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Dissipation of [^{14}C]Acetochlor Herbicide under Anaerobic Aquatic Conditions in Flooded Soil Microcosms

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Acetochlor degradation was studied under anaerobic conditions representative of conditions in flooded soils. Soil−water microcosms were prepared with a saturated Drummer clay loam and made anaerobic by either glucose pretreatment or N_2 sparging. Sparged microcosms consisted of sulfate-amended, unamended, and γ-irradiated microcosms. The microcosms were sampled in triplicate at predetermined time intervals during a 371 day incubation period. Volatile, aqueous, extractable, and bound (unextractable) ^{14}C residues were quantified with liquid scintillation counting and characterized using high-performance liquid radiochromatography (HPLRC) and soil combustion. SO_4^{2-}, Fe(II), CH_4, and pH were monitored. Complete anaerobic degradation of [^{14}C]acetochlor was observed in all viable treatments. The time observed for 50% acetochlor disappearance (DT_{50}) was 10 days for iron-reducing and sulfate-reducing conditions (sulfate-amended), 15 days for iron-reducing conditions (unamended), and 16 days for methanogenic conditions (glucose-pretreated). Acetochlor remained after 371 days in the γ-irradiated microcosms, and metabolites were observed. [^{14}C]Metabolites were detected throughout the study. Formation of one of the metabolites correlated with Fe(II) formation (r^2, 0.83). A significant portion of the ^{14}C activity was eventually incorporated into soil-bound residue (30−50% of applied acetochlor) in all treatments.

KEYWORDS: Anaerobic herbicide degradation; chloroacetamides; acetochlor; flooded soil

INTRODUCTION

The chloroacetamide herbicide acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide) is used for preemergence control of annual grasses and small-seeded broadleaf weeds in corn and soybean (*1*). In 1994, acetochlor was introduced to the market with the aim of partially replacing alachlor, atrazine, butylate, EPTC, 2,4-D, and metolachlor; therefore, a gradual increase in the use of acetochlor was expected (*2*). In its first year of use, acetochlor was the fifth most extensively used corn herbicide in the Midwest (*3*), and by 1996, it was ranked third in use (*4*). Its median concentration and estimated annual load of acetochlor from the Mississippi River Basin to the Gulf of Mexico steadily increased from 1994 to 1998 (*5*).

Persistence of chloroacetamide herbicides in the field varies with soil type, soil−water content, temperature, depth below the soil surface (*6*), and other factors. Like other chloroacetamides, acetochlor is not particularly susceptible to photodecomposition or volatilization (*1*). The U.S. EPA registration document categorizes acetochlor as moderately persistent in the environment and moderately to very mobile in soil (*2*). The water solubility of acetochlor is 223 mg L^{-1} (25 °C), and because of the potential threat to drinking water supplies, the U.S. EPA gave acetochlor a conditional use registration that mandates continued ground and surface water monitoring (*2*). After the first season of use, acetochlor was found in rain and surface water in Minnesota at concentrations between 10 and 250 ng L^{-1} (comparable to other herbicides of the same family) (*7*), and it was detected for the first time in groundwater in 1995 (*8*). Chemical and biological transformations of chloroacetamide herbicides to metabolites have been reported (*9, 10*), and the detection of metabolites in groundwater and surface waters is a concern. Metabolites formed under anaerobic conditions may differ from those identified as products of glutathione conjugation (*11*−*13*). Identification of unknown metabolites is a challenge since these compounds are not commercially available. The estimated half-lives of acetochlor were 6.3 days in a surface soil field study (*10*), 6.9 days in an in vitro aerobic soil study, and 1.1 days in anaerobic sewage sludge (*9*), but there is no specific information available regarding its persistence in anaerobic soil or sediment, particularly with respect to anaerobic microbial processes.

In aerobic soil, acetochlor is transformed to oxanilic acid (OXA), ethanesulfonic acid (ESA), and sulfinylacetic acid by glutathione conjugation (*11*). Kalkhoff et al. (*12*) suggested that

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ESA and OXA are more persistent in the environment than acetochlor. These compounds also have been detected in groundwater more frequently and generally in higher concentrations than acetochlor. Acetochlor is typically applied during the spring and early summer and may be subjected to anaerobic conditions following heavy rainfall on poorly drained surface soils. Anaerobic microbial processes contribute to organic carbon turnover in soil, but their roles in herbicide fate are poorly understood. Anaerobic dissipation of acetochlor and resulting degradation products has not been characterized.

This study was aimed at characterizing acetochlor dissipation and degradation product formation under anaerobic conditions in saturated soil and the corresponding anaerobic microbial activities, including Fe(III) reduction, sulfate reduction, and methanogenesis.

MATERIALS AND METHODS

Chemicals. Uniformly ring-labeled [^{14}C]acetochlor (specific activity, 1.0×10^6 Bq μmol^{-1}; purity, 98% by high-performance liquid radiochromatography, HPLRC) was obtained from Monsanto (St. Louis, MO). Unlabeled acetochlor (purity, 99% by HPLC) was obtained from Chem Service (West Chester, PA). All solvents were Optima grade (Fisher Scientific, Pittsburgh, PA). Purity of ^{14}C and technical acetochlor was determined in our laboratory at the time of use.

Soil. A Drummer clay loam from the taxonomic class fine-silty, mixed, superactive, mesic Typic Endoaquolls (Champaign County, IL) was used. The <2 mm fraction contained 28% sand, 42% silt, 30% clay, and 5.5% organic matter.

Media. The anaerobic mineral salts−trace metals medium was prepared as in Crawford et al. (*13*), except with 0.4 g L^{-1} $MgCl_2 \cdot 6H_2O$ and 0.04 g L^{-1} NH_4Cl. The medium had a background sulfate concentration of 0.18 mM due to elemental salts. In the microcosms prepared according to the EPA protocol (glucose pretreatment), the medium also contained glucose (D-glucose, 10 g L^{-1}). The sulfate-amended treatment included the additions of Na_2SO_4 (7.5 mM) and $TiCl_3$ (a reductant, 30 μM).

Experimental Design. Four different microcosms were prepared in this study for determining the anaerobic fate of acetochlor: (i) glucose-pretreated, aerobic preparation of soil and incubation with glucose before herbicide addition (EPA Protocol N-162-3, *14*); (ii) unamended, anaerobic preparation of soil with N_2 sparging and use of the anaerobic mineral salts−trace metals medium (above); (iii) sulfate-amended, anaerobic preparation of soil and medium with N_2 sparging and sulfate; and (iv) unamended sterile, an abiotic (γ-irradiated) control anaerobically prepared with N_2 sparging and use of the anaerobic mineral salts−trace metals medium.

Soil−water (1:1 w/v) microcosms were prepared in serum bottles (120 mL) using saturated sediment (15 g, 10.7 g air-dried) and anaerobic medium. Except for those prepared according to EPA Protocol N-162-3, microcosms were made anaerobic by displacing the headspace with O_2-free N_2 and were transferred to an anaerobic chamber where the corresponding unamended or amended sterile, anaerobic mineral salts medium, and acetochlor solution were added. All microcosms received [^{14}C]acetochlor (filter sterile, 0.2 μm) to a final concentration of 22 mg acetochlor kg soil^{-1} (3952 Bq biometer^{-1}, prepared by dilution with technical acetochlor in methanol, final concentration of methanol, 2.6 mg g^{-1} soil + water). Methanol is ubiquitous in the environment and is suitable for use in soil herbicide degradation studies. The total concentration of acetochlor corresponds to a field application rate (on an area basis) of 1.60 kg ha^{-1} (*1*). For the glucose-pretreated microcosms (Protocol N-162-3), 1:1 (w/v) soil:glucose anaerobic mineral salts−trace metals medium was added to serum bottles under sterile, aerobic conditions and microcosms were sealed and incubated in the dark (25 ± 3 °C) for 30 days prior to acetochlor addition to allow anaerobic conditions to develop as a function of glucose and O_2 consumption. On day 30, the microcosms were transferred to an anaerobic chamber where the [^{14}C]acetochlor solution was added. All bottles were sealed with either a slotted gray stopper (for sampling days 0−16) or a 2.5 cm thick butyl rubber stopper (for sampling days 24−371) and crimp-sealed. Each stopper had an attached vial (2 mL) containing sterile Tropaelin O, 1 mg L^{-1} in 2 M KOH (1 mL) as a CO_2 collection trap.

Acetochlor incubation treatments included 33 replicates each of glucose-pretreated, unamended, sulfate-amended, and unamended sterile control microcosms. γ-Irradiation (0.5 Mrad, twice in 3 days, Isomedix, Groveport, OH) was performed prior to the application of the [^{14}C]-acetochlor. For each treatment, 33 additional control microcosms were prepared under the same conditions but without the addition of acetochlor. All microcosms were incubated in the dark at 25 ± 3 °C.

Sampling. Triplicate microcosms from each treatment were destructively sampled and analyzed immediately (day 0) after preparation and then after 8, 16, 24, 32, 64, 90, 142, 193, and 371 days.

Headspace Analyses. CH_4 and O_2 levels were determined in headspace samples (0.5 mL) using gas chromatography (GC, GOW-MAC Instrument, Bridgewater, NJ), including a Whased Molesieve Alltech column, isothermal conditions of 100 °C, and Ar carrier (11 mL min^{-1}) (*15*). Estimation of the radioactivity associated with volatile acetochlor and degradation products was assessed on days 24, 32, and 64 (*13*, *16*). Liquid scintillation counting (LSC) was performed using a Packard TRICARB 2900TR Liquid Scintillation Analyzer (Meriden, CT).

Processing Microcosm Slurries. *Determination of ^{14}C Distribution.* $^{14}CO_2$, aqueous, extractable (sorbed), and unextractable (bound) ^{14}C were quantified with LSC after sample processing as described in detail by Crawford et al. (*13*). Aqueous samples were removed and filtered, and aliquots were stored at 4 and −20 °C for HPLRC analysis and sulfate analysis, respectively. Aqueous aliquots for LSC were prepared in duplicate, and one was treated with saturated $BaCl_2 \cdot H_2O$ to precipitate CO_2/HCO_3^-. The non-$BaCl_2$-treated sample represented total aqueous radiocarbon ([^{14}C]acetochlor + ^{14}C degradation products + $H^{14}CO_3^-$). The difference in ^{14}C between the $BaCl_2$-treated and untreated samples was reported as $H^{14}CO_3^-$ (or aqueous $^{14}CO_2$). The pH of the soil solution was determined using Colorphast Indicator Strips (*13*).

A 0.5 g soil sample was removed for Fe(II) analysis, and the remaining soil was extracted with diethyl ether (20 mL, dispensed using a calibrated Brinkmann dispensette pump) with horizontal shaking (20 min) and centrifuged (15 min, 12 000*g*, 4 °C) (*17*). From the supernatant, the organic liquid phase (16.5 mL) was transferred to empty glass vials. An aliquot from the organic phase (0.5 mL) was removed for LSC analysis to quantify extractable ^{14}C. Organic extracts were air-dried and resuspended in methanol (2 mL). Soil samples were air-dried, pulverized (with mortar and pestle), and reextracted two more times following the same procedure, first with ethyl acetate/acetone (95:5, 19 mL) and later with methanol (15 mL), for 48 h each time. After each reextraction, an aliquot of the organic phase was removed for LSC, and the remaining extract was evaporated to dryness and resuspended in methanol (2 mL). Sorbed ^{14}C was calculated as extractable ^{14}C (diethyl ether + ethyl acetate/acetone + methanol) minus soil solution ^{14}C (remaining in the soil following removal of the aqueous fraction). After the last reextraction, soil samples were air-dried and pulverized, and bound (unextractable) ^{14}C residues were quantified by combustion (*13*).

HPLRC Analysis of Parent and Metabolites. The aqueous samples were extracted 2:1 (v/v) with ethyl acetate/acetone, evaporated to dryness, and resuspended in methanol. Because of the low amount of radioactivity contained in these samples, triplicates were combined, and the resulting sample was evaporated and resuspended in methanol for HPLC analysis. Organic extracts from the three soil extractions were combined, evaporated, and resuspended in methanol/H_2O for HPLRC analysis.

Aqueous and extractable [^{14}C]acetochlor and [^{14}C]metabolites were quantified with a HPLRC equipped with a Hewlett-Packard 1050 series autosampler instrument, using both ^{14}C (Packard Radiomatic Flo-one/beta detector) and UV (254 nm) detectors. The composition of the HPLRC mobile phase was 24:35.7:40:0.3 (v/v/v/v) methanol:acetonitrile:H_2O:acetic acid glacial (*18*). Mobile phase was continuously sparged with He and was delivered at 0.5 mL min^{-1} to a C_{18} RP Econosil column (5 μm, 250 mm × 4.6 mm; Alltech Associates, Deerfield, IL). [^{14}C]Acetochlor standards and samples were dissolved in methanol and stored at 4 °C.

Table 1. Concentrations of Terminal Electron Acceptors and Reduced Compounds in the Glucose-Pretreated Microcosm with and without [^{14}C]Acetochlor

	with [^{14}C]acetochlor			without [^{14}C]acetochlor		
time (days)	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]
0	0.08 ± 0.01	274 ± 21	22.2 ± 0.5[b]	0.08 ± 0.004	237 ± 71	22.2 ± 0.1
8	0.08 ± 0.00	245 ± 40	25.4 ± 2.3	0.07 ± 0.001	282 ± 39	22.0 ± 3.0
16	0.08 ± 0.01	388 ± 8	26.4 ± 2.5	0.06 ± 0.012	294 ± 5	39.7 ± 0.3
24	0.09 ± 0.01	335 ± 40	28.9 ± 1.0	0.04 ± 0.004	161 ± 5	60.8 ± 8.9
32	0.08 ± 0.02	293 ± 17	27.9 ± 3.8	0.05 ± 0.009	250 ± 15	32.9 ± 5.6
64	0.41 ± 0.15	287 ± 37	30.7 ± 4.1	0.07 ± 0.011	289 ± 11	44.6 ± 9.2
90	0.48 ± 0.03	360 ± 32	48.6 ± 11.1	0.36 ± 0.131	313 ± 46	65.1 ± 6.3
142	0.17 ± 0.13	406 ± 35	32.9 ± 1.4	0.12 ± 0.074	387 ± 32	52.4 ± 26.3
193	0.34 ± 0.14	451 ± 128	38.1 ± 14.9	0.15 ± 0.108	390 ± 23	52.4 ± 26.3
371	0.04 ± 0.01	472 ± 124	34.2 ± 12.3	0.04 ± 0.009	526 ± 27	68.5 ± 12.9

[a] Concentration was measured in biometer headspace. [b] A loss of 19.5% CH_4 from the headspace occurred when biometers were opened to add acetochlor; thus, 19.5 was added to CH_4 values from acetochlor-treated biometers.

Table 2. Concentrations of Terminal Electron Acceptors and Reduced Compounds in the Unamended Microcosms with and without [^{14}C]Acetochlor

	with [^{14}C]acetochlor			without [^{14}C]acetochlor		
time (days)	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]
0	0.16 ± 0.02	38 ± 7	0 ± 0	0.09 ± 0.06	31 ± 19	0 ± 0
8	0.08 ± 0.02	19 ± 4	0 ± 0	0.07 ± 0.03	11 ± 3	0 ± 0
16	0.06 ± 0.01	71 ± 8	0 ± 0	0.06 ± 0.00	39 ± 11	0 ± 0
24	0.07 ± 0.00	152 ± 21	0 ± 0	0.05 ± 0.00	51 ± 25	0 ± 0
32	0.07 ± 0.01	131 ± 26	0 ± 0	0.04 ± 0.01	54 ± 9	0.4 ± 0.4
64	0.09 ± 0.00	214 ± 52	7.5 ± 1.7	0.10 ± 0.06	100 ± 43	0 ± 0
90	0.07 ± 0.00	283 ± 17	16.5 ± 7.4	0.05 ± 0.01	168 ± 54	1.5 ± 1.5
142	0.07 ± 0.01	232 ± 40	24.0 ± 9.6	0.04 ± 0.01	157 ± 32	2.2 ± 2.2
193	0.06 ± 0.01	326 ± 27	27.0 ± 17.5	0.03 ± 0.00	274 ± 67	0.1 ± 0.1
371	0.05 ± 0.01	572 ± 9	40.7 ± 15.7	0.80 ± 0.77	370 ± 178	11.8 ± 5.9

[a] Concentration was measured in biometer headspace.

Determination of Terminal Electron Accepting Processes. Sulfate, Fe(II), and CH_4 were monitored in all herbicide-treated and untreated microcosms. These parameters were determined in untreated microcosms in order to distinguish microbial activity related to the presence of the herbicide from activity associated with soil organic content. Sulfate concentrations were estimated using a microscale adaption of the barium precipitation method (*13*). Soil Fe(II) formation was analyzed with a microscale adaptation of the ferrozine method (*19*), with absorbance read at 550 nm instead of 590 nm. Microplate spectrophotometry was carried out with a ATTC plate reader model 340 (SLT LabInstruments GesmbH, Grödig, Austria).

Data Analyses. The statistical program SAS/STAT for Windows (*20*) was used to generate means, standard errors, and percent of total radiocarbon applied that was recovered as mineralized, sorbed (parent and metabolites), aqueous (parent and metabolites), and bound residue ^{14}C. The time observed for 50% disappearance of acetochlor (DT_{50}) was read from a plot of time (days) vs percent of applied acetochlor. To estimate degradation rate constants, acetochlor decline curves were fitted to a first-order decay equation using SigmaPlot 8.0 for Windows (SPSS, Chicago), and the nonlinear regression equation for exponential decay, $y = ae^{-kx}$, in which y = % of acetochlor, a = initial % of acetochlor, k = rate constant, and x = time; half-life, $T_{1/2} = 0.693/k$.

RESULTS AND DISCUSSION

Microbial Processes. *Glucose-Pretreated.* These microcosms were flooded and treated with glucose 30 days prior to herbicide addition; thus, day 0 of herbicide treatment represents day 30 of flooding. Fe(II) and CH_4 levels were elevated at the time of herbicide addition (**Table 1**). After herbicide addition, methanogenesis continued until it reached a maximum value on day 90. Sulfate reduction was not evident, and Fe(II) did not increase further until day 90. There was no appreciable difference regarding sulfate and Fe(II) levels between the herbicide-treated and the untreated microcosms. CH_4 accumulated to higher concentrations in untreated microcosms (**Table 1**).

Unamended. Fe(II) formation was evident in acetochlor-amended microcosms just after the initiation of the experiment and continued until the end of the experiment (**Table 2**). CH_4 production was not evident until day 64 and was produced until the end of the study. Fe(II) formation and CH_4 production were also observed in herbicide-free microcosms (**Table 2**), but both processes were slower and less productive than in the herbicide-treated microcosms. Increased Fe(II) and CH_4 formation in the herbicide-treated microcosms suggested that the presence of acetochlor provided an advantage to iron-reducing bacteria and to methanogens. In nonsulfidogenic environments, nonenzymatic Fe(III) reduction is inconsequential to Fe(II) formation as compared with enzymatic Fe(III) reduction by microorganisms (*21*), so it is likely that the observed Fe(II) production was microbially catalyzed. Sulfate reduction was negligible in both herbicide-treated and untreated microcosms. Methanol was also added in the acetochlor-treated microcosms, so the potential for anaerobic growth with methanol and cometabolism of acetochlor was considered. Anaerobic methanol metabolism is accomplished by a specialized group of microorganisms (methylotrophs), including *Hyphomicrobium* spp., which require nitrate for methanol metabolism, and some sulfate reducers, methanogens, and acidogens. Iron-reducing bacteria are not known to metabolize methanol. Methane levels were not consistently higher in the presence of methanol and acetochlor; therefore, the role of methanol in supporting methanogenic growth was unclear.

Sulfate-Amended. Sulfate was depleted before day 24 in the acetochlor-amended microcosms (**Table 3**). In many cases, sulfate reduction was accompanied by FeS precipitation (black

Table 3. Concentrations of Terminal Electron Acceptors and Reduced Compounds in the Sulfate-Amended Microcosms with and without [^{14}C]Acetochlor

	with [^{14}C]acetochlor			without [^{14}C]acetochlor		
time (days)	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]
0	5.45 ± 0.85	12 ± 2	0 ± 0	4.76 ± 0.84	10 ± 1	0 ± 0
8	6.12 ± 1.84	82 ± 28	0 ± 0	5.49 ± 0.34	19 ± 6	0 ± 0
16	4.85 ± 0.30	66 ± 21	0 ± 0	4.03 ± 0.67	12 ± 0	0 ± 0
24	0.05 ± 0.00	241 ± 69	0 ± 0	4.74 ± 0.29	75 ± 19	0 ± 0
32	0.05 ± 0.00	254 ± 21	0 ± 0	6.69 ± 1.21	95 ± 8	0 ± 0
64	0.08 ± 0.01	308 ± 8	16.0 ± 6.8	4.58 ± 0.56	102 ± 38	0 ± 0
90	0.08 ± 0.00	342 ± 22	41.4 ± 2.2	2.10 ± 0.13	239 ± 66	0 ± 0
142	0.09 ± 0.00	420 ± 58	55.2 ± 2.1	1.72 ± 1.63	247 ± 98	0 ± 0
193	0.08 ± 0.00	535 ± 9	16.9 ± 10.7	0.78 ± 0.70	482 ± 6	3.8 ± 2.0
371	0.11 ± 0.10	521 ± 90	25.7 ± 25.7	6.44 ± 0.46	221 ± 37	0 ± 0

[a] Concentration was measured in biometer headspace.

Table 4. Concentrations of Terminal Electron Acceptors and Reduced Compounds in the Unamended Sterile Microcosms with and without [^{14}C]Acetochlor

	with [^{14}C]acetochlor			without [^{14}C]acetochlor		
time (days)	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]	sulfate (mM)	Fe(II) (mg/kg)	% [CH_4][a]
0	0.66 ± 0.04	148 ± 23	0 ± 0	0.60 ± 0.08	149 ± 11	0 ± 0
8	0.30 ± 0.14	131 ± 28	0 ± 0	0.55 ± 0.00	146 ± 23	0 ± 0
16	0.16 ± 0.03	180 ± 13	0 ± 0	0.12 ± 0.01	199 ± 28	0 ± 0
24	0.18 ± 0.02	175 ± 19	0 ± 0	0.13 ± 0.00	158 ± 26	0 ± 0
32	0.20 ± 0.05	138 ± 51	0 ± 0	0.15 ± 0.01	165 ± 5	0 ± 0
64	0.20 ± 0.03	111 ± 18	0 ± 0	0.12 ± 0.02	127 ± 11	0 ± 0
90	0.15 ± 0.04	120 ± 60	0 ± 0	0.14 ± 0.01	136 ± 27	0 ± 0
142	0.19 ± 0.03	60 ± 16	0 ± 0	0.28 ± 0.08	63 ± 13	0 ± 0
193	0.49 ± 0.21	32 ± 14	0 ± 0	0.09 ± 0.00	9 ± 1	0 ± 0
371	1.84 ± 0.92	8.7 ± 0.07	0 ± 0	0.74 ± 0.57	9 ± 0.1	0 ± 0

[a] Concentration was measured in biometer headspace.

particles in the liquid, soil, and on biometer walls), which was noticeable after day 64. Fe(II) formation started just after the initiation of the experiment and continued until day 193. Methanogenesis was evident between days 64 and 142. Fe(II) formation and sulfate reduction were less significant and slower in the nonherbicide microcosms as compared to the acetochlor-amended microcosms (**Table 3**). Growth of sulfate reducers with methanol was also possible. Furthermore, the presence of CH_4 was observed only on day 193 (**Table 3**). The anaerobic nature of the system was evident in the anaerobic processes observed; however, O_2 was detected at levels nearing the detection limit (2.6%) in the headspace of some microcosms. Trace quantities of O_2 are rapidly consumed in sulfate-reducing or methanogenic environments (*22*) and, consequently, likely do not compromise the anaerobicity of saturated soil.

Unamended Sterile. Terminal electron-accepting processes were not observed in the systems sterilized by γ-irradiation (**Table 4**). The presence of elevated levels of Fe(II) in the sterile microcosms was in agreement with a previous study, which found that γ-irradiation liberates soil ions (*23*). The decrease in Fe(II) levels may have been due to oxidation to Fe(III), since O_2 was detected at low levels and is also a byproduct of irradiation.

Microcosm pH. In all herbicide-treated microcosms, pH values ranged from 6.0 or 6.5 to 8.0, whereas in the absence of herbicide pH ranged from 7.0 to 8.0.

Radiocarbon Distribution and Recovery. The profiles of ^{14}C measured in aqueous, sorbed, and bound fractions in the different microcosms demonstrated that radiocarbon initially associated with the aqueous phase underwent increasing sorption to soil over time (**Tables 5**−**8**). Depletion from the sorbed phase corresponded to an increase in bound residue formation. In the viable microcosms, formation of ^{14}C bound residue was both more rapid and more productive than in the sterile treatment, which is consistent with a previous study using dimethenamid, another chloroacetamide herbicide (*13*). Formation of bound residues under both sterile and viable conditions indicates that both abiotic and biological mechanisms are involved. The difference between sterile and viable bound residue formation may reflect the different metabolites formed (**Figure 1**) and their affinities for, or their incorporation into, soil organic matter. Herbicides and their transformation products resemble precursors to soil organic matter, and biological activities are known to catalyze formation of soil organic matter (*24*−*26*). Trapped $^{14}CO_2$ and $H^{14}CO_3^-$ accumulated to 0.07 ± 0.09 and 0.8 ± 1.0% of applied ^{14}C (average of all microcosms and treatments), respectively, indicating that mineralization of the acetochlor benzene ring was negligible. The sum of the three ^{14}C fractions

Table 5. Distribution of Applied ^{14}C in the Glucose-Pretreated Microcosms

time (days)	aqueous[a]	sorbed[a]	$H^{14}CO_3^-$/ $^{14}CO_2$	bound	total recovered
0	39.1 ± 1.4	48.3 ± 2.8	0.4 ± 0.3	5.1 ± 0.3	93 ± 2
8	28.5 ± 1.1	61.4 ± 0.9	0.8 ± 0.5	20.3 ± 0.1	111 ± 2
16	23.9 ± 1.2	48.6 ± 1.2	0.6 ± 0.3	25.7 ± 0.8	99 ± 2
24	23.2 ± 1.2	53.3 ± 1	0.2 ± 0.1	27.6 ± 0.4	104 ± 0
32	25.6 ± 0.1	47.6 ± 3.2	0 ± 0	30.9 ± 1.3	104 ± 4
64	22.9 ± 0.4	41.8 ± 0.6	0.7 ± 0.2	37.2 ± 0.8	103 ± 0
90	23.2 ± 1.2	40.1 ± 1.1	0.1 ± 0	37.6 ± 1.4	101 ± 2
142	23.4 ± 0.5	38.5 ± 1.9	0.1 ± 0	39.6 ± 1.5	102 ± 2
193	21.9 ± 0.2	45.3 ± 0.9	0.8 ± 0.4	35.9 ± 1.3	104 ± 2
371	20.3 ± 1.5	42.5 ± 1.7	2.1 ± 1.5	34.6 ± 2.4	100 ± 3

[a] Includes acetochlor and metabolites.

Table 6. Distribution of Applied ^{14}C in the Unamended Microcosms

time (days)	aqueous[a]	sorbed[a]	$H^{14}CO_3^-$/ $^{14}CO_2$	bound	total recovered
0	56 ± 0.4	39.1 ± 1.5	0.4 ± 0.3	2.6 ± 0.1	98 ± 2
8	28.3 ± 3.1	62.6 ± 4.5	0.6 ± 0.1	19.8 ± 2.6	111 ± 6
16	22.6 ± 4	42.6 ± 8.1	0.4 ± 0.2	28.8 ± 3.7	95 ± 16
32	21 ± 1.4	44.7 ± 1.7	1.1 ± 0.1	37.5 ± 2	104 ± 1
64	18.6 ± 1.3	35.4 ± 1.1	0.2 ± 0.1	41.7 ± 3.3	96 ± 5
90	21.9 ± 2.9	35.4 ± 3	0.2 ± 0.2	47.3 ± 3.3	105 ± 3
142	16.8 ± 0.4	24.4 ± 2.5	0.1 ± 0.1	50.8 ± 1.5	92 ± 2
193	18.5 ± 0.5	37.3 ± 1.5	0.1 ± 0.1	43.8 ± 0.8	100 ± 2
371	20.6 ± 3.4	36.3 ± 1.7	3.5 ± 3.2	40.3 ± 4.6	101 ± 4

[a] Includes acetochlor and metabolites.

Table 7. Distribution of Applied ^{14}C in the Sulfate-Amended Microcosms

time (days)	aqueous[a]	sorbed[a]	$H^{14}CO_3^-$/ $^{14}CO_2$	bound	total recovered
0	49.1 ± 0.9	47.8 ± 1.6	2 ± 0.8	1.3 ± 0	100 ± 1
8	22.6 ± 0.4	54.4 ± 0.4	0.3 ± 0.1	26.4 ± 1.7	104 ± 1
16	18.4 ± 0.7	50 ± 0.1	1 ± 0.2	33.3 ± 0.2	103 ± 1
24	15.5 ± 0.4	38.9 ± 1.2	0.1 ± 0.1	39.1 ± 0.7	94 ± 2
32	14.3 ± 0.5	34.6 ± 1.5	0.2 ± 0.1	42 ± 2	91 ± 4
64	13.6 ± 1.5	39.6 ± 1.1	0.5 ± 0.5	45.5 ± 1.2	99 ± 2
90	14.2 ± 0.3	42.1 ± 0.3	0.4 ± 0.3	44.7 ± 2.5	102 ± 2
142	13.9 ± 0.4	37 ± 0.5	1.1 ± 0.6	50.8 ± 1	103 ± 1
193	14.5 ± 0.6	37.7 ± 0.8	0.2 ± 0.2	42.4 ± 1.9	95 ± 3
371	14.9 ± 0.4	35.5 ± 0.8	0.4 ± 0.3	46.8 ± 0.8	98 ± 1

[a] Includes acetochlor and metabolites.

Table 8. Distribution of Applied ^{14}C in the γ-Irradiated Microcosms

	% of applied ^{14}C				
time (days)	aqueous[a]	sorbed[a]	$H^{14}CO_3^-$/ $^{14}CO_2$	bound	total recovered
0	46.7 ± 5.2	42.1 ± 2.8	1.6 ± 1.5	2.8 ± 0.1	93 ± 9
8	38.2 ± 3.0	54.3 ± 2.6	1 ± 0.9	10.2 ± 0.7	104 ± 7
16	33.1 ± 4.4	56.3 ± 2.3	1.3 ± 1.1	12.7 ± 0.3	103 ± 5
24	32.5 ± 3.6	44.2 ± 3	0.3 ± 0.3	16.9 ± 0.8	94 ± 6
32	31.3 ± 0.9	47 ± 5.3	1.2 ± 0.7	17.6 ± 2	97 ± 3
64	27.4 ± 2.3	45 ± 3.3	0.5 ± 0.3	24.1 ± 1.6	97 ± 3
90	25.1 ± 0.7	34.8 ± 2.6	0.9 ± 0.9	29.6 ± 3.3	90 ± 3
142	20.8 ± 2.5	25.7 ± 2.9	0.2 ± 0.1	35.5 ± 3.3	82 ± 6
193	21.7 ± 0.8	29.6 ± 1.5	5.8 ± 1.7	26.2 ± 1.5	83 ± 3
371	25.8 ± 0.4	31.1 ± 2.9	1.5 ± 0.8	30 ± 0.9	89 ± 3

[a] Includes acetochlor and metabolites.

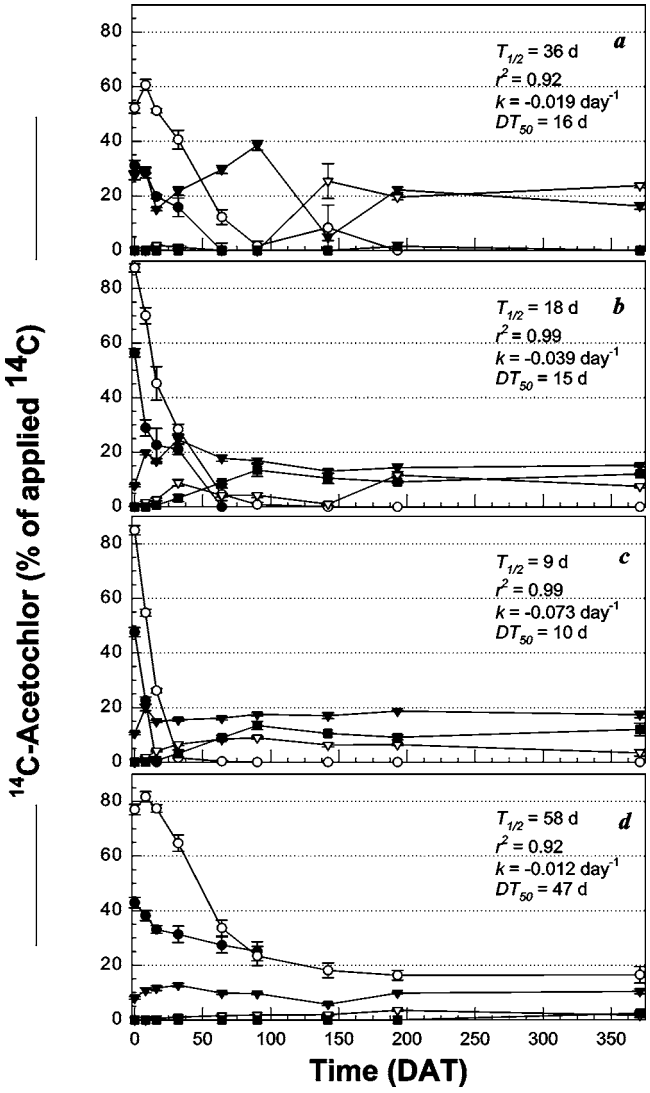

Figure 1. Distribution of [^{14}C]acetochlor and [^{14}C]metabolites in the (**a**) glucose-pretreated, (**b**) unamended, (**c**) sulfate-amended, (**d**) and unamended sterile microcosms. Total acetochlor (○), aqueous acetochlor (●), group 1 metabolites (▼), group 2 metabolites (▽), and metabolite 6 (■). The sorbed radiocarbon fraction is represented in the area between the total and the aqueous acetochlor lines. Error bars represent the standard error of the triplicate average. The aqueous acetochlor graph only spans the number of days that acetochlor was detected using HPLRC. Metabolites were only present at detectable levels in sorbed samples.

was close to 100% of applied ^{14}C (**Tables 5**−**8**), indicating excellent recovery of acetochlor and degradation products. Neither [^{14}C]methane nor [^{14}C]volatiles was detected in the headspace of microcosms.

[^{14}C]Acetochlor Degradation. [^{14}C]Acetochlor was depleted to <2% of the amount applied by day 90 in the glucose-pretreated and unamended microcosms and by day 32 in the sulfate-amended microcosms (**Figure 1a**−**c**). The sterile treatment had >40% of acetochlor remaining at day 90 (**Figure 1d**). The time observed for 50% disappearance of acetochlor (DT_{50}) was shortest in the sulfate-amended (10 days) and unamended (15 days) systems, with the period of acetochlor degradation corresponding to the most substantial increase in Fe(II) formation (**Tables 2** and **3**; **Figure 1b**,**c**). In the unamended and sulfate-amended microcosms, higher levels of Fe(II) were observed in the presence of acetochlor than in acetochlor-free microcosms. These data may suggest that iron-reducing bacteria are involved in acetochlor biodegradation. Because acetochlor was depleted most rapidly under iron- and sulfate-reducing conditions (**Figure 1c**), it appears that iron and sulfate reduction have a synergistic effect in the degradation of acetochlor. The presence of Fe(II) may ameliorate sulfide toxicity through FeS precipitation (*27*). Methanol may have had a role in cometabolism by sulfate reducers.

In a complex environment such as soil, many populations are present that are each capable of one or more types of anaerobic metabolism. The succession of anaerobic processes will proceed from least to most reductive depending on the availability of terminal electron acceptors. Degradation of herbicides and metabolites is likely to proceed both cometabolically with soluble components of soil organic matter and synergistically through combined activities of fermentative microorganisms, denitrifiers, iron reducers, sulfate reducers, and methanogens (again, depending on the availability of terminal electron acceptors), which may rely on each other for production

of suitable substrates for metabolism and disproportionation of fermentative byproducts to CO_2 and CH_4. The results of this study further demonstrate that in a flooded surface soil, iron-reducing processes may occur for many months, resulting in substantial Fe(II) formation. Fe(II)-catalyzed reduction of organic compounds has been described (*19*, *21*, *27*, *28*). The possibility that acetochlor degradation occurred as a result of methylotrophic cometabolism with methanol by methanogens was unlikely since acetochlor degradation preceded substantial methane formation; however, cometabolism of acetochlor and methanol by sulfate reducers was possible. Ultimately, iron reduction had the greatest impact on acetochlor depletion, and iron reduction is not supported by methanol.

In viable microcosms, the aqueous fraction was depleted of acetochlor prior to acetochlor depletion from the sorbed fraction (**Figure 1**). Nonequilibrium between solution and sorbed phase herbicide was expected, since biodegradation typically results in the removal of solution phase herbicide before slow desorption of sorbed herbicide occurs (*25*). In sterile microcosms, the ratio of sorbed to aqueous acetochlor remained nearly 1:1 until 93 days into the study (**Figure 1d**), suggesting that equilibrium existed between the two phases during this period.

Acetochlor depletion curves demonstrated good agreement ($r^2 = 0.92-0.99$) with the nonlinear first-order decay equation, which implies that the loss of substrate (acetochlor) was concentration-dependent. Depletion of a low concentration of chemical by a large number of cells may also follow first-order kinetics (*29*). The order from highest to lowest rate of acetochlor degradation was sulfate-amended > unamended > glucose-pretreated > sterile (**Figure 1**). $T_{1/2}$ and DT_{50} were in agreement for the sulfate-amended and unamended microcosms but varied with the glucose-pretreated and sterile microcosms. This variation may reflect the greater role of abiotic degradation in the latter two microcosms.

[^{14}C]Metabolites. Degradation of [^{14}C]acetochlor corresponded to the formation of up to seven different [^{14}C]-metabolites as indicated by nonparent HPLRC peaks. Identification of [^{14}C]degradation products was beyond the scope of this study, due to the lack of commercially available metabolite standards, and the unwillingness of laboratories that are familiar with herbicide degradation to analyze ^{14}C samples. However, the formation of metabolites and their persistence warrant a discussion of the trends observed in viable and sterile microcosms. To facilitate this discussion, we have categorized the metabolites according to how they appeared as follows: group 1 metabolites were formed instantaneously, immediately upon addition of the acetochlor to the microcosms (HPLRC retention times 12, 10.4, 9.4, and 7.8 min), group 2 metabolites were formed gradually (HPLRC retention times 17.8 and 13.2 min), and one metabolite (6, HPLRC retention time 11.3 min) was formed concomitant to Fe(II) production.

Group 1 metabolites were considered instantaneous and probably formed as a result of abiotic processes because they were present on day 0, and had been sampled and processed immediately after adding the acetochlor to each of the triplicate microcosms in the anaerobic chamber. The time elapsed from acetochlor addition to the removal of the aqueous fraction and addition of organic solvent to the soil fraction was 20 min (15 min of which was centrifugation at 4 °C). Group 1 [^{14}C]-metabolites represented 27% of applied ^{14}C in the glucose-pretreated microcosms on day 0 and 7−10% of applied ^{14}C other three test systems (**Figure 1a−d**). The chemistry of the glucose-pretreated microcosms was very reduced, as indicated by high levels of Fe(II) and methane relative to the other treatments

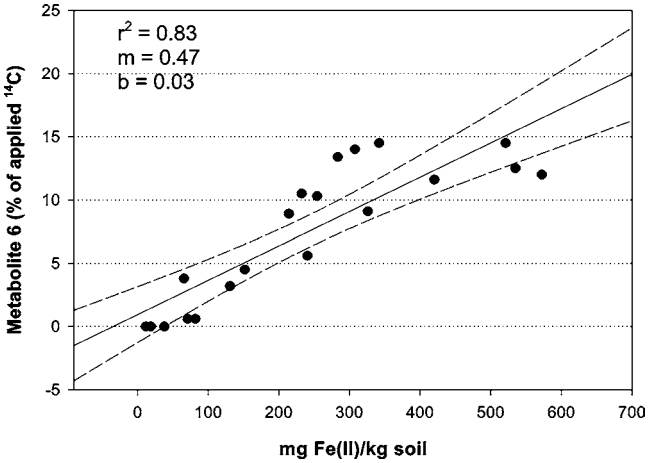

Figure 2. Linear regression (—) of Fe(II) vs [^{14}C]metabolite 6 formation in unamended and sulfate-amended microcosms. The dashed lines represent the 95% confidence interval.

(**Tables 1−4**); thus, it appears acetochlor was unstable under these conditions. Like the glucose-pretreated test systems, irradiated microcosms contained elevated Fe(II) levels and group 1 metabolites at experiment initiation. Previous studies have demonstrated the Fe(II)-catalyzed reduction of trifluralin (*19*) and carbomoyloxime pesticides (*30*). Group 1 metabolites were persistent.

Group 2 metabolites gradually accumulated to the highest levels in glucose-pretreated microcosms (24% of applied ^{14}C; **Figure 1**). Group 2 metabolites were formed at significantly higher levels in viable microcosms (10 and 16% of applied ^{14}C in the sulfate-amended and unamended, respectively) as compared to sterile microcosms (1.5% of applied ^{14}C); therefore, it is likely that the formation of these metabolites was facilitated by biological activity.

Up to 14.5% of the applied [^{14}C]acetochlor was transformed to a metabolite, designated "metabolite 6" (retention time 11.3 min; **Figure 1**), which was observed in the unamended and sulfate-amended microcosms and appeared to be related to Fe(II) formation. A linear regression of Fe(II) concentration vs the degradation product in the unamended and sulfate-amended treatments (**Figure 2**) had an r^2 of 0.82 ($p = 0.05$), which demonstrated a correlation between the Fe(II) production and the formation of this metabolite. The gradual appearance of this metabolite concurrent with Fe(II) formation suggests that this reaction was biologically mediated; however, without further study, we cannot discern the effect of iron reduction on acetochlor degradation as being biological, through direct metabolism of the herbicide by iron reducers, or abiotic, through reduction of acetochlor by microbially catalyzed Fe(II) formation. The prevalence of iron in soils and the favorable conditions for iron-reducing bacteria in soils suggest that iron reduction may have a significant impact on acetochlor in flooded soils.

Metabolite formation in the unamended and sulfate-amended microcosms was similar and differed from the trends in metabolite formation observed in glucose-pretreated and sterile microcosms (**Figure 1**). These results agree with the literature that mechanisms of anaerobic degradation vary with redox conditions, since anaerobic populations utilize organic compounds via different mechanisms. These results also seem to reflect abiotic vs biological degradation mechanisms, since, for example, the levels of metabolites formed instantaneously were highest in the prereduced (glucose-pretreated) microcosms. The sterile microcosms demonstrated the slowest rate of acetochlor degradation and the least amount of metabolites formed.

Formation of metabolite 6, which was correlated to Fe(II) production, was highest in the viable microcosms (**Figure 1**). Anaerobic degradation of the chloroacetamide herbicides dimethenamid (*13*) and alachlor (*31*) has also been demonstrated under anaerobic conditions in soil:water and aqueous systems, respectively. In the dimethenamid study, substantial degradation occurred under iron- and sulfate-reducing conditions. In general, less information is available concerning the behavior of herbicides in anaerobic environments than in aerobic soils. Both iron-reducing and sulfate-reducing bacteria are capable of metabolizing a variety of compounds (*32*, *33*), so it is important to consider their potential in herbicide degradation. The results presented here demonstrate that anaerobic degradation of acetochlor is possible in anaerobic saturated soils. The different trends in biological and chemical processes observed in the glucose-pretreated test system vs those detected in the unamended and sulfate-amended microcosms suggest the pretreatment with glucose selects for methanogens. Reductive dehalogenation is favored under methanogenic conditions (*34*). The unamended system simulates anaerobic herbicide degradation under flooded field conditions, since it permits the natural succession of redox processes that occur in saturated environments that do not have elevated levels of electron acceptors such as sulfate. Knowing which anaerobic processes contribute to herbicide degradation and metabolite formation places the information in an environmental context and may facilitate predictions of herbicide behavior in anaerobic environments.

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