Kinetics and Mechanism of Imazosulfuron Hydrolysis

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Knowledge of the kinetics and pathways of hydrolytic degradation is crucial to the prediction of the fate and transport mechanism of chemicals. This work first describes the kinetics of the chemical hydrolysis of imazosulfuron, a new sulfonylurea herbicide, and evaluates the results to propose a degradation pathway. The hydrolysis of imazosulfuron has been studied in aqueous buffers both within the pH range 1.9−12.3 at ambient temperature (thermostated at 25 ± 2 °C) and at pH 3.6 within the temperature range of 15−55 °C. The hydrolysis rate of imazosulfuron was characterized by a first-order kinetics, pH- and temperature-dependent, and accelerated by acidic conditions and higher temperatures. The calculated half-lives at pH 4.5 and 5.9 were 36.5 and 578 days, respectively. At pH 6.6, 7.4, 9.2, and 12.3 no significant change in imazosulfuron concentration was observed after 150 days. Half-lives were much lower at pH <4 (= imazosulfuron pK_a), at which they ranged from 3.3 to 6.3 days. Moreover, a change in temperature from 15 to 25 °C in acidic conditions (pH 3.6) decreased the half-life of imazosulfuron by a factor of ∼4.0; in any case, a 3−5-fold increase in the rate of hydrolysis was found for each 10 °C increase in temperature. In acidic conditions the only hydrolysis products were the two molecules resulting from the cleavage of the sulfonylurea bridge.

Keywords: *Imazosulfuron; hydrolysis; half-life; pH; temperature*

INTRODUCTION

Recently, an increasing number of pesticides have entered our environment. Many of them are potentially hazardous to human health and to the ecosystem. Indeed, the problem of environmental protection and pollution control has become a major concern. One prerequisite for decision making in environmental protection and pollution control is the ability to identify and measure the xenobiotic materials in our ecosystem. One of the most important aspects in our understanding of the fate of pesticides in the environment is the knowledge of their degradation mechanism. Such degradation processes may lead to the formation of new chemicals with reduced toxicity or, in some cases, increased toxicity, to aquatic biota. Knowledge of hydrolytic degradation pathways and kinetics in the normal pH range of the aquatic environment (5.5−8.0) is crucial in the prediction of the fate and transport mechanism of chemicals (*1*).

Sulfonylurea herbicides are a relatively new class of chemicals used to control weeds and some grasses in cereal crops (*2*). The environmental fate of several sulfonylureas has been reported (*3*−*6*). Their main degradation pathways are chemical hydrolysis and microbial degradation, the former being of utmost importance for the environmental dissipation of these herbicides (*7*).

Imazosulfuron, 1-(2-chloroimidazo[1,2-*a*]pyridin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea (Figure 1), is a new postemergence sulfonylurea herbicide. Like other sulfonylureas (*2*, *8*), it has a strong herbicidal activity against perennial and annual weeds but low mammalian toxicity. It is applied once per growing season and is highly active at low application levels; it is used to control most annual and perennial broadleaved weeds and sedges in paddy rice [75−95 g of active ingredient (ai) ha^{-1}] and turf (500−1000 g of ai ha^{-1}) (*9*). No information is available in the literature on its environmental fate.

The aim of this study was to evaluate the degradation kinetics of imazosulfuron in water (rate constants and half-lives) as a function of pH and temperature by an HPLC method capable of detecting and quantifying simultaneously imazosulfuron and its metabolites.

MATERIALS AND METHODS

Chemicals. Imazosulfuron (Figure 1) and its metabolites, 2-chloroimidazo[1,2-*a*]pyridin-3-sulfonamide (IPSN) and 1-(2-chloroimidazo[1,2-*a*]pyridin-3-ylsulfonyl)-3-(4-hydroxy-6-methoxypyrimidin-2-yl)urea (HMS), were provided by Takeda Chemical Industries, Ltd. The third metabolite, 2-amino-4,6-dimethoxypyrimidine (ADPM), was purchased from Sigma-Aldrich (Milan, Italy) and had a purity of 98%. All were used without further purification. All solvents used were pure analytical HPLC grade solvents (Carlo Erba, Milan, Italy).

Nine buffer solutions were used to study the aqueous hydrolysis of imazosulfuron. Table 1 shows the procedures followed for their preparation.

Hydrolysis Rate Determination. Hydrolysis rates were determined by monitoring the disappearance of imazosulfuron in aqueous buffer solutions within the pH range of 1.9−12.3 (Table 1). To avoid microbial degradation, buffer solutions were

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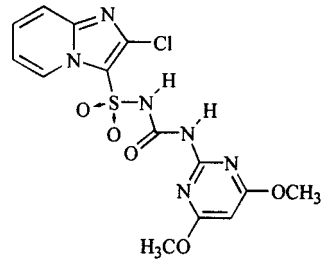

MW = 412.8

pK_a = 4.0

K_{ow} = 1.12

Solubility In water 0.308 g/L (pH 7, 25°C)

Figure 1. Chemical structure and main physicochemical properties of imazosulfuron (data from ref *9*).

Table 1. Buffer Solutions Prepared for the Aqueous Hydrolysis of Imazosulfuron

buffer solution	pH	preparation for 100 mL of buffer solution
A	1.9 ± 0.1	25 mL of 0.2 M KCl + 6.5 mL of 0.2 M HCl
B	2.8 ± 0.1	98 mL of 0.2 M KCl + 2.0 mL of 0.2 M HCl
C	3.6 ± 0.1	5.0 mL of 1 M NaOH + 65 mL of 1 M CH_3COOH
D	4.5 ± 0.1	100 mL of 0.07 M KH_2PO_4
E	5.9 ± 0.1	16 mL of 0.5 M KH_2PO_4 + 1.4 mL of 0.5 M Na_2HPO_4
F	6.6 ± 0.1	8.0 mL of 0.5 M KH_2PO_4 + 4.0 mL of 0.5 M Na_2HPO_4
G	7.4 ± 0.1	2.0 mL of 0.5 M KH_2PO_4 + 6.0 mL of 0.5 M Na_2HPO_4
H	9.2 ± 0.1	5.0 mL of 1 M HCl + 5.5 mL of 2M NH_3
I	12.3 ± 0.1	2.5 mL of 0.2 M KCl + 6.5 mL of 0.2 M NaOH

sterilized by filtration (Millex GS/AP20 0.22 μm, Millipore, Bedford, MA) and all glass apparatuses by autoclaving for 45 min (Tecnoclav 50, Fedegon, Pavia, Italy) at 130 °C. Aseptic techniques were adopted throughout the study to maintain sterility. The effectiveness of the aseptic procedures performed was confirmed by microbiological tests. No microorganisms were isolated from the aqueous solutions remaining at the end of the experiments (150 days).

A stock solution containing 44.0 mg/L of imazosulfuron in acetonitrile was prepared. Triplicate 40.0 mL samples, each containing 2.20 mg/L of herbicide, were obtained by diluting aliquots of 2.0 mL of the stock solution with the appropriate buffer solution. The treated buffer solutions were stored in the dark at ambient temperature (25 ± 2 °C) in centrifuge glass tubes. Another set of triplicate 40.0 mL distilled water samples at pH 3.6, containing 2.20 mg/L of herbicide, was stored in the dark at 15, 25, 42, and 52 °C to test the effects of temperature on hydrolysis. In all trials, the pH of each sample was periodically measured and did not vary by >0.1 unit.

One milliliter from each tube was aseptically removed at appropriate time intervals ranging from 1 h for imazosulfuron solutions at higher temperatures to weeks (150 days) for solutions at pH >4 and stored at −20 °C until use.

Analytical Procedure. Reversed phase HPLC equipped with UV detection has been used to monitor the disappearance of imazosulfuron and the formation of its hydrolytic products in the aqueous samples.

The concentrations of imazosulfuron and its metabolites were determined by a Waters HPLC system (model 600 pump, 486 UV detector operating at 238 nm; Milford, MA) and a computing integrator chromatography workstation (model PU 4810; Philips), equipped with a Prodigy C-18 reversed phase column (250 × 4.6 mm, particle size = 5 μm) from Phenomenex (Torrance, CA). A Security Guard column (Phenomenex) was used as precolumn. The mobile phase was a 50:50 (v/v) mixture of acetonitrile/0.1% (v/v) aqueous acetic acid (pH 3.5). Flow rate was 1.0 mL/min. No extraction or purification step was required prior to analysis.

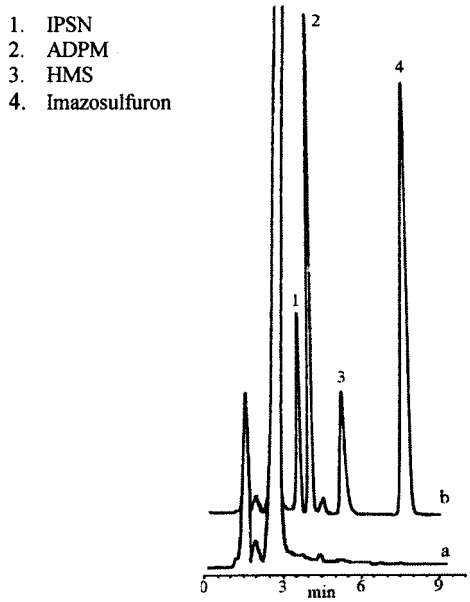

Figure 2. HPLC chromatogram of an aqueous buffer sample (pH 3.6 at 25 ± 2 °C): (a) blank; (b) spiked with a mixture of imazosulfuron and its transformation products (for HPLC conditions, see text).

Under these conditions the retention time of imazosulfuron was 7.6 min, whereas retention times for its metabolites were 5.2 min for HMS, 4.1 min for ADPM, and 3.7 min for IPSN. The isocratic elution allowed a satisfactory chromatographic separation. Blanks, characterized by 40.0 mL of buffer solution with 5% of acetonitrile (v/v), did not exhibit any peak that interfered with the detection of imazosulfuron and/or its metabolites (Figure 2).

Identification and analysis were performed using authentic standards for comparison. Standard acetonitrile solutions of both the herbicide and its metabolites were prepared fresh every week and stored at 4 °C in the dark until use.

Calibration curves for imazosulfuron, ADPM, and IPSN (r^2 > 0.99) were obtained by injecting 20 μL samples of solutions at concentration within the range of 0.025−2.500 μg/mL. The detection limits (LOD) (estimated to be 3 times the background noise) were 50 μg/L for imazosulfuron, 70 μg/L for ADPM, and 100 μg/L for IPSN. The limits of quantitation (LOQ) (estimated to be 10 times the background noise) were 165 μg/L for imazosulfuron, 230 μg/L for ADPM, and 330 μg/L for IPSN. No significant difference in background noise was observed between the analyses of the standards in acetonitrile and those of the matrices analyzed (buffer solutions). Therefore, LOQ limits for the analyses on matrices can be assumed to be the same as those observed for standards.

RESULTS AND DISCUSSION

Hydrolysis Rate. The hydrolysis of imazosulfuron, monitored at different temperatures and pH values, followed first-order kinetics, with significant determination coefficient ($P < 0.05$), thus indicating that the half-life was independent of initial herbicide concentration; this is in agreement with results of preliminary studies at different concentrations (unreported data) and with the data presented by Dinelli et al. (*10*) on other sulfonylureas.

Hydrolysis rates of imazosulfuron showed variation with pH as do those of other sulfonylureas. The observed rate constant, k, and calculated half-lives ($t_{1/2} = \ln 0.5/k$) in acidic conditions are given in Table 2. At pH 1.9 ± 0.1 imazosulfuron degradation was ~10-fold faster than at pH 4.5 ± 0.1 (half-lives were 3.30 and 36.5 days, respectively). The half-life of imazosulfuron at pH 5.9

Table 2. Determination of Rate Constant (k) and Half-Life ($t_{1/2}$) for the Hydrolysis of Imazosulfuron under Acidic Condition

	k^c (days^{-1})	r^2	$t_{1/2}{}^c$ (days)
pH[a]			
1.9 ± 0.1	0.21	0.990	3.30
2.8 ± 0.1	0.16	0.989	4.33
3.6 ± 0.1	0.11	0.988	6.30
4.5 ± 0.1	0.019	0.964	36.5
5.9 ± 0.1	0.0012	0.999	578[d]
temp[b] (°C)			
15 ± 2	0.026	0.942	26.7
25 ± 2	0.11	0.988	6.30
42 ± 2	3.62	0.999	0.191
52 ± 2	13.12	0.997	0.053

[a] Hydrolysis of imazosulfuron in aqueous solution (C_i = 2.2 mg/L) in the dark at ambient temperature (25 ± 2 °C). [b] Hydrolysis of imazosulfuron in aqueous solution (C_i = 2.2 mg/L) in the dark at pH 3.6 ± 0.1. [c] The variability of triplicate samples was no more than 5%. [d] Extrapolated by the regression curve.

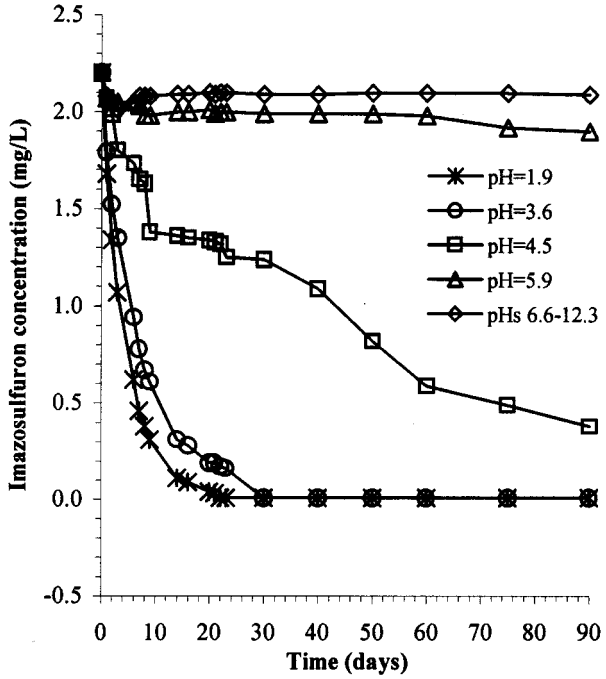

Figure 3. Disappearance of imazosulfuron at different pH values in aqueous solution in the dark at ambient temperature (25 ± 2 °C).

was estimated to be 578 days by extrapolation of the regression curve. Half-lives at pH 6.6, 7.4, 9.2, and 12.3 could not be estimated because imazosulfuron concentration did not change significantly up to 150 days (Figure 3). Therefore, higher pH values seem to strongly reduce the hydrolysis rate of imazosulfuron.

To better characterize the effects of pH on the hydrolysis of imazosulfuron, the rate constant was plotted against H^+ concentration. As can be seen in Figure 4, the k values increase at increasing H^+ concentration. Moreover, the pattern of variations of k values indicates that the major variations of k values occur between pH 5.9 and 3.6, whereas only slight further increases take place between pH 3.6 and 1.9. Considering that k values at pH 6.6, 7.4, 9.2, and 12.3 were not detectable because they were extremely low, and presumably similar to each other, the pattern in Figure 4 could be considered as a part of a sigmoidal curve. Interestingly, the inflection range is approximately located at pH = pK_a of imazosulfuron (pK_a = 4.0), that is, at the pH interval where the maximum of variation of the ratio of neutral form/anionic form occurs. Therefore, the pattern observed supports the hypothesis that only the neutral form of the sulfonylurea bridge is susceptible to hydrolysis, whereas the anionic form is substantially unaffected. These results are in partial agreement with those reported by Vega et al. (*11*), who found a similar pattern for triflusulfuron methyl, which showed in its neutral form a lower, although measurable, hydrolysis rate than in its anionic form.

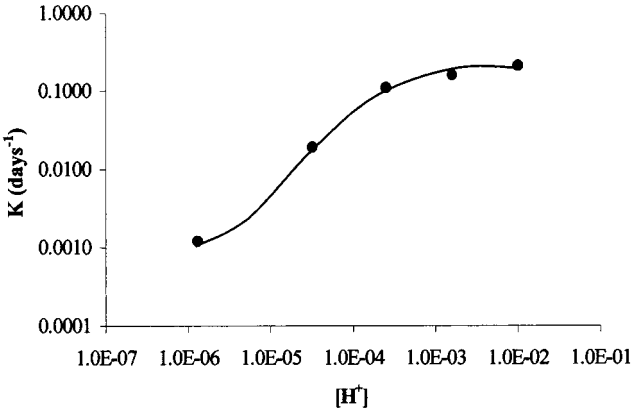

Figure 4. Variation of k with $[H^+]$ for the acidic hydrolysis of imazosulfuron in aqueous solution in the dark at ambient temperature (25 ± 2 °C).

The hydrolysis of imazosulfuron at pH 3.6 (±0.1), monitored in the temperature range of 15−55 °C, showed a marked effect of temperature on degradation rate and half-life (Table 2). A change in temperature from 15 to 25 °C decreased the half-life of imazosulfuron by a factor of ∼4.0.

The effects of temperature on the rate of imazosulfuron hydrolysis were characterized by the Arrhenius equation

$$\ln k = \ln A - E_a/RT$$

where A is the pre-exponential factor typical of the imazosulfuron hydrolysis reaction, E_a is the activation energy (J/mol), R is the universal gas constant (8.314 J/K·mol), and T is the absolute temperature (K).

As can be seen in Figure 5, a linear regression line fitted the experimental points, as expected for the Arrhenius equation and for chemical reactions. The activation energy (E_a), calculated from the slope of the linear regression multiplied by R, was 143.9 kJ/mol, suggesting that imazosulfuron requires a great amount of energy to activate acidic hydrolysis.

The activation entropy, $\Delta S^{\ddagger}$, at ∼300 K was also determined. The pre-exponential parameter, A, is linked to the entropy change occurring during the activation process (activation entropy, $\Delta S^{\ddagger}$), which can be obtained from the equation (*12*)

$$A = e^{kT/h} \exp(\Delta S^{\ddagger}/R)$$

where k is the Boltzmann constant (J/K) and h the Planck constant (6.625×10^{-34} J·s). At −300 K

$$\Delta S^{\ddagger} = 8.314(\ln A - 30.474)$$

The positive value of $\Delta S^{\ddagger}$ (121.72 J/K·mol) indicates that the reaction leads to a less ordered state, for

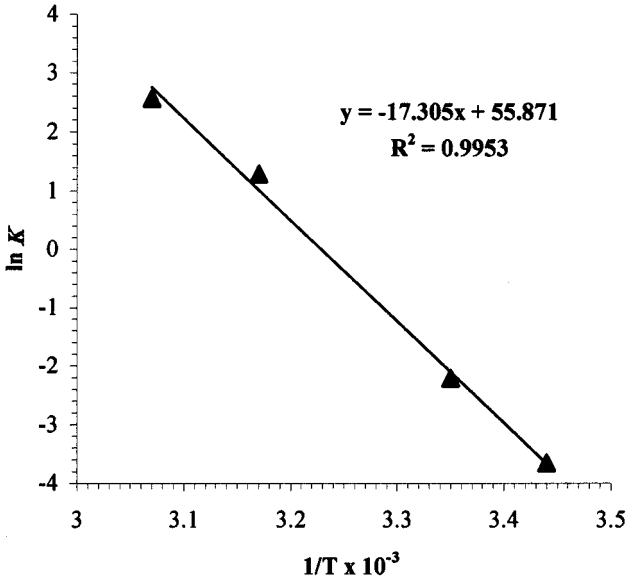

Figure 5. Variation of ln *k* with temperature for the acidic hydrolysis of imazosulfuron (pH 3.6 ± 0.1).

example, the formation of a less constrained transition state than the imazosulfuron molecule.

Hydrolysis Metabolites. The hydrolysis mechanism, which involves the cleavage of the sulfonylurea bridge to yield the corresponding sulfonamide and heterocyclic amine, is very common during the degradation of sulfonylurea herbicides in water (*13*). Nevertheless, alternative pathways of the chemical hydrolysis of sulfonylureas have been observed: rimsulfuron hydrolyzes easily through the contraction of the sulfonylurea bridge (*14*), whereas the cleavage of the sulfonylurea bridge and O-demethylation of the methoxy group of the triazine ring occur for thifensulfuron-methyl (*15*), and metsulfuron-methyl (*16*).

Our previous work on imazosulfuron degradation in soil demonstrated that imazosulfuron can undergo different degradation pathways, yielding—under anaerobic conditions—the O-demethylation product, HMS (*17*). In the present study, there was no chromatographic evidence of such a degradation pathway. The only hydrolysis products of imazosulfuron identified in acidic aqueous solution were ADPM and IPSN, which resulted from the cleavage of the sulfonylurea bridge. Their structures were confirmed by comparing their HPLC retention times with those of authentic standards. The degradation pathway is shown in Figure 6. As can be seen in Figure 7, the decrease in imazosulfuron concentration corresponded to the appearance and evolution of the two different compounds, ADPM and IPSN. For example, 0.202 μmol of imazosulfuron incubated for 4 days at pH 3.6 yielded 0.134 μmol of the remaining imazosulfuron, 0.060 μmol of ADPM, and 0.065 μmol of IPSN. As a matter of fact, equal numbers of ADPM and IPSN moles were formed up to day 14. However, starting from day 14 the total moles of degraded imazosulfuron were not equal to the moles of metabolites recognized, a difference that became more evident in the long run.

Whether imazosulfuron undergoes other degradation pathways producing metabolites not detectable by our HPLC method or whether ADPM and IPSN are subject to a subsequent degradation is still unknown. Nevertheless, we can exclude the occurrence of microbial degradation because aseptic techniques were adopted throughout the study, and no microorganisms were isolated from the aqueous solutions remaining at the end of the experiments.

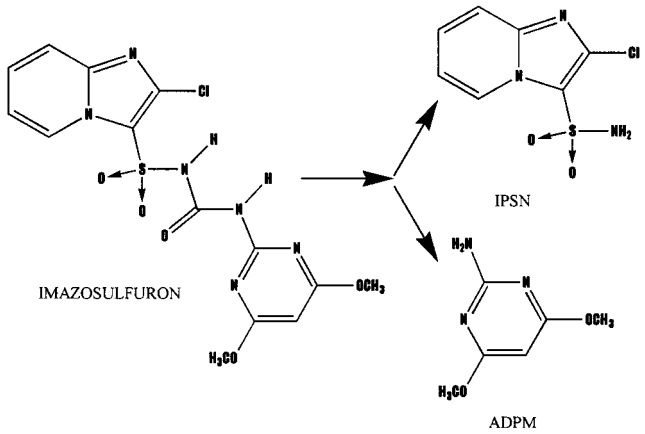

Figure 6. Degradation pathway of imazosulfuron in aqueous solution.

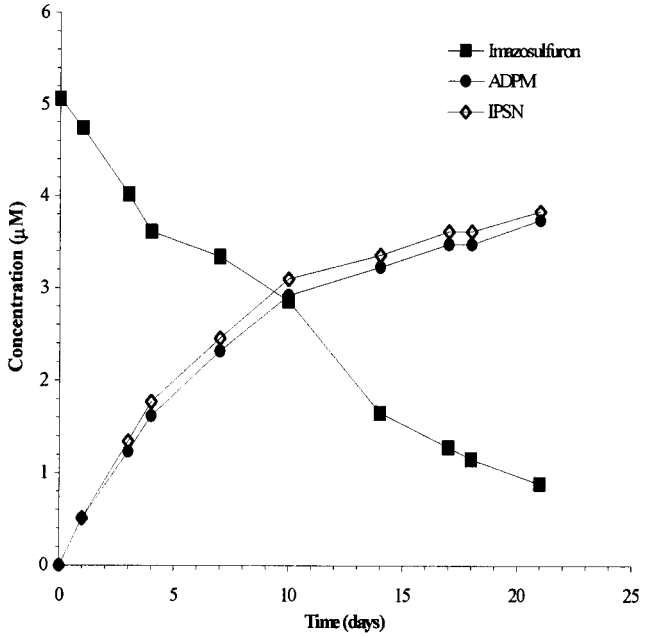

Figure 7. Hydrolysis of imazosulfuron in acidic conditions (pH 3.6 ± 0.1) in the dark at ambient temperature (25 ± 2 °C): formation of ADPM and IPSN.

Conclusions. The results indicate that the rate of imazosulfuron hydrolysis is dependent on pH and temperature. This is in agreement with the findings of Beyer et al. (*7*), who pointed out the importance of both of these conditions of the aqueous medium in controlling the hydrolysis rate of sulfonylureas. It is important to emphasize that at pH and temperature values typical of the aquatic environment (5.5−8.0 and 10−15 °C, respectively) imazosulfuron should exhibit a relatively slow aqueous degradation. Its half-life at pH 5.9 is 578 days, and it tends to become higher at higher pH values, as well as at lower temperature values.

At all pH and temperature values studied the cleavage of the sulfonylurea bridge is the main pathway of degradation of imazosulfuron. Similarly to other sulfonylureas, this reaction appears to be accelerated by acidic and temperature conditions (*18*, *19*); however, contrarily to other sulfonylureas, higher pH values seem to strongly reduce the hydrolysis rate of imazosulfuron.

In fact, alkaline conditions have been demonstrated to promote the hydrolysis of some sulfonylurea herbicides (*14*, *15*, *20*). Therefore, we can conclude that different degradation behaviors can occur within the sulfonylurea family, depending on the chemical structure of the molecule under examination.

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