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PESTICIDE-PESTICIDE INTERACTION IN
ORGANOPHOSPHORUS INSECTICIDE DEGRADATION

BY

PETER E. CHENG

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in Chemistry
South Dakota State University
1984
PESTICIDE-PESTICIDE INTERACTION IN
ORGANOPHOSPHORUS INSECTICIDE DEGRADATION

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of the thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Thesis Advisor

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To My Parents
Mr. and Mrs. Cheng, Shium
Sisters and Brother
Mary, Rose and Joseph
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INTRODUCTION

The widespread use of pesticides during the last two decades has played an important role in the development of today's agricultural system and is one of the major topics of modern science. It is also expected to assist in meeting the nutritional needs of the rapidly expanding populations in the world. The organophosphorus insecticides are among the most toxic pesticides employed for agricultural pest control. However, organophosphorus insecticides are usually rapidly degraded and eliminated. There has been a great amount of study on the chemical, microbial, and physical factors affecting degradation of organophosphorus insecticides, but degradation pathways and behavior have not really been understood which may be due to the variability of the soil selected and the environmental factors.

In this study we attempted to understand the degradation of organophosphorus insecticides, Terbufos, Phorate, Fonofos, and Ethoprop, (1) in presence of antimicrobials such as PCNB, Bronopol, Benomyl which may affect populations at bacteria and fungi, and (2) effects on phosphatase activities in soil with time.
LITERATURE REVIEW

Metabolism of Organophosphate Insecticides

Organophosphates having insecticidal activity can be traced to a basic structure which was first defined by Schrader's (1) acyl formula (Fig 1) in 1963. He predicated that a biologically active phosphates will be obtained when the following conditions are fulfilled:

(a) Either sulfur or oxygen must be directly bound to the phosphorus atom.

(b) $R_1$ and $R_2$ may be alkoxy, alkyl or amino residues

(c) Acyl represents the anion of an organic or inorganic acid such as fluorine, cyanate, thiocyanate, or of other acidic residues.

Schrader's formula and predication demonstrated a successful relationship between structure and activity of organophosphates.

The acute toxicity of organophosphorus insecticides is expressed as the $\text{LD}_{50}$ which means the dosage that is lethal to 50% of the test animals. The oral acute $\text{LD}_{50}$ value for terbufos is 3.5 mg/kg (male mouse); 9.2 mg/kg (female mouse); 4.5 mg/kg (male rat); 9.0 mg/kg (female rat); for phorate it is 2-4 mg/kg (male rat); for fonofos it is 8-17.5 mg tech/kg (male rat); for ethoprop it is 61.5 mg/kg.
Toxicity of organophosphorus insecticides primarily depends upon their structures. Schmidt (17) reported that a switch from P=O to P=S analogous brings a reduction in acute toxicity partly by reducing the phosphorylating activity and partly because in mammals a metabolic oxidative activation of the PS compound to PO must be first to take place, which leaves more time for the hydrolytic detoxification processes. Another reduction in toxicity in mammals can be observed by changing from diethyl phosphates to the dimethyl analogues. The reason for this, Schmidt explained, was the additive effect of enzymatic demethylation by glutathione, which is present in insects only in insignificant quantities.

Oxidation in tissues is one of the major degradation reactions especially of the thiono-phosphates. Usually this reaction does not result in detoxication, on the contrary, it enhances toxic action, due to a changing from P=S form to P=O form (11) in the presence of NADPH₂ and oxygen. Knaak, Stahmann, and Casida (45) assumed that oxygen is converted by the peroxidase-hydrogen donor system into free perhydroxyl radicals, which form activated rapidly-decomposing intermediates with P=S grouping. An important degradative route for phorate is the oxidation to the corresponding sulfoxides and sulfones (12). The same mechanism holds true for terbufos. McBain and Menn (13) reported that in the rat fonofos is first oxidized enzymatically, and then further oxidized to sulfoxides and sulfones. Metabolism of terbufos in corn and sugar beets also produces several toxic metabolites (15), such as sulfoxide, sulfone, oxygen analog, oxygen analog sulfoxide, and oxygen sulfone. However, the hydrolytic metabolites are not significantly toxic.
Electronic Interpretations and Properties of Organophosphorus Insecticides and other Pesticides

In the periodic chart, phosphorus in its fundamental state possesses the electronical configuration $3s^23p^3$. All the organophosphates are derivatives of covalent phosphorus which possess a tetrahedral structure corresponding to $sp^3$ hybridization. To satisfy valence considerations, the phosphorus-oxygen or phosphorus-sulfur bonds in the type $x_3PO$ or $x_3PS$ are double bond. Lewis believed that semi-polar $P - O[S]$ bond should be assumed. Pauling (2) and Collin (3) took account of 3d orbitals of phosphorus and pointed out that there is a $\pi$-bonding system superimposed on the $\sigma$-bonding skeleton. However, from UV-spectra (4), no $n-\pi^*$ transitions can be found, furthermore, no information on the presence of $p_{\pi} - d_{\pi}$ bonding is revealed by IR spectra either.

From spectroscopic investigations, Goubeau (5) reported the results on various phosphorus-sulfur bonds, and the bond order is 2. Using Sieber's method (6) determined from both force constants the bond order for $P-SCH_3$ as 1.16 (approaching the single bond) and for $P-S$ as 1.75, a value considerably greater than that of the single bond.

Since it is impossible to estimate the absolute extent of this $\pi$-component, the most accepted way (7) of depicting these compounds would be four $\sigma$ bonds and an associated $\pi$-component.

Four organophosphorus insecticides, terbufos, phorate, ethoprop, and fonofos, were chosen for this study. Their structures and properties are as follows:
Terbufos, or chemically S-[1,1-Dimethylethyl, thio] methyl o,o diethyl phosphorodithioate.

\[ X = \text{CH}_2\text{-S-C-CH}_3 \]

Phorate, or o,o-Diethyl S-[ (ethyl thio) methyl] phosphorodithioate.

Ethoprop, o-Ethyl s,s-dipropyl phosphorodithioate.

Fonofos, o-Ethyl-s-phenylethylphosphonodithioate

Terbufos, a soil insecticide, sold as Counter® is available in the granular form of 15%. Technical Terbufos is a clear, slightly brown liquid, melting at slightly below \(-15^\circ C\), slightly soluble in water, miscible with xylene, vegetable oils, carbon tetrachloride, alcohols, esters, and ethers. It hydrolyzes under alkaline conditions. At room temperature, technical Terbufos is stable for at least two years.
Terbufos is poisonous by skin contact, eye contact, inhalation or swallowing.

Phorate, a soil and systemic insecticide, also named Thimet, Rampart, and Vegfru is available in the granular form of 10% and 15%. Technical Phorate is a clear liquid, slightly soluble in water (about 50 ppm), miscible with xylene, vegetable oil, carbon tetrachloride, alcohols, ethers, and esters. Phorate (technical) and its formulations are highly poisonous.

Ethoprop, a nematocide-soil insecticide, also named MOCAP prophos, or Jolt is available in granular form of 10%. It is slightly soluble in water, and readily soluble in most organic solvents.

Fonofos, a soil insecticide, also named Dyfonate or N-2790, is available in the granular form of 20% and 10%. It is used for control of corn rootworms, wireworms, cutworms, symphyllins, and other soil pests.

The following section describes the structures and properties of antimicrobials used in this study.

**PCNB**

Chemical name: pentachloronitrobenzene
common name: PCNB, quintozene
Action: Soil fungicide; seed dressing agent
Properties: Crystalline solid melting at 142-145°C, acute oral LD$_{50}$(rat) as aqueous suspension of wettable powder, greater than 12,000 mg/kg. Used for damping-off of cotton; black root and club-root of cabbage, and also combined with Terrazole.
Terrazole

Chemical name: 5-Ethoxy-3-trichloromethyl-1,2,4-thiadiazole

Common Name: Ethazol

Action: fungicide

Properties: Reddish brown liquid, specific gravity 1.500 at 25°C, soluble in ethanol, xylene. Acute oral LD<sub>50</sub>(rat), 1077 mg/kg controls pythium at low rates. Terrazol shows considerable promise as a nitrification inhibitor.

Bronopol

Chemical name: 2-Bromo-2-nitropropan-1,3-diol

Common name: Bronopol, bronocot

Action: Bactericide and bacteriostat active particularly against xanthomonas malvacearum

Properties: Colorless to pale brownish yellow, odorless crystalline solid. MP about 130°C, acute oral LD<sub>50</sub>(rat), 400 mg/kg. Bronopol is used as a seed treatment to cotton gives protection against blackarm disease.

Benomyl

Chemical name: Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate

Common name: Benomyl, Benlate R

Action: Systemic fungicide
Properties: White crystalline solid, acute oral LD$_{50}$(rat), more than 10,000 mg/kg. Used for control of wide range of disease of fruits, nuts, vegetables, field crops.

Fenaminosulf

Chemical name: Sodium [4-(dimethylamino) phenyl] diazene sulfonate
Common name: Lesan
Action: Turf and soil fungicide and seed treatment

Properties: Yellow-brown powder. Soluble in water 2-3% at 25°C. Acute oral LD$_{50}$(rat), 75 mg/kg. Used to protect germinating seed and seedlings.

N-Serve

Chemical name: 2-chloro-6-(trichloromethyl) pyridine
Common name: nitrapyrin
Action: nitrification inhibitor through selective activity against Nitrosomonas bacteria
Properties: White crystalline solid soluble in anhydrous ammonia, xylene. Acute oral LD$_{50}$(rat), 1230 mg/kg. Delays nitrification of ammonium ions in soil when applied with urea and ammonium fertilizer nitrogen sources.
Organophosphorus Insecticides in Soil

A lot of factors can be traced which affect the degradation and persistence of organophosphorus insecticides in the soil, chemically, physically, and environmentally. Among these factors are:

1) adsorption to clay and organic matter
2) soil type
3) volatilization to the atmosphere
4) uptake by soil organisms or plants
5) soil moisture, pH, and temperature
6) microbial degradation
7) chemical degradation
8) photolysis

Of primary importance is the chemical nature of the organophosphorus insecticides and the soil type. These factors may either enhance or reduce degradation rates.

Adsorption: Organophosphorus insecticides, like other chemical molecules, have varying tendencies to be adsorbed to clay or organic matter particles or dissolved in the soil solution. For the most part the adsorption sites on clay or organic matter are negatively charged and constitute the "cation exchange capacity" for a particular soil. For each organophosphorus insecticide, for each soil type, and for each set of soil conditions, a different equilibrium is established between the amount of organophosphorus insecticide adsorbed and the amount dissolved in the soil (27).
Soil type. Soil type is one of the most important factors influencing organophosphorus insecticide sorption equilibria. Of special importance are the clay and organic matter, for these are colloidal and have high cation exchange capacities and high surface areas. Clay and organic matter content in soil can vary from less than 1% in sand to well over 50% in heavy clay or peat soils. Adsorption of organophosphorus insecticides to the negatively charged sites on clay or organic matter can occur by dipole-dipole attraction, hydrogen bonding or by actual ionic bonding if cationic pesticides are involved (38).

Nature of the organophosphorus insecticides. The chemical structure of an organophosphorus insecticide determines sorption equilibria by influencing its direct affinity for the clay or organic matter or by influencing its solubility or affinity for the soil. Ward and Upchurch (39) reported a general but not precise inverse correlation between pesticide solubility and absorption, that 60% of the variation in absorption could be attributed to the effects of structure on solubility, while the remaining variation was due to the effects of structure on the direct affinity of the chemical for the absorbent. In addition to many other factors the persistence of pesticides in soil is influenced by the way they are formulated for application. Granular formulations are usually the most persistent. Wettable powder and dust formulations are often less persistent than emulsifiable preparations.
Soil moisture content. One would expect more pesticide to be absorbed when soils are dry than when they are moist, since decreases in soil moisture should shift sorption equilibria toward greater absorption. This is true in moderately light to very light soils but not in heavy soils. Green and Obien (40) established that the impact of fluctuations in available soil moisture on the amount of atrazine dissolved in the soil solution was inversely related to the absorption capacity of soil.

Soil pH. Most soils have a pH in the 4.5 to 8.0 range. The fate of organophosphorus insecticides in soil varies with pH differences. Primarily because of the influence of pH on sorption phenomena. Smelt and Leistra (33) reported that soil pH and soil type affected the rate of degradation for ethoprop. In this study, the loss of ethoprop corresponded approximately to first-order kinetics, the half-life was about 87 days in a humic sand and a peaty sand with pH values of 5.4 and 4.6 respectively. In a sandy loam and a loam soil with pH values of 7.2 and 7.3 respectively, the half life ranged between 14 and 28 days.

Soil Temperature. Pesticide adsorption in soil is an exothermic process (38). When hydrogen bonds or ionic bonds are formed, heat is given off. Thus, when the soil temperature increases, the input of heat can break some of these bonds and cause the desorption of some pesticide molecules. At higher temperatures the greater solubility of pesticides also results in further shifts in the sorption equilibrium toward more pesticide being available in soil. Verma (31) reported some data of soils treated with phorate at constant temperature of 15, 30 and 45°C in the laboratory. After 24 hour of treatment there was a 69-73%
reduction in residues at all 3 temperatures (7.8, 8.6, 8.7 ppm to 2.1, 2.5 and 2.6 ppm respectively). Up to 33 days of treatment the difference in degradation of residues at different temperatures was not significant. However, observation at 63 and 103 days after treatment indicated significantly less reduction at low temperature (103 days; 0.44, 0.26 and 0.26 ppm). Phorate residues fell below detectable levels in 128, 117 and 109 days at 15, 30, and 45°C respectively. It was concluded that the different temperatures of 15, 30, and 45°C or higher might cause increased long term persistence of phorate. Another supporting test (32) on fonofos and phorate has shown that they are broken down much more rapidly in tropical and subtropical climates.

Volatilization. Several factors influence the tendency of pesticides to volatilize and leave soil as a vapor (41). The structure of the chemical is important because this determines its vapor pressure, as well as its solubility in soil water and its tendency to be adsorbed. Cool, dry conditions in soils with high organic matter or clay content normally result in very little loss of even the most volatile chemicals from the soil, since they are adsorbed tightly. Conversely, warm, moist conditions bring about greater desorption and greater volatilization losses.

Microbial degradation. The primary microorganisms in soil are algae, fungi, actinomycetes, and bacteria. Most of these are dependent on organic compounds for energy and growth. When an organophosphorus insecticide is added to soil and reaches an equilibrium between the soil colloids and the soil, any molecules in the soil are
immediately attacked as potential energy sources. If the chemical is readily available in the soil, any organisms that can adapt to it as an energy source rapidly increase in numbers until they have completely degraded it. After this they may decrease in number (42). Any factors that encourage the growth of degrading microorganisms or that increase the availability of pesticides in the soil enhance the disappearance of the chemical. Soil organic matter can have slightly opposing effects on pesticide persistence. On the one hand it may decrease the availability of the pesticide, but on the other hand it could improve conditions for the growth of the microorganisms. Most other conditions, such as warm temperature, lack of pH extremes, and adequate fertility, encourage microorganisms and increase the desorption and availability of pesticides.

Chemical degradation. Chemical reactions in soil can destroy the activity of some pesticides and activate others. In chemical degradation pH is an important factor, but the exact influence of high or low pH varies for different pesticides. Getzia (43) reported that an organophosphorus insecticide, Diazinon, is broken down more rapidly under acid conditions, but the reverse is true for another organophosphorus insecticide, malathion. The hydrolysis of organophosphorus insecticides follows several patterns, depending upon the type of ester, the solvent, the pH range, temperature, pesticide concentration. According to Schrader (1) rule, insecticidal phosphates must possess an electron-withdrawing substituent at the phosphorus atom. The hydrolysis of the trialkyl phosphates in aqueous alkaline medium is a first-order reaction with respect to both hydroxyl ion and ester. The P-O bond is ruptured.
In the rate determination step the hydroxyl ion attacks the phosphorus. An exchange of oxygen between the phosphoryl group and the hydroxyl ion can be excluded. Larsson (8) pointed out that this process is a one-step reaction, in which the degree to which a group leaves rests solely upon the approach of the hydroxyl ion to the phosphorus atom. Hudson and Green (9) demonstrated this process in Sn2, mechanism as below; analogous to Sn2 attach in carbon chemistry, with a Walden inversion reaction. The transition state (A) is favored by the fact that phosphorus can form the structure of coordination number 5 with the configuration $3s^23p^33d^1$ corresponding to a trigonal bipyramid.

For those organophosphorus insecticides such as terbufos, phosphate, fonofos, and ethoprop which contain sulfur in the thiol form, the effective charge on phosphorus would be expected to be reduced as a result of lower electronegativity of sulfur and the increased polarizability of sulfur in comparison with oxygen. The ability to participate in $\pi$-bonding is diminished also as a result of lower electronegativity of sulfur. The positive character of the phosphorus atom is maintained, favoring a basic attack. Ruzicka and Thomson (10) reported within homologous series the thiol compounds hydrolyze faster than the corresponding oxygen ester.
A study of the oxidation of terbufos has been done by Laveglia (14). Soil samples containing terbufos were incubated at 22°C for up to 6 weeks. Microbial activities were measured via ammonium production, nitrification, carbon dioxide evolution, and sulfur oxidation. Terbufos was rapidly oxidized to its sulfoxide with an approximate 4-5 day half-life. Terbufos sulfoxide reached a peak after 2 weeks of incubation, while terbufos sulfone did not appear until 1 week after the incubation was started.

Waller and Dahm (16) reported that within 2 days after application of phorate to soil, 85% of the added phorate was lost; about 30% by adsorption to the soil, 35% by oxidation to the sulfoxide, and 20% by oxidation to the sulfone.

Photodegradation. Few organophosphorus insecticides are completely resistant to photolysis, but this is probably not a major means of organophosphorus insecticides inactivation or disappearance in soil (44).

Menzer and Fontanilla (36) pointed out that the long persistence of phorate in soils is undoubtedly caused by its low mobility in soil. Phorate after application moves in both horizontal and vertical directions.

Lichtenstein and Fuhremann (37) reported that residues were least persistent when phorate was applied solely to the soil surface. Six days were required until one half of the applied dosage could no
longer be detected in the form of phorate and its metabolites. Residues were more persistent after insecticide had been mixed with the upper 4 to 5 inches of soil, and 30 days were required until one half of applied dosage could no longer be detectable.

Kiigemagi and Terriere (32) suggested that in the first few (2-3) weeks after application, physical factors such as volatilization accounted for most of the pesticides loss (Dyfonate), whereas chemical and biological mechanisms accounted for the losses in latter stages.

A study of persistence, movement, and metabolism of carbon-14 fonofos and carbon-14 phorate has been reported (34). The more water soluble [14C] phorate was more mobile and was metabolized to a greater extent than insecticides of low water solubilities. 14C residues of these two insecticides have different values in different soils.

Another [14C] phorate study (35) reported that phorate was much more persistent under flooded than under nonflooded conditions.

A considerable amount of organophosphorus insecticide analysis has been done by gas-liquid chromatography(GLC) with different kinds of detectors and columns. In Munnecke's (24) work, for example, organophosphorus insecticides were determined by using a gas-liquid chromatograph with a flame ionization detector equipped with a 6% SE 30 glass column. Detector and injector temperatures were 350 and 275°C respectively, and column temperatures range from 190 to 230°C.

Besides gas-liquid chromatography, high-performance liquid chromatography (HPLC) is also available to be used for pesticide analysis. Sparacino and Hines (25) reported that normal phase HPLC
packings give satisfactory results but reverse phase columns give better results. In Visalakshi (26) study, phorate was determined by colorimetric methods in a certain period of time after applying organophosphorus insecticides to soils. Half-lives were also determined by this method. Another study (27) pointed out that the absorption of Dyfonate was studied on humic acid (HA) saturated with various cations (Fe, Al, Cu, Zn, Co, Mn, Ni, Ca, Mg, and H ions). The amount of Dyfonate absorbed was affected by the cation with which the HA was saturated. Thin-layer chromatography is another method for determination of residual amounts of organophosphorus insecticides. Ignatov (46) reported that the best analytical results for phorate analysis were obtained with silica gel in a heptane: acetone 7:1 solvent, and the developing reagent consisting of 0.2% palladium dichloride solution in 0.5% hydrochloric acid. Phorate appears on the chromatogram in the form of a tile red spot with a darker periphery, and $R_f$ 0.61. The method is highly sensitive, the detection limit of 0.5 μg for phorate.

Brown (28) pointed out some improvements in methodology for the determination of residue of phorate and its three principal metabolites. These were (1) chilling the sample during extraction to prevent emulsion formation, (2) using a microcolumn clean up procedure resulting in economy of time and materials, and (3) evaporating under nitrogen to preclude oxidation of phorate.

Solvent systems for extraction vary greatly with organophosphorus insecticides interest. Walgenbach and co-workers (29) used a mixture of chloroform and methanol for phorate and terbufos, benzene
for fonofos. However, Mausd (30) reported that in a number of solvents were studied for extraction, 10% acetone in benzene was the most suitable for phorate and the recovery of phorate determined by gas-liquid chromatography was 90%.

Phosphatase activity. Phosphatases are enzymes that catalyze the hydrolysis of phosphate esters such as P-O-S, P-S-C, P-F, and others. There are classified as acid or alkaline depending on their pH optimum, 5-6 for acid phosphatase, 8-10 for alkaline phosphatase.

Several methods have been proposed for estimation of the phosphatase activity of soils. Kroll and Kramer (19) estimated soil phosphatase activity by determining the phenol released by incubation of soil with phenyl phosphate. Skujins et al (20) assayed soil phosphatase activity by a procedure in which the amount of glycerophosphate hydrolyzed is estimated by analyses for extractable total and inorganic phosphates after incubation. However this method is tedious and time-consuming and the data reported have low precision. Ramirez-Martineg and McLaren (21) reported another method involving fluorimetric assay of the β-naphthylphosphate. However, their method is complicated by sorption of β-naphthol by soil constituents and requires that the capacity of each soil analyzed to sorb β-naphthol be determined and allowed for in calculation of results. The basic differences in these methods are in the substrate used and in the technique employed to measure hydrolysis of the substrate by phosphatase enzymes.
Generally, Kramer and Yerdei's method (22) was a satisfactory method except that the procedure used to extract the phenol released by phosphatase activity did not give quantitative recovery of phenol and that the colorimetric technique used for estimation of phenol was complicated by instability of the color developed. Tabatabai and Bremmer (23) studied the use of other substrates for estimation of soil phosphatase activity which involves use of p-nitrophenyl phosphatase as substrate, permitting rapid and precise assay of soil phosphatase activity. The reaction is as shown below;

\[
\text{p-nitrophenyl disodium} \quad \text{orthophosphate} \quad \text{chromophore}
\]
EXPERIMENTAL

Reagents

A) The standard organophosphorus insecticides and other pesticides were obtained from Environmental Protection Agency, Research Triangle Park, N.C.

For the study of recovery, all pesticides used were the analytical standard grade solution diluted in hexane, benzene, or acetone, and added to 20 grams of soil. The concentrations of all the pesticides were adjusted to 5 ppm. Data for recovery studies are shown Table 2A, 2B.

For the study of degradation, all the pesticides used were applied to 1,000 grams soil as a solution of technical grades in hexane, benzene, or acetone with rolling to ensure thorough mixing. The concentration of all the pesticides used were 10 ppm of technical grade.

The nominal purities of the standards and technical grade pesticides and solvents used are as listed in Table 1.

B) Solvents

Analytical grade hexane, acetone, ethylacetate, methylene chloride, and benzene.

C) Other chemicals for phosphatase activity study

Analytical grade toluene, calcium chloride, sodium hydroxide, p-nitrophenyl disodium orthophosphate (PNP), standard p-nitrophenol solution.
Table 1: The purity of the standards

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Common Name</th>
<th>Standard's purity (%)</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>terbufos</td>
<td>Counter</td>
<td>99.1</td>
<td>Benzene</td>
</tr>
<tr>
<td>phorate</td>
<td>Thimet</td>
<td>95</td>
<td>Benzene</td>
</tr>
<tr>
<td>fonofos</td>
<td>Dyfonate</td>
<td>99.7</td>
<td>Benzene</td>
</tr>
<tr>
<td>Ethoprop</td>
<td>Mocap</td>
<td>97.8</td>
<td>Benzene</td>
</tr>
<tr>
<td>PCNB</td>
<td>PCNB</td>
<td>99.04</td>
<td>Benzene</td>
</tr>
<tr>
<td>ethazol</td>
<td>Terrazole</td>
<td>98.6</td>
<td>Benzene</td>
</tr>
<tr>
<td>Bronopol</td>
<td>Bronopol</td>
<td>-</td>
<td>Benzene</td>
</tr>
<tr>
<td>Benomyl</td>
<td>Benomyl</td>
<td>-</td>
<td>Benzene</td>
</tr>
<tr>
<td>Nitrpyrin</td>
<td>N-Serve</td>
<td>-</td>
<td>Benzene</td>
</tr>
<tr>
<td>Fenaminosulf</td>
<td>Lesan</td>
<td>-</td>
<td>Benzene</td>
</tr>
</tbody>
</table>
Apparatus

a) Gas-liquid chromatography (GLC) was found to be a satisfactory method for pesticide analysis. A Varian 2100 gas chromatograph with a $^{63}$Ni electron capture detector was used. A column of 4% SE-30 + 6% QF-1 at 180°C and a column of 3% SP-2250 DB at 178°C were first used and provided reasonable resolution of peaks for most pesticides (except terbufos with PCNB, fonofos with PCNB) and long retention times. Better resolution of overlapping peaks and shorter retention times have been successfully obtained by utilizing either the column of 1.5% OV-17: 1.95% QF-1 at 190°C or the Varian 3700 equipped with a thermionic specific detector and using a column of 1.5% SP2250:1.95% SP2401.

For the gas chromatography stationary phase and column condition the following were used.

OV-17
QF-1
SP-2250
SP-2401

Condition for Varian 2100:
Column oven temperature = 190°C
Injection port temperature = 220°C
Detector temperature = 295°C
Nitrogen flow rate = 30 ml/min
Attenuator = $4 \times 10^{-10}$ amp full scale
Conditions for Varian 3700:
Column oven temperature = 180°C
Injection port temperature = 220°C
Detector temperature = 250°C
Nitrogen flow rate = 20 ml/min.
Attenuator = 16, range 10

B) To measure the absorbance of p-nitrophenol which is released by incubation of soil with disodium p-nitrophenyl phosphate for the study of phosphatase activity, the Spectronic 88 spectrophotometer was used at wavelength 420 nm.

Pesticides combination applications

For the study, the following pesticides combination applications were examined in two kinds of soils:

Terbufos, Terbufos with PCNB-Terrazole
Phorate, Phorate with PCNB-Terrazole
Phorate, phorate with PCNB, phorate with Terrazole, Phorate with Bronopol, Phorate with Benomyl, Phorate with N-Serve, Phorate with fenaminosulf.

Since PCNB, Bronopol, and Benomyl appeared to have distinctive effective on phorate degradation, they were chosen in further studies.

Fonofos, Fonofos with PCNB, Fonofos with Bronopol Fonofos with Benomyl.

Ethoprop, Ethoprop with PCNB, Ethoprop with Pronopol, Ethoprop with Benomyl.
Soil treatment and conditions

Two different kinds of soils (abbreviated S1 and S2) were obtained from Dr. Duane P. Matthees, Station Biochemistry section, Department of Chemistry, South Dakota State University. Before any treatment, all soils were air-dried for 48 hours, then treated with one of those pesticides combinations respectively. The concentration was obtained at a level of 10 ppm (5 ppm for the recovery study). To ensure pesticide distributing evenly though the soil. (1000 grams with 10 ppm pesticides). All soils were placed in round metal cans and rolled for 20 minutes. All soils right after previous treatments were moistened to approximately 20% with distilled water. Following treatment, soil cans stored in a constant temperature and humidity (22°C, ~90% relative humidity or in the laboratory at 24°C) room either in the Plant Science laboratory or the laboratory of Dr. Duane P. Matthees.

Soils for terbufos and phorate analysis were taken initially at application and weekly after application for measurement of degradation. For fonofos and ethoprop analysis, soils were taken initially and every other week.

Extractions

Fifty (50) grams of treated soil from storage can was first mixed with 100 ml of either ethyl acetate or methylene chloride depending upon the recovery and shaken continuously for an hour. The mixture was then dried and cleaned by passing through a column containing sodium sulfate and washing with two 30 ml portions of ethyl acetate or methylene chloride. Then the ethyl acetate (or methylene chloride) solution
was concentrated by rotary evaporation and concentration to volume under N₂ for analysis. Dilution of the concentrate were made as needed, using hexane as a solvent.

Data handling

In this degradation analysis, the amounts of the pesticides left were calculated using the following equations:

\[
\text{ppm} = \frac{\mu g}{g} = \frac{V \text{ sample in } \mu l \times \mu g \text{ std.} \times A \text{ sample}}{W \text{ sample g } \times \mu l \text{ sample injected } \times A \text{ std.} \times \frac{1}{\text{fraction recovered}}} 
\]

or

\[
\frac{V \text{ sample in } ml \times \text{ng std. } \times A \text{ sample}}{W \text{ sample in g } \times \mu l \text{ sample } \times A \text{ std. } \times \text{recovery factor}} 
\]

\[V = \text{volume in } \mu l \text{ or } ml\]

\[W = \text{the amount of sample used for analysis in gram}\]

\[\text{Std} = \text{standard}\]

\[A = \text{Area under the peak}\]

Phosphatase activity examination

Several methods have been proposed for examination of phosphatase activity of soils. The basic differences in these methods are in the substrate used and in the technique employed to measure hydrolysis of the substrate by phosphatase enzymes.

Place 1 gram of treated soil in a 50-ml Erlenmeyer flask add 4 ml of distilled water, 0.25 ml of toluene and 1 ml of p-nitrophenyl phosphate (0.115M) (PNP) solution and swirl the flask for a few seconds to mix the contents. Stopper the flask and place it in a water bath
container for incubation at 37°C. After 10 minutes, remove the stopper, add 1 ml of 0.5M calcium chloride and 4 ml of 0.5 M sodium hydroxide. Swirl the flask for a few seconds, and filter the soil suspension. Transfer the filtrate to a cuvet and measure its absorbance with a Spectronic 88 spectrophotometer at wavelength 420 nm. Calculate the p-nitrophenol concentration of the filtrate by reference to a calibration curve. Figure 6.

To prepare the calibration curve, dilute 1 ml of the standard (1 g/1) p-nitrophenol solution to 100 ml in a volumetric flask and mix the solution thoroughly. Then pipette 0, 1, 2, 3, 4 and 5 ml aliquots of this diluted standard solution into small volumetric flask, adjust the volumes to 5 ml by addition of distilled water, and proceed as described for p-nitrophenol analysis of the incubated soil sample.

Controls should be also performed with each soil analyzed to allow for color not derived from p-nitrophenol released by phosphatase activity. In order to do that, perform the procedures described above for assay of phosphatase activity but make the addition of 1 ml of PNP solution after the additions of 0.5 M CaCl₂ and 0.5 M NaOH.
RESULTS AND DISCUSSIONS

Gas Chromatographic Analysis

The gas chromatographic analysis of four organophosphorus insecticides - terbufos, phorate, ethoprop, and fonofos as well as two other pesticides (PCNB, Terrazole) were performed by using a Varian 2100 gas chromatograph with a $^{63}$Ni electron capture detector. Column packing and gas chromatographic conditions were described as Experimental Part 2, Apparatus. The retention times for terbufos, phorate, fonofos, ethoprop, PCNB, and Terrazole were 7.8, 2.2, 3.1, 1.9, 2.8 and 1.9 respectively. Because of very sharp and reasonably large peaks obtained, the comparisons of peak heights of pesticides with those of standards were made. All calculations were done according to the equations described in Experimental Part 6, data handling. All standards showed very good linearity at assigned chromatographic conditions.

Recoveries of Standards with Two Different Solvents

Prior to degradation study, Extraction efficiency for each standard was examined by using analytical grade ethyl acetate and methylene chloride. The results are showed in Table 2A and 2B. The polar solvent ethyl acetate, generally gives better recoveries for organophosphorus insecticides which are polar molecules due to electronegative sulfur or oxygen atom bonded to phosphorus, with the exception of phorate. On the other hand, better recoveries were achieved for PCNB and Terrazole by using the less polar solvent methylene chloride. In this degradation study, solvent choice was based upon recovery studies.
Table 2A: Recovery study with two different solvents in Soil 1 for Terbufos, PCNB, Terrazole.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Solvent</th>
<th>Recovery</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbufos</td>
<td>methylene</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>chloride</td>
<td>67.2</td>
<td>79.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>82.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ethyl acetate</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>84.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>91.3</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>85.4</td>
<td></td>
</tr>
<tr>
<td>PCNB</td>
<td>methylene</td>
<td>80</td>
<td>70.6</td>
</tr>
<tr>
<td></td>
<td>chloride</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>76</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>68.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ethyl acetate</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>73.2</td>
<td></td>
</tr>
<tr>
<td>Terrazole</td>
<td>methylene</td>
<td>70.6</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>chloride</td>
<td>85.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ethyl acetate</td>
<td>73.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>63.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>68.8</td>
<td>63.9</td>
</tr>
</tbody>
</table>
Table 2B: Recovery with different solvents in Soil 1 and 2, for Fonofos, Ethoprop, and Phorate

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Soil 1</th>
<th>Soil 2</th>
<th>Soil 1</th>
<th>Soil 2</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Recovery Average</td>
<td>Recovery Average</td>
<td>R</td>
<td>A</td>
</tr>
<tr>
<td>Fonofos</td>
<td>75.3</td>
<td>80.8</td>
<td>80.2</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>73.5</td>
<td>90.4</td>
<td>78.5</td>
<td>74.4</td>
</tr>
<tr>
<td></td>
<td>68.1</td>
<td>72.3</td>
<td>80.0</td>
<td>83.7</td>
</tr>
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<td>Ethoprop</td>
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<td>79.8</td>
<td>85.6</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>75.3</td>
<td>72.6</td>
<td>80.3</td>
<td>82.95</td>
</tr>
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<td></td>
<td>79.9</td>
<td>77.9</td>
<td>71.2</td>
<td>74.5</td>
</tr>
<tr>
<td>Phorate</td>
<td>77.2</td>
<td>66.0</td>
<td>60.5</td>
<td>53.2</td>
</tr>
<tr>
<td></td>
<td>63.4</td>
<td>75.3</td>
<td>62.2</td>
<td>61.9</td>
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<tr>
<td></td>
<td>75.3</td>
<td>70.8</td>
<td>74.2</td>
<td>70.1</td>
</tr>
</tbody>
</table>
Degradation of Terbufos Applications and Phorate Applications in Soil 1

The interactive effects of PCNB-Terrazole on Terbufos and Phorate were examined in Soil 1. It was found that both Terbufos with PCNB-Terrazole application and Phorate with PCNB-Terrazole application degraded more slowly than Terbufos alone or Phorate alone. As shown in Figures 1A and 1B, PCNB-Terrazole increased the persistence of Terbufos and Phorate. Both Terbufos applications degraded steadily. However, concentrations of Phorate applications were decreasing rapidly in the first week from an average of 5.2 to 1.6, approximately at twice the rate of Terbufos alone.

Figures 2A and 2B showed the degradation rates of PCNB and Terrazole. There are two distinctive differences from Terbufos and Phorate applications. First PCNB-Terrazole application degraded more slowly than Terbufos and Phorate. Secondly, all applications with or without pesticides followed almost the same degradation pattern.

Degradation of Phorate Applications in Soil 1 and Soil 2

Figures 3A, 3B, 3C, and 3D showed the degradation patterns for Phorate applications in Soil 1 and Soil 2. Some conclusions can be drawn from these figures. First, phorate applications showed longer persistence in Soil 1 than they did in Soil 2. The half-lives are 7 days and 4 days in Soil 1 and Soil 2 respectively. Second, Phorate showed a more rapid degradation in first week than in the other weeks. This phenomenon is the same as the previous study in which phorate was added with two other fungicides, PCNB and Terrazol, rather than just
FIGURE 1A
Degradation Curve for Terbufos Applications in Soil 1
FIGURE 1B
Degradation Curve for Phorate Application in Soil 1
FIGURE 2A
Degradation Curve for PCNB

PCNB
PCNB (Terrazole ledufos)
PCNB (Terrazole Phorate)
FIGURE 2B
Degradation Curve for Terrazole
FIGURE 3A
Degradation Curve for Phorate Applications in Soil 1

[Graph showing degradation curves for Phorate applications in Soil 1]
FIGURE 30
Degradation Curve for Phorate Applications in Soil 1
FIGURE 3C
Degradation Curve for Phorate Application in Soil 1
FIGURE 3D
Degradation Curve for Phorate Application in Soil 2
one pesticide. Third, Phorate with PCNB application and Phorate with Bronopol application degraded more slowly than phorate alone application and others in Soil 1 while other applications degraded faster than or similar to the phorate alone application. However, all applications in Soil 2 degraded faster than that of Phorate alone application.

From above three results, it is suggested that soil type played a major role in Phorate's degradation.

Degradation of Fonofos Applications in Soil 1 and Soil 2

Among four organophosphorus insecticides Torbufos, Phorate, Fonofos, and Ethoprop, Fonofos showed the longest persistence, 2 ppm remained even after 84 days in Soil 1.

Figures 4A and 4B show the degradation patterns of Fonofos applications in Soil 1 and Soil 2 respectively. Generally, like the previous study, all applications experienced a slower degradation in Soil 1 than in Soil 2. Fonofos with Bronopol application and Fonofos with Benomyl application had a slower degradation than Fonofos alone in both Soil 1 and Soil 2. This coincidence suggested the pesticide-pesticide interaction played an important role on the Fonofos degradation. Bronopol is a bactericide, Benomyl is a systemic fungicide. Both have antimicrobial activity which may affect populations of bacteria and fungi to decrease the rate of degradation of fonofos. However, Fonofos with PCNB, a soil fungicide, showed a faster degradation route than Fonofos alone application.
FIGURE 4A
Degradation Curve for Fonofos Applications in Soil 1

[Graph showing degradation curve for Fonofos applications in soil 1]
FIGURE 4b
Degradation Curve for Fonofos Applications in Soil 2
Degradation of Ethoprop Applications in Soil 1 and Soil 2

Ethoprop applications experienced a longer persistence than Terbufos and Phorate applications. Figures 5A and 5B showed the degradation patterns of each Ethoprop application. As in the Phorate and Fonofos degradation study, all applications of Ethoprop experienced a slower degradation route in Soil 1 than they did in Soil 2. This result suggests again that soil type has a great influence on pesticide degradation. Ethoprop with Bronopol application, as well as Ethoprop with Benomyl application in Soil 2, showed a very rapid degradation in the first week. Both degraded from 11.4 ppm to 2.8 ppm, a loss of 75% rate. Ethoprop with PCNB application in both soils showed a slower degradation than the Ethoprop only application did, while all others were gone very quickly.

Examination of Phosphatase Activity

The calibration curve, Figure 6 was obtained by measuring the absorbance at λ = 420 nm of 0, 1, 2, 3, 4 and 5 ml of 10 µg/ml p-nitrophenol solutions with Spectronic 88 spectrophotometer. It showed good linearity in the range of 20 to 50 mg of p-nitrophenol solution with absorbances from 1.11 to 1.65.

The activities of phosphatase reported in Table 3A, 3B, 3C and 3D are indices of the activity of microflora involved in soil organic phosphatase decomposition. Phosphatase activity was examined at the beginning, the 36th day, the 65th day, and the end, 110th day, after application for fonofos and ethoprop. It was assayed at the beginning, approximately 4 and 10 weeks later, and at the end of the study for
FIGURE 33
Degradation Curve for Ethoprop Applications in Soil 2

Ethoprop
Ethoprop (CRAB)
Ethoprop (unmonitored)
FIGURE 6
Calibration Curve for p-nitrophenol Solution
### Table 3A: Phosphatase Activities in Soil 1

<table>
<thead>
<tr>
<th></th>
<th>Absorbance at First day</th>
<th>µg of p-nitrophenol released</th>
<th>A at 37th day</th>
<th>A at 70th day</th>
<th>A at 110th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>0.7</td>
<td>12</td>
<td>1.09</td>
<td>1.10</td>
<td>1.13</td>
</tr>
<tr>
<td>Fonofos</td>
<td>1.3</td>
<td>30</td>
<td>1.3</td>
<td>1.35</td>
<td>1.58</td>
</tr>
<tr>
<td>Fonofos PCNB</td>
<td>1.0</td>
<td>18</td>
<td>1.3</td>
<td>1.40</td>
<td>1.45</td>
</tr>
<tr>
<td>Fonofos Bronopol</td>
<td>0.9</td>
<td>26</td>
<td>1.29</td>
<td>1.38</td>
<td>1.42</td>
</tr>
<tr>
<td>Benomyl</td>
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<td>35</td>
<td>1.42</td>
<td>1.44</td>
<td>1.42</td>
</tr>
<tr>
<td>Ethoprop</td>
<td>-</td>
<td>-</td>
<td>1.4</td>
<td>1.51</td>
<td>1.86</td>
</tr>
<tr>
<td>Ethoprop PCNB</td>
<td>1.2</td>
<td>24</td>
<td>1.23</td>
<td>1.30</td>
<td>1.46</td>
</tr>
<tr>
<td>Ethoprop Bronopol</td>
<td>1.5</td>
<td>40</td>
<td>-</td>
<td>1.35</td>
<td>1.44</td>
</tr>
<tr>
<td>Benomyl</td>
<td>1.5</td>
<td>40</td>
<td>1.32</td>
<td>1.35</td>
<td>1.39</td>
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</table>

* All values shown in this table are the average value from two (or three) replicates.
* Value was estimated by extending standard graph.
Table 3B: Phosphatase Activity in Soil 2

<table>
<thead>
<tr>
<th></th>
<th>A at 1st day</th>
<th>-µg/g soil/unit time</th>
<th>A at 35th</th>
<th>-µg</th>
<th>A at 63</th>
<th>µg</th>
<th>A at 110th</th>
<th>n&lt;sub&gt;µg&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>Soil</td>
<td>1.01</td>
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<td>1.51</td>
<td>40</td>
<td>1.81</td>
<td>55*</td>
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<tr>
<td>Fonofos PCNB</td>
<td>1.3</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>1.68</td>
<td>47</td>
<td>1.88</td>
<td>62*</td>
</tr>
<tr>
<td>Fonofos Bronopol</td>
<td>1.5</td>
<td>40</td>
<td>1.33</td>
<td>31</td>
<td>1.63</td>
<td>46</td>
<td>1.78</td>
<td>53</td>
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<tr>
<td>Fonofos Benomyl</td>
<td>1.6</td>
<td>46</td>
<td>1.27</td>
<td>29</td>
<td>1.45</td>
<td>38</td>
<td>1.86</td>
<td>60*</td>
</tr>
<tr>
<td>Ethoprop</td>
<td>1.5</td>
<td>40</td>
<td>1.61</td>
<td>46</td>
<td>1.6</td>
<td>46</td>
<td>1.66</td>
<td>49</td>
</tr>
<tr>
<td>Ethoprop PCNB</td>
<td>1.7</td>
<td>52</td>
<td>1.50</td>
<td>40</td>
<td>1.62</td>
<td>47</td>
<td>1.61</td>
<td>46</td>
</tr>
<tr>
<td>Ethoprop Bronopol</td>
<td>1.6</td>
<td>46</td>
<td>1.65</td>
<td>49</td>
<td>1.60</td>
<td>46</td>
<td>1.50</td>
<td>40</td>
</tr>
<tr>
<td>Ethoprop Benomyl</td>
<td>1.2</td>
<td>24</td>
<td>1.51</td>
<td>40</td>
<td>1.66</td>
<td>49</td>
<td>1.90</td>
<td>63*</td>
</tr>
</tbody>
</table>

* All values shown in this table are the average values.
* Value was estimated by extending standard graph.
Table 3C: Phosphatase Activities in Soil 1^A

<table>
<thead>
<tr>
<th></th>
<th>A at 1st</th>
<th>-μg</th>
<th>A at 21st</th>
<th>μg</th>
<th>A at 48th</th>
<th>-μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>1.2</td>
<td>24</td>
<td>1.3</td>
<td>30</td>
<td>1.2</td>
<td>24</td>
</tr>
<tr>
<td>Phorate</td>
<td>1.52</td>
<td>40</td>
<td>1.48</td>
<td>38</td>
<td>1.3</td>
<td>30</td>
</tr>
<tr>
<td>Phorate PCNB</td>
<td>1.31</td>
<td>30</td>
<td>1.64</td>
<td>47</td>
<td>1.29</td>
<td>30</td>
</tr>
<tr>
<td>Phorate Bronopol</td>
<td>1.6</td>
<td>46</td>
<td>1.0</td>
<td>18</td>
<td>1.4</td>
<td>35</td>
</tr>
<tr>
<td>Phorate Benomyl</td>
<td>1.45</td>
<td>37</td>
<td>2.24</td>
<td>-</td>
<td>1.3</td>
<td>30</td>
</tr>
</tbody>
</table>

^A All are average values
Table 3D: Phosphatase Activities in Soil 2

<table>
<thead>
<tr>
<th></th>
<th>A at first</th>
<th>µg</th>
<th>A at 18th</th>
<th>µg</th>
<th>A at 28th</th>
<th>µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>1.4</td>
<td>35</td>
<td>1.6</td>
<td>46</td>
<td>1.2</td>
<td>24</td>
</tr>
<tr>
<td>Phorate</td>
<td>1.62</td>
<td>46</td>
<td>1.62</td>
<td>46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phorate PCNB</td>
<td>-</td>
<td>-</td>
<td>1.28</td>
<td>30</td>
<td>1.38</td>
<td>34</td>
</tr>
<tr>
<td>Phorate Bronopol</td>
<td>1.53</td>
<td>40</td>
<td>1.41</td>
<td>37</td>
<td>1.45</td>
<td>37</td>
</tr>
<tr>
<td>Phorate Benomyl</td>
<td>1.55</td>
<td>41</td>
<td>1.35</td>
<td>33</td>
<td>1.20</td>
<td>24</td>
</tr>
</tbody>
</table>

\(\Delta\) All are average values
phorate applications. Most assays were done with frozen soil. The absorbances of phosphatase-generated p-nitrophenol showed good agreement between fresh soils and frozen soils. Soils without any pesticide treatment had very stable activity from the beginning to the end. Phosphatase activity for phorate-treated soils decreased with time, while phosphatase activities for fonofos and ethoprop applications soils increased with time.
CONCLUSIONS

Degradation of terbufos, phorate, fonofos, and ethoprop applications varies greatly with the soil type and the presence of other pesticides. It was found that there are some interactions of pesticides on these four organophosphorus insecticides either increasing or reducing the rate of degradation. Fonofos with Bronopol application, fonofos with Benomyl application, and phorate with Bronopol application increased persistence. Bronopol and Benomyl have antimicrobial activity which may inhibit the microorganisms in soil and consequently cause decreased degradation. A similar result was also observed in terbufos with a PCNB-Terrazole application as well as phorate with PCNB-Terrazole application. It was noticed that all applications showed faster degradation in Soil 2 than those in Soil 1. Fonofos and ethoprop applications showed longer persistence. On the other hand, terbufos and phorate applications degraded much faster.

According to Tu's observation (18), phorate, fonofos, and ethoprop treated soils reduced the activity of phosphatase in two weeks. However, in this study over 38 days, activity for phorate-treated soils generally decreased with time. In 110-day period of time, the activities of fonofos and ethoprop applications soils increased with time, and the pesticide-treated soils showed higher phosphatase activity than those soils without treatment.
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