Kinetics of Abiotic Hydrolysis of Isoxaflutole: Influence of pH and Temperature in Aqueous Mineral Buffered Solutions

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A kinetic study of the chemical hydrolysis of isoxaflutole [5-cyclopropyl-4-(2-methanesulfonyl-4-trifluoromethylbenzoyl)isoxazole (IFT)], a new herbicide recently developed by Rhône-Poulenc Agro, in buffered, sterile aqueous solutions was carried out in the dark at 295, 308, and 323 K and at nine pH values between 1.8 and 10.1. Samples were analyzed by HPLC-UV. The decrease in IFT concentration was accompanied by an increase in the concentration of its diketonitrile derivative (DKN). Obeying pseudo-first-order kinetics, isoxaflutole hydrolysis increased with increasing pH and temperature: for 295 K and pH 9.3 the rate of degradation was 100-fold faster than at pH 3.8. Using the Arrhenius equation, the rate constants K_{obsd}, activation energies E_a, and entropies $\Delta S^{\ddagger}$ were calculated, and plotting $\log(K_{obsd})$ against pH showed that the effect of pH varied with temperature. According to $\Delta S^{\ddagger}$ values the mechanism of the reaction was found to be different with respect to pH range. The benzoic acid derivative, known as a degradation product of DKN in plants, was not detected in the present study.

Keywords: *Isoxaflutole; diketonitrile derivative; hydrolysis; pH; temperature; kinetics*

INTRODUCTION

Isoxaflutole [5-cyclopropyl-4-(2-methane sulfonyl-4-trifluoromethylbenzoyl)isoxazole (IUPAC name) (IFT)] is a new herbicide recently developed by Rhône-Poulenc Agro and used in corn and sugarcane. It provides efficient control of both grass and broad-leaf weeds after pre-emergence application at a relatively low dose (∼75 g/ha). Preliminary studies (Rhône-Poulenc Agro, personal communication; Rouchaud et al., 1998) showed that degradation of IFT in various media (water, soil, and vegetation) led to a diketonitrile derivative (DKN) [2-cyano-3-cyclopropyl-1-(2-methanesulfonyl-4-trifluoromethylphenyl)propane-1,3-dione] by opening of the isoxazole ring. The DKN is in fact the active herbicide principle and acts by inhibiting the enzyme 4-hydroxyphenylpyruvate dioxygenase (Pallett et al., 1997, 1998; Viviani et al., 1998), which could be isolated in carrot and tobacco (Garcia et al., 1997, 1999). Because of the importance of this enzyme in carotenoid synthesis, its inhibition leads to bleaching symptoms in susceptible weed species, followed by growth suppression and necrosis. In the plant, the DKN is then rapidly converted into 2-methanesulfonyl-4-trifluoromethylbenzoic acid, an inactive benzoic acid derivative (BA).

The objectives of the present study were to determine the influence of physicochemical parameters such as pH and temperature on the rate of isoxaflutole hydrolysis in water and to attempt to propose a mechanism for this reaction. Experiments were carried out at three temperatures (295 ± 2, 308 ± 1, and 323 ± 1 K) and nine pH values between 1.8 and 10.1 in buffered solutions.

Table 1. Preparation of Buffered Aqueous Solutions for the Hydrolysis of IFT

pH	preparation for 100 mL buffer
1.8	25 mL of 0.2 M KCl + 5.3 mL of 0.2 M HCl
3.8	25 mL of 4×10^{-3} M KCl + 5.3 mL of 4×10^{-3} M HCl
5.2	98.8 mL of 6.7×10^{-2} M KH_2PO_4 + 1.2 mL of 6.7×10^{-2} M Na_2HPO_4
6.0	87.7 mL of 6.7×10^{-2} M KH_2PO_4 + 12.3 mL of 6.7×10^{-2} M Na_2HPO_4
7.2	39.2 mL of 6.7×10^{-2} M KH_2PO_4 + 60.8 mL of 6.7×10^{-2} M Na_2HPO_4
8.0	5.5 mL of 6.7×10^{-2} M KH_2PO_4 + 94.5 mL of 6.7×10^{-2} M Na_2HPO_4
8.3	50 mL of 0.1 M H_3BO_3 + 3.97 mL of 0.1 M NaOH
9.3	50 mL of 0.1 M H_3BO_3 + 21.3 mL of 0.1 M NaOH
10.1	50 mL of 0.1 M H_3BO_3 + 43.9 mL of 0.1 M NaOH

MATERIALS AND METHODS

Chemicals. Analytical standards of IFT, DKN, and BA were provided by Rhône-Poulenc Agro (Ongar, U.K.). All solvents, supplied by Carlo Erba, were of HPLC grade. Buffered aqueous solutions were prepared with reagents of purity ≥99% and with sterile and pyrogen-free water (B. Braun Medical S.A.). Because of the low solubility of IFT in water at 293 K (∼6 mg L^{-1}), aqueous solutions containing 5 mg L^{-1} of this compound were prepared from methanolic solutions at 500 mg L^{-1}, giving a methanol content in the studied solutions of 1%. Buffered aqueous solutions were prepared as shown in Table 1.

Degradation in Aqueous Solutions. To simultaneously evaluate the influence of pH and temperature on the hydrolysis of IFT, the above solutions were kept in the dark at three different and constant temperatures (295 ± 2, 308 ± 1, and 323 ± 1 K). Aliquots were sampled at appropriate intervals, depending on the conditions under which the sample was stored, and directly analyzed by HPLC-UV without further processing.

Analytical Procedure. Samples were analyzed by HPLC-UV, using a TSP Spectra system P1000 pump with a Rheodyne 7125 injector fitted with a 20 μL injection loop. The compounds were detected by their UV absorbance at 267 nm (IFT), 290

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Table 2. Linearity of the UV Detector Response and Coefficients of Determination r^2 for IFT and Its Derivatives at 267, 290, and 235 nm

wavelength λ, nm	compd	linear regression area/a.u. = f(C_{ift}/mg·L^{-1})	coefficient of determination (r^2)
267	IFT	$A = 673.3 \, (\pm 16.2)C_{ift} - 1.2 \, (\pm 9.9)$	$0.9997 \, (\pm 2 \times 10^{-4})$
290	DKN	$A = 639.6 \, (\pm 26.7)C_{dkn} - 48.8 \, (\pm 17.0)$	$0.9984 \, (\pm 1.3 \times 10^{-3})$
235	BA	$A = 154.9 \, (\pm 4.7)C_{ba} - 8.8 \, (\pm 4.8)$	$0.9989 \, (\pm 6 \times 10^{-4})$

nm (DKN), and 235 nm (BA) using a Shimadzu SPD-6A UV detector. The stationary phase was a C$_{18}$ Hypersil ODS column 5 μm, 250 mm × 4.6 mm, from Supelco; the mobile phase was ACN/H$_2$O/CF$_3$COOH (45:55:0.5 v/v/v) delivered at a flow rate 1.2 mL min^{-1}. Under these conditions, the retention times of IFT, DKN, and BA were 14.5, 12.9, and 4.4 min, respectively. Identification and analysis were performed by injection of analytical standards for comparison. Data were collected using HP Chemstation software.

Quality Criteria of the Method. The limits of detection (LOD) were 0.1 mg L^{-1} for IFT (267 nm), 0.1 mg L^{-1} for DKN (290 nm), and 0.15 mg L^{-1} for BA (235 nm). The limit of quantification (LOQ) was defined as the sample concentration required to give a signal-to-noise ratio of 6:1 and corresponds to twice the LOD for each compound (0.2 mg L^{-1} for IFT and DKN, 0.3 mg L^{-1} for BA). Linearity of the detector response was evaluated at the three wavelengths used in this study, by injecting solutions containing the three compounds at five different concentrations (for each compound, from C = LOQ to C = 10 × LOQ). Each injection was carried out in duplicate, and the entire procedure was repeated on three additional days. Results are given in Table 2. Absence of interferents was verified by injecting buffered solutions containing none of the studied molecules.

RESULTS AND DISCUSSION

The influence of temperature and pH on the chemical hydrolysis of IFT in buffered, sterile aqueous solutions was studied at three temperatures and nine pH values. Parts a, b, and c of Figure 1 represent the decrease in IFT concentration by plotting ln(C/C_0) against time t at 295, 308, and 323 K, respectively. Under the experimental conditions described above and for a given temperature, IFT is more stable in acid media than in neutral or alkaline media. Thus, after 2 weeks at ambient temperature, almost 70% of IFT remained at pH 5.2, whereas <10% remained at pH 7.2.

For each temperature and pH value, the hydrolysis of IFT followed pseudo-first-order kinetics (Logan, 1996; Jungers, 1958)

$$C = C_0 \exp(-K_{obsd}t) \tag{1}$$

where t is the incubation time (in hours), C the concentration of isoxaflutole (in mol L^{-1}) at time t, C_0 its initial concentration (in mol L^{-1}), and K_{obsd} (in h^{-1}) the observed rate constant of the reaction.

Table 3 shows the values of the various K_{obsd} and half-lives $t_{1/2}$ ($t_{1/2} = \ln 0.5/K_{obsd}$) obtained for every solution under given pH and temperature conditions. The chemical hydrolysis of IFT in the pH range studied exhibited the characteristics of a base catalysis (K_{obsd} increased with increasing pH, whereas the half-life decreased) and the degradation was found to increase with temperature.

The effect of temperature on the rate of isoxaflutole hydrolysis was determined by using the Arrhenius law

$$K_{obsd} = A \exp[-E_a/(RT)] \tag{2}$$

where A is a specific constant of the reaction, E_a is the activation energy in J mol^{-1}, R is the universal gas
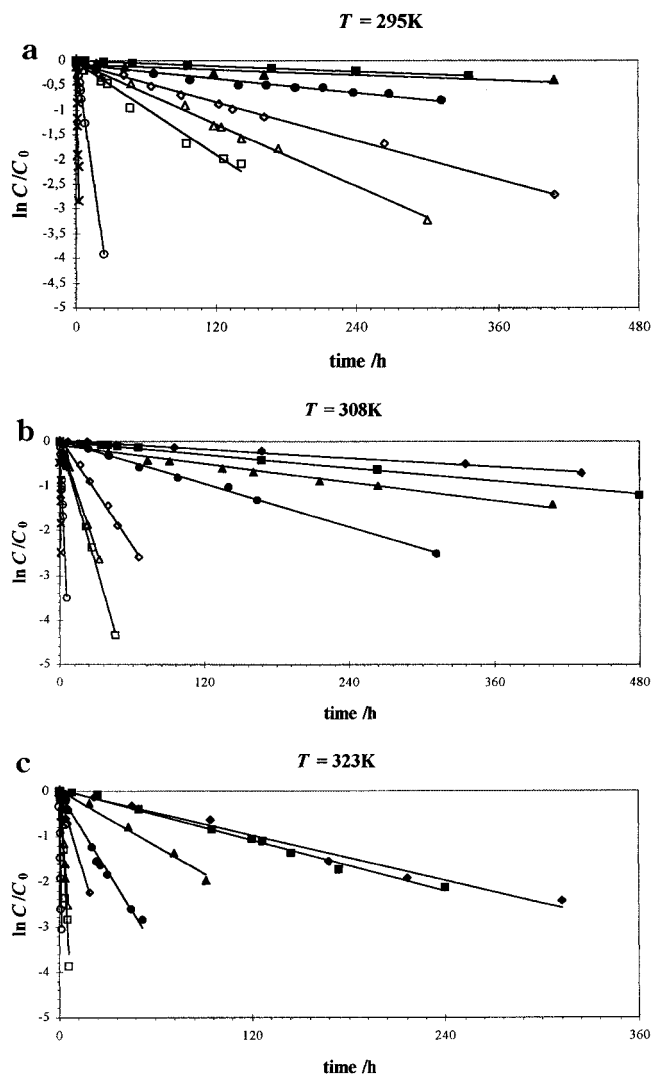

Figure 1. Kinetics of hydrolysis of IFT at (a) 295, (b) 308, and (c) 323 K for pH 1.8 (◆), 3.8 (■), 5.2 (▲), 6.0 (●), 7.2 (◇), 8.0 (□), 8.3 (△), 9.3 (○), and 10.1 (×).

constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature in K. Figure 2 represents the Arrhenius diagram [ln K_{obsd} = f(1/T)] for all pH values except 1.8 and 10.1, from which the values of E_a and activation entropies ($\Delta S^{\ddagger}$) were calculated (see Table 4).

As may be seen in Table 4, activation entropies varied from negative values in acidic media to positive values in basic media, which showed that the mechanism of the reaction was highly pH-dependent. Generally, positive values of $\Delta S^{\ddagger}$ mean that the reaction leads to a less ordered state: this corresponds here to the formation of a transition state that is less constrained than IFT. It is already known that isoxazoles with an unsubstituted 3-position (see Figure 3) have a high reactivity in alkaline media (Speroni and Quilico, 1962; Grünanger and Vita-Finzi, 1991). Because of a highly unequal electronic distribution in the isoxazole nucleus, which

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Table 3. Observed Rate Constants K_{obsd} and Half-Lives $t_{1/2}$ of IFT in Aqueous Solutions at Different pH Values and Temperatures

pH	$T = 295$ K		$T = 308$ K		$T = 323$ K	
	K_{obsd}, h^{-1}	$t_{1/2}$, h	K_{obsd}, h^{-1}	$t_{1/2}$, h	K_{obsd}, h^{-1}	$t_{1/2}$, h
1.8	nd^a	nd	1.6×10^{-3}	433	8.5×10^{-3}	81
3.8	0.9×10^{-3}	770	2.0×10^{-3}	350	8.8×10^{-3}	78
5.2	1.6×10^{-3}	433	3.8×10^{-3}	182	2.0×10^{-2}	35
6.0	2.9×10^{-3}	241	8.0×10^{-3}	87	5.8×10^{-2}	12
7.2	7.3×10^{-3}	95	3.9×10^{-2}	18	1.2×10^{-1}	6
8.0	1.6×10^{-2}	43	8.8×10^{-2}	8	5.7×10^{-1}	1.2
8.3	1.1×10^{-2}	65	8.0×10^{-2}	9	4.5×10^{-1}	1.5
9.3	1.3×10^{-1}	5	5.5×10^{-1}	1.2	2.4	0.3
10.1	1.1	0.6	1.8	0.4	nd	nd

^{a} nd, not determined.
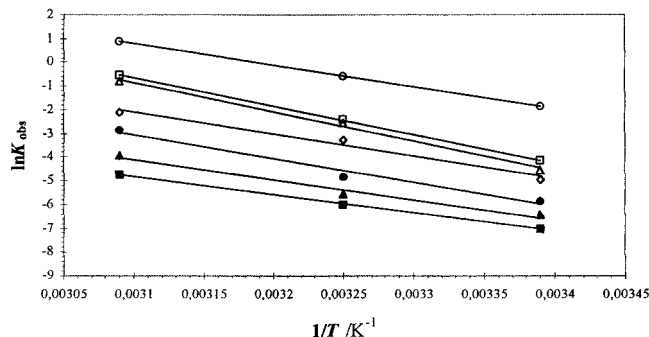
Figure 2. Variation of ln K_{obsd} with temperature for pH 3.8 (■), 5.2 (▲), 6.0 (●), 7.2 (◇), 8.0 (□), 8.3 (△), and 9.3 (○).

makes the carbon-3 electronically deficient, the corresponding hydrogen atom has a high tendency to protonize. The facile opening of the isoxazole ring leads to an enolate ion stabilized by resonance, and the final β-ketonitrile derivative of IFT was formed by addition of a proton and regeneration of the hydroxide ion. The relatively low value of $\Delta S^{\ddagger}$ at pH 9.3 was probably due

Table 4. Arrhenius Equations, Coefficients of Determination r^2, Activation Energies E_a, and Activation Entropies $\Delta S^{\ddagger}$ for Values of pH between 3.8 and 9.3

| pH	equation ln $K_{obsd} = f(1/T)$	r^2	E_a, kJ mol^{-1}	$\Delta S^{\ddagger}$, J mol^{-1} K^{-1}
3.8	ln $K_{obsd} = -7613(1/T) + 19$	0.9982	63.3	-97
5.2	ln $K_{obsd} = -8465(1/T) + 22$	0.9801	70.4	-69
6.0	ln $K_{obsd} = -10037(1/T) + 28$	0.9790	83.4	-20
7.2	ln $K_{obsd} = -9282(1/T) + 27$	0.9784	77.2	-32
8.0	ln $K_{obsd} = -11940(1/T) + 36$	0.9999	99.3	$+49$
8.3	ln $K_{obsd} = -12472(1/T) + 38$	0.9937	103.7	$+61$
9.3	ln $K_{obsd} = -9613(1/T) + 31$	0.9991	79.9	$+1$

to greater experimental error due to the reaction velocity. Under acidic conditions, activation entropies were negative, and this is most likely due to hydrogen bonding between water molecules and the transition state, creating an enolate ion which is more hydrated (and consequently more constrained) than IFT under basic conditions, an effect which is enhanced by the presence of hydronium ions in low-pH solutions. Figure 3 illustrates both proposed mechanisms of the hydrolysis of IFT.

To more precisely characterize the effect of [OH$^-$] on the chemical hydrolysis of IFT, log(K_{obsd}) was plotted against pH (Figure 4). Under our experimental conditions, log(K_{obsd}) showed a linear but discontinuous dependence on pH values. For pH values <3.8, the degradation of IFT was not catalyzed because of the acidity of the medium, and the temperature was the sole parameter influencing the rate of the reaction. The corresponding slopes were close to zero, with log(K_{obsd}) = 0.11(pH) − 3.0 and 0.10(pH) − 2.3 for $T = 308$ and 323 K, respectively. For pH values from 5.2 to 10.1, Table 5 shows the results obtained with the intervals pH 5.2−8.0 and pH 8.0−10.1 considered separately. The influence of pH for each temperature was followed by comparing the slopes from one interval to the other
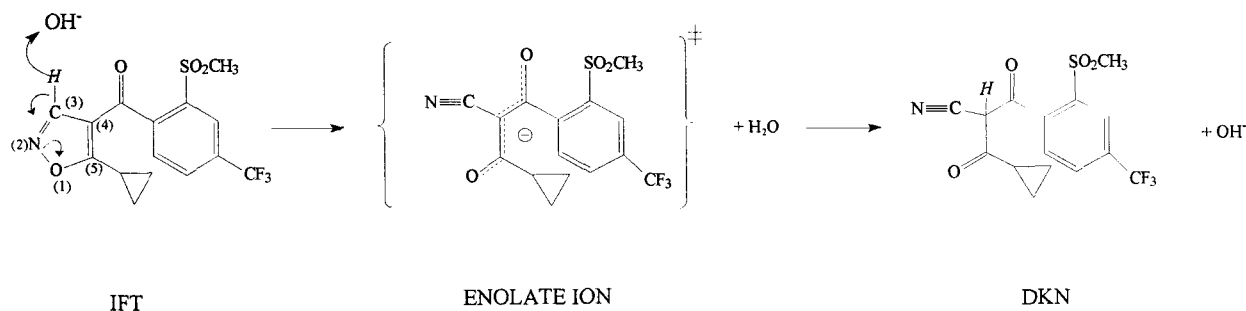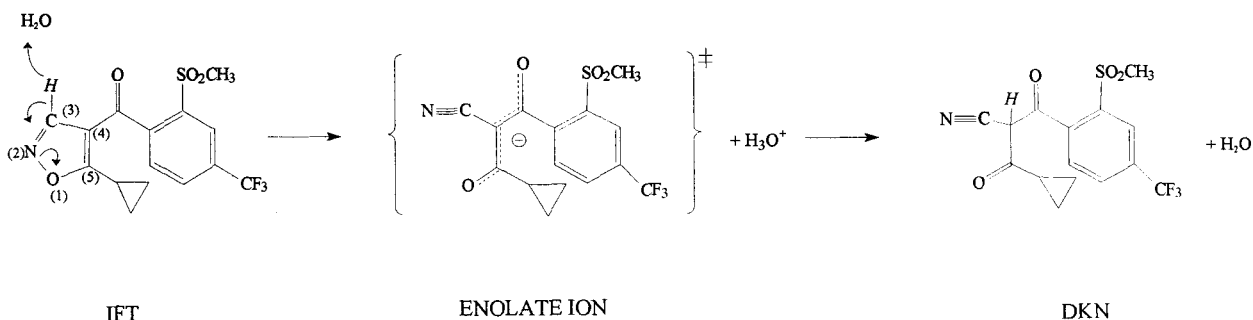
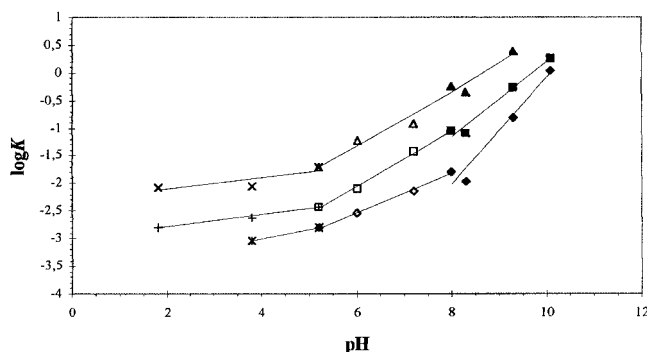**Figure 3.** Hydrolysis reaction mechanisms of IFT under basic and acidic conditions.

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Figure 4. Variation of log K_{obsd} with pH for all pH values at 295 (∗, ◇, ◆), 308 (+, □, ■), and 323 K (×, △, ▲).

Table 5. Linear Regressions log K_{obsd} = f(pH) for the Intervals pH 5.2–8.0 and 8.0–10.1 and for the Three Temperatures 295, 308, and 323 K

temp T, K	pH 5.2–8.0	pH 8.0–10.1
295	log K_{obsd} = 0.35(pH) − 4.6	log K_{obsd} = 0.95(pH) − 9.6
308	log K_{obsd} = 0.50(pH) − 5.0	log K_{obsd} = 0.67(pH) − 6.6
323	log K_{obsd} = 0.48(pH) − 4.2	log K_{obsd} = 0.54(pH) − 4.7

for a given temperature: at 295 K the slope for the interval pH 8.0–10.1 was 2.7 that of the slope for the first interval. At 308 K, this ratio was ∼1.3, and at 323 K the slopes had similar values.

Such a discontinuity in ln K_{obsd} = f(pH) was observed in a study of the hydrolysis of four sulfonylureas (Dinelli et al., 1997), and the authors attributed their findings to different reactivities of dissociated and undissociated forms of these herbicides with respect to water molecules. In the same way, it could be proposed that in aqueous solutions the reactivity of IFT is slightly different with respect to reagents used to prepare the buffers (KH_2PO_4/Na_2HPO_4 and H_3BO_3/NaOH, respectively), which could explain the differences observed between pH 8.0 and 8.3 (see Table 3). This effect seemed to be less important when temperature increased, as may be seen from the values of the slopes presented in Table 5.

In the interval pH 8.0–10.1, log(K_{obsd}) rapidly increased with increasing pH. Although values of K_{obsd} remained lower at 295 K than at 308 K, linear regression revealed a higher slope at 295 K than at 308 K (see Table 5 and Figure 4), which seems to demonstrate that for the highest pH values, the influence of pH became more important than the influence of temperature.

If it is considered that log(K_{obsd}) = a(pH) + b, then K_{obsd} = k_{OH}[OH⁻]^a (where k_{OH} = $10^b/(k_E)^a$ and k_E is the ion product of water). The value of a represents the order of the reaction with respect to the hydroxide ion, and the complete equation of the hydrolysis rate of IFT can be written as follows:

$$v = k_{OH}[OH^-]^a[IFT] \qquad (3)$$

It was previously reported in the literature (Grünanger and Vita-Finzi, 1991) that the hydrolysis of isoxazoles follows second-order kinetics; however, in this study the reaction was found to be second order only at 295 K between pH 8.0 and pH 10.1, where the value of a is close to 1.

CONCLUSION

The experiments, which were carried out over a wide range of pH at three different temperatures, showed that the reaction followed pseudo-first-order kinetics with respect to IFT. Under our experimental conditions the latter was irreversibly converted into its DKN derivative, which was the only degradation product detected. The proposed mechanism of the reaction is a concerted one-stage E2 type (Figure 3), and the sign of $\Delta S^{\ddagger}$ changes with respect to the pH of the medium. The relatively easy hydrolysis of IFT even in acidic medium (pH 5.2 and 6.0) is not surprising because such a reaction has already been observed (Grünanger and Vita-Finzi, 1991). Additional studies currently conducted in aqueous organic buffered solutions tend to show that the nature of the conjugate bases plays an important part in the kinetics of IFT hydrolysis.

The half-life values calculated in this study showed that IFT can be considered as a herbicide of low persistence. However, under natural conditions, the biological degradation must also be taken into account, as physicochemical parameters of the soil may also influence the hydrolysis of IFT; these additional factors may change the values of $t_{1/2}$ determined in this study. Thus, preliminary studies carried out in water contaminated by a *Pseudomonas* sp. bacteria (unpublished data) showed that under such conditions, the rate of IFT hydrolysis is radically different but has not yet been explained. Additional work on different soils will be necessary to further understand the behavior of this new herbicide and its main metabolites in the environment.

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