Flupyrsulfuron Soil Dissipation and Mobility in Winter Wheat Crops

Jean Rouchaud,*,† Olivier Neus,† Karolien Cools,‡ and Robert Bulcke‡

Laboratory of Phytopharmacy, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium, and Research Center for Weed Science, Universiteit Gent, 9000 Gent, Belgium

Residues of the sulfonylurea herbicide flupyrsulfuron were extracted from cropping soils with 0.1 M $NaHCO_3$. The soil extracts were cleaned up by partitioning and repeated thin-layer chromatography. Flupyrsulfuron was transformed by diazomethane into *N*-(4,6-dimethoxypyrimidine-2-yl)-*N*-(3-methoxycarbonyl-6-trifluoromethylpyridine-2-yl)methylamine (**2**), which was analyzed by gas−liquid chromatography with electron capture detection, and confirmation for several samples was made by gas chromatography combined with mass spectrometry. The sensitivity limit was 0.5 μg of flupyrsulfuron kg^{-1} of dry soil. Bioassays using sugar beet as test plant qualitatively confirmed the results of the chemical analyses. Flupyrsulfuron [10 g of active ingredient ha^{-1}] was applied in autumn on plots in two winter wheat crops on a sandy loam soil, the first crop being made in 1996−1997 and the second one in 1997−1998. In the 0−8 cm surface soil layer of both crops, the flupyrsulfuron soil half-lives were 123 and 92 days, respectively. Flupyrsulfuron was also applied post-emergence in March to other plots in the same crops; the half-lives in the 0−8 cm surface soil layer were similar in both seasons, that is, ∼58 days. During all crop trials, flupyrsulfuron remained in the 0−8 cm surface soil layer and was not detected in the 8−10, 10−15, and 15−20 cm surface soil layers. The surface−2 cm soil layer contained the greatest flupyrsulfuron soil concentration, but the residues progressively moved down into the 2−4 and 4−6 cm soil layers. At the winter wheat harvest date for each trial, flupyrsulfuron was not detected in any of the soil layers (<0.5 μg kg^{-1}).

Keywords: *Flupyrsulfuron; sulfonylureas; herbicide; soil; winter wheat crop; persistence; mobility*

INTRODUCTION

Flupyrsulfuron [methyl 2-[[[[4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-6-(trifluoromethyl)-3-pyridinecarboxylate monosodium salt] is a new sulfonylurea herbicide (Figure 1) applied post-emergence on winter wheat at the rate of 10 g of active ingredient (ai) ha^{-1} in autumn or in spring. It provides an excellent control of important grass weeds such as *Alopecurus myosuroides* and *Apera spica-venti*, as well as a wide range of broadleaf weeds (Teaney et al., 1995). Flupyrsulfuron is active through both foliar and root uptake. It is also applied in mixture with metsulfuron-methyl (10 + 5 g of ai ha^{-1}, respectively). Similar to other sulfonylurea herbicides, flupyrsulfuron acts by inhibiting the ALS enzyme system found only in plants. It inhibits the biosynthesis of the essential branched-chain amino acids valine and isoleucine, hence stopping cell division and plant growth (Schloss et al., 1988). Solubility of flupyrsulfuron in water is 63 and 603 mg kg^{-1} at pH 5 and 7, respectively, and the pK_a is 4.9 (Teaney et al., 1995). There is no published information on the persistence and mobility of flupyrsulfuron in soil.

Liquid chromatography (LC) and gas chromatography (GC) after derivatization are the two main techniques used for analysis of sulfonylureas at the 1 μg kg^{-1} or lower level in soil. High-pressure liquid chromatography (HPLC) was first used with the uncommon photoconductivity detector for soil analysis of chlorsulfuron and sulfometuron-methyl (Zahnow, 1982, 1985). A method using HPLC with the commonly available ultraviolet (UV) detection for nine sulfonylureas in soils including flupyrsulfuron has recently been reported (Powley and de Bernard, 1998). The soil extracts were cleaned up by two successive chromatographic steps, the first on a C_{18} cartridge and the second by a silica solid phase extraction (SPE) cleanup. Confirmation of sulfonylurea residue identity has been obtained by combined electrospray LC/mass spectrometry (LC/MS) and LC/tandem mass spectrometry (LC/MS−MS) (Marek and Koskinen, 1996; Li et al., 1996).

Chlorsulfuron soil analysis was made by gas chromatography with electron-capture detection (GC-ECD) after methylation with diazomethane (Ahmad and Crawford, 1990). The soil extract was cleaned up with Florisil, and monomethylchlorsulfuron was detected as such. For the analysis of chlorsulfuron, metsulfuron-methyl, and other sulfonylurea herbicides by GC, the diazomethane methylation conditions were optimized (2 h of reaction in ethyl acetate at 20 °C) to produce the *N*,*N*′-dimethyl derivatives of the sulfonylureas, which were detected as such by GC-ECD, GC with nitrogen-phosphorus detection (NPD), and GC/MS (Klaffenbach and Holland, 1993; Klaffenbach et al., 1993). This method was applied to the measurements of primisulfuron-methyl and metsulfuron-methyl residues in field soils (James et al., 1995). Rimsulfuron soil analysis was made after its transformation by diazomethane into monomethylrimsulfuron; during its GC-ECD analysis, monomethylrimsulfuron was thermally transformed—by SO_2NHCO elimination—into *N*-(3-ethylsulfonyl)-2-pyridinyl)-4,6-dimethoxy-2-pyrimidineamine, which was detected (Rouchaud et al., 1997).

† Université Catholique de Louvain.
‡ Universiteit Gent.

10.1021/jf981017s CCC: $18.00
Published on Web 08/07/1999

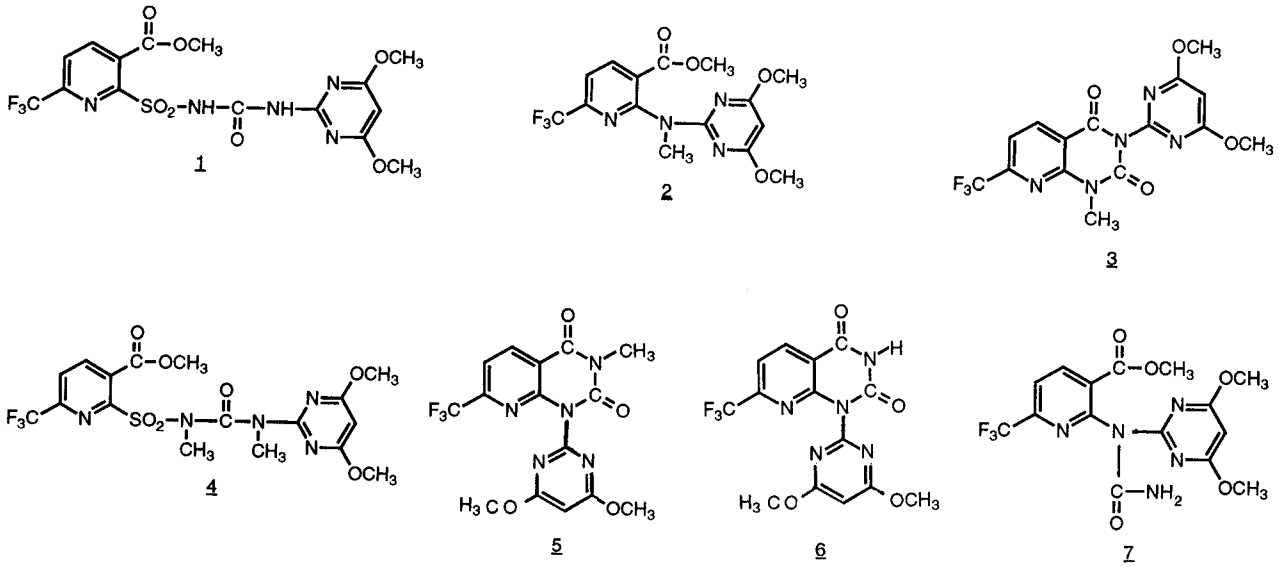

Figure 1. Flupyrsulfuron and its products of diazomethane methylation and degradation.

Table 1. Concentrations of Flupyrsulfuron in the 0−8 cm Soil Layer of the 1996−1997 Winter Wheat Crop, after Application in December 1996

sampling date	days after treatment	cum rainfall, mm	flupyrsulfuron concn,[a] μg kg^{-1}
Dec 18, 1996	6	6	10.8 ± 0.7
Jan 23, 1997	42	19	9.2 ± 0.6
Feb 28, 1997	78	91	7.0 ± 0.5
March 28, 1997	106	112	6.3 ± 0.4
April 30, 1997	139	129	5.6 ± 0.4
May 28, 1997	167	208	4.3 ± 0.3
June 16, 1997	186	278	3.9 ± 0.3
July 18, 1997	218	380	0.5 ± 0.3

[a] Means of four replicates ± SD.

In the present work, a method for analysis of flupyrsulfuron in soils was developed based on GC and GC/MS following thin-layer chromatography (TLC) cleanup and transformation of the extracted residues with diazomethane. The persistence and mobility of flupyrsulfuron have been measured in the soil of several winter wheat crops after autumn or spring applications.

MATERIALS AND METHODS

1996−1997 Winter Wheat Crop with Flupyrsulfuron Application in December 1996 or March 1997. A 120 × 350 m field at Melle, Belgium (clay, 7%; silt, 38%; sand, 55%; organic matter, 2.1%; pH 6.5; sandy-loam soil) was tilled on December 11, 1996. On December 12, 1996, winter wheat (cv. Castell) was sown on the whole field, and four replicate plots (10 × 12 m plot), located at random points in the field, were treated pre-emergence with 10 g of flupyrsulfuron ai ha^{-1} using an assay formulation (water dispersible granules containing 50% flupyrsulfuron sodium salt, DuPont, Belgium) applied in water. Flupyrsulfuron was applied to bare soil surface in 2.5 m wide strips using an air-pressurized knapsack sprayer (Azo Sprayers, Veeze Ede, Holland), equipped with drift guarding flat fan tips with 110° spray angles (Teejet DG 11002), delivering 300 L of water ha^{-1} at 200 kPa of pressure. On March 24, 1997, on another four replicate plots in the same field, the same flupyrsulfuron rate was applied post-emergence. At intervals after flupyrsulfuron application, samples were taken separately (and analyzed separately) in the 0−8 cm surface soil layer of each of the four replicate plots (Tables 1 and 2). For each soil sample, 15 cores (2.5 cm diameter) were taken from each plot at random points, the cores from each replicate plot were bulked together and then stored at −25 °C until analyzed.

Table 2. Concentrations of Flupyrsulfuron in the 0−8 cm Soil Layer of the 1996−1997 Winter Wheat Crop after Post-emergence Application in March 1997

sampling date	days after treatment	cum rainfall, mm	flupyrsulfuron concn,[a] μg kg^{-1}
March 28, 1997	4	3	10.9 ± 0.7
April 30, 1997	37	20	6.8 ± 0.4
May 28, 1997	65	99	5.0 ± 0.3
June 16, 1997	84	169	3.9 ± 0.3
July 18, 1997	116	271	3.0 ± 0.3
Aug 27, 1997	156	316	nd

[a] Means of four replicates ± SD. nd, not detected.

1997−1998 Winter Wheat Crop with Flupyrsulfuron Application in November 1997 or March 1998. A second winter wheat crop was sown at Melle, Belgium, using an adjacent field (clay, 7%; silt, 38%; sand, 55%; organic matter, 1.8%; pH 6.6; sandy-loam soil) in 1997−1998 in the same way as the previous crop. The field was tilled on October 22, 1997, winter wheat was sown on October 23, 1997, and 10 g of flupyrsulfuron ai ha^{-1} was applied on November 21, 1997 on four replicate plots in the field. On March 30, 1998, another four replicate plots in the field were treated in the same way with flupyrsulfuron. At intervals after flupyrsulfuron application, samples were taken separately (and analyzed separately) in the 0−8 cm surface soil layer of each of the four replicate plots (Tables 3 and 4). Soil samples were also taken from the 0−2, 2−4, 4−6, 6−8, 8−10, 10−15, and 15−20 cm surface soil layers, with samples at each depth from two replicate field plots being mixed to give duplicate samples for analysis.

Instrumental Analysis. TLC was carried out using 20 × 20 cm, 0.2 mm layer, silica gel 60 F254 plates (Merck). GC was carried out using a Varian 2700 instrument with ^{63}Ni ECD, injector and detector at 280 °C, and a glass column 1.80 m × 2 mm i.d. containing 5% SE30 on Chromosorb W-HP 80−100 mesh, 210 °C isothermal. Nitrogen was used as carrier gas at 50 mL min^{-1}. The retention time of compound **2** (Figure 1) was 2.7 min. Residues in some extracts were confirmed by GC/MS (VG AutoSpec; Fisons GC 8065). One-microliter injections were made into the GC injector operated in splitless mode at 280 °C. A 15 m capillary column, 0.45 mm i.d., contained SE 54 at 1.0 μm film thickness (Alltech); column oven temperature program was 50 °C (3 min) increasing to 250 °C at 20 °C min^{-1} and maintained at this temperature for 8 min. Helium carrier gas head pressure was 15 psi. The transfer oven was operated at 280 °C. Electron impact ionization parameters were 30 eV, source temperature of 200 °C, full scan of 50−500 amu, and scan rate of 1 scan/s. Chemical ionization was performed with reagent gas isobutane at 0.95 Torr.

Table 3. Soil Concentrations and Mobility of Flupyrsulfuron in the 1997−1998 Winter Wheat Crop after Post-emergence Application in November 1997

sampling date	days after treatment	cum rainfall, mm	flupyrsulfuron concn[a] (μg kg^{-1} of dry soil) in soil layer				
			0−8 cm	0−2 cm	2−4 cm	4−6 cm	6−8 cm
Nov 26, 1997	5	13	10.2 ± 0.7	38.1 ± 1.9	2.7 ± 0.3	nd	nd
Dec 12, 1997	21	67	9.3 ± 0.6	23.3 ± 1.2	13.4 ± 0.7	0.5 ± 0.3	nd
Jan 13, 1998	53	173	7.3 ± 0.5	17.3 ± 1.0	10.9 ± 0.7	1.0 ± 0.3	nd
Feb 18, 1998	89	204	5.1 ± 0.4	9.4 ± 0.7	8.5 ± 0.5	1.5 ± 0.3	1.0 ± 0.3
March 17, 1998	116	269	3.9 ± 0.3	nd	5.2 ± 0.3	9.9 ± 0.7	0.5 ± 0.3
April 23, 1998	153	341	3.3 ± 0.3	nd	3.9 ± 0.3	8.3 ± 0.6	1.0 ± 0.3
May 13, 1998	173	368	3.1 ± 0.3	nd	3.8 ± 0.3	7.5 ± 0.5	1.1 ± 0.3
June 12, 1998	203	484	0.6 ± 0.3	nd	1.0 ± 0.3	1.4 ± 0.3	nd
July 8, 1998	229	531	nd	nd	nd	nd	nd

[a] In the 0−8 cm surface soil layer, means of four replicates ± SD. In the 0−2, 2−4, 4−6, and 6−8 cm surface soil layers, means of two replicates ± SD. nd, not detected. Flupyrsulfuron was not detected in the 8−10, 10−15, and 15−20 cm surface soil layers.

Table 4. Soil Concentrations and Mobility of Flupyrsulfuron in the 1997−1998 Winter Wheat Crop after Post-emergence Application in March 1998

sampling date	days after treatment	cum rainfall, mm	flupyrsulfuron concn (μg kg^{-1} of dry soil)[a] in soil layer				
			0−8 cm	0−2 cm	2−4 cm	4−6 cm	6−8 cm
April 1, 1998	3	0	10.1 ± 0.5	40.4 ± 2.0	nd	nd	nd
April 24, 1998	26	67	9.3 ± 0.7	26.0 ± 1.3	11.2 ± 0.6	nd	nd
May 13, 1998	45	94	7.2 ± 0.4	15.3 ± 0.8	11.3 ± 0.5	2.2 ± 0.3	nd
June 12, 1998	75	210	4.4 ± 0.3	3.2 ± 0.3	8.5 ± 0.4	4.7 ± 0.3	1.2 ± 0.3
July 8, 1998	101	257	3.2 ± 0.3	nd	3.7 ± 0.3	6.6 ± 0.3	2.5 ± 0.3
July 29, 1998	122	291	0.7 ± 0.3	nd	nd	2.8 ± 0.3	nd
Aug 14, 1998	138	298	nd	nd	nd	nd	nd

[a] As in Table 3. Flupyrsulfuron was not detected in the 8−10, 10−15, and 15−20 cm surface soil layers.

Selected ion monitoring (SIM) experiments used the ions 372 (M^+), 341 (M − OCH_3), and 313 (M − CO_2CH_3). When the analysis standards were directly injected into the mass spectrometer, electron impact was at 70 eV (*m/e*; relative abundance, %). IR spectra were recorded by means of the Midac FTIR apparatus with KBr disks (cm^{-1}). The ^1H NMR spectra were recorded by means of the Varian 300 MHz spectrometer (δ, parts per million relative to tetramethylsilane in $CDCl_3$).

Flupyrsulfuron Free Acid 1. For the preparation of flupyrsulfuron **1** (Figure 1) free acid [methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-6-(trifluoromethyl)-3-pyridinecarboxylate], the mixture of the flupyrsulfuron assay formulation (water dispersible granules containing 50% flupyrsulfuron-sodium, 4 g; Du Pont, Belgium) in water (50 mL) was brought to pH 2.0 with 1 M HCL and extracted with ethyl acetate (3 × 200 mL). The ethyl acetate extract was dried (Na_2SO_4) and concentrated to dryness in a vacuum rotary evaporator, and the solid was recrystallized in 1:3 acetone/dichloromethane (v/v), giving 1.78 g of protonated flupyrsulfuron as the free acid (93%): IR 3305, 3184, 2960, 2922, 1748 (CO), 1719 (CO), 1609, 1580, 1506, 1450, 1364, 1296, 1252, 1219, 1205, 1168, 1154, 1094, 981, 807, 738; ^1H NMR 3.92 (s, 6H, pyrimidine−OCH_3), 4.03 (s, 3H, CO_2CH_3), 5.82 (s, 1H, H-5 pyrimidine), 7.42 (br, 1H, NH), 7.93 (d, 1H, H-4 pyridine), 8.38 (d, 1H, H-5 pyridine), 13.1 (br, 1H, SO_2-NH); MS 465 (M^+, 6), 358 (M − SO_2NHCO, 34), 327 (358 − OCH_3, 9), 299 (358 − CO_2CH_3, 100), 268 (299 − OCH_3, 47), 204 [$C_5H_2N(CF_3)(CO_2CH_3)$, 43].

Flupyrsulfuron Methylation Products. Diazomethane (carcinogenic, explosive) was freshly prepared before use. Behind a safety shield, to a 250 mL flask was added a cooled solution of potassium hydroxide (6 g) in water (10 mL), followed by diethyl ether (80 mL), and the stirred mixture was cooled in an ice bath. *N*-Methyl-*N*-nitrosourea (3 g; suspected to be carcinogenic) was added portionwise, after which the reaction mixture was stirred for an additional 15 min with ice cooling. The ether layer containing diazomethane was decanted and immediately used as such for methylation.

To the solution of flupyrsulfuron free acid (0.5 g, 1.08 mmol) in ethyl acetate (100 mL) was added an ethereal solution of diazomethane (70 mL) until persistence of the yellow color. After 2 h at 20 °C, the solution was concentrated to dryness in a vacuum rotary evaporator, and the solid was purified by preparative TLC (3:1 ether/hexane, v/v). Each TLC band was separated and TLC again with the same elution solvent, giving the following main products with a total yield of 83%: compound **2** [*N*-(4,6-dimethoxypyrimidine-2-yl)-*N*-(3-methoxycarbonyl-6-trifluoromethylpyridine-2-yl)methylamine; $R_f = 0.35$; 169 mg, 0.45 mmol, 42%]; compound **3** [1-methyl-2,4-diketo-3-(4,6-dimethoxypyrimidin-2-yl)-7-trifluoromethyl-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine; $R_f = 0.15$; 62 mg, 0.16 mmol, 15%]; compound **4** [*N*,*N*′-dimethylflupyrsulfuron, i.e., 2-[(4,6-dimethoxy-2-pyrimidinyl)methylamino)carbonyl)methylamino)-sulfonyl]-6-(trifluoromethyl)-3-(carbomethoxy)pyridine; R_f = 0.67; 138 mg, 0.28 mmol, 26%] (Figure 1). For methylation of 1 mg or lower amounts of flupyrsulfuron **1** free acid, to the solution of **1** in 5 mL of ethyl acetate was added ∼7 mL of the same solution of diazomethane in ether, until persistence of the yellow color. After 2 h at room temperature, compound **2** was the main product, being formed with a yield >90%.

Spectra of Compounds 2−4. *Compound **2**:* IR 3113, 2954, 2925, 1734 (CO), 1640, 1601, 1564, 1449, 1409, 1386, 1341, 1276, 1233, 1184, 1113, 904, 791; ^1H NMR 3.58 (d, 6H, pyrimidine−OCH_3), 3.72 (s, 3H, NCH_3), 3.93 (s, 3H, CO_2CH_3), 5.12 (s, 1H, H-5 pyrimidine), 7.28 (d, 1H, H-4 pyridine), 8.19 (d, 1H, H-5 pyridine); MS 372 (M^+, 36), 341 (M − OCH_3, 8), 313 (M − CO_2CH_3, 100), 298 (313 − CH_3, 7), 283 (313 − OCH_3 + H, 11), 168 [$C_4HN_2(NCH_3)(OCH_3)_2$, 8], 139 [$C_4HN_2(OCH_3)_2$, 21].

*Compound **3**:* IR 3109, 3071, 2956, 2926, 1730 (CO), 1693 (CO), 1612, 1545, 1449, 1368, 1346, 1282, 1196, 1152, 1095, 1056, 799; ^1H NMR 3.56 (s, 3H, NCH_3), 3.94 (s, 6H, OCH_3), 6.20 (s, 1H, H-5 pyrimidine), 7.59 [d, 1H, H-5 pyrido(2,3-*d*)-pyrimidine], 8.69 [d, 1H, H-6 pyrido(2,3-*d*)pyrimidine]; MS 383 (M^+, 96), 382 (M − 1, 100), 368 (M − CH_3, 13), 353 (M − OCH_3 + H, 15), 325 (M − NCH_3CO − H, 14), 311 (325 − N, 13), 297 (311 − CH_3 + H, 12).

*Compound **4**:* IR 2957, 2926, 2855, 1744 (CO), 1701 (CO), 1595, 1575, 1367, 1300, 1189, 1155, 1130, 1079, 804; ^1H NMR 3.33 (s, 3H, $CONCH_3$), 3.37 (s, 3H, $CON'CH_3$), 3.97 (s, 6H, pyrimidine−OCH_3), 4.02 (s, 3H, CO_2CH_3), 5.78 (s, 1H, H-5 pyrimidine), 7.92 (d, 1H, H-4 pyridine), 8.28 (d, 1 H, H-5 pyridine); MS 493 (M^+, 32), 372 (M − SO_2NCH_3CO, 29), 313 (372 − CO_2CH_3, 12), 268 [$C_5H_2N(CF_3)(CO_2CH_3)(SO_2)$, 15], 225 [$C_4HN_2(OCH_3)_2(NCH_3CONCH_3)$, 78]; 204 [$C_5H_2N(CF_3)(CO_2$-$CH_3)$, 76], 196 [$C_4HN_2(OCH_3)_2(NCH_3CO)$, 100].

Residue Analysis of Flupyrsulfuron in Soil. Soil (100 g) was stirred with 0.1 M $NaHCO_3$ in water (200 mL, 20 min, 20 °C), and the mixture was centrifuged (3000 rpm, 15 min) and the supernatant removed. The extraction was repeated, and the supernatants were combined and washed with dichloromethane (2 × 150 mL). The dichloromethane layer was discarded, and the aqueous phase was brought to pH 2.2 with 1 N hydrochloric acid and extracted two times with ethyl acetate (2 × 200 mL); the ethyl acetate solution was dried (Na_2SO_4), concentrated successively to 40 and 15 mL in a vacuum rotary evaporator (at 30 and 20 °C, and in 1 L and 50 mL flasks, respectively), and then concentrated further to 0.5 mL under a slow stream of nitrogen (20 °C). The extract was applied to a TLC plate along with flupyrsulfuron **1** free acid (~5 μg, i.e., the lowest amount to make the spot visible at the fluorescence) in a separate lane. Elution with 1:1.5 acetone/hexane (v/v) gave a band corresponding to flupyrsulfuron at $R_f = 0.49$, which was scraped off; the silica gel was extracted with acetone (40 mL) in a small column, and the extract was concentrated to 15 mL in a vacuum rotary evaporator at 20 °C and then concentrated further to 1 mL under a slow stream of nitrogen (20 °C). Ethyl acetate (5 mL) was added, and then a solution of diazomethane in ether (7 mL) was added until the yellow color persisted. After 2 h at 20 °C, the solution was concentrated to 0.5 mL under a slow stream of nitrogen (20 °C) and applied to a second TLC plate, together with compound **2** (~5 μg) in a separate lane. Elution with 2:1 ethyl acetate/hexane (v/v) gave a band corresponding to compound **2** at $R_f = 0.55$, which was scraped off and extracted with acetone. The concentrated extract was applied to a third TLC plate. Elution with ether gave a band corresponding to compound **2** at $R_f = 0.51$ which was separated and extracted with acetone. The concentrated extract (between 0.08 and 0.3 mL) was analyzed by GC with confirmation by GC/MS. When the final extract was not sufficiently clean (interferences at the GC or GC/MS analyses), the third TLC step was repeated.

Quantification Standards and Recovery Experiments. For calibration of the GC and GC/MS chromatograms, 10 mg of flupyrsulfuron free acid **1** was dissolved in ethyl acetate (10 mL), and the diazomethane solution in ether (~5 mL) was added until persistence of the yellow color. After 2 h at room temperature, the volume of the mixture was reduced to 5 mL by a current of nitrogen (20 °C), and the volume was adjusted to 10 mL with ethyl acetate. Several dilutions of this solution with ethyl acetate gave calibration solutions containing 0.4−10 × 10^{-9} g of flupyrsulfuron μL^{-1}. Injection of these solutions (external standard) gave curves correlating the GC or GC/MS signals to the amounts of flupyrsulfuron. Calibration was repeated several times during a daily series of analyses. Compound **2** (8 mg) dissolved in ethyl acetate (10 mL) gave a solution, which by dilutions gave several calibration solutions corresponding to 0.4−10 × 10^{-9} g of flupyrsulfuron μL^{-1}. These solutions gave GC and GC/MS calibration curves similar to the ones obtained by methylation of flupyrsulfuron **1**, taking into account that at this level methylation transformed flupyrsulfuron quantitatively into compound **2**. The calibration solutions were kept at −20 °C and were stable for >1 month.

For recovery experiments, soil was taken at Melle in field plots not treated with flupyrsulfuron. The soil was air-dried for 24 h at 20 °C in a ventilated hood. Ten milligrams of flupyrsulfuron free acid **1** was dissolved in 10 mL of acetone. By dilution, a solution in acetone containing 10^{-5} g mL^{-1} was made; 1 mL of this solution was dissolved in 100 mL of water, giving a solution in water containing 10^{-7} g mL^{-1}. Of this solution 0.5, 2, 5, or 10 mL was dissolved in water to make a final volume of 15 mL, and this was mixed with 100 g of soil, resulting in fortification levels of 0.5, 2.0, 5.0, and 10 μg kg^{-1}. The spiked soil samples were kept overnight at 12 °C and extracted. To observe the possible effect of residue aging, some soil samples were kept at 12 °C for 48 h before extraction, the soil humidity being maintained at 15%. Incubation time between 20 and 48 h had no significant influence on flupyrsulfuron recoveries. At the levels of 5, 2, and 0.5 (sensitivity limit) μg of flupyrsulfuron kg^{-1} dry soil, recoveries were 83−97, 81−92, and 74−89%, respectively. Other soil samples containing 5 or 10 μg of flupyrsulfuron kg^{-1} were extracted after 6 days of incubation at 12 °C, with the soil humidity maintained at 15%. The decrease of their recoveries was <5%, relative to the same samples extracted for 20−48 h after incorporation. A soil blank (untreated with flupyrsulfuron and nonfortified with it) and a reagent blank (the whole extraction procedure without soil) were included in each set of samples analyzed.

Table 5. Effect of Known Concentrations of Flupyrsulfuron Free Acid 1 on Sugar Beet Grown in the Greenhouse 15 Days after Planting

μg of flupyrsulfuron kg^{-1} of dry soil	fresh shoot weight, % of untreated ± SD	injury rating[a]
1	96 ± 8	0
2	85 ± 9	2
3	71 ± 8	3
5	47 ± 6	4
7	29 ± 5	7
8	23 ± 5	7
10	19 ± 4	8

[a] Injury ratings 2 weeks after planting: 0 = no visible effect; 1−3 = slight stunting and chlorosis; 4−6 = moderate stunting, yellowing of the leaf edges, and chlorosis; 7−9 = tissue necrotic, stem green, reduced emergence, and shoot growth; 10 = all plants killed.

The limit of quantitation was 0.5 μg of flupyrsulfuron free acid **1** kg^{-1} of dry soil. This was the lowest fortification level evaluated at which acceptable recoveries and precision were obtained. At this quantitation limit and fortification level, the peak of compound **2** was 3 times greater than the background peaks. Moreover, the chromatograms were free of interfering peaks close to the one of compound **2**.

The flupyrsulfuron soil half-lives in the 0−8 cm surface soil layer with their 95% confidence intervals were obtained using the linear regression $\ln y = kt + b$ between the naperian logarithms of the flupyrsulfuron soil concentrations (y = micrograms of flupyrsulfuron per kilogram of dry soil) and the time t (days) following flupyrsulfuron treatment (SAS logicial CMS SAS 5.18, SAS Institute Inc., Cary, NC) (Table 6).

Bioassay. For each field trial, the flupyrsulfuron soil concentrations measured by chemical analysis were confirmed by means of bioassays using sugar beet (*Beta vulgaris*) as test plants (Eskens and Bulcke, 1996; J. Weerts, Ministry of Middle Classes and Agriculture, Brussels, Belgium, personal communication, 1997). Soil was taken from control plots not treated with flupyrsulfuron or any other herbicide. The soil spread in a 3 cm thick layer was dried in the laboratory at 20 °C in a ventilated fume cupboard and screened through a 4 mm sieve. A dispersion of flupyrsulfuron in water was made using the flupyrsulfuron-methyl−sodium assay formulation. Amounts of this dispersion were incorporated in soil to provide soil calibration standards at concentrations of 10, 7, 5, 4, 2, or 1 μg of flupyrsulfuron kg^{-1} of dry soil. The flupyrsulfuron calibration standards and soil sampled from the 0−8 cm surface layer of the field trial plots were adjusted to 13% moisture content. Each soil was potted (150 g), and 10 sugar beet seeds (cv. Victoria G, with 90 g of imidacloprid ha^{-1} in seed dressing) were planted at 1 cm depth. There were four replicate pots for each calibration standard and the trial samples (field replicates). The pots were placed in a greenhouse in a completely randomized design and maintained at 26 ± 4 °C during the day and under a 16 h day with supplemental lighting (250 μeinstein m^{-2} s^{-1}) and at 20 ± 3 °C during the night. Relative humidity was maintained at 35 ± 15%. The soil moisture was maintained at 13%. Germination occurred 5 days after sowing. The effects on sugar beets of the different flupyrsulfuron soil concentrations were qualitatively observed: delay in plant germination and development; stunting; chlorosis; yellowing of the leaf edges; necrosis (Table 5). Shoot fresh weights were determined after severing the plants at the soil line. Each experiment was performed twice: in September 1997 and in September 1998. Qualitative comparisons were made between the field samples (collected at

Table 6. Linear Regression ln $y = kt + b$ between the Naperian Logarithms of the Flupyrsufuron Soil Concentrations ($y = \mu g\ kg^{-1}$ of Dry Soil) in the 0−8 cm Surface Soil Layer and the Time t (days) Following Flupyrsulfuron Treatment [Flupyrsulfuron Soil Half-Lives (Days)]

winter wheat trial	period of application of the linear regression, days	corr coeff	slope, $days^{-1}$	flupyrsulfuron soil half-lives, days[a]
1996−1997 trial: flupyrsulfuron applied in Dec 1996	186	−0.9862	−0.00563	123 ± 6.2
1996−1997 trial: flupyrsulfuron applied in March 1997	116	−0.9884	−0.0116	60 ± 3.6
1997−1998 trial: flupyrsulfuron applied in Nov 1997	173	−0.9868	−0.00757	92 ± 4.6
1997−1998 trial: flupyrsulfuron applied in March 1998	101	−0.9797	−0.0126	55 ± 2.8

[a] ± 95% confidence intervals.

different intervals after flupyrsulfuron application) and the reference soils containing known concentrations of flupyrsulfuron, or no flupyrsulfuron at all (controls). The lower limit of qualititative detection was 2 μg of flupyrsulfuron kg^{-1} of dry soil.

RESULTS AND DISCUSSION

Methylation of flupyrsulfuron at the 0.5 g level generated the mixture of compounds **2**, **3**, and **4** (*N*,*N*′-dimethylflupyrsulfuron) (Figure 1). Compound **3** could be formed by cyclization of the nonisolated *N*-monomethylflupyrsulfuron, with elimination of SO_2. However, 1-(4,6-dimethoxypyrimidin-2-yl)-2,4-diketo-3-methyl-7-trifluoromethyl-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine (**5**) is the regioisomer of compound **3**. Compound **5** is the *N*-methyl derivative of 1-(4,6-dimethoxypyrimidin-2-yl)-2,4-diketo-7-trifluoromethyl-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine (**6**), which is a soil metabolite of flupyrsulfuron (C. R. Powley, DuPont Agricultural Products, Wilmington, DE, personal communication, 1998). Compound **6** should be formed by cyclization of the intermediate *N*-carbamoyl-*N*-(4,6-dimethoxypyrimidin-2-yl)-*N*-(3-methoxycarbonyl-6-trifluoromethylpyridine-2-yl)amine (**7**). Such a urea derivative was observed in the hydrolysis in acidified water and in the soil metabolism of the sulfonylurea rimsulfuron (Schneiders et al., 1993; Rouchaud et al., 1997). The structure **5** in place of **3** for the tetrahydropyrido[2,3-*d*]pyrimidine methylation product of flupyrsulfuron is sustained by the high ^{1}H NMR shift value of the NCH_3 band at 3.56 indicating that the nitrogen is imido rather than amido (C. R. Powley, personal communication, 1998). When 1 mg or lower amounts of flupyrsulfuron were methylated with diazomethane, compound **2** became the main product, being formed with a yield >90%. The quantitative transformation of flupyrsulfuron at the residue level into a derivative measured by GC and GC/MS has been optimized here. Before this analysis method is extended to another sulfonylurea, its efficiency for this sulfonylurea should be verified as the reactivity of each sulfonylurea depends on its chemical structure.

For the soil analysis of flupyrsulfuron, in the present work the aqueous sodium bicarbonate extracts of soil were cleaned by partitioning and three successive TLC steps. For the R_f evaluations, standards of the analytes were applied on separate lanes of the TLC plates. In the soil extract, residues of parent flupyrsulfuron were first isolated from its potential soil metabolites. Methylation of flupyrsulfuron at the 1 mg or lower level quantitatively generated compound **2** (Figure 1), which was formed by elimination of SO_2NCH_3CO from the flupyrsulfuron dimethyl derivative **4**. Compound **2** was measured by GC-ECD in the cleaned up soil extracts with confirmation by GC/MS. The GC and GC/MS chromatograms were free of interfering peaks close to those of compound **2**.

For calibration of the GC and GC/MS signals, the solutions obtained by dilutions of the methylation products of 10 mg of flupyrsulfuron and the solutions made starting with compound **2** gave the same signal/quantity relationships. This confirmed that methylation of low amounts of flupyrsulfuron generated compound **2**. Recovery experiments were made during the 20−48 h period following fortification of soil. Within this period of time, the recoveries did not change significantly, indicating the absence of bonding of the residue to the soil, which could lower the extraction efficiency. Extraction of the spiked soils after 6 days of incubation at 12 °C gave recoveries lower by <5% relative to the recoveries obtained within the 20−48 h period following incorporation. The decrease of the recoveries corresponded to the rate of flupyrsulfuron dissipation in the soil of the trial fields and thus not to the residue aging during this period (Tables 1−4).

In the trial fields, soil samples were collected within the 3−6 days following flupyrsulfuron treatment (Tables 1−4). Recovery experiments made after 1−6 days of incubation at 12 °C showed that the flupyrsulfuron observed in the field during the 3−6 day period following application corresponded approximately to the initial flupyrsulfuron soil concentration. The similar "initial" flupyrsulfuron concentrations measured in the 0−8 cm surface soil layer in all field trials corresponded to the applied rate, the measured mean density of the fresh soil being 1.35 g cm^{-3}. No initial period of fast flupyrsulfuron decomposition in soil was observed; instead, a slow smooth soil dissipation was observed (Tables 1−4). This was confirmed by the bioassays. Dissipation was slower during the winter than during the spring and summer seasons. The biphasic kinetics observed in previous studies occurred mainly with the dissipation of sulfonylurea in soil incubated in the laboratory after incorporation of the herbicide in soil (Thirunarayanan et al., 1985). The absence of an initial fast soil decomposition suggests that in the field flupyrsulfuron is rapidly adsorbed onto the soil and its organic matter. Recovery experiments ascertained that, within the first week following treatment, the efficiency of the extraction method was not altered by short-term aging of the residue in soil. No extrapolation out to the crop end, however, may be done. The possibility of longer term residue aging with stronger bonding to soil was not evaluated by the short-term incubation. The solvent and extraction time used to separate flupyrsulfuron from the soil, however, suggest that the analysis procedure measures about all of the flupyrsulfuron which potentially may become free in soil until the crop end.

In the bioassays, the germination and development of the sugar beet plants sown in the 0−8 cm soil layer

of each of the field trials were compared to those sown in the calibration standards (Table 5). Delay in germination and plant development, stunting, yellowing, degeneration, and necrosis were visually evaluated, the limit of sensitivity being 2 μg of flupyrsulfuron kg^{-1} of dry soil. The magnitude of these symptoms decreased with decreasing flupyrsulfuron soil concentrations. The fresh weight of the sugar beet plants roughly measured these effects as a whole. The bioassays qualitatively confirmed the concentrations measured by chemical analyses (J. Weerts, personal communication, 1997).

There was a linear relationship $\ln y = kt + b$ between the naperian logarithms of the flupyrsulfuron concentrations in the 0−8 cm surface soil layer and the time elapsed since its application (first-order kinetics; Tables 1−4 and 6). This occurred during the main crop period following flupyrsulfuron application, that is, 6 and 3.6 months after the applications made in autumn or spring, respectively. Several parameters changed during each crop trial: seasons, temperature, rains, etc. The apparent kinetics of flupyrsulfuron dissipation in soil thus had no fundamental chemical meaning but was a mathematical means to analyze statistically the results. At the final sampling at the end of the crop, the rate of flupyrsulfuron dissipation was greater than the one predicted by the first-order kinetics. This has also been observed with other herbicides, and the effect has been more pronounced in soils with high levels of organic matter (Rouchaud et al., 1993). The distortion in the kinetics therefore was assumed to be due to adsorption−desorption of the herbicide onto the soil organic matter, although no assay was made to explain the phenomenon.

Flupyrsulfuron soil half-lives were greater when application was made in autumn rather than in spring. When flupyrsulfuron was applied in autumn, its soil half-life in the 0−8 cm surface soil layer was shorter (92 days) in the 1997−1998 trial than in 1996−1997 (123 days) (Tables 1, 3, and 6). This could be related to the greater rainfall in 1997−1998 (531 mm) compared to 1996−1997 (380 mm). When flupyrsulfuron was applied in March, its soil half-life was similar in the 1997 and 1998 trials (60 and 55 days, respectively), rainfall being also similar in both trials (316 and 298 mm, respectively) (Tables 2, 4, and 6).

Despite its solubility in water (603 mg kg^{-1} of water at pH 7.0) and the heavy rain during the winter and spring 1997−1998, flupyrsulfuron remained in the 0−8 cm surface soil layer during all field trials. Flupyrsulfuron was not detected in the 8−10, 10−15, and 15−20 cm soil layers in any of the trials. The 0−2 cm soil layer contained the greatest initial flupyrsulfuron soil concentration, but progressive movement of residues into the 2−4 and 4−6 cm soil layers was observed. The concentration of flupyrsulfuron in the surface layers ensures good herbicide efficacy. Excessive diffusion of flupyrsulfuron into the soil would dilute the flupyrsulfuron soil residue and also reduce herbicidal efficiency. During the 3 months following application of flupyrsulfuron in autumn, it remained in the upper 0−2 and 2−4 cm soil layers; only from March onward did it move down mostly to the 4−6 cm soil layer. When flupyrsulfuron was applied in March, it mostly remained in the 0−2 and 2−4 cm soil layers until July. Some movement occurred into the 4−6 cm soil layer after July. At winter wheat harvest (beginning of August), flupyrsulfuron was not detected in the 0−2, 2−4, 4−6, 6−8, 8−10, 10−15, or 15−20 cm soil layers in any of the trials. The mean rainfall during the trials was between 52 and 70 mm $month^{-1}$, which is normal for Atlantic North Europe. No bromide marker was used in this study to evaluate the net water movement. However, it is known that during the winter season, in this region there is a descending water movement in soil with normal rainfall.

The biological observations made by Cools et al. (1998) confirmed the results of the chemical analyses reported here. An early harvest was made at the beginning of July in some areas of the same 1996−1997 and 1997−1998 winter wheat trials treated in March with 10 g of flupyrsulfuron ha^{-1}. After a superficial soil preparation up to 10 cm depth, sensitive crops were sown: sugar beet (*Beta vulgaris*), oat (*Avena sativa*), turnip (*Brassica rapa*), and lettuce (*Lactuca sativa*). Low flupyrsulfuron soil residues caused light phytotoxicity (stunting) observed in August, especially with sugar beet. Other plots of the same field were sown at the beginning of July with green manures [white mustard (*Sinapis alba*), ryegrass (*Lolium multiflorum*), vetch (*Vicia sativa*), and horseradish (*Armoracia rusticana*], and no phytotoxicity was observed in September.

The sulfonylurea herbicides chlorsulfuron and triasulfuron have uses in winter wheat similar to that of flupyrsulfuron. Chlorsulfuron has a longer soil half-life between 89 and 144 days (Thirunarayanan et al., 1985), and it results in low levels of persistent residues in soil (Anderson and Barrett, 1985). Triasulfuron has a soil persistence similar to that of chlorsulfuron (Kotoula-Syka et al., 1993). In the case of flupyrsulfuron, no residues were detected in the soil at the time of winter wheat harvest from either spring or autumn application. During the cropping season, flupyrsulfuron remained in the 0−8 cm surface soil layer in all of the trials. There is thus no concern about possible injury to succeeding susceptible crops or movement of flupyrsulfuron to deeper soil layers and to groundwater. Flupyrsulfuron remained concentrated in a thin layer near the soil surface, where its high concentration provided high herbicide efficacy.

ACKNOWLEDGMENT

Mass spectra were recorded by C. Moulard (Université Libre de Bruxelles, Brussels, Belgium). We thank J. Weerts (Ministry of Agriculture, Brussels, Belgium) for interesting scientific suggestions (the combined bioassays).

LITERATURE CITED

Ahmad, I.; Crawford, G. Trace residue analysis of the herbicide chlorsulfuron in soil by gas chromatography-electron capture detection. *J. Agric. Food Chem*. **1990**, *38*, 138−141.

Anderson, R. L.; Barrett, M. R. Residual phytotoxicity of chlorsulfuron in two soils. *J. Environ. Qual*. **1985**, *14*, 111−114.

Cools, K.; Callens, D.; Bulcke, R. *Report No. 63 of the results obtained during the 1996−1997 crop season*; Weed Research Center, University of Gent: Gent, Belgium, 1998; 167 pp.

Eskens, B.; Bulcke, R. *Soil activity and persistence of the new sulfonylurea flupyrsulfuron-methyl and triflusulfuron-methyl*; Faculty of Agronomy, University of Gent: Gent, Belgium, 1996; 96 pp.

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

Klaffenbach, P.; Holland, P. T. Analysis of sulfonylurea herbicides by gas−liquid chromatography. 2. Determination

of chlorsulfuron and metsulfuron-methyl in soil and water samples. *J. Agric. Food Chem*. **1993**, *41*, 396−401.

Klaffenbach, P.; Holland, P. T.; Lauren, D. R. Analysis of sulfonylurea herbicides by gas−liquid chromatography. 1. Formation of thermostable derivatives of chlorsulfuron and metsulfuron-methyl. *J. Agric. Food Chem*. **1993**, *41*, 388−395.

Kotoula-Syka, E.; Eleftherohorinos, I. G.; Gagianas, A. A.; Sficas, A. G. Persistence of preemergence applications of chlorsulfuron, metsulfuron, triasulfuron and tribenuron in three soils in Greece. *Weed Sci*. **1993**, *41*, 246−250.

Li, L. Y. T.; Campbell, D. A.; Bennett, P. K.; Henion, J. Acceptance criteria for ultratrace HPLC−Tandem mass spectrometry: quantitative and qualitative determination of sulfonylurea herbicides in soil. *Anal. Chem*. **1996**, *68*, 3397−3404.

Marek, L. J.; Koskinen, W. C. LC/MS Analysis of 4-methoxy-6-methyl-1,3,5-triazin-2-yl- containing sulfonylurea herbicides in soil. *J. Agric. Food Chem*. **1996**, *44*, 3878−3881.

Powley, C. R.; de Bernard, P. A. Screening method for nine sulfonylurea herbicides in soil and water by liquid chromatography with ultraviolet detection. *J. Agric. Food Chem*. **1998**, *46*, 514−519.

Rouchaud, J.; Gustin, F.; Van Himme, M.; Bulcke, R.; Sarrazyn, R. Soil dissipation of the herbicide isoxaben after use in cereals. *Weed Res*. **1993**, *33*, 205−212.

Rouchaud, J.; Neus, O.; Callens, D.; Bulcke, R. Soil metabolism of the herbicide rimsulfuron under laboratory and field conditions. *J. Agric. Food Chem*. **1997**, *45*, 3283−3291.

Schloss, J. V.; Ciskanik, L. M.; Van Dyk, D. E. Origin of the herbicide binding site of acetolactate synthase. *Nature* **1988**, *331*, 360−362.

Schneiders, G. E.; Koeppe, M. K.; Naidu, M. V.; Horne, P.; Brown, A. M.; Mucha, C. F. Fate of rimsulfuron in the environment. *J. Agric. Food Chem*. **1993**, *41*, 2404−2410.

Teaney, S. R.; Armstrong, L.; Bentley, K.; Cotterman, D.; Leep, D.; Liang, P. H.; Powley, C.; Summers, J.; Cranwell, S.; Lichtner, F.; Stichbury, R. DPX KE459: A new sulfonylurea for postemergence grass and broadleaf weed control in cereals. *Proc. Br. Crop Prot. Conf. −Weeds* **1995**, *1*, 49−56.

Thirunarayanan K.; Zimdahl, R. L.; Smika, D. E. Chlorsulfuron adsorption and degradation in soil. *Weed Sci*. **1985**, *33*, 558−563.

Zahnow, E. W. Analysis of the herbicide chlorsulfuron in soil by liquid chromatography. *J. Agric. Food Chem*. **1982**, *30*, 854−857.

Zahnow, E. W. Analysis of the herbicide sulfometuron methyl in soil and water by liquid chromatography. *J. Agric. Food Chem*. **1985**, *33*, 479−483.

Received for review September 14, 1998. Revised manuscript received May 24, 1999. Accepted June 9, 1999. This work was sponsored by the Research and Development Department of the Ministry of Middle Classes and Agriculture, Brussels, Belgium.

JF981017S