JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effects of Moisture, Temperature, and Biological Activity on the Degradation of Isoxaflutole in Soil

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The effects of several environmental factors on the dissipation, transformation, and mineralization of isoxaflutole were investigated in laboratory incubations. In the soil, isoxaflutole hydrolyzed to a diketonitrile derivative, which is the active form of the herbicide. The diketonitrile was then metabolized to an inactive benzoic acid derivative and later into two unknown products, which were found only in small quantities. Degradation of isoxaflutole was faster in soil maintained at −100 or −1500 kPa compared to that in air-dry soil. At 25 °C, the half-lives for isoxaflutole were 9.6, 2.4, and 1.5 days in air-dry, −1500 kPa, and −100 kPa moisture regimes, respectively. A simple Arrhenius expression described the response of isoxaflutole transformation (mineralization and transformation) to temperature in the range of 5 to 35 °C. An activation energy value (E_a) of 67 kJ/mol for isoxaflutole suggested the transformation of the herbicide to the diketonitrile derivative was primarily a chemical reaction. Moreover, biological activity had little effect on the hydrolysis of isoxaflutole, with half-lives of 1.8 and 1.4 days in sterile and nonsterile soil, respectively. However, the transformation of diketonitrile to benzoic acid and the production of the unknown products were greatly reduced in the sterile soil, suggesting one or more biologically mediated processes.

KEYWORDS: Herbicide degradation; herbicide dissipation; HPPD inhibitor; isoxaflutole

INTRODUCTION

Isoxaflutole (5-cyclopropyl isoxazol-4-yl-2-mesyl-4-trifluoromethylphenyl ketone) is a new preemergence herbicide for grass and broadleaf weed control in corn. This herbicide exhibits a new mode of action: inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme found in the pathway for carotenoid biosynthesis, and thus causes bleaching symptomology in susceptible weeds (*1*, *2*). Environmental benefits of isoxaflutole (IFT) include low use rate, rapid dissipation, and very low mammalian toxicity (*3*, *4*). Soil applications of IFT provide control of many weed species important in Midwest corn production, including common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), common lambsquarters (*Chenopodium album* L.), *Amaranthus* spp., kochia (*Kochia scoparia*), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), and velvetleaf (*Abutilon theophrasti*) (*5*).

In plants, soil, or water, a diketonitrile derivative (2-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)-3-oxopropanenitrile) is produced by opening of the isoxazole ring of IFT (**Figure 1**) (*3*, *6*, *7*). Isoxaflutole is a "pro-herbicide", because diketonitrile-IFT (DKN) is the form of the herbicide known to inhibit HPPD in plants (*6*). DKN is thought to be converted to an inactive benzoic acid derivative (2-mesyl-4-trifluoromethyl benzoic acid), which is mineralized in the soil (*3*, *6*). Isoxaflutole

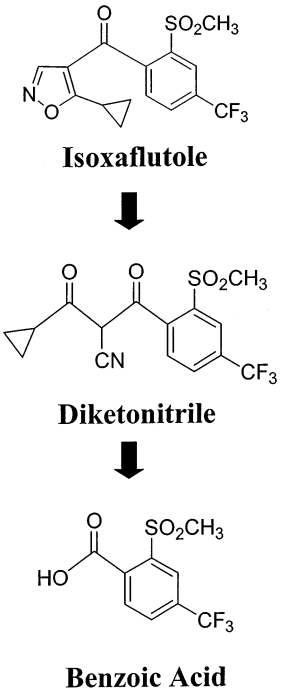

Figure 1. Degradation pathway of isoxaflutole in soil.

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10.1021/jf011486l CCC: $22.00
Published on Web 08/30/2002

and its metabolites have the potential to leach through the soil profile under conditions of high rainfall, but rapid degradation of isoxaflutole under field conditions reduces the likelihood of the parent reaching groundwater (*3*). Lacking, however, are studies that characterize the environmental fate of isoxaflutole under a range of conditions that are likely to be encountered in the field. Also scarce is detailed information on the environmental behavior of the herbicidally active form, DKN.

The objectives of this study were to determine the effects of temperature and moisture on IFT dissipation, transformation, and mineralization in controlled laboratory conditions, with particular attention given to the active DKN product. Biological dependence was also evaluated by examining activation energy for dissipation and the use of a metabolic inhibitor to suppress biological processes.

MATERIALS AND METHODS

Soil. All incubations were conducted in the laboratory using a Drummer silty clay loam (fine-silty, mixed, mesic Typic Endoaquolls) with pH 7.0 and 2.5% organic carbon. Composite samples of moist soil were collected between June and November, 1998, from the top 10 cm (A horizon) of fields which had not previously received an application of IFT. Soil was sieved through a 2-mm screen and stored at 5 °C in thin-walled polyethylene bags. Before the degradation studies were conducted, the soil was allowed to air-dry for at least 24 h.

Chemicals. In all studies, [*phenyl*(U)-^{14}C] IFT with specific activity of 909.1 MBq/mmol (2525 kBq/mg) and >99% radiopurity was used. The stability of the diketonitrile derivative of IFT was evaluated using [*phenyl*(U)-^{14}C] diketonitrile with the same specific activity as IFT and >99% radiopurity. Analytical standards of the parent herbicides, and two major metabolites, referred to herein as the benzoic acid derivative (benzoic acid-IFT) and DKN were used for confirmation of analyte identity. All radiolabeled and analytical grade forms of IFT, DKN, and benzoic acid-IFT were provided by Aventis (previously Rhone-Poulenc). Organic solvents and water were Optima grade, and were purchased from Fisher Scientific (Pittsburgh, PA).

Laboratory Incubations. Soil biometers were created using 480-mL wide-mouth Mason jars with airtight lids as described previously (*8*). The concentration of [^{14}C] isoxaflutole used in each study was 0.054 μg/g (136 Bq/g), equivalent to the field-used rate of 105 g ai/ha, assuming a 15-cm mixing depth in the field. To obtain a water pressure of −100 kPa, the appropriate amount of water was added to the bottom of the jar prior to the addition of air-dry soil. Soil samples (50 g oven-dry weight equiv) were transferred to the Mason jars containing the appropriate concentration of ^{14}C-isoxaflutole in water, and samples were thoroughly mixed after allowing the herbicide to be redistributed by advection for 4 h after treatment. A 20-mL scintillation vial containing 10 mL of 0.2 M NaOH was suspended from the lid above the soil to trap evolved CO_2. Treatments were incubated in the dark for the duration of the study. At 1, 3, 7, 14, 28, and 56 days after isoxaflutole application, 5-gram soil samples (oven-dry weight equiv) were removed and analyzed immediately for extractable and unextractable ^{14}C. Jars were opened to provide aeration, and the trap solution was replaced and analyzed at each sampling time, as well as weekly between longer sampling times. This procedure ensured that the biometer headspace contained sufficient oxygen to support microbial activity during the periods the jar was sealed.

Moisture Study. The moisture treatments included air-dry (25 g H_2O/kg dry soil), −1500 kPa (134 g H_2O/kg dry soil), −100 kPa (247 g H_2O/kg dry soil), and initially air-dry (for 14 d) followed by wetting to −100 kPa. Incubation was performed at 25 °C. For the moisture study only, the soil was treated in bulk with ^{14}C isoxaflutole (136 Bq/mL) in acetone using an air brush (Badger Air-Brush Co., Franklin Park, IL) pressurized with propane. Acetone was allowed to evaporate before moisture treatments were imposed. This procedure minimized isoxaflutole degradation (by avoiding exposure to water) prior to initiation of the study. Treatments with moisture contents of −100 kPa and −1500 kPa were established by adding water as determined by a soil moisture curve for the Drummer soil. In the samples that were initially air-dry, then wet at 14 d, additional sampling times at 15, 17, and 21 days after treatment (DAT) were included.

Temperature Study. Treatments were applied as described above, using a water content that achieved a water pressure of −100 kPa. Biometers were maintained at 5, 15, 25, or 35 °C, and sampled as described above.

Biological Dependence Study. The role of microorganisms in degradation was assessed by comparing results obtained in the presence of a microbial inhibitor in soil incubated at 25 °C. This was achieved with the addition of 3.68 mmol (1000 mg) $HgCl_2$/kg soil in 10 mL of Optima water, which was allowed to move through the soil by advection, and mixed thoroughly after 4 h. Mercuric chloride was chosen as a metabolic inhibitor as it minimizes problems with re-growth, and was shown to produce the fewest changes in soil chemical and physical properties (*9*). In biologically active soil treatments, an equivalent concentration of $CaCl_2$ was added to provide the same background of Cl^{-1}.

Sample Analyses. Soil was extracted by adding 10 mL of ethyl acetate to the 5-g soil samples in 50-mL PTFE (Teflon) centrifuge tubes, which were agitated on a reciprocating shaker for 4 h at 5 °C. After the 4-h agitation, samples were removed and centrifuged at 7800*g* for 10 min at 5 °C. A 1-mL sample of the supernatant was placed in a scintillation vial, evaporated in a vacuum hood, and combined with 15 mL of scintillation cocktail. Liquid scintillation spectrometry (LSS) was used to determine the total quantity of ^{14}C in the extractable phase. A 5-mL aliquot of the supernatant was concentrated by evaporation, and analyzed by thin-layer chromatography (TLC) on silica gel plates in a mobile phase of ethyl acetate/methanol/acetic acid (92:5:3). The TLC system had been previously shown to provide separation and quantification of IFT, the diketonitrile-IFT product, and a benzoic acid-IFT transformation product of diketonitrile-IFT(*2*). The remaining 4 mL of supernatant was discarded and the soil pellet was allowed to dry in a vacuum hood for 3 d. After air-drying, the soil pellet was ground into a fine powder and three subsamples (0.25−0.30 g) from each tube were combusted in a model OX-500 biological oxidizer (R. J. Harvey Instrument Co., Hillsdale, NJ), and the $^{14}CO_2$ released was quantified using LSS. Values obtained from soil combustion analysis were used to verify the fraction of applied ^{14}C in the unextractable phase. To determine the mineralization of radiolabeled materials, the NaOH solutions were analyzed for ^{14}C at each sampling time by adding 1 mL of the solution to 15 mL of scintillation cocktail, and after dark adaptation (to reduce chemiluminescence), radioactivity was counted using LSS.

Ethyl acetate and low-temperature (5 °C) extraction were chosen to achieve efficient recovery of IFT (95% of IFT aged up to 28 days at −5 °C) and to eliminate hydrolysis of the labile compound during the extraction process. Previous work had shown that IFT degraded rapidly to DKN in a solvent system containing water, particularly in the presence of soil (*10*). Ethyl acetate was sufficiently volatile to allow concentration of extracts with negligible degradation. Optimization of extraction recovery for IFT did not guarantee that all products were recovered with the same efficiency, thus the possibility exists that, in addition to bound residues, the unextractable fraction contained some unrecovered degradation products. This would result in an underestimation of quantities of these products that were formed.

Calculations. Half-lives for IFT were calculated based on first-order kinetics using the equation

$$\ln C = \ln C_o - kt \quad (1)$$

where C is the concentration of IFT at time t, C_o is the initial concentration, and k is the first-order rate constant (*11*). The plot of the natural log of the concentration against time provided a regression equation with a slope equal to k. The half-life ($t_{1/2}$) was calculated as follows:

$$t_{1/2} = (\ln 2)/k \quad (2)$$

For each treatment, dissipation of IFT was fit to a first-order rate model to determine the rate constants and half-lives. Measurements taken through the first two half-lives (initial degradation rate) were

used to determine these parameters, as the rate of degradation decreased over the long incubation times, possibly due to decreased microbial activity resulting from prolonged laboratory conditions or bioavailability limitation induced by slow desorption kinetics (*12*−*14*). The use of initial degradation rates was discussed by Lehmann et al. (*15*) in a similar experiment with the herbicide flumetsulam.

Activation energy values (E_a) were used to predict the relative contributions of chemical and biological processes to specific reactions (*16*). Activation energy was calculated from data obtained in the temperature study using the Arrhenius equation

$$\ln k = \ln A - (E_a/RT) \quad (3)$$

where k is the degradation rate constant (as calculated by the first-order kinetics, eq 2), A is an empirical constant, T is temperature (K), R is the universal gas constant (8.3145 J/K•mol), and E_a is expressed in kJ/mol.

Safety. Caution and containment must be employed in the conduct of aerosol application of radiolabeled herbicides because of the potential for respiratory exposure. The use of mercuric chloride for inhibition of biological activity poses an environmental hazard, and thus should be kept to a minimum.

Statistics. To facilitate statistical analysis, all treatments were conducted in triplicate. Data were subjected to analysis of variance using SAS software (SAS Institute, Cary, NC), and means were separated using Fisher's protected LSD at the 5% significance level in the tables and figures.

RESULTS AND DISCUSSION

In the studies reported herein, four radiolabeled polar products were detected in addition to the parent IFT. The primary products (occurring ubiquitously and in high concentrations) were identified as diketonitrile-IFT and benzoic acid-IFT based on LCMS analysis (m/z = 358 and 267, respectively) and confirmed by HPLC co-chromatography with authentic standards. Identity of the IFT and DKN were confirmed in each study using ^{14}C-isoxaflutole and DKN as standards during routine TLC analysis. The R_f values for IFT, DKN, and benzoic acid-IFT were 0.90, 0.63, and 0.28, respectively, and were nearly identical to those reported previously (*2*) using the same TLC procedure. The unknown products, D and E, exhibited R_f values of 0.75 and 0.81, respectively. Insufficient mass was obtained for mass spectroscopic identification of these minor products.

From the analysis of the extractable phase, first-order half-lives for IFT were calculated from the initial IFT dissipation rate for each treatment (as described above). The first-order model adequately described the data (R^2 values ranged from 0.89 to 0.99), and thus the half-lives obtained were used to make the treatment comparisons that follow. Tailing of degradation kinetics in the latter phase of the incubation was attributed to factors other than treatments, such as decreased bioavailability, as described previously (*16*, *18*).

Moisture Study. The distribution of radiolabeled material among extractable, unextractable, and mineralized phases as a function of the four moisture regimes is given in **Table 1**. The total recovery of radiolabeled material was 97% averaged across moisture regimes and sampling days.

Mineralization was detected in the −100 kPa soil at 7 days sampling and in the −1500 kPa soil at 14 days. In both regimes at 56 days, mineralization increased to 9 and 28% of the applied ^{14}C in the −1500 and −100 kPa soils, respectively. No mineralization was detected in the air-dry soil over the duration of the study, likely due to lack of moisture for the initial hydrolysis of IFT to DKN. In the samples that were initially air-dry and subsequently adjusted to −100 kPa at 14 days, mineralization (0.2% of applied ^{14}C) was detected 7 days after wetting (data not shown), and accumulated to 11% by 56 days.

Table 1. Moisture Effects on ^{14}C Distribution (Reported as % ^{14}C Recovered) after the Application of 0.054 μg/mL ^{14}C-Isoxaflutole to the Soil during 54-d Incubations[a]

		soil moisture				
day	pool	air dry	−1500 kPa	−100 kPa	dry, wet[b]	LSD[c]
1	extractable[d]	68.4	60.1	51.6	70.0	3.9
	unextractable[e]	35.4	40.4	44.3	34.0	2.4
	mineralized	0.0	0.0	0.0	0.0	0.0
	total recovered	103.8	100.5	95.9	104.0	
3	extractable	36.5	44.1	41.4	-[f]	1.9
	unextractable	58.9	52.8	54.4	-	1.1
	mineralized	0.0	0.0	0.0	-	0.0
	total recovered	95.4	96.9	95.8	-	
7	extractable	15.1	37.4	35.5	-	1.0
	unextractable	82.1	58.0	58.0	-	1.1
	mineralized	0.0	0.0	0.1	-	0.1
	total recovered	97.2	95.4	93.6	-	
14	extractable	8.3	35.8	33.0	8.3	0.9
	unextractable	88.1	55.3	59.0	87.0	3.3
	mineralized	0.0	0.2	0.8	0.0	0.1
	total recovered	96.4	91.3	92.8	95.3	
28	extractable	13.1	33.1	27.8	35.4	3.9
	unextractable	82.4	61.2	58.2	59.3	3.5
	mineralized	0.0	1.9	6.8	0.6	0.9
	total recovered	95.5	96.2	92.8	95.3	
56	extractable	1.6	21.9	15.0	25.4	2.6
	unextractable	93.4	72.4	58.1	61.2	8.7
	mineralized	0.0	9.4	27.6	11.3	2.8
	total recovered	95.0	103.7	100.7	97.9	

[a] Data include three replications. [b] Samples were maintained air-dry initially, then were wet to –100 kPa after the 14-d sampling. [c] Least significant differences at alpha = 0.05. [d] Includes soil solution and the sorbed phase that could be extracted by agitation with ethyl acetate for 4 h. [e] Determined by oxidation of the soil and trapping ^{14}C. [f] Dash (–) indicates samples were not taken at 3 and 7 day timings because the treatment was identical to the air-dry treatment up to the 14 day sampling time.

The fraction of extractable ^{14}C ranged from 50% in the −100 kPa soil to 70% in air-dry soil at day one, and the remaining ^{14}C was detected in the unextractable phase. As observed for other herbicides (*16*, *18*), the extractable pool decreased over time, with 36 and 33% of the applied ^{14}C remaining extractable in the −1500 and −100 kPa moisture regimes, respectively, at 14 days. In dry soil, the loss of extractability was much more rapid, with only 8% remaining extractable by 14 days. The extraction procedure may have been less efficient for recovery of aged residues in the air-dry soil.

Transformation of IFT to DKN, benzoic acid-IFT, and other products was stimulated by the presence of water (**Figure 2**). In air-dry soil, IFT was slowly transformed to DKN, and other products were not detected until the last sampling time (56 d). In the −1500 kPa soil, transformation of IFT to DKN was much more rapid, with DKN accounting for 68% of the extractable phase at 3 days after treatment. After 7 days, the fraction of DKN began to decline as other products were formed, presumably at the expense of this intermediate. Benzoic acid-IFT was first detected in the extractable phase at 3 DAT and increased throughout the duration of the study to 17% at 56 days. Two unknown products were also detected at low concentrations later in the study, but each accounted for less than 15% of the extractable phase (approximately 5% of the total ^{14}C applied). Unknown products accounted for less than 30% of the extractable phase and less than 10% of the total ^{14}C under all conditions employed in the study. As a result, insufficient mass of either product accumulated to obtain a positive identification. At 56 DAT, the concentrations of benzoic acid and product D were

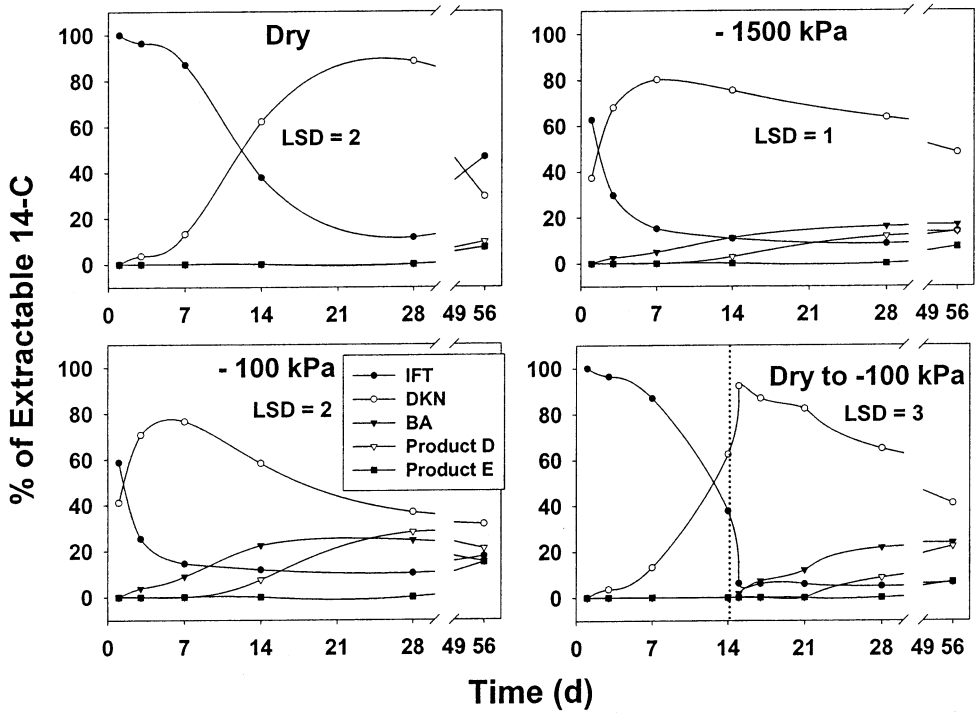

Figure 2. Soil degradation of isoxaflutole to diketonitrile, benzoic acid-IFT, and other products in four different moisture regimes: air-dry, −1500 kPa, −100 kPa, and air-dry for 14 d then wet to −100 kPa. Fisher's protected LSD at alpha = 0.05 is given for each moisture regime.

Table 2. First-Order Half-Lives of Isoxaflutole at Different Moisture Contents[a]

moisture	half-life (days)	rate constant (days^{-1})	R^2
air dry	9.6	0.07	0.95
−1500 kPa	2.4	0.29	0.92
−100 kPa	1.5	0.46	0.99

[a] Initial dissipation through two half-lives was used to determine values.

decreasing as a fraction of the extractable material in the soil maintained at −100 kPa. The half-lives for IFT were 9.6 days in air-dry soil, 2.4 d in −1500 kPa soil, and 1.5 d in −100 kPa soil (**Table 2**). The increase in the rate of conversion of IFT to diketonitrile-IFT with increasing soil moisture observed here may reflect a general feature of the fate of organic chemicals rather than a particular property of IFT, as similar trends have been observed with unrelated compounds such as the herbicides chlorimuron (*19*), cloransulam-methyl (*16*), and flumetsulam (*20*), as well as simple phenolics (*21*), which are degraded by different mechanisms in soil.

Degradation rates began to rapidly increase after the addition of water (−100 kPa) to previously air-dry soil at 14 DAT. One day after re-wetting, the majority of remaining IFT had been transformed to DKN, which accounted for 92% of the extractable phase at 15 days. This rapid transformation represents what could happen when rainfall occurs after application of the herbicide under dry conditions. Diketonitrile-IFT is more soluble than IFT (326 μg/mL versus 6.2 μg/mL), thus it may be more available for plant uptake under wet conditions. The high concentration of available DKN immediately following rainfall may help to explain reported "rechargeable activity" and sporadic cases of crop injury. Similarly, our previous work on the sorption of IFT revealed desorption of the parent compound coupled to hydrolysis to form DKN in solution (*10*). After 15 days, DKN was slowly transformed to benzoic acid-IFT and the unknown products, all of which are likely to be inactive.

Table 3. Temperature Effects on ^{14}C Distribution (Reported as % ^{14}C Recovered) after the Application of 0.054 μg/mL ^{14}C-isoxaflutole to the Soil during 54-d Incubations[a]

		incubation temperature					
		5 °C	15 °C	25 °C		35 °C	
day	pool	IFT	FT	IFT	DKN	IFT	LSD[b]
1	extractable[c]	78.9	63.5	53.9	40.5	41.2	3.6
	unextractable[d]	16.8	33.0	45.1	59.0	58.2	3.8
	mineralized	0.0	0.0	0.0	0.0	0.0	0.0
	total recovered	95.7	96.5	99.0	99.5	99.4	
3	extractable	66.9	47.4	40.3	36.1	34.5	2.1
	unextractable	27.9	51.5	56.9	63.9	63.0	6.6
	mineralized	0.0	0.0	0.0	0.1	0.1	0.1
	total recovered	94.8	98.9	97.2	100.1	97.6	
7	extractable	57.9	42.0	37.8	37.0	33.6	2.0
	unextractable	35.8	54.6	60.5	63.0	63.2	2.6
	mineralized	0.0	0.0	0.1	0.3	0.6	0.1
	total recovered	93.7	96.6	98.4	100.3	97.4	
14	extractable	46.0	36.6	35.4	33.9	30.7	1.6
	unextractable	50.1	59.0	63.1	65.5	63.8	6.0
	mineralized	0.0	0.1	0.8	1.5	2.9	0.2
	total recovered	96.1	95.7	99.3	100.9	97.4	
28	extractable	41.0	36.3	31.9	30.0	24.9	1.5
	unextractable	59.1	64.4	64.2	67.5	67.0	3.6
	mineralized	0.0	0.2	3.1	5.1	8.5	0.3
	total recovered	100.1	100.9	99.2	102.6	100.4	
56	extractable	36.9	24.0	26.0	22.5	20.1	2.1
	unextractable	55.3	72.1	57.1	57.8	58.8	3.9
	mineralized	0.1	0.6	8.5	12.9	16.2	0.3
	total recovered	92.3	96.2	91.6	93.2	95.1	

[a] Data include three replications. [b] Least significant differences at alpha = 0.05. [c] Includes soil solution and the sorbed phase that could be extracted by agitation with ethyl acetate for 4 h. [d] Determined by oxidation of the soil and trapping ^{14}C.

Temperature Study. The total recovery of applied radioactivity averaged 97% across four temperatures when added as IFT, and 99% at 25 °C when added as DKN (all sampling times pooled). The distribution of ^{14}C among the measured pools, however, was profoundly influenced by temperature (**Table 3**; **Figure 3**).

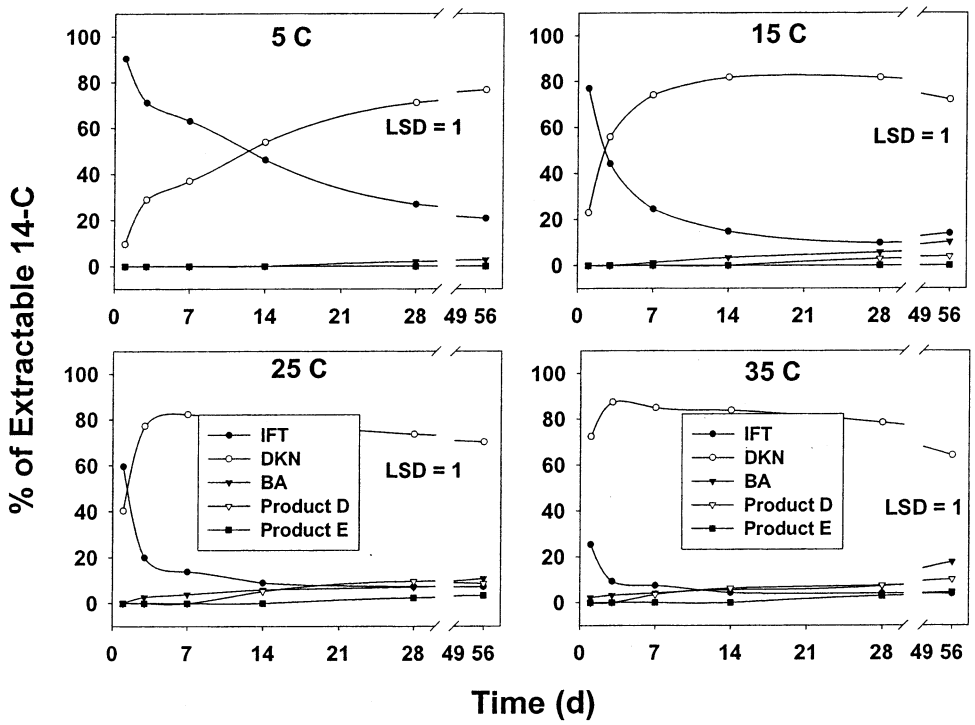

Figure 3. Soil degradation of isoxaflutole to diketonitrile, benzoic acid-IFT, and other products at different temperatures: 5, 15, 25, and 35 °C. Fisher's protected LSD at alpha = 0.05 is given for each temperature.

As expected, the rate of mineralization was positively affected by temperature. After 56 days of incubation, 16% of the applied ^{14}C appeared as CO_2 in the 35 °C treatment, whereas negligible mineralization had occurred in the 5 °C treatment. Mineralization was detected earlier and accounted for a greater proportion of radioactivity when added as ^{14}C-diketonitrile compared to ^{14}C added as IFT at 25 °C. This suggests that conversion to DKN is an important step in the path toward mineralization.

The ^{14}C in the extractable phase accounted for 40 to 80% of the applied radioactivity at the first sampling time, and the amount remaining in this phase over time was inversely related to temperature, owing to the combined effects of material loss (mineralization) and decrease in extractability. Throughout the duration of the study, the radiolabeled material was more efficiently extracted at lower temperatures than at high temperatures, likely due to less efficient extraction of degradation products and formation of bound residues compared to that of parent IFT. By the completion of the study, most of the ^{14}C material occurred in the unextractable phase, which probably included unrecovered transformation products in addition to soil-bound residues.

Composition of the extractable phase at the four incubation temperatures is shown in **Figure 3**. Product formation followed the same temporal sequence (IFT>DKN>benzoic acid-IFT>D>E) at each temperature, however the effect of temperature on the rate of formation of the products resulted in a different distribution of products at each temperature. At 5 °C, IFT transformation was restricted primarily to DKN, which accumulated to 77% of the extractable phase by the 56-d sampling time. Accumulation of the benzoic acid and products D and E was observed when the temperature was increased to 15 °C. The DKN derivative was the primary product at all temperatures throughout the study. The half-lives for dissipation of IFT were 13.9, 3.3, 1.3, and 0.8 d at 5, 15, 25, and 35 °C, respectively (**Table 4**). Similar relationships between temperature and degradation have been demonstrated with flumetsulam (*15*), metribuzin, metolachlor, and fluometuron (*22*).

Table 4. First-Order Half-Lives of Isoxaflutole at Different Temperatures[a]

temperature (°C)	half-life (days)	rate constant (days^{-1})	R^2
5	13.9	0.05	0.95
15	3.3	0.21	0.96
25	1.3	0.54	0.99
35	0.8	0.85	0.89

[a] Initial dissipation through two half-lives was used to determine values.

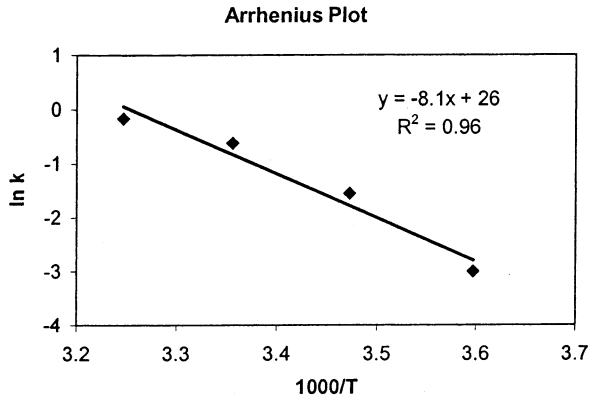

Figure 4. Arrhenius plot of isoxaflutole half-lives based on temperatures of 5, 15, 25, and 35 °C.

The first-order rate constants calculated at each temperature for IFT (**Figure 4**) were used to determine the activation energy (E_a) using the Arrhenius relationship described above (eq 3). On the basis of regression of the rate constant with temperature, an E_a of 67 kJ/mol was calculated for the dissipation of IFT in the soil. Previous studies have suggested that E_a values less than 30 kJ/mol are likely to represent biological mechanisms, whereas values greater than 60 kJ/mol have been reported for chemical reactions in the soil (*16*). It is assumed that catalyzed reactions,

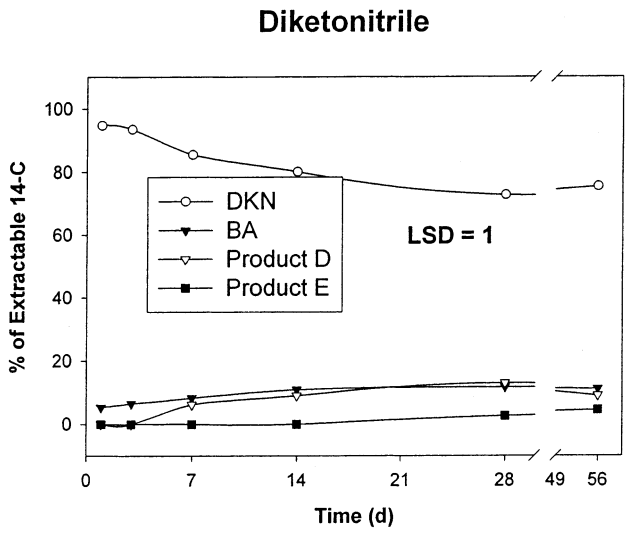

Figure 5. Soil degradation of diketonitrile to benzoic acid-IFT and other products at 25 °C and moisture content −100 kPa. Fisher's protected LSD at alpha = 0.05 is given for the transformation of diketonitrile.

such as enzyme-mediated biological processes, have a lower activation energy requirement, causing them to be less responsive to temperature compared to chemical reactions (*16*, *17*). Our results are consistent with abiotic conversion of IFT to DKN. Formation of minor products was similar at temperatures ranging from 15 to 35 °C, indicating these steps were less temperature responsive.

Fate of DKN. When labeled DKN was introduced into soil at 25 °C, benzoic acid-IFT was detected 1 day after the initiation of the study, with the latter compound accounting for 12% of the extractable phase after 28 d (**Figure 5**). Products D and E were also detected, indicating they arise from the DKN or benzoic acid-IFT, rather than from IFT, which was not present in this study. Product D accounted for 13% of the extractable phase (4% of total ^{14}C) at 28 d, but decreased to 9% (2% of total) by 56 d, while product E reached a maximum of 5% at 56 d (1% of total). The half-life for DKN was not calculated because it appeared to exceed the duration of the study. The results clearly show that DKN is much more stable in soil than the parent.

Biological Activity. A comparison of the ^{14}C recovered in the abiotic control (treated with $HgCl_2$) and the biologically active soil is given in **Table 5**. Total recoveries averaged 97 and 99% across all sampling times for nonsterile and sterile soil, respectively. No significant respiration was detected in the abiotic control (data not shown), verifying the efficiency of the inhibitor. As observed in studies reported above, mineralization was detected at 3 d in the biologically active soil and increased for the duration of the study to 4% of the applied ^{14}C at 56 d. With IFT, the percent of extractable ^{14}C was not significantly different between biologically active and abiotic control soils for most sampling times, indicating the inhibitor did not affect extraction efficiency.

In contrast to the effects of the inhibitor on mineralization, dissipation of IFT was similar in biologically active and abiotic control soils, though slightly faster kinetics occurred in the active soil (**Figure 6**). The IFT half-lives were similar (**Table 6**, 1.8 and 1.4 days, in the presence and absence of the inhibitor, respectively. This result is consistent with results reported above, which indicate that DKN is formed abiotically, and the other three products arise from DKN rather than IFT. Formation of the benzoic acid derivative probably involves microorganisms,

Table 5. Effects of Biological Activity on ^{14}C Distribution (Reported as % ^{14}C Recovered) after the Application of 0.054 μg/mL ^{14}C-Isoxaflutole to the Soil during 54-d Incubations[a]

		soil treatment		
day	pool	nonsterile	sterile	LSD[b]
1	extractable[c]	52.7	55.8	7.9
	unextractable[d]	43.8	41.4	6.9
	mineralized	0.0	0.0	0.0
	total recovered	96.5	97.2	
3	extractable	39.7	41.4	5.6
	unextractable	57.0	56.5	7.2
	mineralized	0.1	0.0	0.0
	total recovered	96.8	97.9	
7	extractable	37.7	36.8	3.0
	unextractable	58.4	63.2	3.4
	mineralized	0.3	0.0	0.1
	total recovered	96.4	100.0	
14	extractable	36.4	32.7	2.5
	unextractable	59.8	66.9	1.1
	mineralized	0.7	0.0	0.2
	total recovered	96.9	99.6	
28	extractable	31.4	30.1	2.7
	unextractable	63.3	70.3	6.6
	mineralized	1.9	0.0	0.3
	total recovered	96.6	100.4	
56	extractable	32.0	31.4	4.5
	unextractable	62.0	69.6	8.6
	mineralized	4.0	0.0	0.4
	total recovered	98.0	101.0	

[a] Data include three replications. [b] Least significant differences at alpha = 0.05. [c] Includes soil solution and the sorbed phase that could be extracted by agitation with ethyl acetate for 4 h. [d] Determined by oxidation of the soil and trapping ^{14}C.

as its production was reduced greatly in the abiotic control soil, with less than 5% benzoic acid-IFT detected at 56 DAT compared to 25% in the nonsterile soil. The absence of Products D and E in the control soil may indicate these products are also the result of biological reactions, or they are abiotically produced from benzoic acid-IFT. Biological processes have been shown to be important in the degradation of many herbicides including chlorsulfuron, triasulfuron (*23*), clomazone (*8*), diallate, triallate (*24*), 2,4-D, trifluralin, diuron, chloramben, and chlorpropham (*25*).

Collectively, the results indicate that the dependence of isoxaflutole dissipation on temperature and moisture explain the compound's "rechargeable activity". The initial degradation step is largely abiotic and produces a soluble product with herbicidal activity. It is expected that early in the growing season, when preemergence herbicides are applied, cooler temperatures will result in increased persistence of IFT, providing longer residual weed control. However, the potential for corn injury may also increase when low temperatures allow the herbicide to persist long enough in the soil to affect corn plants at a susceptible stage. The enzymatic processes involved in metabolizing DKN to inactive forms in the corn plant may also be slower in cool temperatures. The increase in herbicidal activity after a rainfall event may involve coupled desorption of IFT and hydrolysis to form the active DKN (*10*).

Knowledge of the processes involved in the degradation of DKN to inactive metabolites is critical for an understanding of the herbicide's unique residual activity in the soil. Sprague et al. (*7*) demonstrated that preemergence applications of DKN provided activity on corn and velvetleaf equal to that of isoxaflutole. Results presented here show that dissipation of diketonitrile is primarily a biological process and occurs much more slowly than isoxaflutole dissipation. Future research should

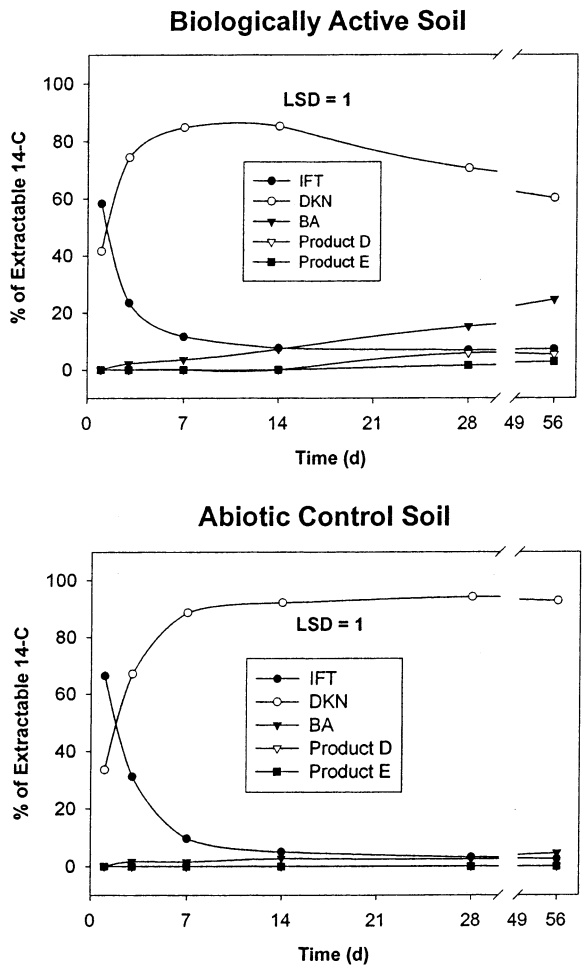

Figure 6. Soil degradation of isoxaflutole to diketonitrile, benzoic acid-IFT, and other products in sterile and nonsterile soil at 25 °C and moisture content −100 kPa. Fisher's protected LSD at alpha = 0.05 is given for sterile and nonsterile soil.

Table 6. First-Order Half-Lives of Isoxaflutole Related to Biological Activity[a]

soil	half-life (days)	rate constant ($days^{-1}$)	R^2
sterile	1.8	0.39	0.99
nonsterile	1.4	0.49	0.99

[a] Initial dissipation through two half-lives was used to determine values.

focus on DKN and the role it plays in residual activity, corn injury, and the potential for carryover to sensitive crops.

ACKNOWLEDGMENT

We appreciate the assistance of Gabe Tarr, Shawn Maskel, and Ryan Hultgren, University of Illinois, in the conduct of this research. We also acknowledge technical advice and the contribution of labeled isoxaflutole and DKN, as well as analytical standards of the herbicide and its products, provided by Aventis. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Received for review November 8, 2001. Revised manuscript received July 16, 2002. Accepted July 19, 2002.

JF011486L