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Metolachlor and Alachlor Breakdown Product Formation Patterns in Aquatic Field Mesocosms

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The transformation of metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] and alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide] in aquatic systems was investigated using outdoor tank mesocosms. Metolachlor and alachlor levels and their ethane sulfonic acid (ESA) and oxanillic acid breakdown products were monitored over time under five experimental treatments (each in quadruplicate). Background water conditions were identical in all treatments with each treatment differing based on the level and type(s) of herbicide present. Treatments included a no-herbicide control, 10 μg/L metolachlor, 25 μg/L metolachlor, 25 μg/L alachlor, and 25 μg/L alachlor plus 25 μg/L metolachlor in combination. The experiment was initiated by adding herbicide(s) to the units to the target concentrations; herbicide and breakdown product levels and other chemical parameters were then monitored for 85 days. In general, metolachlor half-lives were longer than alachlor half-lives under all treatments, although the differences were not statistically significant. Metolachlor half-lives (±95% confidence limits) ranged from 33.0 d (±14.1 d) to 46.2 d (±40.0 d), whereas alachlor half-lives ranged from 18.7 d (±3.5 d) to 21.0 d (±6.5 d) for different treatments. Formation patterns of ESA were similar in all treatments, whereas oxanillic acid formation differed for the two herbicides. Alachlor oxanillic acid was produced in larger quantities than metolachlor oxanillic acid and either ESA under equivalent conditions. Our results suggest that the transformation pathways for alachlor and metolachlor in aquatic systems are similar and resemble the acetochlor pathway in soils proposed by Feng (*Pestic. Biochem. Physiol.* **1991**, *34*, 136); however, the oxanillic acid branch of the pathway is favored for alachlor as compared with metolachlor.

Introduction

Metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] and alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide] are among the most commonly used pre-emergence herbicides for corn and soybean crops in the United States (*2*). Because of their extensive use and their moderate persistence, both herbicides and their breakdown products are accumulating in many agriculturally impacted surface waters and groundwaters (*3*−*7*). The two compounds are also moderately toxic (*8, 9*); therefore, the fate and attenuation of the two herbicides and their products is of extreme practical interest.

Metolachlor and alachlor are structurally similar (see Figure 1) and have similar modes of herbicidal action (*10*). They are both relatively water-soluble (metolachlor = 530 mg/L at 20 °C; alachlor = 240 mg/L at 25 °C) and have moderate octanol−water partition coefficients (metolachlor log K_{ow} = 3.13−3.45; alachlor log K_{ow} = 2.9) (*11*). Although both plants and microbial systems transform these compounds (*10*), neither compound is readily mineralized (*11*, *12*), and partial degradation products tend to accumulate in waters exposed to these two herbicides (*7*). In fact, herbicide breakdown product levels are frequently found at much higher levels than the parent compounds in natural waters. For example, Thurman et al. (*7*) found that a metabolite of alachlor, 2-[(2,6-diethylphenyl)(methoxymethyl)amino]-2-oxoethane sulfonate (alachlor ESA), was present in surface waters at up to 10 times the concentration of alachlor. Similar results have been observed for the metolachlor ESA (*4*). The possible significance of these breakdown products is sufficiently great to warrant the U.S. EPA to include alachlor ESA in its October 1997 Draft Drinking Water Contaminant Candidate List (*13*).

This paper is an extension of studies at the University of Kansas assessing the fate and attenuation of chloroacetinilide herbicides in aquatic systems (*14*). Here we present experimental results on the transformation and breakdown product formation patterns of alachlor and metolachlor. In particular, four herbicide treatments were assessed using field mesocosms (*15*−*17*) to evaluate alachlor and metolachlor decay rates and metolachlor and alachlor ESA and oxanillic acid formation patterns in water columns. The ultimate goal of this study, therefore, was to first assess decay rates and breakdown product formation patterns for these herbicides under quasi-natural field conditions and then to compare product formation patterns among treatments to better delineate transformation pathways for these herbicides in aquatic systems.

Materials and Methods

Field Program. *The Mesocosms.* The mesocosms used for this study were identical to those described previously by Graham et al. (*14*). The basic units were cylindrical, flat-bottomed fiberglass tanks, 3.2 m in diameter and 1.4 m deep. Thirty-two basic tanks were arranged in four rows of eight and placed in a shallow host pond located at the Nelson Environmental Study Area (NESA) of the University of Kansas near Lawrence, KS. Of these 32 tanks, 20 were used for the experiment described in this paper.

The mesocosms were initially filled with 11.3 m^3 of water from a protected source water reservoir at NESA. A surrounding host pond was then filled with water to a depth of 1.2 m to insulate the mesocosms from rapid changes in air

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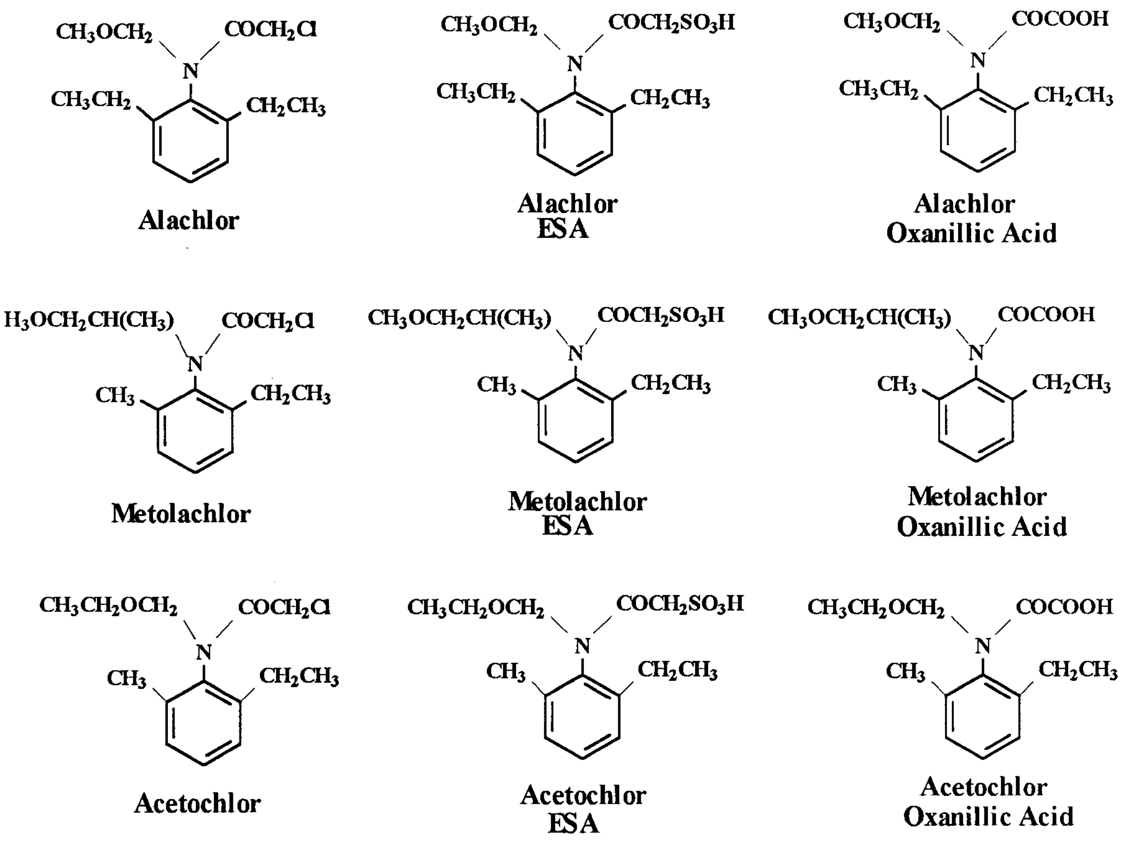

FIGURE 1. Structures of alachlor, metolachlor, acetochlor, and their ESA and oxanillic acid breakdown products.

temperature and to better replicate natural temperature and light conditions. The specific tank treatments in this study were identical to previously reported "aerobic" treatments (*14*). The only major manipulation to the basic tanks was the inclusion of two 39 cm × 53 cm plastic trays in the bottom of each tank that contained benthic sediments from an adjacent uncontaminated pond. These trays were intended to provide a microbial inoculum to the tank waters and to permit some macrophyte growth in the tanks during the study.

The construction and initial setup of the experimental systems was conducted in the early summer of 1998. The tank treatment arrangement for this study was selected using a randomized block design with four replicate tanks being prepared for each treatment and four tanks being retained as no-herbicide controls (see Table 1). All tanks were provided supplemental nitrogen (N) and phosphorus (P) (as KNO_3 and Na_2HPO_4, respectively) to achieve water column nutrient levels typical of regional reservoirs (*18*). The desired total nitrogen (TN) and total phosphorus (TP) levels were maintained continuously throughout the experiment using weekly amendments of inorganic N and/or P; the weekly additions were based on analyzed levels of TN and TP from the previous week's sampling event. The nutrients were added by mixing

TABLE 1. Mean Chemical Conditions under the Five Treatments in the Tank Mesocosms

treatment	k[a] (d^{-1})	half-life[a] (d)	TN[b,c] (mg/L)	TP[b,c] (mg/L)	DOC[b,c] (mg/L)	TOC[b,c] (mg/L)	TIC[b,c] (mg/L)	temp[d] (°C)	pH[d]	conductivity[d] ($m\Omega$/cm)	mean DO[d] (mg/L)	chlorophyll-*a*[b,c] (mg/L)
10 μg/L metolachlor	0.015	46.2	1.16	0.071	17.4	21.1	7.7	26.5	9.6	0.257	13.8	0.026
	(0.013)	(40.0)	(0.16)	(0.011)	(5.5)	(6.2)	(2.6)	(1.4)	(0.2)	(0.017)	(2.1)	(0.017)
25 μg/L metolachlor	0.021	33.0	1.12	0.061	16.6	19.2	7.8	26.6	9.7	0.248	13.5	0.024
	(0.009)	(14.1)	(0.39)	(0.012)	(5.3)	(6.0)	(1.7)	(1.5)	(0.3)	(0.022)	(1.6)	(0.015)
25 μg/L alachlor	0.033	21.0	1.13	0.062	13.7	15.3	7.6	26.6	9.4	0.249	13.4	0.032
	(0.010)	(6.4)	(0.26)	(0.016)	(3.6)	(5.0)	(1.2)	(1.5)	(0.3)	(0.024)	(1.2)	(0.022)
25 μg/L metolachlor[e]	0.020	34.7										
combined	(0.007)	(11.6)	1.17	0.057	13.6	16.2	8.8	26.6	9.4	0.243	12.0	0.027
25 μg/L alachlor[e]	0.037	18.7	(0.29)	(0.011)	(4.1)	(4.9)	(2.3)	(1.5)	(0.3)	(0.012)	(1.5)	(0.008)
combined	(0.007)	(3.5)										
no herbicide control	N/A	N/A	0.98	0.048	13.6	16.5	9.0	26.5	9.4	0.237	12.2	0.020
			(0.14)	(0.006)	(2.9)	(3.7)	(2.7)	(1.5)	(0.3)	(0.008)	(1.8)	(0.009)

[a] Estimated first-order decay coefficient and half-life. Mean values based on estimates from four tanks under each treatment; 95% confidence intervals (%95 CI) are provided in parentheses. [b] TN, TP, DOC, TOC, TIC, and chorophyll-*a* are mean values based on seven measurements over time per tank per treatment. [c] The analytical MDLs for TN, TP, DOC, TOC, TIC, and chorophyll-*a* were 0.1, 0.002, 0.1, 0.1, 1.0, and 0.001 mg/L, respectively. [d] Temperature, pH, conductivity, and mean DO are depth-weighted averages based three samples per sampling event per tank per treatment. [e] Treatment where alachlor and metolachlor were combined.

the powdered reagent grade chemicals with 4 L of the target mesocosm water in a plastic carboy. The dissolved mixture was then dispersed into the tanks by gently stirring with a paddle.

The mesocosms were monitored for one month (July 1998) prior to herbicide addition to allow the mesocosms to develop stable water conditions and to verify that the desired physiochemical water conditions were developing. On August 3, 1998, alachlor and metolachlor were added to the respective tanks. The alachlor amendment was provided using commercial grade Lasso (Monsanto Corp., St Louis, MO), which has an alachlor content of 45.1 wt %. The metolachlor amendment was provided using Dual II (Ciba-Geigy Corp, Greensboro, NC), which has a metolachlor content of 84.4 wt %. The herbicides were emulsified in 1 L of deionized water and dispersed into the tanks with a paddle as described previously (*14*).

Field Sampling and Processing. Field measurements of dissolved oxygen (DO), pH, conductivity, and water temperature were made weekly at three depths (0.3, 0.7, and 1.2 m) using a Water Checker Field Monitor (Horiba Instruments). Depth-integrated water samples for laboratory analysis were also collected weekly using 1.2 m length, 25 mm diameter PVC tubes equipped with a check valve at the bottom end. Typically, the sampling tubes were inserted slowly down into the mesocosm waters. Upon withdrawal, the sampler captured a depth-integrated sample of the whole water column.

Sample processing of the depth-integrated samples was as follows. The samples were typically divided into 500- and 125-mL fractions and separated for specific analyses. The 500-mL samples were stored in acid-washed, amber glass bottles equipped with Teflon-lined caps. The 125-mL samples were stored in amber glass serum bottles equipped with a crimp-sealed, Tygon septum. All samples were maintained in the dark, in coolers with ice packs, prior to their return to the laboratory for analysis.

Upon returning to the laboratory, the 500-mL sample was divided as follows. Ten milliliters was separated for total organic carbon (TOC) analysis, and 250 mL was separated and filtered through prerinsed Whatman GF/F glass fiber filters (particle retention of $>0.7\mu$m) for chlorophyll-*a* (captured particulate) and inorganic ion (filtrate) analyses. The remaining volume was retained for total nitrogen (TN) and total phosphorus (TP) analyses. The filtrate was divided into 125 mL and ca. 10 mL volumes for herbicide and DOC analysis, respectively, and the associated glass fiber filter was retained for chlorophyll-*a* analysis. The filters were stored at −10 °C in aluminum foil for no more than 30 days prior to final analysis. All filtration was performed under low light conditions to minimize degradation of the chlorophyll-*a*. All samples collected for TN and TP analysis were processed immediately. The TOC and DOC samples were acidified to ca. pH 2 with 85% H_2PO_4, stored at 4 °C, and processed within 72 h.

The 125-mL sample was amended with sodium azide at the time of sample collection and used for total inorganic carbon (TIC) analysis. The TIC sample was prepared for analysis by removing 5 mL of liquid via syringe to provide a headspace in the flask. The sample was acidified using 0.5 mL of 7 N HCl to convert the inorganic carbon in the sample to CO_2. Headspace CO_2 levels were then determined using gas chromatography, and TIC levels were calculated using the CO_2 data.

Analytical Procedures. *Herbicide and Herbicide Breakdown Product Analysis.* All herbicide and breakdown product analyses were performed by the United States Geological Survey (USGS) located in Lawrence, KS. Automated GC/MS analysis of alachlor and metolachlor was performed using a Hewlett-Packard model 5890 gas chromatograph and a 5970A mass selective detector (MSD), in selected ion mode (minimum detection level = 0.05 μg/L; MDL), according to procedures described by Scribner et al. (*19*). The analyses of alachlor and metolachlor ESA and alachlor and metolachlor oxanillic acid also were performed at the USGS laboratory. Analyses were performed using a Hewlett-Packard model 1090 high-performance liquid chromatograph (HPLC) equipped with a diode-array detection system (MDL = 0.20 μg/L), which has been described previously (*4*, *20*). The accuracy of this analysis was corroborated by supplemental analysis using a more sensitive HPLC−MS analytical procedure (MDL = 0.05 μg/L) that was developed near the end of the experimental program.

Other Chemical Analysis. DOC and TOC were determined using a Dohrmann total organic carbon analyzer. TP was analyzed spectrophotometrically following wet digestion with acidic potassium persulfate (*21*, *22*). Similarly, TN was measured spectrophotometrically following alkaline persulfate digestion (*21*, *23*). The digests were stored at 4 °C for no longer than 2 days prior to analysis using a Shimadzu UV 160 spectrophotometer.

TIC was determined by analyzing 0.2 mL of the sample bottle headspace gas for CO_2 using a Carle 311 analytical gas chromatograph equipped with a thermal conductivity detector and an Alltech 1.82 m, 0.00635 m diameter, CTR1 column coupled to a Hewlett-Packard model 3392A integrator. Chlorophyll-*a* was analyzed spectrophotometrically following extraction with hot ethanol (*24*).

Data Analysis. *Estimation of Mean Conditions in the Different Mesocosms.* The estimated mean water conditions in each tank were based on a minimum of six samples per mesocosm collected over the course of the experiment. Data from the four replicate mesocosms (per treatment) were then combined to produce overall descriptive means for each treatment. The 95% confidence intervals (95% CI) were calculated from standard errors associated with each mean. Details of the data analysis procedures have been provided elsewhere (*14*).

Estimation of Herbicide Decay Rates and Half-Lives. Curve-fitting techniques were used to estimate herbicide decay rates for each treatment condition. For the purpose of developing decay rate estimates that were comparable with previous studies, the decay rates were estimated based on herbicide data collected from day 15 to day 57 of the experiment (after herbicide addition). Due to problems with the herbicide analysis, the herbicide analytical data collected before day 15 were not used in the curve-fitting analysis or in subsequent mass balances. After day 57, the mesocosms were monitored for an additional 4 weeks (to day 85) to provide long-term data for assessing breakdown product formation patterns.

A first-order decay model fit the decay data most closely. Decay rates and 95% CI for each tank were developed based on the measured herbicide levels over time in each tank ($n > 5$ samples). The decay rates and 95% CI levels for each treatment were then estimated based on cumulative data from all tanks under each treatment. Half-lives for each treatment were calculated directly from the estimated mean herbicide decay rates.

Results

Physical and Chemical Conditions Developed under the Treatments. Table 1 summarizes the mean water conditions observed in the mesocosms under the four treatments and in the no-herbicide control. The table shows that highly reproducible water conditions were developed in all mesocosms. The TN levels for all tanks were consistently in the eutrophic range ($0.65 < \text{TN [mg/L]} < 1.2$) (*25*). TP ($0.030 < \text{TP [mg/L]} < 0.10$) and chlorophyll-*a* ($0.009 <$ chlorophyll-*a* $\text{[mg/L]} < 0.025$) levels were also in the eutrophic range. The mean pH under all treatments was always higher than the

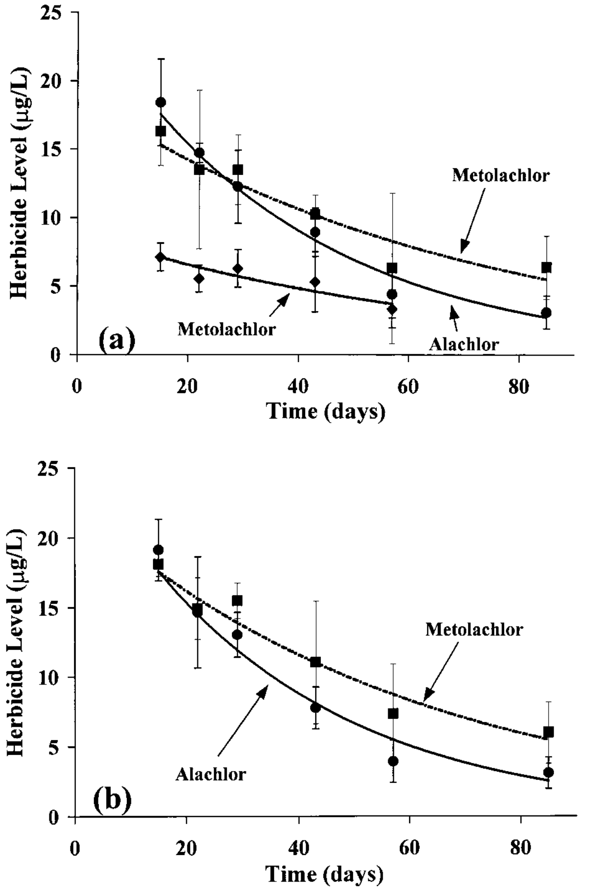

FIGURE 2. Alachlor disappearance curves for the (a) single herbicide treatments and (b) combined herbicide treatment. Each point represents the mean herbicide level based on four samples. Error bars refer to 95% confidence intervals. Trendlines for each treatment are provided.

source water (pH = 8.2), indicating depressed levels of CO$_2$ in solution and high levels of photosynthesis in all treatments. DO levels also were consistently high in all mesocosms and were typically supersaturated (range from 12 to 14 mg/L). In general, the physiochemical data suggest similar conditions among the treatments, with all mesocosms having photosynthesis-dominated, eutrophic waters. No significant differences in water conditions were observed between the no-herbicide controls and the herbicide-amended units.

Herbicide Decay Rates. Figure 2 presents the herbicide decay patterns over time for all treatments. First-order decay coefficients and half-life estimates for each treatment are summarized in Table 1. The longest half-life observed was for the 10 μg/L metolachlor treatment (46.2 days, 95% CI of ±40.0 days). Half-lives for the 25 μg/L metolachlor only and 25 μg/L alachlor only treatments were 33.0 d (±14.1 d) and 21.0 d (±6.4 d), respectively. The half-lives for metolachlor and alachlor in the combined treatment (25 μg/L of each) were 34.7 d (±11.6 d) and 18.7 d (±3.5 d).

The rate of metolachlor disappearance in the low metolachlor treatment (10 μg/L) was about 30% lower than the high metolachlor only treatment, although this difference was not statistically significant (see Table 1). Table 1 also shows that the half-lives for herbicides in the combined treatment were similar to the half-lives observed in the single herbicide treatments (18.7 vs 21.0 days for alachlor and 34.7 vs 33.0 days for metolachlor). No inhibitory or synergistic effects were apparent in herbicide decay when the two herbicides were combined.

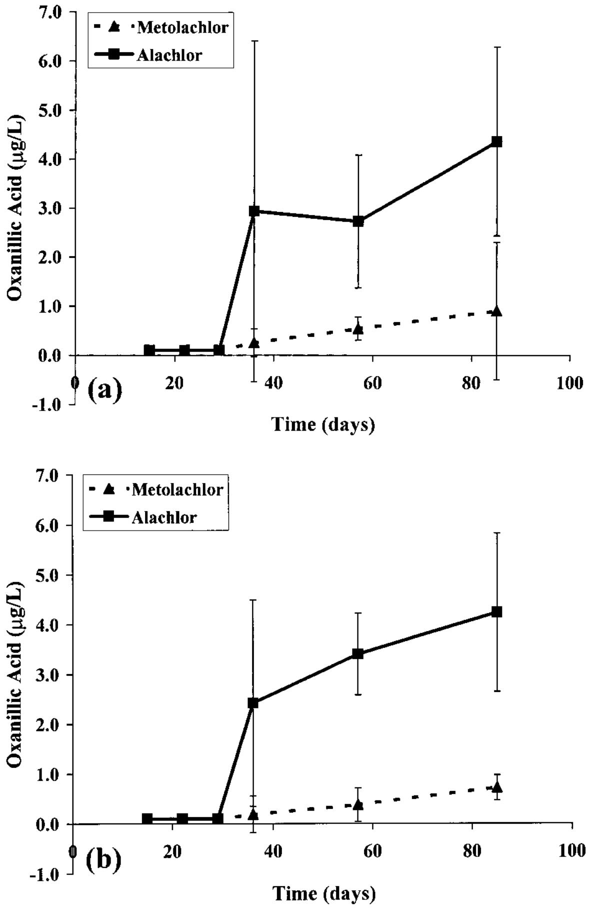

FIGURE 3. Oxanillic acid formation patterns for the (a) single herbicide, 25 μg/L treatments and (b) combined herbicide, 25 μg/L treatment. Each point represents the mean product level based on four samples. Error bars refer to 95% confidence intervals.

Herbicide Breakdown Product Formation Patterns. Figures 3 and 4 summarize the formation patterns of the ESAsand oxanillic acids of alachlor and metolachlor for the three 25 μg/L herbicide-amended treatments. Figure 3a shows that greater quantities of alachlor oxanillic acid were formed as compared with metolachlor oxanillic acid in the single 25 μg/L herbicide treatments. Similarly, Figure 3b shows that alachlor oxanillic acid also was formed in greater quantities than metolachlor oxanillic acid in the combined treatment. In contrast, Figure 4 shows that roughly equal amounts of alachlor ESA and metolachlor ESA were formed under all 25 μg/L herbicide treatments. In general, alachlor oxanillic acid was more readily formed than either herbicide ESA or metolachlor oxanillic acid in our systems.

Table 2 shows that only between 39.2% and 49.0% (depending upon the treatment) of the herbicide carbon present on day 15 was recovered as parent compound, oxanillic acid, or ESA at the end of the experiment (day 85). Note that all concentrations in Table 2 have been normalized to μmol/L to permit direct comparison of parent compound carbon and breakdown product carbon for the mass balance calculations. Interestingly, the fractional recovery of carbon as parent, ESA or oxanillic acid on day 85 was very similar for the two herbicides despite the fact that the parent decay rates were different.

Discussion

Physiochemical Conditions in the Mesocosms and Herbicide Transformation Rates. Two key criteria must be met

TABLE 2. Fractional Herbicide–Carbon Recovery Levels after 85 Days of Exposure

treatment	metolachlor only	metolachlor/alachlor combined: metolachlor	metolachlor/alachlor combined: alachlor	alachlor only
herbicide, day 15[a]	0.89 (0.13)[b]	0.99 (0.047)	1.05 (0.12)	1.01 (0.17)
herbicide, day 85	0.36 (0.051)	0.33 (0.051)	0.17 (0.015)	0.16 (0.045)
oxanillic acid, day 85	0.050 (0.078)	0.040 (0.014)	0.24 (0.088)	0.24 (0.11)
ESA, day 85	0.023 (0.074)	0.021 (0.094)	0.037 (0.027)	0.023 (0.0092)
total herbicide carbon[c]	0.43 (0.074)	0.39 (0.066)	0.45 (0.075)	0.42 (0.87)
% recovered	48.3	39.3	42.9	41.6

[a] Measured herbicide level 15 days after herbicide addition. [b] All concentrations are provided as μmol/L; 95% confidence intervals are provided in parentheses. [c] Total herbicide–carbon present on day 85. This is the sum of the day 85 levels of herbicide, oxanillic acid, and ESA levels (in μmol/L).

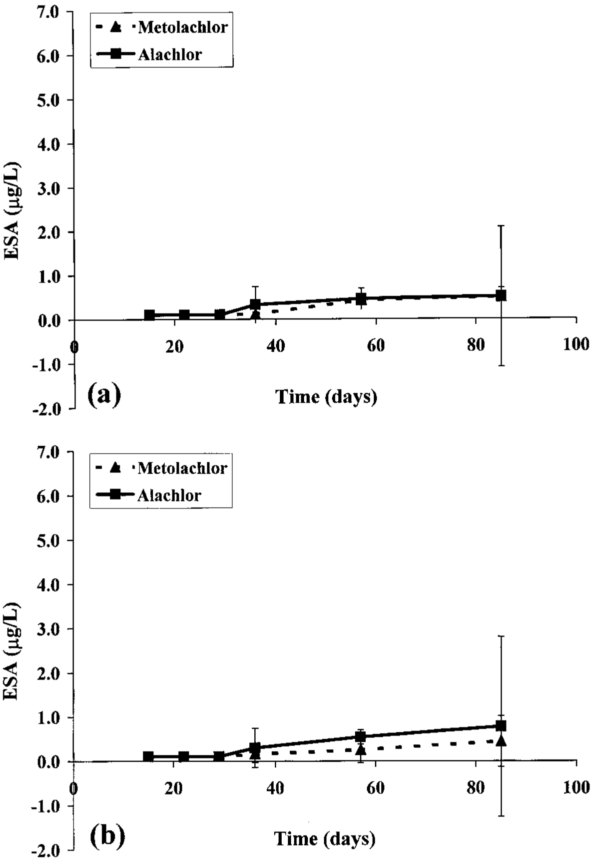

FIGURE 4. Ethane sulfonic acid (ESA) formation patterns for the (a) single herbicide, 25 μg/L treatments and (b) combined herbicide, 25 μg/L treatment. Each point represents the mean product level based on four samples. Error bars refer to 95% confidence intervals.

for the effective use of field aquatic mesocosms for the study of in situ contaminant transformation phenomena. First, the developed water conditions must be realistic and should reflect conditions in natural ecosystems. Second, consistent conditions within treatments and significantly contrasting conditions across treatments must be created. In this experiment, our major experimental goal was to create stable, reproducible eutrophic aerobic water conditions for assessing herbicide transformation phenomena. The data presented in Table 1 indicate that we were successful in creating such environments in this study. The water conditions were well replicated across treatments and were different from past treatments (26).

Metolachlor was more persistent than alachlor in our systems, although the difference in persistence was not statistically significant. The mean metolachlor half-life was 33.8 d as compared with 19.8 d for alachlor at the 25 μg/L initial herbicide levels. This result is consistent with previous observations for metolachlor and alachlor transformation, where alachlor has been found to break down moderately rapidly in aerobic systems (27), whereas metolachlor is more stable and persistent (28). For comparison, Chesters et al. (10) found that metolachlor half-lives ranged from ca. 36 to 182 d in soils as compared with only ca. 7–14 d for alachlor.

Implications of Breakdown Product Formation Patterns. Figures 3 and 4 summarize the formation patterns of alachlor and metolachlor oxanillic acid and ESA for the individual and combined 25 μg/L herbicide treatments. The production level of alachlor oxanillic acid was considerably greater than alachlor ESA in both the individual and combined herbicide treatments. This consistent result between the individual and combined treatments is strong confirmation that the alachlor oxanillic acid is the more prominent breakdown product formed in aquatic systems. In contrast, metolachlor oxanillic acid formation levels were only slightly greater than metolachlor ESA formation levels in all metolachlor treatments (Figure 4). These data suggest that alachlor and metolachlor probably have similar transformation pathways in natural systems; however, the amount of carbon processed through oxanillic acid versus ESA differs as a function of the specific herbicide assessed. This difference in carbon processing might partially explain why metolachlor is more persistent than alachlor or acetochlor in natural systems (see later).

Feng (1) had previously proposed that acetochlor, which is structurally similar to alachlor, was transformed by soil microorganisms via a β-glutamyl transpeptidase-mediated (β-GTP) pathway. In that pathway, it was proposed that acetochlor was initially conjugated with glutathione (GSH) and was subsequently transformed to a relatively stable acetochlor–cysteine conjugate. This conjugate could then be transformed either to acetochlor sulfinylacetic acid, to an alcohol that was subsequently oxidized to acetochlor oxanillic acid, or to a thiol that was oxidized to the acetochlor ESA. The thiol formation reaction was proposed to be catalyzed by cysteine–S conjugate β-lyase (Cβ-lyase), which is a common enzyme in many bacteria (29).

Our data suggest that both alachlor and metolachlor are transformed in aquatic systems through a reaction sequence similar to that of acetochlor in soils; however, our results indicate that the alcohol-mediated transformation step from the herbicide–cysteine conjugate to oxanillic acid occurs more readily for alachlor than metolachlor. Although we do not have a specific explanation for why metolachlor is not significantly processed to oxanillic acid, we can speculate. A hypothetical schematic is provided in Figure 5 (1). It is possible that the comparatively large metolachlor headgroup (relative to either alachlor or acetochlor; see Figure 1) hinders simple displacement reactions that, in turn, limit the rate of formation of metolachlor oxanillic acid. In principle, the processing of the metolachlor–cysteine conjugate proceeds in roughly equal quantities through the alcohol and thiol

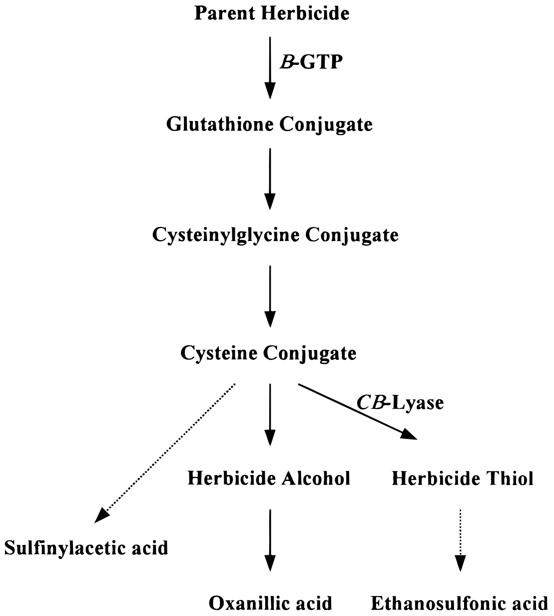

FIGURE 5. Schematic of the proposed transformation pathway for chloroacetanilide herbicides in aquatic systems [after Feng (*1*)]. Suspected enzyme-catalyzed reactions are noted. Dashed lines refer to undefined reaction sequences.

intermediates (also possibly to sulfinylacetic acid), whereas alachlor, which has a smaller headgroup than metolachlor, is primarily processed through a displacement reaction through the alcohol to oxanillic acid. Although it is strictly speculative, it possible that the inability to readily form oxanillic acid might explain why metolachlor has a longer half-life than alachlor and acetochlor. Further experimentation is required to test this hypothesis.

Practical Implications. The issue of nonpoint-source agricultural runoff into streams and lakes is the subject of much regulatory activity in the United States and abroad. The associated regulations must consider the combined effect of both herbicides themselves and nutrients associated with the runoff stream. As this and our previous papers demonstrate (14, 26), both the chemistry of the specific herbicide and the chemistry of the receiving environment can impact the natural processing of herbicides. The results presented here show that breakdown product formation patterns differ for alachlor and metolachlor, even though the two compounds are quite similar structurally. This current paper has provided new data on possible transformation pathways for these two compounds in aquatic systems, although more work is clearly required to determine the relevance of these pathways in natural systems. Studies are ongoing, examining the ecotoxicological impact and transformation characteristics of these herbicides and their breakdown products, and it hoped that an effects-based description of these compounds will be possible in the near future.

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