Kinetics and Hydrolysis Mechanism of Triasulfuron

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The hydrolysis of the sulfonylurea herbicide triasulfuron [(2-(2-chloroethoxy)-*N*-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide] was studied in aqueous buffers of pH values 2, 3, 4, 5, 6, 7, and 9. The reaction was of first-order and pH-dependent. Triasulfuron was more persistent in neutral or weakly basic than in acidic solution. Five metabolites have been isolated and identified. At all pH values studied, the primary pathway of degradation was the cleavage of the sulfonylurea bridge. However, minor degradation pathways have also been observed like O-demethylation and opening of the triazine ring. The product distribution was pH-dependent.

Keywords: *Sulfonylureas; herbicides; triasulfuron; hydrolysis; degradation*

INTRODUCTION

Triasulfuron is a selective sulfonylurea herbicide used at very low rates (10−25 g of active ingredient/ha) for weed control in cereals (Amrein and Gerber, 1985). Sulfonylurea herbicides degrade in soil by chemical hydrolysis and microbial degradation (Beyer et al., 1988). Generally, hydrolysis involves the breakdown of the sulfonylurea bridge to give the corresponding sulfonamide and heterocyclic amine. Nevertheless, alternative pathways were observed for chemical hydrolysis. Rimsulfuron hydrolyzes easily through the contraction of the sulfonylurea bridge (Schneiders et al., 1993), whereas the cleavage of the sulfonylurea bridge and O-demethylation of the methoxy group of the triazine ring occur for thifensulfuron methyl (Cambon and Bastide, 1996). Several studies were devoted to the degradation of triasulfuron in soil (Iwanzik and Amrein, 1988; Martin and Blair, 1988; Walker and Welch, 1989). In particular, Oppong and Sagar (1992) observed triasulfuron degradation in both sterile and nonsterile soils. Triasulfuron was more susceptible to chemical hydrolysis at acidic than neutral or basic pH (Dinelli et al., 1993; Berger and Wolfe, 1996). Dinelli et al. (1995), using capillary electrophoresis, detected five metabolites in the hydrolysis of triasulfuron at pH 4. However, no study concerning the degradation mechanism of triasulfuron and the identity of its byproducts is available. This work describes the kinetics of the chemical hydrolysis of triasulfuron in aqueous buffers in the pH range 2−9 and the nature of degradation products. On the basis of the results, a degradation pathway is proposed.

MATERIALS AND METHODS

Chemicals. Triasulfuron, 2-(2-chloroethoxy)-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide (**1**) (purity 99.5%) was supplied by Ciba-Geigy, Saronno, Italy. Its purity was checked by HPLC. All the solvents were of HPLC grade (Carlo Erba Reagenti, Milano, Italy) and were used without further purification.

2-Amino-4-methoxy-6-methyltriazine (2). Triasulfuron (0.5 g) was dissolved in DMSO (dimethyl sulfoxide, 3 mL). The solution was left at room temperature for 3 days. The precipitate formed was filtered, washed three times with $CHCl_3$, and then dried in a desiccator. Compound **2** was obtained as white crystals (Huffinan et al., 1963) (mp 256−257 °C; 45% yield). MS (*m*/*e*): 140 $(M)^+$, 110, 69, 42; IR (KBr drift) ν cm^{-1}: 1660, 1565, 1372, 1346, 1021.

2-(2-Chloroethoxy)benzenesulfonamide (3). To a solution of triasulfuron (0.5 g) in acetonitrile (5 mL) was added 1 N HCl (1 mL). The solution was stirred at room temperature overnight, and then the crude reaction mixture was concentrated under vacuum. The residue was separated by column chromatography on silica gel (70−230 mesh, Merck) using cyclohexane plus ethyl acetate (1 + 4 by volume) as eluant. By collecting the eluate fractions, each of 50 mL, metabolite **3** was present in the pure state from the third to fifth fraction. These fractions were gathered and evaporated under vacuum to dryness. Compound **3** was obtained as white crystals (mp 118−119 °C; 53% yield). MS (*m*/*e*): 235 $(M)^+$, 186, 173, 156; IR (KBr drift) ν cm^{-1}: 1589, 1481, 1324, 1159, 756.

2-(2-Chloroethoxy)-*N*-[[(4-hydroxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide (4). Metabolite **4** was obtained from triasulfuron according to the procedure proposed by Sabadie (1992) for the analogous byproduct of chlorosulfuron, modified as follows. To a suspension of triasulfuron (0.5 g) in water (50 mL) NaOH (0.1 g, pH = 12.5) was added under stirring. After 16 h, more NaOH (0.1 g) was added and the mixture was stirred at room temperature until clear (about 24 h). Then 1 N HCl was added to the solution up to pH 2.5. After precipitation, metabolite **4** was filtered, washed twice with distilled water, and dried in a desiccator. Compound **4** was obtained as white crystals (Adams et al., 1952) (mp 177−185 °C dec; 90% yield). MS (*m*/*e*): 235, 219, 199, 186, 173, 156; IR (KBr drift) ν cm^{-1}: 1737, 1690, 1591, 1471, 1432, 1361.

2-Amino-4-hydroxy-6-methyltriazine (5). Metabolite **2** (0.2 g) was dissolved in DMSO (2 mL) and aqueous 1 N NaOH (2 mL) was added. The solution was left overnight under stirring at room temperature. The clear reaction mixture was added with 1 N HCl up to neutrality and then concentrated under vacuum. The residue was recovered with water, filtered, washed twice with distilled water, and dried in a desiccator. The byproduct **5** was obtained as white crystals (mp >320 °C; 90% yield). MS (*m*/*e*): 127 $(M - H)^+$, 109, 99, 85, 81, 79; IR (KBr drift) ν cm^{-1}: 1698, 1682, 1611, 1462.

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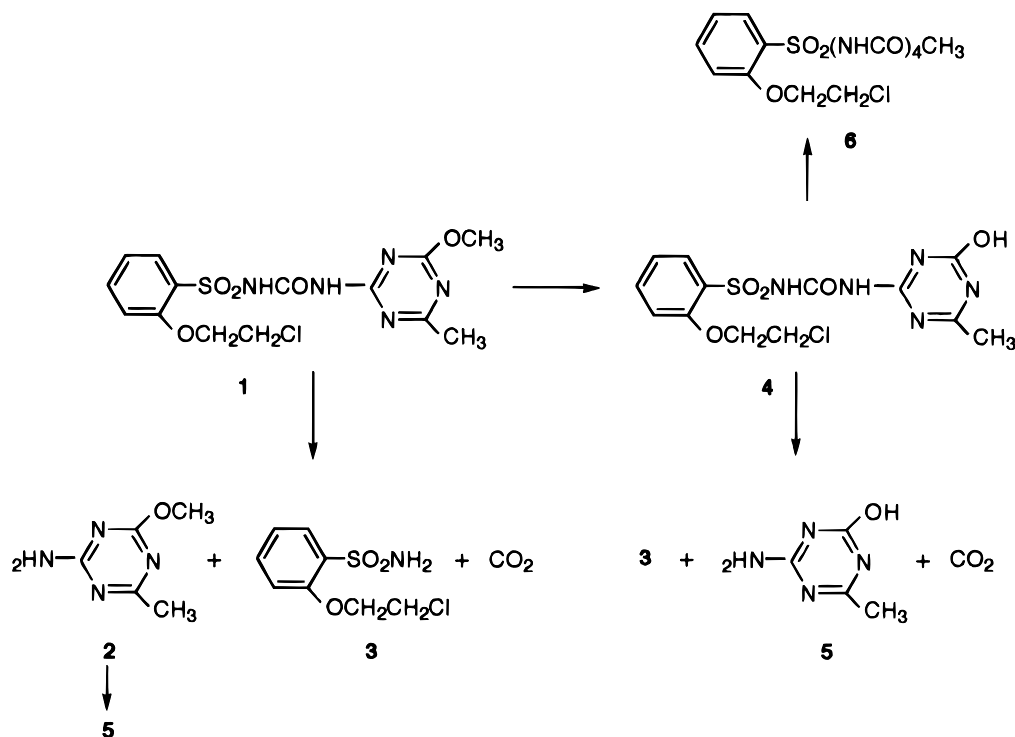

Figure 1. Structures of triasulfuron and its metabolites.

Table 1. ^{1}H NMR Chemical Shifts for Triasulfuron and Its Degradation Products[a]

	δ_H (ppm)						
assignment	**1**[b]	**2**[b]	**3**[b]	**4**[c]	**5**[d]	**6**[b]	**6**[e]
SO_2NH	12.44 (1H, brs)					11.06 (1H, brs)	10.96 (1H, brs)
CONH	10.99 (1H, brs)					11.02 (1H, brs)	10.91 (1H, brs)
						11.25 (1H, brs)	10.32 (1H, brs)
							9.23 (1H, brs)
phenyl	8.02−7.29 (4H, m)		7.85−7.21 (4H, m)	7.04−7.77 (4H, m)		7.98−7.28 (4H, m)	8.03−7.22 (4H, m)
triazine-NH_2		7.36 (2H, brs)					
SO_2NH_2			6.94 (2H, brs)				
CH_2Cl	4.51 (2H, t, J = 5.0)		4.52 (2H, t, J = 5.5)	4.31 (2H, t, J = 5.3)		4.55 (2H, t, J = 5.5)	4.46 (2H, t, J = 5.5)
OCH_3	4.09 (3H, s)	3.90 (3H, s)					
OCH_2	3.94 (2H, t, J = 5.0)		4.15 (2H, t, J = 5.5)	3.83 (2H, t, J = 5.3)		4.07 (2H, t, J = 5.5)	3.97 (2H, t, J = 5.5)
CH_3	2.58 (3H,s)	2.31 (3H,s)		2.09 (3H,s)	2.59 (3H,s)	2.21 (3H,s)	2.02 (3H,s)

[a] J values in Hz. [b] DMSO-d_6. [c] D_2O + NaOD, pH = 13. [d] D_2O + DCl, pH = 3. [e] CD_3CN.

1-[2-(2-Chloroethoxy)benzene-1-sulfonyl]-7-acetyltriuret (6). Metabolite **6** was obtained from triasulfuron according to the procedure proposed by Sabadie (1992) for the analogous byproduct of chlorosulfuron. Triasulfuron (0.5 g) was dissolved in a mixture of methanol (45 mL), water (5 mL), and 37% HCl (0.5 mL). After 4 days, a white precipitate was filtered, washed with water, and dried in an oven at 120 °C. Compound **6** was obtained as white crystals (mp 181−184 °C dec; 35% yield). MS (m/e): 407 $(M - H)^+$, 237, 219, 141, 121; IR (KBr drift) ν cm^{-1}: 1732, 1718, 1707, 1684, 1433, 1225, 1208, 628. Structures of triasulfuron **1** and its metabolites **2**−**6** are reported in Figure 1.

Buffer Solutions. Buffer aqueous 0.01 M solutions were prepared by diluting with sterile deionized water the following chemicals: Normex Carlo Erba Reagenti (Milano, Italy), citrate buffers (pH 2−6); Hydrion Aldrich (Milano, Italy), phosphate buffer (pH 7); Hydrion Aldrich, borate buffer (pH 9).

Hydrolysis Rate Determination. The hydrolysis rate was determined by monitoring the disappearance of triasulfuron in 0.01 M aqueous buffer solutions.

A stock solution containing 2.49 mmol L^{-1} (1 g L^{-1}) triasulfuron in acetonitrile was prepared. Aliquots of 1.2 mL of the stock solution were diluted to 100 mL with the appropriate aqueous buffers of pH 2−6 and 9. The final concentration of triasulfuron in the buffered solutions was 29.89 μM. For the hydrolysis at pH 7, 1.3 mL of stock solution was diluted with phosphate buffer at pH 7 up to of 100 mL; the resulting concentration was 32.59 μM. A drop of toluene was added to each test solution in order to minimize microbial activity (Ballard and Fiskell, 1974). The solutions were maintained in the dark at 25 °C. At appropriate times, depending on the hydrolysis rate, each test solution was analyzed directly by HPLC. All the experiments were run in triplicate.

All the metabolites structures were confirmed by MS, ^{1}H and ^{13}C NMR, and FT-IR.

HPLC. The concentration of triasulfuron and its degradation products present after various intervals of incubation was determined by HPLC (high-performance liquid chromatography). The system was assembled as follows: a Jasco 880-PU Intelligent pump equipped with a Rheodyne 7120 injection valve; a Jasco 875-UV Intelligent UV/VIS detector at 224 nm; a Borwin v 1.21.60 chromatography software; a Spherisorb C_8 analytical column (5 μm, 4.6 × 250 mm) eluting with acetonitrile plus water (40 + 60 by volume, pH = 2.75) at a flux of 1 mL^{-1}. The retention times for triasulfuron and its metabolites, under the chromatographic conditions described previously, were 10.5, 9.3, 6.7, 6.2, 5.5, and 4.5 for the compounds **1**, **6**, **3**, **2**, **5**, and **4**, respectively. The quantitation of triasulfuron and its degradation products was based on an external standard. The detector response was calibrated with reference standards of triasulfuron **1** and its metabolites **2**−**6** obtained as described before. Calculations were based on the average peak areas of the external standards.

Spectroscopic Measurements. NMR (nuclear magnetic resonance) spectra were determined on a Bruker AC-P (300

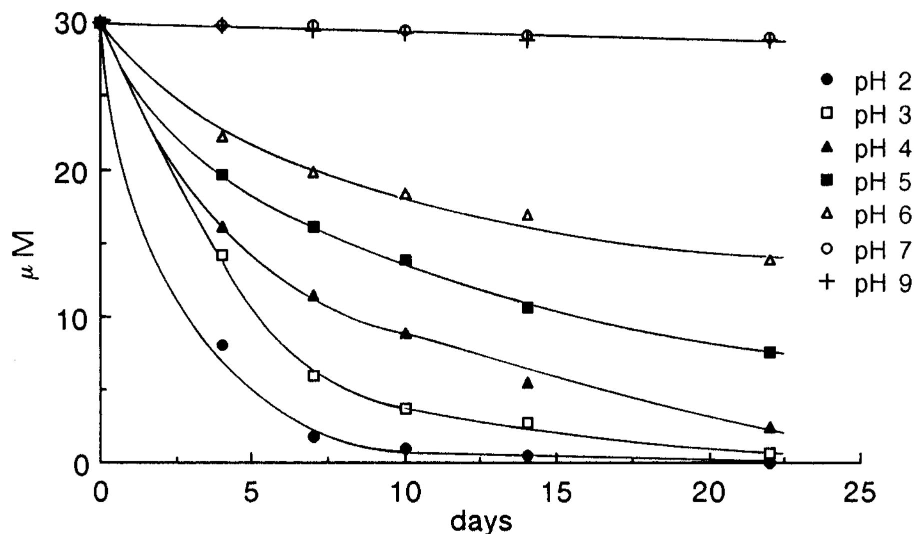

Figure 2. Disappearance of triasulfuron at different pH values.

Table 2. ^{13}C NMR Chemical Shifts for Triasulfuron and Its Degradation Products[a]

	δ (ppm)					
assignment	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[c]	**6**[a]
triazine ring	178.3	176.9		177.9	174.9	
	170.0	170.7		171.0	161.4	
	164.0	168.2		165.3	150.1	
$COCH_3$						172.7
HNCONH	148.3			155.5		150.2
						149.9
						147.7
phenyl	155.2		154.5	158.5		155.2
	135.9		133.8	134.7		135.8
	131.2		131.5	130.7		130.7
	126.1		127.7	130.2		126.2
	120.7		120.7	121.6		120.5
	113.9		113.9	114.8		114.0
CH_2Cl	69.3		68.9	69.8		69.1
OCH_3	55.1	53.7				
OCH_2	42.6		42.6	43.0		42.3
CH_3	25.1	24.8		24.0	23.5	23.8

[a] DMSO-d_6. [b] D_2O + NaOD, pH = 13. [c] D_2O + DCl, pH = 3.

Table 3. Kinetics Data of Hydrolysis of Triasulfuron at Different pH Values

pH	$k_{obs} \times 10^{-3}$ (d^{-1})	$t_{1/2}$ (d)	correl coeff
2	240	2.89	0.999
3	141	4.93	0.997
4	89.1	7.78	0.996
5	50.0	13.8	0.986
6	25.6	27.1	0.954
7	1.41	492	0.969
9	1.79	387	0.993

MHz) NMR spectrometer using Bruker software. The ^1H and ^{13}C shifts of the compounds **1**–**6** are listed in Tables 1 and 2, respectively.

Mass spectra were obtained on a Perkin Elmer SCIEX API III-Plus (atmospheric pressure ionization) spectrometer equipped with an ion spray liquid–mass interface and a VG 7070 E MS spectrometer at 70 eV using electron impact ionization.

Fourier transform IR spectra were recorded at room temperature within the range 4000–600 cm^{-1} using a FT-IR Nicolet Impact 400 spectrophotometer and a Omnic FT-IR software. The IR spectra of triasulfuron and its metabolites were obtained using a DRIFT (diffuse reflectance infrared Fourier transform) device.

RESULTS AND DISCUSSION

Hydrolysis Rate. The hydrolysis of triasulfuron in the pH range 2–9 followed simple pseudo-first-order kinetics (Table 3 and Figure 2). The reaction was quite fast at acid pH and much slower at neutral and basic pH. Noticeably, a drastic decrease of the rate constant was observed from pH 6 to pH 7, most likely due to a mechanicistic change. Since no significant difference

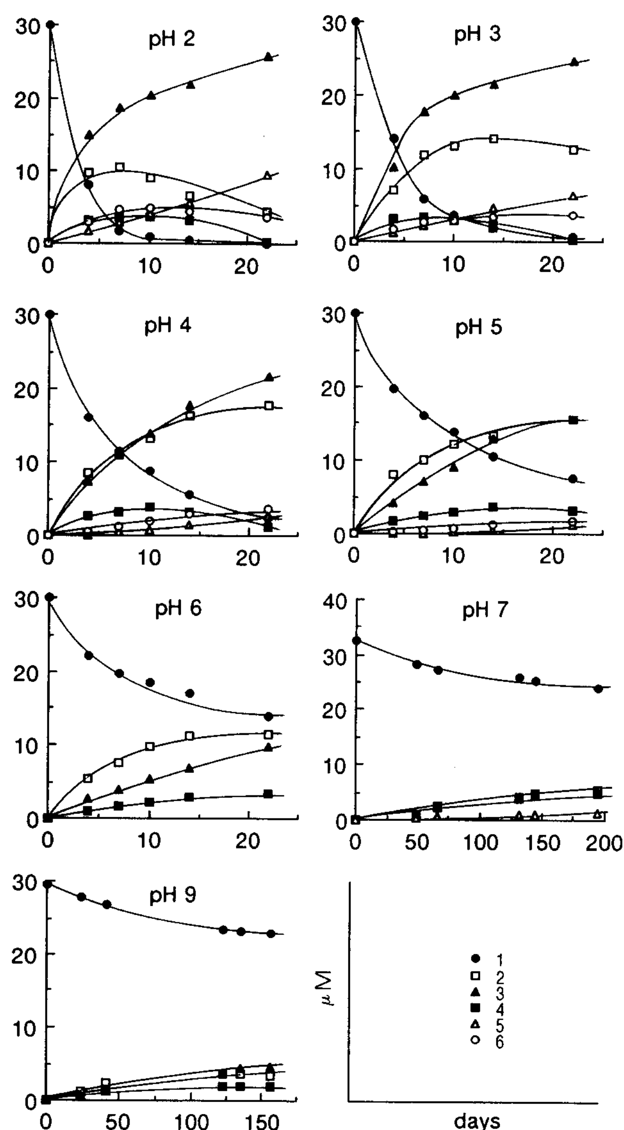

Figure 3. Product distribution at different pH values.

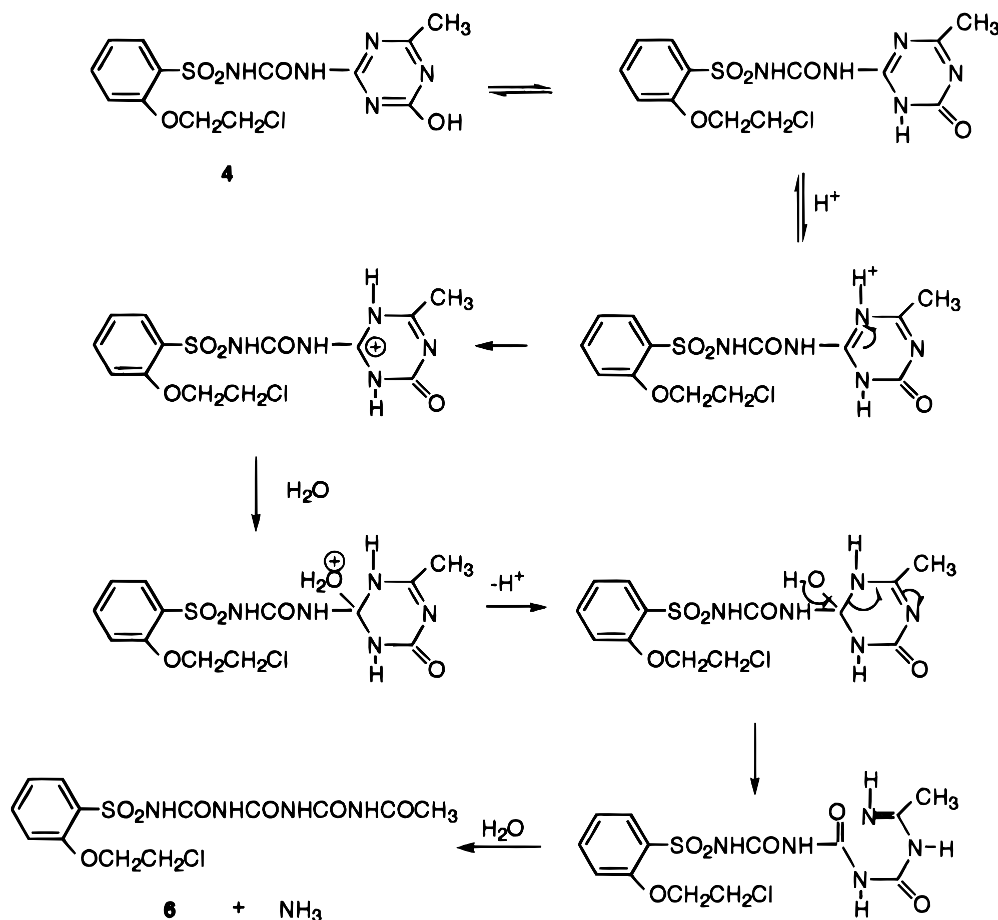

Figure 4. Degradation mechanism of triasulfuron to the acetyltriuret **6**.

was observed on changing either phosphate with acetate or their concentration, an effect of the buffer on hydrolysis can be ruled out. However, due to the complexity of the reactions involved, a deeper discussion on the kinetic aspects of the process is beyond the aim of this work. On the whole, the results are in agreement with those reported by Dinelli et al. (1993). In fact, they indicated that the disappearance of triasulfuron at 25 °C from pH 2 to pH 7.5 was of pseudo-first-order and that triasulfuron was more persistent at neutral or weakly basic than at acidic pH. However, the values of the pseudo-first-order rate constants and half-lives were different from ours, particularly those at acidic values of pH. This discrepancy may be attributed to the fact that the rates were not measured in buffered solutions. Triasulfuron behaves as a weak acid with a pK_a value of 4.5 in water (Worthing and Hance, 1991) due to the dissociation of the NH group within the sulfonamide bridge. Therefore, small changes in pH would affect the neutral/anionic form ratio for the herbicide, particularly near its pK_a. At pH 9, a small increase of hydrolysis rate is observed, probably because of a base-catalyzed reaction.

Hydrolysis Metabolites. Altogether five products were detected by HPLC in hydrolysis experiments and identified as **2**−**6** (Figure 1). Their structures were confirmed by spectral data and by comparison of the HPLC retention times with those of authentic samples obtained by univocal synthesis. Accordingly, Dinelli et al. (1995), by using capillary electrophoresis, detected five triasulfuron metabolites during the hydrolysis at pH 4. However, the nature of the degradation products was not investigated.

The product distribution in triasulfuron hydrolysis at different pH values is reported in Figure 3. In the range of pH 2−5, the products **2** and **3** were the major metabolites with minor amounts of **4**−**6**. Methoxytriazine **2** and sulfonamide **3** arise from the hydrolytic cleavage of the sulfonylurea bridge. Instead, the O-demethylation of methoxy group of triazine ring affords hydroxytriasulfuron **4**. The latter metabolite is unstable in acidic aqueous condition (pH range 2−5) and degrades further through two different pathways: (i) the cleavage of the sulfonylurea bridge to give sulfonamide **3** and hydroxytriazine **5** and (ii) the opening of the triazine ring to yield acetyltriuret **6**. This was confirmed in a separate experiment (not described in the experimental part) in which treating the isolated byproduct **4** at pH 3 caused metabolites **3**, **5**, and **6** to be obtained.

Product **6** is the result of acid-catalyzed hydrolytic breakdown of the triazine ring (Figure 4). This reaction is known and, with the non-substituted triazabenzene ring, affords aldehydic compounds (Quirke, 1984). Accordingly, when triasulfuron is hydrolyzed at pH 2−5, the concentrations of byproducts **3**, **5**, and **6** increased continuously with time, whereas the concentration of **4** increased steadily to a maximum and then decreased thereafter. At pH 2 and pH 3, also methoxytriazine **2** is not stable and analogously to metabolite **4** undergoes an O-demethylation reaction yielding the hydroxytriazine **5**.

At pH 6, triasulfuron is totally in anionic form, less suited to the nucleophilic attack by water; therefore, the hydrolysis rate decreases. Metabolites **2**−**4** are formed, but the absence of an effective acid catalysis does not permit further degradation of products **2** and **4**. In the range of pH 7−9 triasulfuron degrades slowly to give

comparable amounts of **2** and **3** and only a minor amount of byproduct **4**. Under neutral to alkaline aqueous conditions, both the compounds **2** and **4** are stable and do not degrade further.

Conclusions. The results indicate that the rate of hydrolysis of triasulfuron is pH-dependent. At all values of pH studied, the primary pathway of degradation is the cleavage of sulfonylurea bridge. This reaction is catalyzed to a higher extent by acidic than basic conditions. This is in agreement with what is generally reported on the chemical hydrolysis of sulfonylurea herbicides (Beyer et al., 1988). However, minor degradation pathways have also been observed for triasulfuron, like O-demethylation and opening of the triazine ring. Studies carried out on metsulfuron-methyl (Badon et al., 1990), chlorosulfuron (Sabadie, 1992), and thifensulfuron-methyl (Cambon and Bastide, 1996) (three sulfonylureas sharing a methoxy substitution in the triazine ring with triasulfuron) showed hydrolysis pathways similar to those of triasulfuron. For all three sulfonylureas, in addition to their respective sulfonamide and aminotriazine derivatives, products both of O-demethylation and hydrolytic breakdown of the triazine ring were isolated and identified. Therefore, this kind of reactions seems common to methoxytriazine-substituted sulfonylurea herbicides. In the pH range 5.5–8.0, typical of the aquatic environment, only byproducts **2** and **3** arising from the cleavage of sulfonylurea bridge and a minor amount of O-demethylated compound **4** are expected. However, it cannot be excluded that hydrolysis surface catalyzed by soil colloids could promote unexpected reactions. This will be the object of further research.

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