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Fate of Isoxaflutole in Soil under Controlled Conditions

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Isoxaflutole (IFT, 5-cyclopropyl-1,2-oxazol-4yl-α,α,α-trifluoro-2-mesyl-*p*-tolyl ketone) is a new pre-emergence proherbicide used in maize and sugarcane. Its two main derivatives are a diketonitrile derivative, 2-cyano-3-cyclopropyl-1-(2-methanesulfonyl-4-trifluoromethylphenyl)propane-1,3-dione, called DKN, and a benzoic acid derivative, 2-methanesulfonyl-4-trifluoromethylbenzoic acid, called BA. Few data are available of the factors influencing the degradation of IFT in soil, and the purpose of the present work was to determine the relative importance of, and factors affecting, the degradation of IFT in soil. Experiments were conducted on five soils with distinct physicochemical characteristics, at different temperatures and moisture contents in biotic and abiotic conditions. The isomerization of IFT to DKN is rapid, increasing with higher moisture contents and higher temperatures. It depends strongly on pH and is governed by chemical processes. The degradation of DKN to BA appeared to be essentially due to the biological activity of the soil.

KEYWORDS: Soil; degradation; abiotic conditions; biotic conditions

INTRODUCTION

Isoxaflutole (IFT, 5-cyclopropyl-1,2-oxazol-4-yl-α,α,α-trifluoro-2-mesyl-*p*-tolyl ketone) is a new pre-emergence proherbicide (i.e., IFT is not the active ingredient) used in maize and sugarcane and applied at low doses (between 75 and 150 g ha^{-1}) to provide control of grass and broadleaf weeds. In soil, water, and vegetation, IFT is rapidly converted into a diketonitrile derivative, DKN [2-cyano-3-cyclopropyl-1-(2-methanesulfonyl-4-trifluoromethylphenyl)propane-1,3-dione], by opening of the isoxazole ring (*1*; Bayer CropScience France, personal communication). DKN is the active principle of the herbicide and acts by the inhibition of 4-hydroxyphenylpyruvate dioxygenase (4-HPPD), a specific enzyme affecting carotenoid synthesis (*2*, *3*). This inhibition leads to bleaching in susceptible weed species, followed by growth suppression and necrosis. In soil and plants, DKN undergoes degradation, leading to a benzoic acid derivative, BA (2-methanesulfonyl-4-trifluoromethylbenzoic acid), which is biologically inactive (**Figure 1**).

Degradation studies in soils are essential for the evaluation of the persistence of pesticides and their breakdown products. Data on the rate of degradation and on the factors influencing this degradation are extremely important, as they permit the prediction of the levels likely to remain in soil and allow assessment of the potential risk associated with exposure. Fate and transport of pesticides are affected by many factors involving micro-organisms, soil constituents, and physicochemical properties of the compound considered (*4*, *5*). Microbial pesticide metabolization may be influenced by pedoclimatic factors (temperature, moisture content, aeration) and also depend on retention processes (*6*, *7*), which affect the bioavailability of the compound. Abiotic degradation is dependent on physicochemical properties of soil such as the content and quality of clays and the pH of the soil solution (*8*, *9*).

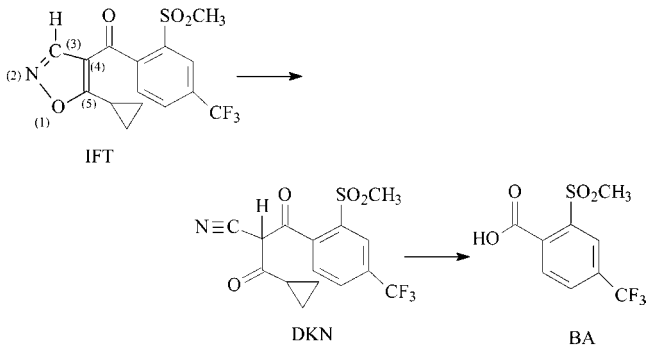

Figure 1. Isoxaflutole (IFT) and its two main derivatives, DKN and BA.

Few data are available on the factors influencing the degradation of IFT in soil. Previous hydrolysis studies have shown that the degradation of IFT in aqueous solutions occurs more rapidly at higher pH and at higher temperatures and that this degradation is dependent on the buffer components (*10*, *11*). The purpose of the present work was to determine the relative importance of, and factors affecting, the degradation of IFT in soil. Experiments were conducted on five soils with different physicochemical characteristics at different temperatures and moisture content levels in biotic and abiotic conditions.

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Table 1. Physical and Chemical Properties of the Five Soils Studied

	B	C	O	M2	M1
FAO[a] classification	clay	clay	sand loam	clay loam	clay loam
pH[b]	8.30	4.83	6.07	8.60	8.00
clay content (%)	46.4	40.9	9.4	19.5	21.9
sand content (%)	32.4	28.9	73.8	48.6	47.1
silt content (%)	21.2	30.2	16.7	32.0	31.1
organic carbon content (%)	1.41	1.68	1.06	0.66	3.15
CEC (cmol/kg)	17	5	2	8	10
Ca^{2+} (cmol/kg)	11	2	1	7	9
K^+ (cmol/kg)	2	0.5	0.2	0.2	0.6
Na^+ (cmol/kg)	<0.1	<0.1	<0.1	<0.1	0.1
Al^{3+} (cmol/kg)	0	1	<0.1	0	0

[a] FAO, Food and Agriculture Organization. [b] pH measured in water, in the absence of pesticide.

MATERIALS AND METHODS

Soils. Five soils (O from south of France, B from west of France, C from Martinique, and M1 and M2 from Mediterranean areas) were chosen on the basis of their different physical and chemical properties (**Table 1**). The soils named M1 and M2 (0−10 cm and 10−20 cm layers, respectively) were sampled in the field where it was proposed to conduct a mobility study (unpublished data). All five soils were air-dried, passed through a 2-mm sieve, and stored at +4 °C before the experiments were carried out. The soil M1 was also used for the study in abiotic conditions after sterilization by autoclaving (three times at 120 °C with 24-h intervals), and the corresponding sterile soil was named M1S.

Chemicals and Materials. IFT, DKN, and BA of analytical standard purity were supplied by Aventis CropScience (Ongar, UK). The organic solvents (propanol-2, methyl alcohol, and acetonitrile for HPLC) and sulfuric acid (96%) were supplied by Carlo Erba, sterile and apyrogenic water by B. Braun Medical S.A., trifluoroacetic acid (99% for analysis) by R. P. Normapur, and (trimethylsilyl)diazomethane (TMSD, 2.00 M in hexane) by Aldrich. Hydrochloric acid (96% supplied by Prolabo) was prepared at 0.10 M by dilution in apyrogenic water. Aqueous spiking solutions of IFT (50, 25, and 2.5 mg/L) were prepared by dilution of 1000 mg/L methanolic solution in sterile water.

Treatment and Sampling. Since preliminary experiments showed that the initial content of IFT did not influence the rate constant of IFT isomerization, this initial content was set to 10 mg/kg of soil for the entire study. All the experiments were conducted in duplicate in the dark with 10 g of soil sample in closed jars. Sampling was modulated according to temperatures of incubation. In any of the incubation conditions, moisture content was regularly verified and adjusted by addition of distilled water when necessary.

Degradation in Biotic Conditions. The influence of moisture content was studied on soil O at 30 °C. The moisture contents were 17% (2 mL of IFT solution at 50 mg/L), 29% (4 mL at 25 mg/L), and 45% (8 mL at 12.5 mg/L), maintained constant throughout the experiment. The influence of temperature was studied in soil M1 at 17% moisture content, and five different temperatures were applied: 10 ± 1, 20 ± 1, 30 ± 1, 40 ± 1, and 60 ± 1 °C. The study of the influence of the physicochemical properties of soil was conducted on the five soils C, O, B, M1, and M2 at 30 °C and with a moisture content of 17%.

Degradation in Abiotic Conditions. The study was conducted on soils M1 and M1S at 30 °C and at 17% moisture content.

Analytical Procedure. A soil sample (10 g) was placed in a centrifugation tube, maintained in an ice bath to prevent any degradation of IFT into DKN, and extracted twice with CH_3CN/H_2O/0.1 M HCl (60/40/0.8 v/v/v) for 20 min with stirring. After centrifugation, the supernatant was removed and kept at −18 °C for 2 h to allow separation of the aqueous and organic phases. The organic phase was removed, and acetonitrile was added to the aqueous phase. The two organic phases were combined and evaporated just to dryness, and the residue was taken up by 3.0 mL of the mobile phase (extract 1). Extract 1 was divided into two aliquots: the first was injected into an HPLC/UV system for IFT and DKN analysis; the second aliquot was used for analysis of BA by GC coupled with an electron-capture detector (ECD) and GC/MS after derivatization, due to an interference on the HPLC/UV chromatogram (**Figure 2**).

Derivatization of BA. A 0.4-mL amount of acidified 2-propanol (C_3H_7OH/0.10 M H_2SO_4, 90/10 v/v) and 0.6 mL of TMSD were added to 0.5 mL of extract 1 and maintained at 35 °C under N_2 flow for 10 min. The volume was then adjusted to 1.5 mL with acetone (extract 2), and the sample was analyzed by GC/ECD, with confirmation by GC/MS.

Instrumental Analysis. Extract 1 was analyzed by HPLC/UV, using a Shimadzu LC-10A pump with an SPD 10AVP autoinjector (injected volume, 20 μL). The compounds were detected by their UV absorbance at 267 nm (IFT) and 290 nm (DKN) using a Shimadzu SPD-10AVP UV detector. The stationary phase used was a C_{18} Hypersil ODS column, 5 μm, 250 mm × 4.6 mm, from Supelco; the mobile phase was $CH_3CN/H_2O/CF_3COOH$ (48/52/0.5 v/v/v), delivered at 1 mL/min. Under these conditions, the retention times of IFT and DKN were 14.6 and 13.7 min, respectively. Identification and analysis were performed by injection of analytical standards for comparison. Data were collected using Star software (Varian). It was verified that no compound interfered with IFT and DKN during chromatographic analysis by injecting a blank soil extract.

Extract 2 was analyzed by gas chromatography coupled with an ECD. The chromatographic conditions were as follows: GC Varian 3350 equipped with a ^{63}Ni ECD; injector temperature, 185 °C; detector temperature, 300 °C; column, HP-1701, 15 m, 0.25 mm i.d., 0.25 μm film thickness; initial oven temperature, 90 °C for 1 min raised at 15 °C/min to 240 °C and held for 25 min; carrier gas, nitrogen at a flow rate of 1.8 mL/min; makeup gas, nitrogen q.s.p. 25 mL/min. In these chromatographic conditions, the retention time of BA was 10.85 min. Confirmation of positive results was done by GC/MS analysis. The chromatographic conditions were as follows: GC HP 5890 connected to an HP MSD 5971A mass spectrometric detector; electron impact mode, 70 eV; splitless injection mode; injector temperature, 175 °C; detector temperature, 280 °C; column, DB 5 JW scientific, 30 m, 0.25 mm i.d., 0.25 μm film thickness; initial oven temperature, 90 °C for 1 min raised at 15 °C/min to 235 °C and held for 15 min; solvent delay, 3 min; carrier gas, helium 5.5 at a flow rate of 1.5 mL/min. In these chromatographic conditions, the retention time of methylated BA was 8.29 min, and the characteristic *m*/*z* ions were 251 and 267 amu (**Figure 3**).

Recovery Studies. To determine the method efficiency for IFT and DKN, untreated soil samples were fortified with known amounts of analytical standards dissolved in water (0.04, 0.2, and 1 mg/kg). The mean recoveries were 77 ± 8% for IFT and 83 ± 6% for DKN. The limit of quantification was defined for HPLC/UV as the sample concentration required to give a signal-to-noise ratio of 6:1. It was evaluated at 0.04 mg/kg for IFT and at 0.05 mg/kg for DKN.

To determine method efficiency for BA, untreated soil samples were fortified with known amounts of analytical standards dissolved in water (0.05, 0.1, and 0.2 mg/kg). The mean recoveries were 98 ± 17%. The limit of quantification was defined for GC/ECD as the sample concentration required to give a signal-to-noise ratio of 6:1. It was evaluated at 0.05 mg/kg of soil.

RESULTS AND DISCUSSION

In all the conditions studied, the decrease of IFT fitted well the pseudo-first-order kinetics model (*12*): $\ln(C/C_0) = -K_{obs}t$, where C_0 is the initial concentration of IFT (in mg/L), C its concentration (in mg/L) at the time t (in h), and K_{obs} the observed rate constant of the reaction, in h^{-1}. K_{obs} values included water catalysis constants, as defined in Beltràn et al. (*11*). Formations of DKN and BA were observed in variable amounts; the formation of BA was quantitatively followed in temperature and abiotic condition studies.

Biotic Conditions. *Influence of Moisture Content.* As may be seen in **Table 2**, the rate of isomerization of IFT increased

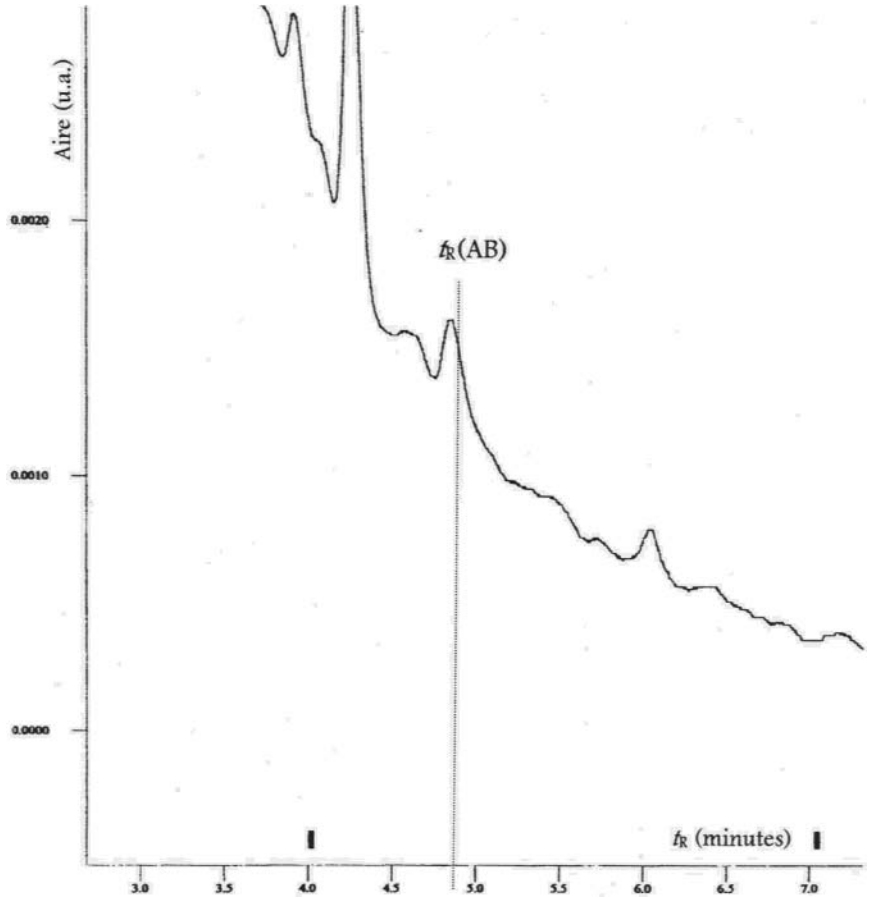

Figure 2. HPLC/UV chromatogram of blank soil (extract 1).

with higher moisture content. The half-life of IFT being $t_{1/2} = (\ln 2/K_{obs})$, a linear correlation could be established between $t_{1/2}$ (in h) and moisture content H (in %): $t_{1/2} = -1.2H + 86.1$ ($r^2 = 0.9865$).

In agricultural conditions of use, these results are of importance since rainfall quantity and frequency will affect the rate of formation of the active ingredient and, consequently, its migration toward deeper layers since K_{oc}(DKN) is about 49 (*13*), as observed by Tingle et al. (*14*) for flumetsulam. Moreover, according to previous results (*10*), for $T = 30$ °C and pH 6, the half-life of IFT in aqueous sterile solution should be 110 h; this value is higher than the half-lives determined here and confirms the catalytic effect of the soil reported by Taylor-Lovell et al. (*15*).

Influence of Temperature. As expected, the rate of decrease of IFT was higher at higher temperatures. To evaluate the role of chemical and biological processes in IFT isomerization, an Arrhenius diagram was drawn (**Figure 4**). The linear relationship between the logarithm of K_{obs} and the inverse of the temperature showed that the destruction of biological microflora at high temperatures did not affect the reaction and confirmed the chemical character of IFT isomerization in soil M1 at 17% moisture content. Activation energy E_a (in J/mol) could be calculated from the Arrhenius law, $K_{obs} = A \exp[-E_a/RT]$, where A is the Arrhenius constant (in h^{-1}) and R the universal gas constant (8.314 J/mol K). The value obtained (81.2 kJ/mol) was similar to that obtained in sterile aqueous solutions [82.4 kJ/mol, (*10*)], indicating that the mechanism of the reaction was the same in both cases.

The formation of BA was quantitatively followed at 20, 30, 40, and 60 °C (**Figure 5**). At 20 and 30 °C, the amount of BA formed increased to a maximum before decreasing to the limit

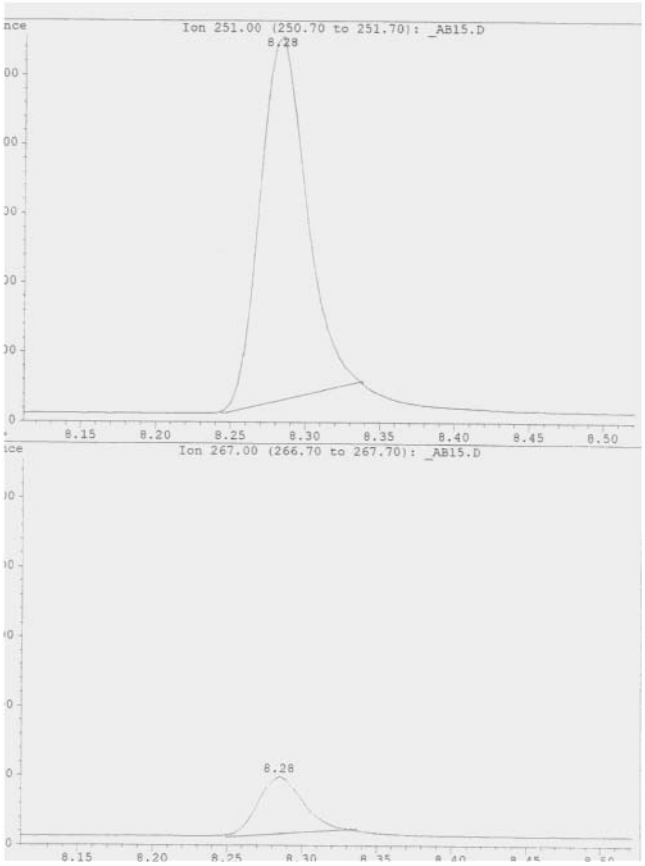

Figure 3. GC/MS chromatogram of methylated BA and ions $m/z = 251$ and 267.

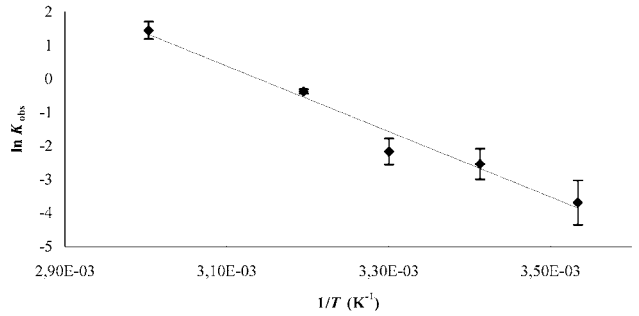

Figure 4. Arrhenius diagram of IFT isomerization in soil M1 at 17% moisture content.

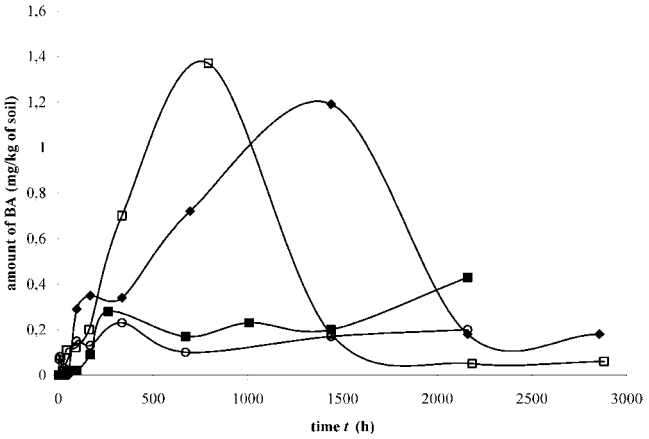

Figure 5. Influence of temperature on the amounts of BA formed in soil M1 under nonsterile conditions and 17% moisture content (◆, 20 °C; □, 30 °C; ■, 40 °C; ○, 60 °C).

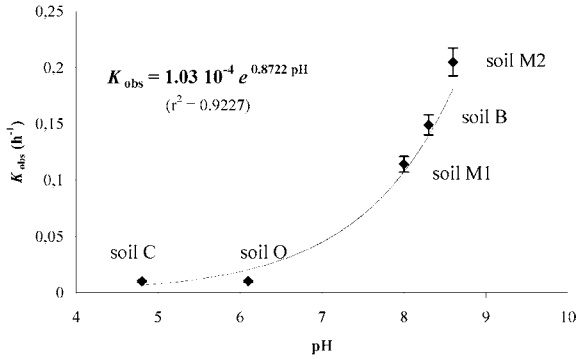

Figure 6. Influence of pH on K_{obs} values of the reaction IFT → DKN at 30 °C (303 K) and 17% moisture content.

of quantification. This maximum was higher at 30 °C than at 20 °C, and occurred sooner (760 vs 1380 h), with a shorter lag phase. At 40 and 60 °C, where biological activity is weakened, the amount of BA formed did not exceed 0.4 mg/kg (at 40 °C),

Table 2. Influence of Moisture Content *H* on K_{obs} Values of the Reaction IFT → DKN at 30 °C under Nonsterile Conditions

	moisture content *H*		
	17%	29%	45%
$K_{obs} \times 10^3$ (h^{-1})	10.3	14.1	21.2
$t_{1/2}$ (h)	67	49	33
r^2	0.9579	0.9904	0.9186

Table 3. Comparison of K_{obs} Values of IFT → DKN and Amount of BA Formed in Soils M1 and M1S at 30 °C and 17% Moisture Content

	soil M1	soil M1S
IFT → DKN		
$K_{obs} \times 10^3$ (h^{-1})	114	95.6
$t_{1/2}$ (h)	6	7
r^2	0.9643	0.9946
DKN → BA		
maximum amount of BA formed (mg/kg of soil)	1.39	not quantifiable

and no maximum could be determined. Thus, even if no kinetic model could be applied to these experimental data, we could conclude that the reaction DKN → BA in our conditions consisted mainly of biological processes. It is well known that at high temperatures, microflora is strongly inhibited and therefore only chemical processes occur, whereas for temperatures below 35 °C both chemical and biological processes take place (*16*). Anyway, the maximum amount of BA formed was 1.39 mg/kg (at 30 °C), which was only 14% of the initial amount of IFT applied to the soil. This fact suggested the possibility of partial mineralization or formation of tightly bound residues, as can be observed with atrazine (*17*, *18*); volatilization is unlikely to have occurred since experiments were conducted in closed recipients.

Influence of the Nature of Soil. After 6 days (t = 143 h), no IFT remained in soils B, M1, and M2, and only 10% of the initial amount of IFT remained in soils C and O. At t = 143 h, the total amounts of molecules recovered were 100% (soil M2), 81% (soil M1), 72% (soil B), 60% (soil C), and 48% (soil O). This fact seems to indicate the formation of hardly extractable residues in the more acidic soils C and O, which is consistent with previous desorption results (*13*). In these soils, low pH could have favored the solubilization of Fe^{2+} ions, involving possible complexation with DKN and BA, and stengthened retention of these compounds on the soil surface. No correlation could be obtained with the physicochemical properties of the soil, such as clay content or organic carbon content, but soil pH had an influence on the rate of the reaction. As may be seen in **Figure 6**, the K_{obs} values (from

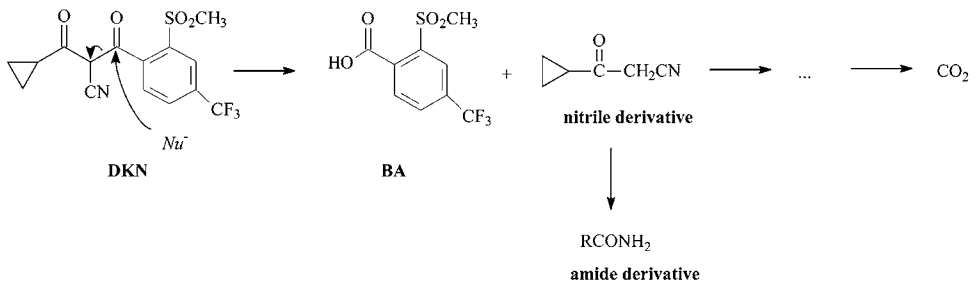

Figure 7. Supposed mechanism of the reaction DKN → BA.

pseudo-first-order kinetics model) of the reaction IFT → DKN were exponentiallydependent on pH. An enhancement of the rate of IFT isomerization was already observed in water and in soil/water systems (*10*, *13*, *15*), and Houot et al. (*19*) reported a nonlinear relationship between the ratio of mineralized atrazine and soil pH. Since one of the IFT commercial products also contains atrazine, it would be interesting to study the influence of soil pH on this formulation.

Abiotic Conditions. The degradation of IFT in sterilized soil M1S at 30 °C and 17% moisture content was compared to that in soil M1 (nonsterile soil) in the same conditions. The chemical character of the isomerization of IFT into DKN was confirmed, as may be seen by the similar values of K_{obs} obtained in both cases (**Table 3**). The degradation of DKN into BA appeared to be essentially due to biological activity, since in nonsterile conditions (soil M1) BA was quantifiable from the fourth day, whereas after 2 months of incubation only traces of this compound could be detected in sterile soil M1S. The presence of BA in small amounts at the end of the experiments indicated that some chemical process could not be totally neglected, as was shown by Mougin et al. (*20*), but the biological degradation was more important than chemical degradation. Biological degradation of molecules containing a nitrile substituent generally involves the formation of an amide intermediate which is transformed into an acid afterward, by attack on the CN group (*21*, *22*). In the case of DKN, the nitrile function CN is protected by the two ketone functions stabilizing the molecule by tautomeric equilibrium (Bayer CropScience France, personal communication), and the formation of BA appears to be due to a nucleophilic attack on the carbon atom in α position of the aromatic ring (**Figure 7**), in a scheme similar to that proposed by Pallett et al. (*23*). The nitrile derivative is certainly rapidly degraded into an amide one, undetectable with the analysis method developed in this study. The biological microflora responsible for the reaction could not be identified in our study, but it is known that the degradation of DKN by fungal strains *Phanerochaete chrysosporium* and *Trametes versicolor* in aqueous medium appeared to be due to extracellular oxidases of the two strains, with other enzymatic systems possibly also involved (*20*).

CONCLUSION

The results obtained in this study showed that the isomerization of IFT into DKN is rapid, depends strongly on pH, and is governed by a chemical process, whereas the degradation of DKN into BA appeared to be essentially due to the biological activity of the soil. Since DKN is the active ingredient of the herbicide and BA its main metabolite, it would be interesting to conduct an extensive study on the parameters influencing the reaction DKN → BA, such as the nature of bacteria and/or fungi able to degrade the active ingredient, or the possibility of adaptation and development of soil microflora due to repeated application of the herbicide. Studies conducted with radiolabeled molecules could also give important information about respective proportions of mineralization and formation of bound residues. The influence of natural conditions on the degradation of IFT in soil is actually being studied in order to complete the results obtained under controlled conditions.

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