Monitoring Stereoselective Degradation of Metolachlor in a Constructed Wetland: Use of Statistically Valid Enantiomeric and Diastereomeric Fractions as Opposed to Ratios

Mohamed Y.Z. Aboul Eish[1,2] and **Martha J.M. Wells**[1,*]

[1]Center for the Management, Utilization, and Protection of Water Resources and Department of Chemistry, Tennessee Technological University, Box 5033, Cookeville, TN 38505 and [2]American University of Sharjah, P.O. Box 26666, Sharjah, UAE

Abstract

Environmentally contaminated aqueous samples are examined for evidence of stereoselective degradation of metolachlor. The unique chemical structure of metolachlor, a chloroacetamide herbicide, consists of four stereoisomers due to axial and/or C-chirality. The degradation of metolachlor is monitored over time in agricultural runoff water that is applied to a subsurface flow constructed wetland. Metolachlor stereoisomers are isolated from aqueous samples by achiral reversed-phase solid-phase extraction and analyzed by normal-phase high-performance liquid chromatography using a chiral stationary phase. The analyses of 64 post-application samples, collected over a period of four weeks, are reported. The samples are filtered (0.45 µm) prior to analysis and thereby represent metolachlor in solution and/or associated with dissolved organic carbon. Sixteen samples demonstrate total racemic metolachlor concentrations greater than 10 ppb. Of these 16 samples, one sample is determined statistically to demonstrate enantioselective degradation. Significant contributions made by this study include the evaluation of stereoselectivity based on mathematically derived fractions, rather than ratios, and statistical evaluation of precision establishing the variability resulting from chromatographic processes versus metabolic processes. The research demonstrates that distribution of metolachlor between the solid phase composed of chemical and/or biological particulates and the aqueous phase is not primarily stereoselective, and that stereoselectively enriched metolachlor does not dominate in the aqueous phase.

Introduction

Metolachlor, a chloroacetamide, is one of the most frequently used pesticides in the United States (1,2). The unique chemical structure of metolachlor (Figure 1) results in four stereoisomers (3,4). Metolachlor has both axial- and C-chirality. C-chirality results from asymmetrical substitution about the C-atom at the alkyl methyl group, and axial-chirality results from hindered rotation around the phenyl-nitrogen bond (5). Stereoisomers of metolachlor were conformationally stable with no significant isomerization detected at ambient temperature (1,4,6,7). A racemic mixture of metolachlor was applied in this study. A formulation of the active *S*-isomers of metolachlor is also currently marketed (*S*-metolachlor). The *S*-configuration of metolachlor exhibits herbicidal activity while the *R*-configuration has fungicidal activity (3,8).

Many herbicides have chiral centers resulting in multiple stereoisomers that are enantiomers, diastereomers, and/or atropisomers. Different stereoisomers of contaminants may be

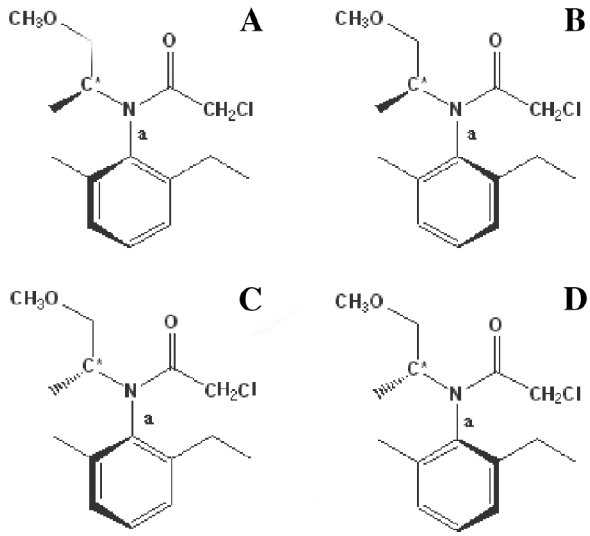

Figure 1. Metolachlor stereoisomers. (A) αR,1S; (B) αS,1S; (C) αR,1R; and (D) αS,1R; axial chirality = a; C-chirality = *.

* Author to whom correspondence should be addressed: email mjmwells@tntech.edu.

preferentially metabolized by microorganisms and differ in biological effects in the environment (9,10). Enantiomerically enriched pesticides are now being introduced as a means of reducing the amount of inactive chemicals introduced into the environment (6). Chiral chromatographic separations are used to investigate the potential for stereoselective degradation. However, analyzing chromatographic data using ratios rather than fractions of chromatographic peaks, and analyzing data without appropriate statistical attention to variability in the chromatographic measurements themselves can lead to erroneous determinations of stereoselective degradation in the environment.

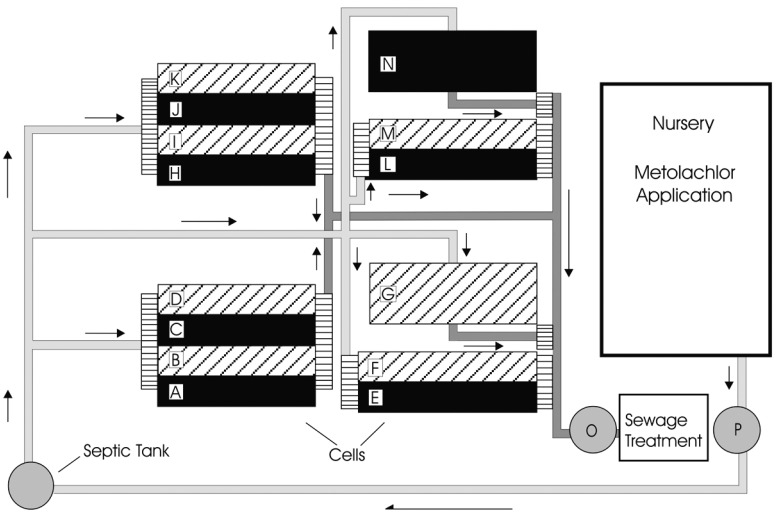

Figure 2. Subsurface flow constructed wetland system. The characteristics of cells A–N are presented in Table I; O and P indicate runoff tanks. Arrows represent effluent flow from the nursery to the wetland. Solid cells are vegetated; hatched cells are not vegetated.

Table I. Characteristics of Subsurface Flow Constructed Wetland Cells

Cell*	Plants†	Depth (cm)	Length (m)	Width (m)	Aspect ratio	Volume of water applied (L/day)	Estimated hydraulic detention time (days)‡
A	y	30	4.9	1.2	4:1	240	2.2
B	n	30	4.9	1.2	4:1	240	2.0
C	y	30	4.9	1.2	4:1	120	4.9
D	n	30	4.9	1.2	4:1	120	3.8
E	y	30	4.9	1.2	4:1	60	12.2
F	n	30	4.9	1.2	4:1	60	7.3
G	n	30	2.4	4.9	1:2	120	7.3
H	y	46	4.9	1.2	4:1	240	3.5
I	n	46	4.9	1.2	4:1	240	3.1
J	y	46	4.9	1.2	4:1	120	7.7
K	n	46	4.9	1.2	4:1	120	6.1
L	y	46	4.9	1.2	4:1	60	19.3
M	n	46	4.9	1.2	4:1	60	11.6
N	y	46	2.4	4.9	1:2	120	19.3

* Data from the literature (16).
† Bulrush (Scirpus validus); y = plants present, and n = no plants.
‡ Based on average flow accounting for evapotranspiration and precipitation.

Historically, determination of stereoselective degradation has been based primarily upon the calculation of enantiomeric ratios (ERs); that is, ER = Peak Area 1/Peak Area 2, derived from the areas of peaks in a chromatogram. However, recent evaluation has called into question the use of ERs for this purpose in favor of the calculation of enantiomeric fractions (EFs); that is, EF = Peak Area 1/(Sum of Peak Areas 1 and 2) (11,12,13). Problems encountered when ER values were used include: (*i*) the ER value was undefined when one of the enantiomers was not detected (11); (*ii*) plots of ER versus the peak area of one enantiomer were nonlinear (11,12); (*iii*) graphical plots of ER misrepresented the data due to the asymmetric, lognormal distribution of the function (12,13); (*iv*) the ER value was more complicated to use in mathematical expressions of fate (12,13); and (*v*) because of the asymmetric nature of the ER value, it was not well described by parametric statistics, such as the mean and standard deviation (12). EF is a bounded, additive scale, ranging from 0 to 1.0 as a normal distribution where EF = 0.5 represents a racemic mixture; therefore, each unit of standard deviation is equivalent for both positive and negative values around 0.5 (12,13). In contrast, ER is an unbounded, multiplicative scale (12). The ER value for a racemate is 1.0, but ER can range from 0 to infinity (13). In addition, mathematically calculating either ER or EF from the other value is questionable (12). All statistical comparisons in this research were based on EF and diastereomeric fraction (DF) values using parametric statistics (i.e., mean, standard deviation, analysis of variance), and ER values are reported without statistical evaluation for comparison to historical data. The majority of studies on stereoselectivity reported to date are based on stereoisomeric ratios, not stereoisomeric fractions. Any chromatographic evaluation that uses a ratio of peak areas will exhibit nonparametric characteristics and should be evaluated with nonparametric statistics.

In this research, degradation of racemic metolachlor was monitored in aqueous samples during field application to a containerized nursery from which the runoff was passed through a subsurface flow constructed wetland. The ornamental plant nursery industry traditionally applies large amounts of pesticides and herbicides to potted plants (14). Subsurface flow constructed wetlands provide a cost-effective treatment technology to reduce the pollution resulting from containerized plant production. The subsurface flow constructed wetland used in this study has been previously demonstrated to remove nutrients and herbicides, including metolachlor, from agricultural runoff (15,16). Metolachlor stereoisomers were isolated from filtered (0.45 µm) aqueous samples by achiral reversed-

phase solid-phase extraction (SPE) and analyzed by normal-phase high-performance liquid chromatography (HPLC) using a chiral stationary phase (17). The chromatographic data were used to determine whether enantiomeric and/or diastereomeric differences in degradation among the stereoisomers of metolachlor were observed in the environmentally contaminated aqueous samples analyzed. Statistical evaluation of variability contributing to analyses by the HPLC process (injection through detection) was necessary to implement decision-making regarding the occurrence of enantiomeric and/or diastereomeric selectivity.

Experimental

Chemicals and reagents

Ethyl acetate (Optima grade), methanol (Optima or HPLC grade), water (HPLC grade), potassium phosphate monobasic, and potassium phosphate dibasic (A.C.S. certified) were purchased from Fisher Scientific, Fair Lawn, NJ. *n*-Hexane (HPLC grade, 95%) and isopropyl alcohol (HPLC grade, 99.9%) were purchased from Acros Organics, Fair Lawn, NJ. Metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methyl) acetamide] standard (minimum purity, 95%) was obtained from Ultra Scientific (North Kingstown, RI).

Study site

Water samples were collected from a subsurface flow constructed wetland (Figure 2) located in Baxter, TN. The constructed wetland was designed by Dr. Dennis B. George and maintained by Drs. George and G. Kim Stearman, associated with Tennessee Technological University (15,16). Treated wastewater from a municipal facility was used to irrigate a containerized ornamental plant nursery. Racemic metolachlor was applied once to the nursery area at the beginning of the study. The effluent from the nursery was directed into the constructed wetland. Discharge from the cells was released to a sewage treatment area.

The constructed wetland (Table I) consisted of 14 cells (465 m^2 total) that differed in design by volume, flow rate, discharge rate, detention time, and the presence or absence of bulrushes (*Scirpus validus*). The cells (labeled A–N) were divided into shallow (30 cm) and deep cells (46 cm). The cells were filled with two types of gravel that differed in size (small gravel, 1.9–2.2. cm; and large gravel, 1.3–3.8 cm). The shallow cells were filled with a 20 cm layer of large gravel, topped with a 10 cm layer of small gravel. The deep cells were filled with a 30 cm layer of large gravel, topped with a 16 cm layer of small gravel.

Aqueous samples (approximately 1 L) were collected from each cell, the septic tank (S), and runoff tanks (O and P) weekly, over a period of four weeks. Cell samples were collected from a standpipe at the side of the cells with a plastic syringe. The syringes were pre-rinsed with the cell effluent. The standpipe drew the samples from the bottom of the cells. Samples from the runoff and septic tanks were collected using a Teflon bailer. Samples were pressure filtered under nitrogen (40 psi) through 0.45 µ plain supported nylon filters (Magna, Osmonics, Inc., Minnetonka, MN). The filtrate was stored at 4°C until analyzed.

Metolachlor recovery

Achiral SPE was used to extract metolachlor from fortified and environmentally contaminated samples using C_{18} Mega Bond Elut sorbent (1.0 g columns, Varian Inc., Harbor City, CA), according to a procedure adapted from Wells et al. (18). Extraction columns were conditioned by passing 10 mL methanol, followed by 10 mL of phosphate buffer (0.1 M, pH 7), through the sorbent. Sample loading of known volumes of approximately 1 L onto the sorbent was performed under vacuum (600 mm Hg). During conditioning and sample adsorption, the column was not allowed to dry. To dry the sorbent, vacuum was applied to the columns for about 10 min after sample adsorption. Metolachlor was desorbed using 10 mL methanol. The methanol was evaporated under nitrogen, and the residue was dissolved in 1 mL ethyl acetate. Samples were allowed to equilibrate 24 h at 4ºC before analysis.

Analysis

Metolachlor standards, fortified samples, and samples collected from the constructed wetland were analyzed at room temperature by chiral HPLC (Model 1050, Hewlett-Packard). Samples (50 µL aliquots) were injected on a chiral normal-phase column, (R,R) Whelko-1 CSP (17), 25 cm × 4.6 mm, derived from 4-(3,5-dinitrobenzamido)tetrahydrophenanthrene covalently bound to 5 µm silica (Regis Technologies, Inc., Morton Grove, IL). The mobile phase consisted of isopropyl alcohol–*n*-hexane (10:90, v/v) delivered at 1.5 mL/min. Ultraviolet absorbance was monitored at 254 nm.

Calculations

Calculation of enantiomer and diastereomer fractions and ratios was based on the areas of the three chromatographic peaks identified in Figure 3. The enantiomeric fraction of Peak 1, EF_1, was determined by:

$$EF_1 = \frac{Area1}{Area1 + Area2 + Area3} \qquad \text{Eq. 1}$$

while the corresponding enantiomeric fraction represented by Peaks 2 and 3, EF_{23}, was calculated as:

$$EF_{23} = \frac{Area2 + Area3}{Area1 + Area2 + Area3} \qquad \text{Eq. 2}$$

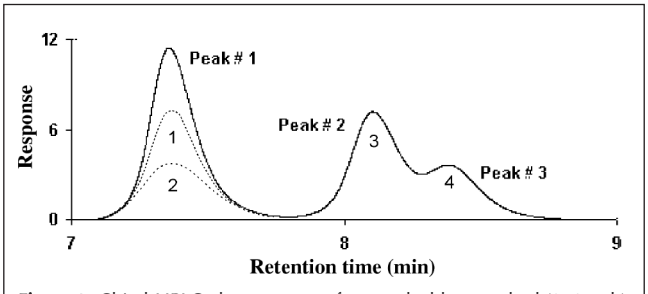

Figure 3. Chiral HPLC chromatogram for metolachlor standard (940 ppb) (solid line). The hypothetical contributions of compounds 1 and 2 to Peak #1 (dotted lines).

The diastereomeric fraction of Peak 2, DF_2, was defined as:

$$DF_2 = \frac{Area2}{Area2 + Area3} \quad \text{Eq. 3}$$

the enantiomeric ratio of Peak 1, ER_1, was determined by:

$$ER_1 = \frac{Area1}{Area2 + Area3} \quad \text{Eq. 4}$$

the diastereomeric ratio of Peak 2, DR_2, was calculated by:

$$DR_2 = \frac{Area2}{Area3} \quad \text{Eq. 5}$$

In each equation, the subscript referred to the peak or peaks occurring in the numerator.

Data interpretation

Chromatograms were obtained in Excel-readable format and were integrated using a Peak Fit program (Version 4, AISN Software, Inc.). The Statistical Analysis System (SAS, Version 8, SAS Institute Inc., Cary, NC) was used to evaluate the data.

Results and Discussion

The enantioselective degradation of metolachlor in spiked and incubated soil samples was reported by Muller and Buser (7) and Polcaro et al. (19). In surface water and rain, Muller and Buser (7) found that the enantiomers of metolachlor were present as racemates such that little stereoselective biological degradation occurred. Buser et al. (6) attributed enantioselective determinations in lake water to an environmental response to the introduction of a nonracemic, *S*-metolachlor, product. Klein et al. (20) determined that metolachlor biodegradation was not enantioselective in water and soil samples.

Chromatographic separation

Metolachlor stereoisomers A and C (Figure 1) and stereoisomers B and D differ from each other in the orientation about the C-chiral center, thereby representing two sets of enantiomers. The pairs of stereoisomers A and B, as well as C and D, differ from each other by the orientation about the axial-chiral center, resulting from restricted rotation about the nitrogen-aryl carbon atom bond, thereby representing two sets of diastereomers. Compounds A and D and compounds B and C differ from each other at both C-chiral and axial-chiral centers and also represent diastereomers.

Buser and Muller (1) used chiral high-resolution gas chromatography (HRGC) and chiral high-performance liquid chromatography (HPLC) to study the chromatographic separation of enantiomers and diastereomers of metolachlor. With chiral HPLC, metolachlor was resolved into three partially resolved peaks with one pair of unresolved stereoisomers. C-chiral enantiomers were more easily resolved than axial-chiral enantiomers (1). Muller et al. (4) separated metolachlor stereoisomers using a combination of achiral and chiral HPLC. Interconversion occurred between diastereomers during GC analysis at an injector temperature of 250–280ºC (4). Klein et al. (20) analyzed metolachlor and two polar metabolites using liquid chromatography–mass spectrometry and capillary zone electrophoresis. Kabler and Chen (21) developed a method for separating and quantitating the diastereomer pairs of metolachlor and *S*-metolachlor in water using SPE and chiral-column liquid chromatography–mass spectrometry–mass spectrometry. Polcaro et al. (19) separated the four metolachlor stereoisomers by chiral HPLC on silica gel coated with a cellulose tris-(3.5-dimethylphenylcarbamate) derivative.

The four stereoisomers of metolachlor are not known to be available as individual standards. Muller et al. (4) analyzed *rac*-metolachlor and *S*-metolachlor, while Polcaro et al. (19) analyzed *rac*-metolachlor and two diastereomeric mixtures. These researchers each used polarimetric detection, chiral chromatography, and deduction to establish the absolute stereochemistry of the stereoisomers of metolachlor.

In this research, chiral chromatography of *rac*-metolachlor standards without polarimetric detection was applied. The best chiral separation achieved for the four stereoisomers of metolachlor (Figure 3) produced only three chromatographic peaks. The greatest degree of separation observed was between Peak 1 and Peaks 2 and 3, differing in retention time by approximately 1 min; the resolution (R_S) between Peak 1 and Peak 2 was equal to 2.0, and between Peak 1 and Peak 3 was equal to 2.1. The value of R_S between Peak 2 and Peak 3 was equal to 0.5. The stereoisomers that spent more time associated with the chiral stationary phase rather than the achiral mobile phase were better separated.

Although only three chromatographic peaks were resolved, it was possible to apply chemical deduction to calculate the *EF* value and one of the two possible *DF* values from the data obtained to examine whether selective degradation was observed. To calculate these values, it was not necessary to establish the absolute stereochemistry of the individual compounds. The only necessary assumption was that enantiomers, being of equal concentration in a racemic sample, exhibit equivalent responses to UV detection (i.e., equivalent peak areas), while diastereomers do not.

In this research, the identities of compounds 1, 2, 3, and 4 as labeled in Figure 3 were not matched to the compounds A, B, C, and D in Figure 1. No attempt was made to establish the absolute stereochemistry of the compounds investigated, because it was not necessary to do so to answer the question of the potential for enantiomeric or diastereomeric selectivity in metolachlor degradation.

The area of Peak 1 was approximately equal to the sum of the areas of Peaks 2 and 3 (EF_1 = 0.489 ± 0.011 and EF_{23} = 0.511 ± 0.011), based upon *rac*-metolachlor in the 10–100 ppb range. Therefore, Peak 1 was deduced to be composed of two unresolved diastereomers, compounds 1 and 2, indicated by dotted lines in Figure 3, that are anticipated to have UV responses equivalent to compounds 3 and 4, respectively. The value for EF_1, comparing Peak 1 to the sum of the areas of Peaks 1, 2, and 3, is therefore the correct parameter for assessing enantioselectivity of metolachlor in the samples.

Continuing the process of chemical deduction, Peaks 2 and 3 were concluded to represent a pair of diastereomers (compounds 3 and 4). Considering only the area of standards for Peaks 2 and

3, the relationship is not enantiomeric because the peak areas are dissimilar and the calculated DF_2 value of nondegraded metolachlor was 0.620 ± 0.024, based upon *rac*-metolachlor in the 10–100 ppb range. A 50/50 racemic relationship did not exist between the areas of the two compounds represented by Peaks 2 and 3 because the compounds were not enantiomeric. Therefore, the value for DF_2 that compares the area of Peak 2 to the combined areas of Peaks 2 and 3 is the correct parameter for assessing the diastereomeric selectivity of compounds 3 and 4, and by analogy, that of compounds 1 and 2.

However, with only three peaks resolved, the relationship of degradation between compounds 1 and 4 and between compounds 2 and 3 cannot be assessed. Because compound 4 is an enantiomer of compound 2, the relationship in peak area in the nondegraded state between compounds 1 and 4 is the same as that between 1 and 2. However, it would be incorrect to assume that the two sets of diastereomers, one set resulting from a single site of axial chirality and the other set resulting from both axial-chirality and C-chirality, should necessarily exhibit the same degradation patterns.

To summarize, in the chromatographic separation achieved in this research, Peak 1 was concluded to result from two unresolved diastereomers while Peaks 2 and 3 were enantiomers of the unresolved diastereomers in Peak 1. Buser and Muller (1) also observed that resolution of C-chiral enantiomers, corresponding to the resolution between compounds 1 and 3 and between compounds 2 and 4 in our research, was easier to achieve than resolution of the diastereomers of metolachlor.

Precision of the HPLC assay of metolachlor stereoisomers

To evaluate the occurrence of enantiomeric and/or diastereomeric selectivity in field samples, the statistical variability inherent in the chromatography of standards, representing variability in the HPLC process from injection through detection, was evaluated (Table II). The stereoisomeric ratios, ER_2 and DR_2, were reported without statistical evaluation. Parametric statistics were used to evaluate the stereoisomeric fractions, EF_1 and DF_2.

Standards ranging over three orders of magnitude were evaluated, from approximately 1 ppb to 1,000 ppb, expressed as racemic metolachlor concentration. The detector response over

Table II. Stereoisomeric Fractions and Ratios of Metolachlor Standards

Racemic Concentration (ppb)	N	EF_1*,†	ER_2	DF_2	DR_2
0.9	3	0.453 ± 0.037[a]	0.834	0.660 ± 0.049[ab]	1.979
5.0	5	0.462 ± 0.032[ab]	0.864	0.618 ± 0.008[b]	1.619
9.4	5	0.483 ± 0.004[ab]	0.935	0.618 ± 0.015[b]	1.621
50	3	0.491 ± 0.013[ab]	0.967	0.616 ± 0.033[b]	1.616
94	5	0.493 ± 0.014[ab]	0.975	0.626 ± 0.030[b]	1.684
940	6	0.497 ± 0.006[c]	0.989	0.692 ± 0.013[a]	2.248

* Mean ± SD.
† Means within each column followed by the same letter are not significantly different (α = 0.01).

Table III. Stereoisomeric Fractions of Field Samples

	EF_1* Time (weeks)					DF_2* Time (weeks)			
Cell	1	2	3	4	Cell	1	2	3	4
A	NA†	NA	NA	NA	A	NA	NA	NA	NA
B	0.491 ± 0.007	0.490 ± 0.008	NA	NA	B	0.634 ± 0.034	0.582 ± 0.023	NA	NA
C	NA	NA	NA	NA	C	NA	NA	NA	NA
D	NA	NA	ND‡	ND	D	NA	NA	ND	ND
E	NA	0.482 ± 0.014	NA	0.508 ± 0.006	E	NA	0.573 ± 0.016	NA	0.612 ± 0.060
F	ND	ND	NA	NA	F	ND	ND	NA	NA
G	ND	ND	NA	ND	G	ND	ND	NA	ND
H	0.494 ± 0.008	0.484 ± 0.025	NA	0.493 ± 0.007	H	0.613 ± 0.022	0.576 ± 0.022	NA	0.614 ± 0.042
I	NA	0.498 ± 0.003	NA	NA	I	NA	0.598 ± 0.006	NA	NA
J	0.492 ± 0.007	0.490 ± 0.012	NA	NA	J	0.594 ± 0.032	0.588 ± 0.011	NA	NA
K	NA	0.489 ± 0.014	NA	NA	K	NA	0.589 ± 0.044	NA	NA
L	ND	ND	ND	–§	L	ND	ND	ND	–
M	ND	NA	NA	–	M	ND	NA	NA	–
N	NA	NA	NA	ND	N	NA	NA	NA	ND
O	0.507 ±0.004	0.506 ± 0.020	NA	ND	O	0.589 ± 0.011	0.585 ± 0.049	NA	ND
P	0.520 ± 0.005**	NA	ND	–	P	0.600 ± 0.012	NA	ND	–
S	0.502 ± 0.007	0.504 ± 0.005	NA	–	S	0.584 ± 0.035	0.575 ± 0.019	NA	–

* The reference EF_1 value of standards = 0.489 ± 0.011, and the reference DF_2 value of standards = 0.620 ± 0.024; mean ± SD.
† Metolachlor detected, but racemic concentration < 10 ppb.
‡ Not detected.
§ Sample lost in analysis.
** Value was significantly different from standards (α = 0.01).

this entire range was nonlinear (i.e., quadratic), which is common in chromatography over a wide range of concentrations. As is typical for variability in concentration measurements, the confidence intervals (not shown) were greater at the lowest and highest concentration levels within the range of 1 to 1,000 ppb.

Based on the analysis of variance (Table II) and the desire for consistency in measuring EF_1 and DF_2, subsequent calculations of the stereoisomeric fractions for samples were conducted by applying the calculated values for standards in the linear range of the detector, between 10–100 ppb. The standard deviation was greater for samples less than 10 ppb and the values at the highest concentration were statistically different (Table II). Statistical evaluation verified the need to assess only chromatographic peaks that occurred in the linear range of the detector.

The results of analysis of variance (Table II) indicated differences in the measurement of stereoisomeric fractions due to concentration. However, concentration differences did not cause inherent variability in the stereoisomeric fractions measured; the variability occurred because standards could not as easily be detected and measured at the extremes of the concentration scale.

Examination of stereoselective degradation in field samples

Field samples (approximately 1 L) were collected. In this screening study, the entire sample was used in each analysis. The field samples were not replicated. Stereoisomeric fractions for field samples for which parametric statistics were conducted are reported in Table III. Table IV presents stereoisomeric ratios for field samples without statistical evaluation for comparison to historical data. Sixty-four post-application samples, collected over a period of four weeks, were analyzed for the stereoisomers of metolachlor. The samples were filtered (0.45 µm) prior to analysis, and thereby represented metolachlor in solution and/or associated with dissolved organic carbon (DOC). The potential for stereoselectivity of metolachlor associated with filtered particulates was not analyzed but should be considered in future studies.

The observation of variability in precision among standards substantiates the need for careful statistical evaluation on which to base the decision of stereoselectivity observed in chromatographic data for field samples. In sixteen samples, the total racemic metolachlor concentrations were greater than 10 ppb. Of the sixteen samples evaluated, one sample, collected from runoff tank P at week 1, was determined by analysis of variance of EF_1 across all samples to demonstrate enantioselective degradation. Sample P at week 1 was the only sample determined to have an EF_1 value that differed from the standard value of 0.489 ± 0.011. The EF_1 values for sample E at 28 d, sample H at 7 and 28 d, sample I at 14 d, sample O at 7 and 14 d, and sample S at 7 and 14 d were not different than sample P at week 1, but were also not different than the EF_1 value for the standard of 0.489 ± 0.011. No samples demonstrated diastereoselective degradation determined by analysis of variance of DF_2 values for samples relative to the reference standard DF_2 value of 0.620 ± 0.024.

Conclusions

Metolachlor stereoisomers were monitored in a subsurface flow constructed wetland optimized for nutrient and herbicide degradation. Significant contributions of this study include the evaluation of stereoselectivity based on mathematical fractions

Table IV. Stereoisomeric Ratios of Field Samples

	ER_1 Time (weeks)					DR_2 Time (weeks)			
Cell	1	2	3	4	Cell	1	2	3	4
A	NA*	NA	NA	NA	A	NA	NA	NA	NA
B	0.964	0.961	NA	NA	B	1.749	1.400	NA	NA
C	NA	NA	NA	NA	C	NA	NA	NA	NA
D	NA	NA	ND	ND	D	NA	NA	ND	ND
E	NA	0.930	NA	1.034	E	NA	1.344	NA	1.617
F	ND†	ND	NA	NA	F	ND	ND	NA	NA
G	ND	ND	NA	ND	G	ND	ND	NA	ND
H	0.976	0.940	NA	0.974	H	1.587	1.365	NA	1.609
I	NA	0.991	NA	NA	I	NA	1.487	NA	NA
J	0.969	0.960	NA	NA	J	1.476	1.429	NA	NA
K	NA	0.959	NA	NA	K	NA	1.453	NA	NA
L	ND	ND	ND	–‡	L	ND	ND	ND	—
M	ND	NA	NA	–	M	ND	NA	NA	—
N	NA	NA	NA	ND	N	NA	NA	NA	ND
O	1.029	1.026	NA	ND	O	1.434	1.432	NA	ND
P	1.085	NA	ND	—	P	1.505	NA	ND	—
S	1.008	1.015	NA	—	S	1.418	1.356	NA	—

* Metolachlor detected, but racemic concentration < 10 ppb.
† Not detected.

as opposed to ratios, and statistical evaluation of the precision of the chromatographic process to determine if stereoselective degradation occurred. Although few studies of the stereoselectivity of metolachlor have been conducted, in the results reported to date, stereoselective degradation was reported to be more prevalent in soil than in water. Because stereoselectivity was not frequently observed in the aqueous phase, it can be inferred that sorption from water of metolachlor by particulate phases does not appear to be stereoselective. However, once metolachlor stereoisomers sorb on mineral or organic chemical particulates and/or bacterial or biofilm solids, stereoselective degradation may occur. If the enantiomer that is not selectively metabolized in the particulate phase is desorbed from solid matter into the aqueous phase, stereoselectivity in the water column would be observed. Metolachlor stereoisomers associated with DOC (< 0.45 µm) may be protected from stereoselective degradation.

References

1. H-R. Buser and M.D. Muller. Environmental behavior of acetamide pesticide stereoisomers. 1. Stereo- and enantioselective determination using chiral high resolution gas chromatography and chiral high performance liquid chromatography. *Environ. Sci. Technol.* **29(8):** 2023–2030 (1995).
2. R.I. Wilson and S.A. Mabury. Photodegradation of metolachlor: Isolation, identification, and quantification of monochloroacetic acid. *J. Agric. Food Chem.* **48:** 944–950 (2000).
3. H. Moser, G. Rihs, and H. Sauter. The influence of atropisomerism and chiral center on the biological activity of metolachlor. *Z. Naturforsch.* **37B:** 451–462 (1982).
4. M.D. Muller, T. Poiger, and H-R. Buser. Isolation and identification of the metolachlor stereoisomers using high-performance liquid chromatography, polarimetric measurements, and enantioselective gas chromatography. *J. Agric. Food Chem.* **49:** 42–49 (2001).
5. E.L. Eliel and S.H. Wilen. Stereochemistry of Organic Compounds. John Wiley and Sons, New York, NY, 1994.
6. H-R. Buser, T. Poiger, and M.D. Muller. Changed enantiomer composition of metolachlor in surface water following the introduction of the enantiomerically enriched product to the market. *Environ. Sci. Technol.* **34:** 2690–2696 (2000).
7. M.D. Muller and H-R. Buser. Environmental behavior of acetamide pesticide stereoisomers. 2. Stereo- and enantioselective degradation in sewage sludge and soil. *Environ. Sci. Technol.* **29:** 2031–2037 (1995).
8. S. Jayasundera, W.F. Schmidt, C.J. Hapeman, and A. Torrents. Influence of the chemical environment on metolachlor conformations. *J. Agric. Food Chem.* **47(10):** 4435–4442 (1999).
9. D.L. Lewis, A.W. Garrison, K.E. Wommack, A. Whittemore, P. Steudler, and J. Melillo. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* **401:** 898–901 (1999).
10. W.J.M. Hegeman and R.W.P.M Laane. Enantiomeric enrichment of chiral pesticides in the environment. *Rev. Environ. Contam. Toxicol.* **173:** 85–116 (2002).
11. H-J. de Geus, P.G. Wester, J. de Boer, and U.A.Th. Brinkman. Enantiomer fractions instead of enantiomer ratios. *Chemosphere* **41:** 725–727 (2000).
12. E.M. Ulrich, D.R. Helsel, and W.T. Foreman. Complications with using ratios for environmental data: comparing enantiomeric ratios (ERs) and enantiomer fractions (EFs). *Chemosphere* **53:** 531–538 (2003).
13. T. Harner, K. Wiberg, and R. Norstrom. Enantiomer fractions are preferred to enantiomer ratios for describing chiral signatures in environmental analysis. *Environ. Sci. Technol.* **34:** 218–220 (2000).
14. D.J. Sutherland, G.K. Stearman, and M.J.M. Wells. Development of an analytical scheme for simazine and 2,4-D in soil and water runoff from ornamental plant nursery plots. *J. Agric. Food Chem.* **51:** 14–20 (2003).
15. M.C. Kemp and D.B. George. Subsurface flow constructed wetlands treating municipal wastewater for nitrogen transformation and removal. *Water Environ. Res.* **69(7):** 1254–1262 (1997).
16. D.B. George, G.K. Stearman, K. Carlson, and S. Lansford. Simazine and metolachlor removal by subsurface flow constructed wetlands. *Water Environ. Res.* **75(2):** 101–112 (2003).
17. C.J. Welch and T. Szczerba. HPLC separation of the enantiomers of commercial agricultural compounds using Whelk-O CSP. *Enantiomer* **3(1):** 37–43 (1998).
18. M.J.M. Wells, D.D. Riemer, and M.C. Wells-Knecht. Development and optimization of a solid-phase extraction scheme for determination of the pesticides metribuzin, atrazine, metolachlor and esfenvalerate in agricultural runoff water. *J. Chromatogr. A* **659:** 337–348 (1994).
19. C. Polcaro, A. Berti, L. Mannina, C. Marra, M. Sinibaldi, and S. Viel. Chiral HPLC resolution of neutral pesticides. *J. Liq. Chromatogr & Rel. Technol.* **27(1):** 49–61 (2004).
20. C. Klein, R.J. Schneider, M.T. Meyer, and D.S. Aga. Enantiomeric separation of metolachlor and its metabolites using LC-MS and CZE. *Chemosphere* **62:** 1591–1599 (2006).
21. A.K. Kabler and S. Chen. Determination of the 1′S and 1′R diastereomers of metolachlor and S-metolachlor in water by chiral liquid chromatography–mass spectrometry/mass spectrometry (LC–MS–MS). *J. Agric. Food Chem.* **54:** 6153–6158 (2006).

Manuscript received February 19, 2007;
revision received June 27, 2007.