Anaerobic Aquatic Degradation of Flumetsulam [*N*-(2,6-Difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide]

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The herbicide flumetsulam [*N*-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide] was investigated in sediment–water systems to ascertain anaerobic aquatic degradation as a potential route for dissipation. In a strongly anaerobic test system (sulfate-reducing or methanogenic) flumetsulam degraded to a dominant product which preferentially partitioned to the water phase; flumetsulam half-life ($t_{1/2}$) was 183 days. The anaerobic degradation product was identified as reduced flumetsulam hydrate [*N*-(2,6-difluorophenyl)-4,5,6,7-tetrahydro-5-hydroxy-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide]. Reduced flumetsulam hydrate distributed among several products in an equilibrium mixture and reverted to flumetsulam upon aeration. Thus, the anaerobic degradation product never accumulated in a weakly anaerobic test system (initially denitrifying). When applied to aerobic soil, the anaerobic degradation product exhibited a $t_{1/2}$ of 2 days compared to a $t_{1/2}$ of 40 days for flumetsulam. Aerobic degradation yielded parent flumetsulam and the primary aerobic degradation product of flumetsulam metabolism. Flumetsulam anaerobic aquatic degradation is viewed as a principally abiotic process occurring only in strongly anaerobic, static environments isolated from the atmosphere. The likelihood of reduced flumetsulam hydrate occurrence and accumulation in the environment is minimal when flumetsulam is used for terrestrial food crops.

INTRODUCTION

The sulfonamide herbicide flumetsulam [*N*-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide] is an acetolactate synthase (ALS)-inhibiting herbicide for broadleaf weed control in soybean and field corn (Kleschick et al., 1992). Field use rates range from 70 to 79 g of ai/ha for preplant incorporated application. Flumetsulam has the following properties at 25 °C: water solubility, 49 mg/L (pH 2.5); pK_a, 4.6; pK_{ow}, 0.21 (Kleschick et al., 1992). The estimated half-life for aerobic soil metabolism ranges from 2 weeks to 4 months across geographically diverse soils of varied texture and organic carbon content (Lehmann et al., 1992). Intermediate breakdown products do not occur at concentrations ≥0.01 ppm when flumetsulam is applied at 0.20 ppm (milligrams per kilogram of oven-dry soil).

Degradation rates and pathways as well as the organisms involved may differ among redox regimes (Berry et al., 1987; Gibson and Sulflita, 1986). Therefore, this study describes degradation in static, strongly anaerobic systems (poised in sulfate-reducing or methanogenic regions) and in weakly anaerobic test systems (initially poised under denitrifying conditions but becoming progressively aerobic with time). The fate of reduced flumetsulam hydrate in an aerobic soil system, where it has not been observed to occur, was investigated as well. Consideration of flumetsulam degradation under these varied redox regimes helps to define the actual importance of anaerobic degradation in the environment.

MATERIALS AND METHODS

Two concurrent series of reaction flasks were established under anaerobic conditions; one series received [^{14}C]pyrimidine-labeled flumetsulam as the test substance, while the second series received [^{14}C]aniline-labeled flumetsulam. The treatment rate was approximately 0.20 ppm (mg/kg oven dry sediment), representing a worst case where a shallow, ponded area of a field is oversprayed at 3 times the maximum label rate for a soil preplant incorporated treatment. All (sediment–water)–series–time combinations were performed in duplicate. The experiment was conducted under static, strongly anaerobic conditions and repeated a second time under weakly anaerobic conditions where systems became increasingly aerobic with time. A third experiment was conducted in which the fate of the anaerobic aquatic degradation product was monitored in aerobic soil.

Chemicals. Test substances were [^{14}C]flumetsulam (i) labeled in the 5 position of the pyrimidine ring (specific activity, 10.9 mCi/mmol; 7.37 × 10^4 dpm/μg) and (ii) labeled uniformly in the aniline ring (specific activity, 28.0 mCi/mmol; 1.89 × 10^5 dpm/μg). These chemicals were 99% radiochemically pure as determined by high-performance liquid chromatography (HPLC) radioassay.

Sediment, Water, and Soil. The sediment and water samples used in this study were obtained from a pond located within an agricultural watershed representative of a geographic region of projected flumetsulam use (Wayside, MS). Samples of sediment and water were collected in the field and transferred to sterilized glass bottles from which headspace was excluded. On receipt, samples were stored in the dark in an incubator at approximately 25 °C until initiation of the study. Samples were collected September 13, 1989, and March 13, 1990, for use in the weakly and strongly anaerobic studies, respectively. Commerce silt loam surface soil (Aeric Fluvaquents; Wayside, MS) was used for the aerobic soil study.

Selected physical and chemical properties of the test sediment, pond water, and aerobic soil are summarized in Table I. Water temperature was measured at sampling. Pond water pH, dissolved oxygen (by oxygen probe), electrical conductivity, and sediment color (by Munsell color charts) were determined on receipt of sediment and water samples. Pond water was decanted from sediment following particle settling, and a sample was analyzed for turbidity and selected metals and ligands. A subsample of air-dry sediment and aerobic soil was analyzed

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Table I. Selected Physical and Chemical Properties of Wayside Sediment and Water for the Weakly and Strongly Anaerobic Systems and for the Aerobic Commerce Soil

	strongly anaerobic sediment	weakly anaerobic sediment	aerobic soil
	Wayside Sediment		
textural class	silt loam	clay loam	silt loam
particle size distribution			
sand, %	20	23	26
silt, %	56	49	56
clay, %	24	28	18
moisture, 0.33 bar			
%, oven-dry soil basis			18.69
CEC, mequiv/100 g	14.5	12.7	11.0
pH (1:1 sediment/water)	5.0	5.7	7.8
organic carbon, %	4.9	5.6	0.48
DTPA-extractable			
Mn, mg/kg	84	70	
Fe, mg/kg		370	
	Water		
temp, °C	25	18	
pH	6.34	6.38	
EC, mmho/cm	0.11	0.34	
dissolved O_2, mg/L	0.13	N/D[a]	
turbidity, NTU	1296	78	
NO_3-N, mg/L	<0.2	<0.2	
total			
Ca, mg/L	27.2	39.4	
Fe, mg/L	22.0	11.2	
Mg, mg/L	8.1	9.68	
Mn, mg/L	0.92	2.31	
P, mg/L	1.19	0.51	
K, mg/L	11.3	15.6	

[a] N/D, not determined.

according to standard methods (Klute, 1986; Page et al., 1982) for pH, percent organic C, particle size distribution, cation-exchange capacity, and extractable Mn and Fe.

Analytical Procedures. *Chromatography.* Reversed-phase gradient HPLC was used for isolation of the parent flumetsulam and degradation products in water-phase samples and sediment-phase extracts. A C18 column was used as the stationary phase, and the mobile phase consisted of water, acetonitrile, and acetic acid. The gradient consisted of initial mobile-phase composition of 84% water, 15% acetonitrile, and 1% acetic acid that changed linearly to 9% water, 90% acetonitrile, and 1% acetic acid over 35 min and then was held at this composition for 10 min. Approximate retention times were 12 and 15 min for reduced flumetsulam hydrate and flumetsulam, respectively, in this system.

Isocratic conditions were utilized for peak separation, cleanup, and fractionation of ^{14}C activity in preparation for degradation product characterization. For isocratic HPLC, the mobile phase was typically 169:30:1 water/acetonitrile/acetic acid and the flow rate was 1 mL/min. A C18 column was used as the stationary phase. Approximate retention times were 18 and 30 min for reduced flumetsulam hydrate and flumetsulam, respectively, in this system.

Aliquots of water were prefiltered through 0.45-μm filters prior to injection. Aliquots of acidified acetone extracts were taken to dryness under a stream of N_2 gas. The residue was brought up in mobile phase and filtered as for water samples prior to injection.

Aquatic Studies. *Preparation of ^{14}C Spiking Solutions.* Appropriate aliquots of [^{14}C]flumetsulam in acetone were delivered to 0.005 M NH_4OH solution and the acetone was evaporated under a stream of N_2 for preparation of the spiking solutions. Aliquots of these solutions delivered to a nominal mass of 50 g of sediment (oven-dry equivalent) resulted in an application of 0.20 $\pm$ 0.02 μg/g. Average ^{14}C applied was (6.75 $\pm$ 0.11) $\times 10^5$ and (7.93 $\pm$ 0.08) $\times 10^5$ dpm for the weakly and strongly anaerobic systems, respectively.

Preparation, Spiking, and Sampling. Sediment was separated from water at the time of study initiation by decantation of water following sediment settling. Subsamples of sediment (with entrained water) were analyzed for air-dry and oven-dry (od) moisture content. Reaction flasks (biometers; Bartha and Pramer, 1965) received combinations of decanted water and sediment (with entrained water) to provide 100 mL of water and 50 g of sediment (od basis). Additionally, treatments for the strongly anaerobic system received 0.5 g of finely ground alfalfa per reaction flask to serve as an easily oxidizable source of reducing power. Reaction flasks were maintained in an incubator in the dark at 25 °C prior to spiking of test materials. Sediment and water prepared in this manner were stored for 8 days prior to spiking to the weakly anaerobic system; a 32-day storage period was used for the strongly anaerobic system to allow for the oxidation of added alfalfa.

At the time of sample spiking, biometers were charged with 100 mL of 0.2 N NaOH for trapping of evolved ^{14}C. Aliquots of spiking solution were delivered to the surface water in each flask. A sweep of N_2 gas delivered to the water surface was used to distribute applied ^{14}C and to flush the headspace in each biometer flask. The flasks were then sealed and returned to incubators, where they were maintained in the dark at 25 °C until sampled.

At each time of sampling, dissolved O_2 and pH (strongly anaerobic system) of the water phase was measured and the color of the sediment phase was noted. (Additionally, E_H was measured for the 360-day sampling of the strongly anaerobic system.) NaOH trapping solution was recovered and analyzed for ^{14}C, which was confirmed as $^{14}CO_2$ by Ba precipitation. Sediment and water were transferred to 250-mL volume, capped, tared polyallomer centrifuge tubes. Sediment was separated from water by centrifugation at 1640*g* for 30 min. Water was decanted to labeled 50-mL capacity plastic bottles for storage, while sediment with entrained water (hereafter referred to as sediment) was retained in the centrifuge tubes for storage. Water and sediment samples were stored frozen when not in use. The efficiency of sample transfer from reaction flasks to centrifuge tubes and the subsequent partitioning of water and sediment were tracked through weighing of the tared centrifuge tubes following the appropriate transfers. Nominal times of sampling were 0, 30, 60, 180, and 360 days after application.

Sediment Extraction. Total extractable [^{14}C]flumetsulam and residues in sediment were determined by hand or robotically using acidified acetone (acetone/acetic acid/water 18:1:1 v/v/v). Acidified acetone has been shown to be the most efficient of a wide variety of extractants for the extraction of flumetsulam and residues from soil matrices.

Extraction involved weighing from 5 to 15 g of moist sediments into centrifuge tubes followed by addition of 15 mL of acidified acetone solution. Capped centrifuge tubes were mechanically shaken for $\geq$1 h. The samples were then centrifuged at about 260*g* and decanted to 50-mL containers. Extraction with 15-mL aliquots of acidified acetone was repeated twice more with 1-h shaking times, and the centrifuged extracts were decanted and combined with the previous fractions. Aliquots of the combined extracts were used for LSC and HPLC/fractional LSC.

Sediment-Bound Residues. Nonextractable bound residues were determined by combustion and trapping of evolved $^{14}CO_2$. Triplicate determinations were performed by hand or robotically. Extracted sediments (air-dry) were weighed ($\approx$1 g) to glass boats for combustion. The $^{14}CO_2$ evolved was trapped in a scintillation vial containing cocktail, and ^{14}C activity was assayed by LSC.

Reduction of Flumetsulam. The conditions of the strongly anaerobic system appeared to be conducive to flumetsulam reduction; thus, investigations were initiated to determine the nature of products generated from flumetsulam reduction with the intent of synthesizing a standard of reduced flumetsulam hydrate. All new products were characterized by ^1H NMR, IR, mass spectrometry, or combustion analysis.

Hydrogen over Palladium on Carbon (Pd/C). A mixture consisting of 0.25 g of Pd/C (10%, Aldrich Lot MX 03715 CX), 3.0 g of flumetsulam, and 50 mL of ethyl acetate was hydrogenated at 3.1 kPa in a Parr bomb for 3 h. The reaction was followed by TLC and HPLC. The reaction was poured onto dry silica gel and eluted with ethyl acetate. The product was collected in 200 mL of solvent and the solvent removed in vacuo to afford 285 mg of white solid.

Lithium Aluminum Hydride (LAH) and Tetrahydrofuran (THF). LAH was added with stirring to a solution of 1.0 g of

Table II. Selected Characteristics of Sediment and Water in Anaerobic Test Systems for Each Time of Sacrifice

	weakly anaerobic system				strongly anaerobic system		
	color						
time of sacrifice, days	sediment	sediment/water interface	dissolved oxygen, mg/L	water pH	sediment color	dissolved oxygen, mg/L	water pH
as recd	N/D[a]	N/D	0.13	6.34	N/D	N/D	6.38
0	N/D	N/D	N/D	N/D	dark gray	0.12	6.61
30	N/D	N/D	1.16	N/D	dark gray	0.31	7.87
60	greenish gray	yellowish red	0.38	N/D	dark gray	0.12	7.98
90	dark greenish gray	dark reddish brown	0.43	N/D	dark gray	0.26	7.67
180	dark gray	dark reddish brown	N/D	N/D	dark gray	0.17	7.57
360	dark gray	yellowish red	6.05	6.06	dark gray	0.22	7.39

[a] N/D, not determined.

flumetsulam and 40 mL of THF at 0 °C. The mixture was warmed to room temperature and stirred under N_2 for 4 days, after which time 40 mL of 1 M HCl and ice were carefully added. The aqueous phase was extracted with 40 mL of diethyl ether followed by 2 × 30 mL of ethyl acetate. The combined extracts were dehydrated over Na_2SO_4 and $MgSO_4$, filtered, and evaporated to a tan solid. Flash chromatography on 230–400-mesh silica gel with ethyl acetate as eluent provided 210 mg of product as white solid.

Aerobic Soil Study. The anaerobic degradation product in aqueous solution which had been isolated and concentrated from the strongly anaerobic system was applied to Commerce soil. For each sample, 1.0 g of soil was weighed into a 25-mL centrifuge tube. Each sample was then treated with 1.03 × 10^4 dpm of isolated degradation product (equivalent to ≈0.1 μg/g on an oven-dried basis). The soil moisture was adjusted to 75% of 0.33 bar with distilled water.

The treated soils were incubated in the dark at 25 °C in biometer flasks under a positive oxygen head of 130 mm of H_2O. Samples were sampled at 0, 1, 2, 4, and 9 days posttreatment. At sampling, three 1-mL portions from the caustic traps were assayed for $^{14}CO_2$ (confirmed by Ba precipitation). Extraction of the soils was conducted with an acidified acetone solution as for sediments using three 5-mL portions of acidified acetone; aliquots were assayed by LSC.

The soil extracts were taken to dryness under N_2 and redissolved in mobile-phase eluent. The concentrate was analyzed by isocratic LC.

Additional Commerce soil was treated with isolated degradation product to generate mass for identification of breakdown products of reduced flumetsulam hydrate occurring in aerobic soil. Duplicate 5.0-g soil samples in 25-mL centrifuge tubes received 1.03 × 10^4 dpm of isolated degradation product, and the soil moisture was adjusted to 75% of 0.33 bar with distilled water. The tube was then placed in a biometer flask with 100 mL of 0.2 N NaOH in the side chamber. The samples were incubated in the dark at 25 °C for 4 days and then sampled. The caustic traps were assayed for ^{14}C activity by LSC. The soils were extracted as for sediments. The extracts were concentrated and fractionated using isocratic LC with fractions assayed for ^{14}C activity by LSC. Metabolite peaks were isolated and concentrated for mass spectral analysis. This study was repeated with 6.16 × 10^5 dpm applied to 5 g of oven-dry soil.

HPLC Comparison with Aerobic Soil Metabolites. A solution of flumetsulam aerobic metabolites was obtained from an aerobic soil metabolism study of flumetsulam. This solution was analyzed by LC under the same conditions as used to analyze the samples from the above kinetic study. This provided a direct comparison of retention times for degradation products from reduced flumetsulam hydrate with those from flumetsulam in aerobic soils.

RESULTS AND DISCUSSION

Characteristics of Aquatic Test Systems. Sediment color, water pH, and dissolved O_2 were monitored for selected times of sampling (Table II). These data indicate marked differences in the degree of anaerobicity maintained in the strongly vs weakly anaerobic systems.

For the weakly anaerobic system, slight reddening of sediment at the sediment/water interface was noted, beginning day 27 of the study, from O_2 diffusing into the biometer flasks. The progressive loss of anaerobicity in the water phase and surface sediment with time of incubation was verified through measurements of dissolved O_2 and color of the sediment. Increased dissolved O_2 in water phase and consequent oxidation of surface sediment (as indicated by increasingly red colors) were noted with time of incubation, while strong anaerobic conditions continued to be exhibited in the sediment (colors remained gray).

The strongly anaerobic system was established under conditions designed to maintain anaerobicity for the duration of the study (through addition of alfalfa in the 32-day preconditioning period). The system remained strongly anaerobic throughout the term of the study as reflected by the measured E_H at 360 days (−201.4 ± 12.6 mV), the low and relatively nonvariable dissolved O_2 concentrations (mean 0.20 ppm, range 0.12–0.31 ppm; at the limit of sensitivity for the instrument), and the maintenance of dark gray sediment color (Table II). The water pH increased from 6.61 on day 0 to 7.98 at day 60 and then declined to 7.39 by day 360; this is likely a consequence of biologically mediated reductive processes occurring under anaerobic conditions (Stumm and Morgan, 1981). The E_{H7}, calculated on the basis of dissolved O_2 and pH, indicated the system was maintained anaerobically (below the limit for O_2 stability); while E_{H7} calculated from platinum electrode measurements at the 360-day sampling indicated that the test system was maintained under sulfate-reducing or methanogenic conditions.

Strongly Anaerobic System. *Recovery and Distribution of Applied ^{14}C.* Distribution of applied ^{14}C for any time of sampling was no different for [^{14}C]aniline- vs [^{14}C]-pyrimidine-labeled flumetsulam under strongly anaerobic conditions; thus, results were averaged by time of sampling. Radiocarbon was associated predominantly with the water phase (Table III); average total ^{14}C activity in the water phase was 56% of applied ^{14}C across all times of sampling. Water-phase ^{14}C activity was associated with parent flumetsulam, which declined from 38 to 18% of applied ^{14}C through day 360, and with reduced flumetsulam hydrate, which increased from 5 to 39% of applied ^{14}C through day 360. Other individual peaks detected by chromatography of water-phase samples accounted for ≤4% of applied ^{14}C on average.

Average total ^{14}C activity in acidified acetone extracts of sediments was 29% of applied ^{14}C across all times of sampling (Table III). Extractable [^{14}C]flumetsulam decreased from 31 to 9% of applied ^{14}C, and reduced flumetsulam hydrate increased from 1 to 13% of applied ^{14}C over the 360-day time course of sampling. Other individual peaks detected by chromatography of extracts accounted for ≤2% of applied ^{14}C on average. The

Table III. Distribution of Applied Radiocarbon in the Strongly Anaerobic Aquatic System[a]

	water				acidified acetone extract[b]						
time of sacrifice, days	flumetsulam	reduced flumetsulam hydrate	other[c]	total	flumetsulam	reduced flumetsulam hydrate	other[c]	total	caustic trap	bound residue	total
0	38 ± 10	5 ± 2	4 ± 2	47 ± 3	31 ± 2	1 ± 1	1 ± 1	33 ± 1	0	7 ± 1	87 ± 15
30	40 ± 2	13 ± 1	3 ± 1	56 ± 2	26 ± 5	3 ± 2	4 ± 1	33 ± 5	0	10 ± 2	99 ± 8
60	36 ± 2	19 ± 1	2 ± 1	57 ± 1	21 ± 3	5 ± 2	4 ± 2	31 ± 2	1 ± 1	11 ± 3	100 ± 4
90	32 ± 3	24 ± 2	3 ± 1	59 ± 3	15 ± 1	9 ± 1	1 ± 1	25 ± 3	1 ± 1	10 ± 2	95 ± 6
180	23 ± 4	30 ± 9	3 ± 1	56 ± 11	17 ± 1	9 ± 3	7 ± 1	33 ± 3	3 ± 5	15 ± 6	107 ± 4
360	18 ± 2	39 ± 2	3 ± 1	60 ± 4	9 ± 1	13 ± 3	2 ± 1	24 ± 4	1 ± 1	11 ± 1	94 ± 3

[a] Reported as mean percent of applied radiocarbon ± standard deviation (two replicates each of pyrimidine- and aniline-^{14}C) for a nominal application of 0.20 μg/g of oven-dry sediment. [b] Includes water entrained following centrifugation and decantation. [c] Consists of numerous peaks, none representing >4% on average of applied radiocarbon.

majority of acidified acetone-extractable activity is associated with sediment-entrained pore water (sediments entrained on average 33% of applied water following centrifugation and decantation).

Average of water-phase and extractable total [^{14}C]-flumetsulam declined from 69 to 27% of applied ^{14}C and reduced flumetsulam hydrate increased from 6 to 52% of applied ^{14}C through 360 days after spiking. Thus, in this strongly anaerobic system, flumetsulam appeared to convert predominantly to a single degradation product. Total ^{14}C associated with any other peaks eluting by HPLC of water and extracts averaged ≤4% of applied ^{14}C for any sampling times, and occurrences were unpatterned in time.

Volatilization was minimal in the strongly anaerobic system. The average activity in caustic traps was ≤3% of applied ^{14}C for any time of sampling (Table III). Average bound residue increased from 7 to 15% of applied ^{14}C over 180 days and then declined to 11% of applied ^{14}C by 360 days. Recovery of applied mass averaged 96 ± 9% across all treatments and times of sampling.

Kinetics of Parent and Degradation Product Fate. As discussed previously, flumetsulam appeared to convert predominantly in the strongly anaerobic system to a single degradation product (reduced flumetsulam hydrate, Table III). An estimate of flumetsulam degradation from the strongly anaerobic system was made through modeling data for average recovery of applied [^{14}C]flumetsulam and reduced flumetsulam hydrate with time as linked, concurrent apparent first-order reactions

$$dC_F/dt = -k_1 C_F$$

$$dC_R/dt = k_1 C_F - k_2 C_R$$

where C_F and C_R represent concentration of the parent flumetsulam and reduced flumetsulam hydrate, respectively, normalized for recovery. Data were subjected to simulation modeling using SimuSolv software (Dow Chemical Co., Midland, MI; Steiner et al., 1986). This model accounted for 94% of the variation in data for flumetsulam and 98% of the variation in data for reduced flumetsulam hydrate (Figure 1). Calculated rate constants were 3.78×10^{-3} (±5% RSD) and 7.84×10^{-4} day^{-1} (±43% RSD) for k_1 and k_2, respectively. Half-life was calculated as $t_{1/2} = 0.693/k_1 = 183$ days for flumetsulam. The half-life for reduced flumetsulam hydrate was not projected since this product accumulated and demonstrated no decline within the 360-day term of the study.

Reductive Chemistry of Flumetsulam. Reduction of flumetsulam using hydrogen over palladium on carbon in ethyl acetate or lithium aluminum hydride in tetrahydrofuran provided a chromatographically separable mixture of enamine 1 and the totally reduced material 2 (Figure 2). Treatment of an acetonitrile solution of the enamine 1 with 1 M hydrochloric acid caused conversion to two more polar materials in an approximate 80/20 ratio as determined via reversed-phase HPLC. Reduction of this mixture with sodium cyanoborohydride provided a good yield of the fully saturated material 2 (Figure 3).

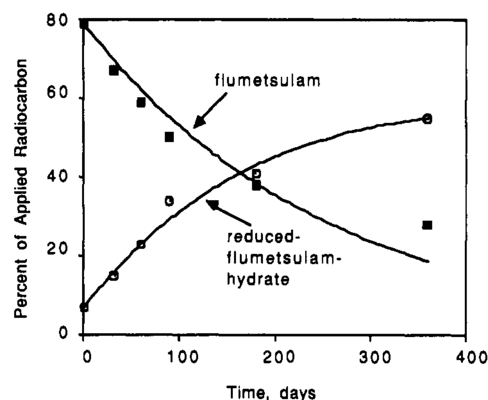


Figure 1. Modeled fit for flumetsulam degradation in the strongly anaerobic aquatic system.

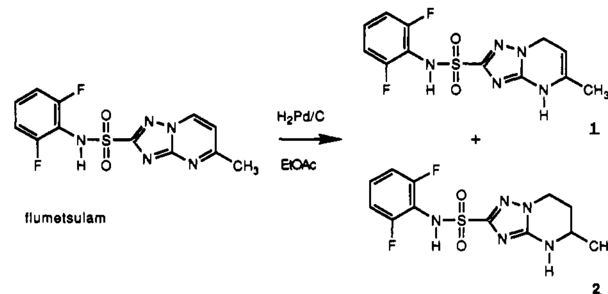


Figure 2. Reduction of flumetsulam to form flumetsulam enamine 1 and totally reduced flumetsulam 2.

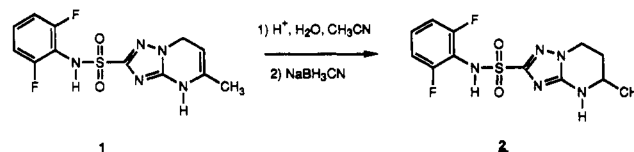


Figure 3. Reduction of flumetsulam enamine 1 to form totally reduced flumetsulam 2.

On the basis of these results the following sequence of events may be operative: Treatment of 1 with aqueous acid caused hydration through the intermediates depicted in Figure 4. The mechanism of enamine hydrolysis and

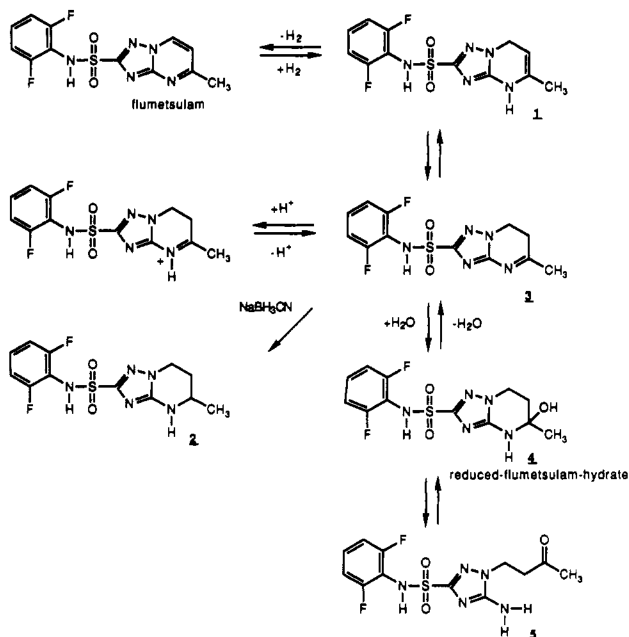


Figure 4. Pathway of flumetsulam reduction and enamine hydrolysis.

the existence of these types of intermediates have been well documented [see, for example, Chapter 3 in Cook (1969)].

Because of this series of equilibria, it was not surprising that more than one new polar material was observed when 1 was exposed to aqueous acid. On the basis of mass spectrometry and infrared data, the hydrate 4 is favored as the major polar form observed by HPLC, although imine 3 and ketoamine 5 cannot be ruled out. Treatment of this equilibrium mixture with sodium cyanoborohydride irreversibly reduced the imine form to the fully saturated material 2 [*N*-(2,6-difluorophenyl)-4,5,6,7-tetrahydro-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide]. All attempts at isolation of 4 from solution caused dehydration, resulting in the formation of a significant quantity of 1. The enamine 1 can also be oxidized back to flumetsulam under standard laboratory conditions; thus, the anaerobic aquatic degradation product is stable only under reducing conditions.

The reductive chemistry of flumetsulam demonstrates the following relative to the strongly anaerobic test system: (1) The likely product of flumetsulam degradation is reduced flumetsulam hydrate (4, Figure 4) [*N*-(2,6-difluorophenyl)-4,5,6,7-tetrahydro-5-hydroxy-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide] on the basis of enamine chemistry and subsequent characterization. (2) The enamine 1, hydrate 4, imine 3, and ketoamine 5 comprise a complex equilibrium mixture; the particular form favored will be highly dependent on conditions under which their formation, isolation, and purification are carried out. The isolation and cleanup methodolgy employed is, thus, expected to change the nature of the anaerobic degradation product. (3) Parent flumetsulam readily re-forms from the enamine under oxidizing conditions; therefore, the generation of reduced flumetsulam hydrate is likely a side reaction. There is little likelihood of reduced flumetsulam hydrate occurring in nature in other than a static anaerobic environment such as that modeled in the strongly anaerobic aquatic test system.

Degradation Product Isolation and Characterization. The 90- and 180-day water-phase samples from the system were used for isolation and characterization of the anaerobic degradation product. Additional degradation product was generated through anaerobic incubation of flumetsulam at elevated concentrations (1–5 μg/g of oven-dry sediment) in sediment–water systems at 35 °C. Concentration was carried out under vacuum at 35–50 °C. The degradation product peak was isolated and partially purified through successive isocratic LC, fractional LSC of subsamples, and combination and reconcentration of the peak cut fractionally by LC. The isocratic conditions used were as described under Chromatography with slight variation in the mobile-phase composition to better effect cleanup. Aliquots of these isolated and concentrated fractions were used for subsequent characterization as described below. Isocratic reversed phase cochromatography of reduced flumetsulam hydrate (day 90 sample) with the reaction mixture obtained from cold flumetsulam reduction with lithium aluminum hydride in tetrahydrofuran was monitored at 240 nm and exhibited four peaks representative of flumetsulam reduction. Peaks represented the hydrate 4, imine 3, and/or ketoamine 5, alone or in combination; the isolate degradation product cochromatographed as a mixture with these peaks. Enamine chemistry favors the hydrate 4 (reduced flumetsulam hydrate) as the dominant product (see Reductive Chemistry of Flumetsulam). Saturated flumetsulam 2 and unreacted flumetsulam exhibited the same retention time, while a late-eluting peak was the enamine 1.

A portion of isolated degradation product (day 180 sample) was reacted with sodium cyanoborohydride and cochromatographed with cold saturated flumetsulam 2 standard. Radiochromatography indicated unreacted degradation product and a single product with an elution time corresponding to that of the saturated flumetsulam.

A portion of the late-eluting peak from isocratic LC of the 90-day aqueous-phase sample was taken to dryness under N_2 and dissolved in acetone. Chemical ionization (CI) negative ion mass spectrometry confirmed it as flumetsulam. Residue from an aqueous fraction containing the earlier eluting peak (anareobic degradation product) was prepared in a similar manner. DP/MS CI negative ion mass spectra of this sample contained fragments (m/z 133 and 197) characteristic of flumetsulam, indicating reversion of the degradation product to parent flumetsulam. Fragments of m/z 135 and 199 were present as well, indicating the presence of either enamine or imine (1 and 3 in Figure 4, respectively). Dehydration/dehydrogenation of the hydrate 4 or ketoamine 5 by direct probe could result in these fragments.

A portion of the degradation product isolated by isocratic LC of aqueous-phase samples from the 35 °C incubation was analyzed by thermospray (TS) liquid chromatography mass spectrometry (LC/MS). The LC/MS TS mass spectra of this sample contained protonated molecular ions at m/z 326, 328, and 346. This is consistent with the presence of flumetsulam, the enamine 1, or the imine 3 (Figure 4) and reduced flumetsulam hydrate 4 with molecular mass 325, 327, and 345, respectively. Electron impact particle beam analysis indicated the presence of fragments consistent with the presence of either the enamine or the imine.

The isolation and characterization of the anaerobic degradation product by the above reactions as well as the reductive chemistry of flumetsulam led to the tentative identification of *N*-(2,6-difluorophenyl)-4,5,6,7-tetrahydro-5-hydroxy-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide (reduced flumetsulam hydrate) as the product of anaerobic aquatic metabolism of flumetsulam.

Table IV. Distribution of Applied Radiocarbon in the Weakly Anaerobic Aquatic Systems[a]

	water				acidified acetone extract[b]						
time of sacrifice, days	flumetsulam	reduced flumetsulam hydrate	other[c]	total	flumetsulam	reduced flumetsulam hydrate	other[c]	total	caustic trap	bound residue	total
0	76 ± 5	0	0	76 ± 5	9 ± 1	0	1 ± 1	10 ± 2	0	9 ± 2	95 ± 4
30	45 ± 4	0	0	45 ± 4	13 ± 4	2 ± 2	2 ± 2	17 ± 6	2 ± 2	28 ± 13	92 ± 7
60	41 ± 5	6 ± 3	7 ± 3	54 ± 2	11 ± 3	2 ± 1	3 ± 3	16 ± 6	6 ± 1	27 ± 10	103 ± 4
90	31 ± 4	3 ± 3	9 ± 5	43 ± 4	10 ± 2	1 ± 0	5 ± 1	16 ± 2	10 ± 2	29 ± 4	98 ± 2
180	22 ± 5	2 ± 1	10 ± 6	34 ± 8	12 ± 2	1 ± 1	5 ± 1	18 ± 3	19 ± 5	31 ± 6	102 ± 8
360	4 ± 2	1 ± 1	4 ± 2	9 ± 4	10 ± 2	0	5 ± 3	15 ± 4	27 ± 5	44 ± 12	95 ± 7

[a] Reported as mean percent of applied radiocarbon ± standard deviation (two replicates each of pyrimidine- and aniline-^{14}C) for a nominal application of 0.20 μg/g of oven-dry sediment. [b] Includes water entrained following centrifugation and decantation. [c] Consists of numerous peaks, none representing >7% on average of applied radiocarbon.

Weakly Anaerobic System. The metabolism of flumetsulam in the weakly anaerobic system was markedly different than that observed in the strongly anaerobic system. Distinct shifts in radiocarbon distribution in water phase and acidified acetone extracts were noted with time as the initially anaerobic system became progressively aerobic. [Dissolved O_2 increased from 0.13 to 6.05 ppm, and sediment colors at the sediment/water interface became progressively redder as a consequence of iron oxidation (Table II).]

Recovery and Distribution of Applied ^{14}C. Distribution of applied ^{14}C occasionally appeared different for [^{14}C]-aniline vs ^{14}C]pyrimidine-labeled flumetsulam under weakly anaerobic conditions (Table IV); but because no consistent differences were noted with time, results were averaged across ring labels for each time of sampling.

Radiocarbon was initially associated predominantly with the water phase (76% of applied at 0 days) but declined to 9% of applied activity by 360 days (Table IV). Water-phase ^{14}C activity was associated with parent flumetsulam, which declined from 76 to 4% of applied ^{14}C through day 360. Reduced flumetsulam hydrate increased to 6% of applied ^{14}C by day 60 but declined to 1% of applied as the system became progressively more aerobic with later times of incubation. Other individual peaks detected by chromatography of water-phase samples accounted for ≤7% of applied ^{14}C on average.

The average activity in acidified acetone extracts of sediment was 15% of applied ^{14}C across all times of sampling (Table IV). Extractable [^{14}C]flumetsulam ranged from 9 to 13% of applied ^{14}C, and reduced flumetsulam hydrate never exceeded 2% of applied ^{14}C over the 360-day time course of sampling. Other individual peaks detected by chromatography of extracts accounted for ≤3% of applied ^{14}C on average.

When the results of water-phase and extractable ^{14}C distribution are combined, average total [^{14}C]flumetsulam declined from 85 to 14% of applied ^{14}C through 360 days. Reduced flumetsulam hydrate increased from 0 to 8% of applied ^{14}C by 60 days but then declined to 1% by 360 days as the system became aerobic.

Volatilization was significant in the weakly anaerobic system; average activity in caustic traps was 27% of applied ^{14}C by 360 days (Table IV). Average bound residue increased from 9 to 44% of applied ^{14}C over 360 days (Table IV). Recovery of applied mass averaged 98 ± 4% across all treatments and times of sampling.

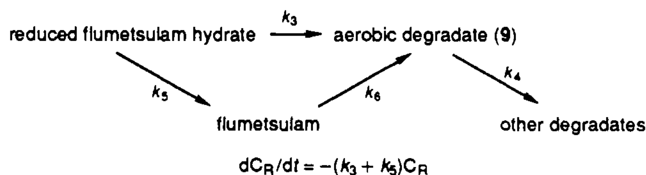
Ion Pairing LC of Early Eluting Peaks. C18 reversed-phase ion pair LC with 0.005 M tetrabutylammonium phosphate of a [^{14}C]pyrimidine-labeled sample from the weakly anaerobic system at 360 days showed the anaerobic metabolite to be completely metabolized, producing at least six fragments (one a possible sulfonamide). The early-eluting fragments are most likely organic acids, since the retention times of organic acids such as acetic, pyruvic, propionic, and oxalic acids are similar to those of the observed peaks. Ion pairing LC of a [^{14}C]aniline-labeled sample, however, indicated a single leading peak to be present. Thus, degradation of flumetsulam in the weakly anaerobic system appears to center on the pyrimidine ring.

The change in redox regime of the weakly anaerobic system with time of incubation is analogous to the changes occurring in an anaerobic environment in contact with the atmosphere, which becomes reaerated with time [that is, with seasonal turnover of lakes and ponds; see, for example, Chapter 9 in Wetzel (1983)]. In this system reduced flumetsulam hydrate formed during an early anaerobic stage but disappeared with subsequent aeration of the system. The oxidative fate of reduced flumetsulam hydrate was more pronounced in an aerobic soil environment (see Aerobic Soil Fate of Reduced Flumetsulam Hydrate). The behavior of reduced flumetsulam hydrate under oxidizing conditions is consistent with the enamine chemistry described earlier (see Reductive Chemistry of Flumetsulam).

Aerobic Soil Fate of Reduced Flumetsulam Hydrate. Aerobic metabolism of reduced flumetsulam hydrate on Commerce silt loam was monitored to better ascertain the nature and magnitude of degradation product occurrence in terrestrial environments. Combined water-phase sample which had been concentrated and then fractionated and purified by preparative LC was used as the source of reduced flumetsulam hydrate.

Reduced flumetsulam hydrate disappeared rapidly in an aerobic soil environment to yield two principle products by reversed-phase HPLC (Table V). An early-eluting polar peak (RT = 9 min) had the same retention time as the primary metabolite of aerobic soil degradation of flumetsulam, while a later eluting peak (RT = 28 min) had the same retention time as flumetsulam. The later eluting peak was confirmed as flumetsulam by GC/MS. Regeneration of flumetsulam from reduced flumetsulam hydrate in aerobic soil is consistent with the chemistry of enamine products from the reduction of flumetsulam (including reduced flumetsulam hydrate) which revert to flumetsulam in an oxidizing environment (see Reductive Chemistry of Flumetsulam).

The loss of reduced flumetsulam hydrate from aerobic soil was modeled as a series of first-order reactions:



The model accounted for 96% of the variation in the data

Table V. Distribution of Applied Radiocarbon in Aerobic Commerce Soil[a]

	acidified acetone extract						
time of sacrifice, days	flumetsulam	reduced flumetsulam hydrate	aerobic degradate	other[b]	caustic trap	bound residue	total
0	2	71	0	16	0	4	93
1	6	43	13	9	<3	15	89
2	9	30	14	6	3	15	77
4	22	12	17	6	6	13	76
9	22	8	16	4	12	19	81

[a] Reported as percent of applied radiocarbon for a nominal application of 0.1 μg/g. [b] Consists of numerous peaks.

and yielded rate constants for reduced flumetsulam hydrate loss of 0.214 (±5% RSD) and 0.150 (±12% RSD) for k_1 and k_3, respectively. The half-life of reduced flumetsulam hydrate in aerobic soil was calculated as

$$t_{1/2} = 0.693/(k_3 + k_5) = 2 \text{ days}$$

The extremely short half-life of reduced flumetsulam hydrate in aerobic soil relative to the half-life of flumetsulam (40 days) means that reduced flumetsulam hydrate occurrence in terrestrial field environments is extremely remote. This is confirmed by aerobic soil degradation studies of flumetsulam, in which reduced flumetsulam hydrate has not been identified.

CONCLUSIONS

In the strongly anaerobic system, both [^{14}C]aniline- and [^{14}C]pyrimidine-labeled flumetsulam exhibited similar patterns of degradation. Flumetsulam degraded to form a single dominant degradation product which preferentially partitioned to the water phase and accounted for 52 ± 1% of applied ^{14}C by 360 days. The half-life ($t_{1/2}$) of flumetsulam in the strongly anaerobic system was 183 days. The anaerobic degradation product was identified as reduced flumetsulam hydrate [*N*-(2,6-difluorophenyl)-4,5,6,7-tetrahydro-5-hydroxy-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide]. This assignment was made on the basis of (i) mass spectra for the degradation product fraction isolated from water-phase samples, (ii) cochromatography with product mixtures obtained from the reduction of flumetsulam with lithium aluminum hydride in tetrahydrofuran, (iii) reaction with sodium cyanoborohydride of the anaerobic degradation product and mixtures of flumetsulam reduction products to form a common end product [*N*-(2,6-difluorophenyl)-4,5,6,7-tetrahydro-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide], and (vi) knowledge of the enamine chemistry involved in flumetsulam reduction. Materials balance was 96 ± 9% of applied radiocarbon; caustic traps contained ≤3% of applied ^{14}C for any time of sampling, and bound residues averaged 11% of applied for the duration of the study.

In the weakly anaerobic system, the anaerobic degradation product never accumulated at >8% of applied ^{14}C. There appeared to be some difference in the pattern of degradation of the [^{14}C]aniline- vs [^{14}C]pyrimidine-labeled flumetsulam. Materials balance was 97 ± 6% of applied radiocarbon; caustic traps and bound residue accounted for 27 and 44% of applied ^{14}C, respectively, by day 360.

When applied to soil and incubated aerobically at 25 °C in the dark, the anaerobic degradation product exhibited a $t_{1/2}$ of 2 days in comparison to a $t_{1/2}$ of ≈40 days for flumetsulam incubated under similar conditions.

Generation of the anaerobic aquatic degradation product of flumetsulam is viewed as a principally abiotic process occurring only in strongly anaerobic, stable (i.e., static) environments isolated from the atmosphere. Thus, the likelihood of flumetsulam hydrate occurrence and accumulation in the environment is minimal when flumetsulam is used for terrestrial food crops.

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