S-Metolachlor, S-MESA

Rac-Metolachlor, Rac-MESA

**Analysis of Metolachlor Ethane Sulfonic Acid (MESA) Chirality in
Groundwater: A Tool for Dating Groundwater Movement in
Agricultural Settings**

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ABSTRACT

To better address how much groundwater contributes to the loadings of pollutants from agriculture we developed a specific dating tool for groundwater residence times. This tool is based on metolachlor ethane sulfonic acid, which is a major soil metabolite of metolachlor. The chiral forms of metolachlor ethane sulfonic acid (MESA) and the chiral forms of metolachlor were examined over a 6-year period in samples of groundwater and water from a groundwater-fed stream in a riparian buffer zone. This buffer zone bordered cropland receiving annual treatments with metolachlor. Racemic (rac) metolachlor was applied for two years in the neighboring field, and subsequently *S*-metolachlor was used which is enriched by 88% with the *S*-enantiomer. Chiral analyses of the samples showed an exponential increase in abundance of the *S*-enantiomeric forms for MESA as a function of time for both the first order riparian buffer stream ($R^2 = 0.80$) and for groundwater within the riparian buffer ($R^2 = 0.96$). However, the *S*-enrichment values for metolachlor were consistently high indicating different delivery mechanisms for MESA and metolachlor. A mean residence time of 3.8 years was determined for depletion of the initially-applied rac-metolachlor. This approach could be useful in dating groundwater and determining the effectiveness of conservation measures.

40 ***One sentence summary:***

41 A mean residence time of 3.8 years was calculated for groundwater feeding a first-order
42 stream by plotting the timed-decay for the *R*-enantiomer of metolachlor ethane sulfonic
43 acid.

1. Introduction

Under the 1972 Clean Water Act, many US waterways are listed as impaired often due to nitrate from agricultural nonpoint sources, resulting in total maximum daily load (TMDL) restrictions (USEPA, 2009). Estimating these loads is done using models that require numerous inputs including knowledge of groundwater contributions. However, significant uncertainties exist in this process especially concerning the residence time of nitrate-N in groundwater (Lindsey et al., 2003). A number of approaches have been utilized to improve these estimates, including isotopic ratio changes in nitrate or water, fortuitous detection of soluble anthropogenic compounds, or gaseous analysis (Lindsey et al., 2003). No selective dating markers exist for groundwater processes in an agricultural setting.

Metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(1-methoxypropan-2-yl)acetamide) is a major-use, pre-emergent herbicide for maize and soybean production and has been registered in the US since 1976 (Fernandez-Cornejo et al., 2014). After application, metolachlor is readily transported into the soil where it is transformed into several compounds, the most abundant being, metolachlor ethane sulfonic acid (2-[(2-ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxoethanesulfonic acid, MESA) (Baran and Gourcy, 2013; Bayless et al., 2008; White et al., 2010). MESA can be found in groundwater wherever metolachlor has been used and it appears to be quite stable in groundwater (Baran et al., 2004; Barbash et al., 1999; Bayless et al., 2008; Denver et al., 2010; Domagalski et al., 2008; Huntscha et al., 2008; Kalkhoff et al., 2012; Krutz et al., 2006; Rebich et al., 2004).

MESA is also more mobile than metolachlor due to its much greater water solubility (MESA: $2.12 \times 10^5 \text{ mg L}^{-1}$; metolachlor: 530 mg L^{-1}) (Bayless et al., 2008) and smaller organic carbon partition coefficient (K_{oc}) values (MESA K_{oc} : $13.8 \pm 2.4 \text{ L kg}^{-1}$; metolachlor K_{oc} : $172 \pm 25 \text{ L kg}^{-1}$, Baran and Gourcy, 2013). All these properties taken together suggest that MESA under the right conditions can be a good indicator of nitrate loadings from agriculture (McCarty et al, 2014). For example, concentrations of MESA were relatively high in the unconfined groundwater of Morgan Creek in Maryland (United States), and MESA loads were well correlated with nitrate-N loads (Domagalski et al., 2008). Most recently, MESA was used to examine the fate of nitrate-N in the Choptank River (Maryland) both upstream and in the downstream estuary (McCarty et al., 2014).

Another interesting feature of metolachlor and MESA is that they both exist as a set of four stereoisomers, Fig. 1. As with many biologically-active compounds, one enantiomer is often more active than the other, and this is true for metolachlor, where the *S*-form is more active than the *R*-form (Blaser et al., 2007). In the US, metolachlor applications were switched to the more active *S*-metolachlor form after 1988. Prior to this time, metolachlor was applied as a racemic mixture (rac), equal amounts of *S*- and *R*-enantiomers. Several studies based on analysis of chiral forms of metolachlor have made use of this unique time marker produced by the abrupt switch to the more-efficacious *S*-metolachlor. The chiral ratios of metolachlor were calculated for samples obtained from Swiss lakes in 1998 and 1999, where use of *S*-metolachlor began in 1997 (Buser et al., 2000). A rapid environmental response to the changed structural-composition of the herbicide was observed in surface waters that received fresh input from runoff enriched in the *S*-enantiomers, whereas, the deeper water in the lakes

retained ratios closer to the racemic composition. A similar approach was used to describe several processes involved in distributing metolachlor in southern Canada (Kurt-Karakus et al., 2010b). There are no reports for chiral analyses of MESA in the literature, nor are there reports where chiral analysis of metolachlor in groundwater samples is conducted.

Although some research has examined the change in chirality of metolachlor, little research has been carried out exploiting the chiral forms of MESA which has a much longer persistence and is observed at larger concentrations in the environment than metolachlor (Baran and Gourcy, 2013; Graham et al., 1999; Huntscha et al., 2008; Kalkhoff et al., 2012). The transformation of metolachlor to MESA is a biotic process involving substitution of the chlorine with a sulfonic moiety (Field and Thurman, 1996), and this occurs without any rearrangements in the chiral carbon centers (Klein et al., 2006). These authors examined enantiomer ratios for metolachlor while also monitoring MESA to establish this non-enantioselectivity; however, others have just examined the disappearance of enantiomers of metolachlor, and there are contradicting reports for chiral selectivity. Ma et al. 2006 showed preferential degradation of the *S*-enantiomers and Mailaard et al. 2016 showed preferential degradation of the *R*-enantiomer while Aboul Eish et al. 2009 found no enantioselectivity. Since there is controversy on this point and no conclusive evidence to contradict us we assumed for this paper that enantiomeric changes in metolachlor commercial products should be reflected in similar chiral shifts in MESA, i.e., the applied product of 88% *S*-metolachlor will produce *S*-MESA that has the same enantiomeric composition.

In this paper, we use the chiral ratios of MESA and metolachlor to describe the groundwater flushing times in a riparian buffer adjacent to cropland that was under continuous culture of *Zea mays* and was treated annually with metolachlor. Using the enantiomeric ratio, the mean residence time was determined for this riparian area. This approach should be useful in dating the groundwater in other areas and in assessing the effectiveness of conservation measures such as installing riparian buffers or wetlands.

2. Methods and materials

2.1 Materials

Standard reference material for racemic (rac) MESA (CGA-354743) and *R*- and *S*-MESA (SYN-502271 and CGA-380168, respectively) were obtained from Syngenta (Greensboro, NC). Racemic metolachlor was obtained from Chem Services (West Chester, PA) 98.8% pure. Internal standards for quantitation were [¹³C] 2, 4-dichlorophenoxyacetic acid, obtained from Cambridge Isotope Laboratories Inc., (Cambridge, Massachusetts) and terbuthylazine, obtained from Dr. Ehrenstorfer, GMBH (Augsburg, Germany). The surrogate standard triphenylphosphate (TPP) was obtained from Supelco Inc. (Bellefonte, Pennsylvania).

Solvents, acetonitrile, and methanol, were high purity, pesticide-grade from Burdick & Jackson (Honeywell International Inc., Muskegon, MI). Carbon-free deionized water (DI water) was obtained from a NANOpure system (Barnstead International, Dubuque, IA). GF/F glass microfiber filters (70 mm diameter, 0.7 µm pore size) were from Whatman Inc., (Florham Park, New Jersey). OASIS HLB cartridges, 500 mg, were

obtained from Waters Inc (Milford, Massachusetts). Liquid chromatography columns were a C-18 Ultrasphere ODS column (4.6 mm x 250 mm, 5 µm) (Beckman Coulter, Chaska, MN) and a Waters Inc. XTerra (X-Terra MS C-18 column, (5 mm x 150 mm, 5 µm) for routine quantitation of MESA and metolachlor. For chiral separation of these compounds, a reverse phase column Chiralpak QN-AX column, (4.6 mm x 150 mm, O-9-[tert-butylcarbanoyl] quinidine immobilized on 5 µm silica gel; Chiral Technologies Europe, West Chester, PA) with a QN-AX guard column (4 mm x 10 mm, 5 µm) was used for MESA; and for metolachlor a normal phase column, Chiralpak AS-H column (250 mm x 6.0 mm, 5 µm; Chiral Technologies Europe) was used.

2.2 Site description

The study area is part of the Lower Chesapeake Bay Long-Term Agroecosystem Research site located at the USDA-Beltsville Agricultural Research Center in Beltsville, Maryland (USA) and is contained within a first-order agricultural watershed in the mid-Atlantic coastal plain. The sub-watershed consists of a 20 ha agricultural field that drains into a riparian zone and a small, non-incised, north-to-south-flowing first order stream. The first-order stream length is 1200 m, at which point it joins a larger, higher order stream, marking the terminus of the study area (Fig. 2). The stream channel was instrumented with five permanent stream sampling/monitoring stations, essentially breaking up the stream into four sections. Numerous piezometers were located within the riparian zone in transects (from field to stream) between the stream stations.

The soils and hydrology of the agricultural field have been examined extensively and details can be found elsewhere (Gish et al., 2005). The underground flow patterns from the field were mapped using ground penetrating radar and digital elevation maps. A restrictive clay lens was found over 80% of the field at a depth of less than 1.8 m. The depth to this restricting clay layer beneath the field varied from 0.9 to 2.7 m below the soil surface. The structure of this lens encourages preferential flow (or movement) of groundwater to the upwelling zones between stations 2 and 3 of the stream (Gish et al., 2005).

The wetland area is forested primarily with red maples (*Acer rubrum*) with an understory of skunk cabbage (*Symplocarpus foetidus*) and jewelweed (*Impatiens pallida*). The riparian soils are mostly Typic Haplosaprists (Johnston silt-loam series), 2 m in depth, underlain by an oxic sand and gravel aquifer; additional details can be found in Angier et al. (2005). As is common with wetland soil, the organic matter content was high ~25%. Stream flow characteristics and groundwater behavior are highly variable in this system. Movement of water and nutrients in this study area is non-horizontal, non-uniform (Angier et al., 2005; McCarty and Angier, 2001). The continuous flow in the stream is maintained by groundwater transfer from the adjoining fields, and preferential pathways play an important role in groundwater delivery to the stream. The area that typically displays the highest concentrations of agrochemicals in surface water (both in the stream channel and in zones of groundwater emergence onto the flood plain) lies between stations 2 and 3. This part of the riparian zone is a groundwater-fed wetland with perennially-saturated surface conditions.

The cropped area was continuously maintained in maize production and was treated with a mixture of metolachlor and atrazine each year (Prueger et al. 2005). Herbicides were applied via spray broadcast onto a bare soil surface shortly after the corn was planted. In 1997 and 1998 rac-metolachlor was applied at a rate of 2.24 kg ha⁻¹, and from 1999 to 2005, S-metolachlor was applied at a reduced rate of 1.41 kg ha⁻¹. The field received water only from natural rain events.

2.3 Water sampling

Stream water and groundwater sampling locations are identified in Figure 2. Groundwater samples within the riparian buffer were obtained from clustered piezometers located as transects moving from the inner edge of the riparian buffer strip to the first order stream and were dispersed from the upper part of the buffer zone near stream station 1 down to the wells near stream station 4, Fig 2. Groundwater samples were obtained by pumping out at least one full well volume, waiting for full recovery, and then collecting the sample with a bailer. In addition, a series of wells outside of the buffer zone and lying in a transect along the edge of the treated field was sampled from 2003 to 2005 (Fig. 2). Typical sample volumes were 1 L, however, when well recharges were slow, volumes were less but none were below 0.5 L.

Stream water samples were obtained at four stations (Fig. 2) using a simple grab sample collection system with a metal bucket. Samples were stored in glass containers and processed within one week of collection. Additional water samples were obtained

by autosamplers set up to collect during selected precipitation events. Typical sample volumes were 0.5 L for the autosampler, while the grab samples were usually 1 L.

2.4 Description of samples selected for chiral analyses.

Six years of sampled groundwater ($n = 19$) and stream water ($n = 42$) were randomly selected for the chiral analyses from the archived collection of extracts from two previous studies. The first examined the fate of nutrients and herbicides in the riparian buffer system (Angier and McCarty, 2008; Angier et al., 2005). All analyzed samples were collected from 1999 to 2005, and most were collected during the growing season. In the second study, selected extracts of groundwater were analyzed that were provided to us from samples collected under the neighboring field (Gish et al. 2005). This second set of extracts were collected between 2003 to 2005 and were taken from wells bordering the down-gradient edge of the field, Fig. 2. The riparian buffer groundwater samples selected for chiral analyses were all located in the major upwelling location of the riparian buffer between stream water sampling stations 2 and 3 (Fig. 2). Stream water samples were obtained from stations 2, 3, 4, and 5 generally during base flow conditions. A few ($< 15\%$) collected during higher flow conditions.

2.5 Sample extraction and cleanup.

Water samples (1 L or 0.5 L) were filtered through GF/F glass microfiber filters prior to solid-phase extraction. Triphenylphosphate (TPP) was added as a surrogate to

each sample prior to extraction to monitor extraction efficiency. The solid phase Oasis extraction cartridges were preconditioned sequentially with 6 mL of methanol, 6 mL of acetonitrile, 6 mL of methanol, and 6 mL of deionized water. The filtered samples were passed through the conditioned cartridges using vacuum suction at a flow rate of 10 mL min⁻¹. Before elution, the cartridges were dried under vacuum for approximately 7 min. Analytes were eluted first with methanol (2 x 6 mL) followed by 6 mL of acetonitrile. Combined eluents were concentrated under nitrogen gas just to dryness and then made up with 0.5 mL of methanol. Just prior to analysis, samples were transferred to injection vials and diluted to 50% with water. Each sample was spiked with 10 µL each of internal standards: [¹³C] 2, 4- dichlorophenoxyacetic acid (100 µg mL⁻¹) and terbutylazine (35.6 µg mL⁻¹).

2.6 Analysis procedures

2.6.1 Initial quantitation

The samples were analyzed to determine the concentrations of metolachlor and MESA using reverse-phase, non-chiral separation and triple quadrupole mass spectrometry detection (Waters 2690 Alliance liquid chromatograph and Micromass Quattro LC triple quadrupole mass spectrometer, Waters Corp., Milford), operated in electrospray mode for sample detection and quantitation. Extracts were separated on a C₁₈ Ultrasphere ODS LC column at 45 °C using the following gradient at 1 mL min⁻¹ of solvent A (30:70 v/v of methanol:1% formic in water) and solvent B (100% methanol):

89:11 (A:B) held for 0.5 min, increased linearly to 5:95 (A:B) over the next 6.5 min, held for 5 min, and then returned to initial conditions. A 6-min equilibration preceded each injection.

Samples collected in 2005 were analyzed using a Waters XTerra LC column at 40 °C using the following gradient at 0.3 mL min⁻¹ of solvent A (30:70 v/v of methanol:1% formic in water), solvent B (100% water)and solvent C (100% methanol): 70:30 (A:B) held for 2 min, increased linearly to 58:42 (A:C) over 2 min, followed by a linear increase to 5:95 (A:C) over 14 min, and then returned to initial conditions. A 7-min equilibration preceded each injection. Injection volumes were 10 µL.

Matrix spiking yielded the following average recoveries with standard deviation: 94 ± 13% (*n* = 20) using 0.2 µg L⁻¹ of metolachlor, 91 ± 8% (*n* = 27) using 2 µg L⁻¹ spiking with MESA, and 78 ± 25% for triphenylphosphate surrogate which was added to all samples. The limit of detection (LOD) depended on sample volume and ranged from 1 to 2 ng L⁻¹ for metolachlor and from 10 to 20 ng L⁻¹ for MESA. These were not exhaustively determined but based on the lowest observable peaks for analytes that could be observed in the lowest concentration standard.

2.6.2 Chiral Analyses

Data included in the current study are a subset of groundwater and stream water extracts selected from our archives. Archived sample extracts were stored at -20 °C in 2.0 mL vials and were stored in the extraction solvent, the same solvent utilized for chiral analyses. Sample stability was verified by reexamining the total concentrations

for metolachlor and MESA; no changes were observed. Prior to chiral analysis, the concentrations of MESA and metolachlor were reviewed so that adjustments in sample volumes could be made to ensure that the injected amounts fell within the range of linearity for quantitation by the triple quadrupole MS system used in the analysis. This was particularly important for MESA, since many samples had to be diluted to keep them within the method range of linearity (less than $1 \mu\text{g mL}^{-1}$). However, the concentrations of metolachlor in many samples were too small resulting in many non-detections for this analyte. Detection limits for the chiral isomers of metolachlor and MESA were approximately two times higher than the detection limits based on non-chiral analyses reported above.

The methods of Kabler (Kabler and Chen, 2006; Kabler, 2006) were used with minor modifications for separation of metolachlor and MESA enantiomers. For MESA, a Chiralpak QN-AX column was used rather than the MAC-MOD ProntoSIL 120-5 Chiral AX QN column used by Kabler (2006). The enantiomers were eluted with a mobile phase of (90:10, v/v) methanol:ammonium acetate (50 mM, pH 5). The column temperature was held at 15 °C using a column chiller, and injection volumes were 10 μL . The mass spectrometer was operated in electrospray negative ionization acquisition mode, and the transition ion pair monitored was $328 > 121 \text{ } m/z$. The order of elution of stereoisomers was similar to Kabler's, although our retention times were shorter (Fig. 3). The elution of the peaks were as follows: a co-elution of both of the two aryl isomers of the *S*-enantiomers of MESA (*aS,1'S* and *aR,1'S*) followed by two fairly-well-resolved peaks the aryl isomers of the *R*-enantiomers (*aR,1'R* and *aS,1'R*).

For metolachlor separation, a Chiralpak AS-H column was used. The mass spectrometer was operated in atmospheric pressure chemical ionization (APCI) positive mode, and the mass transition ion pair was $284 > 252$ m/z . The settings of the mass spectrometer source were corona voltage 20 μA , cone voltage 15 V, argon collision gas energy 17 eV. The source temperature was 300 $^{\circ}C$, and the nitrogen desolvation gas was set to 200 L h^{-1} . The running solvent for LC separation was *n*-hexane/2-propanol (90:10, v/v) at an isocratic flow rate of 1.0 mL min^{-1} and a column temperature of 28 $^{\circ}C$. Chromatograms were similar to those reported by Kabler (2006) with retention times of 5.7 min for (*aS*,1'*S* + *aR*,1'*S*), 7.2 min for (*aR*,1'*R*) and 9.1 min for (*aS*,1'*R*).

Peak areas were integrated for *R*- and *S*-stereoisomers using mass spectrometer software (MassLynx 4.0), manually screened for proper baseline adjustment, and quantified using the authentic standards. Data were stored as processed data in spreadsheet files (Microsoft Excel). Pure standards of the *S*- and *R*-metolachlor forms were not available, so we assumed that the metolachlor standard was racemic with equal amounts of the *R* and *S* forms and that all the enantiomers had similar response factors.

2.7 Computation of enantiomeric ratios

Various models are available for determining the ratio values for the chiral mixtures of metolachlor and MESA. The simplest approach is to express the value as a chiral ratio or the percentage of enantiomeric change after undergoing environmental degradation or selective partitioning. Another approach based on chiral enrichment is

more descriptive and captures the behavior of these stereoisomers as they approach total changeover (Buser et al., 2000; Kurt-Karakus et al., 2010a). In this study, we chose the latter (Kurt-Karakus et al., 2010a) where the ratio data for metolachlor and MESA are presented as *SF* values (*S*-enrichment factor)-values, or the amount of herbicidally-active metolachlor (*S*-metolachlor) divided by total amount of herbicidally-active and -inactive metolachlor (*S*-metolachlor + *R*-metolachlor), Equation 1 (Fig. 1).

$$SF = \frac{[aS,1'S] + [aR,1'S]}{[aS,1'S] + [aR,1'S] + [aR,1'R] + [aS,1'R]} \quad (1)$$

The convenience of this method is that the *SF* value ranges from 0 to 1, and as such, a value of 0.5 represents rac-metolachlor and a value of 0.88 represents the commercial *S*-metolachlor product (Kabler and Chen, 2006).

2.8 Data analyses

SF values were grouped and statistically evaluated using Graph Pad Software Inc, (La Jolla, CA). Curve fitting was performed using the plotting options available in the software. Curves producing the highest coefficient of determination values (R^2) were chosen as the most suitable models to represent the data.

3. Results and discussion

3.1 Description of sample results

In general, total MESA concentration values were much larger, by up to two orders of magnitude, than total metolachlor concentration values, except for some stream samples where metolachlor concentrations were sometimes larger just after metolachlor was applied. In the stream, the mean $\pm$ SD for total MESA concentration was $8.9 \pm 5.0 \mu\text{g L}^{-1}$ (range: $0.010 \mu\text{g L}^{-1}$ to $16 \mu\text{g L}^{-1}$, $n = 30$), whereas the mean total metolachlor concentration was $3.4 \pm 12 \mu\text{g L}^{-1}$ (range: less than the LOD, $0.001 \mu\text{g L}^{-1}$, to $69 \mu\text{g L}^{-1}$, $n = 29$). Metolachlor concentrations were at or near the limit of detection in the riparian buffer groundwater samples, mean $0.04 \mu\text{g L}^{-1}$ ($n = 4$); however, more metolachlor was detected in groundwater from the field again with the larger values observed immediately after application. The mean total metolachlor concentration in groundwater below the field was $5.0 \pm 8.7 \mu\text{g L}^{-1}$ (range: less than $0.001 \mu\text{g L}^{-1}$ to $32 \mu\text{g L}^{-1}$, $n = 18$). The mean total MESA concentrations in the riparian buffer groundwater samples was $14 \pm 5.8 \mu\text{g L}^{-1}$ (range: $4.7 \mu\text{g L}^{-1}$ to $23 \mu\text{g L}^{-1}$) and for the groundwater from the field it was $27 \pm 21 \mu\text{g L}^{-1}$ (range: $8.6 \mu\text{g L}^{-1}$ to $86 \mu\text{g L}^{-1}$, $n = 19$).

3.2 SF values for metolachlor and MESA in stream samples

MESA *SF* values were calculated for all stream samples and were plotted as a function of time after 1999, the year that *S*-metolachlor use began (Fig. 4). A steady, non-linear increase for the MESA *SF* values was observed beginning with 0.54 for the first sample collected 155 days after *S*-metolachlor was first applied and ending up at 0.78 six years after subsequent yearly treatments with the *S*-metolachlor. The kinetic model of this *S*-enrichment in the stream water can be assumed to be first order and is

described by an exponential curve ($R^2 = 0.80$), Fig. 4. MESA formation has been shown to occur in the upper aerobic-layer of the soil column (Baran and Gourcy, 2013; Bayless et al., 2008; Denver et al., 2004; Denver et al., 2010; Domagalski et al., 2008; Huntscha et al., 2008). In a multi-year field and modeling study, Bayless et al. (2008) found that metolachlor was very transient with a simulated half-life of 10 days and that MESA was rapidly formed in the first meter of the soil and was leached to the water table within 1-2 years. This rapid transformation of metolachlor to MESA led to high concentrations of MESA in the water table beneath the field.

In contrast to MESA, the *SF* values for metolachlor in stream water were only weakly correlated to time (Fig. 4a). All the *SF* values were above 0.8 indicating that only recent forms of metolachlor are present. These data also demonstrate that metolachlor and MESA are delivered to the stream via different mechanisms. MESA, which is formed in the vadose zone and is very water soluble, is transported to the stream through groundwater. The slight positive slope for metolachlor in stream water may reflect a very small contribution of parent compound loading from groundwater. But generally metolachlor would be expected to be delivered quickly by overland processes, e.g., runoff during extreme rain events or tree canopy rain washoff (Rice et al., 2016). Metolachlor is not delivered to the stream by leaching to the ground water under the field soil and traveling to the riparian area groundwater (Angier et al., 2005). This is due to its relatively large K_{oc} in comparison to the K_{oc} of MESA and also due to the rapid degradation of metolachlor in the surface soils. These findings demonstrate the utility of enantiomeric measurements for differentiating delivery mechanisms to surface waters.

3.3 *SF* values for MESA in groundwater wells

The *SF* values for MESA in groundwater within the riparian buffer when plotted as a function of years since *S*-metolachlor had a similar-shaped curve to that of the stream samples with an even better coefficient of determination of 0.96 (Fig. 4b). This indicates a tight linkage of the stream water and riparian groundwater as was expected. Previous studies have shown that groundwater exfiltration to the riparian buffer is responsible for generating stream flow under baseflow conditions at this site (Angier and McCarty, 2008).

The groundwater sampled from the wells located in the field just outside the riparian buffer area had *SF* values for MESA that were substantially higher than those collected at the same times as groundwater collected in the riparian buffer (Fig. 5). MESA *SF* values in the shallow groundwater under the field were similar to the *SF* values for metolachlor in these samples. This finding is consistent with a conclusion that these field wells were primarily sampling metolachlor and MESA which had recently moved vertically from the vadose zone into these wells and reflects current applications of *S*-metolachlor. This is in stark contrast to the enantiomeric composition of MESA measured in the riparian buffer zone which represents MESA entrained in the deeper flow paths and reflects multi-year applications of metolachlor.

3.4 Mean residence time of MESA

The change in SF values of MESA in the stream water can be used to calculate a groundwater mean residence time. The inverse of the MESA SF values, which describes the disappearance of the racemic form of metolachlor in groundwater, were plotted as a first-order decay function (Fig. 6). The half-life for the decay represents a mean residence time for racemic metolachlor in groundwater and this value was found to be 3.8 years. This time represents the average time needed for field-applied metolachlor to enter the soil surface, degrade to MESA and to leach through the vadose zone to the deeper groundwater flow paths and then to be transported to the stream. It is important to notice the differences between the deeper water flow paths feeding the stream and riparian buffer wells as compared to the lysimeter data collected from the field, Fig. 5. Delay times for MESA in these field wells largely reflect metolachlor transformations and vertical transport processes in the vadose zone and have a different delay time for MESA than is observed for the stream and groundwater samples collected within the riparian buffer zone which reflect groundwater flow path transport.

3.5 Implications in assessing conservation practices

When a conservation practice is implemented, a lag period often exists before the effects of the conservation practice are observed on surface water chemistry (Hamilton, 2012; Lindsey et al., 2003). This is especially true for groundwater systems as the mean residence time, which can be as long as 20 years, may be required to assess the effectiveness of the practice on surface water chemistry. We have demonstrated previously that MESA is an excellent conservative tracer of agricultural

427 waters, and therefore is an excellent tracer of agricultural nitrate-N (McCarty et al.,
428 2014). Thus, measuring the change in MESA chirality provides a robust method for
429 estimating not only the groundwater mean residence time in an agroecosystem, but the
430 fate of agriculturally-derived nitrate-N as well.

431

DISCLAIMER

Mention of specific products is for identification and does not imply endorsement by the US Department of Agriculture to the exclusion of other suitable products or suppliers.

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ABBREVIATIONS USED

K_{oc} , organic carbon partition coefficient

LOD, limit of detection

MESA, metolachlor ethane sulfonic acid

rac, racemic

SF , S -enrichment factor

TMDL, total maximum daily load

Figure Captions

Figure 1. Metolachlor ($X = Cl$) and MESA ($X = SO_3H$) are made up of two enantiomers due to an asymmetric carbon ($1'$), and each of these isomers has an additional enantiomer (atropisomer) due to hindered rotation about the phenyl-nitrogen bond of metolachlor.

455

456 Figure 2. Planar schematic of sampled region, showing details of well sites and stream
457 sampling points (stations 1 to 4). Axes units are meters. Insert is an aerial photograph
458 showing the locations of field wells lying in a transect along the lower edge of sector B.

459

460 Figure 3. Representative LC chromatogram of a) rac MESA standard and b) S-enriched
461 field sample of groundwater from 2005 separated on Chiralpak QN-AX column and
462 eluted with a mobile phase of (90:10, v/v) methanol:ammonium acetate (50 mM, pH 5).

463

464 Figure 4. Buffer zone groundwater and stream water S-enrichment values for
465 metolachlor and MESA. Curves describing the change in the enantiomeric ratio versus
466 time after the 1999 transition to S-metolachlor are shown a) for stream water and b) for
467 groundwater. The coefficient of determination (R^2) for the curve fits were 0.80 for stream
468 water and 0.96 for groundwater. The dotted horizontal lines are the theoretical S-
469 enrichment values for the racemic product (lower line) and S-enriched product (upper
470 line).

471

472 Figure 5. S-enrichment values for MESA and metolachlor in groundwater sampled from
473 the field bordering the riparian buffer zone. The MESA enrichment curve for buffer-zone
474 groundwater is provided for comparison. The dotted horizontal lines are the theoretical
475 S-enrichment values for the commercial racemic-metolachlor product (lower line) and
476 for the commercial S-metolachlor product (upper line).

477

478 Figure 6. Exponentially-fit decay curve describing the disappearance of racemic MESA
479 in the riparian buffer stream samples. The dotted lines are the theoretical values when
480 commercial racemic metolachlor is converted into racemic MESA (0.5) and the
481 expected S-MESA (0.12) value when degraded from the commercial S-enriched
482 metolachlor product. .

483

$\alpha S, 1'S$

$\alpha R, 1'R$

$\alpha R, 1'S$

$\alpha S, 1'R$

Metolachlor: X = Cl; MESA: X = SO₃H

484
485

486 Figure 1

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490 Figure 2

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Figure 4

Figure 5

Figure 6

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