Enhanced Degradation of Isofenphos by Soil Microorganisms

Kenneth D. Racke and Joel R. Coats*

Laboratory experiments were conducted to investigate the enhanced degradation of isofenphos [1-methylethyl 2-[[ethoxy[(1-methylethyl)amino]phosphinothioyl]oxy]benzoate] in soil and to elucidate the microbiology of this phenomenon. [U-ring-^{14}C]Isofenphos was most rapidly degraded in Iowa cornfield soils that had a history of isofenphos insecticide use. Between 13 and 42% of an applied dose of 5 ppm remained as isofenphos after 4 weeks in soil with isofenphos use history, whereas between 63 and 75% remained in comparable nonhistory soils. Soils with enhanced isofenphos degradation contained an adapted population of soil microorganisms responsible for the degradation observed. Degradation products of isofenphos detected in cultures of adapted soil microorganisms included isopropyl salicylate, $^{14}CO_2$, and polar products. A bacterial strain (*Pseudomonas sp.*) isolated from soil with enhanced isofenphos degradation proved capable of utilizing isofenphos as a sole carbon source.

Enhanced degradation is the phenomenon whereby a soil-applied pesticide is rapidly degraded by a population of microorganisms that has adapted due to previous exposure to the pesticide. This phenomenon has been observed with several herbicides (Newman and Thomas, 1949; Audus, 1951; Fryer and Kirkland, 1970; Harvey and Schuman, 1981), insecticides (Sethunathan and Pathak, 1972; Felsot et al., 1981; Read, 1983), and fungicides (Walker et al., 1986; Yarden et al., 1986). The type of pesticide metabolism involved is microbially beneficial and is accompanied by an increase in the numbers of specific pesticide-degrading soil microorganisms (Kaufman and Edwards, 1983). The practical implication of enhanced degradation is that the pesticide involved may be so rapidly degraded as to reduce its efficacy against the target pest (Felsot et al., 1982).

Isofenphos [1-methylethyl 2-[[ethoxy[(1-methylethyl)-amino]phosphinothioyl]oxy]benzoate] is an organophosphorus insecticide that was registered for use against soil-dwelling pests of corn (Amaze). Failure of isofenphos to control damage caused by larval corn rootworms (*Diabrotica spp.*) resulted in its withdrawal from that market after the 1983 growing season. Although initial reports indicated that isofenphos and its oxon metabolite were rather persistent in soil (Chapman and Harris, 1982; Felsot, 1984), recent evidence indicates that isofenphos is much less persistent after a second consecutive year of its application (Chapman et al., 1986).

The current research was initiated to determine whether enhanced microbial degradation of isofenphos had occurred. These laboratory studies were designed to investigate both the fate and degradation of [^{14}C]isofenphos in soil as well as the microbiology of its degradation.

MATERIALS AND METHODS

Chemicals. Uniformly ring-labeled [^{14}C]isofenphos, technical- and analytical-grade isofenphos, and several of its metabolites were obtained from Mobay Chemical Corp., Kansas City, MO. [^{14}C]Isofenphos was purified by using thin-layer chromatography with development in 4:3 hexane–acetone (R_f 0.77). The obtained radiopurity was greater than 98%. The fungicide cycloheximide [3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide] and the bactericide chloramphenicol [D-(−)-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide] were purchased from Sigma Chemical Co., St. Louis, MO.

Department of Entomology, Iowa State University, Ames, Iowa 50011.

Soils and Treatments. Surface samples of Iowa soils were collected from cornfields at the end of the growing season, sieved to remove debris, and stored in a moist condition at 4 °C. Soils chosen for study included those with isofenphos history, in which isofenphos had not performed well after a second year of use, and comparable soils with no recent insecticide history. Additional soils had received prior applications of other organophosphorus or carbamate insecticides. Properties of soils in which isofenphos degradation was extensively studied (soils I–VI) are listed in Table I.

Soil samples were treated with [^{14}C]isofenphos in acetone at 5 ppm as described by Lichtenstein and Schulz (1959). Aliquots of treated soil were taken for incubation and also for analysis of initial insecticide concentration. Soils that received antimicrobial treatments were treated with aqueous solutions of either chloramphenicol or cycloheximide at 100 ppm. Soil sterilization was by autoclaving at 121 °C for 1 h.

Extraction and Analyses. At the end of the incubation period, [^{14}C]isofenphos residues in soil were extracted twice with acetone–methanol (1:1) and once with acetone–methanol–dichloromethane (1:1:1) and partitioned into dichloromethane as described by Lichtenstein et al. (1973). Aqueous samples of [^{14}C]isofenphos-treated microbial cultures were acidified with concentrated HCl before partitioning.

Isofenphos and its metabolites were characterized by thin-layer chromatography (TLC) and autoradiography, as described by Hsin and Coats (1986). The R_f values for these compounds with development of the chromatogram in hexane–acetone (4:3) were as follows: isopropyl salicylate (0.86), isofenphos (0.77), isofenphos oxon (0.47), salicyclic acid (0.06). Confirmatory analysis was by high-pressure liquid chromatography using a Waters Model 6000A system with 254-nm UV detector. The extracts were dissolved in mobile phase (10% H_2O in methanol) and injected onto a Bondapak C_{18} column (0.6 × 30 cm) with a flow rate of 0.3 mL/min. Retention times for isopropyl salicylate, isofenphos, and isofenphos oxon were 16.2, 14.8, and 13.3 min, respectively. Unextractable, soil-bound [^{14}C]insecticide residues were recovered by combustion to $^{14}CO_2$ in a Packard sample oxidizer. Quantification of ^{14}C, including that present in vapor traps and in alkaline CO_2 traps, was by liquid scintillation counting.

EXPERIMENTAL PROCEDURES

Assay of Enhanced Microbial Degradation of Isofenphos. To determine whether the degradation of isofenphos was affected by soil insecticide history, a soil

0021-8561/87/1435-0094$01.50/0

Table I. Soils Used To Evaluate the Enhanced Degradation of Isofenphos

no.	soil series[a]	isofenphos history[b]	pH	OM	texture			% H_2O ($^1/_3$ bar)
					sand	silt	clay	
I	Tama	no	7.3	3.9	8.2	58.6	33.2	32.9
II	Readlyn	no	6.9	2.9	34.8	36.0	29.2	23.6
III	Canisteo	no	7.4	4.8	30.2	34.0	35.8	31.5
IV	Tama	yes	6.7	3.2	5.8	63.8	30.4	28.7
V	Canisteo	yes	7.2	4.8	25.3	38.3	36.4	31.2
VI	Canisteo	yes	7.4	4.9	27.0	38.9	34.2	32.3

[a] Soils I and IV (Tama) and soils III, V, and VI (Canisteo) were companion soils taken from adjacent fields. [b] For isofenphos soils, includes last 2 years; other soils had no insecticide applications for at least 10 years.

degradation assay similar to that described by Bartha and Pramer (1965) was used. A 25-g portion of each soil, in duplicate, was treated with 5 ppm of [^{14}C]isofenphos (0.1 μCi) and then placed within an 8-oz French square bottle along with an equal volume of sterile silica sand (75 g). The soil–sand mixtures were moistened so that the total water content of each was 15 mL. The presence of the sand in this ratio ensured that all soil–sand mixtures were maintained at approximately field moisture capacity ($^1/_3$-bar soil moisture tension) and thus allowed comparison of isofenphos degradation rates in texturally diverse soils (Keeney and Bremner, 1967). Glass vials containing 0.1 N NaOH were placed in each jar and served as CO_2 traps. The jars were closed tightly and incubated at 25 °C in the dark for 1 week, during which time the CO_2 traps were replaced and analyzed for $^{14}CO_2$ daily. The evolution of $^{14}CO_2$ was used as an indicator of isofenphos degradation.

To determine whether soil microorganisms were involved in the degradation of isofenphos observed, the effect of various antimicrobial treatments on the evolution of $^{14}CO_2$ from [^{14}C]isofenphos-treated soil was investigated. A soil with isofenphos history and in which [^{14}C]isofenphos had been rapidly degraded to $^{14}CO_2$ was chosen for study (soil IV). The [^{14}C]isofenphos degradation assay was used with eight jars of soil. Two soils were autoclaved before isofenphos treatment to determine the effect of sterilization on the observed degradation. Two soils were treated with the fungicide cycloheximide at 100 ppm, and two soils were treated at 100 ppm with the bactericide chloramphenicol. Two soils then remained untreated. The soil degradation assay was conducted as described previously.

Effect of Enhanced Degradation on the Fate of [^{14}C]Isofenphos in Soil. This study determined the fate of [^{14}C]isofenphos as affected by enhanced degradation. Six soils, three with a history of enhanced degradation and three with no recent insecticide history, were treated with 5 ppm of [^{14}C]isofenphos (0.5 μCi) in duplicated 100-g samples and added to 250-mL glass jars (5 cm diameter × 11 cm height). Soils were moistened to field capacity ($^1/_3$-bar soil moisture tension) with distilled water and incubated at 25 °C in the dark by using a flow-through incubation system (Kearney and Kontson, 1976; Ferris and Lichtenstein, 1980). Air was periodically purged from the tightly sealed soil jars through both vapor and CO_2 traps, and this allowed monitoring of [^{14}C]isofenphos degradation to $^{14}CO_2$. Distilled water was added as necessary to replace that lost due to evaporation. After either 1 or 4 weeks, the soil in each jar was analyzed for the presence of [^{14}C]isofenphos and its metabolites as previously described.

Because soil-bound residues represented a major end product of the applied [^{14}C]isofenphos in several soils, a study was also conducted to investigate the potential for further metabolism of these soil-bound residues. Soils that had been incubated for either 1 or 4 weeks with [^{14}C]isofenphos and extracted to remove soluble ^{14}C residues were used. These soils, which contained only bound ^{14}C residues, were then reincubated for 1 week to monitor further bound residue degradation by using the methodology described by Racke and Lichtenstein (1985). The role of soil microorganisms was assessed by reincubating bound residue soil that had been sterilized by autoclaving. The criterion for further degradation of these soil-bound residues was the evolution of $^{14}CO_2$.

Metabolism of [^{14}C]Isofenphos by Soil Microorganisms. The metabolism of [^{14}C]isofenphos was studied in soil-free cultures of adapted soil microorganisms. An aqueous extract of soil microorganisms was prepared by shaking a 50-g sample of soil with 200 mL of basal salts medium (Spain et al., 1980), after which the soil was removed by centrifugation. The soil used as the source of inoculum was one in which enhanced degradation of isofenphos had been confirmed (soil IV) and had been treated with 100 ppm of isofenphos 1 week before use to increase the numbers of isofenphos-degrading microorganisms present in the supernatant. A 75-mL aliquot of this supernatant was measured into each of two glass culture jars (5 cm diameter × 11 cm height) and then treated with 10 ppm of [^{14}C]isofenphos (1.0 μCi) dissolved in 10 μL of acetone. The cultures were aerobically incubated at 25 °C by using a flow-through incubation system (Gorder and Lichtenstein, 1980). During the 36-h incubation period, samples of culture medium were periodically withdrawn and analyzed for the presence of [^{14}C]isofenphos and its metabolites. Vapor traps and CO_2 traps were also monitored periodically for the presence of evolved ^{14}C.

Additional metabolism studies were also conducted as described, but either with 50 ppm of salicylic acid added to the culture in addition to the [^{14}C]isofenphos or with the aqueous soil extract sterilized before insecticide treatment.

Enumeration and Identification of Isofenphos-Degrading Soil Microorganisms. This study examined the specific soil microbial population involved in enhanced degradation of isofenphos. A most-probable-number method was used to enumerate isofenphos-degrading microorganisms in soils I–VI (Lehmicke et al., 1979; Somerville et al., 1985). Soil was serially diluted and placed in media containing [^{14}C]isofenphos at 10 ppm as the sole carbon source. The presence of isofenphos-degrading microorganisms was confirmed by the production of considerable quantities of $^{14}CO_2$ (>20% of applied ^{14}C) by the end of a 3-week incubation period.

For comparison, the total numbers of aerobic bacteria, fungi, and actinomycetes present in these same six soils were determined by standard plate count (Wollum, 1982). An M_{32} agar was employed for culturing soil bacteria and actinomycetes (Ridge and Rovira, 1971), and a rose bengal agar was used for culturing soil fungi (Martin, 1950). Final microbial counts were performed after 1 week, and each determination was performed in duplicate.

To identify the specific soil microorganisms involved in enhanced isofenphos degradation, isolates obtained from the most-probable-number study were streaked on basal salts agar with isofenphos provided as the sole carbon

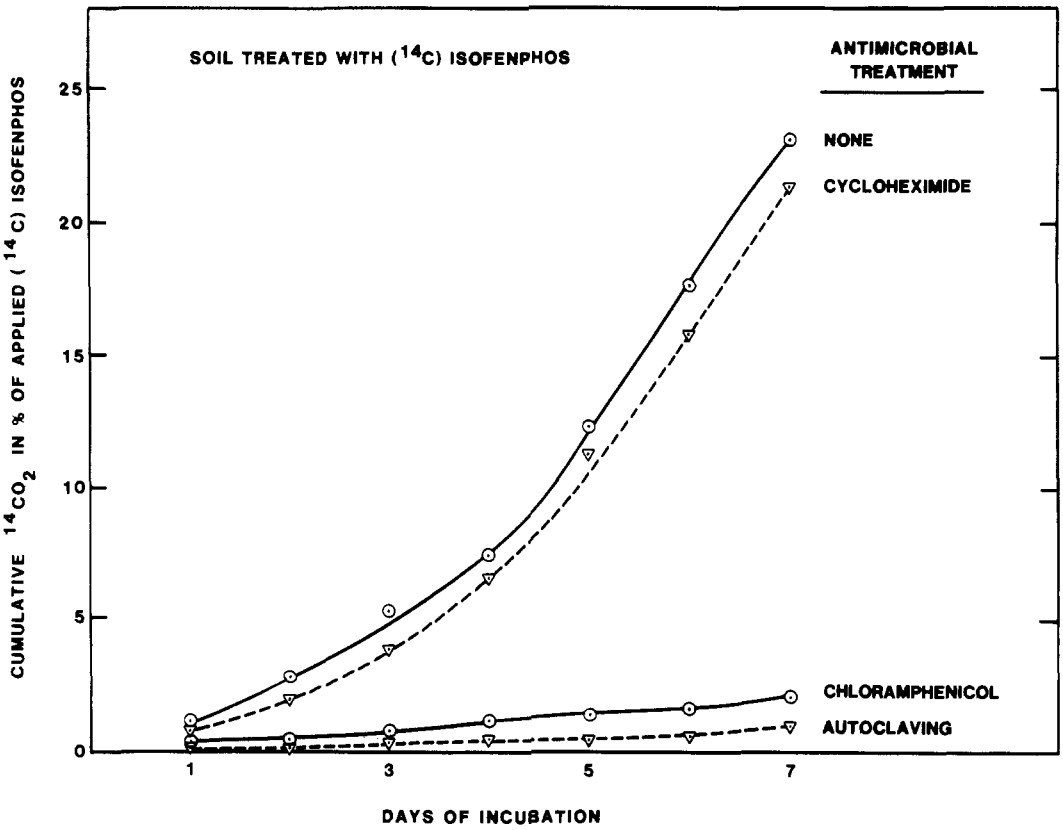

Figure 1. Effect of antimicrobial treatments on the evolution of $^{14}CO_2$ from [^{14}C]isofenphos-treated soil. Results are means of duplicate tests.

source. Colonies growing on this medium were characterized by utilizing standard microbial taxonomic techniques (Skerman, 1967; Buchanan and Gibbons, 1974).

RESULTS AND DISCUSSION

Assay of Enhanced Microbial Degradation of Isofenphos. The soil degradation assay provided an estimate of the rate of [^{14}C]isofenphos degradation in soils with a variety of insecticide histories. Results indicate that less than 3% of the applied insecticide was degraded to $^{14}CO_2$ in 1 week in soils with no recent insecticide history (Table II). In contrast, soils with a history of isofenphos use degraded 32% of the applied [^{14}C]isofenphos to $^{14}CO_2$. Quantities of $^{14}CO_2$ evolved from individual isofenphos history soils were quite variable and ranged from 5 to 56% of applied ^{14}C. This increased degradation rate also occurred in soil treated with 100 ppm of isofenphos 1 week before the assay, with 69% of the subsequently applied [^{14}C]isofenphos degraded to $^{14}CO_2$. Isofenphos degradation in soil was not affected by previous use of any other organophosphorus or carbamate insecticide and was not accelerated in soil in which enhanced degradation of carbofuran had occurred.

The effects of various antimicrobial treatments on the degradation of [^{14}C]isofenphos to $^{14}CO_2$ in isofenphos history soil (soil IV) were investigated. With no treatment applied, there was a rapid, accelerating production of $^{14}CO_2$ from [^{14}C]isofenphos-treated soil during the incubation period (Figure 1). This accelerating degradation is indicative of microbial involvement in the degradation process. The involvement of microorganisms was confirmed by the negligible $^{14}CO_2$ production from soil that had been sterilized by autoclaving. Although the addition of the fungicide cycloheximide did not affect the degradation of [^{14}C]isofenphos, application of the bactericide chloramphenicol had virtually the same effect as autoclaving. This suggests that soil bacteria are mainly responsible for the rapid degradation of isofenphos.

Table II. Evolution of $^{14}CO_2$ from [^{14}C]Isofenphos-Treated Soils (5 ppm)[a] during a 1-Week Incubation as Influenced by Soil Insecticide History

soil insecticide[b] history	no. of soils	$^{14}CO_2$[c] (% appl ^{14}C)
none	5	2.98 ± 0.99
isofenphos	4	31.52 ± 19.28
organophosphorus	8	2.07 ± 0.58
carbamate	6	2.36 ± 1.61

[a] A 25-g aliquot of each soil, in duplicate, was treated with 5 ppm of [^{14}C]isofenphos (0.10 μCi), mixed with 75 g of sand, and incubated for 1 week. [b] Indicates past 2 years of soil insecticide use. Organophosphorus includes terbufos, phorate, ethoprop, and fonofos. Carbamate includes only carbofuran. [c] Represents mean cumulative $^{14}CO_2$ evolved and trapped in 0.1 N NaOH during a 1-week incubation (±SE).

Data indicate that isofenphos is rapidly degraded by microorganisms in soils previously exposed to this insecticide. The observed enhanced degradation of isofenphos is consistent with reports of decreased isofenphos persistence in soils receiving repeated isofenphos applications (Chapman et al., 1986).

Effect of Enhanced Degradation on the Fate of [^{14}C]Isofenphos in Soil. This study was a comparison of the fate and degradation of [^{14}C]isofenphos in control soils (no recent insecticide history) and in isofenphos history soils with enhanced isofenphos degradation. Isofenphos was quite persistent in the control soils (I–III), in which between 63% and 75% of the applied [^{14}C]isofenphos remained after 4 weeks (Table III). This is consistent with earlier observations that determined the half-life of isofenphos in soil to be between 4.5 and 12 weeks (Felsot, 1984; Chapman and Harris, 1982). In soils with enhanced isofenphos degradation (IV–VI), however, only 13% (0.6 ppm) to 42% (2.1 ppm) of the initially applied dose of [^{14}C]isofenphos remained after 4 weeks.

Table III. Effect of Insecticide History on the Fate and Degradation of [^{14}C]Isofenphos in Soil,[a] during a 4-Week Incubation

	^{14}C recovered, % of applied [^{14}C]isofenphos					
	no insecticide history[b]			isofenphos history[b]		
	I	II	III	IV	V	VI
soil						
extractable						
isofenphos	62.77 ± 1.40[c]	74.17 ± 3.35[d]	75.22 ± 0.37[d]	12.88 ± 0.16[e]	24.85 ± 1.11[f]	41.90 ± 0.23[g]
isofenphos oxon	15.16 ± 1.32[c]	11.34 ± 0.01[d]	8.08 ± 0.03[e]	2.79 ± 0.06[f]	4.06 ± 0.01[g]	5.58 ± 0.01[h]
other[i]	0.89 ± 0.42[c]	0.23 ± 0.02[c]	0.75 ± 0.02[c]	0.79 ± 0.37[c]	0.67 ± 0.01[c]	0.62 ± 0.12[c]
bound	9.17 ± 0.11[c]	8.59 ± 0.11[c,d]	7.85 ± 0.21[d]	23.57 ± 0.46[e]	25.79 ± 0.64[f]	15.71 ± 0.35[g]
$^{14}CO_2$[j]	10.00 ± 0.01[c]	5.59 ± 0.04[d]	5.21 ± 0.09[d]	52.38 ± 1.10[e]	34.26 ± 2.76[f]	31.00 ± 0.23[g]
volatiles[k]	0.17 ± 0.05[c]	0.19 ± 0.01[c]	ND[l]	0.41 ± 0.04[d]	ND	ND
total	98.15 ± 0.44[c,d]	100.10 ± 3.16[d]	97.09 ± 0.44[c]	92.80 ± 0.85[c,e]	89.61 ± 4.50[e]	94.80 ± 0.48[c,e]

[a] [^{14}C]Isofenphos was uniformly applied to 100-g portions of soil at 5 ppm (0.5 μCi). [b] Includes previous two growing seasons. [c–h] Means followed by the same letter in each horizontal row are not significantly different at the 5% level (Student–Newman–Keuls test). [i] Includes polar, water-soluble products and traces of other metabolites. [j] $^{14}CO_2$ was trapped in 0.1 N NaOH during the incubation period. [k] Volatile ^{14}C materials were trapped in a vapor trap. [l] ND = not determined.

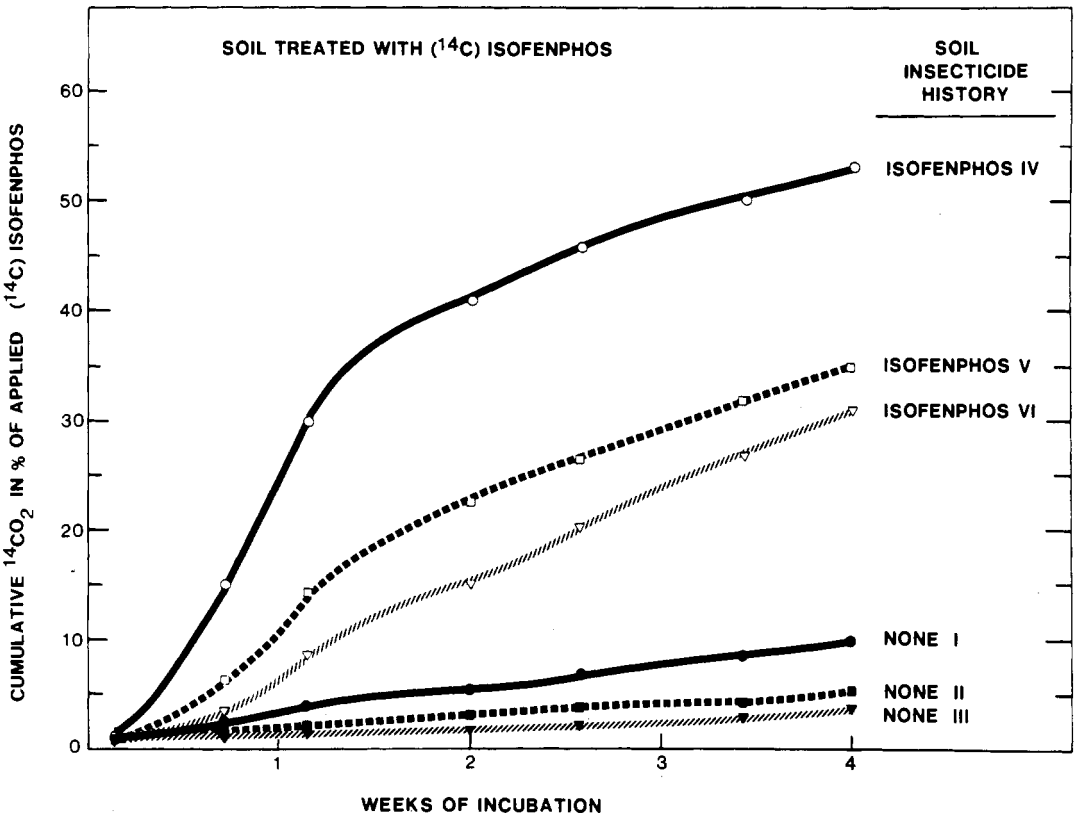

Figure 2. Effect of insecticide history on the evolution of $^{14}CO_2$ from [^{14}C]isofenphos-treated soils. Results are means of duplicate tests.

This decreased persistence could indeed reduce the extended efficacy of isofenphos in soil for a key soil pest of corn, the corn rootworm, against which it is reported to have an LC_{50} in soil of 1.2 ppm (Sutter, 1982).

The distribution of the remaining ^{14}C was quite different in comparing the control soils with the isofenphos history soils. In control soils (I–III), [^{14}C]isofenphos oxon was the major metabolite detected, accounting for up to 15% of the initially applied [^{14}C]isofenphos. In these soils, there was a steady accumulation of this oxidative metabolite, constituting, for example, 6.9 and 15.2% of the applied ^{14}C after 1 and 4 weeks of incubation, respectively (soil I). The accumulation of this persistent metabolite has been reported to occur in soil mainly through nonbiological means (Felsot, 1984). Soil-bound residues and $^{14}CO_2$ were also produced in control soils but generally represented a minor fate of the applied [^{14}C]isofenphos.

The same degradation products of [^{14}C]isofenphos were also detected in isofenphos history soils (IV–VI), but the rate of formation and final distribution of these products were strikingly different. Only small quantities of [^{14}C]-isofenphos oxon were detected, representing, for example, 2.8 and 4.1% of applied ^{14}C after 1 and 4 weeks of incubation, respectively (soil V). A large percentage of the applied [^{14}C]isofenphos was converted to $^{14}CO_2$ and soil-bound residues in these soils. Degradation to $^{14}CO_2$ accounted for one-third to one-half of initially applied [^{14}C]isofenphos during the 4-week incubation period. The most rapid evolution of $^{14}CO_2$ occurred during the first week of incubation (Figure 2).

Large quantities of soil-bound residues were produced in soils in which isofenphos was rapidly degraded, and these residues tended to accumulate largely during the first week of incubation. In general, between 53 and 71% of the total bound residues found after 4 weeks was already present after only 1 week. An experiment conducted to determine whether these residues were susceptible to further degradation revealed that small amounts (<5%)

Table IV. Metabolism of [^{14}C]Isofenphos by a Mixed Culture of Soil Microorganisms[a]

	distribn of ^{14}C in % of recovered after incubation for				
	1 h	6 h	12 h	24 h	36 h
isofenphos	89.0	82.0	69.4	36.3	11.6
isopropyl salicylate	2.4	6.2	4.8	0.4	0.4
polar	5.4	5.5	14.5	30.5	20.5
$^{14}CO_2$	0.0	0.9	2.4	23.9	62.1
other[b]	3.1	5.5	8.8	8.9	5.5

[a] A 75-mL aqueous soil extract, with associated microorganisms, was treated with 5 ppm of [^{14}C]isofenphos (1.0 μCi), in duplicate. [b] "Other" includes that ^{14}C collected in organic vapor traps, as well as traces of salicyclic acid and an unknown metabolite.

of the soil-bound residue could be released upon reincubation. This further degradation, as evidenced by the production of $^{14}CO_2$, proved to be mediated by soil microorganisms. It is possible that some of the $^{14}CO_2$ detected during the incubation of [^{14}C]isofenphos in soil was formed as the result of degradation of soil-bound residues formed. It has been reported that, in general, and increase in insecticide degradation in soil results in an increase in the quantity of soil-bound residues formed (Lichtenstein et al., 1977).

Inasmuch as no other isofenphos metabolites were detected in soils, it is unclear exactly what degradative reactions of isofenphos precede the formation of CO_2 and soil-bound residues. It is likely that the intermediate metabolites formed were rapidly metabolized to other products, which would be consistent with earlier metabolic investigations of enhanced degradation in soil that also failed to detect intermediate metabolites of other insecticides (Rodriguez and Dorough, 1977; Felsot et al., 1981).

Metabolism of [^{14}C]Isofenphos by Soil Microorganisms. [^{14}C]Isofenphos was rapidly degraded by a culture of microorganisms from soil with enhanced isofenphos degradation (Table IV). No degradation was noted in cultures that had been sterilized. As the quantity of [^{14}C]isofenphos in nonsterile systems decreased, a temporary buildup of [^{14}C]isopropyl salicylate was observed, with this hydrolysis product constituting 6% of the recovered ^{14}C after 6 h of incubation. Later in the incubation period, there was also a temporary buildup of polar, water-soluble products. The final product of isofenphos metabolism was the same as that in soil, with $^{14}CO_2$ evolution substantially increasing as the quantities of isofenphos, isopropyl salicylate, and polar products declined. Trace quantities of salicyclic acid and an unknown nonpolar metabolite were also detected in cultures, but no isofenphos oxon.

The appearance of isopropyl salicylate in isofenphos-treated cultures of soil microorganisms implicates a hydrolytic step in the metabolism of isofenphos, which is common with the microbial metabolism of many organophosphorus and carbamate insecticides (Sethunathan and Pathak, 1972; Siddaramappa et al., 1973; Rodriguez and Dorough, 1977; Barik et al., 1979). Because isopropyl salicylate was not detected as a metabolic product during the degradation of isofenphos in soil, it is not certain that this product is formed as a hydrolysis product by soil microorganisms in situ. Insecticide hydrolysis products, however, are difficult to detect in soil (Felsot et al., 1981; Chapman et al., 1985).

Large quantities of polar, water-soluble products of [^{14}C]isofenphos degradation were formed in cultures of microorganisms but were not similarly observed in soil. An experiment therefore was conducted to see whether these polar residues once applied to soil could again be extracted.

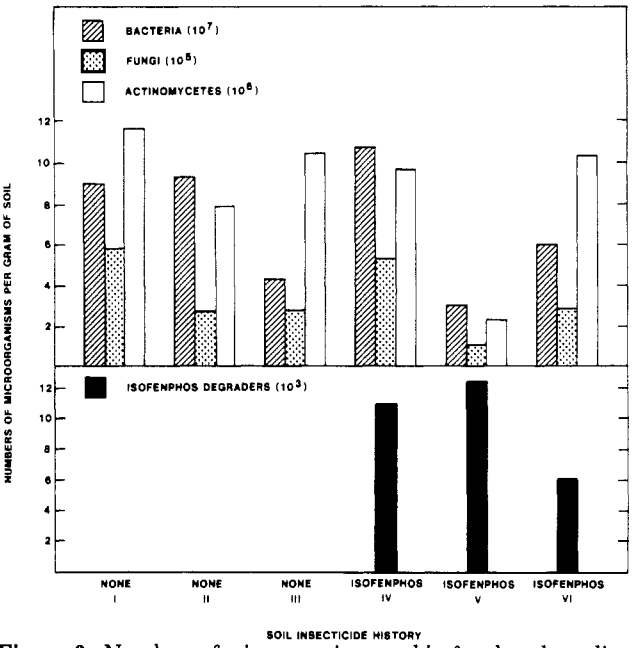

Figure 3. Numbers of microorganisms and isofenphos-degrading microorganisms in soil. Results are means of duplicate determinations.

Within 1 h of application to soil, approximately 90% of applied ^{14}C polar residues were present as unextractable, soil-bound residues. If these same polar residues had been produced in soil, they may have constituted the soil-bound residues detected in the soil degradation study.

Because traces of [^{14}C]salicyclic acid, a secondary hydrolysis product of isofenphos, were detected in isofenphos-treated microbial cultures, a second investigation of isofenphos metabolism was conducted. In this study, 50 ppm of salicyclic acid was added to microbial cultures along with the [^{14}C]isofenphos in an attempt to force the temporary accumulation of any [^{14}C]salicylic acid produced. Although the disappearance rate of [^{14}C]isofenphos was not affected by this addition, the production of $^{14}CO_2$ was delayed as compared with the previous study. The eventual evolution of $^{14}CO_2$ was preceded by a temporary accumulation of [^{14}C]salicyclic acid in the medium, which accounted for 9.8% of recovered ^{14}C after 12 h of incubation. The implication is that salicyclic acid is a probable secondary microbial hydrolysis product of isofenphos. The lack of its accumulation in the previous experiment may be due to its extremely rapid metabolism once formed. Given that some soil bacteria are known to carry degradative plasmids for salicyclic acid metabolism (Chakrabarty, 1972), it is possible that its formation may represent a key factor in the susceptibility of isofenphos to enhanced microbial degradation.

Enumeration and Identification of Isofenphos-Degrading Soil Microorganisms. Results of the microbial most-probable-number determination (Figure 3) indicate that soils in which enhanced isofenphos degradation has occurred contain a population of microorganisms capable of metabolizing this insecticide. The three soils in which isofenphos was rapidly degraded (IV–VI) each contained, per gram, several thousand microorganisms capable of using isofenphos as a sole carbon source. Control soils (I–III) contained no such microbial population. In comparison with the total numbers of microorganisms in these soils (Figure 3), isofenphos-degrading microorganisms represent only a small fraction of the total microfauna present. The presence of an isofenphos-degrading population of soil microorganisms seems to provide a satisfac-

tory explanation for the occurrence of enhanced degradation in these soils. A single application of isofenphos at 100 ppm to control soils (I–III) resulted in the appearance of a similar degradatory population after a 1-week incubation. This suggests that enhanced degradation of isofenphos is an inducible phenomenon. Similarly, Fournier et al. (1981) reported an inducible population of 2,4-D-degrading microorganisms in soils in which enhanced degradation of this herbicide had occurred.

A bacterial strain was isolated from isofenphos-treated culture medium, and it proved capable of using isofenphos as a carbon source. This gram-negative, short rod (length 1.3 μM) metabolized [^{14}C]isofenphos to $^{14}CO_2$ and was identified as a species of *Pseudomonas*. This strain produced a light yellow, nonfluorescent pigment and was both motile and highly aerobic. When given a choice of either isofenphos or glucose as an energy source, the bacteria preferentially chose glucose. Pseudomonads are common soil bacteria capable of metabolizing a wide variety of aromatic compounds (Wheelis, 1975). Several *Pseudomonas spp.* have been isolated from soil that metabolize organophosphorus or carbamate insecticides (Kazano et al., 1972; Siddaramappa et al., 1973; Kilbane et al., 1982), and it is a pseudomonad that has been implicated in the enhanced degradation of carbofuran (Felsot et al., 1981). Also, with salicyclic acid in the microbial pathway of isofenphos degradation, it is noteworthy that some pseudomonads carry the salicylic acid degradative plasmid (Chakrabarty, 1972). Several other bacterial isolates are being evaluated for isofenphos degrading ability.

In conclusion, isofenphos has undergone enhanced degradation in soil due to the induction of an isofenphos-degrading population of microorganisms. Further research is needed to provide a comprehensive understanding of this increasingly important phenomenon if the effective use of biodegradable soil pesticides is to be continued.

ACKNOWLEDGMENT

Special thanks are expressed to D. D. Michael for invaluable help provided with the microbial aspects of this project. Thanks are also expressed to Dr. Jon Tollefson and the corn insects research group for help in locating candidate soils and to Dr. Tom Loynachan for manuscript ideas.

Registry No. CO_2, 124-38-9; isofenphos, 25311-71-1; isopropyl salicylate, 607-85-2; isofenphos oxon, 31120-85-1.

LITERATURE CITED

Audus, L. J. *Plant Soil* **1951**, *3*, 170.
Barik, S.; Wahid, P. A.; Ramakrishna, C.; Sethunathan, N. *J. Agric. Food Chem.* **1979**, *27*, 1391.
Bartha, R.; Pramer, D. *Soil Sci.* **1965**, 100, 68.
Buchanan, R. E., Gibbons, N. E., Eds. *Bergey's Manual of Determinative Bacteriology*, 8th ed.; Williams and Wilkins: Baltimore, 1974.
Chakrabarty, A. M. *J. Bacteriol.* **1972**, *112*, 1391.
Chapman, R. A.; Harris, C. R. *J. Environ. Sci. Health, Part B* **1982**, *B17*, 355.
Chapman, R. A.; Moy, P.; Henning, K. *J. Environ. Sci. Health, Part B* **1985**, *B20*, 313.
Chapman, R. A.; Harris, C. R.; Moy, P.; Henning, K. *J. Environ. Sci. Health, Part B* **1986**, *B21*, 269.
Felsot, A. *J. Environ. Sci. Health, Part B* **1984**, *B19*, 13.
Felsot, A.; Maddox, J. V.; Bruce, W. *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 781.
Felsot, A. S.; Wilson, J. G.; Kuhlman, D. E.; Steffey, K. L. *J. Econ. Entomol.* **1982**, *75*, 1098.
Ferris, I. G.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1980**, *28*, 1011.
Fournier, J. C.; Codaccioni, P.; Soulas, G. *Chemosphere* **1981**, *10*, 977.
Fryer, J. D.; Kirkland, K. *Weed Res.* **1970**, 10, 133.
Gorder, G. W.; Lichtenstein, E. P. *Can. J. Microbiol.* **1980**, *26*, 475.
Harvey, R. G.; Schuman, D. B. *Abstr. Weed Sci. Soc. Am.* **1981**, 124.
Hsin, C. Y.; Coats, J. R. *Pestic. Biochem. Physiol.* **1986**, *25*, 336.
Kaufman, D. D.; Edwards, D. F. In *IUPAC Pesticide Chemistry*; Miyamoto et al., Eds.; Pergamon: New York, 1983; pp 177–182.
Kazano, H.; Kearney, P. C.; Kaufman, D. D. *J. Agric. Food Chem.* **1972**, *20*, 975.
Kearney, P. C.; Kontson, A. *J. Agric. Food Chem.* **1976**, *24*, 424.
Keeney, D. R.; Bremner, J. M *Soil Sci. Soc. Am. Proc.* **1967**, *31*, 34.
Kilbane, J. J.; Chatterjee, D. K.; Karns, J. S. *Appl. Environ. Microbiol.* **1982**, *44*, 72.
Lehmicke, L. G.; Williams, R. T.; Crawford, R. L. *Appl. Environ. Microbiol.* **1979**, *38*, 644.
Lichtenstein, E. P.; Schultz, K. R. *J. Econ. Entomol.* **1959**, *52*, 118.
Lichtenstein, E. P.; Fuhremann, T. W.; Schulz, K. R.; Liang, T. T. *J. Econ. Entomol.* **1973**, *66*, 863.
Lichtenstein, E. P.; Katan, J.; Anderegg, B. N. *J. Agric. Food Chem.* **1977**, *25*, 43.
Martin, J. P. *Soil Sci.* **1950**, *69*, 215.
Newman, A. S.; Thomas, J. R. *Soil Sci. Soc. Am. Proc.* **1949**, *14*, 160.
Racke, K. D.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1985**, *33*, 938.
Read, D. C. *Agric., Ecosyst. Environ.* **1983**, *10*, 37.
Ridge, E. H.; Rovira, A. D. *New Phytol.* **1971**, *70*, 1017.
Rodriguez, L. D.; Dorough, H. W. *Arch. Environ. Contam. Toxicol.* **1977**, *6*, 47.
Sethunathan, N.; Pathak, M. D. *J. Agric. Food Chem.* **1972**, *20*, 586.
Siddaramappa, R.; Rajaram, K. P.; Sethunathan, N. *Appl. Microbiol.* **1973**, *26*, 846.
Skerman, V. B. D. *A Guide to the Identification of the Genera of Bacteria*, 2nd ed.; Williams and Wilkins: Baltimore, 1967.
Somerville, C. C.; Monti, C. A.; Spain, J. C. *Appl. Environ. Microbiol.* **1985**, *49*, 711.
Spain, J. C.; Pritchard, P. H.; Bourquin, A. W. *Appl. Environ. Microbiol.* **1980**, *40*, 726.
Sutter, G. R. *J. Econ. Entomol.* **1982**, *75*, 489.
Walker, A.; Brown, P. A.; Entwistle, A. R. *Pestic. Sci.* **1986**, *17*, 183.
Wheelis, M. L. *Annu. Rev. Microbiol.* **1975**, *29*, 505.
Wollum, A. G. In *Methods of Soil Analysis*; Page et al., Eds.; American Society of Agronomy: Madison, WI, 1982; pp 781–802.
Yarden, O.; Aharonson, N.; Katan, J. Presented at the Sixth International Congress of Pesticide Chemistry, Ottawa, Canada, Aug 1986; paper 6C-24.

Received for review March 26, 1986. Accepted August 25, 1986. Results of this study were presented at the 190th National Meeting of the American Chemical Society, Chicago, IL, Sept 1985; AGRO 38. Funding for this project was provided by grants from the USDA North Central Region Pesticide Impact Assessment Program and the Iowa Corn Promotion Board. Journal Paper No. J-12236 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA.