Degradation of Primisulfuron by a Combination of Chemical and Microbiological Processes

Ilaria Braschi,[†,‡] Alba Pusino,[§] Carlo Gessa,[‡] and Jean-Marc Bollag*[,†]

Laboratory of Soil Biochemistry, Center for Bioremediation and Detoxification, The Pennsylvania State University, University Park, Pennsylvania; Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, Università di Sassari, Sassari, Italy; and Istituto di Chimica Agraria, Università di Bologna, Bologna, Italy

Microbial degradation of the herbicide primisulfuron was investigated using enrichment cultures from contaminated soils and 20 axenic cultures. At neutral pH, no disappearance of the herbicide was detected either in the enrichment cultures or in the growth media of the axenic microbial cultures. During the growth of some of the microbial strains, however, the pH of the medium dropped below 6, resulting in the hydrolysis of primisulfuron. The rate of primisulfuron hydrolysis was clearly pH dependent; primisulfuron was more persistent in neutral or weakly basic solutions than in acidic solutions. After hydrolysis of the herbicide, four products were observed. These were identified as methyl 2-(aminosulfonyl)benzoate, 2-amino-4,6-(difluoromethoxy)pyrimidine, 2-*N*-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoic acid, and 2-(aminosulfonyl)benzoic acid. After hydrolysis, it was found that the fungus *Phanerochaete chrysosporium* mineralized 27 and 24% of ^{14}C-phenyl- and ^{14}C-pyrimidine-labeled products, respectively, after 24 days of incubation. Similarly, *Trametes versicolor* mineralized 13 and 11% of ^{14}C-phenyl- and ^{14}C-pyrimidine-labeled hydrolysis products, respectively. In addition, primisulfuron in a hydrolytically stable solution, at pH 7.0, was rapidly decomposed after ultraviolet irradiation, and two photolysis products were isolated [methylbenzoate and 4,6-(difluoromethoxy)pyrimidin-2-ylurea]. When ^{14}C-phenyl-labeled primisulfuron was exposed to photolysis for 24 h, 32% of the initial radioactivity was recovered as $^{14}CO_2$, whereas no $^{14}CO_2$ was detected if the herbicide was labeled at the ^{14}C-pyrimidine position. Mineralization of ^{14}C-pyrimidine-labeled products of photolyzed primisulfuron by *P. chrysosporium* was ∼25% after 24 days. These results clearly indicate that hydrolysis and photolysis of primisulfuron facilitated microbial degradation.

Keywords: *Sulfonylureas; primisulfuron; herbicide; hydrolysis; photolysis; fungi*

INTRODUCTION

Sulfonylurea herbicides control weeds by inhibiting acetolactate synthase, a key enzyme in the branched-chain amino acid biosynthetic pathways of bacteria, fungi, and higher plants (Blair and Martin, 1988; Beyer et al., 1988). Crop tolerance to sulfonylureas is related to the ability of certain plants to rapidly convert sulfonylureas to herbicidally inactive compounds. Primisulfuron, a sulfonylurea herbicide developed by Ciba-Geigy Corp. under the trade name Beacon, is used for the selective control of many postemergence broadleaf weeds in corn at a use rate as low as 20−40 g/ha (Foy and Witt, 1990). In contrast, conventional herbicides such as metolachlor, alachlor, and atrazine require use rates of 0.5−2.0 kg/ha.

It has been reported that the rate of chemical or biological degradation of sulfonylurea herbicides increased with increasing temperature, increasing soil moisture content, and decreasing soil pH (Blair and Martin, 1988; Beyer et al., 1988). According to Maurer et al. (1987), at soil field capacity of 50%, the half-life of primisulfuron is 30 days at 21 °C and 8 days at 35 °C. Klaffenbach et al. (1995) observed primisulfuron half-lives of 13 and 23 days at different soil−water contents and temperatures.

Studies with other sulfonylureas showed that this group of chemicals is susceptible to nonbiological hydrolysis in acidic soils. Soil microorganisms then act on the hydrolysis products and eventually convert them to carbon dioxide. Berger and Wolfe (1996) and Dinelli et al. (1997) found that primisulfuron was more susceptible to chemical hydrolysis at acidic pH than at neutral or basic pH. In alkaline soils, microbial breakdown of sulfonylureas was reported as the major dissipation mechanism (Anderson and Dulka, 1985; Beyer et al., 1988). No study is available concerning the degradation mechanism of primisulfuron and the identification of its products.

Although photolysis is not considered to be a major degradation process for sulfonylureas, several studies have indicated that photodegradation can be an alternative pathway to chemical hydrolysis (Harvey et al., 1985; Choudhury and Dureja, 1996, 1997). No studies concerning the photodegradation of primisulfuron are available.

* Address correspondence to this author at 129 Land and Water Building, The Pennsylvania State University, University Park, PA 16802 [telephone (814) 863-0843; fax (814) 865-7836; e-mail jmbollag@psu.edu].

† Laboratory of Soil Biochemistry.

‡ Istituto di Chimica Agraria.

§ Dipartimento de Scienze Ambientali Agrarie e Biotechnologie Agro-Alimentari.

10.1021/jf990604q CCC: $19.00
Published on Web 05/03/2000

Joshi et al. (1985) were the first to demonstrate the microbial degradation of chlorsulfuron, a sulfonylurea herbicide, in pure cultures. *Streptomyces griseolus* metabolizes chlorsulfuron via conversion of the chloride group at the phenyl ring to a hydroxyl group and hydroxylation of the methyl group at the heterocycle. Two soil fungi, *Aspergillus niger* and *Penicillium* sp., catalyze the hydrolysis of the sulfonylurea bridge, yielding the corresponding sulfonamide and the heterocycle. In the studies of Joshi et al. (1985), the effect of pH on the hydrolysis of the herbicide was not indicated.

In the present study, we investigated (1) the degradation of primisulfuron in axenic and mixed microbial cultures, (2) the stability of primisulfuron under hydrolytic and photolytic conditions, and (3) the mineralization of the hydrolysis and photolysis products from primisulfuron by fungal cultures.

MATERIALS AND METHODS

Chemicals. Primisulfuron [methyl 2-*N*-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino] carbonyl]amino]sulfonyl]benzoate (CGA-136'872)] with purity of 99.5% was supplied by Ciba-Geigy Corp. (Saronno, Italy, and Greensboro, NC). ^{14}C-Phenyl-labeled primisulfuron (98.1% radiochemical purity with a specific activity of 1.79 kBq/g) and ^{14}C-pyrimidine-labeled primisulfuron (98.2% purity with a specific activity of 1.68 kBq/g) were supplied by Agricultural Division, Ciba-Geigy Corp. (Greensboro, NC). Methyl 2-aminosulfonylbenzoate (CGA-120844) also was provided by Ciba-Geigy. Methyl benzoate was purchased from Aldrich (Milan, Italy), and saccharin was purchased from Sigma Chemical Co. (St. Louis, MO).

Batch Enrichment Cultures. Batch enrichment cultures were carried out using an inoculum from soil contaminated with primisulfuron; the soil samples from Mississippi, Tennessee, and North Carolina were provided by Ciba-Geigy. Soil samples (2 g) were added to 250 mL Erlenmeyer flasks each containing 100 mL of a liquid medium. Soil samples sterilized by 3 Mrad irradiation were mixed with medium to serve as controls. The medium was composed of (per liter of Milli Q water) 0.5 mg of primisulfuron, 0.5 g of NH_4Cl, 0.5 g of $MgSO_4 \cdot 5H_2O$, 3.1 g of Na_2HPO_4, 1 g of KH_2PO_4, 10 mg of $CaCl_2 \cdot H_2O$, 0.5 mg of $MnCl_2 \cdot 4H_2O$, 0.5 mg of $ZnSO_4 \cdot 7H_2O$, 10 mg of $FeCl_3 \cdot H_2O$, 20 mg of yeast extract, and 2 g of glucose (0.2%) and had a pH of 7.1. The medium was filter-sterilized by passage through a 0.22-μm nylon membrane (Fisher Scientific Co., Fair Lawn, NJ). All cultures were incubated at 28 °C on a rotary shaker (100 rpm). The disappearance of primisulfuron from 15-day-old cultures was determined by analyzing the culture filtrate by high-performance liquid chromatography (HPLC). Additionally, a 1-mL sample from each of the 12-day-old cultures was transferred to a new medium; three such sequential transfers were made. An aliquot of the enrichment cultures was removed 15 days after each transfer and analyzed by HPLC for the remaining substrate. Several pure cultures isolated from the enrichment cultures subsequently were tested for their ability to degrade primisulfuron.

Screening for Microorganisms that Degrade Primisulfuron. Twenty axenic cultures, three isolated from the enrichment cultures and the rest obtained from the culture collection of the Laboratory of Soil Biochemistry (The Pennsylvania State University), were tested individually for their ability to degrade primisulfuron in a liquid medium. The medium used was the same as described for the batch enrichment cultures, except that it contained 1% glucose or sucrose. Analysis of substrate disappearance was made with 15-day-old cultures using HPLC.

Hydrolysis of Primisulfuron. The hydrolysis rate was determined by monitoring the disappearance of primisulfuron in buffered solutions that were prepared by diluting the following chemicals: Normex Carlo Erba citrate buffers (pH 4−6), Hydrion Aldrich phosphate buffer (pH 7 and 8), and Hydrion Aldrich borate buffer (pH 9 and 10) (Milano, Italy); the final concentration was 0.01 M. The initial buffer concentration of primisulfuron in the buffered solutions was 28 μM. A drop of toluene was added to each test solution to minimize microbial activity. The solutions were maintained in the dark at 25 °C. At appropriate times, depending on the hydrolysis rate, each test solution was analyzed by HPLC. All experiments were run in triplicate.

Isolation of Hydrolysis Products. Hydrolysis of primisulfuron was carried out to isolate and identify the degradation products. In the first experiment, 250 mg of primisulfuron was dissolved in 100 mL of a solution of acetonitrile and 1 M HCl (1:1, v/v). After 6 days of incubation at 25 °C, the crude reaction mixture was concentrated and extracted three times with dichloromethane. The combined extracts were dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was separated by liquid chromatography (LC) on silica gel using diethyl ether plus ethyl acetate (1:3, v/v) as the eluant. Under these experimental conditions, products **1** and **2** were isolated.

In the second experiment, 250 mg of primisulfuron was dissolved in a 0.5 M NaOH solution. After 24 h, the crude mixture was filtered through a glass fiber (GF) Whatman filter, acidified to pH 5, and then extracted with dichloromethane. The organic phase, after drying over anhydrous sodium sulfate, was concentrated under vacuum. Subsequently, the residue was dissolved in ethanol to crystallize the compound, named product **3**. Finally, 2-(aminosulfonyl)benzoic acid was obtained by heating saccharin in a basic solution according to the procedure proposed by Hemmanda et al. (1994).

Ultraviolet (UV) Irradiation of Primisulfuron. The UV spectrum of primisulfuron is characterized by intense absorption at 240 nm. The absorption is very weak at wavelengths above 300 nm. Solutions contained in a water-cooled quartz flask were irradiated at 254 nm in a Rayonet carousel photoreactor with low-pressure mercury lamps. For wavelengths above 254 nm, a water-cooled borosilicate flask, fluorescent lamps at 300 nm, and black light fluorescent lamps at 366 nm were used.

The photolysis rate was determined by monitoring the disappearance of primisulfuron in 0.01 M aqueous buffer solution that was prepared by diluting Hydrion Aldrich pH 7 phosphate buffer. The initial concentration of primisulfuron in the solution was 4 μM. Control samples were wrapped with aluminum foil and processed the same way. At appropriate times, depending on the photolysis rate, each test solution was analyzed by HPLC. All of the experiments were run in triplicate.

Isolation of Photolysis Products. A photolysis reaction was carried out with primisulfuron to isolate and identify the degradation products. Primisulfuron (200 mg) dissolved in 80 mL of acetonitrile and 20 mL of water was irradiated at 254 nm for 20 min. The crude reaction mixture was concentrated by evaporation under vacuum at room temperature. Products **5** and **6** were detected and isolated by LC on silica gel using ethyl acetate as the eluant.

Mineralization of the Hydrolysis or Photolysis Products from Primisulfuron by Fungal Cultures. To study the mineralization of products from the hydrolysis reaction, solutions containing 1 μM primisulfuron and 1.1×10^2 kBq/L ^{14}C-phenyl-labeled or 1.3×10^2 kBq/L ^{14}C-pyrimidine-labeled primisulfuron, respectively, were hydrolyzed at pH 3 until the disappearance of primisulfuron could be confirmed. After hydrolysis, the total radioactivity of the solutions was measured. The pH was adjusted to 4.5 before the hydrolyzed solution was used for the mineralization studies.

To study the mineralization of products from the photolysis reaction, solutions containing 1 μM primisulfuron and 1.1×10^2 ^{14}C-phenyl-labeled or 1.3×10^2 kBq/L ^{14}C-pyrimidine-labeled primisulfuron, respectively, were photolyzed at 254 nm in pH 7-buffered solution until the primisulfuron disappeared (incubation time = 24 h). Again, after the photolysis reaction, the total radioactivity of the solutions was measured. The solution containing ^{14}C-pyrimidine-labeled products was concentrated under vacuum. The residue was separated by LC on silica gel using diethyl ether and ethyl acetate (1:3, v/v) as the eluant. Products **2** and **6** as well as traces of product **5** were isolated under the described chromatographic conditions.

Table 1. Recovery of Primisulfuron from Batch Enrichment Cultures Inoculated with Herbicide-Contaminated Soils and Grown in a Medium with 0.2% Glucose

inocula	pH [a]	primisulfuron (% of initial) [b]
control, not inoculated	7.1	101.2
Mississippi soil, sterile	6.7	94.7
Mississippi soil, nonsterile	6.8	95.7
Tennessee soil, sterile	6.8	95.3
Tennessee soil, nonsterile	6.6	95.5
North Carolina soil, sterile	6.9	94.8
North Carolina soil, nonsterile	6.9	96.3

[a] pH of 15-day-old cultures. [b] Values presented are means of two samples from 15-day-old cultures.

Solutions containing products **2** and **6** were used for the mineralization studies at the radioactivity concentration of 1.3×10^2 kBq/L.

The fungal cultures were grown in a nitrogen-limited medium (pH 4.5) prepared according to the method of Kirk et al. (1978). The medium contained the hydrolysis and photolysis products. A spore suspension with an optical density of 0.05 at 650 nm was used as the inoculum for *P. chrysosporium*. A small piece of mycelial mat was used as the inoculum for *T. versicolor* and *Fusarium racemosum*. Each flask was stoppered with air exchange glass tubing connected with bacterial filters at both the air inlet and the air outlet. In addition, a Florisil Sep-Pak (Waters Associates, Milford, MA) was placed between the flask and the $^{14}CO_2$ trap to absorb volatile radioactive materials. The fungal cultures were incubated at 30 °C while stationary. During the first 2 days after inoculation, the cultures were incubated under an air atmosphere. On day 3, the cultures were flushed with humidified pure oxygen; thereafter, the oxygen flushing process was repeated every 3 days. After a 24-day incubation, the growth medium was analyzed for radioactivity. All $^{14}CO_2$ evolution studies were performed in triplicate.

Mineralization of the hydrolysis and photolysis products from primisulfuron was determined by monitoring the amount of $^{14}CO_2$ trapped in a glass tube containing 4 mL of 0.5 M NaOH.

Chromatographic Analysis of Primisulfuron and Its Products. A Waters 510 liquid chromatograph equipped with a multiwavelength Waters 490 programmable detector operating at 240 nm and a Waters Baseline 810 chromatography workstation (Milford, MA) was used. The quantitation of primisulfuron and its products was based on an external standard. Calculations were based on the average peak areas of the external standards. Thin-layer chromatography (TLC) was performed on Merck silica gel F254 plates. LC was done using Merck silica gel (Kieselgel 40, 70−230 mesh).

Spectrometric Analysis. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AC-P (300 MHz) spectrometer (Cambridge, MA) using Bruker software. Infrared (IR) spectra were recorded at room temperature within the range of 4000 to 600 cm^{-1} using a Fourier transform (FT)-IR Nicolet Impact 400 spectrophotometer and Omnic FT-IR software. Mass spectra were obtained from electron ionization (EI) or chemical ionization (CI) mass spectrometric analysis using isobutane on a Kratos MS-25 (Manchester, U.K.) or by fast atom bombardment (FAB) xenon atom analysis using glycerol as a matrix on a Kratos MS-9/50 double-focusing mass spectrometer (Manchester, U.K.). Radioactivity was measured in Ecoscint (National Diagnostics, Manville, NJ) scintillation cocktail with a Beta Trac 6895 liquid scintillation counter (Tracor Analytic, Elk Grove, IL).

RESULTS

No primisulfuron disappeared from any of the enrichment cultures that were grown in 0.2% glucose-containing medium (Table 1); this observation was made even after three sequential transfers (data not shown). The pH of these cultures at the time of sampling ranged from 6.6 to 7.1.

Table 2. Screening for Axenic Microorganisms that Biotransform Primisulfuron in Liquid Media (Incubation Time = 15 Days)

	medium with 1% glucose		medium with 1% sucrose	
microorganism	pH	primisulfuron (% of initial)	pH	primisulfuron (% of initial)
control (not inoculated)	7.0	98.9	7.2	99.2
Arthrobacter globiformis			7.3	97.9
Bacillus circulans	4.6	54.5	7.2	95.8
Bacillus megaterium			7.4	96.2
bacterial isolate 1 (from Mississippi soil)	3.8	7.8	6.8	93.9
bacterial isolate 2 (from North Carolina soil)	3.4	6.2	6.9	95.6
bacterial isolate 3 (from North Carolina soil)	3.8	6.0	7.0	94.7
Micrococcus flavus	3.2	7.5	7.2	98.3
Pseudomonas aeruginosa	nd	nd	7.3	95.5
Pseudomonas acidovorans	4.0	10.2	7.1	97.5
Pseudomonas fluorescens	3.3	6.9	7.1	95.6
Pseudomonas putida	3.6	8.4	7.1	94.3
Serratia marcescens			7.3	97.1
Streptomyces sp.	6.7	89.7	7.2	96.8
Chaetomium globosum	5.1	74.9	7.0	94.2
Cunninghamella elegans	5.6	71.1	6.3	87.3
Fusarium racemosum	6.5	85.5	6.5	83.2
Phanerochaete chrysosporium	4.7	60.1	6.6	74.4
Rhizoctonia praticola	5.1	72.0	6.7	85.1
Syncephalastrum racemosum	5.5	74.1	6.5	89.5
Trametes versicolor	4.6	60.0	6.4	75.6

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

pH	$K_{obs} \times 10^{-3}$ ($days^{-1}$)	$t_{1/2}$ (days)	correlation coefficient
4.0	223	3.106	0.997
5.0	36.0	19.23	0.993
6.0	4.46	155.4	0.995
7.0	0.64	1086	0.983
8.0	0.73	951.9	0.992
9.0	0.73	952.9	0.994
10.0	1.65	418.6	0.994

A total of 20 axenic cultures were tested for their ability to degrade primisulfuron. For some microorganisms grown in the medium with 1% glucose, the disappearance of primisulfuron was well correlated with a decrease of pH in the medium (Table 2). Subsequent raising of the pH did not have a reversible effect on the substrate recovery. At pH <4, the recovery of primisulfuron was <10% of the applied chemical. Very little disappearance of primisulfuron was found at pH >6.5. When glucose was replaced by sucrose, there was no obvious change in the pH of the growth medium. As a result, the decrease of the substrate was minimal for most of the microbial cultures except for *P. chrysosporium*, *T. versicolor*, and *F. racemosum*, for which about 26, 24, and 17%, respectively, of primisulfuron was not accounted for after a 15-day incubation.

Because the stability of primisulfuron in the growth medium appeared to be greatly influenced by the pH, we investigated the chemical hydrolysis of primisulfuron in solutions with various pH values; the results obtained confirmed our previous observation (Table 3). The reaction was found to have pseudo-first-order kinetics. The reaction was quite fast at acid pH ($t_{1/2}$ = 3 days at pH 4) and much slower at neutral pH ($t_{1/2}$ = 1086 days at pH 7). At pH 10, an increase in the hydrolysis rate was observed ($t_{1/2}$ = 419 days).

Four products were detected by HPLC analysis after hydrolysis of primisulfuron at various pH values (Figure 1). In the range of pH 4−8, only products **1** and **2** were

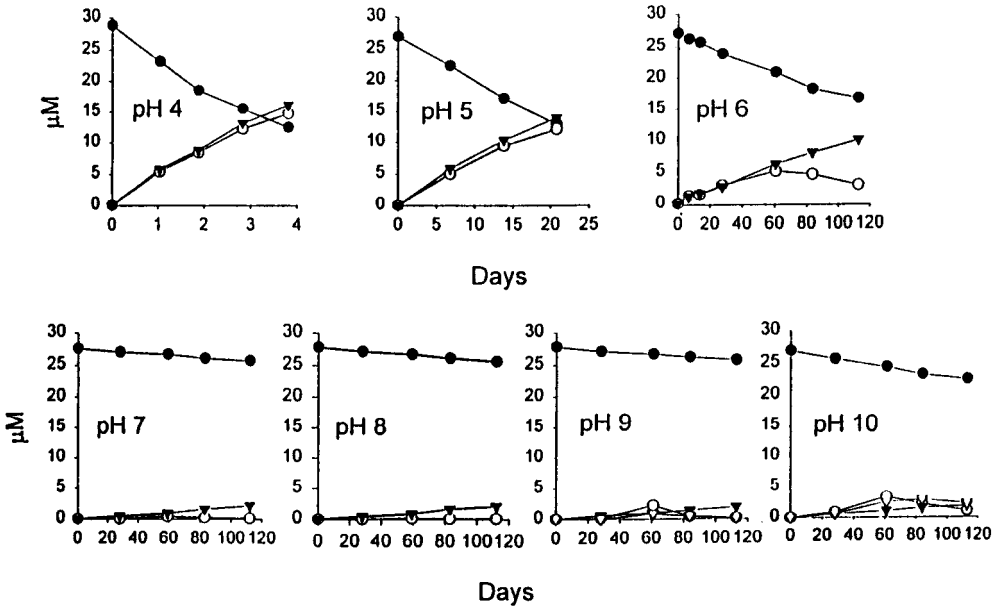

Figure 1. Formation of hydrolysis products from primisulfuron at various pH values: (●) primisulfuron; (○) product 1; (▼) product 2; (▽) product **3**.

Table 4. HPLC Data for Primisulfuron and Its Products

	t_R (min)	
compound	Spherisorb[a]	Nucleosil[b]
primisulfuron	5.1	9.5
product **1**	3.2	
product **2**	4.4	
product **3**	3.5	
product **4**	1.8	
product **5**		7.6
product **6**		3.7

[a] Spherisorb C_8 analytical column (5 μm, 4.6 × 250 mm): flow rate, 1 mL/min; eluant, acetonitrile/water (52:48); pH adjusted to 2.7 with H_3PO_4. [b] Nucleosil 100 C_{18} analytical column (5 μm, 4.6 × 250 mm): flow rate, 0.5 mL/min; eluant, acetonitrile/water (60:40); pH adjusted to 2.7 with H_3PO_4.

Table 5. TLC Data for Primisulfuron and Its Products

	R_f	
compound	eluant[a]	eluant[b]
primisulfuron	0.36	0.84
product **1**	0.59	0.87
product **2**	0.66	0.92
product **3**		0.57
product **4**		0.35
product **5**	0.80	
product **6**	0.92	

[a] Ethyl acetate. [b] Acetonitrile + ethyl acetate + formic acid (15 + 5 + 0.2 by volume).

detected. At pH 9 and 10, in addition to products **1** and **2**, small amounts of product **3** and traces of product **4** (not visible in Figure 1) were found. The analytical columns used for the HPLC analysis, the chromatographic conditions, and the retention times (t_R) of primisulfuron and its hydrolysis products are listed in Table 4. The TLC-developing solvent systems and the R_f of primisulfuron and all of the degradation products are listed in Table 5.

The structure of product **1** was identified by comparing the results of chromatographic analyses (TLC and HPLC) and spectral data [IR and ^{1}H NMR (Table 6)] with those of a standard (CGA-120844); the chemical was identified as methyl 2-(aminosulfonyl)benzoate. Product **2**, which was obtained as white crystals, had the following features: mp 66−67 °C; CI MS, *m*/*z* 228 $(M + H)^+$, 208, 192, 177, 161; IR (KBr) ν 1598, 1481, 1324, 1159, 756 cm^{-1}; and ^{1}H NMR chemical shifts as shown in Table 6. According to these characteristics, product **2** was identified as 2-amino-4,6-(difluoromethoxy)pyrimidine. The following characteristics were determined for product **3**, which was isolated as white crystals: mp 166−167 °C; IR (KBr) ν 1750, 1713, 1614, 1595, 1417, 1337 cm^{-1}; FAB MS, *m*/*z* 455 $(M + H)^+$, 254; and ^{1}H NMR chemical shifts as shown in Table 6. The chemical was identified as 2-*N*-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]-benzoic acid. The chromatographic characteristics (TLC and HPLC) and ^{1}H NMR chemical shift data (Table 6) of product **4** coincided with those of 2-(aminosulfonyl)-benzoic acid. The standard was obtained by direct synthesis according to the procedure proposed by Hemmanda et al. (1994). The structures of primisulfuron and products **1**−**4** are reported in Figure 2.

When the hydrolysis experiment was repeated at pH 3 by adding 1.1×10^2 or 1.3×10^2 kBq/L ^{14}C-phenyl- or ^{14}C-pyrimidine-labeled primisulfuron to 1 μM primisulfuron, no loss of radioactivity was observed (data not shown). When the fungi *P. chrysosporium* and *T. versicolor* were incubated with the hydrolyzed solutions derived from the ^{14}C-phenyl-labeled primisulfuron, 26.7 and 13.2%, respectively, of the initial radioactivity were mineralized after a 24-day incubation. With the products from ^{14}C-pyrimidine-labeled primisulfuron, 24.2 and 10.7%, respectively, of the initial radioactivity were mineralized under the same conditions (Figure 3). No mineralization was observed with *F. racemosum* in the same medium.

In the growth medium of *P. chrysosporium* incubated at pH 4.5 with the ^{14}C-pyrimidine-labeled hydrolysis products, a new metabolite (product **7**) was detected and isolated by HPLC. The EI mass spectrum gave a molecular ion at *m*/*z* 243, where the absence of a protonated molecular ion is due to the presence of an amino group. This pattern was similar to the EI mass fragmentation pattern of product **2** except for 16 units more in the molecular ion. Attempts to isolate product **7** for NMR characterization were not successful due to the low initial substrate concentration (1 μM), but on

Table 6. ^{1}H NMR Chemical Shift for Primisulfuron and Its Degradation Products[a]

	δ_H (ppm)					
assignment	primisulfuron[b]	product **1**[c]	product **2**[c]	product **3**[b]	product **4**[b]	product **6**[c]
SO_2NH	11.48 (1H, br s)			11.98 (1H, br s)		
CONH	10.12 (1H, br s)			10.43 (1H, br s)		8.89 (1H, br s)
phenyl	8.48−7.94 (4H, m)	8.18−7.66 (4H, m)		8.74−8.24 (4H, m)	8.48−7.92 (4H, m)	
($OCHF_2$)	7.88 (2H, t, J = 71.3)		7.37 (2H, t, J = 72.0)	7.93 (2H, t, J = 71.1)		7.64 (2H, t, J = 71.6)
diazine-H	6.61 (1H, s)		5.76 (1H, s)	6.68 (1H, s)		6.26 (1H, s)
diazine-NH_2			5.07 (2H, br s)			7.72 (1H, s)
						6.51 (1H, s)
SO_2NH_2		5.07 (2H, br s)			5.90 (2H, br s)	
CH_3	4.12 (3H, s)	4.01 (3H, s)				

[a] J values in hertz. [b] $(CH_3)_2CO$-d_6. [c] $CDCl_3$.

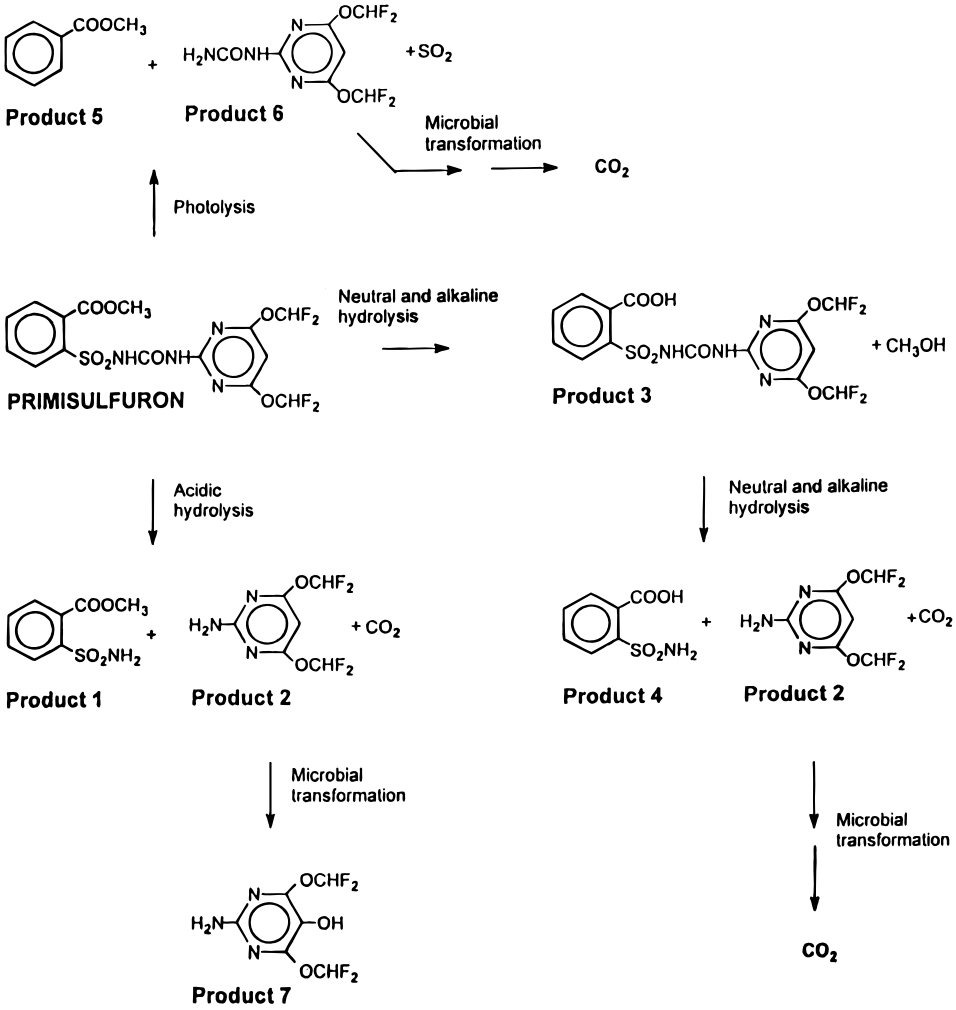

Figure 2. Proposed degradation pathways of primisulfuron.

the basis of the mass spectral data we propose a structure for this product as 2-amino-4,6-(difluoromethoxy)-5-hydroxypyrimidine (Figure 2). Product **7** consisted of <6% of the applied radioactivity, suggesting that microbial hydroxylation of the heterocycle is a relatively minor pathway. Some additional radioactive products were detected at very low concentrations, but no attempt was made to isolate and characterize these products.

In the study of photolysis, changes in the concentration of primisulfuron with time, during irradiation of the solution at 254 nm, suggested that the reaction followed pseudo-first-order kinetics (k_{obs} = 0.069 min^{-1} and $t_{1/2}$ = 10.0 min). No reaction occurred at 300 and 366 nm in solutions at pH 7, and the primisulfuron was recovered almost unchanged after 5 days of irradiation.

After photolysis of primisulfuron at 254 nm, products **5** and **6** were detected by HPLC and TLC analyses (Tables 4 and 5) in almost equimolar quantities. The chromatographic (TLC and HPLC) and spectral data (IR and NMR) of product **5** were compared and found to be identical with those for methyl benzoate. Product **6**, which was obtained as white crystals, had the following features: mp 110−116 °C; CI MS, m/z 271 $(M + H)^+$, 227, 205, 204, 162, 138; IR (KBr) ν 1718, 1636, 1456, 1397, 1339 cm^{-1}; and ^{1}H NMR chemical shifts as shown in Table 6. According to these characteristics, product **6** was identified as 4,6-(difluoromethoxy)pyrimidin-2-ylurea. The structures of products **5** and **6** are presented in Figure 2.

When the photolysis experiment was repeated by adding 1.1 × 10^2 or 1.3 × 10^2 kBq/L ^{14}C-phenyl- or ^{14}C-

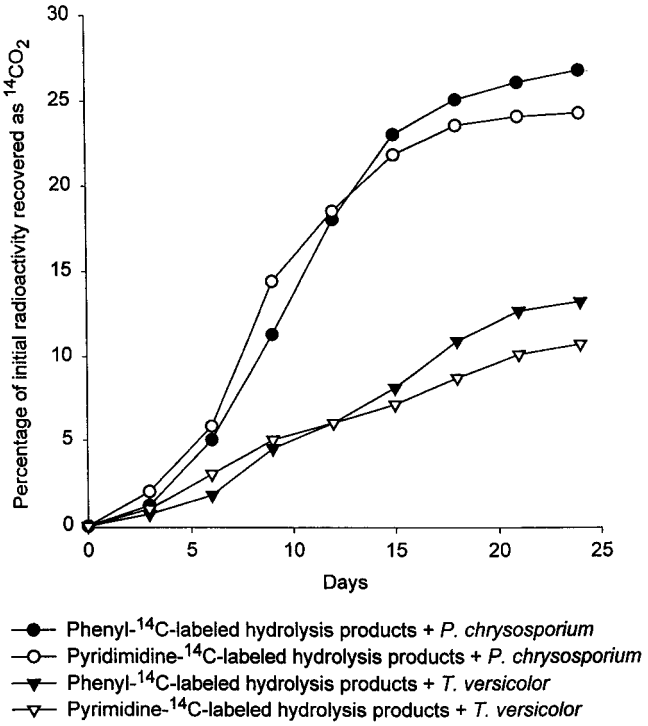

Figure 3. Mineralization of the ^{14}C-phenyl- and ^{14}C-pyrimidine-labeled hydrolyzed solutions by fungi.

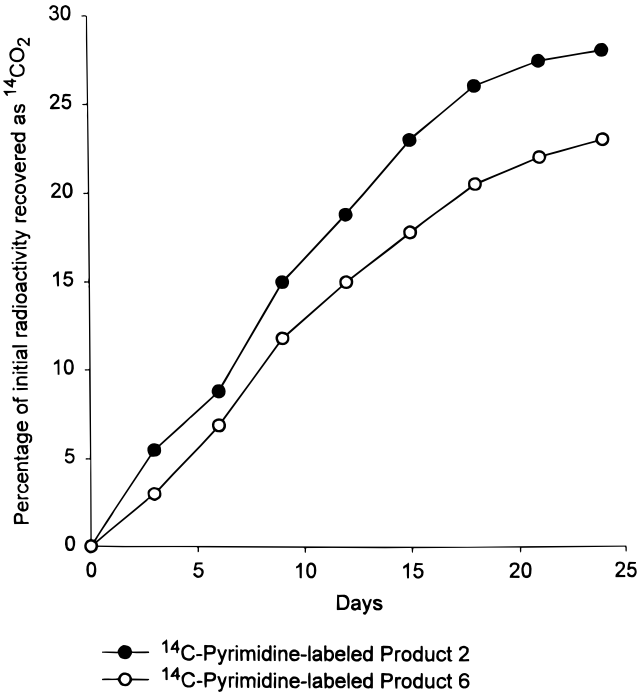

Figure 4. Mineralization of the ^{14}C-pyrimidine-labeled photolysis products by the fungus *P. chrysosporium*.

pyrimidine-labeled primisulfuron to 1 μM primisulfuron, a decrease of 32% of radioactivity was observed in the solution with ^{14}C-phenyl-labeled primisulfuron, but no decrease was observed with ^{14}C-pyrimidine-labeled primisulfuron (data not shown). After photolysis, the solution from ^{14}C-pyrimidine-labeled primisulfuron contained 4,6-(difluoromethoxy)pyrimidin-2-ylurea (product **6**) and 2-amino-4,6-(difluoromethoxy)pyrimidine (product **2**). When the fungus *P. chrysosporium* was incubated with products **2** and **6** for 24 days, the initial radioactivity decreased by 28 and 22%, respectively, due to mineralization (Figure 4).

DISCUSSION

Initial experiments, utilizing both axenic cultures and batch enrichments, indicated that microorganisms do not play a major role in the initial step of primisulfuron degradation. The primisulfuron remained relatively stable in these experiments, except when the pH in the growth media decreased (Tables 1 and 2). Because the stability of primisulfuron was correlated with the pH of the growth media, we investigated the chemical hydrolysis of primisulfuron in aqueous solutions at different pH values. Our results indicate that hydrolysis of primisulfuron is pH-dependent and of first-order kinetics (Table 3); this is in agreement with what was generally reported on the chemical hydrolysis of other sulfonylurea herbicides (Beyer et al., 1988; Braschi et al., 1997). Primisulfuron hydrolysis is accelerated by acidic and, to a lesser extent, alkaline conditions. At pH 10, a small increase in the hydrolysis rate was observed, probably because of a base-catalyzed reaction.

Products **1** and **2**, the major hydrolysis products, are present at all pH values (Figure 1); the two products are generated from the hydrolytic cleavage of the sulfonylurea bridge. Products **3** and **4** are two minor products and are formed at only pH values >8. Primisulfuron acid (product **3**) arises from hydrolysis of the ester group of primisulfuron. Product **4** is formed after alkaline hydrolysis of either the sulfonylurea bridge of product **3** or the ester group of product **1**. On the basis of these degradation products, a hydrolytic pathway is postulated (Figure 2).

Although we did not isolate and identify the products resulting from the chemical hydrolysis through the pH changes in the microbial cultures, we assume that the same products were formed under the same pH conditions. Incubation of the hydrolyzed solution of primisulfuron with *P. chrysosporium* or *T. versicolor* resulted in further metabolism of the hydrolysis products as evidenced by the substantial release of $^{14}CO_2$ ($\sim$25% for ^{14}C-phenyl-labeled and $\sim$12% for ^{14}C-pyrimidine-labeled primisulfuron). The predominant hydrolysis products of primisulfuron at pH 3, methyl 2-(aminosulfonyl)benzoate (product **1**) or 2-amino-4,6-(difluoromethoxy)-pyrimidine (product **2**), can be further transformed microbiologically as demonstrated in Figure 3.

The minor metabolic pathway caused by *P. chrysosporium* in the hydrolyzed solution from ^{14}C-pyrimidine-labeled primisulfuron appeared to be a hydroxylation reaction on the pyrimidine ring that resulted during the formation of product **7** (Figure 2). Hydroxylation of the pyrimidine ring was suggested previously by Beyer et al. (1988), who investigated chlorimuron-ethyl in soybeans and corn and bensulfuron-methyl in rats and goats.

Primisulfuron, which is hydrolytically stable and mostly resistant to biodegradation at neutral pH agricultural soils, was found to be susceptible at pH 7 to fast photolytic degradation at 254 nm irradiation ($t_{1/2}$ = 10 min). The formation of products **5** and **6** by irradiation of primisulfuron indicated the occurrence of a homolytic carbon−sulfur cleavage. This fragmentation is followed by the loss of sulfur dioxide to produce the amine radical. The subsequent hydrogen abstraction from water results in the formation of two products. Pusino et al. (1999) found two analogous products in the photolysis of triasulfuron, another sulfonylurea herbicide. This mechanism has been proposed by several other research groups (D'Souza and Day, 1968; D'Souza

et al., 1970; Pincock and Jurgens, 1979). Sulfonamides are among the most effective chemicals protecting amino groups. This effectiveness arises from their stability in the presence of dilute acids and bases, combined with their easy removal by photolysis. An analogue of product **6**, the monosubstituted urea, was observed during the photolysis of the sulfonylurea herbicide chlorimuron-ethyl in both water and soil (Choudhury and Dureja, 1996, 1997).

No release of radioactivity was observed in a photolysis experiment utilizing ^{14}C-pyrimidine-labeled primisulfuron, suggesting that mineralization of the pyrimidine ring did not occur. On the other hand, a substantial loss of radioactivity (32%) occurred in the case of ^{14}C-phenyl-labeled primisulfuron, indicating that the phenyl ring is susceptible to mineralization by photolysis. Products **2** and **6**, isolated from ^{14}C-pyrimidine-labeled primisulfuron after prolonged photolysis, still contained the pyrimidine ring. This result is consistent with the findings of Harvey et al. (1985), who demonstrated mineralization of ^{14}C-phenyl-labeled sulfometuron methyl during photolysis.

Because the pyrimidine rings are not susceptible to degradation by photolysis, we exposed products **2** and **6** to mineralization by fungi. Incubation of products **2** and **6**, originating from ^{14}C-pyrimidine-labeled primisulfuron after exposure to photolysis, with *P. chrysosporium* resulted in the catabolic metabolism of these substrates as evidenced by the substantial amount of $^{14}CO_2$ released from the growth media (28 and 22%, respectively). It appears that certain products of primisulfuron that are resistant to photolysis can be transformed microbiologically, as demonstrated in Figure 4.

CONCLUSIONS

Results from the present study show that microorganisms do not play a major role in the initial degradation of primisulfuron. Instead, nonbiological hydrolysis and photolysis processes contributed to the degradation of the herbicide. Subsequent degradation of the hydrolysis and photolysis products was observed through the metabolic activity of white-rot fungi. On the basis of the products isolated after hydrolytic or photolytic transformation as well as microbiological degradation, we propose a scheme of primisulfuron transformation pathways as shown in Figure 2.

The importance of combined treatments using physicochemical processes and microbial activity for decontamination of hazardous xenobiotics has received growing attention, and many studies have been concerned with this subject (Kearney et al., 1982; Miller et al., 1988; Amador et al., 1989). However, new technologies of pollution control are still to be developed for in situ application.

ACKNOWLEDGMENT

We thank Ciba-Geigy Corp. (presently Novartis Crop Protection, Inc.) for the supply of chemicals.

LITERATURE CITED

Amador, J. A.; Alexander, M.; Zika, R. G. Sequential photochemical and microbial degradation of organic molecules bound to humic acid. *Appl. Environ. Microbiol.* **1989**, *55*, 2843−2849.

Anderson, J. J.; Dulka, J. J. Environmental fate of sulfometuron methyl in aerobic soils. *J. Agric. Food Chem.* **1985**, *33*, 596−602.

Berger, B. M.; Wolfe, N. L. Hydrolysis and biodegradation of sulfonylurea herbicides in aqueous buffers and anaerobic water-sediment systems: assessing fate pathways using molecular descriptors. *Environ. Toxicol. Chem.* **1996**, *5*, 1500−1507.

Beyer, E. M.; Duffy, M. J.; Hay, J. V.; Schlueter, D. D. Sulfonylureas. In *Herbicide Vol. 3, Chemistry, Degradation and Mode of Action*; Kearney, P. C., Kaufman, D. D., Eds.; Dekker: New York, 1988; pp 118−183.

Blair, A. M.; Martin, T. D. A review of the activity, fate and mode of action of sulfonylurea herbicides. *Pestic. Sci.* **1988**, *22*, 195−218.

Braschi, I.; Calamai, L.; Cremonini, M. A.; Fusi, P.; Gessa, C.; Pantani, O.; Pusino, A. Kinetics and hydrolysis mechanism of triasulfuron. *J. Agric. Food Chem.* **1997**, *45*, 4495−4499.

Choudhury, P. P.; Dureja, P. Phototransformation of chlorimuron-ethyl in aqueous solution. *J. Agric. Food Chem.* **1996**, *44*, 3379−3382.

Choudhury, P. P.; Dureja, P. Studies on photodegradation of chlorimuron-ethyl in soil. *Pestic. Sci.* **1997**, *51*, 201−205.

Dinelli, G.; Vicari, A.; Bonetti, A.; Catizone, P. Hydrolytic dissipation of four sulfonylurea herbicides. *J. Agric. Food Chem.* **1997**, *45*, 1940−1945.

D'Souza, L.; Day, R. A. Photolytic cleavage of sulfonamide bonds. *Science* **1968**, *160*, 882−883.

D'Souza, L.; Bhatt, K.; Madaiah, M.; Day, R. A. Photolysis of dansyl amino acids and dansyl peptides. *Arch. Biochem. Biophys.* **1970**, *141*, 690−693.

Foy, C.; Witt, H. L. Johnsongrass control with DPX-V9360 and CGA-136872 in corn (*Zea mays*) in Virginia. *Weed Technol.* **1990**, *4*, 615−619.

Harvey, J.; Dulka, J. J.; Anderson, J. J. Properties of sulfometuron methyl affecting its environmental fate: Aqueous hydrolysis and photolysis, mobility and adsorption on soils, and bioaccumulation potential. *J. Agric. Food Chem.* **1985**, *33*, 590−596.

Hemmanda, S.; Calmon, M.; Calmon, J. P. Kinetics and hydrolysis mechanism of chlorsulfuron and metsulfuron-methyl. *Pestic. Sci.* **1994**, *40*, 71−76.

Joshi, M. M.; Brown, H. M.; Romesser, J. A. Degradation of chlorsulfuron by soil microorganisms. *J. Agric. Food Chem.* **1985**, *33*, 888−893.

Kearney, P. C.; Plimmer, J. R.; Li, Z.-M. In *Pesticide Residues and Formulation Chemistry*; Greenhalgh, R., Drescher, N., Eds.; Pergamon Press: Oxford, U.K., 1982; pp 397−400.

Kirk, T. K.; Schultz, E.; Connors, W. J.; Bleam, R. D.; Hackett, W. F.; Zeikus, J. G. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. *Arch. Microbiol.* **1978**, *117*, 277−285.

Klaffenbach, T. K. J. P.; Holland, P. T.; Rahman, A. Degradation of primisulfuron-methyl and metsulfuron-methyl in soil. *Weed Res.* **1995**, *35*, 113−120.

Maurer, W.; Gerber, H. R.; Ruffner, J. CGA 136'872: A new post-emergence herbicide for the selective control of *Sorghum spp.* and *Elymus repens* in maize. *BCPC Weeds Proc.* **1987**, *1*, 29−34.

Miller, R. M.; Singer, G. M.; Rosen, J. D.; Bartha, R. Sequential degradation of chlorophenols by photolytic and microbial treatment. *Environ. Sci. Technol.* **1988**, *22*, 1215−1219.

Pincock, J. A.; Jurgens, A. Sulfonamides as photolabile protecting groups for amines. *Tetrahedron Lett.* **1979**, 1029−1030.

Pusino, A.; Braschi, I.; Petretto, S.; Gessa, C. Photodegradation of herbicide triasulfuron. *Pestic. Sci.* **1999**, *55*, 479−481.

Received for review June 4, 1999. Revised manuscript received February 28, 2000. Accepted March 13, 2000. Financial support was provided by the National Research Council of Italy, by the Italian Ministry of University and Scientific and Technological Research (Project ex- 40%), and by the Agricultural Experiment Station at The Pennsylvania State University.

JF990604Q