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# Environmental Behavior of the Chiral Herbicide Haloxyfop. 1. Rapid and Preferential Interconversion of the Enantiomers in Soil

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Supporting Information

**ABSTRACT:** Haloxyfop-methyl is a chiral herbicide that was first introduced as racemate and later replaced by "haloxyfop-P-methyl", mainly consisting of the *R*-enantiomer, which carries the herbicidal activity. We studied the ester cleavage of haloxyfop-methyl and further degradation and chiral inversion of the acid enantiomers in three different soils using enantioselective gas chromatography−mass spectrometry. Our results confirm the rapid ester hydrolysis of haloxyfop-methyl with half-lives of a few hours and indicate that hydrolysis is weakly enantioselective. Further degradation of haloxyfop was slower with half-lives of several days. In all three soils, *S*-haloxyfop was rapidly converted to *R*-haloxyfop. In sterile soil, no degradation and no inversion were observed, indicating that both processes are biologically mediated. In soil where 50% of the water had been replaced by deuterium oxide, significant H−D exchange in haloxyfop was observed, pointing to a reaction mechanism involving abstraction of the proton at the chiral center of the molecule.

**KEYWORDS:** *haloxyfop, soil, enantioselective degradation, chiral inversion, enantioselective GC-MS*

## INTRODUCTION

The aryloxyphenoxy-propionate herbicides (FOPs) control graminaceous weeds in broad-leaved crops such as soybean or sugar beet and are applied postemergent at rates of typically 100−400 g active ingredient/ha. They can be formulated alone or in combination with other herbicides or a safener to enhance selectivity also in cereals.[1] At present, 10 compounds belong to the FOPs, which share a common mode of action by inhibiting acetyl coenzyme A carboxylase (ACCase) and hence the synthesis of fatty acids in sensitive plants.[2] The compounds are applied as esters, which are rapidly hydrolyzed in soil and plants to their herbicidally active acid metabolites.

All FOPs possess an asymmetrically substituted carbon atom in the propionic acid moiety (Figure 1) and are therefore chiral.[3] Initially, the FOPs were developed and introduced in the 1970s and 1980s as racemates.[4] Later, it was recognized that the herbicidal activity is essentially associated with the *R*-enantiomers, and some of the racemic compounds were replaced by the enantioenriched "P" compounds, which offered higher specific activity and lower use rates ("chiral switch"). The suffix "P", as in haloxyfop-P, relates to the fact that all *R*-enantiomers of FOPs show dextro-(+)-rotation of the plane of polarized light.

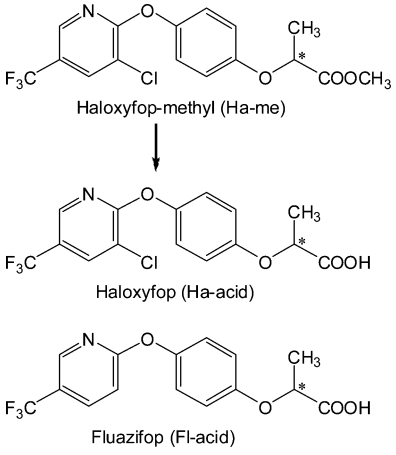


**Figure 1.** Structures of haloxyfop-methyl, the major herbicidally active soil metabolite haloxyfop, and fluazifop, used as internal standard. The asterisk indicates the asymmetrically substituted carbon.

The stereochemistry of substituted propionic acids has been the subject of a series of papers not least because aryl propionic acids (APAs), such as ibuprofen or naproxen, are well-known nonsteroidal anti-inflammatory drugs.[5,6] These drugs, when administered as racemates, are rapidly and almost completely converted to the *S*-enantiomers, which carry the desired therapeutic activity (note that the *S*-configuration in APAs topologically corresponds to the *R*-configuration in FOPs). It is now generally accepted that the chiral inversion of APAs occurs through formation of a (diastereomeric) coenzyme A-thioester with subsequent epimerization of the activated ester by an epimerase.[5,6]

Whereas the mechanism and the rates for the interconversion of the APA enantiomers in mammals are well described and understood, comparatively less is known for the herbicidal FOPs. Plant uptake is mainly through leaves but, after ester cleavage in soil, to a lesser extent also through roots. The enantiomer composition of residual haloxyfop in weeds and crops is unknown and, consequently, the current residue definitions do not state stereochemistry (e.g., "sum of haloxyfop, including haloxyfop-P, its esters, and its conjugates

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Table 1. Characterization of Soils Used for Incubation Experiments

| site name | altitude (m asl) | land use | soil type | sand (%) | silt (%) | clay (%) | pH ($CaCl_2$) | organic carbon (%) | soil moisture[a] (% w/w) (% of MWHC)[b] |
|---|---|---|---|---|---|---|---|---|---|
| Realp | 2120 | alpine pasture | clay | 12.2 | 39.0 | 48.8 | 4.0 | 18.1 | 63−64 |
| | | | | | | | | | 59−60 |
| Neualp | 955 | fertilized pasture | sandy loam | 59.5 | 20.8 | 19.7 | 5.5 | 4.2 | 31−32 |
| | | | | | | | | | 39−40 |
| Dübendorf | 440 | arable land | loam | 40.5 | 32.3 | 27.2 | 7.2 | 1.9 | 19−20 |
| | | | | | | | | | 37−39 |

[a]Soil moisture varied slightly from experiment to experiment due to different water volumes used for spiking. [b]Maximum water-holding capacity.

expressed as haloxyfop"),[7] and residue enforcement methods (e.g., the German official multiresidue method)[8] do not address enantiomer composition. Moreover, the fate of individual stereoisomers in soil is not well understood. Although the rapid enantiomerization of several FOPs, for example, fluazifop, diclofop, and fenoxaprop, in soil was previously described,[3,9−11] a deeper insight into the mechanism and actual conversion rates are still missing.

In this paper, we describe an enantioselective analytical method used to isolate milligram quantities of single enantiomers of haloxyfop (both acid and methyl ester) based on HPLC as well as a method for the enantioselctive analysis of these compounds in soil extracts based on GC-MS. The latter was also used to analyze the compounds in plant extracts as reported in our companion paper.[12] Our results confirm the rapid hydrolysis of haloxyfop-methyl (Ha-me) to haloxyfop (Ha-acid) in soil. We then examine the rapid and preferential interconversion and further degradation of Ha-acid in soil as determined by incubating the racemic compound and the pure *R*- and *S*-enantiomers. Three different soils are used to determine if soil properties have any influence on the enantioselectivity of transformation. Control experiments in sterile soil are performed to determine whether the conversion is biologically mediated. A hypothesis on the mechanism of conversion in soil is postulated on the basis of H−D exchange experiments and mass spectrometric analysis. Mathematic modeling is applied to derive kinetic parameters for *R*/*S* interconversion and degradation in soil.

## MATERIALS AND METHODS

**Reference Materials.** Racemic haloxyfop-methyl and haloxyfop-P-methyl (*rac*-Ha-me and *R*-Ha-me; 99.2 and 99.1%, respectively) were obtained from Dow AgroSciences, Hitchin, UK, and *rac*-haloxyfop (*rac*-Ha-acid, 99.8%) was from Riedel de Haen, Seelze, Germany. Pure *R*- and *S*-enantiomers of Ha-me and Ha-acid were isolated from the racemates by enantioselective HPLC (see below). *rac*-Fluazifop (*rac*-Fl-acid, 94.5%, from Dr. Ehrenstorfer, Augsburg, Germany) and *rac*-fluazifop-methyl (*rac*-Fl-me), prepared by methylation of *rac*-Fl-acid using diazomethane (see below), were used as internal standards. For CAS names of the various compounds used, see Supporting Information Table S2.

**Semipreparative Isolation of *R*- and *S*-Enantiomers of Ha-me and Ha-acid.** Pure *R*- and *S*-enantiomers of Ha-me were prepared from the racemic compound in milligram quantities by enantioselective HPLC according to a method published by CIPAC.[13] The enantiomers were separated on a silica gel column coated with cellulose tricinnamate (Chiralcel OK, 25 cm × 4 mm, 10 μm particle size; Daicel Chemical Industries, Osaka, Japan) at ambient temperature using heptane/isopropanol/methanol (85:5:10) as eluent at a flow rate of 1 mL/min and UV detection at 280 nm. *R*- and *S*-enantiomers of Ha-me eluted baseline separated after 6.7 and 8.8 min, respectively, with peak widths of 1.0 and 1.5 min (chromatogram, see Supporting Information Figure S5).

Pure enantiomers of Ha-acid were prepared using the same column and heptane/isopropanol/acetic acid (95:5:0.1) as eluent at a flow rate of 1 mL/min and UV detection at 280 nm. *R*- and *S*-Ha-acid eluted baseline separated after 13.5 and 20.4 min, respectively (peak widths of 4 and 6 min; chromatogram see Supporting Information Figure S6). A total of 10 injections of *rac*-Ha-me and *rac*-Ha-acid (0.5 mg in 100 μL), respectively, were made, and fractions were collected at the exit of the UV detector while the UV trace was observed. The pooled fractions contained approximately 2 mg of each enantiomer with >99% enantiomeric purity as determined by enantioselective GC-MS after methylation with diazomethane when appropriate (see below). Isolated enantiomers were stored in mobile phase until used for incubation experiments. Aliquots were then evaporated to dryness and redissolved in spiking solution (water or water/methanol, see below).

**Incubation of Ha-me and Ha-acid in Soil.** Soil samples were collected at sampling locations in close proximity to those of the Swiss soil monitoring network (NABO, www.bafu.admin.ch/boden). The soils were selected to cover a wide range of properties, in particular pH and organic carbon (Table 1). Standard equipment was used for sampling soil from the top 10 cm. The soils were kept in the dark at ≈4 °C for no more than 6 months prior to use.

Portions of 2 mm sieved, field-moist soil (100 g) were spread on glass dishes, and the test substance was applied using a small spray bottle (1−2 mL of an aqueous solution containing 100 μg per enantiomer of Ha-acid). For experiments with Ha-me (only with soil Neualp), the spike solution contained 30% methanol as cosolvent to increase the solubility (note the solubility of Ha-me in water is 9.1 mg/L[7]). The resulting spike levels were 1 μg enantiomer/g moist soil and thus high enough to allow for reliable quantification down to 1% of the initial concentration. This spike level is somewhat higher than the concentrations expected from typical application rates in the field of up to 160 g/ha (0.21 μg/g assuming uniform incorporation in the top 5 cm soil layer and a bulk density of 1.5 g/cm$^3$) but is not expected to affect soil microorganisms. Separate experiments were carried out with racemic compounds and with pure *R*- and *S*-enantiomers. The soils were then thoroughly mixed, transferred to Erlenmeyer flasks, closed with air-permeable cellulose plugs, and incubated at 20 °C in the dark for up to 4 days. The moisture of the soils (24−174 g water/100 g dry soil, corresponding to 37−60% of the maximum water-holding capacity) was regularly checked by weighing and kept constant by the addition of distilled water. At appropriate time intervals, aliquots of 10 g of soil were removed for extraction and analysis.

**Sterile Control Experiments.** Abiotic transformation of Ha-acid was studied using a γ-irradiated soil (soil Dübendorf, Table 1) as sterile control. Portions of 200 g of soil in closed screw-cap glass jars were exposed for a few hours to γ-irradiation (dosimeter controlled total dose of 30 kGy) from a commercial $^{60}$Co source (Leoni Studer-Hard, Däniken, Switzerland). The glass jars were kept tightly closed after irradiation until the start of the incubation experiments to ensure sterile conditions. Spiking was done in the same way as for the nonsterile soils except that the soil was kept in the glass jars during spiking. Nevertheless, sterile conditions were broken upon spiking. As the incubation experiments lasted for only a few days, this was not expected to allow for restoration of any substantial biological activity in the soil.

**H−D Exchange Experiments.** Three 100 g portions of 2 mm sieved, field-moist soil (Neualp, Table 1) were spread on glass dishes


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and allowed to dry to approximately 50% of their initial water content. The evaporated water was replaced by dropwise addition of deuterium oxide (Fluka, Buchs, Switzerland) to achieve an approximate 1:1 ratio of $H_2O/D_2O$. As $D_2O$ can be toxic to soil microorganisms, a larger proportion was avoided. The soils were then spiked in the same way as the other soils (separate experiments with *rac*-Ha-acid and with pure *R*- and *S*-enantiomers). Soil moisture was maintained by the addition of a 1:1 mixture of distilled water and deuterium oxide.

**Extraction and Derivatization.** Soil samples from experiments with Ha-acid (10 g) were collected from the incubation vessels in 20 mL glass scintillation vials. Immediately after weighing, 10 mL of methanol and 100 μL of a methanolic solution of *rac*-Fl-acid (0.1 μg/μL) were added and the vials closed and vigorously shaken by hand for approximately 1 min and then stored at −20 °C until further processed (typically within 1 day). The vials were centrifuged at a relative centrifugal force (RCF) of 720 for 5 min (rotor with a radius of 16 cm, 2000 rpm), and the clear supernatants were removed into 30 mL glass vials, diluted with 10 mL of distilled water, and acidified to pH ≈1 by the addition of 4 drops of 5 M sulfuric acid. The extracts were then partitioned twice with 5 mL of dichloromethane, and the combined organic extracts were allowed to evaporate to dryness overnight in a fume hood. The extracts were then redissolved in a few drops of methanol and methylated by the addition of approximately 300 μL of an ethereal diazomethane solution until the yellow color of the diazomethane persisted (see ref 14 and cautionary note therein). The reaction mixtures were allowed to stand for 15 min and then carefully evaporated to dryness in a stream of air, and the dry residues were dissolved in 5 mL of hexane. An aliquot of 1 μL was used for GC-MS analysis. Absolute recoveries over the extraction and derivatization procedure, determined in soil Neualp, were 71% for both Fl-acid and Ha-acid, respectively (spike level, 1 μg/g moist soil). This indicates that both compounds show very similar behavior and that Fl-acid is thus an appropriate internal standard for Ha-acid. The method precision, expressed as relative standard deviation of multiple extractions, on average was 3.2% (range, from 0.3 to 5.9%).

In exploratory experiments with Ha-me, the same extraction procedure was used. In contrast to the experiments with Ha-acid, prior to the extraction the soil samples were fortified with 10 μg of *rac*-Fl-acid in 100 μL of methanol and 10 μg of *rac*-Fl-me in 100 μL of ethyl acetate as internal standards. The samples were derivatized with diazoethane (for a description of the preparation, see ref 12) rather than diazomethane, to allow for the simultaneous determination of parent Ha-me as well as Ha-acid formed during incubation. An aliquot of 1 μL was used for GC-MS analysis. Absolute recoveries over the extraction and derivatization procedure were 68 and 71% for Fl-me and Ha-me, respectively (spike level, 1 μg/g moist soil).

Several control experiments were conducted to ensure that no artifacts were produced during extraction and derivatization. Selected samples were spiked only with Fl-acid and then derivatized with diazomethane or diazoethane to confirm that no (<1%) ethyl ester was formed upon methylation and vice versa. Samples spiked only with Fl-me were derivatized with diazoethane to confirm that no transesterification occurred (<1% Fl-et present in ethylated soil extract dissolved in ethyl acetate). Finally, soils freshly spiked with single isomers of Ha-me and Ha-acid were processed to confirm that no hydrolysis and no enantiomerization occurred during sample preparation.

**Enantioselective GC-MS Analysis.** A Quattro Micro GC-MS/MS system (Waters Corp., Milford, MA, USA) connected to a HP6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) fitted with a PAL autosampler (CTC Analytics, Zwingen, Switzerland) was used in the electron ionization (EI, 70 eV, 180 °C) mode. Full-scan EI mass spectra ($m/z$ 35−435, 0.5 s/scan, nominal mass resolution) were recorded for analyte detection and identification. Analysis of the methyl and ethyl esters was carried out in the selected ion monitoring mode for optimum sensitivity (cycle time = 0.5 s) using the $m/z$ values listed in Table 2.

A custom-made 20 m GC column coated with OV 1701 containing 15% (w/w) permethyl-β-cyclodextrin with a film thickness of 0.1 μm (BGB Analytics, Boeckten, Switzerland) was used for separation. The

**Table 2. GC-MS Masses Used for Determination of Target Compounds, Retention Times of the Enantiomers, and Chromatographic Resolution Achieved with the Permethyl-β-Cyclodextrin Column**

| target compound | $m/z$ (all $M^+$) | retention times (min) | resolution |
|---|---|---|---|
| fluazifop-methyl (Fl-me) | 341.1 | 38.5/38.7 | 1.4 |
| fluazifop-ethyl (Fl-et) | 355.1 | 39.9/40.1 | 1.3 |
| haloxyfop-methyl (Ha-me) | 375.1 | 41.3/41.5 | 1.3 |
| haloxyfop-$D_1$-methyl | 376.1 | 41.3/41.5 | 1.3 |
| haloxyfop-ethyl (Ha-et) | 389.1 | 42.7/42.9 | 1.2 |

GC conditions were as follows: split/splitless injection (240 °C, 48 s splitless time); temperature program, 50 °C for 2 min, increase to 120 °C at a rate of 25 °C/min and to 220 °C (upper temperature limit for this type of columns) at 2 °C/min. Helium was used as carrier gas at a flow rate of 2 mL/min. The column showed excellent stability, low bleed, and good and constant, nearly baseline, resolution of Ha-me, Ha-et, Fl-me, and Fl-et enantiomers (Table 2).

**Kinetic Evaluation of Experimental Data.** All kinetic analyses of experiments were performed using the modeling software Aquasim, version 2.1b.[15] Degradation and isomerization were implemented as first-order processes based on the conceptual model given in Figure 2,

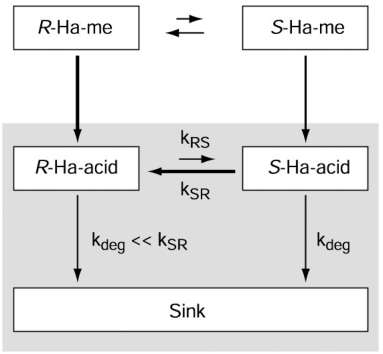


**Figure 2.** Reaction scheme and definitions of reaction rate constants used in the model calculations. The shaded area highlights the portion that was covered by model calculations.

and the corresponding rate constants were determined by fitting the modeled curves to measured data. The rate equations used for curve fitting were

$$d[S]/dt = k_{RS} \times [R] - (k_{SR} + k_{deg}) \times [S]$$

$$d[R]/dt = k_{SR} \times [S] - (k_{RS} + k_{deg}) \times [R]$$

Initial enantiomer concentrations were not fixed, but were fitted as additional parameters, with the following exceptions: the initial concentration of *S*-Ha-acid in the experiment with enantiopure *R*-Ha-acid was set to zero (and vice versa), and equal initial concentrations of *R*- and *S*-Ha-acid were assumed in the experiments with *rac*-Ha-acid.

Fitting was done in several steps. First, all rate constants, including separate degradation rate constants for each enantiomer, were fitted individually for each experiment. This approach did not yield satisfying solutions for the degradation rate constants because isomerization (particularly $S \rightarrow R$) is substantially faster than degradation. It is thus not possible to determine whether the *S*- or the *R*-enantiomer is more rapidly degraded because a small change in the isomerization rate constants would compensate for that difference. Therefore, a more robust fitting approach was used assuming that both enantiomers are degraded with an equal rate constant, $k_{deg}$. Isomerization rate constants $k_{RS}$ and $k_{SR}$, respectively, were fitted for each soil simultaneously with data from the experiments with *rac*-Ha-acid and the pure *R*- and *S*-


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enantiomers, using the enantiomer fractions[16] ([R]/([R] + [S]) and [S]/([R] + [S]), respectively) rather than individual concentrations as fit targets. The fitted values for $k_{RS}$ and $k_{SR}$ were then fixed, and $k_{deg}$ values were determined for each individual experiment by fitting of the total concentrations (sum of *R*- and *S*-enantiomers).

## RESULTS AND DISCUSSION

**Analytical Considerations.** For the sensitive and accurate enantioselective determination of Ha-me and Ha-acid in soil, chiral GC with mass spectrometric detection was used. Ha-acid and Fl-acid (internal standard) were converted to the respective methyl esters using diazomethane. In the initial experiments with Ha-me, selected samples were derivatized with diazo*ethane* to allow for simultaneous detection of Ha-acid (as ethyl ester) and Ha-me. Experiments with sterile soil showed that the extraction and derivatization procedures did not change the enantiomer ratios of Ha-acid.

Several chiral GC columns were evaluated for resolution of haloxyfop and fluazifop enantiomers. Whereas a dimethyl-*tert*-butyldimethylsilyl-*β*-cyclodextrin column had shown excellent enantiomer resolution of various phenoxypropionic acid herbicides,[17] this selector was found to provide insufficient chromatographic resolution of haloxyfop and fluazifop enantiomers. Eventually, a column coated with OV 1701 containing 15% (w/w) permethyl-*β*-cyclodextrin provided best resolution and allowed elution of the target analytes below 200 °C and, thus, well below the temperature limit of the column of 220 °C (Figure 3).

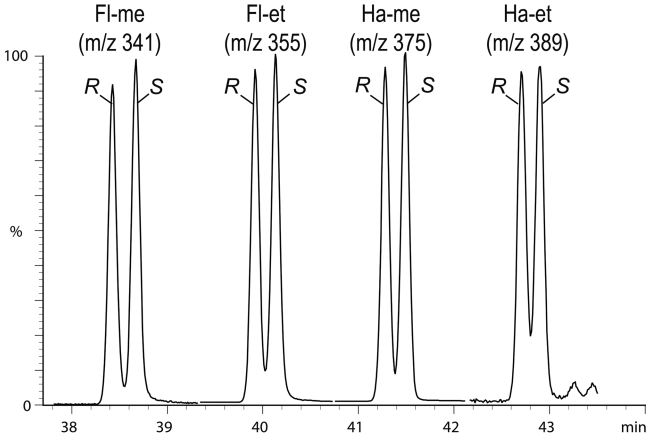


**Figure 3.** EI SIM chromatograms of racemic reference compounds of the methyl and ethyl esters of fluazifop (internal standard) and haloxyfop, analyzed on the enantioselective permethyl-*β*-cyclodextrin column.

The same enantiomer elution order (*R* prior to *S*) was observed for Ha-me, Ha-et, and Fl-me on this chiral selector. It is interesting to note that the same elution order was observed for ibuprofen, when considering its reverted absolute configuration at the *α*-carbon atom (*S* prior to *R*).[18] This indicates a common principle toward chiral recognition of the propionic acid moiety by this cyclodextrin derivative.

Whereas the enantiomer composition of Ha-acid was expected to change during incubation, the racemic fluazifop, serving as an internal standard, theoretically should always show enantiomer ratios of 1:1. The actual ratios for *rac*-Fl-acid (analyzed as methyl and ethyl esters) on a new GC column were indeed very close to 1:1, but with aging of the column, they were shifted toward a small excess in the later eluted *S*-enantiomer (Figure 3), which is commonly observed in enantioselective GC. In this particular case, the shift of apparent peak area ratios was small (approximately 0.9:1.1), and it was not due to peak tailing. Using the observed apparent ratio of the internal standard it was possible to apply a correction factor to the experimental data and thus to obtain accurate enantiomer ratios for haloxyfop.

**Exploratory Soil Incubations of Ha-me.** In initial experiments, *rac*-Ha-me or its pure enantiomers were incubated for 0, 2, and 4 h in soil Neualp (Table 1). In Figure 4 we show

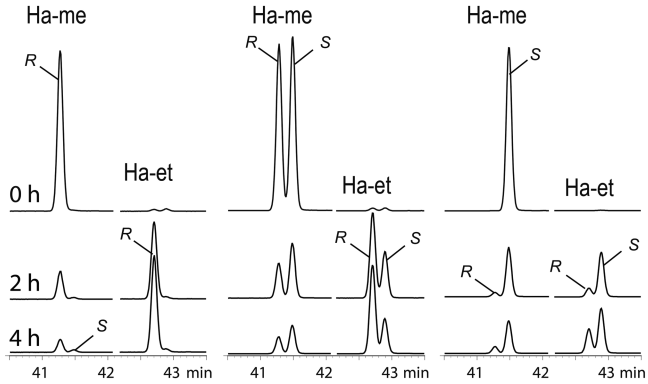


**Figure 4.** EI SIM chromatograms (*m*/*z* 375 + 389) of extracts of soil Neualp spiked with *R*- (left), *rac*- (middle), and *S*-Ha-me (right) immediately after spiking (top) and 2 h (middle) and 4 h later (bottom). Signals are normalized to the peaks for Fl-me used as internal standard. Note the rapid disappearance of Ha-me and the concurrent formation of Ha-acid (analyzed as ethyl ester).

chromatograms from these incubations. In all three experiments, Ha-me rapidly disappeared with half-lives of <2 h, and approximately 75% of the applied Ha-me was transformed to Ha-acid. The chromatograms from the incubation with *rac*-Ha-me indicate that degradation is enantioselective with faster dissipation of the *R*-enantiomer (residual composition, $R < S$). Degradation of the enantiopure compounds was associated with formation of small amounts of the antipode, indicating that enantiomerization occurred, although at a much slower rate compared to degradation.

This initial set of experiments confirmed that rapid degradation of Ha-me was leading to Ha-acid, consistent with previous studies.[7,19] Indeed, the 0 h ("quasi-zero") samples already showed the presence of some small amounts of free acid (analyzed as ethyl esters, Figure 4), whereas standards of Ha-me contained no detectable amounts of free acid. Apparently, the short time (a few minutes) required for preparation (spiking, mixing of soil, sampling) was sufficient to form free acid by hydrolysis of the methyl ester. In the 2 h sample, then, the presence of acid was significant, thus clearly indicating rapid hydrolysis of the methyl ester to the free acid. In a review,[20] the properties of microbial carboxy esterases were described and enantioselective cleavage of, for example, substituted phenoxypropionic acids was reported. Apparently, these esterases are widespread in soil microbiology, which could explain the fast ester cleavage of Ha-me.

Closer inspection of the signals for Ha-acid (analyzed as its ethyl ester) in Figure 4, from incubation of *rac*- and particularly from *S*-Ha-me, indicates that the proportion of *R*-Ha-acid formed is larger than would be expected from the stereochemical composition of the precursor. Furthermore, the proportion of the *R*-enantiomer increases from the 2 h to the


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4 h sample. This indicates that Ha-acid enantiomers formed in soil are rapidly interconverted, with preference for the *R*-enantiomer. This interconversion appears to be faster than the actual degradation of Ha-acid, as its impact on the enantiomer composition is clearly observed. As Ha-acid is the active metabolite of Ha-me and is present in soil much longer than Ha-me, its enantiomer composition determines the residual herbicidal activity. Therefore, we investigated the degradation and enantiomerization of Ha-acid in soil in more detail.

**Incubation of *rac*-, *R*-, and *S*-Ha-acid in Three Different Soils.** Separate incubation experiments were conducted with racemic and enantiopure Ha-acid to investigate possible enantioselective degradation as well as conversion of one enantiomer to the other. From the experiment with Ha-me it was expected that changes in enantiomeric composition are fast. Therefore, short initial sampling intervals of 4 h were chosen, except for soil Dübendorf (Table 1), for which 1 day intervals were used.

In all three soils, the enantiomer composition of spiked Ha-acid changed rapidly as indicated by the chromatograms shown in Figure 5. In fact, the enantiomer ratio after 8 h of incubation was almost independent of the initial enantiomer composition. The change in composition was faster than the overall dissipation of Ha-acid in soil. It involved the formation of *R*- from *S*-Ha-acid and, to a lesser degree, also the reverse reaction. The enantiomer composition eventually approached a steady state, where the concentration of the *R*-enantiomer was about 10 times that of the *S*-enantiomer. The composition in the three soils, expressed as enantiomer fraction (here defined as concentration of *S*-enantiomer divided by total concentration), is shown in Figure 6. From the curves it is suggested that the final enantiomer composition is independent of the initial composition and very similar in all three soils, whereas the rate at which this composition was achieved differed slightly (curve fitting, see below).

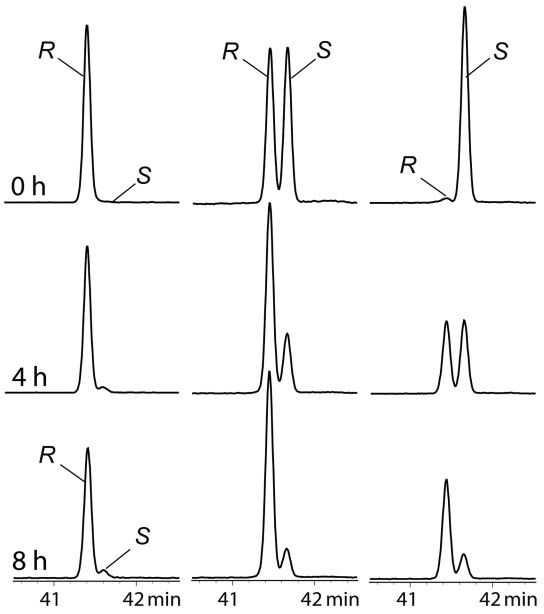


**Figure 5.** EI SIM chromatograms ($m/z$ 375) of extracts of soil Neualp spiked with *R*- (left), *rac*- (middle), and *S*-Ha-acid (right) immediately after spiking (top) and 4 h (middle) and 8 h later (bottom), analyzed after methylation. Signals are normalized to the peaks for fluazifop used as internal standard. Note the rapid disappearance of *S*-Ha-acid and the concurrent formation of the *R*-enantiomer.

In the sterile soil, no degradation and no change in enantiomer composition were observed over the first 4 days of incubation, indicating that degradation as well as enantiomerization in soil are microbially mediated processes. After prolonged incubation, some conversion was observed, probably due to regrowth of microorganisms as sterile conditions were broken with spiking and sampling.

**Kinetic Modeling of Degradation and Enantiomerization of Ha-acid in Soils.** The experimental data in the three nonsterile soils were fitted using the conceptual model shown in Figure 2. In total, three rate constants were fitted to describe the conversion of *R*- to *S*-Ha-acid and vice versa, as well as overall degradation. The actual degradation of Ha-acid was found to be much slower than enantiomerization. Consequently, the degradation rate constants could not be fitted individually for each enantiomer and were thus assumed to be equal for *R*- and *S*-Ha-acid. This does not necessarily mean that the enantiomers were degraded equally quickly, but rather that any differences in degradation rates would be compensated by the rapid enantiomerization.

As an example, the fitting of degradation and enantiomerization of Ha-acid in soil Neualp is shown in Figure 7, where the fitted curves describe the observed behavior (formation and dissipation of enantiomers as well as overall degradation) correctly. The kinetic parameters thus derived for this and the two other soils are shown in Table 3. Overall, degradation was faster in soil Neualp (half-lives = ln $2/k_{deg}$, 2.3−4.0 days) than in the other two soils (6.0−9.3 days). Degradation in the experiments with *rac*-Ha-acid was consistently slower than in experiments with the pure enantiomers. This may be due to the fact that *rac*-Ha-acid was spiked with a slightly lower water volume, and the soil was thus a little less moist (Table 1).

The rate constants for conversion of *R*- to *S*-Ha-acid ($k_{RS}$) were only somewhat higher than the degradation rate constants in the same soils, whereas the rate constants for the reverse reaction were substantially (≈20 times) higher.

Due to rapid enantiomerization, a steady-state enantiomeric composition was achieved within 1−2 days of incubation (Figure 6). From the enantiomerization rate constans ($k_{RS}$ and $k_{SR}$) equilibrium constants ($K_{enant}$) for the enantiomerization of Ha-acid can be calculated as

$$K_{enant} = k_{RS}/k_{SR}$$

The value of $K_{enant}$ corresponds to the enantiomer ratio *S*/*R* at steady state. As shown in Table 3, the values for $K_{enant}$ ranged from 0.061 to 0.082 and were thus very similar in all three soils.

This is in contrast to the situation when a chiral compound is degraded enantioselectively, but with no apparent enantiomerization, such as with the chiral fungicide metalaxyl.[21] In that case, the enantiomer ratio (ER) changes with incubation time according to the equation

$$\ln(ER) = \ln(ER_0) - \Delta k \times t$$

where $\Delta k$ is the difference of the degradation rate constants of the two enantiomers and $ER_0$ is the enantiomer ratio at time zero. This equation indicates that the enantiomer ratio will not reach a steady state at any given time.

In a recent paper on diclofop-methyl,[10] a herbicide that is structurally closely related to Ha-me, the authors used the above equation to evaluate their experimental data. However, they did not take into consideration the rapid enantiomeriza-


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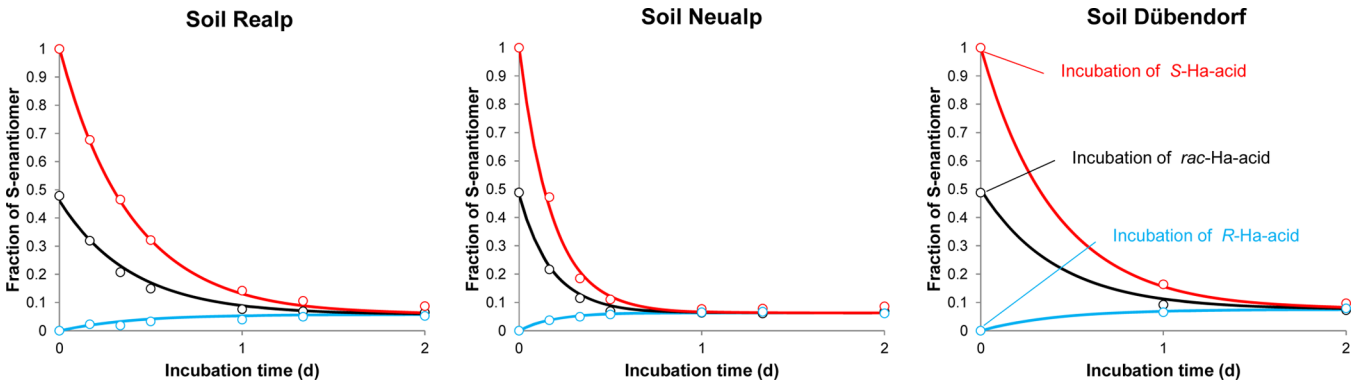


**Figure 6.** Change of enantiomeric composition of Ha-acid in soils, expressed as enantiomer fraction (concentration of *S*-enantiomer divided by total concentration) during incubation of racemic and enantiopure Ha-acid. Lines indicate the fitted curves. Note that the final composition is independent of the initially applied substance.

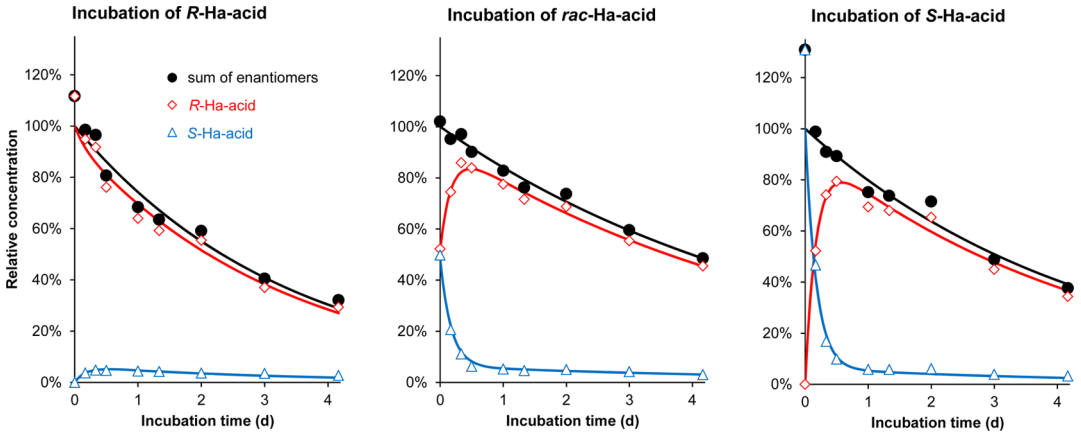


**Figure 7.** Concentrations of *R*-, *S*-, and total Ha-acid in soil Neualp after spiking of *R*- (left), *rac*- (middle), and *S*-Ha-acid (right). Curves were fitted according to the conceptual model shown in Figure 2.

**Table 3. Degradation and Enantiomerization Rate Constants (± Standard Deviation Calculated by the Software) of Ha-acid in Three Soils Determined in Experiments with *rac*-Ha-acid and the Pure *R*- and *S*-Enantiomers Using the Kinetic Scheme Shown in Figure 2[a]**

| soil | spiked with | $k_{deg}$ (day$^{-1}$) | $k_{RS}$ (day$^{-1}$) | $k_{SR}$ (day$^{-1}$) | $k_{RS}/k_{SR}$ |
|---|---|---|---|---|---|
| Dübendorf | *R*-Ha-acid | 0.103 ± 0.012 | 0.184 ± 0.015 | 2.25 ± 0.11 | 0.082 ± 0.008 |
| | *rac*-Ha-acid | 0.080 ± 0.012 | | | |
| | *S*-Ha-acid | 0.115 ± 0.013 | | | |
| Neualp | *R*-Ha-acid | 0.298 ± 0.029 | 0.353 ± 0.086 | 5.21 ± 0.46 | 0.068 ± 0.018 |
| | *rac*-Ha-acid | 0.173 ± 0.017 | | | |
| | *S*-Ha-acid | 0.225 ± 0.020 | | | |
| Realp | *R*-Ha-acid | 0.107 ± 0.002 | 0.147 ± 0.073 | 2.40 ± 0.26 | 0.061 ± 0.031 |
| | *rac*-Ha-acid | 0.074 ± 0.022 | | | |
| | *S*-Ha-acid | 0.113 ± 0.028 | | | |

[a]Note that $k_{deg}$ values were fitted for each experiment individually, whereas $k_{RS}$ and $k_{SR}$ were fitted simultaneously for all experiments with a particular soil.

tion of diclofop acid. To test the applicability of our approach for evaluation of Ha-acid to other, similar cases, we used the data for diclofop acid from ref 10 and applied the same conceptual model as for Ha-acid to derive the kinetic parameters. The resulting plots and kinetic parameters are shown in the Supporting Information (Figures S1−S4; Table S1). Indeed, acceptable fits for diclofop acid were obtained in this way from those previously published data.

**Comparison of Haloxyfop Behavior in Soil with That of Other Phenoxy Acids.** Isomerization of chiral pesticides in the environment was described for numerous compounds from different chemical families.[22] Rapid conversion of enantiomers with clear preference for the *R*-enantiomer was also observed for other FOPs, for example, for fluazifop,[9] diclofop,[3,10] and fenoxaprop.[3] It is also consistent with field experiments with Ha-me, where it was shown that the efficacy of the substance against weeds depended strongly on the enantiomeric


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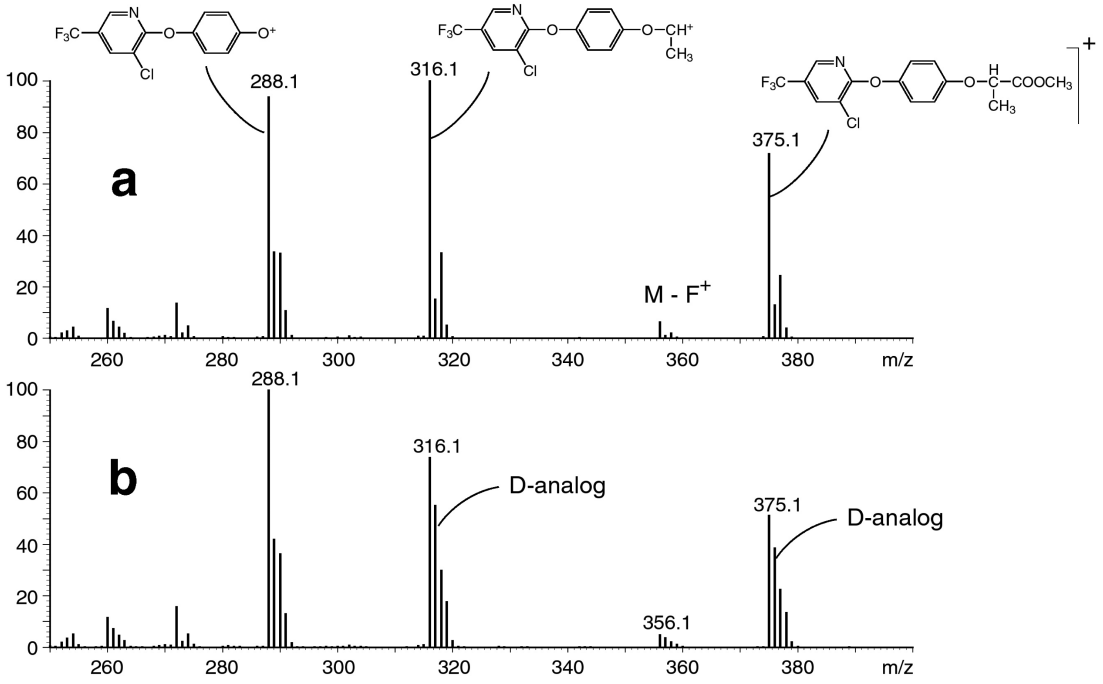


**Figure 8.** Partial mass spectra of (a) Ha-me (reference compound) and of (b) Ha-acid after 4 h of incubation in soil in the presence of $D_2O$ and subsequent methylation. Note the incorporation of a single deuterium into Ha-acid as indicated by the changed isotope profiles of the molecular and the major ions at $m/z$ 375 and 316, respectively (panel b).

composition when applied postemergent (thus applied to the leaves), whereas it was practically independent of the enantiomeric composition when applied pre-emergent (and is thus taken up from soil).[11] In the latter study, residues extracted from soil 7 days after application of Ha-me consisted mainly of *R*-Ha-acid.

Conversion of enantiomers was also observed for phenoxypropionic acid herbicides such as mecoprop and dichlorprop.[17,23] However, in contrast to our results with Ha-acid, degradation appeared to be faster than enantiomerization.[17] The enantioselectivity of degradation/enantiomerization of mecoprop and dichlorprop varied over a wide range and apparently changed with soil pH values (see ref 24 and references therein), whereas no pH dependence with respect to the preferential formation of the *R*-enantiomer was observed with Ha-acid in the three soils studied here.

**Mechanistic Considerations from H−D Exchange Experiments.** Full-scan MS clearly indicated the incorporation of deuterium when Ha-acid was incubated in soil where water was partially replaced with $D_2O$ (H/D ratio of about 1:1). The EI mass spectra of Ha-me (Figure 8), Ha-et, and Fl-me are very similar in that all three show intense molecular ions and agree in their major fragmentation pathways. Ha-me ($M^+$ = 375) and Ha-et ($M^+$ = 389) yield the same major fragment ions at $m/z$ 316 (loss of $COOCH_3$ and $COOC_2H_5$, respectively) and $m/z$ 288 (loss of $CHCH_3COOCH_3$ and $CHCH_3COOC_2H_5$, respectively). No significant differences were observed in the spectra of the enantiomers.

As shown in Figure 8, the mass spectrum of Ha-acid after incubation in $D_2O$-containing soil and analyzed as methyl ester clearly shows changed isotopic patterns of $M^+$ (note increased signal of $m/z$ 376) and for the main fragment ion at $m/z$ 316 (increased signal of $m/z$ 317) due to the incorporation of a single D into the molecule. The exact location of D was deduced from the fact that the isotopic pattern of fragment ion $m/z$ 288 (loss of the complete propionic acid side chain) was still the same as with the native compound. This locates the D label at one of two carbons (C-2 or C-3) of the propionic acid moiety, most likely at C-2 (the methin-H), in the same sense as was observed with the propionic acid herbicide mecoprop.[23] The amount of D incorporation, estimated from the signal intensities at $m/z$ 375 and 376 and when corrected for $^{13}C$ contributions, was about 35%.

The H−D exchange presumably follows a scheme such as outlined in Figure 9 via a planar transitional enolate structure

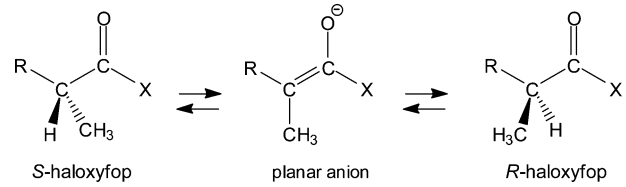


**Figure 9.** Proposed mechanism for the conversion of the enantiomers of Ha-me and Ha-acid in soil. "X" depicts a methoxy, a hydroxy, or a possible intermediate coenzyme A ester, analogous to that proposed for aryl propionic acid isomerization in mammalian metabolism.[6]

(back/front addition of H/D). However, the exchanged proton located next to the carboxylic acid moiety is only weakly acidic. Consequently, this reaction requires enzymatic activity such as in biologically active soils. For APAs it was shown that epimerization proceeds via formation of a coenzyme A thioester.[6] The enzyme that catalyzes epimerization of the coenzyme A esters of APAs in rats (2-arylpropionyl-coenzyme A epimerase) was isolated from rat liver[25] and was subsequently shown to be closely related, if not identical, to the 2-methylacyl-coenzyme A racemases present in peroxisomes and mitochondria,[26] which are involved in the metabolism of branched-chain fatty acids.[27] Although the epimerase is not enantioselective,


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the net formation of an excess of one enantiomer results from enantioselective formation of the coenzyme A thioester.[6]

It is interesting to note that in the WHO JMPR evaluation of haloxyfop[28] the reviewer concluded that in mammals Ha-me undergoes rapid ester cleavage in vivo, followed by an equally rapid and almost complete conversion of *S*- to *R*-Ha-acid. There seems to be a striking similarity in the findings from the studies in rodents, dogs, and monkeys with our finding of the rapid transformations of Ha-me and Ha-acid in biologically active soils. This may indicate that the same biochemical processes are involved in soil as in mammalian conversion of haloxyfop enantiomers, and these may even be very closely related to those involved in the conversion of APA enantiomers.

In contrast to these similarities in behavior of Ha-acid enantiomers in mammalian and soil metabolism, no conversion of Ha-acid enantiomers and no enantioselectivity in degradation of Ha-me or Ha-acid were found in plants.[12] Consequently, the residues in plants most likely reflect the stereoisomer composition of the compound that was applied (uptake by leaves) or that was formed in soil (root uptake).

## ■ ASSOCIATED CONTENT

### Supporting Information

Figures S1−S6 and Tables S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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