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Iowa State University, Ph.D., 1975
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Interaction of three Iowa surface soils with the organophosphorus insecticide Counter®

by

James Gary Laveglia

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

Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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For the Major Department

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For the Graduate College

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Ames, Iowa

1975

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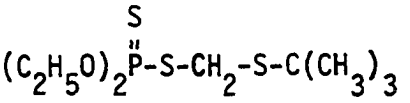
STRUCTURAL FORMULAS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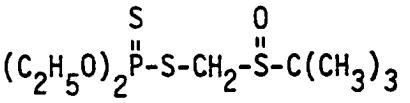
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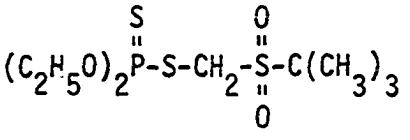
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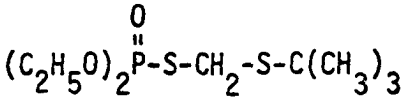
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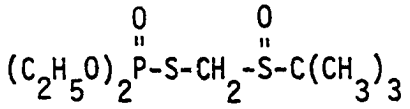
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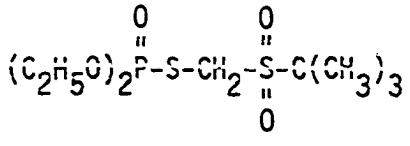
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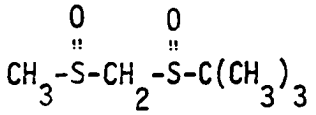
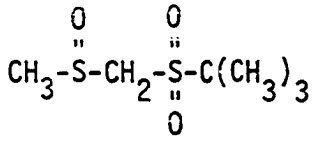

Metabolite A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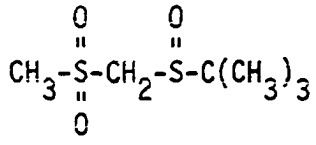
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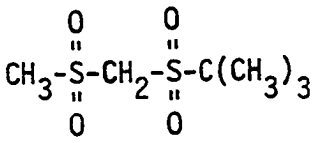


Metabolite 13

INSECTICIDE NOMENCLATURE

aldicarb (Temik®) 2-methyl-2-(methylthio)propionaldehyde O-(methyl=carbamayl)oxime

aldrin 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene

BHC 1,2,3,4,5,6-hexachlorocyclohexane

carbofuran (Furadan®) 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl=carbamate

carbophenothion (Trithion®) S-[[(p-chlorophenyl)thio]methyl] O,O-di=ethyl phosphorodithioate

chlordane 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene

chlorfenvinphos (Birlane®) 2-chloro-1-[(2,4-dichlorophenyl)vinyl] di=ethylphosphate

chlorpyrifos (Dursban®) O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)phos=phorothioate

crotoxyphos (Ciodrin®) alpha-methylbenzyl (E)-3-hydroxycrotonate di=methylphosphate

DDD (Rhothane®) 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane

DDT 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane

demeton (Systox®) mixture of O,O-diethyl S-(and O) [2-(ethylthio)=ethyl] phosphorothioates

diazinon O,O-diethyl O-(2-isopropy-4-methyl-6-pyrimidinyl) phosphoro=thioate

dieldrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octa=hydro-1,4-endo-exo-5,8-dimethanonaphthalene

dimethoate (Cygon®) O,O-dimethyl phosphorodithioate S-ester with 2-mercapto-N-methylacetamide

disulfoton (Di-Syston®) O,O-diethyl S-[2-(ethylthio)ethyl] phosphoro=dithioate

endrin 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene

fensulfothion (Dasanit®) O,O-diethyl O-[4-(methylsulfinyl)phenyl] phosphorothioate

fonophos (Dyfonate®) O-ethyl S-phenyl ethylphosphonodithioate

heptachlor 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene

lindane γ-1,2,3,4,5,6-hexachlorocyclohexane

malathion S-[1,2-bis(ethoxycarbonyl)ethyl] O,O-dimethyl phosphorodithioate

methidathion (Supracide®) S-[(2-methoxy-5-oxo-Δ^2-1,3,4-thiadiazolin-4-yl)methyl] O,O-dimethyl phosphorodithioate

methoxychlor 1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane

mexacarbate (Zectran®) 4-dimethylamino-3,5-xylyl methylcarbamate

parathion (Thiophos®) O,O-diethyl O-p-nitrophenyl phosphorothioate

phenamiphos (Nemacur-P®, Bayer 68,138) ethyl 3-methyl-4-[(methylthio)phenyl] (1-methylethyl) phosphoramidate

phorate (Thimet®) O,O-diethyl S-[(ethylthio)methyl] phosphorodithioate

propoxur (Baygon®) o-isopropoxyphenyl methylcarbamate

SMDC (Vapam®) sodium methyldithiocarbamate

thionazin (Zinophos®) O,O-diethyl O-2-pyrazinyl phosphorothioate

toxaphene chlorinated camphene containing 67-69% of chlorine

trichlorfon (Dipterex®, Dylox®) dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate

trichloronat (Agritox®, Bayer 37,289) O-ethyl O-[(2,4,5-trichlorophenyl)ethyl] phosphonothioate

REVIEW OF LITERATURE

Although Counter®, O,O-diethyl S-[(tert-butylthio)methyl] phosphorodithioate, is a new soil insecticide expected to be used extensively for control of corn rootworms, no published literature is available dealing specifically with Counter. Pertinent literature is available involving the effects of other insecticides on soil microorganisms, as well as metabolism of organophorous insecticides in the soil.

Influence of insecticides on soil microorganisms

Ammonium production

Tu (1970) studied the effect of 4 organophosphorus insecticides (Bayer 37,289, diazinon, chlorpyrifos, and Zinophos®) at 10 and 100 ppm on ammonification. In most instances the insecticides increased ammonium production. The most pronounced stimulatory effect occurred with 100 ppm of diazinon and Zinophos, and with 10 ppm of Bayer 37,289, which increased ammonium production by 66 to 100%. Mineralization of the added peptone was significantly stimulated from 16% with 10 ppm of Bayer 37,289 to 34% with 10 ppm of chlorpyrifos. The accumulation of ammonium showed clearly that the ammonifiers are not sensitive to these insecticides.

Helweg (1972) reported ammonification was not drastically altered by 10, 100, and 1000 ppm concentrations of technical chlorfenvinphos. Singh and Gulati (1972) showed the quantity of ammoniacal nitrogen was less in phorate and disulfoton-treated samples than in controls. Of the organophosphorus insecticides, demeton is probably one of the more toxic

chemicals to soil microorganisms because it inhibited ammonification in soils at below field rates (Audus 1970).

The effects of aldrin on ammonification at 1000 ppm were minor and irregular (Fletcher and Bollen 1954). Even at extremely high concentrations, 10,000 to 20,000 ppm, aldrin exhibited no deleterious effects (Brown 1954, Jones 1956). Ammonium production was considerably increased by the delta and gamma isomers of BHC at 1000 ppm, while alpha and beta-BHC had no influence (Bollen et al. 1954). Jones (1956) presented definite evidence that DDT, chlordane, and BHC became inhibitory to the microorganisms liberating ammonia at concentrations greater than 2000 ppm. It has been reported by others (Wilson and Choudhri 1946, Bollen et al. 1954, Brown 1954) that ammonification proceeds normally when up to 10,000 ppm of DDT were applied to the soil. Ross (1952) found a stimulatory effect of DDT when applied at rates up to 120 ppm.

Nitrification

Tu (1970) found that the effect on nitrification of all 4 organophosphorous insecticides tested (Bayer 37,289, diazinon, chlorpyrifos, and Zinophos) was depressive in most cases at 2 weeks, and still depressive, but to a lesser extent, at 3 weeks. The insecticide concentrations used were 10 and 100 ppm. The decreased effect of the insecticides on nitrification in some samples after 3 weeks indicated that either the insecticides underwent transformation and detoxification in soil during the 3-week period, or that the nitrifiers had adapted to the insecticides.

Bartha et al. (1967) tested 3 insecticides (parathion, malathion,

and phorate) at 150 ppm for 6, 12, and 18 days. It was found again that the ability of these insecticides to retard nitrification decreased with time. Hubbell et al. (1973) reported parathion at 2 ppm reduced nitrification by about 25% during the first 8 weeks.

Lin et al. (1972) studied the effect of 6 organophosphorus insecticides (trichlorfon, Dyfonate®, Bayer 37,289, carbophenothion, chlorpyrifos, and Nemacur-P®) and 3 carbamates (propoxur, aldicarb, and carbofuran) on soil nitrification. No inhibition was found at normal field rates (5 ppm) of application. Some instances of inhibition were observed at 50 and 500 ppm.

Helweg (1972) reported that chlorfenvinphos at 10 and 100 ppm concentrations had no influence on nitrification, while at 1000 ppm nitrification was inhibited completely. Phorate and disulfoton at 100 ppm also inhibited nitrification (Singh and Gulati 1972).

In general, lower doses, or normal field application rates of the organochlorine insecticides showed no effect on nitrification. Martin et al. (1959) reported that the oxidation of ammonium proceeded in a normal manner in 2 soil types when 8 organochlorine insecticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, lindane, and toxaphene) were applied at approximate annual application rates (5 to 20 ppm).

DDT, when applied at rates up to 1000 ppm, resulted in no deleterious effects to the nitrification process (Smith and Wenzel 1947, Ross 1952, Jones 1956, Bartha et al. 1967, Gaur and Pareek 1971, Hubbell et al. 1973). Eno and Everett (1958) reported a slight stimulatory effect

at 1 month after application of DDT at 12, 50, and 100 ppm. High application rates of DDT (1000 to 20,000 ppm) inhibited nitrate production as much as 90% (Brown 1954, Jones 1956).

Bardiya and Gaur (1970) reported lindane to have no effect on nitrification at 1 to 25 ppm concentrations. At higher dose levels, 100 and 1000 ppm, nitrification was inhibited for 3 weeks, but returned to normal by week four. Brown (1954) also showed no effect for lindane at low dose levels, but high concentrations (10,000 ppm) inhibited nitrification.

Dieldrin, at 200 ppm, exhibited a 20% reduction in nitrate production, while at 20,000 ppm a 90% reduction was observed (Jones 1956). Brown (1954) also found high rates of dieldrin (1000 and 10,000 ppm) inhibited nitrification. Normal field rates had no effect on nitrification (Brown 1954, Bardiya and Gaur 1970). At 250 ppm, Bartha et al. (1967) reported that dieldrin caused a significant increase in the amount of nitrate produced.

Aldrin showed no inhibition of nitrification at rates up to 1000 ppm (Ross 1952, Brown 1954, Fletcher and Bollen 1954, Jones 1956, Eno and Everett 1958, Shaw and Robinson 1960, Bardiya and Gaur 1970). Jones (1956) observed a 50% reduction in nitrification when aldrin was applied at 2000 ppm.

Brown (1954) reported inhibition of nitrification from chlordane at 1000 and 10,000 ppm and heptachlor at 10,000 ppm. Concentrations of chlordane ranging between 2000 and 20,000 ppm reduced nitrate production from 75 to 100% (Jones 1956). No effect was demonstrated when heptachlor

or chlordane were applied at rates of 200 ppm or less (Ross 1952, Brown 1954, Jones 1956, Eno and Everett 1958, Shaw and Robinson 1960).

Carbon dioxide evolution

Tu (1970) studied the effect of 4 organophosphorus insecticides on soil respiration. Oxygen uptake was not suppressed with any of the insecticides (Bayer 37,289, diazinon, chlorpyrifos, and Zinophos) at 2 dose levels (10 and 100 ppm). Oxygen consumption was greater at the 100 ppm level than at the 10 ppm level. It was proposed that the increased oxygen consumption was due to microorganisms oxidizing the insecticides.

Bartha et al. (1967) reported the effect of 3 organophosphorus insecticides (malathion, parathion, and phorate) at 150 and 1500 ppm on carbon dioxide evolution. Malathion produced an increase in carbon dioxide evolution. This was accounted for by the extensive oxidation of the insecticide to a relatively nontoxic compound. In the case of parathion there was an initial increase followed by a gradual decrease in carbon dioxide evolution. Phorate exhibited the same initial increase, but it was followed by a rapid decrease to lower inhibitory levels than parathion. A suggested explanation for this complex relation of carbon dioxide evolution to time was that it may be due to any one or a combination of the following mechanisms: 1) an insecticide acts to uncouple oxidative phosphorylation in a manner analogous to 2,4-dinitrophenol, 2) an insecticide lacking antimicrobial action is oxidized in part and transformed to a stable and toxic product, 3) an insecticide that is selectively toxic inhibits carbon dioxide production by sensitive microorganisms but is subject to oxidation without detoxification by other

members of the microbial population that are resistant to its initial action.

DDT at 137.5 ppm and BHC at 275 ppm, when added to the soil, did not appreciably influence microbial respiration (Bollen et al. 1954). Eno and Everett (1958) reported no significant change in soil respiration when 10 organochlorine insecticides (heptachlor, chlordane, methoxychlor, lindane, aldrin, toxaphene, dieldrin, DDD, DDT, and BHC) were incubated for 21 days at dose levels of 12.5, 50, and 100 ppm. Inhibition of carbon dioxide evolution by 20% was noted by Bartha et al. (1967) for endrin and dieldrin at 250 and 2500 ppm. Fletcher and Bollen (1954) observed a slight but definite increase in carbon dioxide evolution from soil when treated with aldrin at 200 and 1000 ppm.

Sulfur oxidation

Tu (1970) reported no influence of Bayer 37,289 at 10 and 100 ppm, and diazinon, chlorpyrifos, and Zinophos at 10 ppm on sulfur oxidation. Diazinon at 100 ppm increased sulfur oxidation by 15% while chlorpyrifos and Zinophos decreased sulfur oxidation 17 and 12%, respectively.

Jones (1956) demonstrated toxic effects to sulfur-oxidizing organisms using 3 organochlorine insecticides. DDT, when added to soils high in organic matter, produced no residual injury at concentrations as high as 10,000 ppm. In sandy soils low in organic matter, a distinct injury occurred when DDT was added at 2000 ppm. Chlordane was more toxic than DDT. The toxicity was evident even in fertile soils at concentrations of 200 ppm. BHC was also toxic to sulfur-oxidizing organisms. A significant inhibition of the action of these microorganisms was noted at

200 ppm. It was pointed out that sulfur-oxidizing bacteria once injured may remain so even after the insecticide has disappeared. When methoxychlor, dieldrin, aldrin, and endrin were added to fertile loam soils no significant injury was observed.

Cellulose decomposition

Audus (1970) reported that most of the insecticides have no effect on the numbers or activities of cellulolytic organisms in the soil until they inhibit at very high application rates. Cellulose breakdown was inhibited by chlordane, dieldrin, and demeton at 20, 200, and 40 times normal application rates, respectively. Bollen et al. (1970) observed no effect on tree litter decomposition when Zectran® was applied at normal field rates. Martin et al. (1959) tested the decomposition of walnut leaves in the presence of aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, lindane, and toxaphene at annual application rates (1 to 20 ppm) and found the walnut leaves decomposed at about the same rate in the treated soils as in the check samples.

In general, the organochlorine insecticides at normal field rates (1 to 20 ppm) do not inhibit the 5 microbial functions mentioned. Even at slightly elevated rates (100 to 500 ppm) no inhibition occurred. The microbial functions were inhibited only at extremely high concentrations (1000 to 20,000 ppm). The organophosphorous insecticides have not been tested at concentrations as high as 10,000 ppm. At field application rates (1 to 5 ppm) there is usually no inhibitory effect. This is not true, especially for nitrification, if the dosage is raised slightly (10 to 50 ppm). The nitrifiers sometimes recover 2 or 3 weeks after

insecticide application.

Insecticide metabolism in soil

Organophosphorus insecticides are less stable in soil than organochlorine insecticides. This instability is due to biological and/or nonbiological reactions. The rates of malathion (Konrad et al. 1969), Ciodrin® (Konrad and Chesters 1969), and diazinon (Konrad et al. 1967) degradation in soils were related directly to the extent of adsorption, suggesting that degradation occurred by a chemical mechanism (hydrolysis) which was catalyzed by adsorption. Factors influencing adsorption were pH, organic matter, and clay content. Konrad et al. (1969) reported malathion degradation was rapid (50 to 90% in 24 hours depending on soil type) in both sterile and nonsterile soil systems. Hydrolysis did not occur in acid systems, but was rapid at pH 11 (99% in 1 day). This hydrolysis resulted in the formation of thiomalic acid and dimethyl thiophosphoric acid as final products with accumulation of diethyl thiomalate as an intermediate. Walker and Stojanovic (1973) indicated microbiological degradation of malathion predominated over chemical mechanisms. Malathion abatement in all cases was more rapid under nonsterile than sterile conditions.

Konrad et al. (1967) found hydrolysis of diazinon was the major degradative pathway in soils. Formation of the hydrolysis products 2-isopropyl-4-methyl-6-hydroxypyrimidine and diethyl thiophosphoric acid was adsorptive rather than acid-catalyzed. Degradation rates were 11, 7, and 6% per day for Poygan sicl, Kewaunee c, and Ella ls, respectively. Bro-Rasmussen et al. (1968) studied the disappearance of diazinon in 2

different soils, with 2 different diazinon concentrations, at 2 levels of water content, and in steam-treated and untreated soils. They found that all 4 factors influenced the degradation of diazinon in soil, the greatest effect occurring with steam-treatment, which suggested microorganisms play an important role in the disappearance of diazinon. Diazinon was degraded equally in autoclaved and nonautoclaved soils (Getzin 1968). Degradation increased at lower pH, higher temperature, and higher soil moisture. In sterilized soils, Sethunathan and MacRae (1969), found the disappearance of diazinon was rapid in acidic clay (pH 4.7) but very slow in other clay (pH 6.6) and clay loam (pH 7.6) soils.

Zinophos was degraded in the soil primarily by microorganisms (30 to 70%) depending upon the environmental conditions (Getzin 1968). Higher temperatures and soil moisture levels accelerated decomposition. An increase in soil pH from 4.3 to 8.1 enhanced the biological breakdown of Zinophos by apparently providing a suitable environment for microorganisms metabolizing this insecticide.

Phorate is rapidly oxidized in soil to its sulfoxide and sulfone (Getzin and Chapman 1960, Getzin and Shanks 1970, Lichtenstein et al. 1973). At 0 days after treatment, Getzin and Chapman (1960) reported 11 to 13% of the detected radioactivity as oxidation products. Phorate sulfone was not degraded rapidly and was present 2 months after application of phorate to the soil surface (Lichtenstein et al. 1973).

Degradation of parathion in soil increased with the concentration of parathion, a rise in temperature, the time of exposure to ultraviolet

light, an increase in relative humidity, incubation time, and a rise in soil pH (Chopra and Khullar 1971). Lichtenstein and Schultz (1964) reported 30% of the parathion added to a loam soil was lost within 12 days after application. Degradation was either by hydrolysis to p-nitrophenol and diethylthiophosphoric acid or by reduction to its amino form depending on populations of soil microorganisms. In autoclaved and dry soil parathion persisted for a relatively long time. Paraoxon was hydrolyzed within 12 hours after its application at 20 ppm to loam soil. During that time the amount of paraoxon constantly decreased while the amount of p-nitrophenol increased.

In a sandy loam soil dimethoate has a half-life of 2.5 days when rainfall occurs after application, whereas the half-life is about 4 days under draught conditions (Bohn 1964). Duff and Menzer (1973) applied dimethoate to 3 soil types and found conversion to dimethoxon was faster in the more moist soils, and levels were generally greater. Dimethoate carboxylic acid was the only hydrolytic metabolite identified.

Chlorfenvinphos was added to a mineral soil and a fen soil at the beginning of June (Suett 1971). Half the insecticide in the mineral soil had disappeared in 9 weeks, while 50% degradation in the fen soil occurred in 18 weeks. At the end of the season, 20 to 30% of the chlorfenvinphos remained in the mineral soil, while 40 to 50% remained in the fen soil. Chlorfenvinphos metabolism was examined in 4 soils by Beynon and Wright (1967). After 4 months, besides the remaining chlorfenvinphos, the major breakdown products were; 2,4-dichloroacetophenone, 1-(2',4'-dichlorophenyl) ethane-1-ol, and desethyl chlorfenvinphos.

Menzer et al. (1970) found that soil type had a greater influence on disulfoton degradation than temperature. Disulfoton metabolism in upland and paddy soils was studied by Takase et al. (1972). Rapid oxidation to disulfoton sulfoxide and disulfoton sulfone was observed in upland soils. These oxidative metabolites were stable and still displayed insecticidal activity. Oxygen analogs were also formed, but in very small amounts. Disulfoton also undergoes oxidation in paddy soil, but at a much faster rate. In sterilized soil, disulfoton is oxidized and degraded to a slight extent. From this, it was concluded that activities of soil microorganisms are interrelated with the oxidation, stability, and decomposition of disulfoton in soil.

Harris et al. (1971) reported that Dasanit® was degraded rapidly in the soil to Dasanit sulfone which was also insecticidally active. In mineral soil Dasanit disappeared relatively quickly, accompanied by a corresponding increase in sulfone. Biological activity persisted at a high level because of the joint action of the 2 compounds. After 20 weeks the amount of Dasanit present in the soil as Dasanit and Dasanit sulfone was approximately 3 and 38%, respectively, of the initial Dasanit application. After 32 weeks, all biological activity had disappeared while the residue levels were more or less the same. In soil high in organic matter Dasanit was also converted rapidly to the sulfone, however no toxicity occurred in any of the bioassays.

Studies were conducted to determine the persistence of Trithion® in 3 California loamy soils (Menn et al. 1960). The degradation of Trithion was fastest in Yolo silty clay loam and slowest in Sorrento

loam. Although the organic matter content of the Sorrento and Yolo loams was nearly equal, they differed greatly in their effect on degradation of Trithion. The soil pH had no apparent effect on the persistence of Trithion, whereas it appeared that the higher clay content in the Yolo silty clay loam contributed to Trithion degradation. The persistence of Trithion increased significantly in soil that was autoclaved or fumigated with Vapam®. Its longer persistence in autoclaved and fumigated soil apparently resulted from a partial destruction of the soil microorganisms which may prevent degradation of Trithion.

The persistence of methidathion in 4 soils of western Washington was observed by Getzin (1970). Degradation was rapid in all 4 soils. Fifty percent of the initial applications decomposed in less than 2 weeks, and more than 90% of the insecticide disappeared within 16 weeks. When soils were treated with ^{14}C-methidathion 40 to 66% of the radioactivity was expired as $^{14}CO_2$ after 16 weeks. In fumigated soils 50% of the initial insecticide applications still remained after 16 weeks and less than 3% of the radioactivity was expired as $^{14}CO_2$ which suggests that microorganisms are primarily responsible for the degradation of methidathion in soil.

Toxicity of selected organophosphorus insecticides

Phorate, demeton, and disulfoton, being closely related structurally to Counter, are extremely toxic insecticides. Gaines (1969) reported the oral LD_{50} to female rats for phorate, demeton, and disulfoton as 2.5, 1.1, and 2.3 mg/kg, respectively. Clark et al. (1955) found the interperitoneal LD_{50} to mice was 1 to 4 mg/kg for both phorate and Counter.

DuBois et al. (1956) compared the oral LD_{50} of demeton, demeton sulfoxide, and demeton sulfone using rats. All 3 compounds appeared to be equally toxic giving values of 1.7, 2.3, and 1.9 mg/kg, respectively.

Waller (1972) studied the dermal ED_{50} (the amount of a chemical that causes an observable effect in 50% of the population tested) of phorate and its 5 metabolites using southern corn rootworm larvae. Phoratoxon (POS) was the most toxic of the compounds tested, 2.1 mg/kg; followed by phorate sulfoxide (PSO) and phoratoxon sulfone ($POSO_2$), 3.1 mg/kg; phoratoxon sulfoxide (POSO), 3.3 mg/kg; phorate (PS), 3.5 mg/kg; and phorate sulfone (PSO_2), 4.2 mg/kg. It is probable that Counter and its metabolites would demonstrate the same toxicities relative to each other as did demeton and phorate.

PART I: INFLUENCE OF COUNTER ON MICROBIAL ACTIVITIES IN THREE IOWA SURFACE SOILS[1]

[1]Published in Environ. Entomol. 3:528-533. 1974.

ABSTRACT

Five laboratory tests were performed using 3 Iowa surface soils (Clarion, Harps, and Webster) to determine the influence of Counter on microbial activities. The insecticide was applied to 10-g soil samples at rates of 0, 1, 10, 50, and 100 ppm. The 1 ppm rate is equivalent to a field application rate of 1 lb/acre. Soil samples were incubated at 22 $\pm$ 1 °C for as long as 6 weeks and samples were removed for analyses at weekly intervals. Microbial activities in the 3 soils were measured by ammonium production, nitrification, carbon dioxide evolution, sulfur oxidation, and cellulose decomposition. There were 276 analytical mean values for soil samples receiving insecticide treatment. Of these, 48 values were significantly (P=0.05) higher than control values (0 insecticide treatment), 15 values were significantly lower than control values were significantly lower than control values, and 213 values were not significantly different from control values. From the data presented it was concluded that Counter has no inhibitory influence on soil microbial activities.

INTRODUCTION

Approximately 5 million acres of Iowa farmland were treated with about 7 million pounds of soil insecticides in 1973. Because of the prevalent use of soil insecticides, it is necessary to study their impact on soil microorganisms. There have been numerous reports dealing with the influences of insecticides on microbial activities in various soils (e.g., reviews by Bollen 1961 and Audus 1970).

Counter is an effective new soil insecticide that is expected to be used extensively. Five laboratory tests were used to determine the influence of Counter on soil microorganisms: ammonium production, nitrification, carbon dioxide evolution, sulfur oxidation, and cellulose decomposition. These were chosen because they measure a range of microbial activities vital to the fertility of the soil.

MATERIALS AND METHODS

Three Iowa surface soils (Table 1) were obtained from the Iowa State University Agronomy and Agricultural Engineering Research Center located about 6 miles west of Ames, Iowa. These soils were chosen because they make up 20% of Iowa's total soil area. The other 80% is made up of many soils, none of which represents more than 10% of the total. The samples were dug from the upper 6 in. of soil and kept at 4° C in plastic bags. The Clarion and Webster soils had not been treated with an insecticide or herbicide for 10 years; the Harps soil had been treated with amiben 5 years previously. The soils were air-dried and crushed to pass a 2-mm sieve. The soil analyses reported in Table 1 were made as follows: pH by glass electrode (soil:water ratio, 1:2.5); organic carbon by the method of Mebius (1960); total nitrogen by a semimicro-Kjeldahl procedure (Bremner 1965); and particle-size distribution (sand, silt, and clay) by the pipet method of Kilmer and Alexander (1949).

The Counter was 96.7% pure and supplied gratis by the American Cyanamid Company, Princeton, N.J. The required amounts of insecticide were weighed in disposable, glass micro-sampling pipets and transferred quantitatively with acetone to 200-ml volumetric flasks. The acetone was evaporated with nitrogen. Fifty milliliters of water and 2 ml of an ethanolic 0.1% (w/w) Triton® X-100 solution were added to each volumetric flask. The insecticide was solubilized with vigorous shaking, and additional water was added to make 200 ml. The soil-treatment solutions were used in all 5 tests with the addition of peptone for ammonium

Table 1. Analyses of 3 Iowa surface soils; each value is the mean of 4 analyses

Soil	pH	Organic carbon (%)	Total nitrogen (%)	Sand (%)	Silt (%)	Clay (%)
Clarion	5.7	2.77	0.146	39	39	22
Harps	7.0	4.16	0.224	24	43	33
Webster	6.3	3.78	0.178	29	45	26

production and ammonium sulfate for nitrification. Details of these additions are given under the respective methods of analysis. Five treatments were used: 0, 1, 10, 50, and 100 ppm, which correspond to field application rates of 0, 1, 10, 50, and 100 lb of Counter/acre. These rates are based on 1 X 10^6 lb soil/acre in a soil volume of 3 in. in depth and 1 acre in area.

In all tests, 10 g of crushed, air-dried, soil were placed in 8-oz, French square bottles. Three milliliters of the treatment solution were added which brought the soil to about 50% water holding capacity and the bottles sealed with rubber stoppers; this kept moisture loss at a minimum. Air was introduced into the system 3 times a week.

There were 2 replicates, 2 samples per replicate, 3 soils, 5 treatments, and 4, 5, or 6 incubation periods as indicated for the specific analyses. Analysis of variance was performed on each soil type for each test. Least significant difference values were calculated between the various treatments and the control or 0 treatment. The data are reported as analytical mean values consisting of 2 replicates/treatment and 2 samples/replicate. A total of 1380 analyses were performed to obtain the values reported for this study.

Ammonium production

The method of Bremner and Keeney (1965) was used for ammonium, nitrate, and nitrite determinations. Because anaerobic conditions were not used, some of the ammonium produced was converted to nitrate; therefore, the nitrate and nitrite values were combined with the ammonium value. The samples were prepared as follows. Peptone (1.333 g) used

as a nitrogen source was added to each of the 4 volumetric flasks containing the insecticide. Peptone was also added to a fifth 200-ml volumetric flask containing only 50 ml of water and 2 ml of ethanolic 0.1% Triton X-100. This was the control or 0 treatment. The contents of the 5 volumetric flasks were diluted to 200 ml, and 3 ml of this solution were added to the soil in the French square bottles and the bottles stoppered. The bottles contained 10 g of soil, 20 mg of peptone, 3 ml of water, and 0, 0.01, 0.10, 0.50, or 1.00 mg of insecticide. The incubation extended over a 4-week period at 22 $\pm$ 1°C, and ammonium, nitrate, and nitrite were extracted simultaneously at weekly intervals with 2M KCl. Four incubation periods gave a total of 240 samples for analyses.

Nitrification

The method of Bremner and Keeney (1965) was used for nitrate and nitrite determinations. The two values were added to indicate total nitrification. The insecticide treatment solutions were prepared and used exactly as described for ammonium production, except that 0.667 g of ammonium sulfate was added to each 200 ml volumetric flask as an ammonium source. The incubation extended over a 4-week period at 22 $\pm$ 1°C; nitrate and nitrite were simultaneously extracted at weekly intervals with 2M KCl, and the extract was analyzed by steam distillation followed by titration with a standard sulfuric acid solution (Bremner and Keeney 1965). Four incubation periods gave a total of 240 samples for analyses.

Carbon dioxide evolution

The method of Bundy and Bremner (1972) was modified for the

determination of carbon dioxide evolved from aerobic incubation of soil. The procedure was the same except no acid was added to the soil to decompose the carbonates to carbon dioxide. The soil-treatment solutions were prepared and applied as described in the second and third paragraphs of Materials and Methods. In this test no fortification material was added to the soil before addition of the 3 ml of treatment solution. Five-milliliter beakers were suspended in the French square bottles. These beakers contained 2M KOH that absorbed carbon dioxide; the beakers were emptied 3 times a week, and the solutions were titrated with HCl. The incubation extended over a 4-week period at 22 $\pm$ 1°C. Because the soil was not extracted, the same soil sample was incubated for 4 weeks. There were 60 individual samples titrated 3 times a week for a total of 180 analyses.

Sulfur oxidation

Determination of sulfate was accomplished by the methods of Tabatabai and Bremner (1970, 1972). Twenty milligrams of sieved (0.125-mm screen) sulfur were added to each soil sample and mixed thoroughly before adding 3 ml of the treatment solutions described in the second and third paragraphs of Materials and Methods. The incubation extended over a 6-week period at 21 $\pm$ 1°C, and sulfate was extracted with 0.135 mM (0.15%) $CaCl_2$ at weekly intervals starting with the second week. The last set of samples was extracted at 6 weeks and 2 days after the beginning of incubation. Five incubation periods gave a total of 300 samples for analyses.

Cellulose decomposition

Cellulose is decomposed to glucose, which is readily metabolized by microorganisms to carbon dioxide. The method of Bundy and Bremner (1972) was modified as for the carbon dioxide evolution analyses. Twenty milligrams of Whatman cellulose powder were added to each soil sample and mixed thoroughly before addition of 3 ml of the treatment solutions as described in the second and third paragraphs of Materials and Methods. The incubation extended over a 6-week period at 22 $\pm$ 1°C. There were 60 individual samples titrated 3 times a week for a total of 180 analyses.

RESULTS AND DISCUSSION

The results are shown in Figs. 1 through 5 and Table 2. Each point in the figures is the mean of 4 analyses (2 replicates, 2 samples/replicate). There were 276 analytical mean values for soil samples receiving insecticide treatment. Of these, 48 values were significantly (P=0.05) higher than control values (0 insecticide treatment), 15 values were significantly lower than control values, and 213 values were not significantly different from control values (Table 2).

Ammonium production

Of the 48 analytical mean values for soil samples receiving insecticide treatment, 16 were significantly higher than control values, and 32 were not significantly different from control values (Table 2, Fig. 1). Stimulation of ammonification was reported by Tu (1970) when 4 organophosphorus insecticides (Bayer 37,289, diazinon, chlorpyrifos, and Zinophos) were tested at 10 and 100 ppm. Some of the stimulatory values were 20 to 30% greater than the controls. Demeton inhibited ammonification in soils at rates less than field usage (Audus 1970). Many organochlorine insecticides have also stimulated ammonium production (Ross 1952, Brown 1954, Jones 1956). Bollen et al. (1954) suggested that this increase in ammonium production is caused by a stimulation of physiological activity in microorganisms because of the intervention of small amounts of insecticide in enzyme systems involved in nutrition. Even extremely high concentrations (1000 to 20,000 ppm) of some of the organochlorine insecticides produced little change in ammonium production

Table 2. Significant effects (P=0.05) of Counter on microbial activities in 3 Iowa surface soils. A=increase in ammonium production[a]; n,N=decrease or increase in nitrification[a]; ca,CA=decrease or increase of carbon dioxide evolution[a]; s,S=decrease or increase in sulfur oxidation[b]; and ce,CE=decrease or increase in cellulose decomposition. A blank space indicates no significant effect

Counter added (ppm)	Incubation time (weeks)					
	1	2	3	4	5	6
			Clarion			
1				ca		S
10						S
50	CA	CA	CA	CE	S,CE	S,CE
100	CA	CA	A,CA	CA,CE	CE	CE
			Harps			
1			ce	ce	ce	ce
10	n	A	ce	ce	ce	ce
50	CE	A,CE	A	A		
100	CA,CE	A,CA,CE	A,N,CA	A,N,CA		S
			Webster			
1				N		s,ce
10		A	A	N,ce	ce	ce
50		A	A	A,N		
100		A	A	A,N		

[a]Not tested at weeks 5 and 6.

[b]Not tested at week 1.

Figure 1. Effect of Counter on ammonium production in 3 Iowa surface soils; 10 g of soil were mixed with 20 mg of peptone and incubated at 22 C. Ammonium was extracted with 2M KCl and the extract analyzed by steam distillation followed by titration with a standard H_2SO_4 solution. Each point is the mean of 4 analyses (2 replicates, 2 samples/replicate).

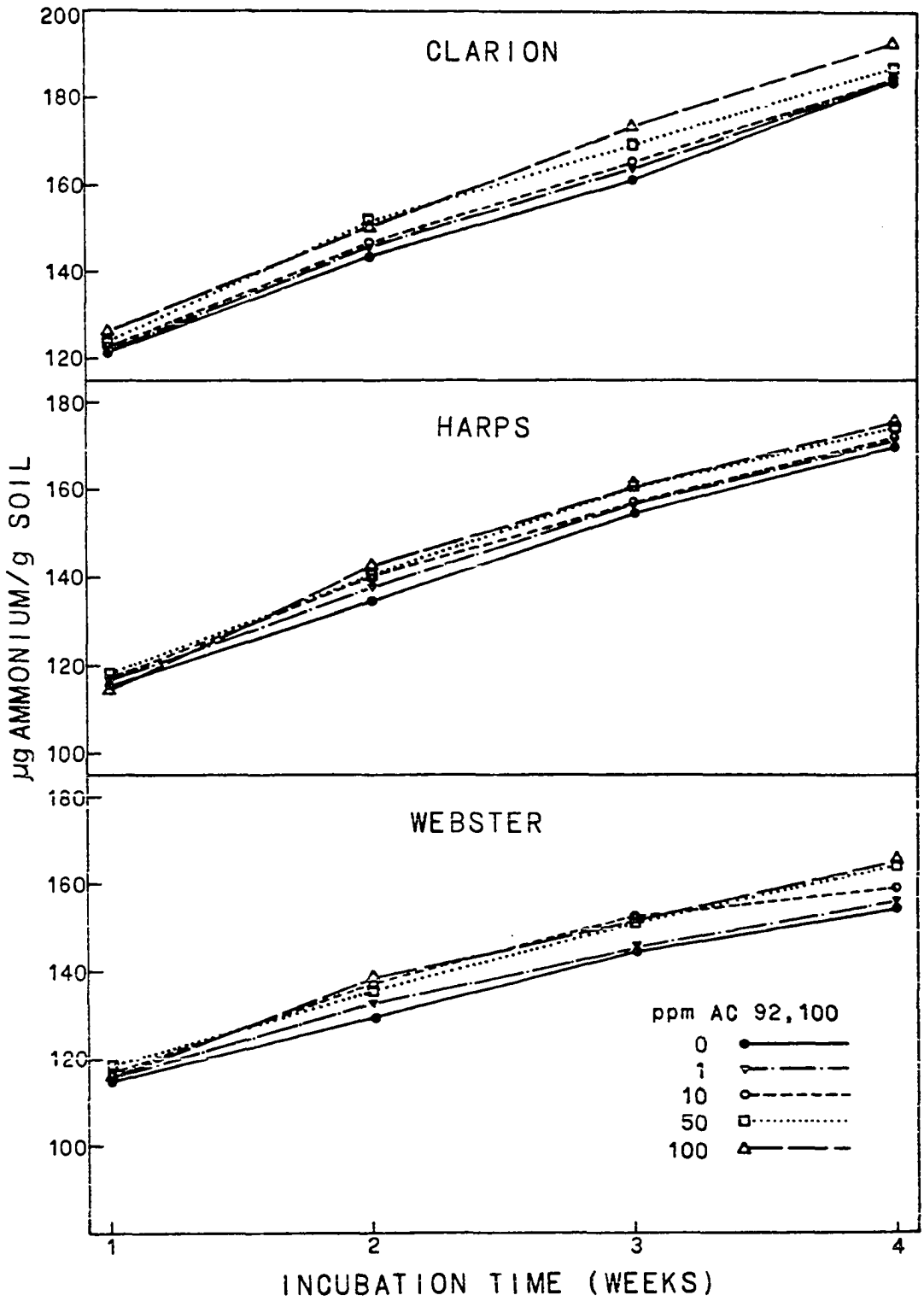
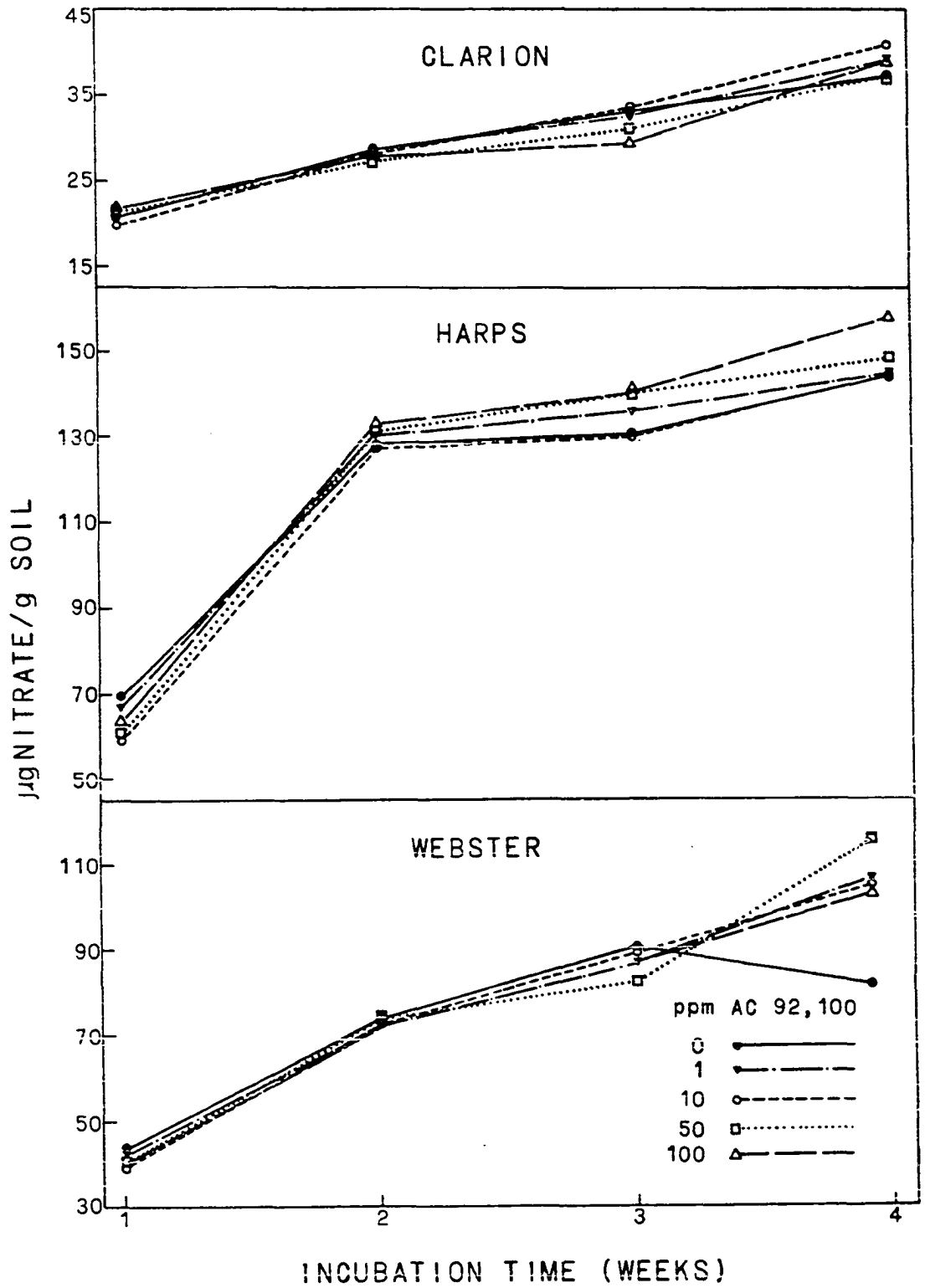

CLARION
HARPS
WEBSTER
200
180
160
140
120
100
µg AMMONIUM/g SOIL
ppm AC 92,100
0
1
10
50
100
1
2
3
4
INCUBATION TIME (WEEKS)

Figure 2. Effect of Counter on nitrification in 3 Iowa surface soils; 10 g of soil were mixed with 10 mg of ammonium sulfate and incubated at 22 C. Nitrate was extracted with 2M KCl and the extract analyzed by steam distillation followed by titration with a standard H_2SO_4 solution. Each point is the mean of 4 analyses (2 replicates, 2 samples/replicate).


CLARION
HARPS
WEBSTER
45
35
25
15
150
130
110
90
70
50
110
90
70
50
30
1
2
3
4
µgNITRATE/g SOIL
INCUBATION TIME (WEEKS)
ppm AC 92,100
0
1
10
50
100

(Fletcher and Bollen 1954, Jones 1956). Endrin, however, at 2000 ppm and higher concentrations inhibited ammonification (Jones 1956).

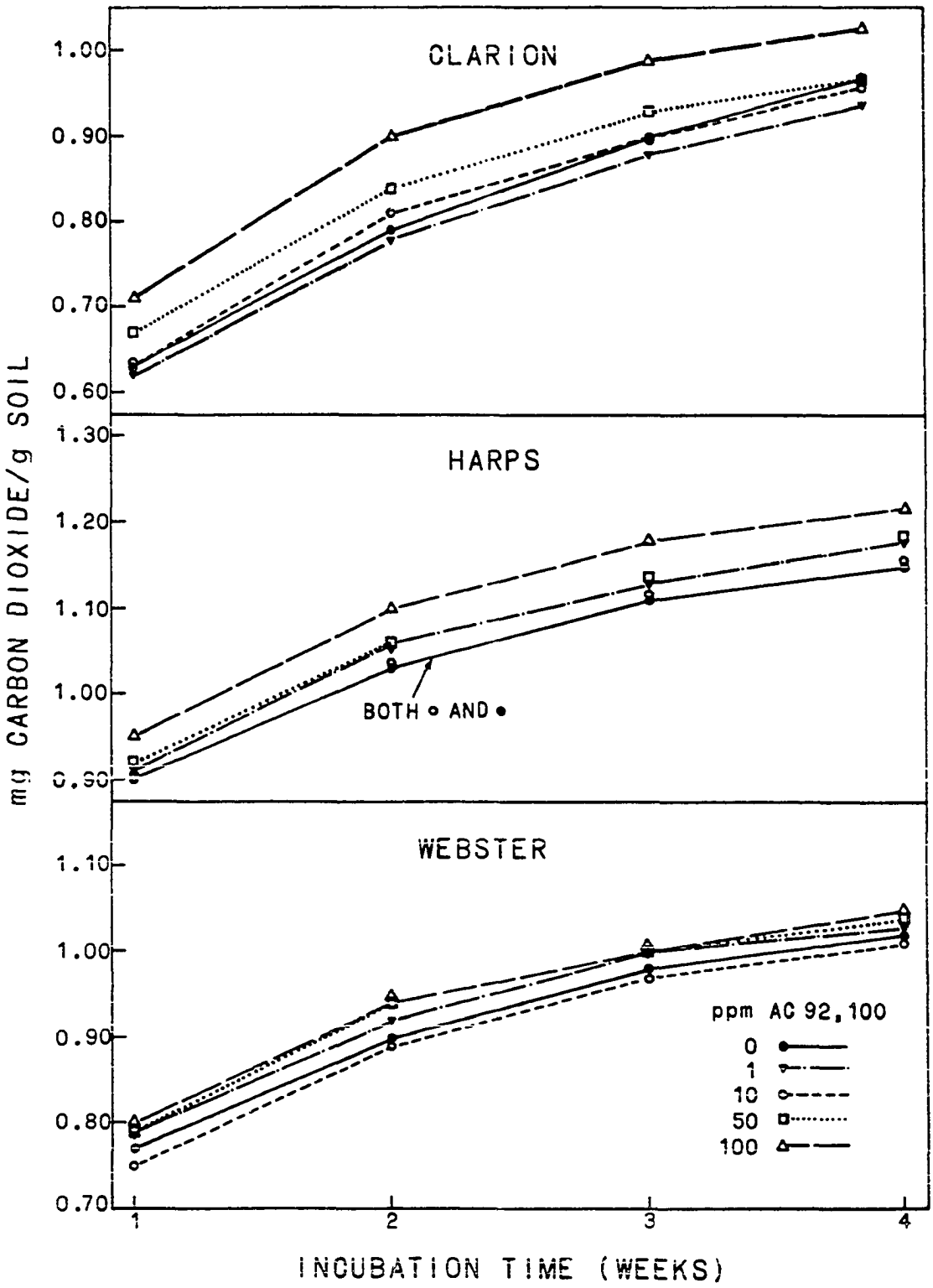
Nitrification

Of the 48 analytical mean values for soil samples receiving insecticide treatment, 1 was significantly decreased, 6 were significantly increased, and 41 were not significantly different from control values (Table 2, Fig. 2). Bartha et al. (1967) found that malathion, parathion, and phorate, each at 150 ppm, retarded nitrification in soil samples incubated to 18 days with these insecticides. Garretson and San Clemente (1968) reported that malathion at 1000 ppm delayed nitrification with liquid cultures of Nitrobacter agilis and parathion at 10 ppm gave complete inhibition. These studies were made in a 2-week period. Organochlorine insecticides exhibited no inhibitory effects on nitrification when tested at field rates (Ross 1952, Martin et al. 1959, Shaw and Robinson 1960). At 1000 ppm, aldrin, dieldrin, and chlordane were inhibitory (Brown 1954, Jones 1956). Although nitrifying microorganisms seem to be more sensitive initially to insecticides than ammonifiers, the nitrifiers usually recover in 3 to 4 weeks.

Carbon dioxide evolution

Of the 48 analytical mean values for soil samples receiving insecticide treatment, 1 was significantly decreased, 11 were significantly increased, and 36 were not significantly different from control values (Table 2, Fig. 3). Some carbon dioxide might have been produced by oxidation of the added Counter. At the 100 ppm level of Counter only

Figure 3. Effect of Counter on carbon dioxide evolution in 3 Iowa surface soils; 10 g of soil were incubated at 22° C. Carbon dioxide was collected with 2M KOH and the solution titrated with a standard HCl solution. Each point is the mean of 4 analyses (2 replicates, 2 samples/replicate).


CLARION
HARPS
WEBSTER
BOTH ○ AND ●
ppm AC 92,100
0
1
10
50
100
mg CARBON DIOXIDE/g SOIL
INCUBATION TIME (WEEKS)
1.00
0.90
0.80
0.70
0.60
1.30
1.20
1.10
1.00
0.90
1.10
1.00
0.90
0.80
0.70
1
2
3
4

0.0375 mg of carbon dioxide/g of soil would be produced if all the carbon in Counter were oxidized to carbon dioxide. If one assumes an average carbon dioxide value of 1 mg of carbon dioxide/g of soil, the carbon dioxide formed by addition of Counter would add 3.75% to the carbon dioxide recovered from the 0 ppm values. The carbon dioxide recovered from the 100 ppm level was as high as 113.9%. Correspondingly smaller amounts would be derived from 1, 10, and 50 ppm of Counter. The changes in carbon dioxide in Fig. 3 owing to insecticide treatments suggest that oxidation of Counter is probably a minor cause of any increases in carbon dioxide production.

Bartha et al. (1967) observed that 150 and 1500 ppm concentrations of parathion and phorate in Nixon sandy-loam caused an increase and then a decrease of respiration by soil microorganisms. These workers proposed that oxidation of these insecticides to more toxic compounds (e.g., desulfuration of parathion and phorate and oxidation of the thioether sulfur of phorate) caused this fluctuation in respiration. Counter is a homologue of phorate; although increased respiration was noted in all 3 soils treated with 100 ppm of Counter (Fig. 3), no decrease was observed as reported for phorate. This lack of a decline in respiration with Counter could be due to the low concentrations of Counter as compared with the higher concentrations of parathion and phorate used by Bartha et al. (1967). Different soils could also be a major factor. Tu (1970) reported an increased oxygen uptake by soil during the first 80 hr of incubation with Bayer 37,289, diazinon, chlorpyrifos, or Zinophos at concentrations of 10 and 100 ppm. Oxygen consumption was

proportional to the concentration of insecticide. Bollen et al. (1970) reported that Zectran, a carbamate insecticide, had no effect on respiration.

Organochlorine insecticides at field application rates had no effect on microbial respiration (Bollen et al. 1954, Fletcher and Bollen 1954, Eno and Everett 1958). Bartha et al. (1967) found slightly depressed respiration rates with a number of organochlorine insecticides at high concentrations.

Sulfur oxidation

Of the 60 analytical mean values for soil samples receiving insecticide treatment, 1 was significantly decreased, 5 were significantly increased, and 54 were not significantly different from control values (Table 2, Fig. 4). Tu (1970) reported little change in sulfur oxidation in the presence of 10 ppm of Bayer 37,289, diazinon, chlorpyrifos, or Zinophos after 4 weeks of incubation. Treatment of soil with 100 ppm of each of these insecticides caused no effect with Bayer 37,289, increased sulfur oxidation with diazinon, and decreased sulfur oxidation with chlorpyrifos and Zinophos. Jones (1956) found that 20 ppm of chlordane or benzene hexachloride had no effect on sulfur oxidation; at 200 ppm, both insecticides decreased sulfur oxidation.

Cellulose decomposition

Of the 72 analytical mean values for soil samples receiving insecticide treatment, 12 were significantly decreased, 10 were significantly increased, and 50 were not significantly different from control values

Figure 4. Effect of Counter on sulfur oxidation in 3 Iowa surface soils; 10 g of soil were mixed with 20 mg of sulfur and incubated at 21° C. Sulfate was extracted with 0.15% $CaCl_2$ and the extract analyzed turbidimetrically. Each point is the mean of 4 analyses (2 replicates, 2 samples/replicate).

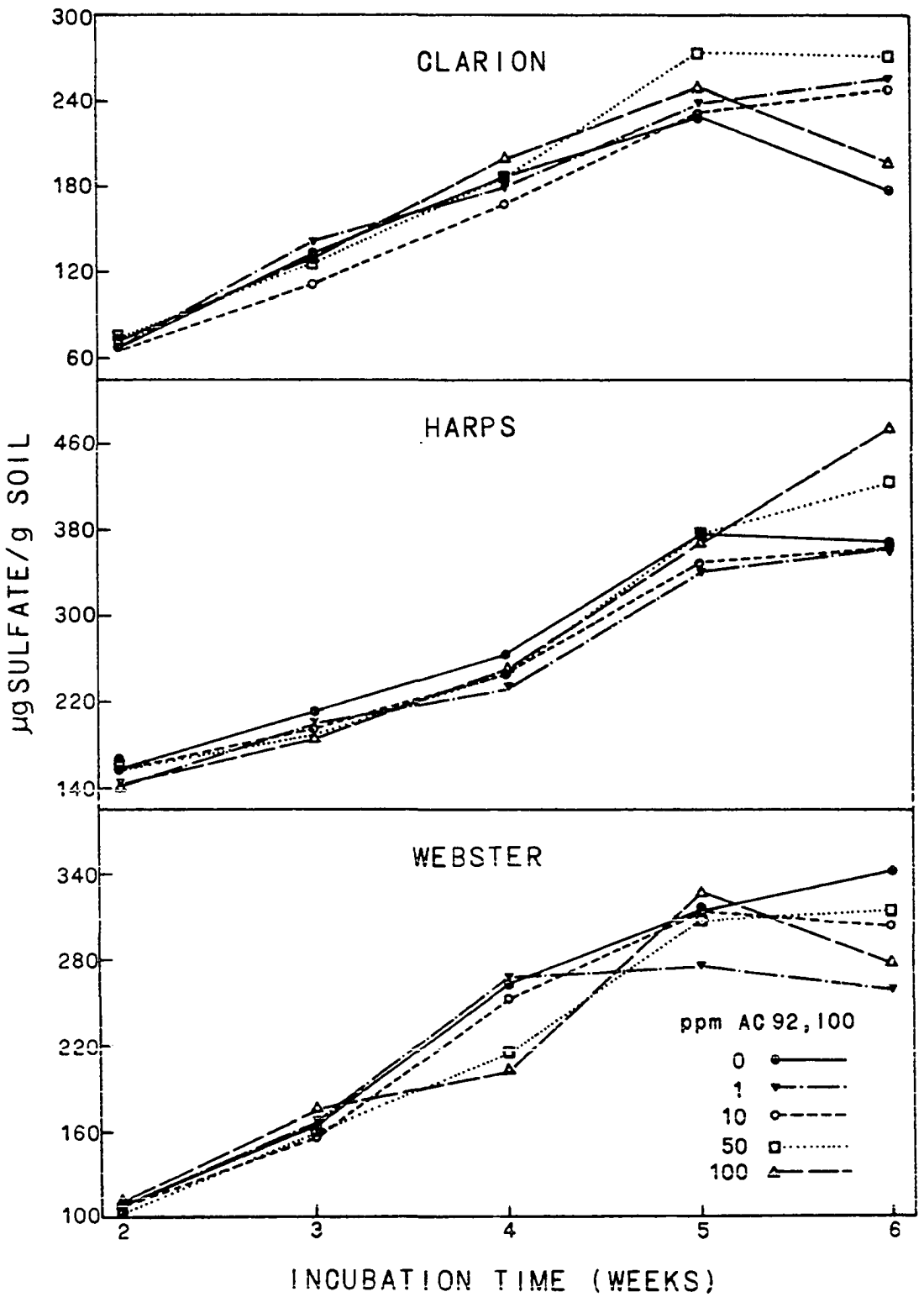
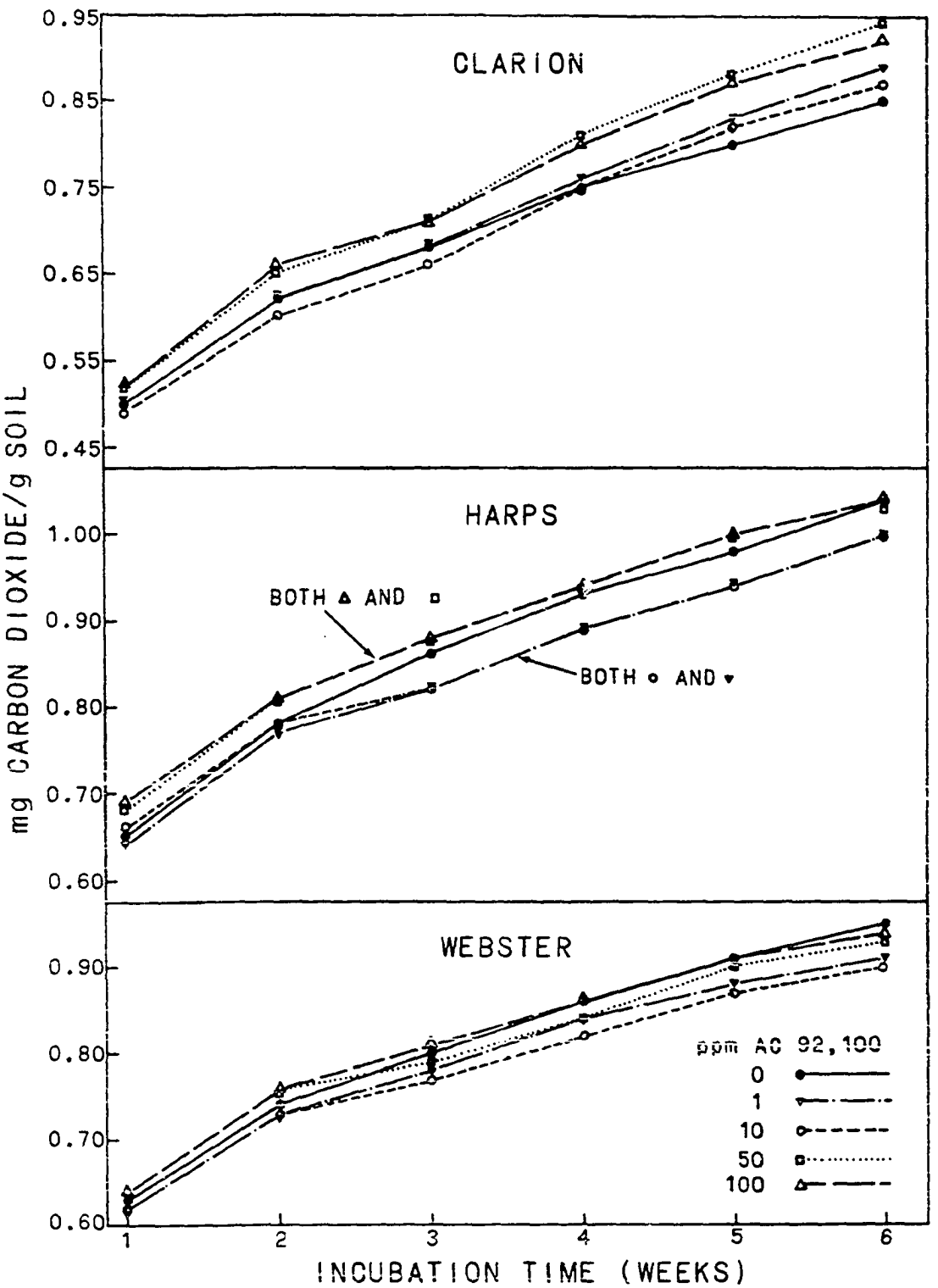

CLARION
HARPS
WEBSTER
300
240
180
120
60
460
380
300
220
140
340
280
220
160
100
2
3
4
5
6
µg SULFATE/g SOIL
INCUBATION TIME (WEEKS)
ppm AC 92,100
0
1
10
50
100

Figure 5. Effect of Counter on cellulose decomposition in 3 Iowa surface soils; 10 g of soil were mixed with 20 mg of cellulose powder and incubated at 22 C. Carbon dioxide was collected with 2M KOH and the solution titrated with a standard HCl solution. Each point is the mean of 4 analyses (2 replicates, 2 samples/replicate).


CLARION
HARPS
WEBSTER
BOTH ▲ AND □
BOTH ○ AND ▼
ppm AC 92,100
0
1
10
50
100
mg CARBON DIOXIDE/g SOIL
INCUBATION TIME (WEEKS)
0.95
0.85
0.75
0.65
0.55
0.45
1.00
0.90
0.80
0.70
0.60
1
2
3
4
5
6

(Table 2, Fig. 5). Audus (1970) stated "most of the insecticides have no effect on the numbers or activities of cellulolytic organisms in the soil until they inhibit at very high rates." Bollen et al. (1970) reported no effect on tree litter decomposition when Zectran was applied at normal field rates.

From the data presented it was concluded Counter has no inhibitory influence on soil microbial activities. Even at concentrations 50 and 100 times normal field rates (50 and 100 ppm) no inhibitory values were found (Table 2). In some cases these higher concentrations had a stimulatory influence. Of the 48 significantly stimulatory values, 41 or about 85% were in the 50 and 100 ppm concentrations. There have been reports of inhibition of microbial activities attributed to high concentrations of insecticides (Brown 1954, Jones 1956). These concentrations were so excessive, greater than 1000 ppm, that their practical value is rather obscure.

PART II: OXIDATION OF COUNTER IN THREE IOWA SURFACE SOILS

ABSTRACT

Laboratory tests were performed to determine the fate of Counter in 3 Iowa surface soils (Clarion, Harps, and Webster). The insecticide was applied to 10-g soil samples at a rate of 1 ppm, which is equivalent to a field application rate of 1 lb/acre. Soil samples were incubated at 22 $\pm$ 1°C for 0, 1, 3, 7, 14, and 21 days. Counter was rapidly oxidized to its sulfoxide. The parent compound had a half-life of 4 to 5 days. Counter sulfoxide reached a peak after 2 weeks of incubation, and Counter sulfone did not appear until 1 week after the incubation was started. Significant differences (P=0.01) were found in the ability of the 3 soils to degrade Counter. Counter sulfoxide and Counter sulfone had no inhibitory influence on soil microbial activities.

INTRODUCTION

Because of the prevalent use of soil insecticides and a concern over the persistence of insecticides in the environment, a study was undertaken of the fate of Counter in 3 Iowa soils. Counter is a new soil insecticide that is expected to be used extensively.

Although organophosphorus insecticides are not as persistent in the environment as are the organochlorines, a wide variability in the half-lives of organophosphorus insecticides in soil does exist. Menzie (1972) lists 9 organophosphorus insecticides with soil half-lives ranging from 2 days for phorate to 290 days for disulfoton. With the diminished use of organochlorine insecticides, which offered protection from insect pests for at least 1 growing season, farmers are depending more heavily on organophosphorus insecticides. The latter, in most instances, offer short-term protection. To have optimal protection against soil insects, both the insecticides and the insect pests must be thoroughly understood. It is, therefore, economically important, scientifically rewarding, and environmentally sound to understand exactly what becomes of insecticides in the soil.

MATERIALS AND METHODS

Three Iowa surface soils (Table 1) were obtained from the Iowa State University Agronomy and Agricultural Engineering Research Center located about 6 miles west of Ames, Iowa. The soil analyses reported in Table 1 are described in Part I under Materials and Methods.

The ^{14}C-Counter was 94% pure, with a specific activity of 7.7 millicuries/millimole, and supplied gratis by the American Cyanamid Company, Princeton, N.J. The ^{14}C-Counter was further purified by preparative-layer chromatography. Precoated E. Merck PLC silica gel (2.0 mm) 20 X 20 cm plates (without fluorescent indicator) were obtained from Brinkmann Instruments, Inc., Des Plaines, Ill. Five milligrams of ^{14}C-Counter were dissolved in acetone and applied to the plate. The solvent system used was methanol:chloroform:toluene (10:95:95 by volume) (American Cyanamid Company 1974). The ^{14}C-Counter was located by using autoradiography. The silica gel containing the ^{14}C-Counter was removed and placed in an 8-oz French square bottle. Fifty milliliters of acetone were added, and the bottle was shaken for 15 minutes on a Fisher-Kahn shaker. The acetone was transferred to a 150-ml Buchner funnel fitted with a medium fritted disk, lined with 2 sheets of Whatman #1 filter paper, and containing 1 in. of anhydrous sodium sulfate. A partial vacuum was used to aid the filtering process. The filtrate was evaporated to dryness by use of a Buchler portable flash-evaporator (Buchler Instruments, Fort Lee, N.J.). The resulting ^{14}C-Counter was 98% pure. This was determined by 3 dimensional thin-layer chromatography and liquid scintillation counting of the ^{14}C-Counter, which is described on

pages 45 through 48. The American Cyanamid Company had previously discovered an impurity in the ^{14}C-Counter. This impurity was a by-product that was produced when the labeled compound was synthesized and accounted for 11.5% by weight of the radioactive preparation. An adjustment was made for this impurity when the ^{14}C-Counter was weighed to make a 1-ppm solution.

The required amount of ^{14}C-Counter was weighed and transferred quantitatively with acetone to a 50-ml volumetric flask. Additional acetone was added to make 50 ml. A 1-ppm treatment was used, which corresponded to a field application rate of 1 lb of Counter/acre. This rate is based on 1 X 10^6 lb soil/acre in a soil volume of 3 in. in depth and 1 acre in area.

In all tests, 10 g of crushed, air-dried, soil were placed in 8-oz, French square bottles. One-half milliliter of the acetone-^{14}C-Counter solution was added evenly to the soil. The acetone was allowed to evaporate. Three milliliters of water were added, which brought the soil to about 50% water-holding capacity, and the bottles sealed with rubber stoppers; this kept moisture loss at a minimum. Air was introduced into the system 3 times a week.

There were 2 replicates, 2 samples per replicate, 3 soils, 1 treatment, and 6 incubation periods. Analysis of variance was performed on the data from samples analyzed for each incubation period. If significance was found, least significant difference values were calculated for mean differences between soils.

Extraction

The incubations extended over a 3-week period at 22 ± 1°C, and extractions were made at 0, 1, 3, 7, 14, and 21 days. Extractions were performed by first adding 5 ml of water to the 8-oz, French square bottles that contained the soil and the ^{14}C-Counter. Fifty milliliters of a hexane:acetone solution (2:1 v/v) were added, and the bottles were shaken for 15 minutes on a Fisher-Kahn shaker. The resulting mixtures were transferred to 250-ml centrifuge tubes. The French square bottles were rinsed once with 20 ml of hexane:acetone solution, and this rinsing solution was transferred to the centrifuge tubes. After a 5-minute centrifugation at 3500 rpm, 50 ml of the hexane:acetone layer were transferred to 150-ml Buchner funnels fitted with a medium fritted disk, lined with 2 sheets of Whatman #1 filter paper, and containing 1 in. of anhydrous sodium sulfate. A partial vacuum was used to aid the filtering process. One gram of Norit-A decolorizing carbon was added to the filtrates. After 5 minutes, the filtrates and carbon mixtures were filtered by using the Buchner funnel procedure just described. The second filtrates were evaporated to dryness with a Buchler portable flash-evaporator. The residues were redissolved in 1 ml of acetone.

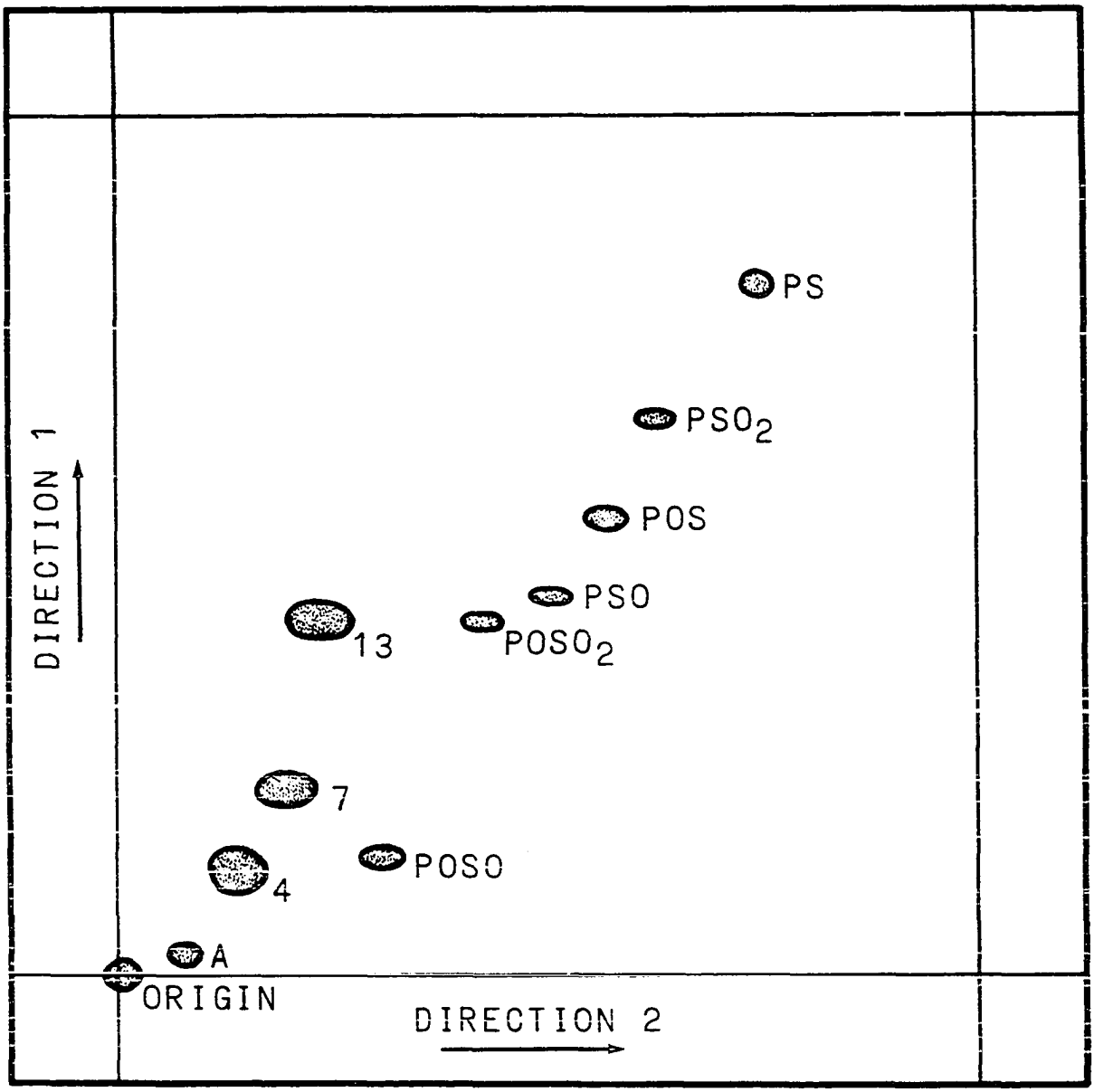
Liquid-scintillation counting and co-chromatography

Twenty microliters of the acetone solutions containing the dissolved residues were transferred to scintillation vials, and the acetone was evaporated with nitrogen. Fifteen milliliters of Aquasol® (New England Nuclear, Boston, Mass.) were added to the scintillation vials. The radioactivity was counted by a Model 3003 Packard Tri-Carb Liquid

Scintillation Spectrometer (Packard Instrument Co., Downers Grove, Ill.).

One-half milliliter of the acetone solution containing the dissolved residue was transferred to a 5-ml beaker. Counter, plus all the known metabolites of Counter, were added to the beaker. This included: 50 µl of Counter (PS); 50 µl of Counter sulfoxide (PSO), O,O-diethyl S-[(tert-butylsulfinyl)methyl] phosphorodithioate; 50 µl of Counter sulfone (PSO_2), O,O-diethyl S-[(tert-butylsulfonyl)methyl] phosphorodithioate; 50 µl of the oxygen analog of Counter (POS), O,O-diethyl S-[(tert-butylthio)methyl] phosphorothiolate; 100 µl of the oxygen analog of Counter sulfoxide (POSO), O,O-diethyl S-[(tert-butylsulfinyl)methyl] phosphorothiolate; 75 µl of the oxygen analog of Counter sulfone ($POSO_2$), O,O-diethyl S-[(tert-butylsulfonyl)methyl] phosphorothiolate; 50 µl of metabolite A, S-[(tert-butylsulfinyl)methyl] methyl sulfoxide; 50 µl of metabolite 4, S-[(tert-butylsulfonyl)methyl] methyl sulfoxide; 50 µl of metabolite 7, S-[(tert-butylsulfinyl)methyl] methyl sulfone; and 50 µl of metabolite 13, S-[(tert-butylsulfonyl)methyl] methyl sulfone. These compounds were supplied by the American Cyanamid Company. Metabolite designations A, 4, 7, and 13 are those assigned by American Cyanamid workers. The structural formulas of these compounds are given on page iv and v. The acetone was evaporated until the remaining quantity could be spotted easily on a precoated E. Merck TLC silica gel (0.25 mm) 20 X 20 cm plate. The chromatograms were run using toluene in direction 1, followed by methanol:chloroform:toluene (10:95:95) in direction 2, and nitromethane:acetonitrile:toluene (25:65:110) in the original

Figure 6. Thin-layer chromatogram of the organosoluble metabolites of Counter. The solvent system used was toluene in direction 1, followed by methanol:chloroform: toluene (10:95:95) in direction 2, and nitromethane: acetonitrile:toluene (25:65:110) in the original direction. The chromatogram was exposed to iodine vapors, and the resulting spots were circled.


PS
PSO2
POS
PSO
13
POSO2
7
POSO
4
A
ORIGIN
DIRECTION 1
DIRECTION 2

direction (Fig. 6). The chromatograms were exposed to iodine vapors, and the resulting spots circled. Autoradiograms were made by exposing Kodak Blue Brand medical X-ray film to the chromatograms for 10 days. The spots on the developed film were identified by placing the film on the chromatograms. The unkown spots on the film were matched with the known circled areas on the chromatograms. The circled areas on the chromatograms were removed and radioassayed by liquid-scintillation counting.

RESULTS AND DISCUSSION

Extraction

Total extraction values are shown in Table 3. In all 3 soils, the extractable portion of radioactivity decreased as incubation time increased. The increasing quantities of unextracted radioactivity with respect to time were caused by the radioactivity binding to the soil. It has been shown that the organic matter content was primarily responsible for the amount of mevinphos bound by various soils (Getzin and Chapman 1959). Getzin and Chapman (1960) also demonstrated with a sandy, a silt loam, and a muck soil that 14, 20, and 40%, respectively, of the phorate added were bound to the soils at 0 time. In this study, the organic matter of the soils used was not as varied as in the Getzin and Chapman (1960) studies. There was no significant difference (P=0.01) in the radioactivity extracted between soils at a specific incubation period.

Oxidation of Counter

Although all the circled areas on the chromatograms corresponding to Counter and its 9 metabolites were radioassayed, only the metabolites that made up at least 1% of the total extracted radioactivity are included in Table 4. POS, POSO, $POSO_2$, and metabolites A, 4, 7, and 13 never were present in amounts greater than 1%. The major metabolites of Counter in soil were the oxidative products, Counter sulfoxide (PSO) and Counter sulfone (PSO_2) (Table 4). It has been reported that phorate in the soil was rapidly oxidized to phorate sulfoxide and phorate

Table 3. Total extraction values in 3 Iowa surface soils at 6 incubation periods

Days after treatment	Per cent of radioactivity	
	Extracted	Not extracted
	Clarion	
0	81	19
1	59	41
3	55	45
7	45	55
14	38	62
21	36	64
	Harps	
0	80	20
1	73	27
3	68	32
7	51	49
14	36	64
21	31	69
	Webster	
0	80	20
1	68	32
3	65	35
7	38	62
14	38	62
21	28	72

Table 4. The fate of Counter applied to 3 Iowa surface soils at 6 incubation periods. Significant differences (P=0.01) between soils for Counter, Counter sulfoxide, and Counter sulfone at a specific incubation period are designated by the same letter appearing after each value. If no letter appears, there is no significant difference

Days after treatment	Per cent of extracted radioactivity				
	Counter	Counter sulfoxide	Counter sulfone	Total oxidation products	Contaminants
			Clarion		
0	77	16	0	16	5
1	65a	29a	0	29	5
3	47ab	49a	0	49	4
7	16a	74	4	78	5
14	6a	78	13	91	2
21	4a	60	34	94	1
			Harps		
0	73	19	0	19	6
1	79a	15a	0	15	4
3	73a	22a	1	23	3
7	46ab	44	1	45	7
14	18ab	74	4	78	2
21	9ab	80	8	88	2
			Webster		
0	72	21	0	21	5
1	72	20	0	20	6
3	62b	33	1	34	3
7	30b	61	2	63	5
14	9b	78	11	89	2
21	6b	69	23	92	1

sulfone (Getzin and Chapman 1960, Lichtenstein 1966, Suett 1971, Lichtenstein et al. 1973). Because Counter closely resembles phorate in structure, these results might have been expected. It was assumed that the oxidation of Counter, like phorate, was a 2-step reaction; rapid oxidation to Counter sulfoxide, and a much slower oxidation of Counter sulfoxide to Counter sulfone. For this reason, total oxidation products (the summation of Counter sulfoxide and Counter sulfone) also are listed in Table 4. At 14 and 21 days after treatment, the sulfoxide or the sulfone values alone could not account for the decrease in Counter. The decreasing Counter values are accounted for by the increasing values of the total oxidation products.

The most rapid degradation of Counter occurred in Clarion soil, followed by Webster, then Harps. There was a significant difference ($P = 0.01$) in the amount of Counter present between Clarion and Harps soils for all incubation periods except 0 day, and between Webster and Harps soils for all incubation periods except 0, 1, and 3 days. These differences in degradative abilities between soils were probably due to a combination of factors. It is known that insecticides persist longer in soils of high organic matter than in soils of low organic matter (Getzin and Chapman 1960, Lichtenstein 1966). Of the 3 soils used in this study, Clarion had the least amount of organic carbon, followed by Webster, then Harps (Table 1). The order of these 3 soils with respect to amount of organic carbon corresponds to the ability of the 3 soils to degrade Counter.

In one of the first studies on insecticide metabolism by isolated

soil microorganisms, Ahmed and Casida (1958) found that phorate was metabolized by a yeast, alga, and bacteria. Many investigators have found since that a multitude of soil microorganisms are capable of metabolizing a variety of insecticides (Matsumura and Boush 1971, Helling et al. 1971).

An increase in soil pH has been shown to enhance the breakdown of diazinon (Getzin 1968, Sethunathan and MacRae 1969). Of the soils used in this study, Clarion had the lowest pH, followed by Webster, then Harps (Table 1). The order of the 3 soils with respect to pH again corresponds to the order of the 3 soils with respect to degradation of Counter. The lower pH of the Clarion soil may have provided a more suitable environment for the microorganisms metabolizing Counter.

Effects on soil microorganisms

Laveglia and Dahm (1974) reported that Counter had no inhibitory influence on soil microbial activities. After 21 days, less than 10% of the extracted radioactivity was Counter (Table 4). It seems probable that Counter sulfoxide and Counter sulfone also have no effect on soil microbial activities, since after the second week of incubation, these compounds were prevalent in the soil. Only in 1 instance, cellulose decomposition in Harps soil, was there a possibility of an inhibitory effect due to the oxidative metabolites of Counter.

It is concluded that, under the conditions of these soil tests, Counter is rapidly oxidized to Counter sulfoxide. At the end of about 1 week, Counter sulfone appears and increases steadily over the next 2 weeks. By the end of 3 weeks, Counter is practically absent from the soil.

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