JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Degradation of Flumioxazin in Illuminated Water−Sediment Systems

Atsushi Shibata,* Rika Kodaka, Takuo Fujisawa, and Toshiyuki Katagi

Environmental Health Science Laboratory, Sumitomo Chemical Company, Ltd., Takatsukasa 4-chome, Takarazuka, Hyogo 665-8555, Japan

Supporting Information

ABSTRACT: The aerobic aquatic metabolism of flumioxazin was studied in two water−sediment systems under illumination and in darkness to investigate its degradation profiles. ^{14}C-Flumioxazin separately labeled at the 1- and 2-positions of the tetrahydrophthalimide moiety or uniformly labeled at the phenyl ring was applied to a overlying water at a rate equivalent to 600 g ai/ha by assuming uniform distribution in the water layer to a depth of 100 cm. Flumioxazin was rapidly degraded at 20 °C in the overlying waters irrespective of irradiation with half-lives of 0.1−0.4 day. Both various modes of liquid chromatography−mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy analyses showed four major degradates under irradiation. Two of them were formed via successive hydrolysis of the cyclic imide ring, and the others were 2-arizidinone derivatives via photoinduced rearrangement. The presence of sediment under illumination greatly reduced the formation of these degradates and accelerated their degradation. The partitions of flumioxazin and its degradates to the bottom sediment not only reduced their fractions in the water layer subjected to hydrolysis and photolysis but also enhanced their microbial degradation in the sediment. The illuminated water−sediment systems were considered to more adequately represent the behavior of flumioxazin and its degradates in the environment than the corresponding studies of aqueous photolysis and water−sediment in darkness.

KEYWORDS: flumioxazin, water−sediment system, photodegradation, hydrolysis, natural water

INTRODUCTION

Flumioxazin (**1**), 2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1*H*-isoindole-1,3-(2*H*)-dione, is a herbicide for pre-emergence control of a broad spectrum of weeds in several crops such as soybean, peanut, and fruit trees.[1,2] The behavior of **1** has been intensively studied in water,[3,4] soil,[5−7] and rat.[8,9] The hydrolysis rate of **1** increased with an increase in pH as its half-lives were 4.1 days, 16.1 h, and 17.5 min at pH 5.0, 7.0, and 9.0, respectively. The primary product was an anilic acid derivative (**2**), which was subsequently degraded to the corresponding dicarboxylic acid (**3**) and aniline (**4**) via opening of the imide ring followed by cleavage of the amide linkage.[3] Kwon et al. have reported the photodegradation of **1** with the respective half-lives of 41.5 and 4.9 h at pH 5 and 7, and one unique photoproduct was suggested only by mass spectrometry to be formed via hydrolytic cleavage of the amide linkage in the benzoxiazinone ring.[4] The moderate soil degradation of **1** was reported with the half-life of 12.9−17.9 days in the laboratory experiments at 15 and 25 °C and 10.6−32.1 days in the field site.[5,6]

Incidentally, pesticide applied to the agricultural fields may reach various water bodies via spray-drift and runoff events and successively undergoes degradation processes such as hydrolysis, photolysis, and microbial transformations together with partitioning to suspended matters and bottom sediment. To know the behavior of pesticides in the aqueous environment, a water−sediment study in darkness is officially required in the European Union (EU) registration of pesticide[10] in accordance with Organisation for Economic Co-operation and Development (OECD) Guideline 308,[11] and the one under illumination is conditionally required for a photolabile pesticide with direct photolysis half-life of a few days and formation of a photoproduct above 10%. However, a limited number of studies have been conducted in the latter case because a fixed test design is not yet available.[12,13] Other than the data for official requirement, many researches have conducted water−sediment studies in darkness and/or illuminated condition for various chemical classes of pesticides.[14−25] Especially in clear and shallow water bodies, the degradation pathway under illumination becomes very important for photolabile compounds, and if excessive amounts of photounique products are formed, their fate in the aqueous environment needs to be clarified in relation to the ecotoxicological assessment.

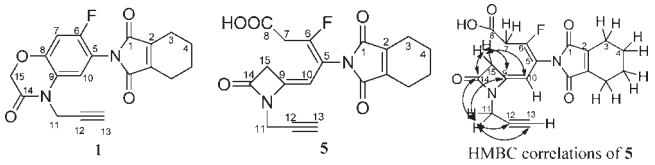
The objective of this study is to clarify the effect of illumination and bottom sediment on the dissipation of **1** and the possible accumulation of its degradates using a water−sediment system under illumination in comparison with those in the aqueous photolysis in natural water and water−sediment in darkness. We also identified the definitive structures of major photoproducts of **1** using liquid chromatography−electrospray ionization−mass spectrometry (LC-ESI-MS) and NMR spectroscopy in conjunction with direct chromatographic comparison with the reference standards.

MATERIALS AND METHODS

Chemicals. The nonradiolabeled authentic standards of flumioxazin (**1**) and its degradates, *N*-[7-fluoro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-3,4,5,6-tetrahydrophthalamic acid (**2**), 3,4,5,6-tetrahydrophthalic acid (**3**), and 6-amino-7-fluoro-4-(2-propynyl)-2*H*-1,4-benzoxazin-3(4*H*)-one (**4**), were prepared in our laboratory according

Received: June 26, 2011
Revised: September 12, 2011
Accepted: September 15, 2011
Published: September 15, 2011

Table 1. NMR Data for Degradate 5 in CD_3CN[a]



C	^1H (multiplicity, J)	^{13}C	H−H COSY correlated H	HMQC correlated C	HMBC correlated C
1		196.1			
2		143.5			
3	2.34 (m)	20.8	H-4	20.8	C-2, 4
4	1.77 (br s)	21.9	H-3	21.9	C-2, 3
5		113.3			
6		152.6 (d, 1019)			
7	3.59 (d, 23.2)	35.1	H-15	35.1	C-5, 6, 8
8		170.3			
9		138.5			
10	5.72 (m)	92.5	H-15	92.5	C-5, 6, 9
11	4.14 (d, 2.4)	30.1	H-13, 15	30.1	C-9, 12, 13, 14
12		77.2			
13	2.63 (t, 2.4)	73.9	H-11		C-11
14		166.1			
15	3.20 (br s)	43.8	H-7, 10, 11	43.8	C- 9, 10, 14

[a] ^1H, H−H COSY, HMQC, and HMBC NMR spectra are shown in the Supporting information (Figures S1−S4).

to the reported method.[3] Degradate **5**, *N*-(2-propynyl)-4-[4-carboxy-3-fluoro-2-(3,4,5,6-tetrahydrophthalimido)-2-butenylidene]azetidine-2-one, was photochemically prepared in our laboratory by using the following method. A 50% aqueous acetontrile solution of **1** (260 mg) using 10 mM acetic acid buffer at pH 5 was continuously irradiated under stirring for 7 days with a 500 W xenon arc lamp (UIV-5150XE, Usio, Japan). The light intensity was maintained almost constant throughout the study with its irradiance at 300−400 nm of 2.144 MJ/m^2/day on average. After removal of acetonitrile under reduced pressure, the aqueous layer was extracted four times with 200 mL of ethyl acetate. The combined organic phase was evaporated to dryness and purified by silica gel (Merck, Darmstadt, Germany) column chromatography with chloroform/methanol (9:1, 6:1, and 4:1, v/v) to obtain the fraction of **5** (total yield, 14.3%). The chemical structure of **5** was confirmed by LC-ESI-MS and ^1H and ^{13}C NMR spectroscopy with various pulse sequences (Table 1 and Supporting Information, Figures S1−S4). Degradate **6**, *N*-(2-propynyl)-4-[4-carboxy-3-fluoro-2-(2-carboxy-1-cyclohexenecarbonylamino)-2-butenylidene]azetidine-2-one, was conveniently prepared by hydrolysis of **5** in borate buffer at pH 9 for 24 h. Its chemical structure was confirmed with LC-ESI-MS. The chemical purity of each nonradiolabeled standard was determined to be >96% by high-performance liquid chromatography (HPLC). The chemical structures of **1** and its potential degradates **2**−**6** are shown in Figure 1.

^{14}C-**1** separately labeled at the 1- and 2-positions of the tetrahydrophthalimide moiety ([THP-^{14}C]-**1**) or uniformly labeled at the phenyl ring ([Phe-^{14}C]-**1**), as shown in Figure 1, was prepared in our laboratory according to the reported method.[3] Each labeled compound was purified by TLC development in ethyl acetate/hexane (3:1, v/v) prior to use. Radiochemical purity was >97% as determined by HPLC. The specific activityies of [THP-^{14}C]-**1** and [Phe-^{14}C]-**1** were 13.2 and 12.6 MBq mg^{-1}, respectively. Other reagents were of the purest grade commercially available.

Spectroscopy. ^1H and ^{13}C and, for two-dimensional experiments, H−H correlated spectroscopy (COSY), ^1H-detected multiple-quantum coherence spectoscopy (HMQC), and ^1H-detected multiple-bond heteronuclear multiple-quantum coherence spectrum (HMBC) NMR spectra were measured with a Varian Unity 400 FT-NMR spectrometer operating at 400.45 MHz with a 5 mm PFG ATB probe. LC-ESI-MS spectra in positive and negative ion modes were obtained by a Waters Micromass ZQ spectrometer equipped with Waters Separation Module 2695 and Photodiode Array Detector 2996 as liquid chromatograph. Samples dissolved in acetonitrile were manually injected into an ionization source through a Sumipax ODS A-212 column (150 mm × 6 mm

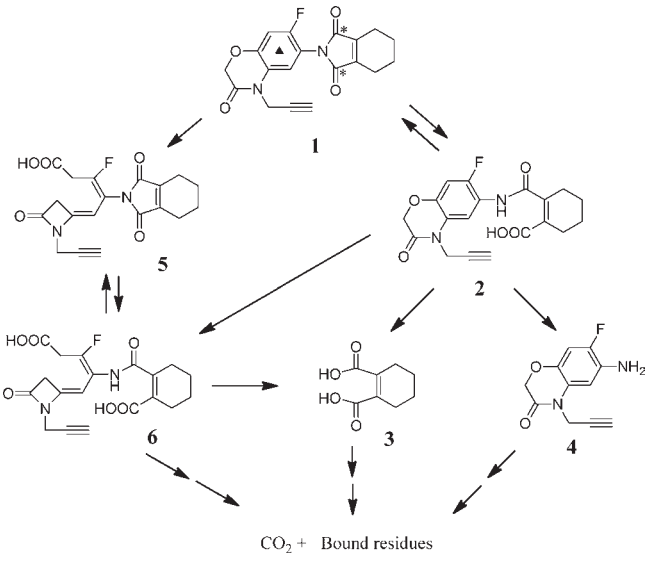


Figure 1. Proposed degradation pathway of **1** in water−sediment systems under illumination. ∗ and ▲ indicate the ^{14}C-labeled positions of **1**.

Table 2. Characteristics of Bottom Sediments and Overlying Water Used in This Study

location	Kasai pond, Hyogo, Japan	Calwich Abbey Lake, Derbyshire, U.K.
sediment		
USDA textural class	silty clay loam	silt loam
sand %	14	27
silt %	56	68
clay %	30	5
organic carbon %	1.9	4.9
pH (H_2O)	6.8	7.9
overlying natural water		
suspended solids (mg L^{-1})	42.9	16.2
pH	6.9	7.9

i.d., 5 μm, SCAS Co., Ltd., Japan) at a flow rate of 1.0 mL min^{-1} using the following gradient system with acetonitrile (A) and 0.05% formic acid in water (B): 0 min, % A—% B, 20—80; 40 min, % A—% B, 60—40; 50 min, % A—% B, 95—5; 51 min, % A—% B, 20—80.

HPLC Analysis. HPLC was carried out by using a Shimadzu LC-20AT pump linked in series with a SPD-20A UV—vis detector and a Perkin-Elmer Radiomatic 150TR radiodetector equipped with a 500 μL liquid cell. An Ultima-Flo AP (Packard Instrument Co.) was utilized as a scintillator. A Sumipax ODS A-212 column was employed for an analytical purpose at a flow rate of 1.0 mL min^{-1}. The following gradient systems were used for the analysis: (method 1) acetonitrile (A) and 5 mM tetrabutylammonium phosphate (PIC-A reagent, Waters) (B), 0 min, % A—% B, 5—95; 20 min, % A—% B, 50—0; 45 min, % A—% B, 90—10; 53 min, % A—% B, 5—95; (method 2) acetonitrile (solvent A) and 0.01% trifluoroacetic acid (solvent B), 0 min, % A—% B, 20—80; 40 min, % A—% B, 60—40; 50 min, % A—% B, 95—5; 51 min, % A—% B, 20—80. Retention times of **1** and its degradates **2**—**6** in methods 1 and 2 were as follows: 33.3 and 38.6 min (**1**); 25.4 and 23.4 min (**2**); 20.3 and 7.7 min (**3**); 24.9 and 15.7 min (**4**); 26.5 and 27.8 min (**5**); 24.1 and 12.1 min (**6**), respectively.

Radioassay. Radioactivity in aqueous solution and sediment extracts was determined by liquid scintillation counting (LSC) with a PerkinElmer model 3110TR liquid scintillation spectrometer equipped with an automatic external standard in low-potassium glass vials, using 10 mL of Emulsifier Scintillator Plus (Perkin-Elmer Inc.). Unextractable sediment residues were air-dried at room temperature and then powdered by a mill mixer MM301 (Retsh, Germany), and its portion was combusted using a PerkinElmer model 307 Sample Oxidizer prior to LSC measurements, as described previously.[19,22]

Metabolism Study. Two types of bottom sediment and overlying water were individually collected from Kasai pond (Hyogo, Japan) and Calwich Abbey Lake (Derbyshire, U.K.) and stored in a refrigerator at 4 °C until use. Their physical and chemical properties are summarized in Table 2. The water—sediment samples of Kasai (pH 6.9) and Calwich Abbey (pH 7.9) were chosen to clarify differences in the behavior of **1** in relation to the pH of the overlying water. Each sample of water and sediment was passed through 0.2 and 2 mm sieves prior to use, respectively.

A sediment sample equivalent to approximately 12 g on a dry weight basis was taken into a two-necked cylindrical glass vessel (4.4 cm i.d.) to a depth of 2 cm, and then the overlying water was added to each vessel to a depth of 6 cm. After a preincubation period of at least 2 weeks, approximately 20 μL of ^{14}C-**1** acetonitrile solution was applied to the water surface in each vessel at a rate of 5.47 μg/vessel, using a microsyringe, which is equivalent to the field application rate of 600 g ai/ha by assuming its uniform distribution in the water layer to a depth of 100 cm.[11] To investigate the effect of illumination, the glass vessel

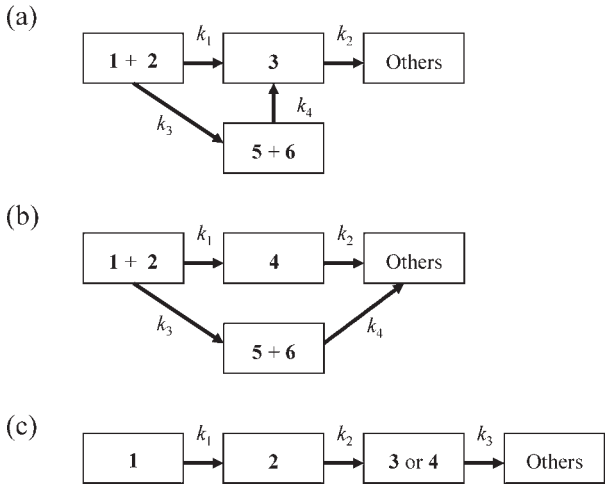


Figure 2. Compartment model used for kinetic analysis of degradation of [THP-^{14}C]-**1** (a) and [Phe-^{14}C]-**1** (b) under illumination and in darkness (c).

including each water—sediment system was immersed in a water bath maintained at 20 ± 2 °C and set on an orbital shaker (MK-200D, Yamato Scientific, Japan) at 20 rpm to moderately mix the water phase without disturbance of the sediment. Humidified air was continuously passed over the water surface to ethylene glycol and alkaline traps in sequence for collection of organic volatiles and carbon dioxide, respectively, in the same manner as previously reported.[19,22] Each vessel was irradiated using a 2 kW xenon arc lamp (UXL-25SC, Usio, Japan) set above the vessels, and UV light with wavelengths below 290 nm was cut off by a Pyrex glass plate. The light intensity was maintained almost constant throughout the study with its irradiance at 300—400 nm of 0.672 MJ/m^2/day on average. Illumination was continued for 8 h per day during the experiment, which was equivalent to the daily irradiance of natural sunlight in Tokyo (35° N, April—June). In the case of dark control, the system was set on an orbital shaker (SCS-20N, Sanki Seiki Co., Ltd., Japan) and maintained at 20 ± 2 °C in an incubator (SR-30VE2, Nagano Science Co., Ltd., Japan) without illumination.

The overlying water and associated sediment were separately analyzed in duplicate at days 0, 1, 3, 7, 14, and 30 for [THP-^{14}C]-**1** and at days 0, 3, 7, 14, and 30 for [Phe-^{14}C]-**1** after application. At each sampling, the pH and oxidation—reduction potential (ORP) values of the water and sediment layers were individually monitored by an F-22 pH-meter equipped with pH and ORP electrodes (Horiba, Ltd., Japan). In addition, the dissolved oxygen content (DO) in the water layer was measured with a DO-8F DO-meter (Horiba, Ltd.). The overlying water was collected by decantation and directly radioassayed by LSC, followed by HPLC cochromatography with the corresponding nonradiolabeled reference standards. The sediment was stepwise extracted three times with acetone/water (5:1, v/v) and then with acetone/0.1 M HCl (9:1, v/v) by a mechanical shaker for 10 min. The portion of each combined extract was individually radioassayed by LSC and concentrated in vacuo for HPLC analysis. The chemical identity of each radiolabeled compound was confirmed by comparing its HPLC retention time with those of the nonradiolabeled reference standards. The unextractable sediment residues were air-dried and combusted for radioassay. The selected unextractable residues were further fractionated by alkaline for characterization of radioactivity.

To examine the effect of sediment on the photolytic and hydrolytic profiles of **1**, its behavior in the corresponding overlying water was examined in the presence and absence of illumination by using [THP-^{14}C]-**1** under conditions identical to the above. The overlying water was analyzed in duplicate up to 30 days after application. At each sampling, the

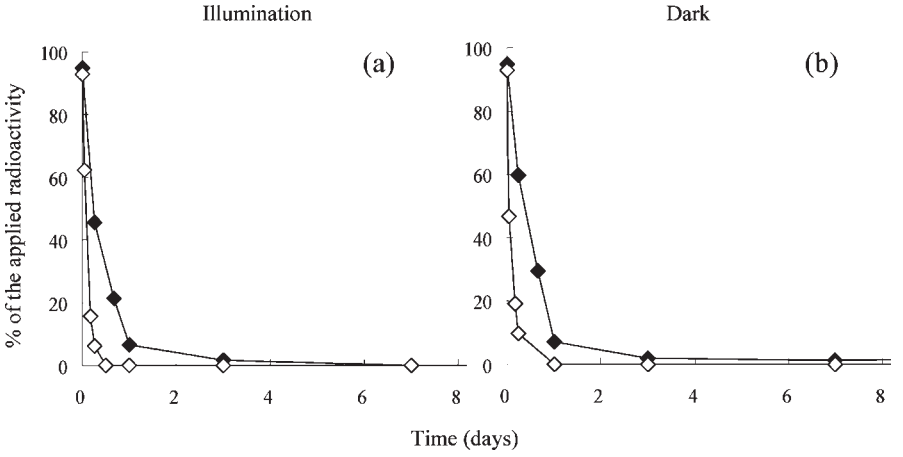


Figure 3. Degradation of [THP-^{14}C]-**1** in natural water under illumination (a) and in darkness (b): (◇) Kasai; (◀) Calwich Abbey.

Table 3. DT_{50} and DT_{90} Values of 1 in Natural Water and Water−Sediment Systems[a]

			illumination			darkness		
			DT_{50} (days)	DT_{90} (days)	χ^2	DT_{50} (days)	DT_{90} (days)	χ^2
Kasai	THP	natural water only	0.3	0.9	6.6	0.4	1.2	9.5
		overlying water in w−s system	1.5	5.1	1.2	1.1	3.8	10.3
		total w−s system	2.6	8.5	5.6	2.3	7.6	21.3
	Phe	overlying water in w−s system	0.9	3.1	3.3	2.1	6.9	4.2
		total system	1.6	5.3	11.9	3.6	11.9	21.4
Calwich Abbey	THP	natural water only	0.1	0.2	2.2	0.1	0.2	12.8
		overlying water in w−s system	0.2	0.5	0.0	0.2	0.5	3.9
		total w−s system	0.2	0.7	5.7	0.2	0.7	19.3

[a] The data were fitted to single first-order equations using FOCUS_DEGKIN version 2. DT_{50} value = 0.693/dissipation rate constant (k), DT_{90} value = 2.303/dissipation rate constant (k).

pH, ORP, and DO values were individually measured. The overlying water was directly radioassayed by LSC and HPLC cochromatography.

Calculations. The dissipation rate constant, half-life (DT_{50}), and period of 90% dissipation (DT_{90}) of **1** were estimated assuming single first-order kinetics by using the FOCUS degradation kinetics software FOCUS_DEGKIN version 2.[26] χ^2 tests were used to evaluate differences between observed and calculated values for the SFO fit. By taking into account the previous hydrolytic degradation of **1**[3] and the chemical structures of photodegradates **5** and **6**, a kinetic analysis for degradates based on the assumed compartments was conducted using the ModelMaker program (version 4, ModelKinetix, U.K.), as shown in Figure 2. The data were fitted to single first-order equations, and the coefficients of determination (r^2) were obtained from the regression analysis.

RESULTS

Photolysis and Hydrolysis in Natural Water. The degradation of [THP-^{14}C]-**1** in the two natural waters is shown in Figure 3. [THP-^{14}C]-**1** rapidly dissipated in both natural waters with half-lives of 0.1−0.3 day under illumination and 0.1−0.4 day in darkness, respectively (Table 3). The formation and decline of the major degradates are shown in Figure 4 (distribution ^{14}C, Supporting Information, Tables S1 and S2). Degradates **2** and **3** were detected as the major ones irrespective of illumination, whereas **5** and **6** were detected only under illumination. Under illumination, the maximum amounts of **2**, **3**, **5**, and **6** were 80.3, 59.1, 11.0, and 50.8% AR for the Kasai water and 82.4, 57.4, 2.1, and 48.5% AR for the Calwich Abbey water, respectively. **2** was a major degradate in the first three days and, thereafter, **5** and **6** were formed to their maxima at 7−14 days with a concomitant decrease of **2**. The photodegradates **5** and **6** gradually decreased toward the end of the study. More rapid conversion of **1** to **6** at a higher pH in the Calwich Abbey water resulted in less formation of **5**. The amount of **3** gradually increased during the studies to 46.9−59.1% AR at 30 days after treatment. Detailed kinetic analysis on the equilibrium between **1** and **2**, that is, opening and recyclization of the imide ring, has clarified that two opposite reactions proceed in relatively similar rates at pH 7.0−8.0[3] and, thus, it was considered difficult to precisely estimate the rates of forward and back reactions between **1** and **2** or between **5** and **6** for the tested waters at pH 6.8 and 7.9. Therefore, we conveniently allocated each of the two related compounds to the single compartments as (**1** + **2**) and (**5** + **6**) for the irradiated samples (Figure 2). The half-lives under illumination for the compartments (**1** + **2**), **3**, and (**5** + **6**) were individually estimated for Kasai and Calwich Abbey waters as 2.7/2.3, 38.4/18.2, and 12.4/2.5 days, respectively (Table 4). In the dark control, **2** and **3** were the major degradates with maximum amounts of 93.9−95.6 and 10.3−35.9% AR at 1−3 and 30 days, respectively, and each half-life was estimated to be

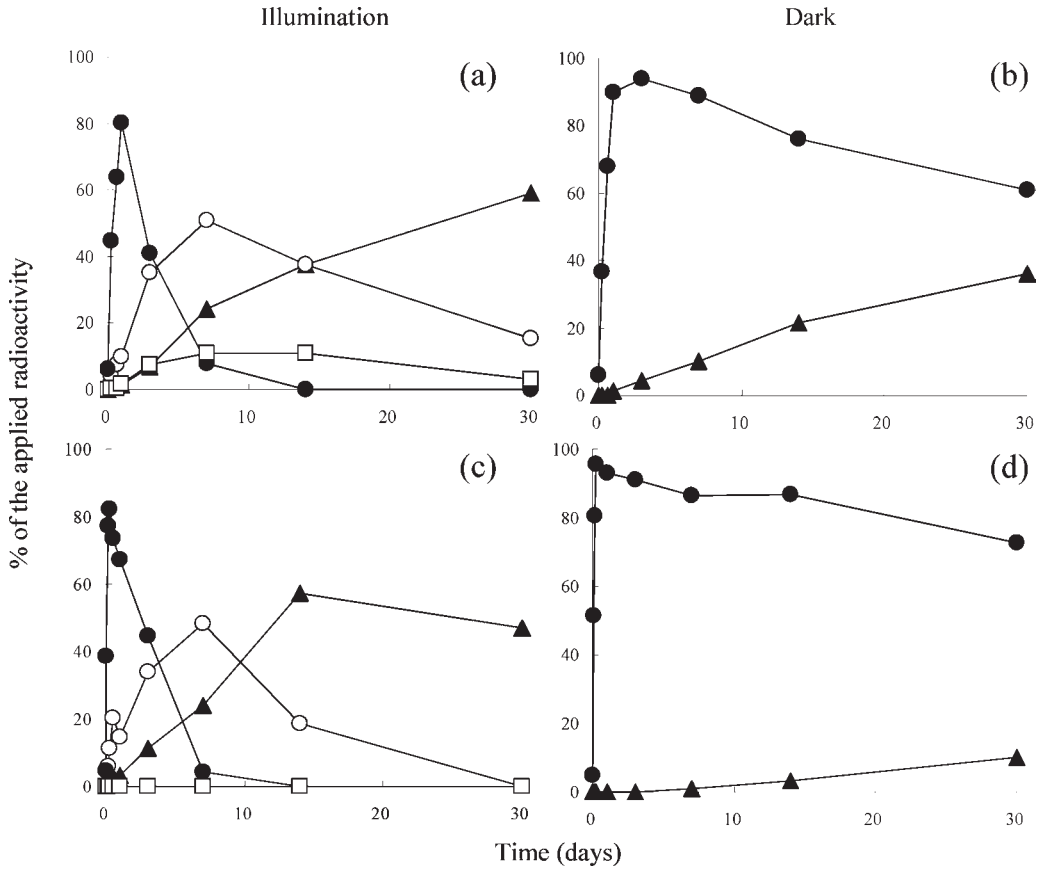


Figure 4. Formation and decline of degradates **2**−**6** in Kasai and Calwich Abbey natural waters under illumination (a and c) and in darkness (b and d): (●) **2**; (▲) **3**; (□) **5**; (○) **6**.

Table 4. DT_{50} Values (Days) of 1 and Its Degradates 2−6 in Natural Water and Water−Sediment Systems[a]

			illumination					darkness				
			1 + 2	**3**	**4**	**5 + 6**	r^2	**1**	**2**	**3**	**4**	r^2
Kasai	THP	natural water only	2.7	38.4	na	12.4	0.986	0.4	38.7	87.5	na	0.991
		overlying water in w−s system	2.2	0.6	na	10.2	0.972	1.4	3.5	0.7	na	0.883
		total w−s system	4.0	1.1	na	7.1	0.985	2.2	7.2	1.5	na	0.863
	Phe	overlying water in w−s system	2.1	na	0.1	4.9	0.994	2.5	2.4	na	0.3	0.834
		total system	3.4	na	0.6	3.2	0.992	3.7	6.4	na	0.8	0.874
Calwich Abbey	THP	natural water only	2.3	18.2	na	2.5	0.977	0.1	62.4	5.6	na	0.858
		overlying water in w−s system	2.1	2.7	na	1.8	0.921	0.3	16.0	0.5	na	0.986
		total w−s system	3.0	4.3	na	2.3	0.962	0.2	35.0	1.3	na	0.993

[a] The DT_{50} values were estimated with ModelMaker program version 4, by assuming the first-order kinetics for each reaction in each compartment model. DT_{50} value = 0.693/rate constant (k). Compartment models a and b were used for the illuminated conditions with THP and Phe labels, respectively, and compartment model c was used for the dark conditions, as shown in Figure 2. na, not applicable.

38.7−62.4 and 5.6−87.5 days. There were several unknown fractions in "others", but none exceeded 9.1% AR (Supporting Information, Tables S1 and S2), and $^{14}CO_2$ gradually increased to 9.0−14.8% AR at 30 days under illumination, whereas negligible amounts of other unknowns and CO_2 were produced in darkness.

Degradation in the Water−Sediment Systems under Illumination and in Darkness. The measured values of pH, ORP, and DO in the overlying water or sediment throughout the studies are listed in Tables 5−7. The pH values of both phases did not significantly change during the incubation period in each system. Aerobicity of the water phase and anaerobicity of the sediment phase were demonstrated to be mostly maintained from the results of ORP and DO values. The distribution of ^{14}C in the water−sediment systems under both illumination and darkness is summarized in Tables 5 and 6 for Kasai systems and in Table 7 for Calwich Abbey ones. Under illumination, [^{14}C]-**1** rapidly dissipated with half-lives of 0.9−1.5 and 0.2 days for the

Table 5. Distribution of [THP-^{14}C]-1 and Its Degradates 2−6 in the Kasai Water−Sediment System under Illumination and in Darkness[a]

	% of the applied radioactivity								
	illumination						darkness		
	0 days	1 day	3 days	7 days	14 days	30 days	1 day	3 days	30 days
volatiles (CO_2)	na	nd	0.3	4.2	8.3	24.0	nd	0.1	8.3
water phase	90.4	78.5	60.9	40.7	12.6	7.7	78.9	74.2	24.1
1	87.1	54.5	23.2	3.4	1.1	nd	53.3	11.9	0.5
2	3.3	14.3	17.2	5.3	0.0	5.6	21.3	55.9	15.5
3	nd	5.6	10.9	9.4	5.4	nd	3.9	6.4	5.7
5	nd	2.6	5.4	10.2	2.6	nd	nd	nd	nd
6	nd	0.5	3.3	9.8	2.7	1.7	nd	nd	nd
other unknowns	nd	1.1	0.7	2.6	0.8	nd	0.4	nd	2.4
sediment phase	7.9	22.1	38.7	48.0	71.1	10.9	19.2	25.6	53.9
1	7.1	15.5	18.7	9.8	5.0	5.7	14.4	12.9	6.7
2	nd	2.0	4.4	4.0	5.9	1.8	1.0	7.3	6.5
3	nd	nd	0.6	0.9	nd	1.2	nd	nd	2.5
5	nd	nd	0.7	2.0	2.0	nd	nd	nd	nd
6	nd	nd	nd	0.3	0.9	0.2	nd	nd	nd
other unknowns	0.3	nd	0.4	4.0	3.7	1.9	1.1	nd	1.2
bound residues	0.6	4.0	13.8	27.0	53.6	48.0	2.0	4.1	36.9
total	98.3	100.6	99.9	92.9	92.0	90.6	98.1	99.9	86.2
water phase									
pH	6.7	6.7	6.9	6.6	6.8	6.8	6.8	7.0	6.9
DO (mg/L)	7.6	6.0	6.2	6.7	5.6	5.9	6.6	6.1	5.4
ORP (mV)	187	160	151	147	190	205	163	119	119
sediment phase									
pH	6.7	6.5	6.8	6.5	6.7	6.7	6.8	6.9	6.7
ORP (mV)	−209	−220	−237	−222	−227	−213	−181	−178	−215

[a] Each value is described as the mean value of duplicate samples. na, not analyzed; nd, not detected. Data of 0, 7, and 14 days in darkness are not shown (full data are shown in the Supporting information, Table S5).

water phase and 1.6−2.6 and 0.2 days in the total system of Kasai and Calwich Abbey, respectively, with corresponding total recoveries of 89.6−100.6 and 80.9−97.7% AR (Tables 5−7). Similarly to the aqueous photolysis, the degradation rates of **1** in the Calwich Abbey system were faster than those in the Kasai one. Degradates **2**, **3**, **5**, and **6** were detected as major degradates with their maximum amounts (total system) of 32.8, 11.5, 12.2, and 17.7% AR individually at 3, 3, 7, and 7 days in the case of the Kasai system, but they markedly decreased to 7.4, 1.2, 0.7, and 1.9% AR at the end of the study. In the Calwich Abbey system, **2**, **3**, and **6** were produced at 70.8, 30.8, and 13.4% AR at 1, 7, and 3 days, each of which decreased to 3.2, 12.4, and 0% AR at 30 days. The half-lives of each compartment (**1** + **2**), **3**, **4**, and (**5** + **6**) were estimated, as listed in Table 4. Overall, compared to the photodegradation in the natural waters, the presence of the bottom sediment greatly retarded the formation of **5** and **6** in the water phase from 50.6−61.8 to 7.0−24.6% AR in total after 7 days. Incidentally, the total recovery in darkness was 86.2−101.0% AR for the Kasai system and 94.1−98.5% AR for the Calwich Abbey system. The half-lives of **1** were 0.2−2.1 days in the water phase and 0.2−3.6 days in the total system. Degradates **2** and **3** formed via hydrolysis were dominantly detected with the maximum amounts (total system) of 44.4−88.9 and 4.4−10.3% AR at 3−14 and 14 days, respectively. The estimated half-lives of **2**, **3**, and **4** based on the compartment model are listed in Table 4. The unknown fractions in "others" exceeded neither 10% AR nor >5% AR in at least two sequential measurements under any conditions tested in this study. The volatile ^{14}C was detected only in the alkaline trap as $^{14}CO_2$, which gradually increased to 7.5−24.5% AR at day 30 under illumination and to 2.5−8.3% AR at day 30 in darkness. The bound residues gradually increased to 40.4−55.3 and 15.8−61.4% AR at the end of the study under light and dark conditions, respectively. The alkaline fractionation of the bound residues showed that the radioactivity was mainly distributed in the fluvic acid and humic acid fractions under both light and dark conditions for the Kasai systems, whereas no similar trend was observed for the Calwich Abbey ones (Supporting Information, Table S6).

Identification of Photoproducts 5 and 6. The chemical structure of one major photoproduct has been previously proposed in the photodegradation of **1** in buffers at pH 5 and 7[4] to be [4-(cyclohex-1-ene-1,2-dicarboxyimido)-3-fluoro-6-(*N*-prop-2-ynylamino)phenoxy]acetic acid, possibly via hydrolytic cleavage of the amide linkage in the benzoxiazinone ring. Because this chemical structure was proposed only on the basis of its molecular weight of 372 obtained with LC-ESI-MS, we have attempted to

Table 6. Distribution of [Phe-^{14}C]-1 and Its Degradates 2−6 in the Kasai Water−Sediment System under Illumination and in Darkness[a]

	% of the applied radioactivity							
	illumination					darkness		
	0 days	3 days	7 days	14 days	30 days	3 days	14 days	30 days
volatiles (CO_2)	na	1.7	7.4	8.8	24.5	0.3	1.1	2.5
water phase	95.3	64.1	43.0	30.3	3.8	56.4	37.6	4.4
1	91.6	9.1	2.6	nd	nd	30.6	2.7	nd
2	2.3	27.9	6.1	nd	nd	17.3	33.9	3.7
4	nd	1.2	0.8	nd	nd	4.9	nd	0.7
5	nd	9.4	7.2	1.7	nd	nd	nd	nd
6	nd	11.1	17.4	6.7	nd	nd	nd	nd
other unknowns	1.4	5.4	8.9	21.9	3.8	3.6	1.0	nd
sediment phase	2.1	34.0	45.2	50.5	68.6	44.3	61.2	89.7
1	2.0	13.4	8.4	4.2	3.6	8.5	13.5	16.9
2	nd	4.9	3.8	2.9	2.1	18.6	10.5	5.6
4	nd	0.9	2.5	2.0	nd	1.1	3.3	3.1
5	nd	1.2	1.4	nd	0.7	nd	nd	nd
6	nd	nd	0.3	nd	0.2	nd	nd	nd
other unknowns	nd	1.9	4.1	5.7	6.6	1.8	3.6	2.8
bound residues	0.1	11.8	24.7	35.6	55.3	14.4	30.3	61.4
total	97.4	99.8	95.6	89.6	96.9	101.0	99.9	96.6
water phase								
pH	6.7	6.7	6.9	7.0	6.2	6.8	6.7	6.2
DO (mg/L)	5.3	5.3	5.8	5.1	5.0	5.1	6.2	5.5
ORP (mV)	268	245	179	160	229	260	160	217
sediment phase								
pH	6.5	6.5	6.8	6.6	6.3	6.8	6.1	5.3
ORP (mV)	−254	−255	−239	−221	−152	−363	−115	11

[a] Each value is described as the mean value of duplicate samples. na, not analyzed; nd, not detected. Data of 0 and 7 days in darkness are not shown (full data are shown in the Supporting information, Table S6).

confirm the chemical identification of photochemically prepared **5** by using not only LC-MS but also ^{1}H and ^{13}C NMR with various pulse sequences. As a result, the chemical structure of **5** was determined to be *N*-(prop-2-ynyl)-4-[4-carboxy-3-fluoro-2-(3,4,5,6-tetrahydrophthalimido)-2-butenylidene]azetidine-2-one, as shown in Table 1. The pseudo ion of **5** was observed at 371 $[M - H]^-$ and 373 $[M + H]^+$ in negative and positive ion modes, respectively, which were identical to the ones previously reported.[4] The comparison between ^{1}H and ^{13}C NMR spectra of **1** and **5** clarified the tetrahydrophthalimide and prop-2-ynyl moieties remained intact, whereas drastic changes occurred in the phenyl ring as the signals of C-7, C-8, C-9, and C-10 shifted toward a higher magnetic field (Supporting Information, Figure S1). With regard to the phenyl ring, C-8 with no protons in the HMQC spectrum was most likely to be assigned as a carboxyl carbon from its chemical shift at 170.3 ppm suggesting the phenyl ring cleavage between C-8 and C-9, and a comprehensive analysis of the HMBC spectrum established the chemical structure of the fragment consisting of C-8−C-7−C-6−C-5−C-10−C-9. Furthermore, in the HMBC spectrum, the olefinic C-9 at 138.5 ppm and amide C-14 at 166.1 ppm both showed crosspeaks to two different methylene protons H-11 and H-15 and, additionally, the proton H-11 show crosspeaks to acetylene carbons C-12 at 77.2 ppm and C-13 at 73.9 ppm (Table 1 and Supporting Information, Figures S3 and S4). These results strongly suggested the structure originating from the phenyl ring cleavage followed by formation of the new four-member ring, 2-arizidinone. Because **6** could be produced by mild alkaline hydrolysis of **5**, its chemical structure was determined as the anilic acid derivative of **5**, which was formed by the opening of cyclic imide. The molecular weight of **6** was determined to be 390 by LC-MS analysis in both positive and negative ion modes with its pseudo ions detected at 389 $[M - H]^-$ and 391 $[M + H]^+$.

DISCUSSION

The half-lives of **1** in the overlying water in the presence and absence of sediment were 0.2−1.5 and 0.1−0.3 days under illumination and 0.2−2.1 and 0.1−0.4 days in darkness, respectively, which suggested the insignificant contribution to the dissipation of **1** due to its rapid hydrolysis (Table 3), whereas the degradation profiles were greatly dependent on illumination. The faster degradation of **1** in Calwich Abbey than Kasai samples could be explained from higher alkalinity of the water layer, because hydrolysis of the imide linkage is a predominant process irrespective of light or sediment. The degradation pathway of **1** in

Table 7. Distribution of [THP-^{14}C]-1 and Its Degradates 2−6 in the Calwich Abbey Water−Sediment System under Illumination and in Darkness[a]

	% of the applied radioactivity								
	illumination						darkness		
	0 days	1 day	3 days	7 days	14 days	30 days	1 day	3 days	30 days
volatiles (CO_2)	na	nd	0.4	2.1	7.2	7.5	nd	0.1	7.8
water phase	95.4	79.3	71.1	57.1	39.5	17.3	81.1	82.4	41.3
1	86.0	1.1	nd	nd	nd	nd	0.9	1.2	nd
2	6.4	60.9	30.1	6.4	1.9	0.8	75.8	76.1	33.9
3	nd	2.9	15.9	28.8	23.0	7.9	nd	0.9	nd
5	nd	nd	nd	nd	nd	nd	nd	nd	nd
6	nd	9.9	11.6	7.0	1.7	nd	nd	nd	nd
other unknowns	nd	4.5	13.5	14.9	12.8	8.7	4.4	4.2	7.4
sediment phase	2.3	16.3	25.9	33.4	43.1	56.1	14.7	16.0	46.5
1	2.2	2.3	2.1	1.8	1.2	nd	1.8	0.8	6.5
2	nd	9.9	12.3	5.8	nd	2.4	11.2	12.8	20.4
3	nd	nd	0.5	2.0	6.6	4.5	nd	nd	0.3
5	nd	nd	nd	nd	nd	nd	nd	nd	nd
6	nd	1.1	1.8	1.7	6.2	nd	nd	nd	nd
other unknowns	nd	1.4	3.1	5.1	2.9	8.7	0.9	1.4	3.5
bound residues	0.1	1.7	6.2	16.9	26.2	40.4	0.7	1.1	15.8
total	97.7	95.6	97.5	92.5	89.8	80.9	95.8	98.5	95.6
water phase									
pH	7.9	7.9	7.8	8.0	8.4	8.2	7.9	8.0	8.1
DO (mg/L)	4.9	2.6	2.1	2.9	6.0	2.9	4.5	2.6	4.9
ORP (mV)	106	83	103	87	96	115	31	70	90
sediment phase									
pH	7.4	7.5	7.3	7.7	7.7	7.7	7.5	7.5	7.6
ORP (mV)	−246	−272	−265	−273	−259	−302	−245	−263	−313

[a] Each value is described as the mean value of duplicate samples. na, not analyzed; nd, not detected. Data of 0, 7, and 14 days in darkness are not shown (full data are shown in the Supporting information, Table S7).

the illuminated water−sediment system was proposed, as shown in Figure 1. **1** underwent hydrolytic opening of the imide ring to form **2** followed by cleavage of the amide linkage to produce **3** and **4**. Direct photolysis is considered to operate in the formation of **5** and **6** because **1** and **2** individually have an absorption maximum in water at 290 and 297 nm, with its shoulder extending above 300 nm (Supporting Information, Figure S8). It is suggested that the photoexcited **1** and **2** in water react with a hydroxyl ion at the C-8 carbon on the phenyl ring, which simultaneously induces its cleavage with subsequent molecular rearrangement to form the four-member ring, 2-arizidinone. Because **2**, having an absorption maximum similar to that of **1**, was rapidly formed by hydrolysis of **1**, **6** may be dominantly produced by the above photoreaction directly from **2**. Some pesticides are known to be susceptible to photolysis, forming a product having a very unique chemical structure via bond cleavage and rearrangement by direct and indirect photolysis.[27,28] The fission of the phenyl ring and successive rearrangement to produce the five-member ring have been reported in the photolysis studies of halogenated phenols and attributed to photooxidation and photoreduction.[27,29] There is a possibility that indirect photolysis with activated oxygen species such as hydroxyl radical produced by humic substances and clay mineral in natural water[30,31] is involved to some extent in the formation of **5** and **6**, but it was not confirmed in this study.

With regard to the major degradates, **2**, **3**, **5**, and **6** were much less produced in the overlying water in the water−sediment system under illumination (Tables 5−7) than observed in natural water under light. The maximum amounts of **2**, **3**, **5**, and **6** produced in the former test system as compared with those in the latter one were lower by factors of 0.35, 0.18, 0.90, and 0.34 for Kasai, and those of **2**, **3**, and **6** were 0.74, 0.50, and 0.24 for Calwich Abbey, respectively. With respect to the dissipation rate, **2**, **3**, **5**, and **6** degraded more quickly in the overlying water in the water−sediment system under illumination than observed in the natural water under irradiation (Table 4). The presence of sediment under illumination accelerated the degradation of the compartment (**5** + **6**) in the total system by a factor of 1.1−1.7 as compared with the water-only system, whereas much faster dissipation of **3** by a factor of 4.2−35.0 was observed. The partition to the bottom sediment and microbial degradation therein were most likely to be involved for less production of degradates and their acceleration on dissipation in the systems, which can be precisely confirmed by comparing the dark control results between the natural water and water−sediment. As expected, less production of **2** and **3** in the overlying water by

factors of 0.60−0.79 and 0.29−0.33 and more rapid degradation in the total system by factors of 1.8−5.4 and 4.3−58.0, respectively, were obtained. It has been reported that the faster dissipation of pesticide in a water layer resulted from the increase of organic matter content in sediment,[21] and partition of the compounds between the water and sediment phase depended on their partition coefficient (K_{oc}) values.[23] In this study, although two sediments with different organic carbon contents were used, large differences in the partitioning trend of **1** and its degradates were not observed. This may be due to rapid hydrolysis of **1** producing the degradates with low K_{oc} values, which will be less affected with respect to the partitioning to the sediment. The K_{oc} value of **1** is in the range of 116−200 by batch equilibrium method,[5] whereas the K_{oc} values of its degradates of **2**, **3**, **5**, and **6** are estimated to be 18, 27, 319, and 512, respectively, by the EPI-Suite program.[32] In addition, the enhancement on decomposition of the degradates by illumination can be suggested from the increase of radioactivity transformed to sediment-bound residue and carbon dioxide in comparison with the dark condition. All of the above consequences clearly indicated that the balance between the partitioning and abiotic/biotic degradation is deeply involved in the contribution of reducing the production of each degradate. Incidentally, aniline compound **4** was scarcely detected in the system according to the results of the Kasai water−sediment system treated with [Phe-^{14}C]-**1** (Table 6), whereas the corresponding degradate **3** produced by the complete cleavage of the imide ring amounted to its total maximum of 11.5% AR for the one using [THP-^{14}C]-**1** (Table 5). To the contrary, other minor unknowns reached 27.6% AR at maximum in the phenyl label treated system after 14 days and decreased to 10.4% AR with a concomitant increase of bound activity, which indicated the rapid transformation of **4** to various kinds of minor degradates followed by their binding to the sediments. These degradation processes may proceed via oxidation of the amino group followed by polymerization, as typically known for an aniline,[33] and in such a case, the quantity of each degradate produced will be variable on each occasion but relatively in low amount.

In conclusion, the results in our illuminated water−sediment study are considered to represent the fate of **1** and its degradates in the aqueous environment where various important phenomena in nature are more precisely included in the test system than the individual aqueous photolysis and water−sediment study in darkness. Our results suggest that **1** and its degradates are rapidly degraded with half-lives of less than several days and do not persist in the aqueous environment.

■ ASSOCIATED CONTENT

Supporting Information. Full data of the ^{14}C distribution in natural water (Tables S1 and S2), in water−sediment systems in darkness (Tables S3−S5), the radioactivity in the bound residues (Table S6), ^{1}H and ^{13}C NMR spectra with various pulse sequences NMR spectra of **5** (Figures S1−S4), representative HPLC radiochromatograms of water layer and sediment extracts in metabolism study (Figures S5−S7), and UV−visible absorption spectra of **5** and **6** (Figure S8). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +81 797 74 2073. Fax: +81 797 74 2132. E-mail: shibataa@sc.sumitomo-chem.co.jp.

■ REFERENCES

(1) Yoshida, R.; Sakaki, M.; Sato, R.; Haga, T.; Nagano, E.; Oshio, H.; Kamoshita, K. S-53482 − a new *N*-phenyl phthalimide herbicide. *Brighton Crop Prot. Conf.—Weeds* **1991**, *1*, 69–75.

(2) Vencil, W. K. *Herbicide Handbook*, 8th ed.; Weed Science Society of America: Lawrence, KS, 2002; pp 200−201.

(3) Katagi, T. Hydrolysis of *N*-phenylimide herbicide flumioxazin and its anilic acid derivative in aqueous solutions. *J. Pestic. Sci.* **2003**, *28*, 44–50.

(4) Kwon, J. W.; Armbrust, K. L.; Grey, T. L. Hydrolysis and photolysis of flumioxazin in aqueous buffer solutions. *Pest Manag. Sci.* **2004**, *60*, 939–943.

(5) Ferrell, J. A.; Vencill, W. K. Flumioxazin soil persistence and mineralization in laboratory experiments. *J. Agric. Food Chem.* **2003**, *51*, 4719–4721.

(6) Alister, C.; Rojas, S.; Gómez, P.; Kogan, M. Dissipation and movement of flumioxazin in soil at four field sites in Chile. *Pest Manag. Sci.* **2008**, *64*, 579–583.

(7) Ferrell, J. A.; Vencill, W. K.; Xia, K.; Grey, T. L. Sorption and desorption of flumioxazin to soil, clay minerals and ion-exchange resin. *Pest Manag. Sci.* **2005**, *61*, 40–46.

(8) Tomigahara, Y.; Matsui, M.; Matsunaga, H.; Isobe, N.; Kaneko, H.; Nakatsuka, I.; Yoshitake, A.; Yamada, H. Metabolism of 7-fluoro-6-(3,4,5,6-tetrahydrophthalimido)-4-(2-propynyl)-2*H*-1,4-benzoxazin-3(4*H*)-one (S-53482) in rat: 1. Identification of a sulphonic acid type conjugate. *J. Agric. Food Chem.* **1999**, *47*, 305–312.

(9) Tomigahara, Y.; Onogi, M.; Kaneko, H.; Nakatsuka, I.; Yamane, S. Metabolism of 7-fluoro-6-(3,4,5,6-tetrahydrophthalimido)-4- (2-propynyl)-2*H*-1,4-benzoxazin-3(4*H*)-one (S-53482, flumioxazin) in the rat: II. Identification of reduced metabolites. *J. Agric. Food Chem.* **1999**, *47*, 2429–38.

(10) European Commission. Working document, Guidance document on aquatic ecotoxicology Sanco/3268/2001.rev.4; European Commission, Brussels, Belgium, 2002.

(11) OECD guideline for the testing of chemicals 308. Aerobic and anaerobic transformation in aquatic sediment systems; April 2002.

(12) European Commission. Pyriproxifen, Existing active substance (3rd stage), Draft assessment report; European Commission, Brussels, Belgium, 2006; http://dar.efsa.europa.eu/dar-web/provision (accessed March 2011).

(13) European Commission. Bensulfuron Methyl, Existing active substance (3rd stage), Draft assessment report; European Commission, Brussels, Belgium, 2007; http://dar.efsa.europa.eu/dar-web/provision (accessed March 2011).

(14) Katagi, T. Behavior of pesticides in water-sediment systems. *Rev. Environ. Contam. Toxicol.* **2006**, *187*, 133–251.

(15) Kodaka, R.; Sugano, T.; Katagi, T.; Takimoto, Y. Aerobic aquatic metabolism of fenitrothion and its oxon analog in water-sediment systems. *J. Pestic. Sci.* **2002**, *27*, 235–241.

(16) Kodaka, R.; Sugano, T.; Katagi, T.; Takimoto, Y. Comparative metabolism of organophosphorus pesticides in water-sediment systems. *J. Pestic. Sci.* **2003**, *28*, 175–182.

(17) Aajoud, A.; Ravanel, P.; Tissut, M. Fipronil metabolism and dissipation in a simplified aquatic ecosystem. *J. Agric. Food Chem.* **2003**, *51*, 1347–1352.

(18) Rice, P. J.; Anderson, T. A.; Coats, J. R. Effects of sediment on the fate of metolachlor and atrazine in surface water. *Environ. Toxicol. Chem.* **2004**, *23*, 1145–1155.

(19) Kodaka, R.; Sugano, T.; Katagi, T. Metabolism of uniconazole-P in water-sediment systems under illumination. *Environ. Toxicol. Chem.* **2006**, *25*, 310–316.

(20) Kwon, J. W.; Armbrust, K. L. Degradation of chlorothalonil in irradiated water/sediment systems. *J. Agric. Food Chem.* **2006**, *54*, 3651–3657.

(21) Kwon, J. W.; Armbrust, K. L. Laboratory persistence and fate of fluoxetine in aquatic environments. *Environ. Toxicol. Chem.* **2006**, *25*, 2561–2568.

(22) Kodaka, R.; Sugano, T.; Katagi, T. Degradation of esfenvalerate in illuminated water-sediment system. *J. Pestic. Sci.* **2009**, *34*, 27–36.

(23) Kodaka, R.; Swales, S. E.; Lewis, C.; Katagi, T. Effect of illumination on degradation of pyriproxyfen in water-sediment system. *J. Pestic. Sci.* **2011**, *36*, 33–40.

(24) van der Kolka, J. W. H.; Crum, S. J. H. Laboratory microecosystems as physical models for predicting the fate of pesticides in aquatic systems. *Sci. Total Environ.* **1993**, *134*, 1429–1437.

(25) Carton, P. R.; Allen, R. The use of a compartment model for evaluating the fate of pesticides in sediment/water system. *Brighton Crop Prot. Conf.—Pest Dis.* **1994**, 1349—1354.

(26) FOCUS Degradation Kinetics Workgroup. Degradation Kinetics Spreadsheet V2, Degradation Kinetics Software; European Commission, Brussels, Belgium; http://focus.jrc.ec.europa.eu/dk/software.html (accessed March 2011).

(27) Burrows, H. D.; Canle, M.; Santaballa, J. A.; Steenken, S. Reaction pathways and mechanisms of photodegradation of pesticides. *J. Photochem. Photobiol. B: Biol.* **2002**, *67*, 71–108.

(28) Katagi, T. Photodegradation of pesticides on plant and soil surfaces. *Rev. Environ. Contam. Toxicol.* **2004**, *182*, 1–195.

(29) Rayne, S.; Forest, K.; Friesen, K. J. Mechanistic aspects regarding the direct aqueous environmental photochemistry of phenol and its simple halogenated derivatives. A review. *Environ. Int.* **2009**, *35*, 425–37.

(30) Katagi, T. Photodegradation of esfenvalerate in clay suspensions. *J. Agric. Food Chem.* **1993**, *41*, 2178–2183.

(31) Dimou, A. D.; Sakkas, V. A.; Albanis, T. A. Trifluralin photolysis in natural waters and under the presence of isolated organic matter and nitrate ions: kinetics and photoproduct analysis. *Photochem. Photobiol. A* **2004**, *163*, 473–480.

(32) Estimation Programs Interface Suite for Microsoft® Windows, v 4.00; U.S. Environmental Protection Agency, Washington, DC; http://www.epa.gov/oppt/exposure/pubs/episuite.htm (accessed March 2011).

(33) Parris, G. E. Environmental and metabolic transformations of primary aromatic amines and related compounds. *Residue Rev.* **1980**, *76*, 1–30.