (Allison, 1965).

Carbofuran Extraction from Soil and Extract Cleanup

A widely used method for carbofuran extraction (Cook, 1973) is shown in Figure 1. When this extraction procedure was used in this study, a cleanup procedure modified from Nelson and Cook (1980) was substituted for the Nuchar-attaclay cleanup step. The modified cleanup step was done in a 19.5 by 0.23-cm glass column packed with successive layers of 1 cm of sea sand, 10 cm (16.5 g) of Florisil deactivated to 2.5% moisture, and 0.5 cm of sea sand. The column was prewetted with hexane, and the sample was transferred to the column with 10 ml of ethyl acetate:hexane (1:9). An additional 140 ml of ethyl acetate:hexane (1:9) was passed through the column and discarded. The sample flask was rinsed with 10 ml of ethyl acetate:hexane (3:7) that was used with an additional 140 ml of ethyl
Collect soil sample
Pass through sieve, mix, determine dry weight
Weigh soil (50 g/sample)
Reflux (1 h, 600 ml 0.25 N HCl), filter

Soil, discard

0.25 N HCl, cool (1 h, -10°C)
Add sodium lauryl sulfate (250 mg)
Extract with dichloromethane (3 X 600 ml)

0.25 N HCl phase, discard

Dichloromethane phase, dry (anhyd. Na₂SO₄)
Concentrate (ca. 135 ml)
Cleanup (Nuchar-attaclay column, 150 ml ethyl acetate:hexane 80:20 eluate)
Concentrate (ca. 25 ml), add benzene (100 ml)
Concentrate (under 1 ml), adjust to volume (1 ml benzene)
Analyze (GLC with DTC)

Figure 1. Flow diagram depicting the carbofuran extraction and direct analysis procedure described by Cook (1973)
acetate:hexane (3:7) for carbofuran elution from the column. In some ex-
periments, a different extraction procedure (Figure 2) modified from
Tweedy and Kahrs (1978) was used. The modifications included the elimina-
tion of cleanup steps and the use of a different analysis procedure.
There was no difficulty measuring either carbofuran or atrazine in the un-
cleaned extracts, but long retention-time contaminants made analysis very
slow. Both the Cook (1973) and modified Tweedy and Kahrs (1978) proce-
dures are time consuming, and the procedure described by Cook (1973) also
requires large-volume glassware, and large quantities of solvent per gram
of soil extracted. These were major limitations because it was necessary
to extract large numbers of samples to determine carbofuran residue levels
in field-collected samples. In an effort to simplify the procedure and
use available extraction and analysis equipment as much as possible, an
extraction procedure was modified from Möllhoff (1975a). The modified
procedure (Figure 3) produced extracts from 60-g portions of the composite
soil samples that could be analyzed by GLC with an ECD. Each 60-g portion
of soil was weighed into a 250-ml glass centrifuge bottle and blended 3
times for 5 min with 100-ml aliquots of methanol:0.1 N HCl (1:1) on a
Servall Omn-Mixer or a Gilchrist No. 22 blender. The extract was
separated from the soil by centrifuging for 5 min at 800 X g. The ex-
tract was decanted into a 500-ml separatory funnel and the soil was dis-
carded. Carbofuran was removed from the 3 combined extracts by shaking
them 3 times for 30 sec with dichloromethane (100, 50, and 50 ml, respec-
tively). The 3 dichloromethane extracts were combined and then evaporated
Collect soil sample
Pass through sieve, mix, determine dry weight
Weigh soil (50 g/sample)
Reflux (1 h, 150 ml acetonitrile:water 9:1), filter

Soil, discard

Remainder of extract, discard

Acetonitrile:water, measure volume

1/5 of extract volume, add water (300 ml) and water saturated with NaCl (20 ml)
Extract with dichloromethane (2 X 25 ml)

Acetonitrile:water phase, discard

Dichloromethane phase, dry (anhyd. Na₂SO₄), rinse Na₂SO₄ with dichloromethane (25 ml)
Evaporate (dryness), adjust to volume (10 ml ethyl acetate)
Analyze (GLC with TSD)

Figure 2. Flow diagram depicting a modification of the extraction and analysis procedure described by Tweedy and Kahrs (1978)
Collect soil sample

Pass through sieve, mix, determine dry weight

Weigh soil (60 g/sample)

Extract with methanol:0.1 N HCl
(1:1, 3 X 100 ml, 5 min)
centrifuge (3 X 800 g, 5 min)

Soil, discard

Methanol:0.1 N HCl, extract with dichloromethane
(100, 50, and 50 ml)

Methanol:0.1 N HCl phase, discard

Dichloromethane phase, concentrate (ca. 5 ml)

Cleanup (Florisil column, 150 ml ethyl acetate:hexane 35:65 eluate)

Evaporate (dryness)
adjust to volume (25 ml benzene)

Form derivatives (0.5 ml extract, 3.4 μl pyridine,
10 μl heptafluorobutyric anhydride)

Adjust to volume (5 ml hexane),
extract with pH 6 buffer (2 ml)

Analyze solvent phase
(GLC with ECD)

Figure 3. Flow diagram depicting the carbofuran extraction method modified from Möllhoff (1975a) and carbofuran derivative analysis procedure used in these studies
to ca. 5 ml on a rotary evaporator at 30°C and partial vacuum. The aqueous phase was discarded.

Cleanup of these extracts was accomplished with a glass column, 22 mm (ID) by 30 cm, packed with successive layers of 10-g anhydrous Na$_2$SO$_4$, 11-g Florisil$^R$ (activated for 1 h at 110°C), and 20-g anhydrous Na$_2$SO$_4$. The packing was wetted with 25 ml of hexane. The dichlormethane extract was added to the column. The eluent was 150 ml of ethyl acetate:hexane (35:65). The eluate was collected in a 500-ml flask and evaporated just to dryness under partial vacuum on a rotary evaporator at 30°C. The residue was made to 25 ml with benzene and stored at -18°C until analyzed.

Later in my experimental work, a thermionic specific detector (TSD) was purchased. The specificity of the TSD for nitrogen-containing compounds made it possible to develop an easier carbofuran extraction and analysis procedure (Figure 4). Approximately 400 g of soil were placed in a 0.95-l glass jar for extraction. Field-collected cores were first stirred with a spatula to break apart soil chunks, then transferred to 2 jars to maintain the 400 g/jar ratio. Additions of 100 ml of 0.25 N HCl (approximately 0.0625 mM HCl/g soil) and 200 ml of ethyl acetate were made to each extraction jar. The jars were sealed and placed on a Fisher-Kahn shaker (280 oscillations/min, 32-mm stroke distance) for 15 min. The ethyl acetate was decanted into a graduated cylinder. Extracts of cores that were separated into 2 extraction jars were combined in a single 1000-ml graduated cylinder. When soil chunks were apparent after initial extraction, it was necessary to break apart the chunks before further extraction. The soils were extracted 2 additional times by shaking for 15
Collect soil sample

Break apart soil aggregates
transfer to extraction jars
(400 g/jar)

Add 0.25 N HCl (100 ml)

Extract with ethyl acetate
(200, 150, and 150 ml, 15 min)

Soil,
determine dry weight
Discard

Ethyl acetate,
mix, measure volume

Remainder of extract,
discard

200 ml of extract,
dry (anhyd, Na₂SO₄)
Analyze (GLC with TSD)

Figure 4. Flow diagram depicting the carbofuran extraction and direct analysis procedure developed for these studies
min with 150 ml of ethyl acetate. The oven-dried weight of the soil was determined, and the soil was discarded. The total volume of ethyl acetate extract was recorded, the extract was mixed, 200 ml were transferred to a 237-ml storage bottle, and the remainder of the extract was discarded. The ethyl acetate was dried by placing 8 to 10 g of anhydrous Na₂SO₄ in the storage bottle. The extracts were stored at -18°C until analyzed. No cleanup procedure was required on these extracts prior to analysis by GLC with a TSD. In some laboratory experiments this procedure had to be slightly modified for use on smaller soil samples. Enough HCl solution was added to these samples to adjust the soil-moisture level to between 30% and 55%. The concentrations of the HCl solutions were adjusted to the 0.0625 mM HCl/g of soil ratio used in the larger samples. The smaller samples were extracted with 50 ml, 40 ml, and 40 ml of ethyl acetate, and the entire extract was saved for analysis.

Extracts that also were analyzed by HPLC were evaporated to dryness, and the residues were dissolved in 5 ml of dichloromethane. The dichloromethane was cleaned with the Florisil column procedure shown in Figure 3. The eluate was evaporated to dryness and the residues were dissolved in 5 ml of dichloromethane. Carbofuran in the dichloromethane and 2 additional 5 ml dichloromethane rinses of the evaporation flask was removed by adsorption when the solvents were passed through a silica SEP-PAK R (Waters Associates, Inc., Milford, MA). Carbofuran was removed from the SEP-PAK with 4 ml of methanol that was used for HPLC analysis.
Analytical Techniques

Gas-liquid chromatography (GLC)

Analysis of a derivative of carbofuran with an electron-capture detector (ECD) is described below. Depending on the carbofuran concentration, 0.1 to 0.5 ml of benzene extract was pipetted into a 5-ml volumetric flask. Benzene was used when necessary to make the extract to 0.5 ml. Derivative formation was accomplished by adding 3.4 µl of silylation-grade pyridine (Pierce Chemical Co., Rockford, IL), 10 µl of heptafluorobutyric anhydride (HFBA, Pierce Chemical Co.), shaking for 10 sec, and allowing the mixture to stand for 8 to 16 h at room temperature (19 to 24°C). Just before analysis, the mixture was diluted to 5 ml with hexane, transferred to an 8-ml vial, and shaken for 30 sec with 2 ml of 1 M phosphate buffer (pH 6).

Derivatives of 4 analytical-grade carbofuran standards, ranging from 1.2 to 3.0 µg of carbofuran/0.5 ml of benzene, were prepared with each group of soil extract derivatives. Analyses were run on the hexane-benzene phase without dilution or drying. If the carbofuran concentration in a soil extract fell outside of the standard curve, a different volume of the soil extract was used for derivative formation. When this occurred, derivatives of carbofuran standards were formed in equal volumes of solvent. Similar methods were used to form 3-hydroxycarbofuran and 3-oxocarbofuran derivatives in soil extracts and as standards.

The HFBA derivatives of carbofuran, 3-hydroxycarbofuran, and 3-oxocarbofuran were measured on a Varian, model 3700, GLC equipped with a $^{63}$Ni ECD. A 2-mm (ID) by 210-cm Pyrex$^R$ column, silanized with Sylon-CT$^R$
(Supelco, Bellefonte, PA), packed with 3% Apiezon N on 60/80 mesh Chromosorb® G, and treated with 50 µl of Silyl-8® (Pierce Chemical Co.) was used for analysis. Operating conditions were: injection port, column, and detector temperatures 200°, 170°, and 270°C, respectively; and nitrogen gas flow 26 ml/min.

The retention times of HFBA derivatives of 3-hydroxycarbofuran, carbofuran, and 3-oxocarbofuran were ca. 3.0, 4.4, and 5.6 min, respectively. Analyses were done on 0.7 to 1.4 ng of 3-hydroxycarbofuran, 1.2 to 3.0 ng of carbofuran, and 0.7 to 2.1 ng of 3-oxocarbofuran with a GLC voltage attenuation of 1280. Injection volumes ranged from 2 to 8 µl.

All results are based on the average peak height of at least 3 injections. Standard curves were prepared by plotting average peak height against ng of insecticide. The total volume of carbofuran in the soil extract was calculated by using the GLC standard curve and dilution factors. Carbofuran in the soil samples (in ppm) was calculated by using the total amount of carbofuran extracted and the dry weight of the soil extracted.

Direct analysis of carbofuran was done with a TSD. Many of the extracts contained both carbofuran and atrazine. These pesticides were not separated by most GLC columns tested. Tests of various lengths and diameters of glass columns deactivated with Sylon-CT showed that carbofuran peaks were greatly reduced or absent when columns larger than 95 cm by 2 mm (ID) were used. Although it might have been possible to overcome this effect by silylation of the packing material, all additional tests were run with 95 cm by 2 mm (ID) columns. Apiezon N was the only liquid phase that provided acceptable separation between atrazine and carbofuran.
(Table 1). Chromosorb W(HP) was a better support than Chromosorb G, because it produced columns with more theoretical plates. Apiezon N was coated on both of these supports by fluidized drying (Leibrand and Dunham, 1973). The short retention times that occurred with many of the packings were lengthened at lower column temperatures and slower rates of carrier-gas flow, but resolution between atrazine and carbofuran was not improved significantly. The retention times on the Apiezon N column and minimum detectable quantities of carbofuran, atrazine and some of their metabolites are shown in Table 2. These and the other analyses were done on a Varian model 3700 GLC with a 200°C injector port, 250°C detector, 175°C column oven, 28 ml/min nitrogen carrier-gas flow, 6 ml/min hydrogen gas flow, 160 ml/min air flow, 4 TSD bias voltage, and 3.90 TSD bead current. GLC voltage attenuations between 1 and \( 8 \times 10^{-12} \) A/mV were used.

Chromatograms obtained from extracts of treated and untreated soils are shown in Figure 5. Although it was not confirmed by other techniques, it is possible that the unknown peak consisted of deethylated and (or) de-isopropylated atrazine. The Apiezon N column would no longer separate carbofuran from the unknown peak or the 2 atrazine metabolites after use followed by storage at room temperature without nitrogen gas flowing through it. For that reason, it was necessary to pack a new column every time the column was removed from the GLC oven.

**Liquid scintillation counting (LSC)**

Total radiocarbon in 50 µl of extract was measured on a Packard model 3315 liquid scintillation spectrometer by using 15 ml of Handifluor (Mallinckrodt, St. Louis, MO) and 20-ml polyethylene vials. Counts were
Table 1. GLC retention times of carbofuran and atrazine

<table>
<thead>
<tr>
<th>Liquid phase</th>
<th>Support</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>% load</td>
<td>Material</td>
</tr>
<tr>
<td>Apiezon N</td>
<td>10</td>
<td>Chromosorb W(HP)</td>
</tr>
<tr>
<td>Apiezon N</td>
<td>3</td>
<td>Chromosorb G</td>
</tr>
<tr>
<td>SE-30</td>
<td>3</td>
<td>Gas Chrom Q</td>
</tr>
<tr>
<td>DC-200</td>
<td>10</td>
<td>Chromosorb W(HP)</td>
</tr>
<tr>
<td>OV-101</td>
<td>4</td>
<td>Gas Chrom Q</td>
</tr>
<tr>
<td>OV-3</td>
<td>3</td>
<td>Supelcoport</td>
</tr>
<tr>
<td>QF-1</td>
<td>5</td>
<td>Gas Chrom Q</td>
</tr>
<tr>
<td>OV-210</td>
<td>5</td>
<td>Gas Chrom Q</td>
</tr>
<tr>
<td>Carbowax 20-M</td>
<td>7</td>
<td>Gas Chrom Q</td>
</tr>
<tr>
<td>EGSS-X</td>
<td>2</td>
<td>Gas Chrom Q</td>
</tr>
</tbody>
</table>
Table 2. The retention times of carbofuran, atrazine, and some of their metabolites (95 cm by 2 mm column packed with 10% Apiezon N on 100/120 mesh Chromosorb W(HP)), and the minimum quantities detectable with a TSD

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Minimum detectable quantity (pg)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deisopropylated atrazine</td>
<td>3.20</td>
<td>60</td>
</tr>
<tr>
<td>Deethylated atrazine</td>
<td>3.42</td>
<td>200</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>4.06</td>
<td>120</td>
</tr>
<tr>
<td>3-oxocarbofuran</td>
<td>5.45</td>
<td>200</td>
</tr>
<tr>
<td>Atrazine</td>
<td>5.90</td>
<td>60</td>
</tr>
<tr>
<td>3-hydroxycarbofuran</td>
<td>7.48</td>
<td>300</td>
</tr>
</tbody>
</table>

\(^a\)Twice the noise level at a voltage attenuation of \(1 \times 10^{-12}\) A/mV. Minimum detectable quantities were about one-third the values listed when determined on a new bead by using a bead current of 2.50.

corrected for background, spectrometer efficiency, and chemical quench.
The channels ratio method (Herberg, 1965) was used to determine quench correction. All results were the average of duplicate analyses.

**High-pressure liquid chromatography (HPLC)**

Extracts were injected on a Waters Associates HPLC consisting of a model U6K injector, model 6000A pump, \(\mu\)Bondapak \(^R\) \(C_{18}\) column, and model 440 UV absorbance detector. The mobile phase was 60% methanol in water (v/v) with a flow of 1 ml/min. Detection was made at 254 nm. Peak heights of carbofuran in soil extracts were determined in comparison with peak heights of carbofuran standards.
Figure 5. GLC chromatograms obtained at a voltage attenuation of 8 X 10^{-12} A/mV using a TSD with a new bead operated at a bead current of 2.50. (A) 1 μl of extract of Canisteo soil treated with 4.8 ppm of carbofuran and 1.0 ppm of atrazine, 1 = ethyl acetate, 2 = unknown, 3 = 3.83 ng carbofuran, 4 = 0.95 ng atrazine; (B) 1 μl of extract of untreated Canisteo soil, 1 = ethyl acetate, 2 = unknown, 4 = 0.34 ng atrazine.
Thin-layer chromatography (TLC) and autoradiography

Samples were spotted on precoated, silica gel F-254 plates. The plates were developed twice vertically with diethyl ether:hexane (3:1). Carbofuran and metabolite standards were visualized by spraying the plates with a solution containing 50-mg p-nitrobenzenediazonium tetrafluoroborate, 20-ml methanol, and 20-ml glacial acetic acid, then spraying with 5% NaOH in methanol (wt/v). Visualization was also achieved by autoradiography. This was accomplished by exposing Kodak\textsuperscript{R} X-ray film for 4 to 6 days, then developing and fixing the film.

Experimental Series

Recovery experiments

An experiment was run to test both the efficiency of carbofuran extraction from soil with the method modified from Möllhoff (1975a, Figure 3), and the carbofuran derivative analysis method. A 60-g (wet weight) aliquot of each of the 11 soils shown in Table 3 was weighed into a separate 250-ml glass centrifuge bottle. Each soil sample received 326 \( \mu \)g of \( ^{14} \)C-carbofuran in 1.0 ml of acetone. The soils were mixed for 30 min on a jar roller to distribute the chemicals and evaporate the acetone. The \( ^{14} \)C-carbofuran concentrations ranged from 6.05 to 6.75 ppm on a soil dry weight basis. The samples were analyzed by both LSC and GLC with an ECD after formation of HFBA derivatives. An additional experiment was done to test the efficiency of extraction of 2 carbofuran metabolites, 3-hydroxycarbofuran and 3-oxocarbofuran. Two 60-g (wet weight) samples from Harlan (Table 3) were treated with 290 \( \mu \)g of 3-hydroxycarbofuran and 290
Table 3. Properties of soils used in 1977 field tests and some laboratory tests

<table>
<thead>
<tr>
<th>Field location</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marshalltown</td>
<td>6.0</td>
<td>3.8</td>
<td>6</td>
<td>72</td>
<td>22</td>
</tr>
<tr>
<td>Newell</td>
<td>7.8</td>
<td>5.5</td>
<td>26</td>
<td>56</td>
<td>18</td>
</tr>
<tr>
<td>Nashua</td>
<td>6.8</td>
<td>3.9</td>
<td>27</td>
<td>52</td>
<td>21</td>
</tr>
<tr>
<td>Keswick</td>
<td>6.5</td>
<td>4.3</td>
<td>4</td>
<td>71</td>
<td>25</td>
</tr>
<tr>
<td>Laurens</td>
<td>5.6</td>
<td>5.3</td>
<td>40</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Winterset</td>
<td>6.3</td>
<td>3.6</td>
<td>2</td>
<td>73</td>
<td>25</td>
</tr>
<tr>
<td>Cedar Falls</td>
<td>6.2</td>
<td>4.6</td>
<td>18</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>Sanborn</td>
<td>6.0</td>
<td>4.7</td>
<td>4</td>
<td>69</td>
<td>27</td>
</tr>
<tr>
<td>Harlan</td>
<td>5.0</td>
<td>3.4</td>
<td>5</td>
<td>69</td>
<td>26</td>
</tr>
<tr>
<td>Wellsburg</td>
<td>6.0</td>
<td>4.1</td>
<td>20</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Rembrandt</td>
<td>8.1</td>
<td>6.2</td>
<td>44</td>
<td>41</td>
<td>15</td>
</tr>
</tbody>
</table>

μg of 3-oxocarbofuran, each in 1.0 ml of acetone. The concentration of each of these metabolites was 6.0 ppm on a soil dry weight basis. The soils were mixed on a jar roller for 30 min, extracted, and the extracts were analyzed by GLC with an ECD.

Two experiments were done to establish the efficiency of carbofuran extraction from soil with ethyl acetate (Figure 4). Four 480-g (wet weight) aliquots of Kenyon-Floyd soil from Nashua and 4 aliquots of Canisteo soil from Newell (Table 4) were weighed into 0.95-l glass jars. Two of the Nashua and 2 of the Newell samples were used as untreated controls to check for carbofuran residues initially in the soil. The 4 remaining samples were treated with 2000 μg of carbofuran in 1.0 ml of
Table 4. Properties of soils used for laboratory tests

<table>
<thead>
<tr>
<th>Collection location</th>
<th>Soil type</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nashua</td>
<td>Kenyon and Floyd</td>
<td>6.1</td>
<td>3.8</td>
<td>26</td>
<td>51</td>
<td>23</td>
</tr>
<tr>
<td>Newell</td>
<td>Canisteo</td>
<td>7.8</td>
<td>5.2</td>
<td>20</td>
<td>59</td>
<td>21</td>
</tr>
<tr>
<td>Ames</td>
<td>Webster</td>
<td>7.6</td>
<td>3.6</td>
<td>42</td>
<td>40</td>
<td>18</td>
</tr>
<tr>
<td>Ames</td>
<td>Clarion</td>
<td>5.1</td>
<td>2.5</td>
<td>48</td>
<td>34</td>
<td>18</td>
</tr>
</tbody>
</table>

methanol and mixed for 20 min on a jar roller. The carbofuran concentration in the 4 treated samples was 4.8 ppm on a soil dry weight basis. The samples were extracted and analyzed by GLC with a TSD. Water was removed from the ethyl acetate extract after the solvent volume was measured. The volume is most easily measured as described, and less drying agent (anhydrous Na$_2$SO$_4$) is necessary. If significant amounts of water are removed by the drying step, however, the sample volume may be changed prior to analysis, thus causing the calculations to be in error. To test this possibility, four 503-g (wet weight) aliquots of Kenyon-Floyd soil from Nashua (Table 4) were weighed into 0.95-l glass jars. Each sample was treated with 2000 µg of carbofuran in 1.0 ml of acetone and mixed for 20 min on a jar roller. The carbofuran in these soils was 5.0 ppm on a soil dry weight basis. Two of the 4 samples were extracted as previously described. Extracts of the other 2 samples were dried with anhydrous Na$_2$SO$_4$ before measurement of the extract volume. All extracts were analyzed by GLC with a TSD.
A factorial experiment was done to determine if soil acidity and moisture levels would affect carbofuran extraction by the ethyl acetate method (Figure 4). Acidity was tested by 2 different factors in the experiment. The actual soil pH was altered by using either acidic Kenyon-Floyd soil from Nashua, or basic Canisteo soil from Newell (Table 4). In addition, the soil pH was lowered by adding 100 ml of moisture as 0.25 N HCl or left unaltered by adding 100 ml of moisture as glass-distilled water. The third factor tested in the experiment was the soil-moisture levels. The initial soil-moisture levels were adjusted to 5% (air-dried) or 25%. As previously mentioned, an additional 100 ml of water or 0.25 N HCl were added during the extraction, so the final soil-moisture levels were 30% or 50%. Duplicate samples of each of the 8 combinations of 3 factors were set up by weighing the appropriate wet weight of soil into sixteen 0.95-l glass jars to make 400-g (dry weight) samples. The 16 samples were treated with 2000 μg of carbofuran in 1.0 ml of acetone, mixed for 20 min on a jar roller, and extracted in the appropriate manner. An additional sample of each soil type was adjusted to the high soil-moisture level, treated with 100 ml of 0.25 N HCl and extracted as a control without carbofuran treatment. Extracts were analyzed by GLC with a TSD.

Soil extracts from a factorial experiment similar to the previously described experiment were analyzed with a second analysis technique to verify the GLC, TSD method. This experiment consisted of duplicate samples of the 4 combinations of the Kenyon-Floyd and Canisteo soils (Table
and low and high initial moisture levels. The initial moisture levels were 7% or 26% in Kenyon-Floyd soil and 9% or 29% in Canisteo soil. Single samples (415 g dry weight) of the 4 treatment combinations were treated with 2000 μg of carbofuran in 1.0 ml acetone. The 4 remaining samples were left untreated to test for carbofuran initially present in the soil. The samples were mixed and extracted with ethyl acetate (Figure 4) as previously described. The extracts were analyzed by GLC and after additional sample cleanup by HPLC.

The efficiency of carbofuran extraction from soil with ethyl acetate (Figure 4) could be dependent on the concentration of carbofuran in the soil. To test this possibility, ten 503-g wet weight aliquots of Kenyon-Floyd soil from Nashua (Table 4) were weighed into 0.95-l glass jars. Groups of 2 soil samples were left untreated as controls, or treated with the appropriate quantity of carbofuran in 2.5 ml of acetone to make carbofuran concentrations of 0.1 ppm, 1.0 ppm, 10 ppm, and 100 ppm on a soil dry-weight basis. The samples were mixed for 20 min on a jar roller prior to extraction. The extracts were analyzed by GLC with a TSD.

As a final check on the ethyl acetate extraction procedure (Figure 4), 2 experiments were done comparing that procedure to 2 established extraction procedures. The first method-comparison experiment was done with field-aged carbofuran residues. Carbofuran (as Furadan 10G) was applied at the rate of 11.5-g carbofuran/100 linear meters in an 18-cm band over the buried corn seeds in four 30.5-m long rows at Nashua. One 10.5-cm wide by 7.5-cm deep soil core was collected from each of the 4 rows 48 days after treatment with carbofuran. The cores were placed individually in
polyethylene bags marked with the collection location, and stored at 0°C. Each core was passed through a 2.38-mm sieve, mixed by rolling on a sheet of paper, and six 60-g (wet weight) aliquots were removed for extraction. Two of each 6 soil aliquots were extracted with ethyl acetate (Figure 4) with slight modifications to account for the small sample size. Two of each 6 soil aliquots were extracted with 0.25 N HCl by a procedure slightly modified from Cook (1973, Figure 1). Two of each 6 soil aliquots were extracted with acetonitrile:water (9:1) according to a procedure shortened from Tweedy and Kahrs (1978, Figure 2). All extracts were analyzed by GLC with a TSD.

The second method-comparison experiment was done with laboratory-treated soil, and was analyzed as 2 separate parts. The first part of the experiment dealt with carbofuran extraction from freshly-treated soil, and the second part dealt with extraction of aged carbofuran residues. One factor in each part of the experiment was extraction with ethyl acetate (Figure 4), or 0.25 N HCl (Figure 1), or acetonitrile:water (9:1, Figure 2). A second factor in each part of the experiment was soil from Nashua or from Newell (Table 4). Six samples of the 6 combinations of treatments were set up by weighing the appropriate quantity of wet soil into 118-ml jars to provide 50 g (dry weight) of soil. Duplicate samples of the 6 combinations of treatments were extracted in the appropriate manner for use as untreated controls. The remaining 24 samples were treated with 22.5 mg of Furadan 10G (equivalent to 2.25 mg of carbofuran), and mixed for 20 min on a jar roller. Duplicate carbofuran-treated samples of the 6 combinations of treatments were brought to 50% moisture and immediately
extracted in the appropriate manner. The remaining samples were main­
tained at 50% moisture by adding water to correct for weight loss each 5
days during the 15-day incubation period. To reduce water loss, the
latter samples were covered with Parafilm$^R$ that had a central hole large
enough to accommodate a pipet tip for water additions. The temperature
was maintained at 22 to 27°C. All extracts were analyzed by GLC with a
TSD.

Carbofuran persistence in soil

Persistence of carbofuran in the field was established at 2 locations
in Iowa during 1979. Two rows adjacent to the Newell and Nashua plots
that were used for 1979 residue and efficacy experiments were planted with
corn and treated with carbofuran in the same manner described for those
plots (see Pages 51 and 52). Properties of these soils are shown in Table 5. Soil samples were collected at frequent intervals, extracted with
ethyl acetate, and analyzed directly by GLC as previously described.
Rainfall information also was collected on the farms at Newell and Nashua
by university personnel stationed at those locations.

It was anticipated that the release of carbofuran from Furadan 10G
granules and from adsorption sites in the soil would be related to soil-
moisture levels and therefore to rainfall. The rate of carbofuran release
into the available compartment where the insecticide could be leached or
degraded (Hamaker and Goring, 1976) might be a very important factor con­
trolling carbofuran persistence in a field situation. For that reason,
water was drained through soil treated with Furadan 10G or technical-
grade carbofuran in the laboratory to determine the amount of carbofuran
Table 5. Properties of carbofuran-treated soil used in 1979 field tests; results are averages of all replicates

<table>
<thead>
<tr>
<th>Field location</th>
<th>Experiment^</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newell</td>
<td>P</td>
<td>8.0</td>
<td>5.3</td>
<td>18</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>Nashua</td>
<td>P</td>
<td>7.1</td>
<td>3.9</td>
<td>30</td>
<td>52</td>
<td>18</td>
</tr>
<tr>
<td>Mechanicsville</td>
<td>E</td>
<td>6.1</td>
<td>3.6</td>
<td>2</td>
<td>72</td>
<td>26</td>
</tr>
<tr>
<td>Stanwood</td>
<td>E</td>
<td>6.6</td>
<td>3.1</td>
<td>2</td>
<td>73</td>
<td>25</td>
</tr>
<tr>
<td>Tipton</td>
<td>E</td>
<td>6.8</td>
<td>2.9</td>
<td>6</td>
<td>75</td>
<td>19</td>
</tr>
<tr>
<td>Ames</td>
<td>E</td>
<td>6.7</td>
<td>2.9</td>
<td>45</td>
<td>38</td>
<td>17</td>
</tr>
<tr>
<td>Newell</td>
<td>E</td>
<td>7.5</td>
<td>5.3</td>
<td>22</td>
<td>55</td>
<td>23</td>
</tr>
</tbody>
</table>

^P = carbofuran persistence experiment; E = carbofuran residues and efficacy experiment.

released into the water at various rainfall levels. Furadan 10G was the formulation used in the previously described field tests, and technical-grade carbofuran might represent carbofuran adsorbed to soil particles after initial release from the formulation. Eight soil tensiometers modified from Rose (1966, p. 145) were set up as shown in Figure 6, and 82.1 g (dry weight) of Kenyon-Floyd soil from Nashua (Table 4) were added to each tensiometer. This soil was chosen to reduce the possibility of alkaline hydrolysis of carbofuran. The distance between the soil surface and the water reservoir surface (No. 10, Figure 6) was adjusted to 150 cm. The soil was flooded and allowed to drain to the equilibrium moisture level. Preliminary experiments with these tensiometers and the Kenyon-Floyd soil showed that the equilibrium was reached at 24.9±2.4% moisture. Each of the 4 treatment combinations of Furadan 10G or technical-grade
Figure 6. Soil tensiometer: (1) 150-ml, medium-porosity, fritted-disk funnel; (2) soil; (3) 1.27-cm bore-diameter Teflon tubing; (4) glass connector; (5) 0.32-cm bore-diameter Teflon tubing; (6) glass connector; (7) 0.95-cm bore-diameter Teflon tubing; (8) glass-distilled water; (9) 500-ml separatory funnel; (10) distance between the soil surface and the water reservoir surface (150 cm)
carbofuran and 25 or 150 ml of water were randomly assigned to 2 soil tensiometers. The soil was loosened with a spatula and 22.5 mg of Furadan 10G or 2.25 mg of technical-grade carbofuran in 1.0 ml of acetone were mixed with the soil by stirring.

Preliminary analysis of carbofuran in three 22.5 mg quantities of Furadan 10G showed 101.3±1.6% of the expected quantity of carbofuran, thus indicating that the treatment doses were accurate. The surface area of the soil in the tensiometers was 35.3 cm$^2$, and the 22.5 mg of Furadan 10G represented approximately the same dose that would fall on a 35.3 cm$^2$ area in a treated band in a field. The 25 and 150-ml quantities of glass-distilled water were dripped evenly across the soil surface at the rate of 25 ml/15 min. On a surface area basis this represented 0.71 and 4.25 cm of precipitation. The soils were covered with Parafilm to reduce evaporation. Small holes were placed in the Parafilm to allow some air movement. The soils were drained for 48 h to allow all 8 samples to return to near equilibrium moisture levels. The soil was scraped from the fritted-disk funnel (No. 1, Figure 6), and the water was drained into the separatory funnel (No. 9, Figure 6). The fritted-disk funnel was rinsed with 0.25 N HCl and ethyl acetate before using those liquids for extraction of the soil. The water phases were extracted 3 times with 100 ml of ethyl acetate. The ethyl acetate was dried over anhydrous Na$_2$SO$_4$, concentrated on a rotary evaporator, and adjusted to 25 ml in a volumetric flask. The water and soil extracts were analyzed by GLC with a TSD.

A longer term experiment was also run to determine how carbofuran degradation relates to soil-moisture levels. If the amount of carbofuran
released into the water phase is the major factor controlling the rate of carbofuran degradation, carbofuran persistence should decrease with increased soil-moisture levels. To study this possibility, 82.1 g (dry weight) of Kenyon-Floyd soil from Nashua (Table 4) containing 17% moisture was weighed into twenty-eight 0.425-l wide-mouth glass jars that provided a 35.3 cm² surface area. Half of the samples were treated with 22.5 mg of Furadan 10G, and the remainder were treated with 2.25 mg of technical-grade carbofuran in 1.0 ml of acetone. The soil was mixed with the insecticide by rolling on a jar roller for 20 min. The samples were covered with Parafilm with a central hole large enough to accommodate a pipet tip for water addition, weighed, and allowed to stand for 48 h for complete acetone evaporation. Four of the 28 samples were extracted with ethyl acetate and analyzed directly by GLC to establish initial recovery levels. The remaining 24 samples were set up so that 4 samples were given each of the 6 treatment combinations of formulated or technical carbofuran, and 17%, 30%, or 50% soil moisture. Half of each group of 4 samples were extracted 7 days and half 17 days after the initial extractions. Water additions were made every 7 days to maintain the correct soil-moisture level in each sample, and temperature was maintained at 22 to 27°C. All samples were adjusted to 50% moisture prior to extraction and direct determination of residues by GLC with a TSD.

Finally an experiment was run to determine if there are differences between soils in the rate of carbofuran degradation when high soil-moisture levels are used. Kenyon-Floyd soil from Nashua, Canisteo soil from Newell, and Webster and Clarion soils from Ames (Table 4) were used in
this experiment. This selection of soils included acidic and basic soils, and soils with and without previous carbofuran treatment. Quantities of 82.1 g (dry weight) of each soil were weighed into twelve 0.425-l wide-mouth glass jars, treated with 22.5 mg of Furadan 10G, mixed by rolling for 20 min on a jar roller, covered with Parafilm with a central hole large enough to accommodate a pipet tip, and weighed. The soil was adjusted to 50% moisture and maintained at that level by water additions each 5 days. The temperature was maintained at 22 to 27°C. Duplicate samples of each soil were extracted 0, 5, 10, 15, and 20 days after treatment, and analyzed by GLC with a TSD.

Carbofuran residues and efficacy

In 1977, 11 fields were selected throughout Iowa where the history of insecticide use on these fields was known (Table 6). At least two fields were selected in each insecticide history grouping; when possible, one field was located in a glacial-till soil, and one was located in a loessal soil. The soil properties of these fields are shown in Table 3. Carbofuran (as Furadan 10G) was applied, 11.5-g carbofuran/100 linear meters, at planting time in an 18-cm band over the buried corn seeds and incorporated in the top 2.5 cm of soil by dragging a chain behind the planter. The distance between corn rows varied between 71 and 92 cm, depending on the planting equipment available. All fields were planted between April 26 and May 10, 1977. Farmers at Marshalltown, Keswick, Winterset, Cedar Falls, Sanborn, and Wellsburg used Northrup King 585, Trojan 119A, Pioneer 3369, Pioneer 3780, DeKalb XL25, and Acco 4561 seed corn, respectively. The remaining fields were planted with DeKalb XL44 seed corn by
Table 6. Information on fields in Iowa used during 1977 to study carbafuran degradation and efficacy (Gorder et al., 1980)

| Field location | Insecticide history | Parent material of soil
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Marshalltown</td>
<td>no insecticide 1969-1976</td>
<td>loess</td>
</tr>
<tr>
<td>Newell</td>
<td>no insecticide 1976</td>
<td>glacial till</td>
</tr>
<tr>
<td>Nashua</td>
<td>no insecticide 1975-1976</td>
<td>glacial till</td>
</tr>
<tr>
<td>Keswick</td>
<td>carbofuran 1972-1976</td>
<td>loess</td>
</tr>
<tr>
<td>Laurens</td>
<td>carbofuran 1972-1976</td>
<td>glacial till</td>
</tr>
<tr>
<td>Winterset</td>
<td>phorate 1972-1976</td>
<td>loess</td>
</tr>
<tr>
<td>Cedar Falls</td>
<td>phorate 1971-1975, fonofos 1976</td>
<td>loess/glacial till</td>
</tr>
<tr>
<td>Sanborn</td>
<td>carbofuran 1972-1975, terbufos 1976</td>
<td>loess</td>
</tr>
<tr>
<td>Harlan</td>
<td>carbofuran 1972-1974, fonofos 1975-1976</td>
<td>loess</td>
</tr>
<tr>
<td>Wellsburg</td>
<td>carbofuran 1972-1975, terbufos 1976</td>
<td>loess/glacial till</td>
</tr>
<tr>
<td>Rembrandt</td>
<td>carbofuran 1974-1975, fonofos 1976</td>
<td>glacial till</td>
</tr>
</tbody>
</table>

\(^a\)As indicated by Oschwald et al. (1965).

university personnel using a John Deere model 71B 4-row unit planter with a modified Nobel\textsuperscript{R} metering unit for insecticide application (Hills et al., 1972). Carbofuran-treated and untreated areas were established in each field. Fields at Marshalltown, Keswick, Winterset, Sanborn, and Wellsburg had adjacent, equal-sized, carbofuran-treated and untreated areas 4 to 12 rows wide that ran the entire length of the field. Only 730 to 2,200 linear meter portions of these areas were studied. At Newell, Nashua, Laurens, Cedar Falls, Harlan, and Rembrandt, carbofuran was applied to 730 to 1,460 linear meters in 4 to 8 rows bordering 0.4 to 0.6-ha soil-insecticide study areas. Untreated areas at Newell, Nashua, and Cedar Falls consisted of four 30.5-m rows randomly located within the soil-insecticide study areas. At Laurens, Harlan, and Rembrandt, un-
treated areas consisted of 4 pairs of rows randomly located within the soil-insecticide study areas. Corn roots and soil in these areas were sampled as previously described. The upper soil layers were extracted with methanol:0.1 N HCl (Figure 3) and analyzed by GLC with an ECD after derivative formation. The lower soil layers were extracted with ethyl acetate (Figure 4) and analyzed directly by GLC with a TSD.

For 1979 field experiments, 6 fields were selected in Iowa that contained moderate to high numbers of corn rootworm eggs in the spring of 1979. It was hoped that these fields would contain corn rootworm larval populations adequate for determination of insecticide efficacy. Properties of some of these soils are shown in Table 6. Plots at each location were set up as randomized complete blocks. Each 122-m long plot was divided into 4 blocks that were 30.5 m long. The 30.5-m rows in each block were planted with corn and received a planting-time insecticide treatment or were left untreated. Blocks always included an 18-cm wide band treatment of carbofuran applied as Furadan 10G at the rate of 11.5-g actual carbofuran/100 linear meters and an untreated control, as well as additional insecticide treatments. At Mechanicsville, Stanwood, Tipton, and Nashua, there were 7 additional insecticide treatments in each block. Each treatment at these locations was applied to 2 adjacent rows. At Ames and Newell, there were 52 additional insecticide treatments in each block. Each treatment at these locations was applied to a single row. Corn was planted, and all chemicals were applied by using a John Deere model 71B 4-row unit planter with a modified Nobel metering unit for insecticide application (Hills et al., 1972). All fields were planted be-
between May 9 and 21, 1979 with Pioneer 3780 seed corn. The distance between rows was 96.5 cm at Stanwood, Tipton, and Mechanicsville; and 76.2 cm at Ames, Newell, and Nashua. Corn roots and soil in these areas were sampled as previously described. The soil samples were extracted with ethyl acetate (Figure 4) and analyzed directly by GLC.
RESULTS AND DISCUSSION

Recovery Experiments

LSC analyses showed that soil extracts made with methanol:0.1 N HCl contained 96.4±2.5% of the applied $^{14}$C-carbofuran. GLC analyses of these extracts showed that 93.9±4.7% of the carbofuran was recovered. These recoveries showed that the extraction procedure was effective, and the good agreement between the LSC and GLC results showed that the GLC procedures were effective for analyzing carbofuran in soil extracts. GLC analyses of extracts from samples treated with the 2 carbofuran metabolites showed recoveries of 67.5±2.8% for 3-hydroxycarbofuran and 80.2±3.7% for 3-oxocarbofuran. All peaks interfering with GLC analysis of these chemicals were removed by the cleanup procedure (Figure 3). Stable HFBA derivatives of carbofuran were obtained only when they were formed using freshly-opened HFBA. Unsatisfactory results seemingly were caused by the formation of heptafluorobutyric acid by the reaction of HFBA with water, similar to a reaction described by Bose (1977). This problem was not solved by efforts to exclude moist air from the HFBA. After derivative formation, drying of the organic solvent with anhydrous $\text{Na}_2\text{SO}_4$ promoted instability of the derivative. This effect was minimized by not drying the solvent, keeping the solvent in contact with the pH 6 buffer, and rapid analysis.

GLC analyses showed that ethyl acetate soil extracts contained 96.1±1.1% of the applied carbofuran, and untreated soil extracts were free of carbofuran. When soil extracts were passed through anhydrous $\text{Na}_2\text{SO}_4$ to
remove water prior to measurement of the extract volume, recovery was 97.7±0.8% of applied carbofuran as opposed to 99.0±1.4% when the extracts were dried in the storage bottle after volume adjustment. These recoveries suggest that drying the extract in the storage bottle might give recoveries that are slightly greater than the actual recovery, but the difference is very small and not significant in this experiment \( (T_{0.05,2}) \). These experiments show that ethyl acetate does an excellent job of extracting carbofuran from soil.

The effects of various factors on the recovery of carbofuran by the ethyl acetate extraction technique are shown in Table 7. There were no soil type or pH adjustment effects, but there were significant soil-moisture effects \( (F_{0.05,1,9}) \). Extraction was poorer from the low moisture Canisteo soil than from the high moisture Canisteo soil \( (F_{0.05,1,4}) \), while no soil moisture effect was seen in the Kenyon-Floyd soil. At the low moisture level, the Canisteo soil was noticeably dryer than the Kenyon-Floyd soil. This was apparently due to a difference in the water-holding capacities of the 2 soils. The dryness of the low-moisture Canisteo soil caused poorer separation between the soil and ethyl acetate so that more ethyl acetate was left in the soil after décantation. This was observed by recovery of 96.3±1.0% of the ethyl acetate added to the 4 high-moisture Canisteo samples, but only 77.0±0.5% of the ethyl acetate added to the 4 low-moisture Canisteo samples. As noted in Table 7, the low-moisture Canisteo samples were extracted a fourth time. An average of 5.3% of applied carbofuran was removed in this extract, but extraction levels were still below the high-moisture samples.
Table 7. The recovery of carbofuran from soil as dependent on soil type, pH, and soil moisture; results are means ± s.d. of duplicate tests

<table>
<thead>
<tr>
<th>Soil type</th>
<th>pH adjustment</th>
<th>Soil moisture</th>
<th>Carbofuran recovered (% of applied)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenyon-Floyd</td>
<td>None</td>
<td>Low</td>
<td>96.1±2.1</td>
</tr>
<tr>
<td>Kenyon-Floyd</td>
<td>None</td>
<td>High</td>
<td>97.9±0.1</td>
</tr>
<tr>
<td>Kenyon-Floyd</td>
<td>Acid</td>
<td>Low</td>
<td>97.3±0.4</td>
</tr>
<tr>
<td>Kenyon-Floyd</td>
<td>Acid</td>
<td>High</td>
<td>98.0±0.3</td>
</tr>
<tr>
<td>Canisteo</td>
<td>None</td>
<td>Low</td>
<td>93.3±1.8*</td>
</tr>
<tr>
<td>Canisteo</td>
<td>None</td>
<td>High</td>
<td>99.1±1.8</td>
</tr>
<tr>
<td>Canisteo</td>
<td>Acid</td>
<td>Low</td>
<td>92.9±2.5*</td>
</tr>
<tr>
<td>Canisteo</td>
<td>Acid</td>
<td>High</td>
<td>97.8±0.3</td>
</tr>
</tbody>
</table>

^a None = 100 ml of glass-distilled water; acid = 100 ml of 0.25 N HCl.

^b Low = 5% moisture before pH adjustment, 30% after; high = 25% moisture before pH adjustment, 50% after.

^c Extracted 4 times with ethyl acetate.

* Significantly lower than the high-moisture Canisteo soils at the 5% level.

This experiment showed that soil type and pH at extraction time do not influence extraction of carbofuran with ethyl acetate. In subsequent experiments, 0.25 N HCl was used to adjust soil moisture, because it could potentially have a beneficial effect on the extraction of aged carbofuran soil residues. The Kenyon-Floyd soil results showed that good carbofuran extraction occurs over a wide range of soil-moisture levels. The Canisteo soil results showed that in some soils poorer carbofuran extraction may occur at low soil-moisture levels. It also is possible to have soil-
moisture levels that are too high for easy decantation of ethyl acetate. In subsequent experiments, a standard volume of 0.25 N HCl was usually added to adjust soil moisture in field-collected soil samples, but some adjustment was necessary in very wet or dry samples.

HPLC with an UVD confirmed the absence of carbofuran in untreated Kenyon-Floyd and Canisteo soils. Carbofuran recoveries in percentage of applied were 98.9, 100.8, 87.4, and 85.1 by GLC and 92.4, 108.4, 89.9, and 84.3 by HPLC for the low moisture Kenyon-Floyd, high moisture Kenyon-Floyd, low moisture Canisteo, and high moisture Canisteo samples, respectively. A. analysis of differences between methods paired by sample showed that the GLC and HPLC results were not significantly different (T\text{0.05,3}). The HPLC results were corrected for losses that occurred in cleanup steps that were not used prior to GLC analyses. The correction (1.141 X % of applied carbofuran recovered after cleanup) was determined by the analysis of carbofuran standards before and after cleanup. The GLC technique was preferred over the HPLC technique because it could be used without extract cleanup.

The recovery of carbofuran from soil samples treated with 0.1, 1.0, 10, or 100 ppm ranged between 96.3±0.8% (1.0 ppm) and 93.7±0.0% (10 ppm) of applied carbofuran. The results were best fitted by a line with the equation Y = 95.4 - 0.7 \log_{10} \text{concentration in ppm}. The slope of the line was not significantly different from zero (5% level). These results show that between 0.1 ppm and 100 ppm the concentration of carbofuran in soil has little or no effect on the percentage of carbofuran extracted with ethyl acetate.
Table 8 shows that ethyl acetate extracted carbofuran residues from field-collected soil samples treated with Furadan 10G as well as the other 2 techniques tested \((F_{0.05,2,12})\). The coefficients of variation (s.d./mean X 100, cv) for the results in Table 8 average 38%. This value is much higher than the cv's in previous recovery experiments in which samples were treated with carbofuran in the laboratory. This suggests that carbofuran distribution between subsamples of field-collected soil samples rather than extraction and measurement errors is responsible for the variation. The ethyl acetate and acetonitrile:water (9:1) extracts both contained atrazine that was also analyzed. The atrazine concentrations ranged around 1 ppm with a cv of only 3%. This shows that carbofuran is not distributed as evenly as other chemicals in soil. For that

Table 8. The recovery of carbofuran by 3 extraction methods from 4 soil samples collected from Nashua 48 days after treatment with carbofuran; results are means ± s.d. of duplicate tests expressed as ppm of carbofuran on a soil dry weight basis

<table>
<thead>
<tr>
<th>Soil sample number</th>
<th>Extraction techniques</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethyl acetate(^a)</td>
<td>Acetonitrile: water (9:1)(^b)</td>
<td>0.25 N HCl(^c)</td>
</tr>
<tr>
<td>1</td>
<td>0.54±0.35</td>
<td>0.55±0.23</td>
<td>0.52±0.11</td>
</tr>
<tr>
<td>2</td>
<td>0.26±0.10</td>
<td>0.32±0.10</td>
<td>0.21±0.18</td>
</tr>
<tr>
<td>3</td>
<td>0.60±0.01</td>
<td>0.55±0.11</td>
<td>0.35±0.09</td>
</tr>
<tr>
<td>4</td>
<td>0.21±0.08</td>
<td>0.22±0.09</td>
<td>0.09±0.04</td>
</tr>
</tbody>
</table>

\(^a\) Method described in the Materials and Methods section.

\(^b\) Method modified from Tweedy and Kahrs (1978).

\(^c\) Method described by Cook (1973).
reason, relatively large amounts of soil must be analyzed to estimate accurately carbofuran levels. Subsamples were used in this experiment because of sample size limitations of the 0.25 N HCl and acetonitrile: water (9:1) procedures. The ethyl acetate procedure, however, can be used to extract an entire soil sample in less time than a subsample can be extracted with the other techniques. The ethyl acetate procedure was preferred because it was faster and had greater sample size flexibility.

Table 9 shows that 0.25 N HCl was significantly worse for extracting carbofuran from soil freshly-treated with Furadan 10G than the other 2 techniques (2 tests, $F_{0.05,1,6}$). There were no significant soil effects ($F_{0.05,1,6}$) or method-soil interactions ($F_{0.05,2,6}$) in the 0 day results. The methods were not different in samples aged 15 days ($F_{0.05,2,6}$), but the soils were different ($F_{0.05,1,6}$). This reflects differences in the capacities of the 2 soils to degrade carbofuran. The rapid breakdown is similar to that in subsequent experiments and will be discussed later. This experiment, however, shows that the ethyl acetate procedure was effective for extraction of carbofuran from high-moisture soil samples treated with Furadan 10G in the laboratory.

In summary, the recovery experiments showed that carbofuran is effectively extracted from a variety of soils with methanol:0.1 N HCl, ethyl acetate, acetonitrile:water (9:1), and 0.25 N HCl. The ethyl acetate procedure was developed independently of other available procedures. Recently, Kadoum and Mock (1978) and Klaassen and Kadoum (1979) used a procedure based on ethyl acetate for the extraction of pesticide mixtures including carbofuran from water-saturated soils in irrigation-tailwater
Table 9. The recovery of carbofuran by 3 extraction methods from 2 soils, containing 50% soil moisture, that were extracted immediately after treatment or 15 days after treatment; results are means ± s.d. of duplicate tests expressed as carbofuran recovered in % of applied

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th>Soil type</th>
<th>Extraction technique</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ethyl acetate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Acetonitrile:</td>
<td>0.25 N HCl&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95.8±7.2</td>
<td>82.2±10.2</td>
<td>70.6±0.8&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>Kenyon-Floyd</td>
<td>90.5±8.3</td>
<td>82.5±0.4</td>
<td>83.9±4.0&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>Canisteo</td>
<td>3.7±3.5</td>
<td>2.2±0.2</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>15</td>
<td>Canisteo</td>
<td>7.9±1.0</td>
<td>3.9±0.4</td>
<td>7.1±4.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Method described in the Materials and Methods section.

<sup>b</sup>Method modified from Tweedy and Kahrs (1978).

<sup>c</sup>Method described by Cook (1973).

<sup>*</sup>The 0.25 N HCl method was significantly worse than the other methods on 0-day samples at the 5% level.

pits and farm ponds. Their procedure differed in that moisture was not adjusted prior to extraction, and acetone was used in the first of 3 solvent extractions. Their extracts also required cleanup prior to analysis by GLC with a CCD. My procedure was considerably shorter than their procedure, and showed that the ethyl acetate technique was equally or more effective than 2 other widely used extraction techniques on both field-treated and laboratory-treated samples. The ethyl acetate procedure was superior in that it was faster and could be used on larger soil samples. Large sample sizes should give more accurate residue levels in field studies because carbofuran was unevenly distributed in field-collected samples. The concentration of carbofuran in the soil and the soil pH
during extraction did not affect carbofuran extraction with ethyl acetate. Low soil moisture reduced ethyl acetate extraction of carbofuran, but a wide range of moisture levels were acceptable. Carbofuran was accurately analyzed in soil extracts as its HFBA derivative (XV, Appendix B) by GLC with an ECD, and in its underivatized state (V, Appendix B) by GLC with a TSD.

Carbofuran Persistence in Soil

Soil sampling sites to study carbofuran persistence in the field were selected randomly. Nine large-diameter soil cores (10.5 cm) were collected on each collection date and all the soil in each core was extracted and analyzed separately. It was subsequently discovered that only 1 of 2 study rows at Newell was properly treated, so Newell results are based on fewer samples. The intensive sampling was done to obtain accurate mean residue levels and a good measure of sample to sample residue variation despite uneven carbofuran distribution in soil (Table 8).

Carbofuran was not detected in soil at the Newell or Nashua locations prior to carbofuran treatment in 1979. This result was expected because these fields were not treated with insecticide in 1978. Carbofuran residue levels and precipitation at Newell and Nashua are shown in Figures 7A and B, respectively. The large sample to sample residue variations (standard deviation lines, Figures 7A and B) show the uneven distribution of carbofuran in soil and the need to analyze a large number of samples. Mean residue levels were above 1 ppm for 6 weeks at Newell (Figure 7A), but only for 1 week at Nashua (Figure 7B). The difference in persistence
Figure 7. Carbofuran soil residue levels in ppm (calculated on the basis of soil dry weight) and precipitation in cm measured during 1979 at (A) Newell (residue levels are means ± s.d. of 6, 2, 5, 4, 6, 3, and 3 samples for the respective dates); and (B) Nashua (residue levels are means ± s.d. of 9, 9, 9, 9, 9, and 3 samples for the respective dates)
is not explained by prior history of soil insecticide treatment because both soils had the same history. The difference also is not explained by increased carbofuran persistence with decreased soil pH (Getzin, 1973) because the soil at Nashua (pH 7.1) had a lower pH than the soil at Newell (pH 8.0, Table 5). It is interesting that at each location over 4 cm of precipitation fell shortly before collecting the samples that showed reduced carbofuran levels (Figures 7A and B).

Because carbofuran has a relatively high water solubility (250 ppm, Caro et al., 1974, to 700 ppm, Cook, 1973) and poor adsorption to soil (Freundlich \( k \) value = 0.51, Caro et al., 1974), it is possible that carbofuran could be physically removed from the 0 to 7.5-cm soil layer with surface runoff water or water leaching through the soil. Caro et al. (1973a) found that no more than 2% of applied carbofuran (also applied as Furadan 10G) was removed by surface runoff water under normal field situations. Therefore, it was anticipated that leaching through the soil was the most likely direction of carbofuran movement. Soil cores collected 22 weeks after carbofuran application were taken to a depth of 37.5 cm to check for downward carbofuran movement. No carbofuran was found, however, below the 0 to 7.5-cm soil layer at Nashua or Newell. This indicates that carbofuran did not leach, or if it did leach, it was rapidly degraded in the lower soil layers. Perhaps carbofuran losses (Figures 7A and B) were due to precipitation-stimulated degradative mechanisms rather than leaching. This idea is supported by the concept that both biological and nonbiological degradative mechanisms operate on pesticides in the soil solution (Hamaker and Goring, 1976), but probably do not operate on the
formulated product. Effects of soil moisture on leaching and degradation were investigated in the following experiments.

When 0.71 or 4.25 cm of simulated precipitation were added to carbofuran-treated soils in tensiometers (Figure 6), carbofuran was divided between the soils and water reservoirs as shown in Figure 8. There was significantly less carbofuran in the water reservoirs of tests treated with Furadan 10G than of tests treated with technical-grade carbofuran (F_{0.01,1,4}). This shows that the 10G formulation is effective in reducing the release of carbofuran into the water solution and thereby reducing its leaching potential in the field. Results of the technical-grade carbofuran tests suggest that carbofuran moves very easily with the water solution. The amount of carbofuran leached into the water reservoirs increased significantly at the higher precipitation level with both formulations (F_{0.01,1,4}). The total carbofuran recovered was significantly less in high precipitation samples than in low precipitation samples (F_{0.01,1,4}). The reason for this was not determined, but it is more likely that losses were associated with the water than with the soil. It is not possible to relate directly the quantities of carbofuran found in the water reservoirs to quantities leached in field experiments because the soil depth was about 2.5 cm in this experiment as opposed to 7.5 cm in the field-collected samples. It should be fair to say, however, that substantially less carbofuran was leached from the 0 to 7.5-cm soil layer in the field than appeared in the tensiometer water reservoirs in the Furadan 10G tests. If carbofuran released from the 10G formulation makes the insecticide available to degradative mechanisms, 0.71 cm of precipitation
Figure 8. Carbofuran distribution between a 2.5-cm deep soil layer and an underlying water reservoir as affected by the carbofuran formulation and the quantity of simulated precipitation added to the soil surface; results are means of duplicate tests.
would not allow much degradation in comparison with 4.25 cm of precipitation. The amount of carbofuran in the water reservoirs was probably less than the amount available to degradative mechanisms because some dissolved carbofuran probably remained in the soil.

The influence of soil-moisture levels on the rate of carbofuran degradation when Kenyon-Floyd soil treated with Furadan 10G was maintained at 3 different but constantly-held soil-moisture levels in containers that did not allow leaching is shown in Figure 9. Very little degradation occurred in any treatments during the first 7 days after application and treatments were not significantly different \( F_{0.05,2,3} \). At 17 days, however, significantly different amounts of carbofuran remained in the 3 treatments (2 tests, \( F_{0.01,1,3} \)). There was a definite decrease in carbofuran persistence at increased soil-moisture levels. This is in agreement with the suggestion by Talekar et al. (1977) and Caro et al. (1973a) that carbofuran appears to have less persistence in high-moisture soils in the field. The 30% and 50% soil moisture curves each had a lag period preceding a period of very rapid carbofuran degradation.

The lag period in these decay curves strongly suggests that carbofuran decay was due to a microbial process. Pesticide metabolism by non-microbial means are first order reactions that do not have a lag period (Kearney et al., 1969). Release and therefore degradation of carbofuran probably became progressively greater at higher moisture levels similar to the increased release at the higher precipitation level in the previous experiment. The soil-moisture level probably also influenced the ability of
Figure 9. The degradation of carbofuran applied to Kenyon-Floyd soil as Furadan 10G when incubated at 3 different soil moisture levels; each point is the mean of duplicate samples.
microorganisms to utilize carbofuran through effects on available oxygen and soil water potential.

Despite the apparent involvement of microorganisms in carbofuran degradation, 3-hydroxycarbofuran and 3-oxocarbofuran (II and III, Appendix A), as described in soil by Caro et al. (1973a), were not found in any soil extracts. A likely possibility is that carbofuran was degraded to carbofuran phenol (IV, Appendix A) as shown in Appendix C. Getzin (1973) used both $^{14}$C-(carbonyl)-carbofuran and $^{14}$C-(ring)-carbofuran to show that after incubation for 4 weeks over 50% of the ring carbons (but not the carbonyl carbons) were bound to Ritzville silt loam following extraction with acetone:benzene (1:1) and 0.25 N HCl. Getzin also showed that $^{14}$C-carbofuran phenol was bound to soil faster and to a greater extent than $^{14}$C-(ring)-carbofuran, and suggested that the bound material was carbofuran phenol. That metabolite was found at levels equivalent to 26% of applied carbofuran in flooded soil (Siddaramappa et al., 1978).

Venkateswarlu and Sethunathan (1979) also found carbofuran phenol in flooded soils, but they found that soil-bound radiocarbon increased at the expense of the carbofuran phenol fraction when the samples were aerated by shaking. These studies suggest that carbofuran phenol is an important metabolite in soil, and that it is not extractable from aerobic soils. Carbofuran phenol is not sensitive to the TSD used in my analyses, so it would not be detected even if it were extracted. Therefore, carbofuran degradation to carbofuran phenol may explain the shape of the degradation curves and the absence of detectable carbofuran metabolites.
The persistence of technical-grade carbofuran also was tested at 17%, 30%, and 50% moisture levels in Kenyon-Floyd soil. Again, very little degradation occurred during the first 7 days after application and the treatments were not significantly different \((F_{0.05,2,3})\). The 17 day recoveries were 95.2±0.1%, 52.0±27.9%, and 79.8±1.3% of applied carbofuran at 17%, 30%, and 50% soil moisture, respectively. The 17% and 30% moisture tests did not differ from similar samples treated with Furadan 10G (Figure 9, \(T_{0.05,2}\)). The 30% soil moisture comparison has little meaning, however, due to the large difference between replicates in samples treated with technical-grade carbofuran. There was significantly more degradation of carbofuran when it was applied as Furadan 10G in 50% moisture samples \((T_{0.01,2})\). It was anticipated that carbofuran applied as Furadan 10G would degrade at an equal or slower rate than technical-grade carbofuran (Ahmad et al., 1979) because of less carbofuran release into the soil solution (Figure 8). The opposite occurred at 50% moisture, suggesting that the formulation had affected the microorganisms that degraded carbofuran in 50% moisture soil. Acetone used to treat the soil with technical-grade carbofuran might have had a negative effect on microorganisms. High carbofuran concentrations in microenvironments around carbofuran granules or microbial utilization of inactive ingredients in the 10G formulation might have had a stimulatory effect. If there was a stimulatory effect due to the 10G formulation, it might be possible to make carbofuran more persistent in high-moisture soils by formulating it in a different manner.
Results in Figure 10 show how carbofuran degraded in 4 soils treated with Furadan 10G and incubated at 50% soil moisture. Very little carbofuran degradation and no significant difference between treatments occurred in the 0 and 5-day samplings (2 tests, F<sub>0.05,3,4</sub>). The treatments were significantly different in the 10 through 20-day samplings (3 tests, F<sub>0.01,3,4</sub>). The differences between soils were due to slower degradation in the Clarion soil than in the other 3 soils (3 tests, F<sub>0.01,3,4</sub>). The shapes of the curves again suggested that carbofuran was degraded by microorganisms in all 4 soils. It appears that carbofuran degrading microorganisms are common in soils and are frequently capable of reducing carbofuran levels to below 25% of the applied amounts in just 10 days if the soil is maintained at the proper moisture level. Since maximum corn rootworm larval feeding ends about 70 days after insecticide application, wet soil conditions are definitely a serious threat to effective corn rootworm control with carbofuran.

Soils that had been treated with carbofuran in previous years (Figure 10) did not show more rapid degradation of carbofuran than other soils. This suggests that an annual treatment with carbofuran is not enough to maintain the carbofuran-degrading microbial population and thereby eliminate the lag time. Even if this were not the case, these results suggest that the absence of a lag time would only reduce persistence by about 5 days. Therefore, prior use of carbofuran in a field should have little or no effect on persistence of carbofuran in that field. Getzin (1973) showed a direct relationship between increased carbofuran persistence and decreased soil pH. The shape of the decay
Figure 10. The degradation of carbofuran applied as Furadan 10G in 4 soils with different pH values and prior carbofuran treatment histories. All soils were maintained at 50% soil moisture; each point is the mean of duplicate samples.
curves and the absence of significant differences in results owing to pH values (Figure 10) indicate that soil pH did not directly influence carbofuran persistence in this experiment. It is possible, however, that pH influenced carbofuran degradation in Clarion soil through the effect of soil pH on microorganisms in that soil. The 50% soil moisture used in this experiment probably had a different influence on oxygen tension in the 4 soils due to differences in soil water-holding capacities. The Clarion soil apparently had the lowest water-holding capacity of the 4 soils because it was the most flooded. It is very possible that flooded conditions in the Clarion soil as opposed to the other 3 soils was responsible for the slower degradation in that soil.

In summary, both laboratory and field tests suggest that soil moisture, as opposed to soil pH and prior treatment of the soil with carbofuran, has the greatest influence on carbofuran persistence. Degradation, that was probably microbial, with carbofuran phenol (IV, Appendix A) as a possible major metabolic product, increased with higher amounts of soil moisture. Leaching of carbofuran to lower soil layers also was suspected, but no carbofuran was detected in the lower soil layers. Laboratory studies showed that carbofuran leaching from the 10G formulation is much reduced over leaching from the technical product. Despite the reduced rate of carbofuran release into the soil solution, carbofuran from Furadan 10G was degraded faster than the technical product in high-moisture soil. For that reason, persistence might be improved with a new formulation. When Furadan 10G was incubated in 4 high-moisture soils, 3 of the soils
degraded carbofuran to low levels within 10 days. That type of rapid
carbofuran degradation could result in rootworm control problems.

Carbofuran Residues and Efficacy

Correctly applied carbofuran should result in 6.0 to 7.6 ppm of
carbofuran in the upper (0 to 7.5 cm) soil layer on the day of application
for soils with bulk densities between 1.1 and 1.4 g/cm³. In 1977, devia­
tions at the Sanborn (64.8 ppm) and Wellsburg (0 ppm) fields were obviously
due to application errors, and data from these 2 fields are not included in Table 10. Initial residue levels at the other fields ranged
between 2.5 and 24.7 ppm.

Carbofuran residues present from 4 to 10 weeks after insecticide
application provide an estimate of the effective level of carbofuran for
rootworm control because it is during this 6-week period that damaging
populations of corn rootworm larvae are present in Iowa cornfields. Mean
carbofuran residues in the 4, 6, and 10-week samples from the upper soil
layer in 9 fields and estimates of root damage are summarized in Table 10.
All residue values were greater than 2 ppm during the critical 4 to 10-
week period. These residue levels are higher than the 0.5 ppm LC₅₀ of
carbofuran in soil for third stage western corn rootworm larvae (G.
Sutter, Northern Grain Insects Research Laboratory, Brookings, SD, personal communication). Paired analysis of root-damage ratings showed that
carbofuran provided significant root protection (T₀.₀₁,₈). The per­
centage control was calculated after subtracting 1.0 from all root-damage
ratings so that values up to a theoretical 100% control could be calcu-
Table 10. Rootworm effective carbofuran residues and performance results at 9 fields in Iowa during 1977 (Gorder et al., 1980)

<table>
<thead>
<tr>
<th>Field location</th>
<th>Soil pH</th>
<th>Carbofuran-treated areas</th>
<th>Untreated areas</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Residues, ppm</td>
<td>Root rating</td>
<td>Root rating</td>
</tr>
<tr>
<td>Marshalltown</td>
<td>5.96±0.14</td>
<td>3.9±2.3</td>
<td>2.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Newell</td>
<td>7.95±0.13</td>
<td>2.4±1.9</td>
<td>3.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Nashua</td>
<td>6.65±0.51</td>
<td>2.8±1.5</td>
<td>2.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Keswick</td>
<td>6.57±0.25</td>
<td>3.2±1.9</td>
<td>2.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Laurens</td>
<td>5.54±0.05</td>
<td>3.2±1.8</td>
<td>2.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Winterset</td>
<td>6.41±0.51</td>
<td>3.3±3.7</td>
<td>1.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Cedar Falls</td>
<td>6.05±0.05</td>
<td>8.8±3.9</td>
<td>2.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Harlan</td>
<td>5.00±0.17</td>
<td>4.1±4.0</td>
<td>2.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Rembrandt</td>
<td>8.19±0.02</td>
<td>3.4±2.3</td>
<td>2.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

^Fields are grouped according to insecticide history as described in Table 6.

^The mean and s.d. from 4, 6, and 10 week upper soil layer samples.

^Based on a scale of 1 to 6, with 1 = no damage and 6 = severe damage.

^\[(\text{root rating in untreated area-1}) - (\text{root rating in carbofuran-treated area-1})\] x 100.

^\[(\text{root rating in untreated area-1})\]

^Roots were dug from soil-insecticide study areas, soil samples were taken in the border rows.
lated. There was about 47% more root injury in the untreated control areas than in the treated plots. As in previous experiments, no significant differences were found in residues or percentage control between fields that had different insecticide use histories (2 tests, $F_{0.05,3,5}$), and pH did not have a major effect on carbofuran persistence.

The possibility of carbofuran leaching to lower soil layers was studied by analyzing lower (7.5 to 15 cm) soil layers. The initial sampling showed that the lower soil layers contained up to 6 ppm of carbofuran in 1 field, and over 1 ppm in 7 of 9 fields, suggesting contamination from the upper soil layer during sampling. Carbofuran in the lower soil layer samples, however, decreased with time so that residues in all 4, 6, and 10-week samples were 1 ppm or below. Since residue levels in lower soil layer samples did not increase as levels in the upper layers decreased, there was apparently little carbofuran movement from the upper to the lower soil layers. Leaching might have been less than normal due to May and June precipitation that ranged from 3.2 to 17.8 cm below normal at the 9 locations. As shown in previous experiments, the dry conditions might also have reduced carbofuran degradation, thus accounting for high residue levels in both soil layers. Carbofuran losses that did occur were probably due to degradation rather than leaching. Again, no 3-hydroxy-carbofuran or 3-oxocarbofuran (II and III, Appendix A) were detected.

Fields used in the 1979 study were not chosen because of previous insecticide use, but the Mechanicsville, Stanwood, and Ames fields were treated with carbofuran in 1978. Soil samples taken in all fields prior to carbofuran treatment in 1979 showed 0.01 ppm of carbofuran in repli-
cates 1, 3, and 4 at Mechanicsville and 3 at Ames. Carbofuran was not detected in the other soil samples before carbofuran treatment. Five of the 6 fields studied in 1979 had adequate corn rootworm larval populations for efficacy tests; the Nashua field did not. For that reason, Nashua was dropped from the efficacy tests and results do not appear in Table 11. The carbofuran treatment levels (0-wk residues, Table 11) were more consistent than in 1977, and no fields were eliminated because of improper treatment. The residue levels were probably more consistent because all fields were treated by university personnel, and because more soil samples were analyzed to give more precise mean residue levels. All residue levels, however, were slightly below the theoretical 6.0 to 7.6 ppm range. The cause(s) of this was not determined.

The 10-week carbofuran residue levels (Table 11) were present through the period of damaging corn rootworm larval populations (4 to 10 weeks after treatment). All residue levels were 0.2 ppm or below. These levels were well below 0.5 ppm, so effective control of feeding damage was not expected. Paired analysis of root-damage ratings showed that carbofuran did provide significant root protection ($T_{0.01, 19}$) despite the low residue levels. Results in previous experiments showed that carbofuran residue levels can decrease very rapidly in a short period of time. For that reason, it is possible that the 10-week residue levels were much lower than the residues that were present in the 4 to 10-week critical period. There was an average of 23% control in 1979. This was significantly less control than the 47% control that occurred in 1977 ($T_{0.01, 27}$). The residue data (Residues, Table 10, and 10-wk residues, Table 11) reflect this
### Table 11. Carbofuran soil residue levels and corn root damage ratings obtained during 1979 at 5 fields in Iowa

<table>
<thead>
<tr>
<th>Field location</th>
<th>Replicate</th>
<th>Soil pH</th>
<th>0-wk residues&lt;sup&gt;a&lt;/sup&gt;</th>
<th>10-wk residues&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Root rating&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Root rating&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% control&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanicsville</td>
<td>1</td>
<td>6.0</td>
<td>2.47±1.13</td>
<td>0.12±0.05</td>
<td>2.6</td>
<td>3.7</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.2</td>
<td>3.90±0.82</td>
<td>0.08±0.04</td>
<td>3.2</td>
<td>3.6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.3</td>
<td>3.33±1.36</td>
<td>0.20±0.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6</td>
<td>3.6</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.0</td>
<td>5.26±2.07</td>
<td>0.11±0.11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.6</td>
<td>4.1</td>
<td>48</td>
</tr>
<tr>
<td>Stanwood</td>
<td>1</td>
<td>6.7</td>
<td>2.99±0.67</td>
<td>0.07±0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.8</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.7</td>
<td>2.89±1.18</td>
<td>0.10±0.08</td>
<td>3.8</td>
<td>4.3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.2</td>
<td>2.98±2.01</td>
<td>0.16±0.11</td>
<td>3.7</td>
<td>4.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.6</td>
<td>3.05±1.19</td>
<td>0.11±0.08</td>
<td>4.0</td>
<td>4.3</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means ± s.d. in ppm of 9 determinations.

<sup>b</sup>Based on a scale of 1 to 6, with 1 = no damage and 6 = severe damage.

<sup>c</sup>(root rating in untreated area-1) - (root rating in carbofuran-treated area-1) / (root rating in untreated area-1) x 100.

<sup>d</sup>Results are based on 8 samples.

<sup>e</sup>Results are based on 6 samples.
Table 11. (Continued)

<table>
<thead>
<tr>
<th>Field location</th>
<th>Replicate</th>
<th>Soil pH</th>
<th>Carbofuran-treated areas</th>
<th></th>
<th>Untreated areas</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-wk residues</td>
<td>10-wk residues</td>
<td>Root rating</td>
<td>Root rating</td>
</tr>
<tr>
<td>Tipton</td>
<td>1</td>
<td>7.0</td>
<td>1.19±0.77</td>
<td>0.02±0.02</td>
<td>3.1</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.7</td>
<td>2.37±1.59</td>
<td>0.02±0.02</td>
<td>3.3</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.7</td>
<td>1.21±0.92</td>
<td>0.02±0.01</td>
<td>2.7</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.8</td>
<td>1.72±0.74</td>
<td>0.03±0.02</td>
<td>2.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Ames</td>
<td>1</td>
<td>8.0</td>
<td>0.98±0.37</td>
<td>0.01±0.01</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.9</td>
<td>1.02±1.24</td>
<td>0.02±0.01</td>
<td>2.4</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.3</td>
<td>3.25±0.99</td>
<td>0.01±0.01</td>
<td>2.0</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.5</td>
<td>1.83±0.67</td>
<td>0.02±0.01</td>
<td>2.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Newell</td>
<td>1</td>
<td>8.2</td>
<td>2.14±0.78</td>
<td>0.00</td>
<td>2.6</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.5</td>
<td>3.43±2.03</td>
<td>0.03±0.02</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.1</td>
<td>2.37±1.09</td>
<td>0.06±0.01</td>
<td>3.0</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8.0</td>
<td>1.66±0.58</td>
<td>0.00</td>
<td>3.0</td>
<td>3.5</td>
</tr>
</tbody>
</table>
result. Carbofuran persistence changed more between years than between fields. The difference between years might have been related to a paucity of rainfall in 1977 as opposed to 1979. The effect of soil moisture on carbofuran residue levels was shown in previous experiments.

Possible effects of prior treatment of soil with carbofuran were not studied in this 1979 experiment. The 10-week residue levels (Table 11) were negatively correlated (5% level) with soil pH. This indicates more rapid carbofuran degradation at high pH, as was shown by Getzin (1973). The relatively small difference between low and high 10-week residue levels, however, indicates that pH was not as important as soil-moisture levels that apparently caused the difference between 1977 and 1979 residue levels. Again, 3-hydroxycarbofuran and 3-oxocarbofuran (II and III, Appendix A) were not detected, so carbofuran may have degraded to carbofuran phenol (IV). The possibility of carbofuran leaching to lower soil layers was not investigated in this study.

In summary, carbofuran residue and efficacy tests showed that carbofuran provided significant root protection in both 1977 and 1979. Protection was much greater in 1977, however, and that result agreed with greater residue levels in that year. The differences in residue levels were much larger between years than between fields in the same year, apparently due to soil-moisture conditions. Carbofuran persistence was not greatly affected by previous soil insecticide treatments or soil pH in 1977. Soil pH, however, seemed to have a minor influence on 1979 residue levels. Carbofuran was not leached to lower soil layers in 1977, and leaching was not studied in 1979. Carbofuran metabolites were not detected in any soil
samples, suggesting that carbofuran degraded to carbofuran phenol (IV, Appendix A), a metabolite that would not be extracted by procedures used in these studies.


ACKNOWLEDGMENTS

I am very grateful to Dr. Paul A. Dahm for his very constructive guidance throughout the research and his efforts to help me improve my writing abilities and supply me with useful information. I would like to extend special thanks to Dr. Jon J. Tollefson for the work that he did to make the field research possible and for the guidance that he provided. I would also like to thank Drs. John M. Bremner (Agronomy), Joel R. Coats (Entomology), David F. Cox (Statistics), Larry P. Pedigo (Entomology), and Fred D. Williams (Bacteriology) for their invaluable suggestions in various parts of this study. I deeply appreciated the materials and advice supplied by Drs. Ronald F. Cook, Robert L. Gates, and Robert A. Robinson at FMC Corporation, Middleport, NY. I am indebted to my wife, Nancy, as well as Randall Conley, Valynda Simonton, Charles M. Sires, and most members of the corn insects research group for assisting me with portions of this research. I am deeply appreciative of the support that I received from Nancy and members of our families.
APPENDIX A: STRUCTURES OF CARBOFURAN AND METABOLITES
<table>
<thead>
<tr>
<th>Common Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbofuran</td>
<td><img src="image" alt="Structure I" /></td>
</tr>
<tr>
<td>3-hydroxycarbofuran</td>
<td><img src="image" alt="Structure II" /></td>
</tr>
<tr>
<td>3-ketocarbofuran or 3-oxocarbofuran</td>
<td><img src="image" alt="Structure III" /></td>
</tr>
<tr>
<td>carbofuran 7-phenol</td>
<td><img src="image" alt="Structure IV" /></td>
</tr>
</tbody>
</table>
APPENDIX B: CARBOFURAN ANALYSIS METHODS
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Detected Product</th>
<th>Detector</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td><img src="image" alt="Structure V" /></td>
<td>DTC</td>
<td>Cassil et al. 1969</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCD</td>
<td>Cassil et al. 1969</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AFID</td>
<td>Ahmad et al. 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TD</td>
<td>Nelson and Cook 1980</td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Structure VI" /></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Structure VII" /></td>
<td>ECD</td>
<td>Holden et al. 1969</td>
</tr>
</tbody>
</table>
See text for full names
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Detected Product</th>
<th>Detector</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CF}_3\text{(CF}_2\text{)}_x\text{C-O-C(F}_2\text{)}_x\text{CF}_3 ) ( x=0, 1, \text{ or } 2 )</td>
<td>XIV</td>
<td>ECD</td>
<td>Seiber 1972</td>
</tr>
<tr>
<td>CH(_3)C-O-C(_3)CH(_3)</td>
<td>XVI</td>
<td>AFID</td>
<td>Seiber 1972</td>
</tr>
<tr>
<td>CH(_3)C-O-C(_3)CH(_3)</td>
<td>XVI</td>
<td>CCD</td>
<td>Lawrence 1976</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>Chapman and Robinson 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>XV</td>
<td>FID</td>
<td>Knaak et al. 1970</td>
</tr>
</tbody>
</table>
See text for full names
APPENDIX C: "PATHWAYS OF CARBOFURAN BREAKDOWN IN SOIL"
Arrow widths suggest the relative importance of the reaction in soil. Dotted arrows are used where the reaction sequence and metabolic agents are not known.