

Degradation of Isazofos in the Soil Environment[†]

L. Somasundaram,* K. Jayachandran,[‡] E. L. Kruger,[§] K. D. Racke,[§] T. B. Moorman,[‡]
T. Dvorak,[§] and J. R. Coats[§]

Department of Entomology, Iowa State University, Ames, Iowa 50011, and National Soil Tilth Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50011

Field and laboratory studies were conducted to investigate the degradation of the organophosphorus insecticide isazofos (*O*-5-chloro-1-isopropyl-1*H*-1,2,4-triazol-3-yl *O,O*-diethyl phosphorothioate) in soil. In a 6-year field study, soil pH was an important factor influencing the degradation of isazofos inasmuch as an increased rate of degradation was observed in soils with previous isazofos applications and pH of 6.9 or more. A laboratory study of [¹⁴C]isazofos confirmed the rapid degradation of this insecticide in high pH soils. No increased degradation rate, however, was observed in sterilized high-pH soils. The availability of isazofos to microorganisms, based on sorption of isazofos to soil, seems to be an important factor influencing the degradation of isazofos in soil. Sorption coefficients were negatively correlated with isazofos degradation rate.

INTRODUCTION

Isazofos (*O*-5-chloro-1-isopropyl-1*H*-1,2,4-triazol-3-yl *O,O*-diethyl phosphorothioate) (Figure 1) is a broad-spectrum organophosphorus insecticide and nematicide that effectively controls soil-inhabiting pests of agricultural crops and turf (Toba and Turner, 1979; Hamlen et al., 1979; Niemczyk and Krueger, 1987). At 20 °C, the solubility of isazofos in water is 150 mg/L and its vapor pressure 1.3×10^{-4} mmHg (Ciba-Geigy Corp., 1983). Field research and laboratory research on bioefficacy and degradation of isazofos indicate its short persistence in soil (Sears and Chapman, 1979; Toba and Turner, 1979; Hamlen et al., 1979; Chapman and Harris, 1982; Niemczyk and Krueger, 1987; Bowman, 1990, 1991, 1993). The half-life of isazofos ranges from 0.5 to 5.0 weeks (Chapman and Harris, 1982; Bowman, 1993). Because of the relatively short persistence of isazofos, the potential either to develop high residue levels in soil or crops or to leach is minimal (Chapman and Harris, 1982; Bowman, 1990).

Although the rapid disappearance of isazofos in soils has been well documented, little is known about the mechanisms of degradation or the factors influencing its degradation. The potential of isazofos to undergo enhanced biodegradation, a phenomenon in which adapted soil microorganisms make use of the pesticide and/or its metabolite(s) as an energy or nutrient source (Somasundaram et al., 1990), has not been studied. Because isazofos persists only briefly in soils, its potential to undergo enhanced degradation after repeated application is an important issue. The objectives of the current study were to investigate isazofos persistence in soil as affected by its prior treatment under both field and laboratory conditions and to elucidate the factors influencing its degradation in soil.

* Author to whom correspondence should be addressed. Present address: Du Pont Agricultural Products, Experimental Station, Wilmington, DE 19880-0402.

[†] Journal Paper No. J-14919 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. 2306.

[‡] National Soil Tilth Laboratory.

[§] Iowa State University.

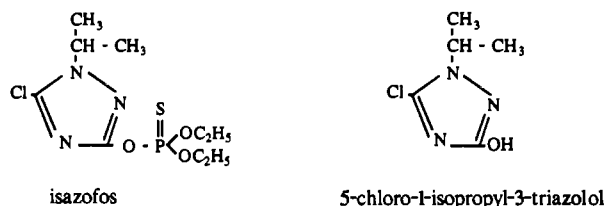


Figure 1. Chemical structures of isazofos and 5-chloro-1-isopropyl-3-triazolol.

MATERIALS AND METHODS

Field Studies. The study site was located in the Nicollet-Clarion-Webster soil association. The soil is a loam with 40% sand, 35% silt, and 25% clay. Organic matter (OM) content and pH of soils from the experimental plots are given in Table I. Because the field selected had been in an annual corn-soybean-oat rotation, no soil insecticide had been used for corn rootworm control before this study. The field was in continuous corn throughout the 6-year study. Isazofos 10G was applied at a rate of 0.61 oz of active ingredient (ai) per 1000 row-ft in a 7-in. T-band ahead of the closing wheels. The 40 × 40 ft experimental plots were separated by untreated plots of equal size. The study was initiated in 1984, with treatment of one plot. The plot treatments were planned in such a way that, during each growing season for 5 years, isazofos was applied to each previously treated plot and to one new plot for the first time. In 1989, at the end of the sixth and final year of the field study, the treatments included soils with 0-6 years of isazofos history.

Residue Analysis. Soil samples were collected at 0, 1, 3, 6, and 10 weeks posttreatment. On each sampling date, five soil cores (4-in. diameter by 4-in. depth) were collected from the center four rows of treated plots. The cores were frozen until processed. Air-dried composite samples were sieved, and two 100-g subsamples were used for residue analysis. The soil was extracted three times with 200 mL of hexane/acetone (3:2), with the extract filtered between extractions. The recovery of isazofos by this method was >88%, and the recovery spikes were performed at a range of 0.1-6.0 ppm to adequately cover the range of field residues reported. The filtrate was concentrated on a rotary evaporator and brought to a volume of 10 mL. The concentration of isazofos in the extract was determined by using a Varian 3740 gas chromatograph. The operating parameters were as follows: column, 2 mm × 90 cm i.d. glass column packed with 10% DC 200/2% OV-225 on 80/100-mesh Chromosorb maintained at 210 °C; detector, nitrogen-phosphorus, 250 °C; carrier gas, nitrogen (25 mL/min); H₂ flow rate, 4.5 mL/min; injector temperature, 240 °C; injector volume, 3 μL. The minimum detectable concentration of isazofos was 0.1 ppm, and the retention time

Table I. Properties of Soils Used^a

years of isazofos application	1986		1987		1988		1989	
	pH	OM	pH	OM	pH	OM	pH	OM
0	7.2	3.0	6.9	3.1	6.7	4.5	NA	NA
1	6.4	4.1	7.9	2.7	5.9	3.3	6.4	2.4
2	6.6	4.9	6.2	3.1	7.4	2.5	6.2	2.8
3	7.4	3.6	6.3	4.7	6.0	3.5	7.4	2.0
4	X	X	6.7	3.9	6.2	4.5	6.1	3.1
5	X	X	X	X	6.9	3.5	6.2	3.5
6	X	X	X	X	X	X	7.0	2.5

^a X, plot treatment not available at this time; NA, not analyzed.

was 1.7 min. The method of quantification was by using an external standard.

Laboratory Treatment and Soil Incubation. Soils were collected from the experimental plots at the end of the growing season, sieved to remove debris, and stored at 4 °C until used. The storage time ranged from 0 to 6 months. To allow comparison of microbial and chemical degradation, a portion of the field-collected soil was autoclaved at 121 °C for 1 h. A 50-g dry weight portion of each soil was treated, in duplicate, with 5 ppm of ¹⁴C-labeled (sp act. 34.6 μCi/mg; 98% radio purity) isazofos. The treating solution made in acetone was applied with a pipet uniformly all over the soil, and air was passed through the jar containing the soil to evaporate the acetone. Aliquots of treated soils were then taken for incubation and for analysis of initial isazofos concentration. Each treatment was replicated twice, and the treated soils were placed in 8-oz French square bottles. Soils were moistened to field capacity (1/3 bar soil moisture tension) with distilled water, and glass vials containing 10 mL of 0.1 N NaOH were placed in each bottle to trap the ¹⁴CO₂ evolved. The bottles were closed tightly and incubated at 25 °C for 3 weeks. The CO₂ traps were replaced daily for the first week and on alternate days for the next 2 weeks and were subsequently analyzed for ¹⁴CO₂ by liquid scintillation counting.

Analyses. At the end of the incubation period, [¹⁴C]isazofos residues were extracted twice with 150 mL of acetone/methanol (1:1) and once with 150 mL of acetone/methanol/dichloromethane (1:1:1). The resultant extract was rotary evaporated to about 60 mL and then partitioned three times into 50 mL of dichloromethane. The organic and aqueous fractions were analyzed by liquid scintillation counting. The organic fraction was subsequently rotary evaporated to about 3 mL and rediluted to 10 mL with dichloromethane. The presence of isazofos and its metabolites in the organic fraction was identified by thin-layer chromatography (TLC) and autoradiography techniques. Normal-phase silica gel plates were used and developed with chloroform/ethyl acetate/hexane/acetic acid (12:12:6:1) for a distance of 14 cm and air-dried. Nonradioactive standards of isazofos (*R_f* = 0.89) and 5-chloro-1-isopropyl-3-triazolol (*R_f* = 0.49) were run for comparative purposes. Autoradiography was performed using X-Omat Kodak diagnostic film. A film was placed in contact with each plate for 3 weeks and then developed. The radioactivity was quantified by scrapping the silica gel areas corresponding to standards into 15 mL of scintillation cocktail. The identity of the metabolite was confirmed by HPLC using the method described under Sorption Studies. The retention time for the triazolol metabolite was 2.16 min. Unidentified minor metabolites in the TLC system and radioactivity in the aqueous fraction of the partitioning process were grouped as "others" to obtain the mass balance. Unextractable, soil-bound ¹⁴C residues were determined by combusting pellets, three per replicate, made of 0.5 g of soil and 0.1 g of hydrolyzed starch in a Packard sample oxidizer. Evolved ¹⁴CO₂ was trapped in 10 mL of Carbo-Sorb E. The samples were added with 10 mL of Permafluor, and the radioactivity was quantified using a LKB 1217 Rack Beta liquid scintillation counter. All counts were corrected for background and quenching, if any.

Estimation of Microbial Biomass and Enumeration of Isazofos-Degrading Soil Microorganisms. Soil microbial biomass was determined for 1988 and 1989 soils by using the methods described by Tate et al. (1988) and Wu et al. (1990). Duplicate samples were used for fumigation and incubation

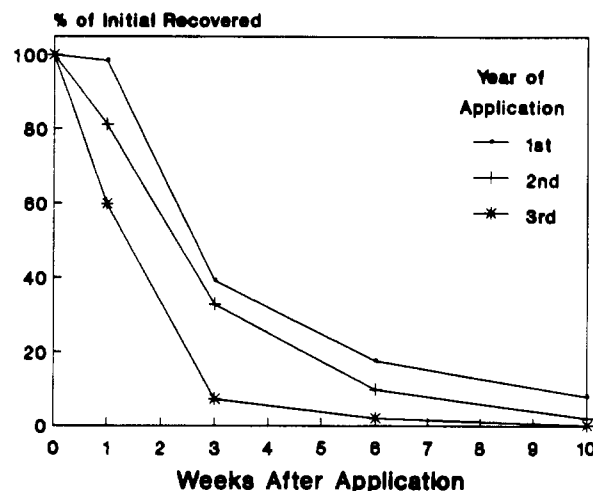


Figure 2. Isazofos field persistence (1986).

without fumigation. The samples were analyzed in a Dohrman DC180 automatic carbon analyzer.

To quantify the populations of soil microorganisms capable of degrading isazofos, a most-probable-number method was used (Lehmick et al., 1979). A mineral salts medium (Kaufman and Kearney, 1965) was prepared and autoclaved at 121 °C for 15 min. A stock solution of trace elements (Zeyer and Kearney, 1982) was also prepared and added to the mineral salts medium. One gram (dry weight basis) of the soil was placed in 9 mL of sterile water, vortexed thoroughly, and serially diluted. One milliliter of each dilution was added to 4 mL of mineral salts medium. A control was also maintained, in which 1 mL of sterile water was added to the 4 mL of medium. The initial concentration of isazofos in all treatments was 10 ppm. All samples were incubated at 28 °C for 2 weeks, filter sterilized, and analyzed for the presence of isazofos using the methods described under Sorption Studies. Five more controls were prepared and stored at 4 °C.

Sorption. Three grams (dry weight) of 1988 soil in a glass centrifuge tube was equilibrated with 15 mL of 0.01 M CaCl₂ (pH approximately 5.8) containing 1.5 ppm of isazofos for 24 h in a shaker and then centrifuged at 1900g for 30 min. The study was replicated four times and conducted at room temperature (25 ± 1 °C). The supernatant was collected, and a Waters HPLC equipped with a UV detector was used to analyze for both isazofos and its triazolol metabolite. The operating parameters were as follows: column, Waters Nova-Pak C₁₈ (8 × 100 mm; 4-μm particle size); injection volume, 50 μL; mobile phase, acetonitrile/water (60:40); detector wavelength, 210 nm; flow rate, 1.6 mL/min. External standards of isazofos were used, and the retention time was 7.35 min. The minimum detectable concentration of isazofos was 0.5 ppm. The detector response was linear in the range 0.5–10 ppm. These were used to quantify isazofos in the soil solution following equilibration. Solution concentrations were above 0.5 ppm in all samples, and the triazolol metabolite was not observed.

RESULTS AND DISCUSSION

Degradation of Isazofos As Influenced by Previous Applications. The residue analysis for the field experiment was conducted for four growing seasons beginning in 1986. The laboratory metabolism studies were conducted for soils collected in 1986, 1987, and 1988. The persistence of isazofos under field conditions is expressed as percent of initially applied isazofos remaining at specific sampling intervals (Figures 2–5). For the laboratory study, persistence is expressed as the percent of applied [¹⁴C]-isazofos remaining at the end of the 3-week incubation. A simple correlation analysis was conducted to determine the influence of soil pH on the persistence of isazofos at 0, 1, 3, 6, and 10 weeks after field application and at the end of the 3-week laboratory incubation. The same

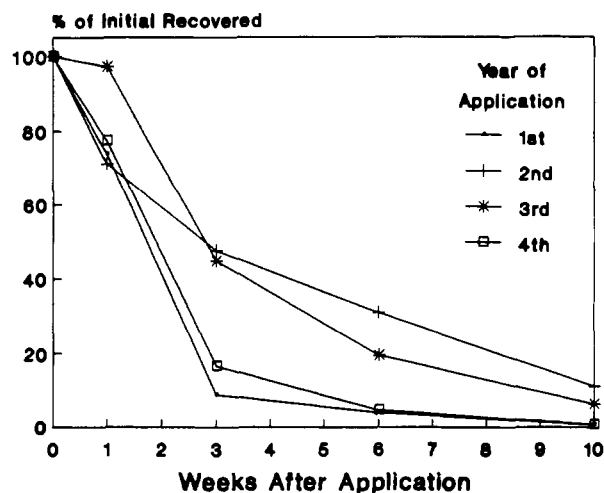


Figure 3. Isazofos field persistence (1987).

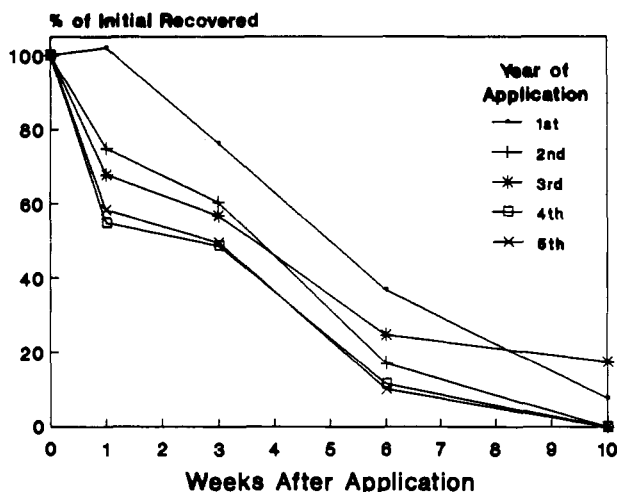


Figure 4. Isazofos field persistence (1988).

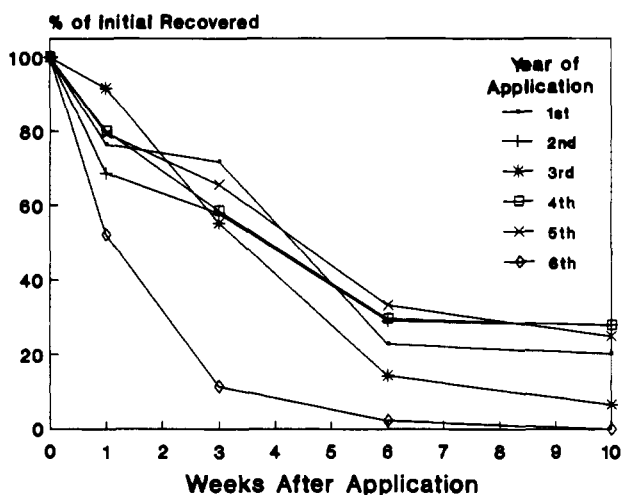


Figure 5. Isazofos field persistence (1989).

statistical treatment was used from year to year. The results of these studies will be discussed by year.

1986. The field treatments consisted of soils applied with isazofos for 1, 2, and 3 years. Residue data indicate that field residues of isazofos declined rapidly from soil treated with isazofos for the third consecutive year (Figure 2). Three weeks after application, less than 10% of the applied isazofos remained in the 3-year soil, compared with 39 and 33% in 1- and 2-year treatments, respectively. Moreover, in the laboratory studies isazofos was least

persistent in the soil with 3 previous years of isazofos application (Table II). Only 2% of the applied parent insecticide was recovered at the end of the 3-week incubation period. This increased degradation resulted in the production of greater amounts of the hydrolysis metabolite 5-chloro-1-isopropyl-3-triazolol (CGA 17193), soil-bound residues, and $^{14}\text{CO}_2$ than in other treatments. No such increase in degradation of isazofos was evident in the soils with either one or two previous isazofos field applications. In all soils, the major extractable metabolite was the hydrolysis product, 5-chloro-1-isopropyl-3-triazolol. Persistence and accumulation of this metabolite in soil has been described (Bowman, 1991).

The increased degradation of isazofos in the 3-year soil indicates that isazofos could have undergone enhanced biodegradation. But because a significant increase in degradation rate was not evident in other soils with an isazofos history, the increased rate of degradation observed in the 3-year soil may have been due to inherent differences between that soil and others. The soil with a 3-year history of isazofos had a higher pH than did the soils with either a 1- or 2-year history (Table I). Isazofos was also less persistent in the laboratory treatment with no prior isazofos exposure than in other treatments, and the insecticide-free soil also had a higher pH. A significant inverse relation ($P < 0.10$) was observed between pH and isazofos persistence under both field and laboratory conditions. Thus, isazofos is probably relatively unstable at high pH values.

1987. Three weeks after application, residues of isazofos declined dramatically in soils with 4 years of previous applications (Figure 3). Similarly, in the soils not previously exposed to isazofos more than 90% of the insecticide applied disappeared within the first 3 weeks. In a recent study, isazofos disappeared quite quickly ($\text{DT}_{50} = 0.53$ week) when applied for the first time to a Vittoria loam soil (Bowman, 1993). The rapid degradation of isazofos in soil treated for the first time in the current study as well as that of Bowman (1993) suggests that the microorganisms were able to degrade isazofos with little or no lag period. In this study, pH was the major difference between 1- and 4-year and 2- and 3-year soils (Table I). As in our 1986 field studies, correlation analysis indicated an inverse relationship between soil pH and isazofos persistence. The reduced persistence of isazofos in first and fourth year treatments is because of high pH values.

Among the five different treatments (0–4-year histories) studied in the laboratory, more than one-third of the initially applied isazofos persisted in soil with no prior exposure to isazofos (Table III). In all other treatments, less than 10% of the applied [^{14}C]isazofos was recovered. This increased degradation of isazofos in history soils resulted in greater amounts of both 5-chloro-1-isopropyl-3-triazolol (23–30%) and soil-bound residues (40–47%). The reduced persistence of isazofos in the 1-year soil corroborates our findings from the field, which confirm the susceptibility of isazofos to high pH. The instability of isazofos under alkaline conditions has been reported (Ciba-Geigy Corp., 1983).

The increased rate of degradation observed in high-pH soils under both field and laboratory conditions indicates that isazofos is prone to significant hydrolytic degradation with increasing soil pH. The half-lives of isazofos in solution hydrolysis studies at 20 °C were 85, 48, and 19 days at pH 5, 7, and 9, respectively (Worthing, 1991). These data support our findings on the instability of isazofos with increasing soil pH. Soil bacteria play a major role in the microbial metabolism of many pesticides, and the

Table II. Degradation of [¹⁴C]isazofos in Soil As Affected by Its Previous Use (1986)^a

	¹⁴ C recovered in % of applied [¹⁴ C]isazofos after X years of isazofos application			
	X = 0	X = 1	X = 2	X = 3
isazofos	17.9 (0.4)	24.9 (2.1)	37.0 (0.3)	1.9 (0.3)
5-chloro-1-isopropyl-3-triazolol	10.1 (0.1)	7.6 (0.7)	4.3 (0.1)	10.3 (0.6)
soil-bound	49.1 (1.6)	45.6 (0.2)	37.4 (2.2)	54.6 (0.5)
¹⁴ CO ₂	5.5 (0.1)	6.6 (0.5)	7.6 (0.3)	9.9 (0.1)
others ^b	10.6 (1.8)	12.2 (2.0)	5.4 (0.5)	16.6 (0.3)
total	93.2 (0)	96.9 (5.1)	96.1 (1.8)	93.3 (1.8)

^a Results after a 3-week incubation are the means of duplicate test. Numbers in parentheses represent standard error. ^b Includes traces of other metabolites, water-soluble products, and volatile products other than ¹⁴CO₂.

Table III. Degradation of [¹⁴C]isazofos in Soil As Affected by Its Previous Use (1987)^a

	¹⁴ C recovered in % of applied [¹⁴ C]isazofos after X years of isazofos application				
	X = 0	X = 1	X = 2	X = 3	X = 4
isazofos	33.5 (0.8)	2.8 (0.1)	9.2 (0.4)	9.3 (0.4)	3.2 (0.1)
5-chloro-1-isopropyl-3-triazolol	18.6 (0.8)	29.8 (0.9)	24.0 (0.2)	23.0 (0.3)	23.5 (0.5)
soil-bound	28.1 (0.1)	47.3 (0.5)	42.7 (0.8)	40.3 (1.3)	40.8 (0.5)
¹⁴ CO ₂	3.5 (0.4)	3.4 (0.2)	6.2 (0)	6.3 (0.4)	7.1 (0.2)
others ^b	4.6 (1.7)	6.5 (0.6)	5.8 (0.1)	7.1 (0.2)	8.3 (0.6)
total	88.3 (2.0)	89.8 (2.3)	87.9 (1.3)	86.0 (0.8)	82.9 (1.5)

^a Results after a 3-week incubation are the means of duplicate tests. Numbers in parentheses represent standard error. ^b Includes traces of other metabolites, water-soluble products, and volatile products other than ¹⁴CO₂.

Table IV. Degradation of [¹⁴C]isazofos in Soil As Affected by Its Previous Use (1988)^a

	¹⁴ C recovered in % of applied [¹⁴ C]isazofos after X years of isazofos application					
	X = 0	X = 1	X = 2	X = 3	X = 4	X = 5
isazofos	66.7 (1.7)	63.1 (1.1)	5.3 (0.2)	79.0 (2.0)	62.5 (0.6)	7.3 (0.3)
5-chloro-1-isopropyl-3-triazolol	5.2 (0.6)	6.3 (0.3)	26.9 (0.3)	4.2 (1.3)	5.3 (0.2)	21.3 (1.2)
soil-bound	12.9 (0.7)	12.3 (0.5)	44.9 (2.6)	8.1 (0.4)	17.2 (0.4)	43.7 (1.2)
¹⁴ CO ₂	1.2 (0)	0.7 (0)	2.7 (0)	0.3 (0)	0.9 (0.1)	4.7 (0.1)
others ^b	3.7 (0.2)	4.6 (0.7)	12.6 (0.2)	3.9 (0.9)	5.2 (0.1)	13.9 (1.2)
total	89.7 (3.2)	87.0 (0.2)	92.4 (2.5)	95.5 (3.8)	91.1 (1.4)	90.9 (3.8)

^a Results after a 3-week incubation are the means of duplicate tests. Numbers in parentheses represent standard error. ^b Includes traces of other metabolites, water-soluble products, and volatile products other than ¹⁴CO₂.

bacterial activity is generally reduced when the soil pH falls below 6.0 (Walker, 1987). High pH favors both bacteria and biological degradation, as well as chemical degradation by hydrolysis. Similar observations of the influence of pH on pesticide persistence have been made by Somasundaram et al. (1987) and by Walker (1987).

Although the soil with no prior exposure to isazofos had a pH slightly higher than that of soil with 2–4-year histories, isazofos persisted well in the previously untreated soil. The instability of isazofos seems to be influenced by high soil pH or by a combination of high pH and repeated applications.

1988. The field residue data for 1988 are presented in Figure 4. Three weeks after application, 76% of applied isazofos remained in soils to which isazofos had been applied for the first time, as compared with 49–60% in other treatments. Throughout the 10-week field study, isazofos was less persistent in 2-, 4-, and 5-year treatments than in 1- and 3-year soils. The degradation of isazofos was positively correlated with soil pH value obtained for 1988 sampling ($P < 0.10$).

At the end of the 3-week laboratory incubation, more than two-thirds of the applied [¹⁴C]isazofos was recovered in soils with no prior exposure (Table IV). Sixty-three to 79% of the applied isazofos persisted in soils with 1-, 3-, or 4-year histories. A dramatic decrease in the persistence of isazofos was observed in soils previously treated for 2 and 5 consecutive years, with less than 10% of applied isazofos remaining in those two treatments. Soils with 2- and 5-year histories of isazofos had high pHs, 7.4 and 6.9, respectively, compared with that of other treatments studied (Table I). These data constitute a strong linear

trend ($R^2 = 0.73$, $P < 0.05$) of decreasing persistence of isazofos with increasing pH. The rapid degradation of isazofos in high-pH soils is similar to our findings in field and laboratory studies in 1986 and 1987. In 1987, reduced persistence of isazofos was observed in soil treated for the first time; that soil had a pH of 7.9.

Two different patterns of degradation products were formed in aggressive (2- and 5-year) and normal (1-, 3-, and 4-year) soils (Table IV). Rapid degradation of isazofos in aggressive soils produced more of the triazolol metabolite (21.3–26.9% in aggressive soils compared with 4.2–6.3% in normal soils). The greater levels of hydrolysis metabolite formation in aggressive soils are consistent with the activity of microbial esterases documented to hydrolyze a variety of organophosphorus insecticides (Munnecke et al., 1982). The aggressive soils also produced increased levels of water-soluble products and ¹⁴CO₂ relative to the normal soils. Those products were apparently formed as products of microbial metabolism. ¹⁴CO₂ evolution, however, was still a relatively minor component of total ¹⁴C, which suggests that soil microorganisms are not well adapted to metabolize the triazole ring. The triazolol metabolite is probably the major component in the formation of bound residues. Thus, in aggressive soils, in which great quantities of the triazolol are produced, increased bound residue levels are expected. These differences in the quantity of degradation products formed in aggressive soils and in normal soils are consistent with our findings from 1986 and 1987 laboratory studies.

1989. Field studies were conducted for the sixth and final year during the 1989 growing season. The treatments included soils applied with isazofos for 1–6 years. Three weeks after field application, only 11% of applied isazofos

Table V. Effect of Autoclaving on the Degradation of [¹⁴C]Isazofos (1988)^a

	¹⁴ C recovered in % of applied ¹⁴ C[isazofos] after X years of isazofos application					
	X = 0	X = 1	X = 2	X = 3	X = 4	X = 5
isazofos	73.2 (1.2)	83.5 (7.0)	71.9 (1.5)	81.7 (2.8)	76.8 (0.4)	86.9 (2.8)
5-chloro-1-isopropyl-3-triazolol	2.5 (0.5)	3.8 (1.6)	3.1 (0.3)	3.5 (0.4)	3.7 (0.9)	4.7 (0.2)
soil-bound	7.7 (0.3)	7.7 (0.1)	12.1 (0.9)	7.7 (0.4)	9.5 (0.3)	11.2 (0.4)
¹⁴ CO ₂	0.4 (0.3)	0.1 (0)	0.1 (0)	0.2 (0)	0.1 (0)	0.1 (0)
others ^b	2.1 (0.3)	3.0 (0.2)	3.0 (0.3)	2.2 (0.1)	3.5 (0.1)	1.9 (0.6)
total	85.9 (2.6)	98.1 (41.3)	90.2 (2.4)	95.3 (2.9)	93.6 (1.7)	104.8 (4.0)

^a Results after a 3-week incubation are the means of duplicate tests. Numbers in parentheses represent standard error. ^b Includes traces of other metabolites, water-soluble products, and volatile products other than ¹⁴CO₂.

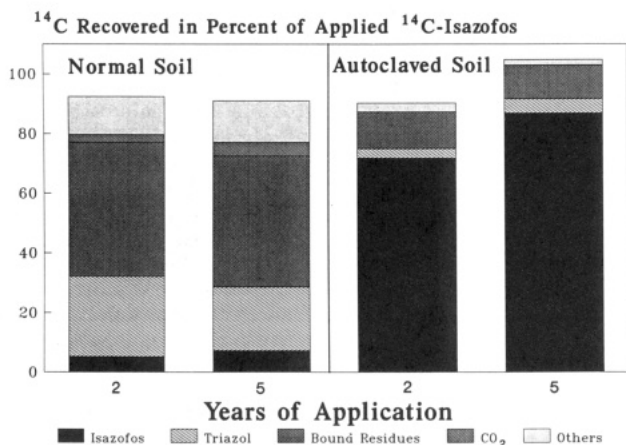


Figure 6. Effect of sterilization on isazofos degradation.

remained in soils previously treated with this chemical for 6 consecutive years as compared with 55–72% in other treatments (Figure 5). This dramatic decrease in the persistence of isazofos, however, was not observed in soil treated for 5 continuous years. In the fifth year of treatment, 66 and 33% of isazofos remained 3 and 6 weeks after application, respectively. The greater persistence of isazofos in the 5-year soil reveals that prior treatment is not the primary factor influencing isazofos degradation in soil. The pH is the major difference between 5- and 6-year soils, and the higher pH in the latter may explain the increased degradation rate. The rate of degradation was also relatively high in the 3-year treatment (as compared with 1-, 2-, 4-, and 5-year treatments), and this soil had a high pH (7.4). Three- and 6-year soils were collected from the same plots that resulted in increased degradation of isazofos in 1988 (2- and 5-year treatments) and in 1987 (1- and 4-year treatments). A significant correlation ($R^2 = 0.94$ and 0.92 , $P < 0.05$) was observed between soil pH and isazofos remaining in soil after 6 and 10 weeks of the field study.

Role of Microorganisms. The increased degradation of isazofos was observed only in two treatments (for 2- and 5-year soils in 1988), and no correlation existed between the rate of degradation and the years of isazofos application. To identify the mechanism of degradation in 2- and 5-year soils, the two soils were autoclaved, and complete metabolism studies were conducted in comparison with nonautoclaved soils. Seventy-two to 87% of the applied isazofos remained in all soils upon completion of the 3-week laboratory incubation (Table V). No increase in the degradation of isazofos was observed in the autoclaved 2- and 5-year soils (Figure 6). The increased persistence of isazofos in sterilized soils illustrates the role of microorganisms in its degradation. Our findings and previous research (Bowman, 1993) confirm that microbial degradation is the major route of isazofos degradation in soil.

Table VI. Amount of Microbial Biomass Carbon in 1988 and 1989 Soils

years of isazofos application	extractable C, ^a μg g ⁻¹ of soil	
	1988	1989
0	238	284
1	128	214
2	191	163
3	140	271
4	297	212
5	258	249
6	X ^b	194

^a Results are the means of duplicate samples. ^b Plot treatment not available at this time.

The correlation between greater degradation of isazofos in soil and soil pH as observed in both field and laboratory studies does not establish the contributions of chemical or biological mechanisms. Both pathways are probably operative, and both would result in the formation of triazole metabolite. The persistence of isazofos in the autoclaved soil (Figure 6) indicates that chemical hydrolysis is a minor component. The effect of pH on isazofos degradation may be indirect, in that it can alter microbial activity and biomass.

Estimation of Soil Microbial Biomass. The extractable carbon varied from 140 to 297 μg g⁻¹ of soil for 1988 treatments and from 163 to 284 μg g⁻¹ for 1989 treatments (Table VI). Although there was variation among the treatments studied, no strong correlation ($R^2 = 0.33$ and 0.45) was observed between pH and biomass for either year. The biomass data reflect the total microbial activity, and the lack of correlation of these data with pH (and thus isazofos degradation) can be attributed to the presence of specific isazofos-degrading microorganisms in the soils studied.

Enumeration of Isazofos-Degrading Soil Microorganisms. Two weeks after the addition of 10 ppm of isazofos to the mineral salts medium (containing no serial dilution of the test soils), only 0.78 ppm of isazofos remained. The pH of the mineral salts medium was 7.2. The degradation of isazofos in the mineral salts medium was similar to our field and laboratory findings on the degradation of isazofos in the pH range 7.0–8.0. No isazofos was recovered in 10⁻¹ or 10⁻² dilutions of the 2-year soil. The concentration of isazofos increased with increases in dilution (data not given), confirming the role of microorganisms in the degradation of isazofos. To determine whether the degradation of isazofos occurred because of the high concentration of the mineral salts medium, studies were conducted with 1/10 the original concentration of the mineral salts medium. Isazofos degradation was fast in that low concentration also. Increased degradation of isazofos was also observed in the controls kept at 4 °C. Because of the instability of isazofos in the mineral salts medium, differentiation between MPN tubes containing degraders and those without degraders became increas-

Table VII. Sorption of Isazofos by 1988 Soils

years of isazofos application	K_d , ^a mL/g	years of isazofos application	K_d , ^a mL/g
0	1.43 ^{ab}	3	1.84 ^a
1	2.82 ^a	4	2.78 ^a
2	0.94 ^b	5	0.81 ^b

^a Mean values followed by the same letter are not significantly different ($P > 0.05$) as determined by the analysis of variance and LSD procedures.

ingly difficult for the more dilute MPN tubes. Therefore, specific values were not calculated.

Sorption Studies. The degradation of isazofos in high pH soils under both laboratory and field conditions and the role of microorganisms (as illustrated by the autoclaved soils) in those soils indicate that a pH-microbial interaction may be responsible for the decreased persistence of isazofos in some soils. To investigate the influence of soil pH on the availability of isazofos to microorganisms, sorption studies were conducted with 0-5-year soils collected at the end of the 1988 growing season. Although the experimental plots were adjacent and in close geographic proximity, the adsorption coefficient (K_d) ranged from 0.81 to 2.82 (Table VII). The variability in adsorption coefficient for a pesticide within a field has been demonstrated (Wood et al., 1987). A significant correlation existed between the degradation of isazofos (under both field and laboratory conditions) and K_d values. The K_d values of 0.94 and 0.81, respectively, for 2- and 5-year soils, were lower than in the other soils. An inverse correlation was observed between pH and K_d values for isazofos ($R^2 = 0.71$, $P < 0.05$). Similar effects of pH on adsorption of isazofos have been observed (Ciba-Geigy Corp., 1983). The effect of pH on the adsorption of several pesticides to soil has been well documented (Renner et al., 1988; Clay et al., 1988). The increased availability of isazofos to microorganisms in high pH soils may also contribute to the rapid degradation observed in such soils.

In summary, the degradation of isazofos was most rapid in soils with relatively high pH (pH ≥ 6.9). An interaction between soil pH and microbial degradation of isazofos was observed. Soil pH seemed to be a more important factor influencing the degradation of isazofos than did number of years of pretreatment. High soil pH could influence the degradation of isazofos by providing an environment relatively conducive to the growth of bacteria that degrade isazofos and/or by increasing the availability of isazofos to degraders.

ACKNOWLEDGMENT

This project was partly supported by the Ciba-Geigy Corp. We thank Homer LeBaron, Steve Dumford, and Chuck Kern of Ciba-Geigy for their cooperation. We gratefully acknowledge the assistance of Jim Oleson and Jon Tollefson in conducting the field studies.

LITERATURE CITED

Bowman, B. T. Mobility and persistence of alachlor, atrazine and metolachlor in plainfield sand, and atrazine and isazofos in honeywood silt loam, using field lysimeters. *Environ. Toxicol. Chem.* 1990, 9, 453-461.

Bowman, B. T. Use of field lysimeters for comparison of mobility and persistence of granular and EC formulations of the soil insecticide isazofos. *Environ. Toxicol. Chem.* 1991, 10, 873-879.

Bowman, B. T. Mobility and persistence of isazofos in granular and microencapsulated formulations in two soils, using field lysimeters. *Pestic. Sci.* 1993, in press.

Chapman, R. A.; Harris, C. R. Persistence of isofenphos and isazofos in a mineral and an organic soil. *J. Environ. Sci. Health* 1982, B17, 355-361.

Ciba-Geigy Corp. "Technical release on CGA-12223"; Agricultural Division, Greensboro, NC, 1983.

Clay, S. A.; Koskinen, W. C.; Allmaras, R. R.; Dowdy, R. H. Differences in herbicide adsorption on soil using several pH modification techniques. *J. Environ. Sci. Health* 1988, B23, 559-573.

Hamlen, R. A.; Scjroeder, W. J.; Beavers, J. B. Toxicity and persistence of surface applied and soil incorporated insecticides against *Diaprepes abbreviatus* larvae. *J. Econ. Entomol.* 1979, 72, 131-134.

Kaufman, D. D.; Kearney, P. C. Microbial degradation of isopropyl-N-3-chlorophenyl carbamate and 2-chloroethyl-N-3-chlorophenylcarbamate. *Appl. Microbiol.* 1965, 13, 443-446.

Lehmicke, L. G.; Williams, R. T.; Crawford, R. L. ¹⁴C-most-probable-number methods for enumeration of active heterotrophic microorganisms in natural waters. *Appl. Environ. Microbiol.* 1979, 38, 644-649.

Munnecke, D. M.; Johnson, L. M.; Talbot, H. W.; Barik, S. Microbial metabolism and enzymology of selected pesticides. In *Biodegradation and Detoxification of Environmental Pollutants*; Chakrabarty, A. M., Ed.; CRC Press: Boca Raton, FL, 1982; pp 1-32.

Niemczyk, H. D.; Krueger, H. R. Persistence and mobility of isazofos in turfgrass thatch and soil. *J. Econ. Entomol.* 1987, 80, 950-952.

Renner, K. A.; Meggitt, W. F.; Penner, D. Effect of soil pH on imazaquin and imazethapyr adsorption to soil and phytotoxicity to corn. *Weed Sci.* 1988, 36, 78-83.

Sears, M. K.; Chapman, R. A. Persistence and movement of four insecticides applied to turfgrass. *J. Econ. Entomol.* 1979, 72, 272-274.

Somasundaram, L.; Coats, J. R. Influence of pesticide metabolites on the development of enhanced biodegradation. In *Enhanced Biodegradation of Pesticides in the Environment*; Racke, K. D., Coats, J. R., Eds.; American Chemical Society: Washington, DC, 1990; pp 128-140.

Somasundaram, L.; Racke, K. D.; Coats, J. R. Effect of manuring on the persistence and degradation of soil insecticides. *Bull. Environ. Contam. Toxicol.* 1987, 39, 579-586.

Somasundaram, L.; Coats, J. R.; Racke, K. D. Degradation of pesticides in soil as influenced by the presence of hydrolysis metabolites. *J. Environ. Sci. Health* 1989, B24, 457-478.

Tate, K. R.; Ross, D. J.; Feltham, C. W. A direct extraction method to estimate soil microbial C: Effects of experimental variables and some different calibration procedures. *Soil Biol. Biochem.* 1988, 20, 329-335.

Toba, H. H.; Turner, J. E. Chemical control of wireworms on potatoes. *J. Econ. Entomol.* 1979, 72, 636-641.

Walker, A. Further observations on the enhanced degradation of iprodione and vinclozolin in soil. *Pestic. Sci.* 1987, 21, 219-231.

Wood, L. S.; Scott, H. D.; Marx, D. B.; Lavy, T. L. Variability in sorption coefficients of metolachlor on a captina silt loam. *J. Environ. Qual.* 1987, 16, 251-256.

Worthing, C. R. *The Pesticide Manual*, 9th ed.; British Crop Protection Council: Surrey, U.K., 1991; p 502.

Wu, J.; Joergensen, R. G.; Pommerning, B.; Chaussod, R.; Brookes, P. C. Measurement of soil microbial biomass C by fumigation-extraction-An automated procedure. *Soil Biol. Biochem.* 1990, 22, 1167-1169.

Zeyer, J.; Kearney, P. C. Microbial degradation of par-chloroaniline as sole carbon and nitrogen source. *Pestic. Biochem. Physiol.* 1982, 17, 215-223.