Isolation and Identification of the Metolachlor Stereoisomers Using High-Performance Liquid Chromatography, Polarimetric Measurements, and Enantioselective Gas Chromatography

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Because of the presence of two chiral elements (an asymmetrically substituted carbon and a chiral axis), the herbicide metolachlor consists of four stereoisomers stable at ambient temperature with *aSS*-, *aRS*-, *aSR*-, and *aRR*-configurations (*aSS*, the isomer with a*S*,1′*S*-configuration, etc.). Metolachlor, initially introduced into the market as the racemic product containing all four stereoisomers, is currently being replaced worldwide by *S*-metolachlor, the product enantiomerically enriched with the herbicidally active 1′*S*-isomers (*aSS*, *aRS*). The isomer-specific analysis of metolachlor requires not only enantioselective ("chiral") analytical techniques but also suitable reference compounds. In this study, two of the four metolachlor isomers were isolated from *rac*-metolachlor in enantio- (*ee* > 98%) and diastereomerically pure forms by a combination of achiral and chiral high-performance liquid chromatography (HPLC). The two isomers were identified as the *aSS*- and the *aRR*-isomers by polarimetric measurements, in reference to previous data. The two isomers were then thermally equilibrated to 1:1 mixtures of the *aSS*/*aRS* and *aRR*/*aSR* diastereomers, respectively, so that analytical data of all four metolachlor isomers became available; they were then used to identify these isomers in technical products by chiral high-resolution gas chromatography (HRGC). The kinetics of the thermally induced interconversion of the atropisomers was studied and the consequences, such as for GC analysis, are discussed. A comparison of on-column and split/splitless injection indicated that the latter technique results in significant isomerization prior to separation and, therefore, cannot be used for accurate isomer analysis.

Keywords: *Metolachlor stereoisomers; enantioselective analysis; chiral switch; thermal conversion; chiral stability*

INTRODUCTION

Many agrochemicals are chiral and thus consist of two or more stereoisomers, sometimes with widely differing biological activities (*1*, *2*). Although the desired biological activity may be contained in just one or a few isomers, these chemicals are often synthesized and used as isomer mixtures. More recently, however, some isomer mixtures (e.g., racemates) were replaced by single-isomer or enantiomerically enriched compounds (*3*, *4*). The benefits of such products are related to their higher specific activities which lead to lower application rates and thus smaller amounts of chemicals released into the environment.

An example of a synthetic chiral pesticide is metolachlor (2-chloro-*N*-[2-ethyl-6-methylphenyl]-*N*-[2-methoxy-1-methylethyl]acetamide). Metolachlor is an important selective herbicide for use in corn and other crops to control a variety of broad-leaved weeds (*3*, *5*, *6*). The presence of a chiral axis and an asymmetrically substituted carbon atom in the structure of metolachlor yields four stereoisomers stable at ambient temperature: *aSS*; *aRS*; *aSR*; *aRR* (*aSS*, the isomer with a*S*,1′*S* configuration, etc., see Figure 1). Because of the partial double-bond character of the (N−CO) amide bond, additional isomers are possible (*7*, *8*). These isomers, however, are not stable at ambient temperature (half-life, <1 d at 20 °C).

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The herbicidal activity of metolachlor resides primarily in the two diastereomeric 1′*S*-isomers (*aSS* and *aRS*)(*9*). Metolachlor was initially introduced into the market as the racemic product containing all four stereoisomers. More recently, however, *rac*-metolachlor is being replaced worldwide by *S*-metolachlor, the herbicide enantiomerically enriched with the two 1′*S*-isomers (*10*). This replacement is expected to result in lower environmental concentrations and in a changed enantiomer/isomer composition of metolachlor in the residues.

The determination of the exact stereoisomer composition of metolachlor in technical products, and in environmental residues, is still complex and challenging. Enantiomers are especially difficult to analyze because they possess identical chemical and physicochemical properties (excepting optical rotation and reactivity in a chiral environment) and require enantioselective ("chiral") analytical techniques, such as enantioselective high-performance liquid chromatography (HPLC) or enantioselective high-resolution gas chromatography (HRGC)(*11*). These techniques rely on chiral auxiliary compounds (chiral selectors) where interactions are difficult to predict (*12*). Furthermore, isomer-specific analyses require suitable reference compounds of known stereoisomer composition, including enantioenriched or enantiopure compounds which often are commercially unavailable.

In this study, we report on the application of chiral and achiral HPLC for the separation, isolation, and

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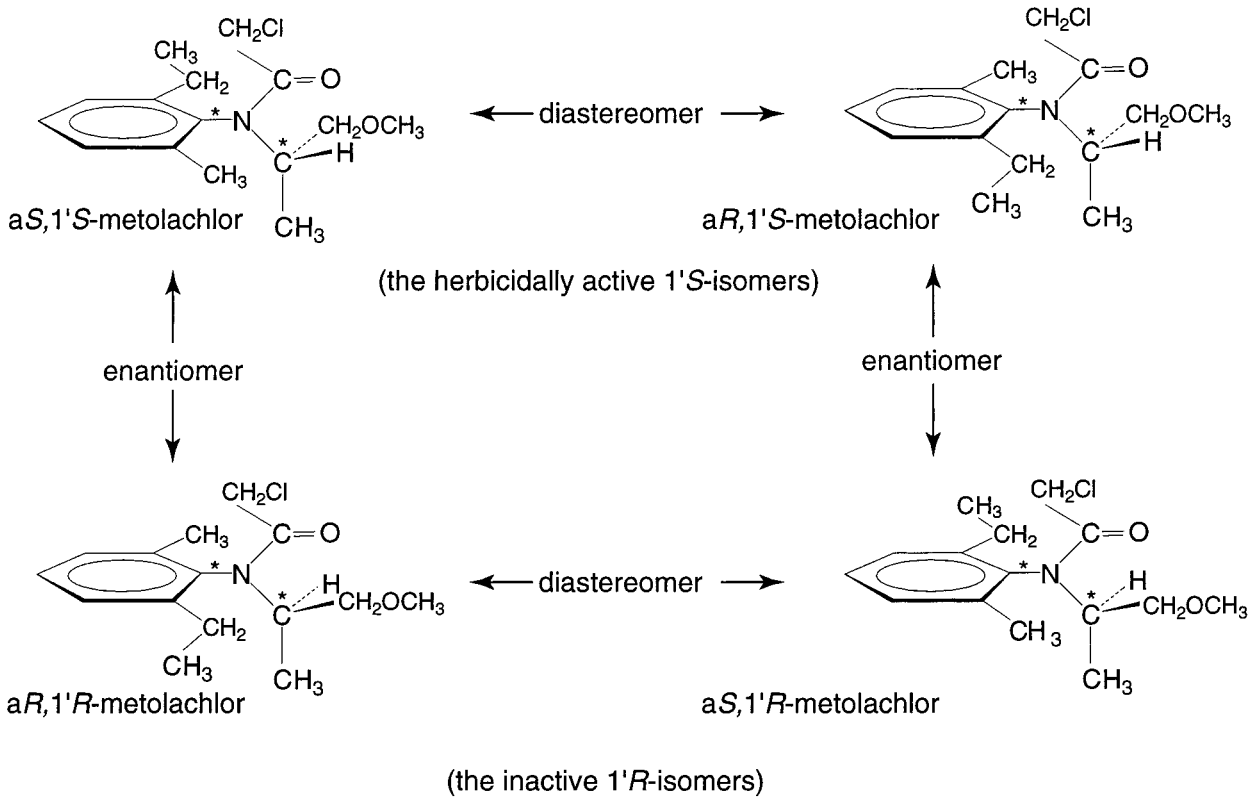

Figure 1. Structures (absolute configurations) of the four stereoisomers of metolachlor (asterisks denote the chiral elements). Also indicated are the diastereomeric and enantiomeric relationships between isomers.

identification of small (mg) quantities of individual stereoisomers from *rac*-metolachlor. Two individual isomers were isolated in high enantiomeric and diastereomeric purity and the absolute configurations were assigned through polarimetric measurements in reference to previous data (*9*). The two isomers were thermally equilibrated to 1:1 mixtures of the related diastereomers, and then all isomers were used for peak assignment in the HRGC analyses. The kinetics of the thermal interconversion of the atropisomerically related compounds were studied, and consequences for enantioselective GC analysis were discussed. The methods and techniques outlined, and the consequences discussed, show new and relevant aspects for the isomer-specific analysis of metolachlor when using enantioselective HRGC, and are important to differentiate and apportion the relative contributions of sources (racemic versus nonracemic) of the compounds on the basis of stereoisomer composition of environmental residues.

MATERIALS AND METHODS

Standard Material. Analytical standards (purities, >99%) of *rac*-metolachlor and *S*-metolachlor were obtained from Novartis AG (Basle, Switzerland), and stock solutions at concentrations of 1−10 mg/mL were prepared in methanol or the mobile phases later used in HPLC. For HRGC analysis, dilutions in ethyl acetate at concentrations of 1−10 ng/μL were prepared. $^{13}C_6$-*rac*-Metolachlor, used as an internal standard in the GC−MS analyses, was from Cambridge Isotope Laboratories (Cambridge, MA).

Semipreparative HPLC of Metolachlor Diastereomers and Enantiomers. The HPLC system consisted of a Jasco model PU-910 pump (Jasco Corp., Tokyo, Japan), connected to a Jasco model AS-1555 autosampler, a Jones model 7955 column chiller (Jones Chromatography, Hengoed, Wales, UK) set to 10 °C, and a Jasco model UV-975 UV−VIS detector set to 210 nm. Separations were performed at 10 °C because of an improved resolution at lower temperatures.

Metolachlor diastereomers were separated on a nonenantioselective ("achiral") Hypercarb PH (7-μm, 10 cm × 4.6 mm i.d.; Shandon HPLC, Life Sciences International, Runcorn, Cheshire, UK, England) graphitized carbon HPLC column operated with 1.0 mL/min acetonitrile/water (1:1). Samples (1−20 μL of 0.1−1% solutions in the mobile phase) with amounts as high as a few hundred μg were injected. The metolachlor diastereomers eluted at retention times of 14.2 and 15.8 min, respectively. Fraction collection was done manually from multiple injections by observing the UV signal and changing the collection vials accordingly. The purity of the fractions was checked with HPLC using the same column system. The two diastereomers were isolated from the mobile phase by extraction with *n*-hexane (3x), the extracts were evaporated to dryness at ambient temperature overnight, and then used for subsequent enantiomer separation after redissolution in *n*-hexane.

Metolachlor enantiomers were separated on a chiral Chiralcel OD-H (5-μm, 25 cm × 4.6 mm i.d.; Daicel Chemical Industries, Tokyo, Japan) column operated with 0.5 mL/min *n*-hexane/2-propanol (98:2). Samples (1−100 μL of 0.1−1% solutions in *n*-hexane) were injected and fractions were collected as described above.

Polarimetric Measurements. Optical rotation was measured on a Perkin-Elmer model 241 Polarimeter (Perkin Elmer, Norwalk, CT) fitted with a temperature (25 °C) controlled 500-μL cell (path length, 10 cm). A mercury lamp together with the respective filters provided monochromatic light with the necessary wavelength of 365 nm for the measurements. Samples were made up in methanol at concentrations of 1−20 mg/mL and the resulting rotations were read from the display. The instrument provided readouts of optical rotation with a precision of 0.001°. Specific rotations were calculated as $[\alpha]_{365} = \alpha_m/(c \times l)$ where α_m is the optical rotation measured, c is the concentration in g/mL, and l is the length of the cell in decimeters (unit, 10^{-1} deg cm^2 g^{-1}).

Analysis by HRGC−Mass Spectrometry (MS). Metolachlor was analyzed by GC−MS under electron-impact ionization (EI) full-scan and selected-ion-monitoring (SIM) conditions, as previously described (*7*). The instrument was a VG Tribrid mass spectrometer (VG Fisons, Manchester, England) with a Carlo Erba HRGC 5160 Mega gas chromatograph (Carlo Erba, Milan, Italy) equipped with cold on-column (OC) and split/splitless (SSL) injectors. For analysis, 1−2 μL samples containing 0.5−2 ng metolachlor (and ^{13}C-analog) in ethyl acetate were injected. Metolachlor and its ^{13}C-analogue were monitored by SIM using the ions at *m/z* 238 and 244, respectively. A 25-m PS086−BSCD (BSCD, *tert*-butyldimethylsilyl-β-cyclodextrin; relative amount, 50%) column (BGB Analytik, Adliswil, Switzerland) was used with OC injection at 70 °C. For some comparative analyses, SSL injection at 250 and 280 °C was used (splitless time, 60 s; glass liner, 80 × 4 mm i.d). The column was temperature programmed: 70 °C for 2 min, then at 20 °C/min to 120 °C, then at 3 °C/min to 230 °C, followed by an isothermal hold at 230 °C. Metolachlor eluted at ≈ 200 °C using this temperature program. Enantiomer composition was expressed as enantiomer excess (*ee*), defined as the excess of one enantiomer over the other (example, see eq 1), or the excess of the 1′*S*-isomers over the 1′*R*-isomers (*EE*, see eq 2, and *13*):

$$ee = \frac{[\mathrm{aSS}] - [\mathrm{aRR}]}{[\mathrm{aSS}] + [\mathrm{aRR}]} \quad (1)$$

$$EE = \frac{([\mathrm{aSS}] + [\mathrm{aRS}]) - ([\mathrm{aSR}] + [\mathrm{aRR}])}{[\mathrm{aSS}] + [\mathrm{aRS}] + [\mathrm{aSR}] + [\mathrm{aRR}]} \quad (2)$$

where [aSS] is the concentration of the *aSS*-isomer, etc. *rac*-Metolachlor has an *EE* of 0, and *S*-metolachlor, with ≈90% of 1′*S*-isomers and ≈10% of 1′*R*-isomers, has an *EE* of ≈0.8.

Thermal Interconversion (Equilibration) of Metolachlor Isomers. Typically, 20-μL aliquots of sample with amounts as low as a few ng of metolachlor and internal standard, were placed in small vials prepared from 50-μL micropipets (80 × 1 mm)(*7* and *14*). The solvent was removed in vacuo (warming with the fingertips) and exchanged for *iso*-octane (20 μL). The vials were then dipped into dry ice, flame-sealed under vacuum, and heated in a GC oven for periods of 5 to 120 min at 200 °C. After being rapidly cooled, the vials were opened, and the *iso*-octane evaporated in vacuo and exchanged for ethyl acetate (20 μL). Aliquots of 2 μL were then analyzed by GC−MS.

RESULTS AND DISCUSSION

Separation and Isolation of Metolachlor Stereoisomers using Achiral and Chiral HPLC. The four stereoisomers can be grouped into two diastereomeric pairs of enantiomers, whereby the *aSS*- and the *aRR*-isomers constitute one pair, and the *aRS*- and the *aSR*-isomers constitute the other pair (Figure 1). Achiral HPLC of *rac*-metolachlor resulted in two well resolved peaks when analyzed using the Hypercarb column (Figure 2a). The two peaks correspond to the two enantiomer pairs, *aSS*/*aRR* and *aRS*/*aSR*, but with, at this stage, unknown elution order. Chiral HPLC of *rac*- and *S*-metolachlor resulted in three to four peaks, when analyzed using the Chiralcel OD-H column, whereby the last-eluted isomers were only marginally resolved, indicating some but not full enantiomer resolution (Figure 2b). Therefore, none of these columns alone was capable of resolving metolachlor sufficiently into all four isomers on a semipreparative scale. However, single isomers were isolated by a combination of achiral and chiral HPLC, as outlined next.

Semipreparative fractionation of *rac*-metolachlor was achieved using the achiral Hypercarb column and the two enantiomers pairs were isolated (isolates *c1* and *c2* for the earlier- and later-eluted pair, respectively) in mg quantities from multiple injections. Reanalysis of the isolates using the same column indicated (diastereomer) purities of >99% and ≈95% for isolates *c1* and *c2*, respectively. The lower purity of isolate *c2* (the later-eluted component) resulted from the tailing of peak 1 into peak 2. Each isolate is expected to consist of a racemic mixture of two enantiomers, because the isolation was started with *rac*-metolachlor.

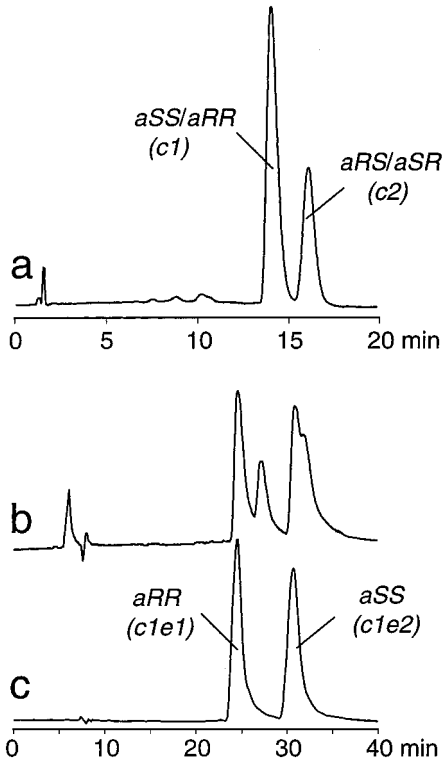

Figure 2. HPLC chromatograms of *rac*-metolachlor, analyzed using the achiral Hypercarb column (a), and the chiral Chiralcel OD-H column (b), and of isolate *c1*, the first-eluted enantiomer pair, analyzed using the chiral column (c). Note the diastereomer resolution in panel a, and the enantiomer resolution in panel c. Also denoted are the isomer assignments eventually deduced (see text).

Isolate *c1*, containing the enantiomer pair of higher purity, was then subjected to semipreparative HPLC for further isomer resolution using the chiral Chiralcel OD-H column. The HPLC chromatogram revealed two peaks in an 1:1 ratio corresponding to the two expected enantiomers (Figure 2c). The first- and second-eluted isomers were collected as isolates *c1e1* and *c1e2*, respectively, and isolated in mg quantities from multiple injections. Reanalysis indicated purities (*ee*) of > 98% and ≈ 95%, respectively. Isolate *c1e2* was then subjected to a second chromatography using the same column system. Finally, both isomers were obtained in high purity (*ee* > 98%). Because of the isolation procedure used, the two isomers isolated have an enantiomeric relationship and were eventually identified as *aSS*- and *aRR*-metolachlor based on polarimetric measurements and the HRGC and HPLC data in relation to *S*-metolachlor as outlined below. The other two isomers, *aRS*- and *aSR*-metolachlor, can be isolated from fraction *c2*, if that should be desirable.

Polarimetric Measurements of Metolachlor. The optical rotations of all four metolachlor isomers, correlated with the absolute configurations, were reported

Table 1. Specific Rotations $[\alpha]_{365}$ of Metolachlor and Individual Stereoisomers

isomer/compound	specific rotation ($[\alpha]_{365}$)[a] (10^{-1} deg cm^2 g^{-1})	concentration[b] (g/mL)	reference
aSS-metolachlor	+ 27 ± 1 (+28 ± 1)	0.0273	Moser et al. (*9*)
aRS-metolachlor	− 43 ± 1 (−49 ± 1)	0.0276	Moser et al. (*9*)
aSR-metolachlor	+ 43 ± 1 (+49 ± 1)	0.0196	Moser et al. (*9*)
aRR-metolachlor	− 27 ± 1 (−28 ± 1)	0.0220	Moser et al. (*9*)
rac-metolachlor	± 0.0 ± 0.1	0.0196	this study
S-metolachlor	− 0.7 ± 0.1	0.0194	this study
isolate *c1e1* (= *aRR*)	− 33 ± 1	0.0016	this study
isolate *c1e2* (= *aSS*)	+ 36 ± 1	0.0013	this study

[a] Corrected values are given in parentheses. They were calculated by taking the optical purities as listed by Moser et al. (*9*) into account. [b] Concentrations in methanol.

by Moser et al. (*9*). In Table 1, we list these data and we include corrected values for the magnitude of these rotations, taking the reported isomer purities into account. As shown, *aSS*- and *aSR*-metolachlor are dextrorotatory, and *aRS*- and *aRR*-metolachlor are levorotatory. The absolute configurations of the two metolachlor isomers in isolates *c1e1* and *c1e2* were then assigned on the basis of these data. To allow a direct comparison with the data reported (*9*), we measured optical rotations at the same wavelength (365 nm) and used the same solvent (methanol) as these authors.

The polarimetric measurements showed the isomers in isolates *c1e1* and *c1e2* to be levo- ($[\alpha]_{365} = -33 \pm 1 \cdot 10^{-1}$ deg cm^2 g^{-1}) and dextrorotatory ($[\alpha] = +36 \pm 1 \cdot 10^{-1}$ deg cm^2 g^{-1}), respectively (Table 1). Because of the differences between the absolute values for the specific rotations of our samples and those reported by Moser et al. (*9*; see Table 1), which likely reflect experimental differences and errors in the measurements (low concentrations), the assignment of the absolute configuration could not be done by polarimetric measurements alone. However, unambiguous assignments were possible based on the HRGC analysis in comparison to *S*-metolachlor, as shown below.

rac-Metolachlor, when measured under these conditions, showed zero optical rotation ($[\alpha]_{365} = 0 \pm 0.1 \cdot 10^{-1}$ deg cm^2 g^{-1}), as expected from its racemic composition. Surprisingly, *S*-metolachlor showed only a very small optical rotation ($[\alpha]_{365} = -0.7 \pm 0.1 \cdot 10^{-1}$ deg cm^2 g^{-1}). This finding indicated that the rotation due to the dextrorotatory isomers (*aSS* plus *aSR*) in *S*-metolachlor is about balanced by that of the levorotatory isomers (*aRS* plus *aRR*), see below.

Stereoisomer Assignment of Metolachlor using Polarimetric and HRGC Data. *rac*-Metolachlor and *S*-metolachlor gave single peaks and showed no diastereomer resolution when analyzed using various achiral GC columns (*7*). In contrast, chiral GC using cyclodextrin derivatives provided some, but incomplete, resolution of the stereoisomers (*7*). Full isomer resolution of metolachlor is challenging because two chiral elements are involved, which likely require different selector−analyte interactions. Even though the interaction of metolachlor with BSCD, the chiral selector eventually chosen, leads to acceptable resolution between corresponding axial- (*aSS*/*aRS*, *aSR*/*aRR*) and corresponding C-chiral isomers (*aSS*/*aSR*, *aRS*/*aRR*), respectively, it results in coelution of two of the isomers, as discussed below.

The HRGC chromatogram of *rac*-metolachlor showed partial resolution into three peaks with coelution of two isomers as a broad peak (peak 2) when analyzed using the chiral PS086-BSCD column (Figure 3a). *S*-Metolachlor showed the same three peaks but with changed isomer ratios (Figure 3b). The isolates *c1e1* and *c1e2*, when analyzed using this column, resulted in single peaks with the isomer in isolate *c1e2* eluted as peak 1, and the isomer in isolate *c1e1* eluted as peak 3 (Figure 3c,d). EI mass spectra of the two isomers, expectedly, were identical, and identical to those of the isomers eluted as GC peak 2 from *rac*- and *S*-metolachlor (data not shown).

The polarimetric data (Table 1), and the HRGC peak elution sequence reported above, indicate the isomer eluted as peak 1 (Figure 3) to have either *aSS*- or *aSR*-configuration (dextrorotation), and the isomer eluted as peak 3 to have either *aRR*- or *aRS*-configuration (levorotation). These possibilities can be distinguished based on the fact that *S*-metolachlor contains a higher concentration (≈90%) of herbicidally active 1′*S*-isomers (*aSS* and *aRS*) than of inactive 1′*R*-isomers (*aSR* and *aRR*), as reported by the manufacturer and Blaser et al. (*10*). The isomer eluted as peak 1 is the major component in *S*-metolachlor (see chromatogram in Figure 3b), and therefore must have *aSS*- and not *aSR*-configuration. Similarily, the isomer eluted as peak 3 is a minor component of *S*-metolachlor and therefore must have *aRR*- and not *aRS*-configuration. The isomers in isolates *c1e1* and *c1e2* thus have *aRR*- and *aSS*-configuration, respectively.

When the single isomers were heated to 200 °C in *iso*-octane, each one eventually formed a 1:1 mixture of two isomers (Figure 3e,f), whereby the two isomers are corresponding atropisomers with *aSS*- and *aRS*-configuration (the two 1′*S*-isomers) from one of the samples (*c1e2*), and with *aRR*- and *aSR*-configuration (the two 1′*R*-isomers) from the other sample (*c1e1*). Co-injection of these mixtures indicated that the later-eluted isomer from the thermalized isolate *c1e2* (*aRS*-metolachlor) and the earlier-eluted isomer from the thermalized isolate *c1e1* (*aSR*-metolachlor) were almost but not exactly coeluting, showing a small though significant difference in retention time. In this way, analytical data on all four metolachlor isomers was obtained. The isomer elution sequence on PS086-BSCD eventually deduced from these data is peak 1, *aSS*-; peak 2, *aSR*- (earlier eluted component) and *aRS*- (later-eluted component); and peak 3, *aRR*-metolachlor (Figure 3). Apparently, the enantiomer elution sequence is *aS* prior to *aR* (retention times, *aSS* < *aRS* and *aSR* < *aRR*) and 1′*S* prior to 1′*R* (retention times, *aSS* < *aSR* and *aRS* < *aRR*), and thus in the same sense for both chiral elements. This leads to a much larger enantioresolution for the enantiomer pair *aSS*/*aRR* than for the pair *aSR*/*aRS*, as shown in Figure 3a.

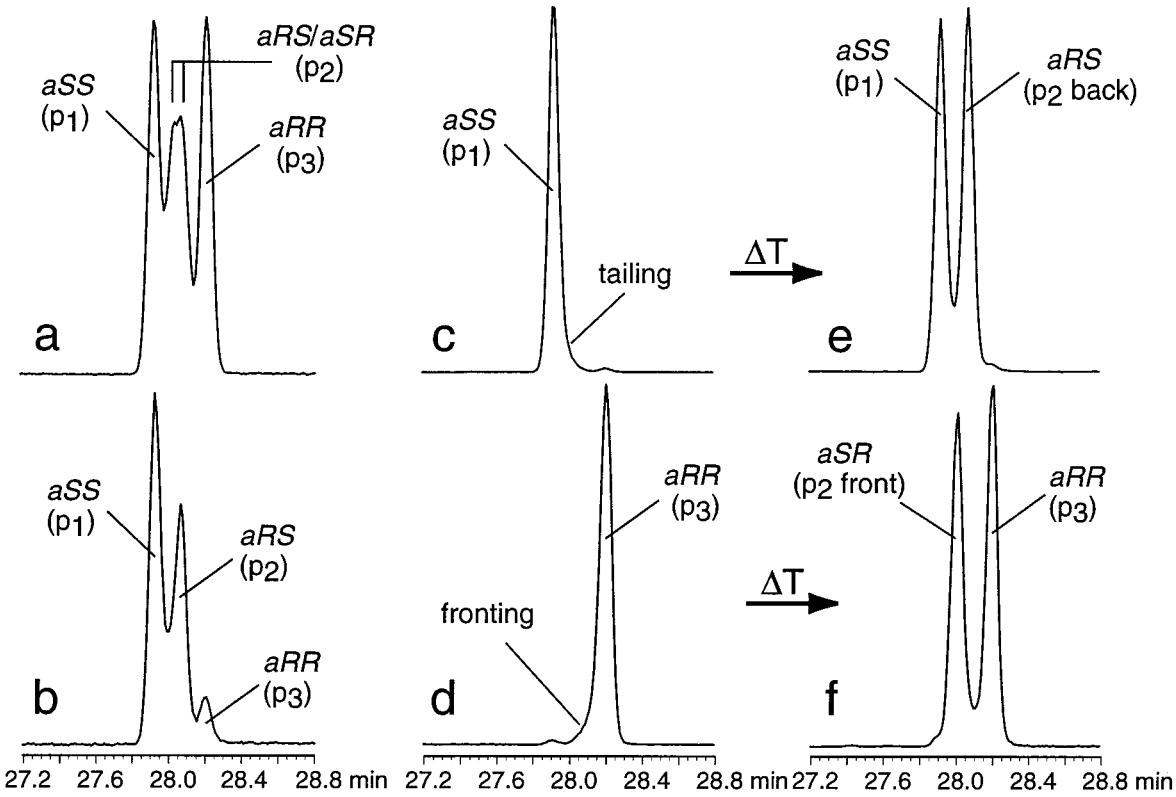

Figure 3. HRGC SIM chromatograms (*m/z* 238) of *rac*-metolachlor (a), *S*-metolachlor (b), isolate *c1e2* (*aSS*-metolachlor) (c), isolate *c1e1* (*aRR*-metolachlor) (d), thermally equilibrated *c1e1* (e), and thermally equilibrated *c1e2* (f), analyzed using the chiral PS086-BSCD column. Note the presence of the corresponding atropisomers in the thermally equilibrated isolates.

Stereoisomer Composition of *rac*- and *S*-Metolachlor from HRGC and Polarimetric Data. The stereoisomer composition of metolachlor in technical products and in environmental residues cannot be determined directly from the HRGC chromatograms shown in Figures 3a,b because of the incomplete isomer resolution. However, the composition can be determined if further conditions are known such as the excess of the 1′*S*- over the 1′*R*-isomers (e.g., *EE* values of 0 and ≈0.8 for *rac*-and *S*-metolachlor, respectively), determined from thermally equilibrated samples (*14*).

The HRGC chromatogram of *rac*-metolachlor (Figure 3a) showed peak area ratios p_1:p_2:p_3 of ≈1:1.2:1, where p_1 is the peak area of peak 1, etc. *rac*-Metolachlor thus contains a higher concentration of the enantiomer pair *aSS*/*aRR* (peak 1 and 3) than of the enantiomer pair *aRS*/*aSR* (peak 2), and its diastereomer composition, ([*aSS*] + [*aRR*])/([*aRS*] + [*aSR*]), as calculated from the peak area ratio (p_1 + p_3)/p_2, is ≈1.7:1. Because *rac*-metolachlor contains equal concentrations of enantiomers ([*aSS*] = [*aRR*]; [*aRS*] = [*aSR*]; *EE* = 0), the stereoisomer composition is ≈32% *aSS*, ≈18% *aRS*, ≈18% *aSR*, and ≈32% *aRR*. Other batches of *rac*-metolachlor showed diastereomer compositions ranging from ≈1:1 to 2:1 with the pair *aSS*/*aRR* present at equal or higher concentration than the pair *aRS*/*aSR*, likely as a result of different conditions during synthesis (*7*).

The HRGC chromatogram of *S*-metolachlor (Figure 3b) showed a clear excess of the first-eluted *aSS*- over the last-eluted *aRR*-isomer, and thus a nonracemic composition with peak area ratios p_1:p_2:p_3 of ≈0.54:0.39:0.07 which indicates a diastereomer composition of ≈1.5:1. The stereoisomer composition, calculated from these ratios, and taking the enantiomer excess *EE* = 0.78 into account (*14*), is ≈54% *aSS*, ≈35% *aRS*, ≈4% *aSR*, and ≈7% *aRR*.

The small but significant optical rotation ($[\alpha]_{365}$ = $-0.7 \pm 0.1 \cdot 10^{-1}$ deg cm^2 g^{-1}) of *S*-metolachlor with ≈ 90% 1′*S*-isomers confirms that both 1′*S*-isomers (*aSS* and *aRS*) are present and suggests that the dextrorotatory *aSS*-isomer (lower specific rotation) is present in a higher concentration than the levorotatory *aRS*-isomer (higher specific rotation), in agreement with our chromatographic data ($p_1 > p_2$, see Figure 3b). The individual contributions of each isomer to the net rotation of *S*-metolachlor, calculated by multiplying concentrations (c_i) and corrected $[\alpha]_{365}$ values (Table 1) were +15.12 *aSS*, −17.15 *aRS*, +1.96 *aSR*, and $-1.96 \cdot 10^{-1}$ deg cm^2 g^{-1} *aRR*. Because these rotations are expected to be additive, as indicated by the zero net rotation of *rac*-metolachlor, the net rotation of *S*-metolachlor then calculated from these data is $[\alpha]_{365}$ = $-2.03 \pm 1 \cdot 10^{-1}$ deg cm^2 g^{-1}, and thus in reasonable agreement with the experimental value ($[\alpha]_{365} = -0.7 \pm 0.1 \cdot 10^{-1}$ deg cm^2 g^{-1}). Theoretically, the optical rotation of *S*-metolachlor with ≈90% *aSS*/*aRS* (the 1′*S*-isomers) and ≈10% *aRR*/*aSR* (the 1′*R*-isomers) could vary between +29 and $-47 \cdot 10^{-1}$ deg cm^2 g^{-1} when assuming the product to contain exclusively dextrorotating isomers (*aSS* and *aSR*) and levorotating isomers (*aRS* and *aRR*), respectively, and using the corrected values of Moser et al. (*9*) in Table 1 for calculation.

Kinetics of the Thermal Interconversion of Metolachlor Atropisomers. The atropisomerism of metolachlor depends on the hindered rotation about the phenyl−nitrogen bond. The energy barrier to rotation for metolachlor (E_r) was determined at 154 ± 13 kJ M^{-1} using NMR measurements (*9*). At ambient temperatures, energy barriers >100−120 kJ M^{-1} result in conformationally stable atropisomers. At higher temperatures (200 °C), however, the metolachlor isomers rapidly interconvert through internal rotation about the

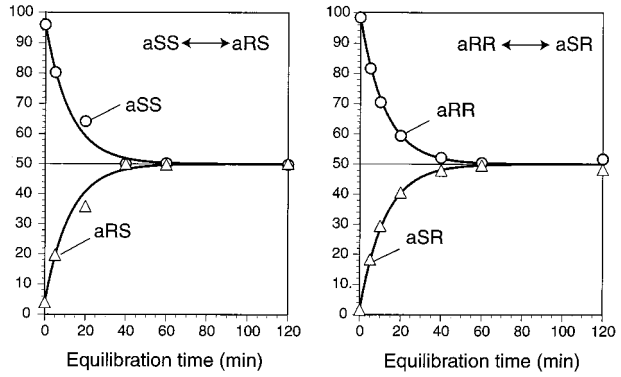

Figure 4. Interconversion of *aSS*- and *aRR*-metolachlor (left and right panels, respectively) to the 1:1 diastereomer mixtures through thermal equilibration at 200 °C. Measured and modeled data using $k = 0.04$ min^{-1}.

phenyl−nitrogen bond (*7*). The rate of interconversion, k_i (h^{-1}; $k_i = \ln 2/\tau$), is a function of temperature according to eq 3:

$$\ln k_i = \ln A - E_r/(RT) \qquad (3)$$

where A is a constant (frequency factor), R is the gas constant of 8.134 J M^{-1} K^{-1}, and T is the absolute temperature (K). In this process, the C-chirality of metolachlor is not affected and thermal equilibration (interconversion) of an isomer will not lead to the corresponding enantiomer but to the other diastereomer such as to *aRS* from *aSS* (*7*).

The two isomers isolated, *aSS*- and *aRR*-metolachlor, were subjected to the thermal treatment (200 °C) for up to 2 h. From both single-isomer compounds, eventually, 1:1 mixtures with the corresponding atropisomers were obtained (see chromatograms in Figure 3e,f). *aSS*-Metolachlor, eluted as peak 1, showed the formation of the later-eluted component of peak 2, thus identified as *aRS*-metolachlor; *aRR*-metolachlor, eluted as peak 3, showed the formation of the earlier-eluted component of peak 2, thus identified as *aSR*-metolachlor. The data confirmed that the C-chirality was not affected because *aRR*-metolachlor (peak 3) was not formed from *aSS*-metolachlor (peak 1), and vice-versa.

$$aSS \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} aRS \qquad k_1 \approx k_{-1} \qquad (4)$$

In Figure 4a,b we plotted the data from these experiments. The data showed rapid interconversion with rate constants k_i of ≈0.04 min^{-1}. Expectedly, the rates for the conversion *aSS* → *aRS* and *aRR* → *aSR* are identical within experimental error. For the conversion of one isomer into the equilibrium state, where both diastereomers are present in a ratio 1:1, the rate is $k = 2\ k_i$ ≈0.08 min^{-1}. This rate corresponds to a half-life ($\tau = \ln 2/k$) of ≈ 9 min, indicating >98% interconversion in 60 min at 200 °C.

For comparison, and to extrapolate conversion rates to other temperatures, we have plotted the data for the two isomers and the data reported for *rac*-metolachlor by Moser et al. (*9*) in Figure 5. The slope of this plot corresponds to an E_r value of 127 kJ M^{-1}. The lower value compared to the value (154 ± 13 kJ M^{-1}) reported by Moser et al. (*9*) likely reflects methodical differences, experimental error, or differences in the reaction conditions (solvents). Nevertheless, the plot is useful to estimate reaction rates at temperatures other than those used in the experiments, and to evaluate possible consequences in GC and GC−MS analyses.

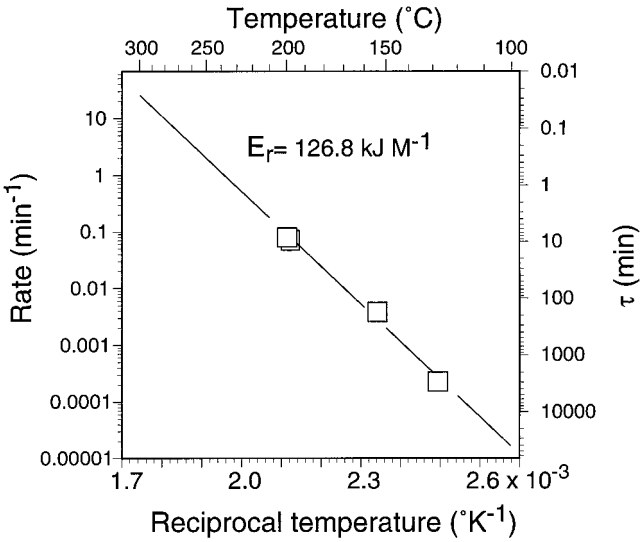

Figure 5. Plot of the interconversion rates (min^{-1}) of metolachlor atropisomers as a function of temperature, as measured in this study (200 °C) and in the study by Moser et al. (*9*; 128 and 154 °C). Right side scale, half-life, $\tau = \ln 2/k$ (min). The slope corresponds to an energy barrier to rotation (E_r) of 126.8 kJ M^{-1}.

A much lower energy barrier and even "free rotation" of metolachlor atropisomers at ambient temperature in some solvent systems was reported by Jayasundera et al. (*15*). Because "free rotation" of metolachlor would have considerable consequences for this and other work, the NMR experiment of Jayasundera et al. was repeated, and also cross-checked using the two isomers isolated and chiral GC−MS. In both cases, no evidence for "free rotation" was obtained (*13*).

Consequences of Atropisomerism to GC and GC−MS Analysis. In principle, atropisomers may interconvert during GC analysis because generally higher temperatures are encountered during these analyses. Three possibilities should be distinguished: (i) interconversion prior to GC separation, (ii) interconversion during GC on the column, and (iii) interconversion following GC separation.

First, interconversion may occur during injection in a heated injector such as when using SSL injection. For injector temperatures of 250−280 °C, routinely used by some laboratories for metolachlor (*16*), rate constants corresponding to half-lives in the range of 5−20 s are extrapolated from the plot in Figure 5. Because mean residence times of the analytes in SSL injectors are up to 60 s, considerable interconversion is expected, and pure *aSS*- and *aRR*-metolachlor analyzed using SSL injection actually resulted in ≈25% and ≈45% conversion of one isomer into its diastereomer at 250 and 280 °C, respectively (chromatograms not shown). It is thus clear that under these conditions SSL cannot be used for the isomer-specific analysis of metolachlor.

Second, there is a potential for interconversion during GC analysis on the GC column because metolachlor elutes at about 200 °C from the PS086-BSCD column. For rapidly interconverting isomers, single peaks are expected (peak coalescence), such as is the case for the related chiral herbicide dimethenamid with a lower energy barrier to rotation (*7*). For less rapidly interconverting isomers, characteristic peak elution profiles with inter-peak plateaus are observed, as reported for certain

biphenyl derivatives (*17*). For metolachlor, with an elution temperature of 200 °C and a temperature programming rate of 3 °C/min, a cumulative (integrated) reaction time (t) equivalent to ≈5 min at 200 °C is estimated from the rates plotted in Figure 5, corresponding to an interconversion of ≈15% of one atropisomer into the other. A careful inspection of the chromatograms of the single isomers (Figure 3c,d) revealed a consistent peak tailing of *aSS*-metolachlor (peak 1), where the corresponding atropisomer is later-eluted, and a consistent peak-fronting of *aRR*-metolachlor (peak 3), where the corresponding atropisomer is earlier-eluted. These effects are independent and in addition to those from injection via a heated injector. However, isomer-specific analyses of metolachlor are possible despite some interconversion on the GC column, as shown for the single isomers and the thermally equilibrated samples (see Figure 3c−f).

Third, interconversion of metolachlor diastereomers may occur following GC separation such as in the GC−MS interface and in the ion source of the mass spectrometer. Conversion in the interface (temperature in this study, 230 °C, in other studies higher), however, is unlikely because the actual residence times in the interface are very short (milliseconds). Furthermore, interconversion would occur following actual separation of the isomers and thus not affect peak profiles and isomer ratios. Interconversion in the ion source during ionization (EI), on the other hand, is more likely because of the high (70 eV) electron energies involved, possibly resulting in complete randomization. If this is the case, all metolachlor isomers would yield identical mass spectra and show identical response.

Use of Single Isomers in Environmental Studies. Single isomers are not only required as reference compounds in isomer-specific analyses, they are also required for a comprehensive evaluation of the environmental behavior and fate of chiral pesticides, where the underlying processes such as uptake and metabolism in plants, and microbiological degradation in soil and water, are often enantioselective. Whereas some of these data can be obtained from studying the racemic products or isomer mixtures, a detailed knowledge of chemical and biological enantiomerization/racemization (chiral stability), and its magnitude relative to other processes, can only be obtained by studying single isomers. Laboratory investigations to approach such problems are feasible with relatively small amounts of compounds, such as were isolated in this study.

In recently published work, the metolachlor isomers isolated in this study were used as qualitative standards, in combination with the chiral HRGC method described here, to determine the stereoisomer composition of technical products and of environmental residues (*14*). The environmental response to the replacement of racemic with enantiomerically enriched *S*-metolachlor was also studied and the method was used to apportion the relative contributions of racemic versus nonracemic (*S*-) metolachlor in surface waters. The next years will likely see a series of additional pesticides presently still produced and used as isomer mixtures being replaced by single-isomer compounds (*1*, *2*, *4*, *18*). Up to now, case-by-case procedures were used to evaluate the risk reduction and benefits of such replacements. We feel that an integrated approach, similar to the one presented by Testa and Trager (*19*) for pharmaceutical compounds, would be benefical, and that the study presented may contribute to a better understanding of the processes involved.

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