Chapter 8

Enhanced Degradation of S-Ethyl *N,N*-Dipropylcarbamothioate in Soil and by an Isolated Soil Microorganism

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Enhanced degradation of EPTC occurs in soil after repeat applications of EPTC. Studies were conducted to evaluate the mechanisms of enhanced degradation in soil and by an isolated soil microorganism (*Rhodococcus* sp.). Inoculation of a soil without a history of EPTC treatment with 1.0% (w/w) of a soil with enhanced EPTC degradation capabilities increased the rate of EPTC degradation in the previously untreated soil. Degradation of ^{14}C-labelled (1-propyl position) and unlabelled EPTC by the microbial isolate yielded *N*-depropyl EPTC (a product of α-propyl hydroxylation) and EPTC-sulfoxide (sulfur oxidation). It is proposed that initial reactions of soil microorganisms involve both hydroxylation and sulfoxidation, resulting in products that are further metabolized to CO_2. The hydroxylation reaction is thought to be dominant when degradation of EPTC occurs at enhanced rates.

Enhanced pesticide degradation refers to the phenomenon whereby a pesticide is degraded at an increased rate in soil previously treated with the pesticide, or a compound of similar structure, as compared to its rate of degradation in a comparable untreated soil. To date, more than 25 different agricultural pesticides have been reported to have the potential for enhanced degradation in soil including pesticides classified as herbicides, insecticides, and fungicides (Racke, this volume). The earliest report of enhanced degradation of a carbamothioate pesticide was that by Rahman et al. (1) in New Zealand where the herbicide EPTC (*s*-ethyl *N,N*-dipropyl carbamothioate) failed to give adequate weed control in fields after repeat applications. Since that initial report, one of the most

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widely studied enhanced degradation systems has been that of EPTC in soil.

Enhanced EPTC degradation in soil is supposedly caused by soil microorganisms which appear to acquire additional degradative capabilities (2-4). Although microbial involvement in enhanced pesticide degradation has been recognized, the mechanisms responsible for this phenomenon remain unresolved. Two theories have been proposed to explain microbial involvement in enhanced degradation (5). The chance mutation theory postulates the presence of mutant microorganisms which become dominant with the addition of a substrate in absence of competition. The lag period normally observed before degradation begins is the time required for the mutant population to build to an appreciable level. The adaptive enzyme theory postulates adaptive enzymes, induced by pesticide additions, are produced by microorganisms. The lag phase is the period required to fully develop the adaptive enzyme potential.

While many soils contain the necessary ingredients (i.e. a susceptible pesticide, an adaptable microorganism, and a suitable environmental condition) (6), enhanced degradation is not always observed. This observation has been difficult to explain, although one possible reason is the inability to obtain similar but untreated (control) soils to establish baseline degradation rates. Equally puzzling is how to account for the persistence of EPTC degradative ability in a soil that no longer contains the pesticide to serve as substrate for the microorganisms. Possibly the enzymes induced by EPTC may also act on other substrates thus maintaining the microorganisms' ability to rapidly exploit EPTC as a substrate (7). Transfer of degradative genes on plasmids among microorganisms is another mechanism whereby the survival of the EPTC degradative system may continue from one year to another (8). A microorganism, isolated from soil that had been repeatedly exposed to EPTC, contained four plasmids, one of which mediated the degradation of EPTC (9). Extracellular enzymes may also result in the maintenance of enhanced pesticide degradation in soil. Many extracellular enzymes are active against functional groups commonly contained within soil pesticide molecules. Some extracellular enzymes maintain their activity in soil for long periods, yielding a "steady-state" soil enzyme component unrelated to current microbial proliferation.

To sort out the relative importance of these, or additional hypotheses for explanation of enhanced EPTC degradation in soil, it is important that the microorganisms involved be identified and that the biochemical pathway(s) by which they degrade EPTC be determined. Although several different pathways have been proposed for carbamothioate degradation in soil, current information is based on studies conducted in animal and plant systems (10-12). Carbamothioate degradation in mammals involves conversion to a sulfoxide which further undergoes cleavage at the carbamoyl bond (13). In plants, EPTC was hypothesized to undergo hydroxylation at the carbon alpha to the sulfur, followed by carbamate cleavage (14). Plant studies have also determined that EPTC degradation involves hydrolysis at the ester linkage to form mercaptan, amine and CO_2 (15).

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Lack of information concerning the microorganisms and the mechanisms involved in enhanced EPTC degradation has seriously limited attempts to control the rapid breakdown of this herbicide, and other carbamothioate pesticides, in soil. This study was initiated to 1) evaluate enhanced EPTC degradation in field and laboratory soils, 2) isolate soil microorganism(s) active in degrading EPTC and 3) determine the biochemical pathway(s) of EPTC degradation by the isolated microbes.

Materials and Methods

Soils. Characteristics of the three surface (0 to 15 cm sample depth) soils used in this study are given in Table I. The Brookston soil was collected from a field located near Canal Winchester, Ohio that had previously been treated with Eradicane for 1, 2, 3, and 4 consecutive years or had remained untreated. The Plano and Dothan soils were collected from sites in Wisconsin and South Carolina, respectively, which had histories of EPTC and butylate (*s*-ethyl-*N*,*N*-diisobutyl carbamothioate) use.

Table I. Soil Characteristics

Soil			Organic
Series (texture)	Subgroup	pH	carbon (%)
Brookston (clay loam)	Typic Argiaquoll	7.5	4.5
Plano (silt loam)	Typic Argiudoll	5.9	3.8
Dothan (sandy loam)	Plinthic Paleudult	5.5	3.0

Chemicals. Technical EPTC and butylate and ^{14}C-1-propyl EPTC (specific activity, 35 mCi/mole) were supplied by the Stauffer Chemical Company. The radiopurity of the ^{14}C-EPTC was greater than 97% as determined by thin layer chromatography (TLC). Millimole quantities of unlabelled and ^{14}C-labelled EPTC-sulfoxide and EPTC-sulfone were synthesized by the method of Casida et al. (16). Purification was accomplished by TLC with ultraviolet radiation (UV) detection.

Analytical Procedures. Gas chromatographic (GC) analyses of EPTC and metabolites were performed using glass columns containing 5% carbowax 20M on Chrom WHP and 3% OV-17 on Supelcoport (100/200 mesh). A Varian 3700 gas chromatograph with a nitrogen specific thermoionic detector was used.

Gas chromatographic/mass spectrometry (GC/MS) results were obtained at the Ohio State University Chemical Instrumentation Center using a Finnigan 4021 GC/MS instrument. Both electron impact and chemical ionization were performed on samples following separation of compounds by a gas chromatograph equipped with capillary columns containing 3% OV-17 or 5% carbowax 20M.

Thin-layer chromatography (TLC) for separation of EPTC and metabolites was accomplished using silica gel 60 F_{254} chromatoplates developed with hexane-acetone (6:1), hexane-ether (4:1) or hexane-ethyl acetate (3:2). Detection of unlabelled and or

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radiolabelled pesticide and metabolites was by UV adsorption and autoradiography, respectively. Quantitation of ^{14}C-activity was accomplished by liquid scintillation counting (LSC) of scraped sections of gel.

Non-protein thiol content in the culture media, after precipitation of protein using 0.2M trichloroacetic acid, was assayed using Ellman's reagent (17), with 2-mercaptomethanol used as a standard.

Microbial Isolate. A microbial isolate capable of growing on EPTC as a sole carbon and energy source was isolated from Jimtown loam soil (Typic Argiaquoll; organic carbon, 4.5%; pH, 7.5) by an enrichment batch culture technique. One gram field-moist soil was added to 50 ml basal salt medium (BSM) (18) supplemented with 100 or 200 mg L^{-1} EPTC (BSME). At 10-day intervals, 1 ml of inoculum was transferred into a flask containing fresh media. The flasks were plugged with cotton and incubated at 27°C on a rotary action shaker at 250 rpm. After four transfers, 0.1 ml dilutions were plated out on nutrient agar (NA) plates. Individual isolates were purified and screened for their ability to grow on basal salt agar plates with EPTC as the carbon source (BSAE). Isolates went through three transfers on BSAE plates and were then tested for their ability to grow in BSME. Isolate JE1 was retained for all subsequent studies based on its ability to rapidly degrade EPTC and to utilize EPTC as a sole carbon source. JE1 has been tentatively identified as *Rhodococcus* sp. on the basis of its morphology and cell wall composition.

Metabolic Studies. To measure EPTC and butylate degradation in soil, aliquots of air-dried Brookston soil were treated with 4 mg kg^{-1} of EPTC or butylate and incubated at 25°C in the dark. At various intervals, samples were removed from the incubator and EPTC or butylate remaining in the soil was extracted with 10:3 toluene:water. The toluene layer was analyzed for EPTC or butylate by GC. The minimum detectable level of EPTC or butylate was 0.05 mg kg^{-1} soil and the average recovery exceeded 90%.

The minimum inoculum level of an EPTC history soil which results in enhanced degradation was evaluated by adding 0.1, 1.0, 10 and 100% (w/w) of a 3-yr history Brookston soil to a non-history Brookston soil. EPTC was added at a rate of 4 mg kg^{-1} soil and the amount remaining after various time intervals was measured as previously described.

The effect of various antibiotics on enhanced EPTC degradation was investigated by treating aliquots of the 3-yr history Brookston soil with kanamycin, streptomycin, chloramphenicol, or cycloheximide at a rate of 100 mg kg^{-1} soil. The samples were then treated with EPTC (10 mg kg^{-1} soil) and the remaining EPTC was extracted and measured after 0, 3, and 7 days as previously described.

Rhodanese assays were conducted on EPTC and butylate history soils as described by Tabatabai and Singh (19).

The experimental design for all experiments conducted on the soils was a randomized block design with three replicates. Separate analyses of variance were performed at each sampling time. Mean separations were determined using the Least Significant Difference

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(LSD) test only when the F-test was significant at the 0.05 level of significance.

Microbial isolate JE1 in BSME (100 mg EPTC L^{-1}) was grown on a rotary shaker at 27°C to midlog phase (2-3 days; $O.D._{600}$, 0.06-0.08). An aliquot of the culture was treated with EPTC (50 mg L^{-1}), and EPTC remaining in solution was measured at 2 h intervals. An aliquot containing a similar amount of EPTC but no microbiol cells was used as a control. A midlog culture was also treated with ^{14}C-1-propyl EPTC (100 mg L^{-1}) and, at 8 h intervals, the headspace above the culture was swept into NaOH and the ^{14}C-activity was measured by LSC.

For identification of metabolites formed during EPTC degradation, the same procedure was used with the exception being the final EPTC concentration was adjusted to 100 mg L^{-1} after the culture had reached the midlog phase. At 8 h intervals, for 32 h, samples were asceptically removed and centrifuged. The supernatants were extracted with toluene, and then analyzed for undegraded EPTC remaining by GC and TLC. The remainder of the toluene extract was concentrated to approximately 0.5 ml under nitrogen and analyzed for EPTC and metabolites using GC or TLC and liquid scintillation counting. The aqueous fraction was lyophilized, dissolved in methanol, and analyzed by GC, TLC (hexane-ether, 9:1 or hexane-acetone, 6:1) and LSC.

Results

Metabolic Studies in Soil. Enhanced degradation of EPTC in a Brookston soil was evident after only a single field application of EPTC (Table II). The rate of EPTC degradation in soil after one year of EPTC use was not significantly different ($P<0.05$) from rates observed in history soils treated for 2, 3, or 4 consecutive years. Butylate, a carbamothioate with structure similar to EPTC, was degraded more rapidly in a 4-year history Brookston soil than in the same soil with no prior history of EPTC use (Figure 1). The degradation of butylate, however, was slower than that of EPTC applied at the same rate.

Table II. Degradation of 4 mg EPTC kg^{-1} in Brookston soil treated with Eradicane for 1, 2, 3 or 4 consecutive years

Previous Eradicane use	Days after application				
	0	3	7	10	14
Years	- - -EPTC remaining, mg kg^{-1} soil- - -				
0	3.86	3.63	2.76	2.41	1.31
1	3.87	1.82	0.64	0.44	0.34
2	3.84	1.73	0.78	0.33	0.30
3	3.82	1.87	0.55	0.27	0.22
4	3.86	1.78	0.74	0.36	0.29
LSD(0.05)†	NS	0.49	0.44	0.12	0.45

† LSD = least significant difference values calculated when the F-test was significant at $P \leq 0.05$.

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An experiment was conducted to determine the minimum amount of inoculum of an enhanced soil required to bring about the development of enhanced degradation in a non-history soil. At all sampling times, an inoculum level of 1.0% or higher resulted in significantly ($P<0.05$) higher degradation rates than the uninoculated non-history soil (Figure 2).

To examine the class of microorganism responsible for enhanced EPTC degradation in the Brookston soil, various antibiotics were applied at a rate of 100 mg kg^{-1} soil. Chloramphenicol, an antibacterial compound, greatly inhibited EPTC degradation. The amount of EPTC remaining after 7 days was greater with this treatment than in a soil not previously exposed to EPTC (Table III). Cycloheximide, streptomycin, and kanamycin had little effect in arresting the enhanced degradation of EPTC in the Brookston soil.

Table III. Effect of various antibiotics on degradation of EPTC (10 mg kg^{-1} soil) in a 3-year Eradicane history Brookston soil

Treatment	Days after application 0	3	7
	EPTC remaining, mg kg^{-1} soil		
None (non-history soil)	9.47	8.56	7.47
None (3-year history soil)	9.44	5.86	1.80
Cycloheximide	9.88	5.24	1.56
Streptomycin	9.46	4.32	1.75
Kanamycin	9.32	4.93	1.86
Chloramphenicol	9.79	8.99	8.75
LSD (0.05)†	NS	0.79	0.86

† LSD = least significant difference values calculated when the F-test was significant at $P \leq 0.05$.

The correlation between rhodanese activity and rates of EPTC degradation in enhanced and non-history soils was investigated because of observations by Reed et al. (20) that bacterial isolates capable of growing on EPTC and butylate also exhibited high rhodanese activity. Mixed results were obtained with two out of the four EPTC history samples having significantly ($P<0.05$) higher rhodanese activities than the non-history soil (Table IV). Of the three soils tested, none had significantly higher rhodanese activity in the butylate history soils as compared to the non-history soil. In fact, with the Dothan A and B soil samples, significantly lower rhodanese activity levels were observed in the butylate history soils.

Metabolic Studies with Microbial Isolate JE1. EPTC was found to be efficiently metabolized by JE1 (Figure 3). Growth of JE1 was associated with the degradation of EPTC over an 8 h period. In contrast, EPTC levels remained constant over the same 8 hour period in the uninoculated control. Degradation of ^{14}C-labelled EPTC and appearance of metabolites into an aqueous or organic soluble fraction was also measured. An initial rise in ^{14}C-activity in the

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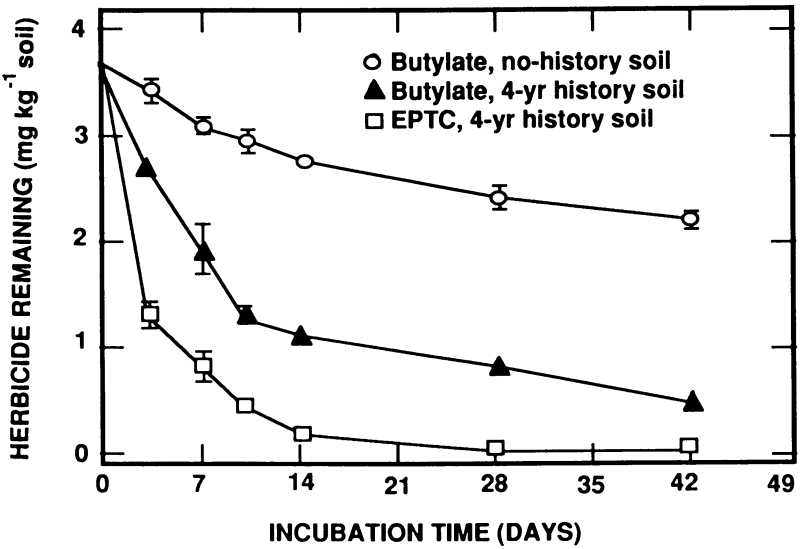

Figure 1. Degradation of butylate (4 mg kg^{-1}) and EPTC (4 mg kg^{-1}) in a Brookston soil treated with Eradicane for four consecutive years. Bars denote standard deviations.

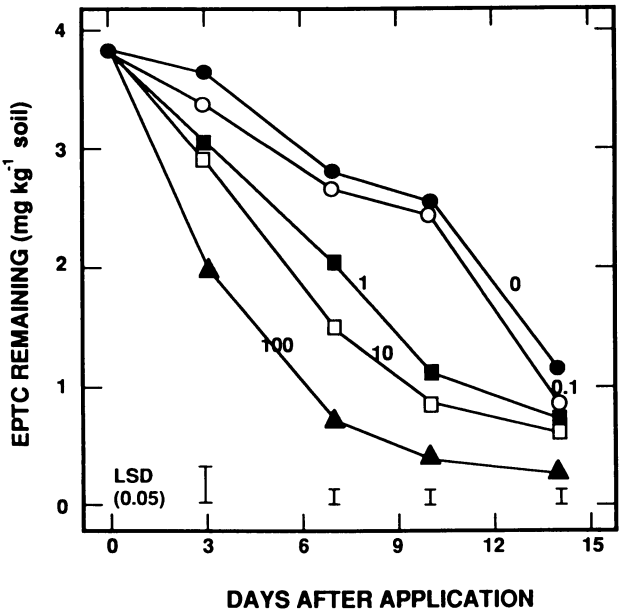

Figure 2. Degradation of EPTC (4 mg kg^{-1}) in a non-history Brookston soil inoculated with various percentages (w/w) of the same soil which had been treated with Eradicane for three consecutive years.

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Table IV. Rhodanese activity of soils with enhanced degradation rates of EPTC and Butylate

Soil history	Rhodanese activity in soil specified, nmoles g-1 h^{-1}			
	Plano	Dothan A	Dothan B	Brookston
Non-history	564	330	730	400
EPTC	688	397	940	355
Butylate	682	292	520	-
LSD (0.05)†	140	8	207	10

† LSD = least significant difference values calculated when the F-test was significant at $P \leq 0.05$.

aqueous fraction was observed followed by a decline to initial levels (Figure 4). The decrease in ^{14}C-activity of the organic fraction was coupled with rapid evolution of $^{14}CO_2$.

Examination of the organic fraction during EPTC degradation using TLC and autoradiography revealed four metabolites (Metabolites 1, 2, 3 and 5; Figure 5) distinct from the parent molecule. Three of the four metabolites did not accumulate appreciably as incubation proceeded suggesting they were rapidly converted to other compounds by JE1. Metabolite 5, however, gradually accumulated with time as the concentration of EPTC declined. After 24 h, 24% of the initial ^{14}C-activity in EPTC was present as Metabolite 5.

Metabolite 1 (Figure 5) was tentatively identified as EPTC-sulfoxide, based on similar chromatographic characteristics with standards. Additional confirmation using GC/MS was not possible because this compound is thermally unstable. Metabolites 2 and 3, which were less polar than EPTC sulfoxide (Metabolite 1), had chromatographic characteristics similar to the hydroxylated EPTC at the β- and α-propyl position (14) although further attempts to confirm this were not made.

Metabolite 5 was the least polar of the metabolites contained in the organic fraction of JE1 cultures actively degrading EPTC. It was tentatively identified as *N*-depropyl EPTC on the basis of its chromatographic properties on thin-layer plates (Figure 5). When GC analysis (3% OV-17 column) was applied for characterization of the organic fraction, Peak #1 was observed to substantially increase in area with incubation time (Figure 6). GC/MS analysis of the organic fraction using a 3% OV-17 capillary column confirmed the identity of this compound as being N-depropyl EPTC. Three other metabolites in the organic fraction were identified by GC/MS when a 5% carbowax 20M column was used. These compounds were propionaldehyde, *s*-methyl formic acid and *s*-ethyl formic acid.

Additional GC analyses of the aqueous fraction of JE1 cultures actively degrading EPTC indicated the presence of traces of propylamine, but no dipropylamine. Also, a strong mercaptan-like odor developed in JE1 cultures actively degrading EPTC suggesting the formation of a mercaptan. Non-protein thiol content (17) was found to accumulate in the media as the incubation progressed.

Evidence that EPTC may be hydroxylated at the a-carbon of the ethyl group to yield α-hydroxy-ethyl EPTC was also observed. However, α-hydroxy-ethyl EPTC is unstable and yields carbonyl

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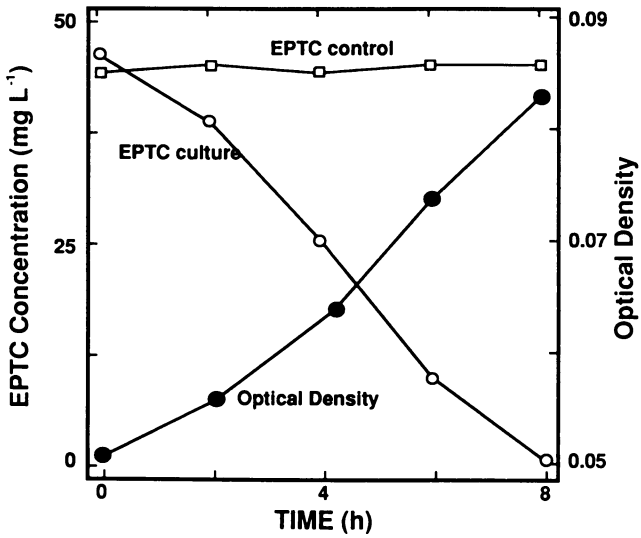

Figure 3. Degradation of EPTC (50 mg L^{-1}) and growth of microbial isolate JE1 in minimal salt media.

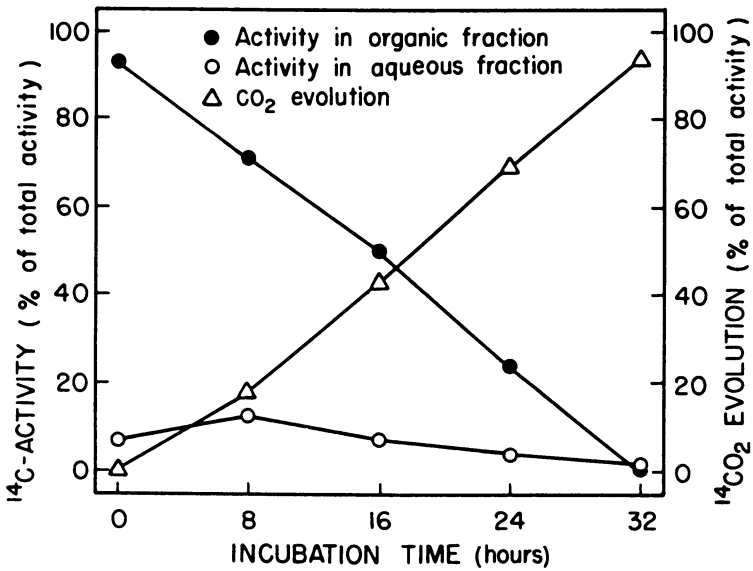

Figure 4. Fractionation of ^{14}C-label during metabolism of ^{14}C-1-propyl EPTC (100 mg L^{-1}) by microbial isolate JE1 in minimal salt media.

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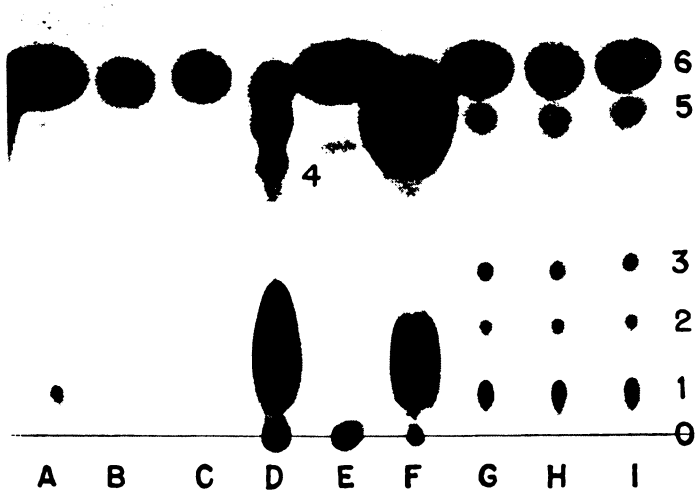

Figure 5. Autoradiogram of metabolic products of EPTC produced by microbial isolate JE1. Thin-layer chromatograph was developed with hexane/ethyl acetate (3:2). A, 8 h incubation; B, 0 h incubation; C, sterilized control; D, E, F, m-chloroperoxy benzoic acid + EPTC; G, H, I, 24 h incubation. Metabolites: 1, EPTC-sulfoxide; 2, 3, unknowns; 4, EPTC-sulfone; 5, *N*-depropyl EPTC; 6, EPTC.

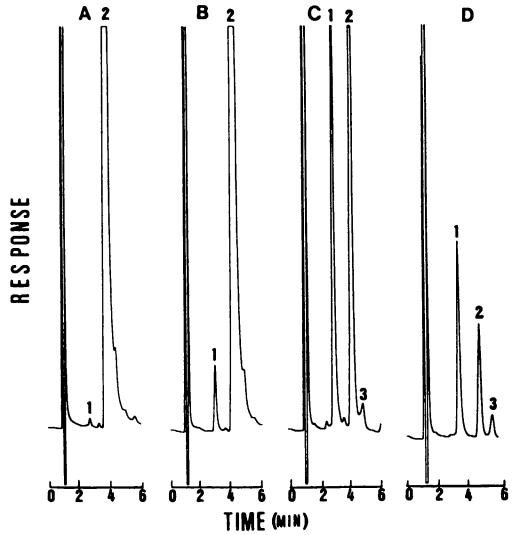

Figure 6. Gas chromatograms of JE1 metabolic products after A, 0 h; B, 8 h; C, 16 h; D, 24 h incubations. Separation on 3% OV-17 glass colums. Peaks: 1, *N*-depropyl EPTC; 2, EPTC; 3, unknown.

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sulfide (COS) and acetaldehyde when it decomposes. The presence of this unstable intermediate was, therefore, implicated by detection of acetaldehyde (16). The extent of this hydrolysis during EPTC degradation as compared to the other reactions taking place could not be assessed, however, because of a lack of EPTC labelled in the appropriate position.

Discussion

Metabolic Studies in Soil. Enhanced EPTC degradation in the Brookston soil after only a single field application (Table II) is consistent with observations made by Obrigawitch et al. (21) and Schuman and Harvey (22). Cross enhancement between EPTC and butylate also occurred in the Brookston soil (Figure 1). However, butylate degradation was slower than that of EPTC. Butylate has been found to give acceptable weed control in EPTC history fields where EPTC has failed (21, 23-24). One possible explanation may be that butylate degrades to form a stable metabolite that has herbicidal activity and can thus provide control even when the parent molecule has disappeared (12-13).

The inoculation experiment results (Figure 2) indicate that only small amounts of soil with enhanced degradation capabilities for a specific pesticide have the potential to induce enhanced degradation in non-history soils. Similar results were reported by Engvild and Jensen (25) and more recently by Yarden et al. (26). Yarden et al. (26) demonstrated that 2% of a soil with enhanced degradation of benzimidazol-2-yl carbamate (MBC) was sufficient to cause a rapid increase in the rate of degradation of this same compound in a non-history soil. Development of enhanced degradative capabilities in non-history soils through inoculation with enhanced soils may be due to a direct amplification of the pesticide degrading population. Thus, a previous application of a pesticide may not be the only means of causing a development of enhanced degradation. A possible consequence is that any movement of an enhanced soil by wind or water may lead to misleading conclusions when comparing degradation in a field repeatedly treated with a pesticide against a supposedly non-enhanced edge or fence row soil sample.

Microorganisms active in degrading EPTC in the Brookston soil were very sensitive to chloramphenicol (Table III). Chloramphenicol exhibits broad spectrum activity against both gram positive and gram negative bacteria. Cycloheximide, an effective inhibitor of fungi, did not affect the rate of EPTC degradation. Kanamycin and streptomycin, which are more effective against gram positive than gram negative bacteria, also did not alter the rate of EPTC degradation. This may lead to the conclusion that the active degraders in the Brookston soil belong to the gram negative class of bacteria. These results are inconsistent with the types of microorganisms we have isolated from soil and which are capable of growing on EPTC as the sole carbon/energy source. In almost all cases, gram positive microorganisms were obtained, and attempts by Tam et al. (9) to isolate an EPTC-degrading microorganism also yielded a gram positive bacterium. Further research to characterize

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the microorganisms responsible for enhanced EPTC degradation in soils is obviously needed.

Rhodanese assays were conducted on EPTC and butylate-history soils and non-history soils (Table IV). The methodology to measure rhodanese activity in soil is thoroughly documented and, if correlated with the rate of EPTC or butylate in soil, would provide a rapid means of identifying soils with enhanced degradative capabilities. However, the results were inconclusive and limit the use of the rhodanese assay for such a purpose. The difficulty in developing a simple enzyme assay for identification of soils with enhanced EPTC degradation, as opposed to several of the organophosphate herbicides, is due to the lack of information on the mechanisms involved in EPTC degradation.

Metabolic Studies with Microbial Isolate JE1. EPTC is rapidly metabolized by microbial isolate JE1 which utilizes it as its sole carbon/energy source (Figure 3). The degradation products were identified either by TLC or GC/MS analysis. Hydroxylation and sulfoxidation were found to be important reactions leading to the mineralization of EPTC by soil microorganisms. The rapid evolution of $^{14}CO_2$ from ^{14}C-1-propyl EPTC suggests that mineralization of this portion of the herbicide molecule is very rapid.

The relative amounts of the *N*-depropyl EPTC both early in the incubation period and later suggest that hydroxylation of the α-propyl carbon of the *N,N*-dialkyl moiety is a major route in the microbial metabolism of EPTC. Hydroxylation of the other carbons of the *N,N*-dialkyl portion of the carbamate was found to be a less preferred route compared to the hydroxylation of the α-propyl carbon. Sulfoxidation of the carbamate may be second in the importance to the hydroxylation reactions observed.

On the basis of the metabolic products identified and their relative amounts formed during incubation, a degradative pathway involving hydroxylation and sulfoxidation mechanisms is proposed (Figure 7). EPTC is first hydroxylated at the α-propyl carbon to form α-hydroxy-propyl EPTC. This compound is unstable and breaks down to form *N*-depropyl EPTC and propionaldehyde. The *N*-depropyl EPTC is further metabolized to *s*-ethyl formic acid and propylamine. The *s*-ethylcarboxylic acid is then demethylated to *s*-methyl formic acid. This product is hypothesized to degrade to form CO_2 and methyl mercaptan.

Although no conclusive identifications were made, the chromatographic characteristics of the other minor metabolites found in the organic fraction suggest that hydroxylations of carbons other than the α-propyl carbon may occur during degradation of EPTC.

The availability of different pathways for the metabolism of EPTC by these microbes may account for the very efficient degradation of this carbamothioate by soil microorganisms. It may also explain why dietholate slows down degradation of EPTC in enhanced soils without having any effect on the normal EPTC degradation (27-29). A possible explanation for enhanced EPTC degradation may be that in soil, where EPTC has been used repeatedly, all available pathways become operative. The addition of dietholate, which has been theorized to inhibit the hydroxylation pathway (30-31), may lessen the importance of this pathway,

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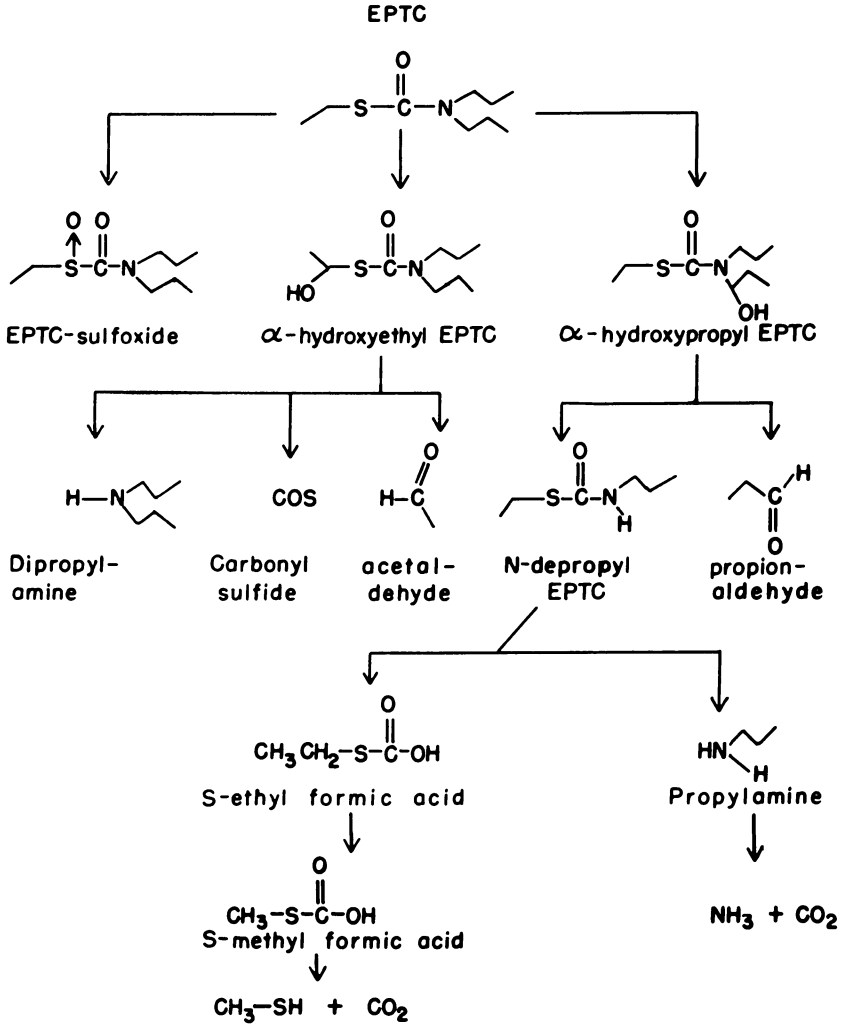

Figure 7. Proposed pathways for the metabolism of EPTC by microbial isolate JE1.

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resulting in a decrease in the rate of degradation of EPTC in the enhanced soil.

Conclusions

Development of enhanced degradation of EPTC in a Brookston soil was rapid, being evident after only a single year treatment. The application of the soil rhodanese assay to identify soils with enhanced EPTC capabilities was not successful. Studies with Brookston soil also indicated that addition of only a small amount of an enhanced soil (0.1%) to a non-enhanced soil was capable of increasing EPTC degradation rates in the non-enhanced soil.

A microorganism (tentatively identified as *Rhodococcus* sp.) capable of utilizing EPTC as a sole carbon/energy source was isolated from soil. Metabolism of labelled (1-propyl position) and unlabelled EPTC by this microorganism yielded two major metabolites, *N*-depropyl EPTC and EPTC-sulfoxide. It is proposed that initial reactions in EPTC metabolism involve hydroxylation of the α-hydroxy-propyl EPTC. Decomposition of this unstable compound yields *N*-depropyl EPTC which is subsequently metabolized to CO_2. Sulfoxidation of EPTC is a less important initial reaction. A possible explanation of enhanced EPTC degradation in soil is that the pathway involving hydroxylation of the α-propyl carbon becomes the major route of degradation. In a non-enhanced soil, however, this pathway is active only at low levels and the sulfoxidation reaction is the major route of EPTC degradation.

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