Effect of Temperature and Moisture on the Degradation and Sorption of Florasulam and 5-Hydroxyflorasulam in Soil

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The degradation rate and sorption characteristics of the triazolopyrimidine sulfonanilide herbicide florasulam and its principal degradation product 5-hydroxyflorasulam (5-OH-florasulam) were determined as a function of temperature and moisture in three different soils. The half-life for degradation of florasulam ranged from 1.0 to 8.5 days at 20−25 °C and from 6.4 to 85 days at 5 °C. The half-life for degradation of 5-OH-florasulam ranged from 8 to 36 days at 20−25 °C and from 43 to 78 days at 5 °C. The degradation rate of both compounds was strongly influenced by temperature, with activation energies ranging from 57 to 95 kJ/mol for florasulam and from 27 to 74 kJ/mol for 5-OH florasulam. Soil moisture content had negligible impact on the degradation rate. Apparent (nonequilibrium) sorption coefficients for florasulam and 5-OH-florasulam at 0 days after treatment (DAT) were 0.1−0.6 L/kg and increased linearly with time for both florasulam and 5-OH-florasulam ($r^2 > 0.90$) to levels as high as 12−23 L/kg. Heats of adsorption were calculated on one soil as a function of time. Heat of adsorption values for both florasulam and 5-OH-florasulam increased as incubation time increased and the amount of each compound decreased; values were near 0 kJ/mol initially and increased to a maximum of 91 and 66 kJ/mol for florasulam and 5-OH-florasulam, respectively.

Keywords: *Florasulam; 5-hydroxyflorasulam; 5-OH-florasulam; aerobic soil metabolism; sorption; aerobic soil degradation; apparent* K_d

INTRODUCTION

Florasulam (*N*-(2,6-difluorophenyl)-5-methoxy-8-fluoro-(1,2,4)-triazolo-[1,5c]-pyrimidine-2-sulfonanilide) is a postemergence herbicide used in Europe and undergoing registration in Canada for the control of broadleaf weeds in cereals and pasture; it's mode of action is through acetolactate synthase (ALS) inhibition. Florasulam is a member of the triazolopyrimidine family of chemistry, which also includes metosulam, flumetsulam, cloransulam-methyl, and diclosulam. It is highly selective to wheat, barley, and turf, but very active on weeds in the plant families *Compositeae*, *Polygonaceae*, *Caryophyllaceae*, *Rubiaceae*, and *Crucifereae*. Florasulam is a low use rate herbicide, with proposed maximum application rates of 7.5 g/ha in Europe and 5.0 g/ha in Canada. The vapor pressure of florasulam is 1×10^{-5} Pa and its pK_a is 4.54 at 23 °C. Because of its ionizable nature, its aqueous solubility is a strong function of pH, ranging from 84.0 mg/L at pH 5, to 6.36 g/L at pH 7, and to 94.2 g/L at pH 9 (Richardson, 1995; Richardson, 1996).

The fate of florasulam applied to soil is clearly of interest to determine its impact on the environment. Determination of the environmental fate of herbicides also involves the evaluation of potential for occurrence in groundwater. Degradation and sorption are the principal processes controlling herbicide mobility in soil and are often the focus of leaching potential assessments (Jury et al., 1986; Gustafson, 1989). The degradation and sorption of organic molecules are coupled processes (Scow and Johnson, 1997), where an increase in sorption generally results in a decrease in microbial degradation (Ogram et al., 1985; Sims et al., 1991; Sims et al., 1992; Krieger et al., 1998). Sorption of many pesticides to soil frequently increases with residence time in soil, resulting in changes in degradation rates and mobility potentials (McCall and Agin, 1985; Cox et al., 1998, Cox and Walker, 1999).

This study examined the sorption−degradation relationships of florasulam and its principal soil degradation product, 5-hydroxyflorasulam (5-OH-florasulam). This study determined key predictors of mobility (degradation rate and sorptivity) for florasulam and 5-OH-florasulam at environmentally relevant concentrations and examined the temporal variations in sorptivity of each compound. This study also determined the effects of temperature and moisture on the degradation and time-dependent sorption of florasulam and 5-OH-florasulam in aerobic soil systems. The degradation and sorption of the 5-OH-florasulam degradate characterized from in situ formation should yield a more accurate representation of the fate of this molecule in the environment than that obtained from direct dosing to soil.

MATERIALS AND METHODS

Test Material and Reference Compounds. Florasulam radiolabeled in the triazolopyrimidine ring structure was obtained from the Specialty Synthesis group of Dow AgroSciences (Indianapolis, IN). The specific activity of the test material was 9.07×10^{11} Bq/mol (1.51×10^{11} dpm/g). Standard solutions were assayed by liquid scintillation counting (LSC) and high-performance liquid chromatography (HPLC) to check

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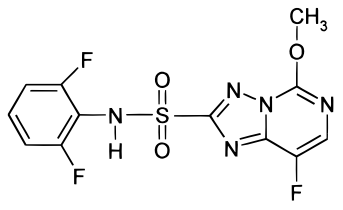
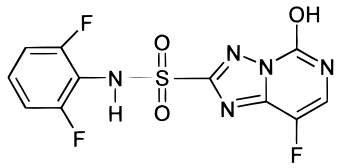


florasulam



5-OH-florasulam

Figure 1. Structures of florasulam and 5-OH-florasulam.

activity and radiochemical purity prior to use in dosing solutions. The radiochemical purity ranged from 96.2 to 98.4% prior to dosing of the test systems. Nonradiolabeled florasulam and 5-OH-florasulam were used as reference substances in this study. The structures of florasulam and 5-OH-florasulam are shown in Figure 1. Radiolabeled 3,4-dichlorobenzoic acid [carbonyl-^{14}C] (DCBA) was used to determine soil biological activity. DCBA was also obtained from the Specialty Synthesis group at Dow AgroSciences; its radiochemical purity was 98.5% and specific activity was 4.22×10^{11} Bq/mol (1.33×10^{11} dpm/g).

Test Systems. The study was conducted using three soils representative of the use regions for florasulam in Europe and anticipated use regions in Canada; physicochemical information on each soil is given in Table 1. Soil moisture holding capacity (MHC) was determined by saturating a column of soil in a Buchner funnel with water and draining the soil overnight; all other soil moisture measurements were performed with a pressure plate instrument. The Marcham sandy clay loam was used in a previous aerobic soil metabolism study with florasulam (Jackson et al., 2000), the Cuckney sand was used in sorption and lysimeter studies, whereas the Naicam-Hoodoo clay loam is representative of the anticipated major use area in Canada. Two separate samples of the Marcham soil with slightly different physical and chemical characteristics were used. Moist, field-sampled soils at a workable consistency were passed through a 2-mm sieve and stored at 4 °C prior to use.

Florasulam was applied at a rate of 25.6 ng/g to the Marcham test systems at 10 and 20 °C (40% MHC) and the Naicam-Hoodoo test systems (5, 10, 20, and 35 °C at 40% MHC), and at a rate of 15.0 ng/g to the Marcham and Cuckney test systems at 5, 15, and 25 °C (40% MHC) and 20 °C (0, 0.05, and 15 bar). Soil moisture was not adjusted during the course of the experiment. These application rates are equivalent to an approximate field use rate of 17.0 and 10.0 g/ha, respectively, assuming a 5-cm incorporation depth. Samples were analyzed at time points from 0 to 189 days after treatment (DAT).

The Marcham 10 and 20 °C test systems and the Naicam-Hoodoo test systems (approximately 50 g oven dry weight, adjusted to 40% MHC) were incubated in biometer flasks equipped with a side chamber containing 100 mL of 0.2 M NaOH solution for the collection of $^{14}CO_2$ released by microbial mineralization. A slight positive pressure of O_2 was supplied to the biometer flask to replace O_2 removed during oxidation of organic matter. The Marcham and Cuckney test systems maintained at 5, 15, and 25 °C (approximately 50 g oven dry weight, adjusted to 40% MHC) and 20 °C (approximately 50 g oven dry weight, adjusted to 0, 0.05, 15 bar moisture) were incubated in closed glass jars with small holes perforated in the lids to allow gas exchange.

Analytical Methods. At each sampling point, a subsample of the 0.2 M NaOH trapping solution (for test systems incubated in biometers) was analyzed for ^{14}C by LSC. The soil was transferred with 100 mL of 0.01 M $CaCl_2$ to a centrifuge bottle, extracted for 10 min on a mechanical shaker, then centrifuged. The aqueous supernatant was decanted and labeled as the aqueous extract. The soil was then extracted with $3 \times 75-100$ mL portions of an acidified organic extraction solvent (80:19:1 acetonitrile/water/*o*-phosphoric acid). These extractions were also performed on a mechanical shaker, with the first extraction being at least 1 h and the subsequent extractions at least 0.5 h. The sample was centrifuged and the extraction fluid decanted between each extraction. The three combined extracts were brought to a volume of 250 mL and labeled as the organic extract. Subsamples of the aqueous extract and organic extract were analyzed for ^{14}C by LSC. (Note: For the Naicam-Hoodoo test systems at 5, 10, and 35 °C, the aqueous extraction step was not performed.) The two-phase extraction system was used to calculate apparent (nonequilibrium) sorption coefficients (K_d) for florasulam and 5-OH-florasulam.

The aqueous extract was acidified to pH 2 with 0.1 M HCl prior to cleanup on a C_{18} solid-phase extraction (SPE) cartridge; this was done to ensure that the analytes were in their neutral charge form. The SPE cartridge was rinsed with methanol and conditioned with acidified water before the sample was loaded. The entire aqueous sample was passed through the SPE cartridge, collected, and frozen. The retained components of the sample were eluted from the cartridge with methanol. The frozen aqueous portion of the sample was freeze-dried, then reconstituted in acidified water and acidified acetonitrile. Freeze-drying the sample allowed the large-volume aqueous sample to be concentrated to a smaller volume after reconstitution. The methanol-eluted components of the original aqueous extract were then combined with the reconstituted freeze-dried sample; this sample was concentrated and filtered prior to analysis by HPLC.

Approximately 100 mL of the organic extract was concentrated by evaporating the organic solvent. The remaining aqueous component of the organic sample was passed through a C_{18} SPE cartridge that had been rinsed with methanol and conditioned with acidified water. The sample that passed through the SPE cartridge was collected and frozen. The retained components of the sample were eluted from the cartridge with methanol. The frozen aqueous portion of the sample was freeze-dried, then reconstituted in acidified water and acidified acetonitrile. The methanol-eluted components of the original organic extract were then combined with the reconstituted freeze-dried sample; this sample was concentrated and filtered prior to analysis by HPLC.

For the Marcham 10 and 20 °C test systems and the Naicam-Hoodoo test systems (5, 10, 20, 35 °C and 40% MHC), aliquots (typically $500-1000$ μL) of the samples were injected onto a Hewlett-Packard Hypersil ODS HPLC column (200 mm $\times$ 4.6 mm, 5 μm particles). An aliquot was also injected directly into an LSC vial and used as a recovery spike. Sample components were separated using one of two linear binary mobile phase gradients: (1) 90% A at time 0, ramped to 50% A at 25 min and to 0% A at 35 min; or (2) 100% A at time 0 and held for 10 min, ramped to 50% A at 25 min, and to 0% A at 35 min, where A = 0.5% acetic acid in water and B = 0.5% acetic acid in acetonitrile. The flow rate was 1.0 mL/min. Eluent fractions were collected at 1-min intervals for up to 40 min. Each fraction was added to 5 mL of Ultima Gold XR scintillation cocktail and assayed for ^{14}C by LSC using a Packard TR2000 liquid scintillation counter. Radiochromatograms were reconstructed using Microsoft Excel.

For the Marcham and Cuckney test systems at 5, 15, and 25 °C (40% MHC) and 20 °C (0, 0.05, and 15 bar), aliquots (typically $200-1500$ μL) of the samples were injected onto a Spherisorb ODS2 (250 mm $\times$ 4.6 mm, 5 μm particles) HPLC column. Sample components were separated using a binary gradient where the solvent composition was 100% A at time 0, held at 100% A for 7 min, ramped linearly to 50% A at 25 min, to 0% A at 35 min and held for 5 min. Solvent A was 2% acetic acid in water and solvent B was 2% acetic acid in acetonitrile. The flow rate was 1.0 mL/min. Detection was with a Packard 500TR flow-through radioactivity detector.

Table 1. Physical and Chemical Characteristics of the Soils Used in This Study. Two Different Samples of the Marcham Soil (a and b) Were Used

	soil			
property	Cuckney	Marcham (a)	Marcham (b)	Naicam-Hoodoo
source country	England	England	England	Canada
pH	6.9	7.6	7.7	7.5
CEC[b] (cmol(+)/kg)	nd[a]	nd[a]	17.82	25.76
organic matter (%)	nd[a]	nd[a]	2.94	9.15
textural class	sand	sandy clay loam	sandy clay loam	clay loam
sand (%)	78.5	53.8	59.2	25.2
silt (%)	12.1	20.3	15.6	43.6
clay (%)	9.4	25.9	25.2	31.2
biomass (μg C/g soil)	117	265	nd[a]	nd[a]
% gravimetric mass moisture content at pressure (bar)				
moisture holding capacity (%)	30.9	53.6	53.7	77.7
0	33.7	40.6	nd[a]	nd[a]
0.05	19.2	25.4	nd[a]	nd[a]
15.0	7.8	15.5	nd[a]	nd[a]

[a] Not determined. [b] Cation exchange capacity.

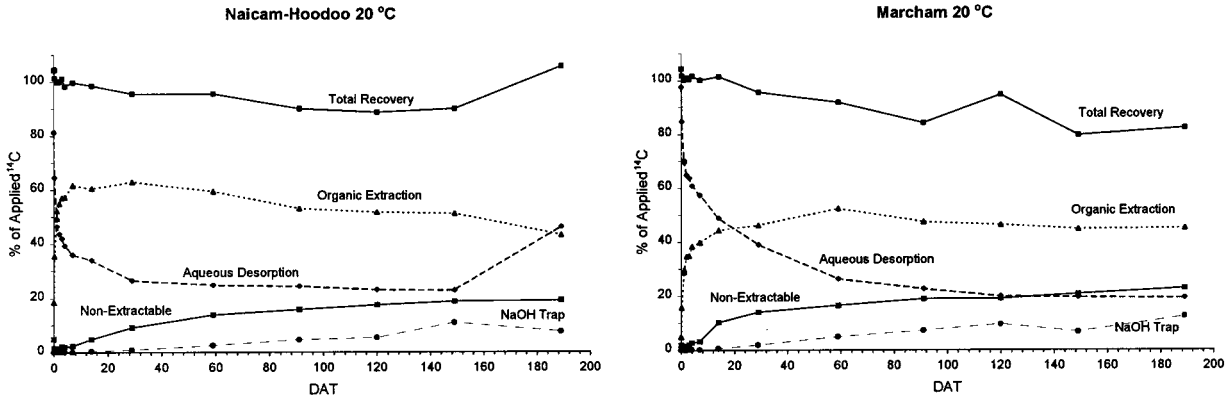


Figure 2. Distribution (%) of radiocarbon as a function of time and soil type for samples incubated at 20 °C and 40% MHC.

The amount of nonextractable radioactivity associated with the soil was determined by combustion of the residual soil sample. Duplicates of each extracted, air-dried soil were combusted either manually or by robot. A small portion of the sample was weighed into a glass boat and combusted using a Harvey OX-400 or OX-500 (Hillsdale, NJ) biological oxidizer. The generated $^{14}CO_2$ was collected in scintillation cocktail and analyzed by LSC. Efficiency of each oxidizer was determined by combusting soil blanks and spiking the generated vials with a known volume of a ^{14}C-picloram standard. The same standard was then spiked onto soil replicates, which were combusted. The efficiency was calculated by dividing the recovered radioactivity from the soil spikes by the radioactivity in the vial spikes. This correction factor was applied to each sample analyzed by combustion on that instrument on that day. Combustion samples were collected in Harvey scintillation cocktail plus Permafluor.

The identity of florasulam and 5-OH-florasulam in the soil extracts was confirmed using liquid chromatography−mass spectrometry (LC−MS) with electrospray ionization (ESI) and also by correlation of retention times with authentic standards. LC−MS experiments were performed on a Finnigan TSQ 700. Sample components were separated using similar chromatographic conditions as above. The column effluent for LC−MS experiments was split between a flow-through radioactivity detector and the mass spectrometer to allow the correlation of ^{14}C with peaks in the mass chromatograms.

Soil Microbial Viability. The microbial viability of the soil was measured at 0, 91, and 197 DAT by dosing test systems containing Naicam-Hoodoo soil (at 20 °C, 40% MHC) or Marcham soil (at 10 and 20 °C, 40% MHC) with ^{14}C-DCBA. These test systems were prepared and incubated in an identical manner to the test systems used to measure the degradation rate of florasulam; however they were not dosed with radiolabeled florasulam. The degradation rate of ^{14}C-DCBA to $^{14}CO_2$ was measured and compared at the various time points to give a relative measure of the biological activity of the test systems. Aliquots of the NaOH trap were assayed for $^{14}CO_2$ 192 h after dosing with ^{14}C-DCBA. A subset of the soils in the microbial viability experiment was also dosed with nonradiolabeled florasulam at 0 DAT in order to determine the effect of florasulam on the biological activity of the test system.

RESULTS AND DISCUSSION

Distribution of Radiocarbon and Material Balance. The distribution of ^{14}C between the NaOH traps, aqueous desorption solutions, organic soil extracts, and nonextractable material for the Marcham and Naicam-Hoodoo samples incubated at 20 °C (40% MHC) is shown in Figure 2. The average material balance across all samples at all temperatures was 100.0 ± 4.0%, and no decrease in material balance was observed over time, indicating that no volatile degradates (other than CO_2) were formed and that there was no dissipation of florasulam by volatilization. The distribution of radiocarbon over time was similar on the Cuckney soil at 40% MHC and the Marcham and Cuckney soils at 0, 0.05, and 15 bar moisture.

A maximum of 12.4% of the applied ^{14}C was observed as $^{14}CO_2$, while nonextractable residues increased with time to a maximum of 32.0% of the applied ^{14}C observed (across all soil types, temperatures, and moistures). The amount of nonextractable material was correlated with temperature, with the amount of nonextractable material increasing from 10.1 to 21.0% at 97 DAT on the Marcham soil as the temperature increased from 5 to 25 °C; similar effects were observed on the other soils.

Table 2. Distribution of Florasulam and 5-OH-Florasulam as a Function of Time, Soil Type, and Temperature; Soil Moisture Was 40% of the Moisture Holding Capacity for Each Soil

Florasulam

	Cuckney			Marcham					Naicam-Hoodoo			
DAT	5 °C	15 °C	25 °C	5 °C	10 °C	15 °C	20 °C	25 °C	5 °C	10 °C	20 °C	35 °C
0	98.4	95.9	98.4	98.7	100.6	96.8	100.8	100.0	95.3	96.6	90.9	96.3
0.2	–[a]	–	–	–	–	–	91.4	–	–	–	94.9	84.6
1	95.9	66.0	47.0	81.9	–	82.7	76.4	60.2	–	–	81.5	61.4
1.2	–	–	–	–	–	–	79.1	–	–	–	83.6	57.1
2	–	–	–	–	–	–	65.8	–	–	–	77.1	44.8
2.2	–	–	–	–	–	–	–	–	–	–	–	43.5
3	50.5	43.7	13.1	54.2	88.7	41.1	58.4	14.3	95.0	86.6	73.2	34.6
4	–	–	–	–	–	–	18.9	–	–	–	64.4	–
7	61.9	40.8	1.1	63.8	74.7	56.7	34.7	18.3	88.2	82.8	54.6	–
14	17.7	4.6	0.7	55.2	62.3	39.4	18.9	12.6	83.3	73.2	38.7	–
17	–	–	–	–	–	–	–	–	–	–	–	8.8
28	–	–	–	–	–	–	–	–	78.1	67.9	–	–
29	–	–	–	–	–	–	9.3	–	–	–	22.3	–
30	6.7	0.0	0.0	37.0	41.5	18.0	–	1.4	–	–	–	–
58	–	–	–	–	–	–	–	–	66.7	46.3	–	–
59	–	–	–	–	–	–	4.6	–	–	–	12.6	–
63	11.1	1.3	0.8	30.2	–	5.1	–	1.2	–	–	–	–
91	–	–	–	–	–	–	2.6	–	–	–	7.0	–
92	–	–	–	–	15.9	–	–	–	–	–	–	–
97	2.7	0.0	0.0	18.6	–	4.8	–	0.0	–	–	–	–
120	–	–	–	–	–	–	8.0	–	–	–	6.0	–
149	–	–	–	–	–	–	1.9	–	–	–	4.9	–
189	–	–	–	–	8.9	–	1.5	–	–	–	4.3	–

5-OH-Florasulam

	Cuckney			Marcham					Naicam-Hoodoo			
DAT	5 °C	15 °C	25 °C	5 °C	10 °C	15 °C	20 °C	25 °C	5 °C	10 °C	20 °C	35 °C
0	0.0	7.4	0.0	0.0	0.1	1.43	0.1	0.56	0.0	1.1	1.8	1.4
0.2	–	–	–	–	–	–	4.1	–	–	–	2.9	8.1
1	1.9	29.5	49.2	15.5	–	24.7	17.7	34.8	–	–	10.8	33.0
1.2	–	–	–	–	–	–	17.8	–	–	–	12.4	33.7
2	–	–	–	–	–	–	27.2	–	–	–	16.6	43.5
2.2	–	–	–	–	–	–	–	–	–	–	–	44.3
3	44.6	50.0	72.1	43.7	9.4	63.4	34.1	68.6	2.8	6.7	21.5	51.8
4	–	–	–	–	–	–	50.1	–	–	–	24.9	–
7	34.6	56.5	73.7	15.6	20.5	32.3	49.9	49.9	5.3	11.2	34.5	–
14	63.1	51.7	36.1	40.4	28.3	52.4	50.1	42.2	9.8	19.8	41.0	–
17	–	–	–	–	–	–	–	–	–	–	–	56.9
28	–	–	–	–	–	–	–	–	14.0	26.1	–	–
29	–	–	–	–	–	–	37.3	–	–	–	43.6	–
30	36.2	63.3	25.2	64.1	42.0	36.7	–	14.1	–	–	–	–
58	–	–	–	–	–	–	–	–	24.3	37.7	–	–
59	–	–	–	–	–	–	16.8	–	–	–	32.6	–
63	58.8	29.4	6.5	54.1	–	23.8	–	6.1	–	–	–	–
91	–	–	–	–	–	–	8.5	–	–	19.6	–	–
92	–	–	–	–	38.7	–	–	–	–	–	–	–
97	41.3	15.2	13.3	55.8	–	28.9	–	14.1	–	–	–	–
120	–	–	–	–	–	–	5.0	–	–	–	14.3	–
149	–	–	–	–	–	–	5.0	–	–	–	9.6	–
189	–	–	–	–	22.2	–	3.3	–	–	–	11.8	–

[a] Indicates that no sample was collected at that time point. Values are the sum of the desorbable and extractable pools and are expressed as a percentage of the amount of florasulam applied. Each data point represents a single sample.

The aqueous extractable pool of ^{14}C declined from >80% of the applied ^{14}C at 0 DAT to approximately 20% of the applied ^{14}C by 189 DAT, suggesting a substantial decrease in the bioavailability of the remaining pool of ^{14}C. (The aqueous extractable pool of material is assumed to be readily bioavailable.) The organic extractable pool reached a maximum level of approximately 60% of the applied ^{14}C, but had begun to decline by 189 DAT to approximately 45% of the applied ^{14}C.

Degradation Profiles. The amount of florasulam and 5-OH-florasulam present at each sampling point is shown in Tables 2 and 3; typical radiochromatograms of the aqueous and organic extracts are shown in Figure 3. The 5-OH metabolite of the parent molecule is the primary degradate of florasulam; degradation is predominantly by microbial metabolism rather than abiotic degradation. Details of the metabolic pathway of florasulam in soil after conversion to 5-OH-florasulam are reported elsewhere (Jackson et al., 2000). The 5-OH-florasulam metabolite reached a maximum level of 73.7% of applied at 7 DAT in the Cuckney soil (at 25 °C), 68.6% of applied at 3 DAT in the Marcham soil (at 25 °C), and 56.9% of applied at 17 DAT in the Naicam-Hoodoo soil (at 35 °C). The levels of 5-OH-florasulam declined to 13.3, 11.8, and 3.3% of applied at 97 DAT in the Cuckney soil (at 25 °C), and 189 DAT in the Naicam-Hoodoo and Marcham soils (at 20 °C), respectively.

Soil Microbial Viability. The change in the biological activity of the test systems over time was measured by incubating test systems for set periods of time, then

Table 3. Distribution of Florasulam and 5-OH-florasulam as a Function of Time, Soil Type, and Soil Moisture; All Soils Were Maintained at 20 °C

	florasulam						5-OH-florasulam					
	Cuckney			Marcham			Cuckney			Marcham		
DAT	0 bar	0.05 bar	15 bar	0 bar	0.05 bar	15 bar	0 bar	0.05 bar	15 bar	0 bar	0.05 bar	15 bar
0	87.1	82.7	95.0	81.4	81.2	82.9	13.6	18.5	4.5	18.2	13.4	16.5
1	73.5	65.2	58.6	77.4	77.4	74.1	26.8	31.0	35.8	27.7	20.0	20.4
3	70.2	50.0	47.5	68.5	57.9	46.8	29.6	47.4	50.0	31.8	39.0	36.3
7	37.9	23.2	20.0	32.5	31.6	22.3	54.1	65.6	70.1	53.8	60.7	68.2
14	17.8	7.7	24.6	10.2	20.7	18.9	55.9	67.8	64.7	52.6	66.1	66.0
30	0.0	9.3	5.7	2.6	4.7	5.6	58.4	33.4	55.0	33.1	47.6	52.3
58	0.0	0.0	0.0	2.5	0.0	3.1	40.3	22.3	34.6	31.5	27.6	44.6
99	0.8	0.0	0.0	3.0	2.8	1.9	22.7	14.9	33.0	21.6	12.7	17.6

[a] Values are the sum of the desorbable and extractable pools and are expressed as a percentage of the amount of florasulam applied. Each data point represents a single sample.

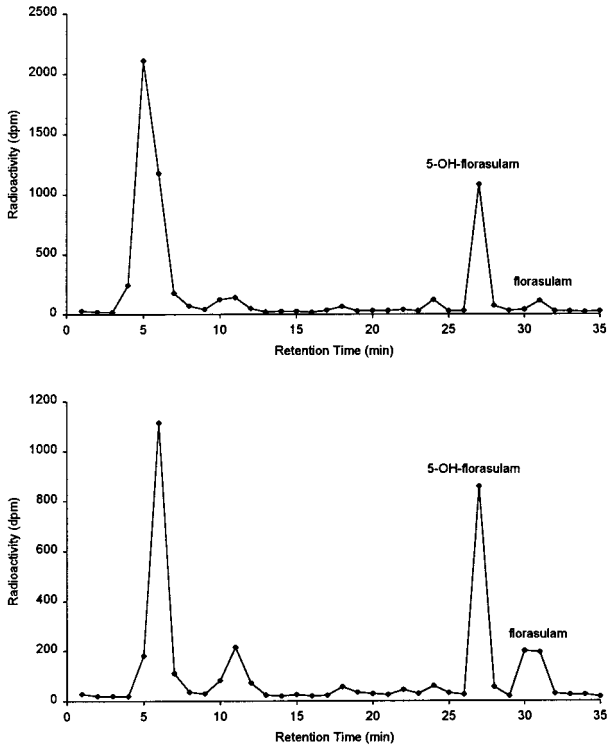


Figure 3. Typical HPLC radiochromatogram of florasulam and degradates from 91 DAT aqueous (top) and organic (bottom) extracts from Naicam-Hoodoo soil (20 °C).

dosing with ^{14}C-DCBA and measuring the production of $^{14}CO_2$ via microbial degradation; results are shown in Table 4. These test systems were either dosed with nonradiolabeled florasulam at 0 DAT or were not dosed with any test material. The ability of the test systems to degrade ^{14}C-DCBA to $^{14}CO_2$ declined by approximately 30−40% over the 197 day incubation period at 20 °C, indicating that the biological activity of the test system decreased with increasing incubation time. The decrease in the biological activity of the test system occurred principally in the first 90 days of the incubation period, although the decline did continue through 197 days. The effect was more pronounced at 10 °C, where the decline in degradation after 197 DAT was more than 60%. The differences in decline in soil biological activity between the untreated soils and those treated with nonradiolabeled florasulam are not considered to be significant; therefore florasulam does not appear to have a significant effect on the soil microbial viability at the rates used in this study.

The decline of the biological activity of the test system likely contributed to a decreasing degradation rate of florasulam and 5-OH-florasulam with increasing incubation time. This effect will be more significant for the slower degrading 5-OH-florasulam than for florasulam, and also at lower temperatures where overall degradation rates were slower. This suggests that degradation rates measured after 90 DAT in this study will be strongly affected by the decrease in biological activity of the test systems, and will underestimate degradation rates in natural systems where the level of biological activity should not exhibit this systematic downward temporal bias. Therefore, the degradation rates predicted in this study, particularly the DT_{90} values, may be longer than actual DT_{90} values observed in the environment. The biometer test systems used in this study are useful for maintaining mass balance, but caution must be used when correlating long-term degradation in biometers with predicted degradation in the environment. The static (with respect to nutrient and moisture cycling) biometer system is clearly not representative of processes that occur over these time periods in the soil environment.

Table 4. Amount of $^{14}CO_2$ Produced (Expressed as a Percent of the Applied ^{14}C) from the Degradation of ^{14}C-DCBA (Over a 192 h Incubation Period) at Each Timepoint

		% $^{14}CO_2$ produced incubation time (days)			
soil/temp	soil treatment	0	91	197	degradation change (%)
Naicam-Hoodoo					
20 °C	untreated	46	44	29	−38
20 °C	florasulam	44	25	32	−27
Marcham					
20 °C	untreated	54	38	32	−40
20 °C	florasulam	46	34	32	−30
10 °C	untreated	30	14	12	−61
10 °C	florasulam	29	15	9	−68

[a] Soil treatment indicates whether the test system had been left untreated or dosed with nonradiolabeled florasulam at 0 DAT. The degradation change column indicates the change in the amount of formation of $^{14}CO_2$ from ^{14}C-DCBA between 0 and 197 DAT.

Degradation Kinetics. Degradation of florasulam was described by a one-compartment first-order exponential decay model:

$$[\text{florasulam}]_t = [\text{florasulam}]_0 e^{-kt}$$

where $[\text{florasulam}]_t$ is the percent of florasulam remaining at time t, k is the rate constant, and $[\text{florasulam}]_0$ represents the initial amount of florasulam (assumed to be 100%). Nonlinear regressions were performed

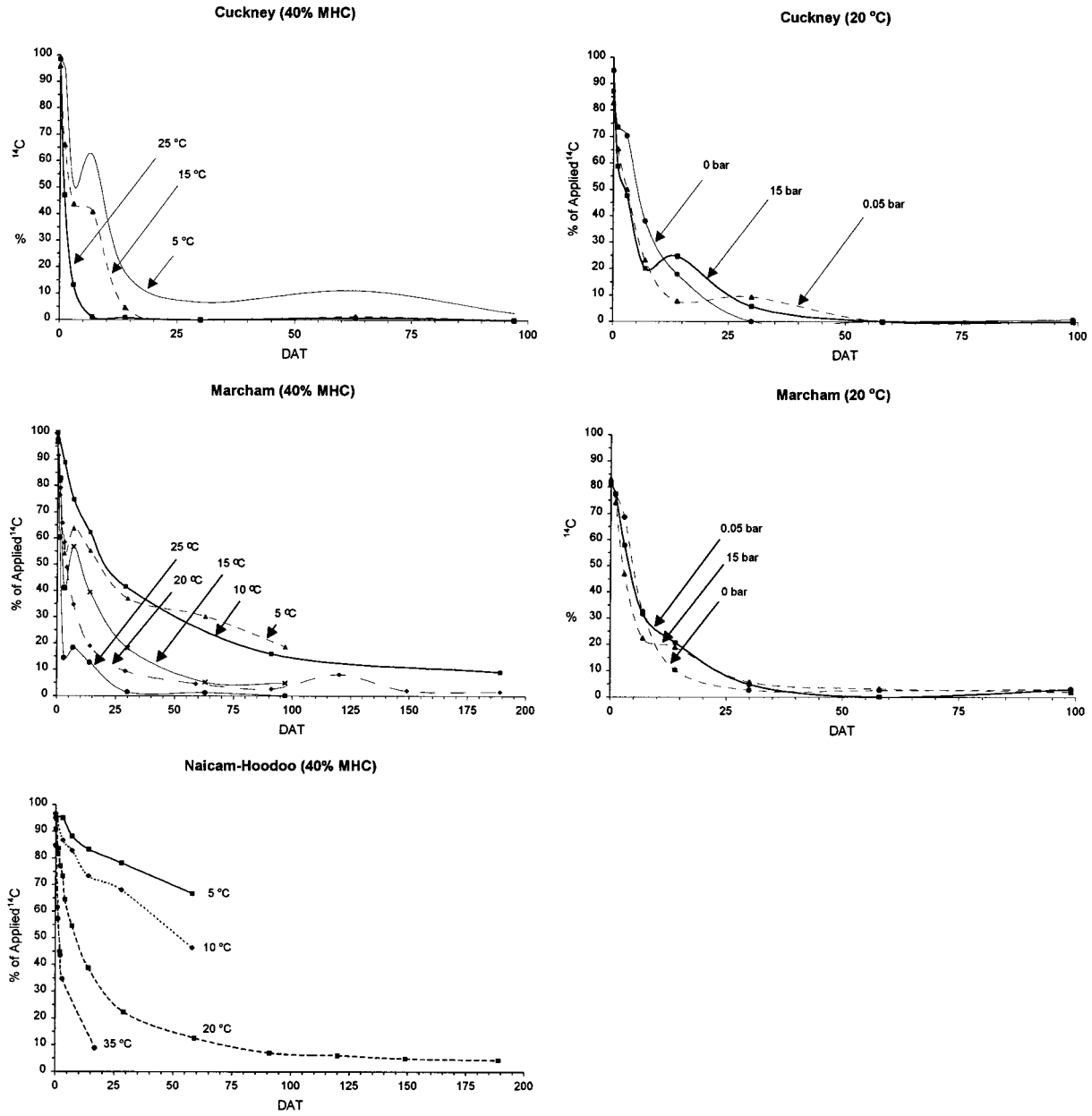


Figure 4. Degradation kinetics of florasulam in aerobic soil systems as a function of soil type, temperature (at 40% MHC), and soil moisture (at 20 °C).

using the Solver function in Microsoft Excel to calculate the first-order rate constants. The rate constant was varied to minimize the sum of the squared residuals between the actual and predicted florasulam concentration at each time point. Because of the use of the simple first-order kinetics model, the terms DT_{50} and half-life can be used interchangeably. The degradation of florasulam as a function of temperature and soil type is shown in Figure 4; the calculated rate constants were used to estimate the time required for 50 and 90% degradation (DT_{50} and DT_{90}, respectively) of florasulam (Table 5). Calculated DT_{50} values for florasulam ranged from 1.0 to 8.5 days at 20 and 25 °C and from 6.4 to 85 days at 5 °C.

Examination of the residuals from the exponential decay fit for each soil reveals that this simple model underestimates the initial rate of decay and overestimates the degradation rate at later time points. The net result is an overprediction of DT_{50} and an underprediction of DT_{90} values; these errors suggest that the degradation of florasulam was actually biphasic in nature. However, the high correlation coefficients for the regressions imply that the absolute magnitude of the errors is small and the model is adequate for predicting the dissipation of florasulam in aerobic soil systems.

The change in 5-OH-florasulam concentration is dependent on both its rate of formation and rate of decay. Applying the first-order rate law for consecutive reactions gives:

$$\frac{\mathrm{d}[\text{5-OH-florasulam}]}{\mathrm{d}t} = k_{\text{florasulam}}\,[\text{florasulam}] - k_{\text{5-OH-florasulam}}[\text{5-OH-florasulam}]$$

where $k_{\text{florasulam}}$ is the rate constant for degradation of florasulam (or for the formation of 5-OH-florasulam) and $k_{5-\text{OH}-\text{florasulam}}$ is the rate constant for the degradation of 5-OH-florasulam. This derivation assumes that flo-

Table 5. Degradation Kinetics and Estimated DT_{50} and DT_{90} Values for Florasulam and 5-Hydroxy-Florasulam as a Function of Soil Type, Temperature (at 40% MHC), and Soil Moisture (at 20 °C)

	florasulam				5-OH-florasulam			
	k (day^{-1})	r^2	DT_{50} (days)	DT_{90} (days)	k (day^{-1})	r^2	DT_{50} (days)	DT_{90} (days)
Cuckney (40% MHC)								
5 °C	0.109	0.927	6.4	21	0.014	0.580	49	164
15 °C	0.209	0.949	3.3	11	0.024	0.848	29	98
25 °C	0.727	1.000	1.0	3.2	0.063	0.956	11	37
Marcham (40% MHC)								
5 °C	0.038	0.845	18	61	0.009	0.732	78	258
10 °C	0.031	0.992	23	75	0.013	0.953	54	179
15 °C	0.093	0.876	7.4	25	0.030	0.358	23	76
20 °C	0.171	0.992	4.1	14	0.047	0.978	15	50
25 °C	0.532	0.964	1.3	4.3	0.085	0.937	8.1	27
Naicam-Hoodoo (40% MHC)								
5 °C	0.008	0.966	85	283	0.016	0.986	43	142
10 °C	0.015	0.967	46	152	0.014	0.990	48	161
20 °C	0.082	0.991	8.5	28	0.026	0.944	27	88
35 °C	0.408	0.975	1.7	5.6	0.044	0.936	16	52
Cuckney (20 °C)								
0 bar	0.137	0.983	5.1	17	0.020	0.948	35	116
0.05 bar	0.241	0.982	2.9	9.5	0.035	0.944	20	66
15 bar	0.258	0.934	2.7	8.9	0.019	0.924	36	121
Marcham (20 °C)								
0 bar	0.156	0.977	4.5	15	0.035	0.879	20	66
0.05 bar	0.224	0.951	3.1	10	0.020	0.966	34	114
15 bar	0.224	0.998	3.1	10	0.020	0.916	34	114

rasulam degrades directly to 5-OH-florasulam with no intermediates or side reactions; we believe this to be an accurate assumption based on the simple reaction pathway (degradation via either *O*-demethylation or substitution of a hydroxy group for the methoxy group), the rapid degradation rate of florasulam, and the large yields of 5-OH-florasulam observed. To determine the degradation rate of 5-OH-florasulam, its predicted concentration at each sampling time was calculated using the equation:

$$[\text{5-OH-florasulam}]_t = [\text{florasulam}_0]\left(\frac{k_{\text{florasulam}}(e^{-k_{\text{florasulam}}t} - e^{-k_{\text{5-OH-florasulam}}t})}{k_{\text{5-OH-florasulam}} - k_{\text{florasulam}}}\right)$$

The rate constants for 5-OH-florasulam degradation were calculated using the Microsoft Excel Solver function to minimize the sum of the squared residuals for all sampling times and thus determine the rate constant that provided the line of best fit for the data. The rate constants previously determined for florasulam were used as inputs for the calculation of the 5-OH-florasulam degradation rates and the initial concentration of florasulam was set to 100% of the applied material. The degradation of 5-OH-florasulam as a function of temperature and soil type is shown in Figure 5; the model was used to estimate the time required for 50 and 90% degradation (DT_{50} and DT_{90}, respectively) of 5-OH-florasulam (Table 5). Calculated DT_{50} values for 5-OH-florasulam ranged from 8 to 36 days at 20 and 25 °C and from 43 to 78 days at 5 °C.

The degradation rate of each compound showed a strong temperature dependence, with DT_{50} values increasing by a factor of 2−6 for each 10 °C temperature decrease. The temperature dependence of the degradation rate can be described by determining the activation energy of the degradation. The activation energy was determined with the Arrhenius equation:

$$k = Ae^{-E_a/RT}$$

where k is the rate constant, E_a is the activation energy of the reaction, R is the gas constant, T is the absolute temperature, and A is a constant. Taking the natural log of both sides of this equation gives:

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

Plotting ln k vs $1/T$ and performing a best fit linear regression gives a line with a slope of $-E_a/R$.

Activation energies were determined using rate constants determined from simple first-order kinetics; results are presented in Table 6. Because soil moisture potential (between 0 and 15 bar) did not have a substantial impact on the degradation rate of either florasulam or 5-OH-florasulam (Table 5), the rate constants determined at various moistures were averaged to give a single rate constant at each temperature for the activation energy calculation. This prevented the large number of rate constants measured at 20 °C from having a disproportionate impact on the linear regression for determination of the activation energy.

It is unclear why the activation energy for degradation of florasulam on the Cuckney sand soil was lower than that observed on the finer-textured Naicam-Hoodoo and Marcham soils. The calculated activation energy values for florasulam are greater than the activation energy of 50.8 kJ/mol determined for structurally related herbicide flumetsulam over a similar temperature range (Lehmann et al., 1993), and indicates that florasulam will have a larger temperature effect on its degradation rate. The calculated activation energy for the degradation of 5-OH-florasulam varied substantially, but was lower than that for florasulam on each soil, indicating a decreased temperature dependence relative to florasulam.

Sorption. Apparent (nonequilibrium) sorption coefficients (K_d, L/kg) were determined for florasulam and 5-OH-florasulam at each sampling point on the Naicam-Hoodoo test systems at 20 °C and the Marcham test systems at 10 and 20 °C using the results of the separate aqueous and organic extractions; results are shown in

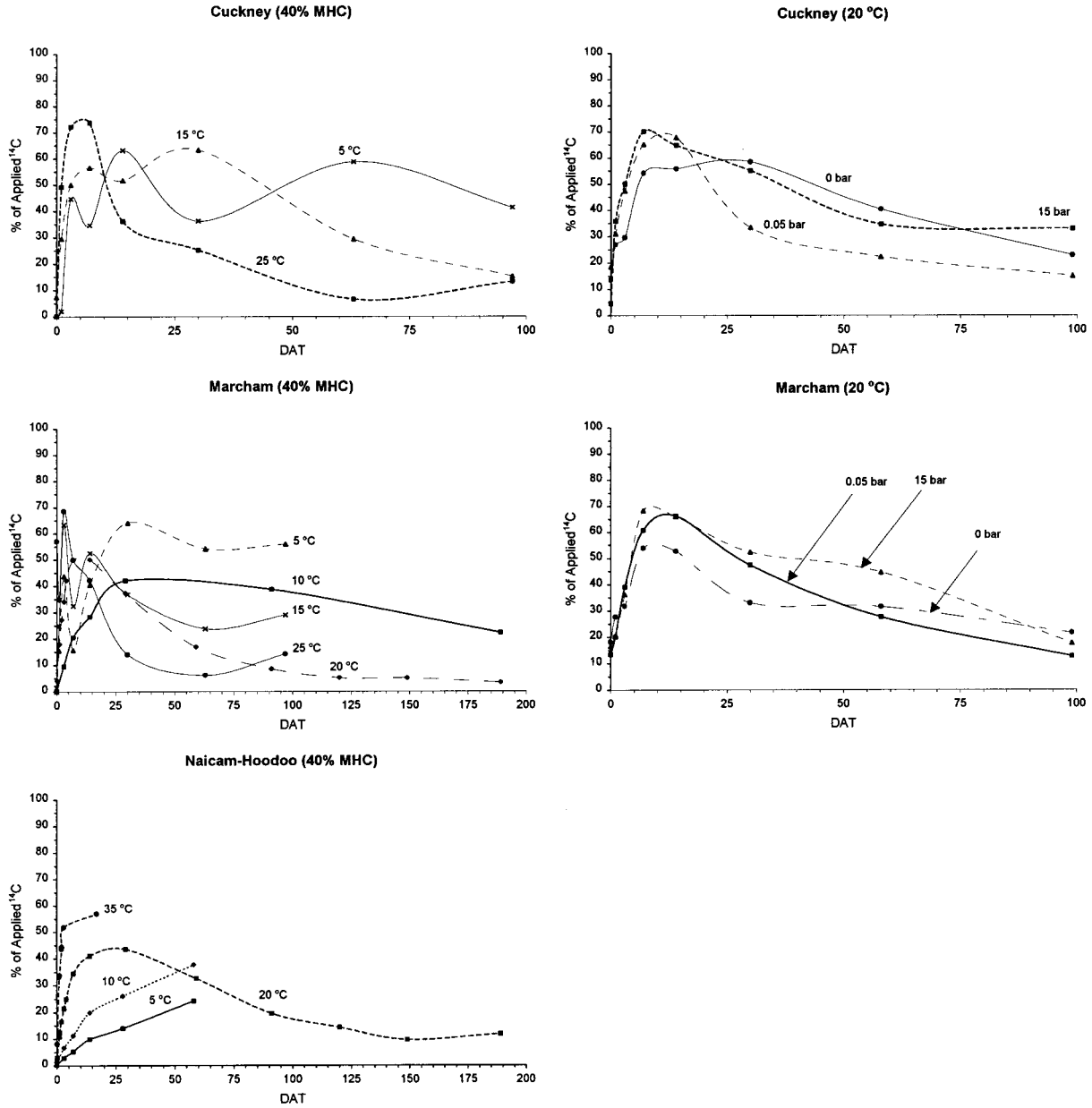


Figure 5. Degradation kinetics of 5-OH-florasulam in aerobic soil systems as a function of soil type, temperature (at 40% MHC), and soil moisture (at 20 °C).

Table 6. Activation Energies for the Aerobic Soil Degradation of Florasulam and 5-OH-Florasulam

	A	E_a (kJ/mol)	r^2
florasulam			
Cuckney	4.81×10^9	-57.0 ± 20.2	0.798
Marcham	5.53×10^{16}	-97.8 ± 18.5	0.903
Naicam-Hoodoo	5.49×10^{15}	-94.9 ± 5.5	0.993
5-OH-florasulam			
Cuckney	4.21×10^6	-45.4 ± 15.0	0.933
Marcham	6.43×10^{11}	-74.0 ± 11.4	0.725
Naicam-Hoodoo	1.39×10^3	-26.6 ± 5.3	0.928

[a] The variation given in the activation energy is 1 SD.

Table 7. Apparent K_d values were calculated assuming that the material in the aqueous extract had been present in the soil solution, whereas the material removed with the subsequent organic extraction represented material that had been sorbed to the soil matrix. The concentration in the soil was obtained by dividing the mass of material extracted with organic solvent by the soil dry weight. The apparent K_d is the ratio of the soil concentration (μg/g) divided by the soil solution concentration (μg/mL). Only extractable material was used to determine apparent sorption coefficients; nonextractable material was assumed to be chemically distinct from either florasulam or 5-OH-florasulam. Apparent K_d values therefore represent what the actual partitioning of a chemical in soil at a given time. Apparent (nonequilibrium) K_d values differ from equilibrium K_d values which describe the partitioning of a chemical in soil after equilibrium has been achieved; apparent K_d values can be useful in understanding the relationship of sorption and degradation over time in a soil (Krieger et al., 1998).

Sorption increased linearly with time for both florasulam and 5-OH-florasulam ($r^2 > 0.90$). Initial sorptivities for florasulam ranged from 0.1 to 0.5 L/kg and increased to 23 L/kg by 91 DAT. Sorption coefficients for 5-OH-florasulam also increased with time, from an initial value of 0.6 L/kg up to as high as 12.5 L/kg at 149 DAT. The initial sorptivities compare well with

Table 7. Apparent (Nonequilibrium) Sorption Coefficients (K_d, L/Kg) for Florasulam and 5-OH-Florasulam on Naicam-Hoodoo Soil at 10 °C and on Marcham Soil at 10 and 20 °C (All at 40% MHC)

	florasulam				5-OH-florasulam			
	Naicam-Hoodoo	Marcham		ΔH_{ads}	Naicam-Hoodoo	Marcham		ΔH_{ads}
DAT	20 °C	10 °C	20 °C	kJ/mol	20 °C	10 °C	20 °C	kJ/mol
0	0.52	0.11	0.10	−3.6	0.61			
0.2	1.17		0.39		1.55		0.53	
1	2.47		0.93		1.91		0.67	
1.2	2.53		0.88		2.11		0.78	
2	2.99		1.33		2.13		0.81	
3	3.32	0.55	1.34	61.7	2.16	0.73	0.76	2.9
4	3.72		1.51		2.53		0.96	
7	4.45	0.99	1.88	44.3	2.83	1.03	0.99	−2.8
14	6.17	1.46	3.61	62.3	2.92	0.95	1.18	14.6
29	11.84	1.86	6.97	91.1	4.17	1.24	1.60	17.5
59	18.03		19.20		5.71		3.34	
91	23.17	3.36			6.73	1.69	4.42	66.4
120					9.60		6.12	
149					12.48		6.22	
189					4.98		9.21	
m	0.246	0.032	0.303		0.042	0.010	0.043	
r^2	0.974	0.910	0.978		0.645[a]	0.903	0.988	

[a] r^2 is 0.972 without 189 DAT data point. Sorption coefficients were calculated only where the amount of material in each pool was at least 0.5% of the applied radiocarbon. The slope (*m*) and correlation coefficient (r^2) of a simple linear regression of apparent K_d vs DAT is given at the bottom. Heat of adsorption values were calculated where sorption information was available at both 10 and 20 °C.

those observed in a batch equilibrium adsorption/desorption study, where average batch adsorption K_d values were 0.46 ± 0.37 (range 0.08−0.94 L kg^{-1}) and 0.38 ± 0.25 L kg^{-1} (range 0.16−0.72 L kg^{-1}) for florasulam and 5-OH-florasulam, respectively. Freundlich 1/*n* values for florasulam and 5-OH-florasulam were 0.93 ± 0.05 and 0.95 ± 0.08, respectively, over a concentration range of 7−1300 ppb, suggesting that some of the observed increase in sorption over time may be due to a decrease in concentration. However, most of the nonlinearity in the Freundlich isotherms was at concentrations significantly greater than those measured in this study (≤25 ppb). Average adsorption coefficients for florasulam at 7 and 13 ppb were 0.51 ± 0.46 and 0.55 ± 0.39 L/kg, respectively, and 0.39 ± 0.30 and 0.44 ± 0.29 L/kg for 5-OH-florasulam at the same concentrations (Ostrander, 1996). Therefore, we expect that the contribution of a decrease in concentration due to degradation to the increase in the apparent (nonequilibrium) sorption coefficients to be negligible.

Equilibrium sorption decreases with increasing temperature for most organic compounds (Ten Hulscher and Cornelissen, 1996); however, apparent (nonequilibrium) K_d values were uniformly higher for florasulam and 5-OH-florasulam at 20 °C than at 10 °C on both soils. Chiou et al., (1979) suggested that increased sorption at higher temperatures can be expected for molecules that exhibit decreasing solubility at higher temperatures. These data are not available for florasulam, but assuming that all of the applied florasulam partitioned into the pore water would yield a concentration of ca. 0.1 μg/mL (well below the minimum solubility of 84 μg/mL at pH 5), it is unlikely that aqueous solubility is a limiting factor in the temperature dependence of the sorption of florasulam. The rate of increase of apparent (nonequilibrium) K_d values (as measured by the slope of a simple linear regression of apparent K_d vs DAT) at 10 °C was approximately 10−25% the rate of increase at 20 °C.

Heats of adsorption (Table 7) were calculated from a Clausius−Clapeyron type of equation:

$$\Delta H = \frac{-R \ln \frac{K_{d,2}}{K_{d,1}}}{\frac{1}{T_2} - \frac{1}{T_1}}$$

where ΔH is the heat of adsorption, R is the gas constant, and $K_{d,1}$ and $K_{d,2}$ are the apparent (nonequilibrium) sorption coefficients at temperatures T_1 and T_2 (Hamaker and Thompson, 1972). The heat of adsorption at early time points is small, which is consistent with the low initial sorption coefficients observed in this study and a batch equilibration study (Ostrander, 1996). The low initial heat of adsorption and the larger heat of adsorption at later time points are consistent with a sorption model containing fast and slow components (Ten Hulscher and Cornelissen, 1996; Brusseau et al., 1989) and the observed changes in the microbial degradation rate. Florasulam and 5-OH-florasulam are initially present on the soil surface on sites with varying desorption energies depending on the nature of the sorption interaction. Over time, molecules that are present on sites with lower desorption energies will degrade more rapidly because they are more likely to desorb into the soil solution where they will be available for microbial degradation. The measured average desorption energy increases with time because the population of molecules present on the lower energy sites is depleted more rapidly than those on higher energy sites. The increase of the heat of adsorption with time implies that the equilibrium between the sites of higher energy and lower energy is slow; if equilibrium was rapid, the average desorption energy would remain constant as molecules exchanged between higher and lower energy sites of adsorption. The net impact is a decrease in the degradation rate because fewer molecules have enough energy to desorb from the surface into the soil solution. The impact on soil mobility is that the tendency for these molecules to migrate through the soil profile will decrease with soil contact time and will be overestimated by simple transport models using time-invariant sorption coefficients.

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