DOI:10.1021/jf103537a

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Enantioselective Environmental Behavior of the Chiral Herbicide Fenoxaprop-ethyl and Its Chiral Metabolite Fenoxaprop in Soil

Yanfeng Zhang, Donghui Liu, Jinling Diao, Zeying He, Zhiqiang Zhou, Peng Wang,* and Xuefeng Li*

Department of Applied Chemistry, China Agricultural University, Beijing 100193, China

The enantioselective degradation behavior of fenoxaprop-ethyl (FE) and its chiral metabolite fenoxaprop (FA) in three soils under native conditions was investigated. Two pairs of enantiomers were analyzed by high-performance liquid chromatography (HPLC) with an amylose tri-(3,5-dimethylphenylcarbamate) (ADMPC) chiral column. The degradation of racemic FE in three soils showed the herbicidally inactive *S*-(−)-enantiomer degraded faster than the active *R*-(+)-enantiomer. FE was configurationally stable in soils because no interconversion to the respective antipodes was observed during incubation of the enantiopure *S*-(−)- or *R*-(+)-FE. The main metabolites of FE were confirmed as FA and 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB), and the formation of the chiral metabolite FA showed enantioselectivity in soils. The degradation of rac-FA was also enantioselective with the *S*-(−)-FA preferentially degraded: the half-life ($t_{1/2}$) of the *S*-form in the three soils ranged from 2.03 to 5.17 days, and that of *R*-form ranged from 2.42 to 20.39 days. The inversion of the *S*-(−)-enantiomer into the *R*-(+)-enantiomer occurred in two of the three soils when the enantiopure *S*-(−)- and *R*-(+)-FA were incubated. The data from sterilized control experiments indicated that the enantioselectivity of FE and FA was attributed to microbially mediated processes.

KEYWORDS: Enantioselectivity; chiral; fenoxaprop-ethyl; fenoxaprop; enantiomer; inversion

INTRODUCTION

Most enantiomers have identical physicochemical properties and abiotic degradation rates, but they may deviate dramatically in biological activity, toxicity, and microbial degradation rates when individual enantiomers interact with other chiral molecules (*1*−*4*). Furthermore, other reports describe that different enantiomers in racemates are enantioselectively metabolized (*4*−*6*). Enantioselectivity plays an important role in the environmental fate and ecological risks of a chiral compound, as many environmental processes are enantioselective (*7*, *8*). As a result, the enantiomeric composition may be changed by enantioselective degradation or enantiomerization over time. So efforts should be made to define the mode of action and elucidate the metabolic pathways of each enantiopure stereoisomer by using enantioselective analysis. Information with respect to differences in stereoselective dissipation kinetics and environmental behavior of individual enantiomers will help us improve our understanding of the pesticide risk to humans, animals, and the environment.

Fenoxaprop-ethyl (FE, **Figure 1**), (±)-ethyl 2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoate, a selective aryloxyphenoxypropionate (AOPP) herbicide, is registered for postemergence control of various annual and perennial grass weeds in dicotyledonous crops as well as grains (*9*). FE works by blocking acetyl coenzyme A carboxylase (ACCase) found in plant chloroplasts, which effectively inhibits lipid synthesis in target plants (*10*, *11*). This inhibition leads to chlorosis in susceptible weed species, followed by growth suppression and exsiccation. FE is not persistent in the environment and its degradation mainly follows two different mechanisms under different conditions, which may yield new chemicals (*12*). Because of the breakdown of the ester bond of FE, the parent compound is hydrolyzed to its corresponding acid fenoxaprop (FA), 2-[4-(6-chloro-2-benzoxazolyloxy) phenoxy] propionic acid, which is still biologically active (*13*). By another mechanism, FE (and FA) undergoes degradation forming 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) by cleavage of the ether linkage of FE (*14*, *15*). Sometimes both pathways are concurrent in neutral conditions (*12*). FE and FA are chiral, but CDHB is achiral.

FE (and FA) has an asymmetrically substituted C-atom and thus consists of a pair of enantiomers with *S*-(−) and *R*-(+) absolute configurations and optical rotations (*16*), but the herbicidal activity mostly originates from the *R*-(+)-enantiomer. Studies showed that enantiomers of α-HCH, metolachlor, metalaxyl, benalaxyl, and lactofen behaved significantly differently during biodegradation and bioaccumulation in a variety of ecosystems (*2*, *5*, *17*−*19*). Moreover, enantiomerization of chiral compounds, such as dichlorprop and mecoprop, was found under certain conditions (*20*). Wink et al. elucidated that no racemization of FE took place, but the inversion of *S*-FA to *R*-FA was observed (*21*). Therefore, information on the environmental fate and ecotoxicological effect of each enantiomer of the parent compound FE or chiral metabolite FA should be reevaluated (*22*).

*To whom correspondence should be addressed. E-mail: wangpeng@cau.edu.cn (P.W.) or lxf1966@263.net (X.L.).

 Published on Web 12/01/2010

Enantioselective dissipation in soils has been observed for various chiral pesticides, and some studies suggested that soil properties that have a major impact on the activity of the soil microbial community could influence chiral signatures (*8*, *19*). Therefore, degradation studies on the stereochemical aspects of FE and its main chiral metabolite FA in three soils were investigated under native and sterilized conditions using enantioselective high-performance liquid chromatography (HPLC). Meanwhile, this paper also monitored the chiral stability of FE and FA in soil with enantiopure compounds.

MATERIALS AND METHODS

Chemicals and Materials. Racemic-fenoxaprop-ethyl (rac-FE, 98.0%) and *R*-(+)-fenoxaprop-ethyl {*R*-FE, optical purity 98.0%} were kindly supplied by Institute for Control of Agrichemicals, Ministry of Agriculture of China. Racemic-fenoxaprop (rac-FA, 97.5%), *R*-(+)-fenoxaprop (*R*-FA, optical purity 97.5%) and 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB, 98.0%) were obtained from the hydrolysis of their corresponding parent compounds (*23*). *S*-(−)-Fenoxaprop-ethyl (*S*-FE, optical purity 99.0%) and *S*-(−)-fenoxaprop (*S*-FA, optical purity 99.0%) were collected manually from multiple injections (50 μL) of the corresponding racemic mixture by observing the UV-signal on an Agilent HPLC with a chiral column (250 mm × 4.6 mm (i.d.). Ethyl ether, *n*-hexane, 2-propanol (analytical grade), and trifluoroacetic acid (TFA) were from Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other chemicals and solvents were purchased from commercial sources and were analytical grade.

Soil Samples. Three soil samples representing diversely physicochemical properties were collected at 0−10 cm plow layer from geographically distinct agriculture regions of China. All samples were air-dried at room temperature, homogenized, passed through a 2-mm sieve, and stored in the dark at 4 °C. More details on soil sites and specific physicochemical characteristics (particle size, texture, pH, and organic carbon) are presented in **Table 1**.

Incubation in Soils under native Conditions. Separate incubation experiments were conducted with the racemate and the pure *S*-(−)- and *R*-(+)-compounds, respectively, using 50-mL polypropylene centrifuge tube covered with aluminum foil. Approximately 5 g of soil (dry weight equivalent) was fortified with 50 μg (50 μL of a 1000 μg/mL stock solution in acetone) of rac-, *S*-(−)-, or *R*-(+)-FE and rac-, *S*-(−)-, or *R*-(+)-FA, respectively, and allowed to air-dry for 5 min before being homogenized thoroughly for 5 min (fortification level of 10 μg/g, experiments SN1-SN6, SY1-SY6, and SC1-SC6, respectively). The soil samples were rehydrated and incubated at 25 °C in the dark, and sample weights were controlled regularly by weighing for water content (about 60% of the field holding capacity (w/w)) in order to compensate for loss of water by addition of deionized water. Three replicate samples were removed from each treatment at different time intervals and immediately transferred into a freezer (−20 °C). Triplicate samples were taken immediately after fortification (t = 0) to determine the recovery and reproducibility of extraction in the soil (see below). Blank determinations of the soil prior to fortification revealed no FE, FA, or CDHB present.

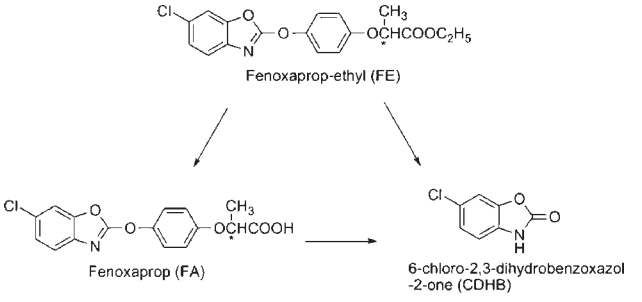

Figure 1. Chemical structures of fenoxaprop-ethyl and its primary metabolites (fenoxaprop and CDHB). Chiral center is denoted by an asterisk (*).

Incubation in Soils under Sterilized Conditions. To determine if the enantioselective dissipation was a result of microbially mediated transformations, a set of soil samples were also prepared and subjected to sterilization treatment, which was achieved by autoclaving the samples twice at 121 °C for 60 min with 24-h intervals to eliminate microbial activity. Portions of 10 g of soil were placed into a separate 50-mL flask and fortified with 100 μg of rac-FE or rac-FA aseptically (spike level, 10 μg/g). Each flask was treated with the nonsterilized samples with addition of sterile water, which was then sealed with film to maintain sterile conditions.

Extraction of Incubated Soil Samples. Samples were thawed at room temperature (half of each sterilized soil sample was removed and placed into 50 mL polypropylene centrifuge tubes, which was equivalent to the native one) and then extracted with ethyl acetate (25 mL) followed by 200 μL of 6 M HCl for efficient extraction of FA. The tube was stirred on a vortex shaker for 3 min, ultrasonically extracted for 10 min, and centrifuged at 4000 rpm for 5 min. Then the organic layer was filtered through anhydrous sodium sulfate for dehydration. The same extraction step was repeated twice with 25 mL of ethyl acetate. The extracts were combined and reduced to near dryness on a vacuum rotary evaporator at 40 °C and then dissolved in 0.5 mL of 2-propanol. An aliquot (20 μL) was analyzed by HPLC.

Enantioselective HPLC Analysis. An Agilent 1200 series HPLC system (Agilent Technology) equipped with a G1322A degasser, a G1311A quat pump, a G1329A automatic liquid sampler, a G1314B variable wavelength UV detector, and Agilent Chemstation software was used. A method for the simultaneous enantiomeric determination of the FE and FA enantiomers and the major metabolite CDHB in soil samples was set up. The baseline separation of these compounds by an amylose tri-(3,5-dimethylphenylcarbamate) (ADMPC) chiral column was obtained with a mobile phase of *n*-hexane/2-propanol/trifluoroacetic acid (TFA) (95:5:0.1, v/v/v) with a flow rate of 1.0 mL/min. TFA was added into the mobile phase for the determination of FA and CDHB as a mobile phase acidic additive. Wavelength for UV detection was 230 nm and temperature for chromatographic separation was 20 °C. These compounds gave five separate peaks, which were as follows: *S*-(−)- and *R*-(+)-FE, CDHB, and *S*-(−)- and *R*-(+)-FA, and the elution order and the identities were established by comparison with the retention time of the enantiopure standards of the FE and FA and the standard of CDHB.

The standard solutions of *S*-(−)-FE, *R*-(+)-FE, *S*-(−)-FA, *R*-(+)-FA, and CDHB (0.5, 1, 5, 10, and 50 μg/mL each compound) for linearity were prepared by diluting the stock standard solution. Calibration curves were generated by plotting peak area of each compound versus their concentration. Recovery estimation was carried out over three concentration levels (adding the standard solutions in 5 g free soil sample to get final concentrations of 0.1, 1.0, 10 μg/g). Recoveries of these compounds were determined immediately after fortification. Experiments showed that the recovery of the above procedure was >90% for FE, FA, and CDHB in soils. The limit of quantification (LOQ) for these compounds in all samples was found to be 0.05 μg/g on the basis of an acceptable RSD of 20%.

Kinetics Analysis. When the degradation fits well to the first-order kinetics, k_S or k_R is the individual degradation rate constant. Corresponding rate constant k is determined from the linear plots

Table 1. Properties and Locations of Tested Soils

	particle-size					
soil site[a]	sand (%)	silt (%)	clay (%)	soil texture	pH (water)[b]	C_{org} (%)
Neimenggu Chifeng (SC)	74.4	21.9	3.7	sandy loam	8.1	1.9
Shandong Yanzhou (SY)	41.9	52.6	5.5	silt loam	6.9	1.1
Jiangxi Nanchang (SN)	30.2	28.1	41.7	clay	5.0	0.7

[a] The sites in China. [b] Suspension of soil in water, 1:2.5 (w/w).

of ln[S] or ln[R] versus time t (*19*):

$$\ln[S] = \ln[S]_{t=0} - k_S \times t \quad (1)$$

$$\ln[R] = \ln[R]_{t=0} - k_R \times t \quad (2)$$

where [S] and [R] are the concentrations at time t, and k_S and k_R are the degradation rate constants of S- and R-enantiomers, respectively.

If degradation is enantioselective ($k_S \neq k_R$), enantiomer composition (ER) of the residues is changing with time, and ER may be expressed as a function of time (t) in the relationship (*5*)

$$ER_t = [S]/[R] = ER_0 \times e^{(k_R - k_S)} = ER_0 \times e^{\Delta k} \quad (3)$$

where ER_0 is the initial ER value, and Δk is the difference between k_S and k_R, which reflects the rate at which ER deviates from ER_0 over time. The above relationship can be further expressed in a linear form after logarithmic transformation of ER (*4*):

$$\ln(ER_t) = \ln(ER_0) + \Delta k \quad (4)$$

A plot of ln(ER) versus t can be used to determine the rate difference Δk and enantioselectivity.

RESULTS AND DISCUSSION

Degradation of FE in Soil. The rapid disappearance of both enantiomers of FE with time was observed in experiments SN1-SN3, SY1-SY3, and SC1-SC3 when the racemate, the enantiopure S-(−)- and R-(+)-FE were incubated under nonsterilized conditions. **Figure 2a−c** showed the chromatograms of the elution of FE from samples of experiment SN1 (incubation with rac-FE) after 0, 0.5, and 20 days, respectively. An initially racemic composition of FE was confirmed, and a slightly faster decrease of S-(−)-FE in comparison to the R-(+)-FE indicated a preferential dissipation of the S-(−)-enantiomer by the soil's enzymatic systems during the incubation. Enantioselective degradation led to relative enrichment of R-(+)-enantiomer which is active against herbs. Degradation in experiment SN1 thus showed an enantioselectivity in the same sense as in experiments SY1 and SC1.

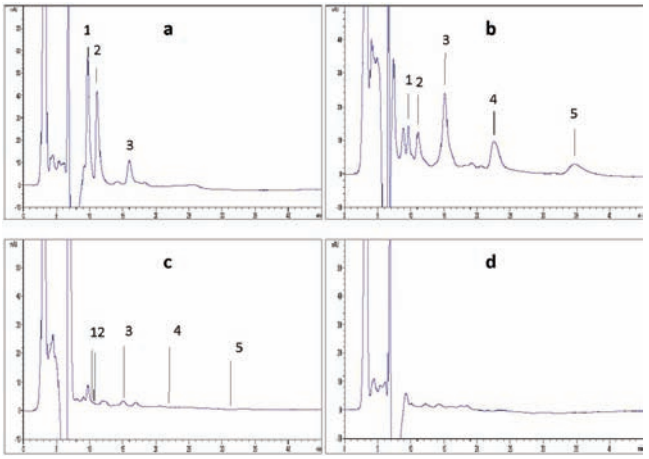

Figure 2. Chromatograms of enantioselective degradation of rac-FE in soil (expt SN1): (**a**) 0 day; (**b**) 0.5 day; (**c**) 20 days; (**d**) blank soil. Peaks: 1, S-(−)-FE; 2, R-(+)-FE; 3, CDHB; 4, S-(−)-FA; 5, R-(+)-FA.

The data of the residual concentrations on two enantiomers were used for estimating the enantiomeric ratio (ER) values (**Table 2**) in these experiments. The ER values of FE from experiment SN1 consistently changed from the initial value of 0.98 (racemic) to 0.42 (concentration [S] < [R], **Figure 3a**). A t test between the means of the ER values of spiked soil and ER = 0.98 yielded a p value of 0.031. Using eq 4, a plot of ln(ER) versus incubation time t was linear for rac-FE (**Figure 3d**). The rate difference Δk calculated from this plot was -0.821 day^{-1}. The ER values of FE from experiment SY1 consistently decreased with time from the initial value of 0.98 to 0.75. A t test between the means of the ER values of spiked soil and ER = 0.98 yielded a p value of 0.012. A plot of ln(ER) versus incubation time t was also linear (R^2 = 0.75) for rac-FE and the rate difference Δk was -0.067 day^{-1}. The ER values of FE from experiment SC1 decreased with time from the initial value of 0.98−0.58. A t test between the means of the ER values of spiked soil and ER = 0.98 yielded a p value of 0.031. A plot of ln(ER) versus incubation time t was linear (R^2 = 0.99) for rac-FE, and the rate difference Δk calculated from this plot was -0.251 day^{-1}. In sum, the degradation of FE in the three soils was enantioselective.

In microbially inhibited experiments (rac-FE) with autoclaved soil samples, ER values also remained close to 1.00, the same as the FE standard. Thus, the enantioselective degradation of FE in native soil samples was predominantly achieved by a biotic pathway.

Chiral Stability of FE in Soil. The incubation experiments group 2 (SN2, SY2, SC2) and group 3 (SN3, SY3, SC3) spiked with enantiopure S-(−)- and R-(+)-FE, respectively. The data documented the high enantiomeric purity of S-(−)- and R-(+)-FE prior to incubation and indicated degradation of

Table 2. Enantiomer Ratio (ER = S/R) of rac-FE and rac-FA metabolite in Experiment SC1, SY1, and SN1

	SC1		SY1		SN1	
time (days)	ER[a] rac-FE	ER rac-FA	ER rac-FE	ER rac-FA	ER rac-FE	ER rac-FA
0	0.98 ± 0.01		0.98 ± 0.01		0.98 ± 0.01	
0.08	0.97 ± 0.01	0.94 ± 0.08	0.93 ± 0.01	0.99 ± 0.08	0.87 ± 0.01	1.96 ± 0.08
0.17	0.93 ± 0.01	0.90 ± 0.08	0.92 ± 0.01	0.76 ± 0.08	0.82 ± 0.01	1.54 ± 0.08
0.33	0.82 ± 0.05	0.88 ± 0.07	0.89 ± 0.05	0.76 ± 0.01	0.73 ± 0.05	1.45 ± 0.07
0.50	0.77 ± 0.05	0.86 ± 0.07	0.82 ± 0.05	0.75 ± 0.07	0.57 ± 0.05	1.21 ± 0.07
1	0.69 ± 0.01	0.78 ± 0.06	0.79 ± 0.01	0.66 ± 0.07	0.42 ± 0.01	0.98 ± 0.06
2	0.58 ± 0.01	0.72 ± 0.10	0.75 ± 0.01	0.61 ± 0.06		0.95 ± 0.08
3		0.62 ± 0.10		0.40 ± 0.10		0.84 ± 0.08
5		0.56 ± 0.08		0.33 ± 0.08		0.81 ± 0.08
7		0.35 ± 0.08		0.17 ± 0.08		0.72 ± 0.07
10		0.31 ± 0.05		0.10 ± 0.07		0.70 ± 0.07
15		0.30 ± 0.07		0.07 ± 0.07		
20		0.29 ± 0.06		0.05 ± 0.06		
25				0.03 ± 0.06		

[a] Values represent the mean ± SD.

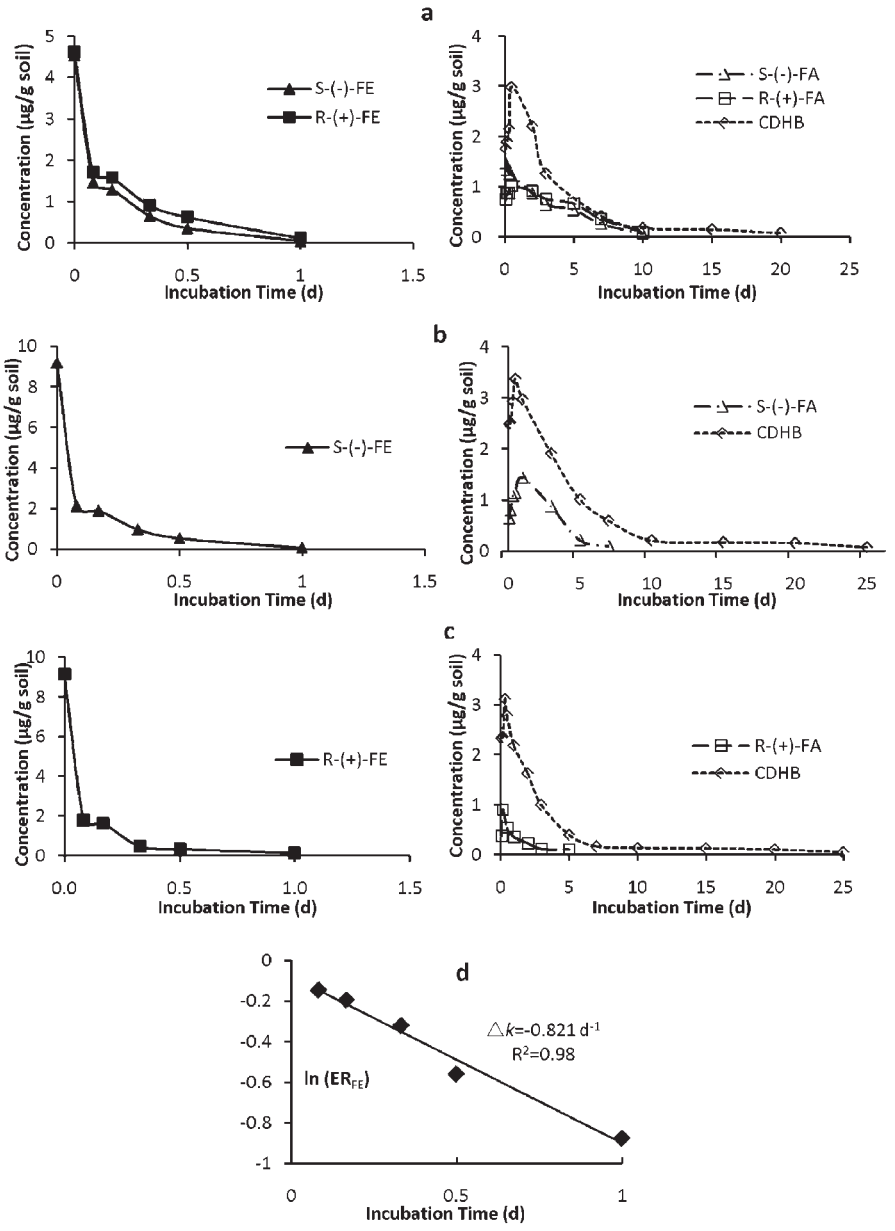

Figure 3. Degradation of FE in soil (expt SN1, SN2, and SN3 with rac-, *S*-(−)-, *R*-(+)-FE, respectively. Note the concurrent rapid formation and slower degradation of CDHB and FA arising from FE): (**a**) degradation of rac-FE (expt SN1); (**b**) degradation of *S*-(−)-FE (expt SN2); (**c**) degradation of *R*-(+)-FE (expt SN3); (**d**) the plot of ln(ER) from experiment SN1 (incubation of rac-FE) versus incubation time *t* showing a linear relationship.

S-(−)- and *R*-(+)-FE from samples of experiments SN2 and SN3 (**Figure 3b**,**c**), respectively. Experiments group 2 (SN2, SY2, SC2) and group 3 (SN3, SY3, SC3) revealed no formation of the *S*-(−)-FE from *R*-(+)-FE throughout the incubation time and vice versa. These data showed that inversion was insignificant compared to degradation indicating that FE configuration was stable in these soils. The results were attributed to rapid hydrolysis and different microbial community.

Formation of the Two Metabolites of FE. FA and CDHB, arising from breakdown of the ester bond and cleavage of the ether linkage, respectively, were the main metabolites of FE detected in this study. The chromatograms in **Figure 2** and the data plotted in **Figure 3a**−**c** showed that the persistent decrease of the unchanged parent compound was accompanied by the concurrent increase and subsequent decrease of FA and CDHB in Jiangxi soils. The hydrolysis of the rac-FE which yielded predominately *R*-(+)-FA residue and the ER values of acquired FA which consistently decreased with time (**Table 2**) showed enantioselectivity in the formation of the chiral metabolite FA in soils. The same formation trend was observed in the other two soil samples.

In Jiangxi soil samples spiked with rac-FE (expt SN1), the amounts of FA and CDHB detected during the initial day of incubation were about 2.22 and 1.76 μg/g, respectively. After further incubation, the concentration of FA reached a maximum of 2.25 μg/g at day 0.50 and then decreased to 0.17 μg/g at day 10. However, the concentration of CDHB reached a maximum of 2.99 μg/g at day 0.50 and then decreased to 0.08 μg/g at day 20. From *S*-(−)- and *R*-(+)-FE (expts SN2 and SN3, respectively) only the formation of the respective FA along with CDHB is observed, and these data indicated that the ester cleavage of *S*-(−)- and *R*-(+)-FE proceeded with retention of configuration, contrary to the activity of the other two soils.

In Shandong soil samples spiked with rac-FE (expt SY1), the amounts of FA and CDHB detected during the initial day of

Table 3. First-Order Rate Constant (k), Half-Life ($t_{1/2}$), and Correlation Coefficient (R^2) for the Degradation of Enantiomers FA in Soils

soil origins	experiment (incubated compound)	enantiomer	k (day^{-1})	$t_{1/2}$ (days)[a]	R^2
Neimenggu Chifeng soil	SC4	*S*-(−)-FA	0.134	5.17 ± 0.05	0.91
	(rac-FA)	*R*-(+)-FA	0.034	20.39 ± 0.03	0.96
	SC5 (*S*-(−)-FA)	*S*-(−)-FA	0.154	4.50 ± 0.07	0.96
	SC6 (*R*-(+)-FA)	*R*-(+)-FA	0.053	13.08 ± 0.08	0.95
Shandong Yanzhou soil	SY4	*S*-(−)-FA	0.280	2.48 ± 0.01	0.94
	(rac-FA)	*R*-(+)-FA	0.153	4.53 ± 0.06	0.95
	SY5 (*S*-(−)-FA)	*S*-(−)-FA	0.234	2.96 ± 0.03	0.98
	SY6 (*R*-(+)-FA)	*R*-(+)-FA	0.132	5.33 ± 0.04	0.92
Jiangxi Nanchang soil	SN4	*S*-(−)-FA	0.342	2.03 ± 0.01	0.96
	(rac-FA)	*R*-(+)-FA	0.286	2.42 ± 0.01	0.96
	SN5 (*S*-(−)-FA)	*S*-(−)-FA	0.290	2.39 ± 0.02	0.98
	SN6 (*R*-(+)-FA)	*R*-(+)-FA	0.242	2.86 ± 0.03	0.97

[a] Values represent the mean ± SD.

incubation were about 2.05 and 1.67 μg/g, respectively. After further incubation, the concentration of FA reached a maximum of 6.75 μg/g at day 2 and then decreased to 2.65 μg/g at day 25. Meanwhile, the concentration of CDHB reached a maximum of 4.04 μg/g at day 1 and then decreased to 0.82 μg/g at day 20. From *R*-(+)-FE (expts SY3) only the formation of the respective *R*-(+)-FA was observed, whereas from *S*-(−)-FE (expts SY2), both *R*-(+)-FA and *S*-(−)-FA appeared, indicating that the ester cleavage of *R*-(+)-FE proceeded with retention of configuration, whereas the ester cleavage of *S*-(−)-FE underwent inversion of the absolute configuration.

In Neimenggu soil samples spiked with rac-FE (expt SC1), the amounts of FA and CDHB detected during the initial day of incubation were about 0.81 and 0.60 μg/g, respectively, and then degraded slightly. After further incubation, the concentration of FA reached a maximum of 9.25 μg/g at day 0.5 and then decreased to 1.21 μg/g at day 25. However, the concentration of CDHB reached a maximum of 0.91 μg/g at day 0.33 and then decreased to 0.12 μg/g at day 25. From *R*-(+)-FE (expts SC3) only the formation of the respective *R*-(+)-FA was observed, whereas from *S*-(−)-FE (expts SC2), both *R*-(+)-FA and *S*-(−)-FA appeared, indicating that the ester cleavage of *R*-(+)-FE occurred with retention of configuration, but the ester cleavage of *S*-(−)-FE occurred with inversion of the optical configuration.

Enantioselective Degradation of the Chiral Metabolite FA in Soil. The degradation from the experiments group 4 (SN4, SY4, SC4), group 5 (SN5, SY5, SC5), and group 6 (SN6, SY6, SC6) with rac-, *S*-(−)-, *R*-(+)-FA, respectively, fitted a first-order kinetics decay model and the data were plotted respectively versus time t. Rate constants and R^2 were listed in **Table 3**. In **Figure 4a**−**c**, the chromatograms from the incubation of the rac-FA (expt SC4) in Neimenggu soil illustrated significant degradation of both enantiomers but with a clearly more rapid degradation of *S*-(−)- than *R*-(+)-FA, leading to residues enriched in *R*-(+)-enantiomer. Data from expt SY4 and expt SN4 showed similar degradation in Shandong soil and Jiangxi soil with varying degrees of enantioselectivity.

In Neimenggu soil samples, the ERs from the incubation of rac-FA (expt SC4) showed a continuous decrease with time from the initial value of 0.98 (racemic) to about 0.03 (concentration [*S*] < [*R*], **Figure 5a**). A plot of ln(ER) versus time t was linear and the slope indicated a rate difference Δk ($k_{Racid} - k_{Sacid}$) of −0.093 day^{-1} (**Figure 5d**), which was in reasonable agreement with the above data ($k_{Racid} - k_{Sacid} = -0.100$ day^{-1}). A t test between the means of ER values of spiked soil and ER = 0.98 yielded a $p < 0.001$. All of them indicated that the degradation of FA was highly enantioselective. In Jiangxi soil samples, the ERs from the incubation of rac-FA (expt SN4) showed a continuous decline with time from the initial value of 0.98 to about 0.69. A plot of ln(ER) versus time t was linear and a rate difference Δk was −0.055 day^{-1}, which was in reasonable agreement with the above data ($k_{Racid} - k_{Sacid} = -0.056$ day^{-1}). A t test between the means of the values of spiked soil and ER = 0.98 yielded a p value of 0.029. These indicated some degree of enantioselectivity. In Shandong soil samples, the ERs from the incubation of rac-FA (expt SY4) showed a continuous fall with time from the initial value of 0.98 to about 0.40. ln(ER) and time t was fitted with a linear correlation and the slope indicated a rate difference Δk of −0.104 day^{-1}, which was in reasonable agreement with the above data ($k_{Racid} - k_{Sacid} = -0.127$ day^{-1}). A t test between the means of the values of spiked soil and ER = 0.98 yielded a p value of 0.030. Enantioselectivity also existed in the degradation of FA.

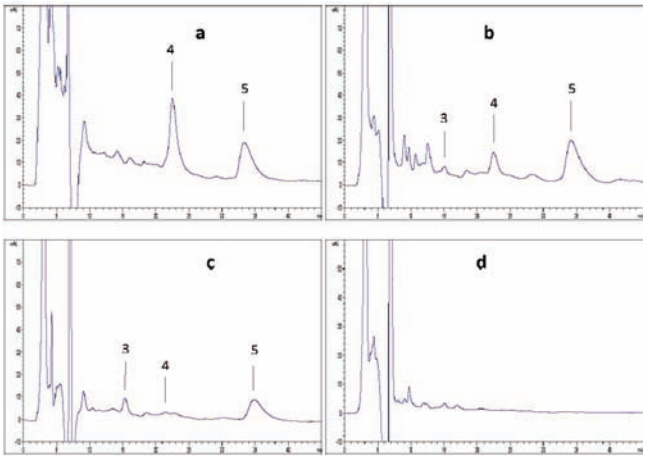

Figure 4. Chromatograms from the incubation of rac-FA in soil (expt SC4) under nonsterilized conditions: (**a**) 0 day; (**b**) 2 days; (**c**) 20 days; (**d**) blank soil. Peaks: 3, CDHB; 4, *S*-(−)-FA; 5, *R*-(+)-FA.

ES, which was defined as ES = $(k_S - k_R)/(k_S + k_R)$, is a measure of enantioselectivity (*1*). The fact that the two enantiomers of FA degraded at different rates ($k_{Sacid} > k_{Racid}$) and the ES value of 0.09, 0.29, and 0.56 were calculated from these rates in experiment SN4, SY4, and SC4, respectively, indicated low to high enantioselectivity of FA in the three soils, and *S*-(−)-FA degraded faster than *R*-(+)-FA. The highest ES was observed in Neimenggu soil with the highest pH (pH 8.1, ES = 0.56), and the lowest ES was found in Jiangxi soil with the lowest pH (pH 5.0, ES = 0.09). In addition, the ES in Neimenggu sandy loam soil is higher than that in Jiangxi clay soil, namely, the ES changed toward less positive values with decreasing sand content and increasing clay content. In control experiments (rac-FA) with

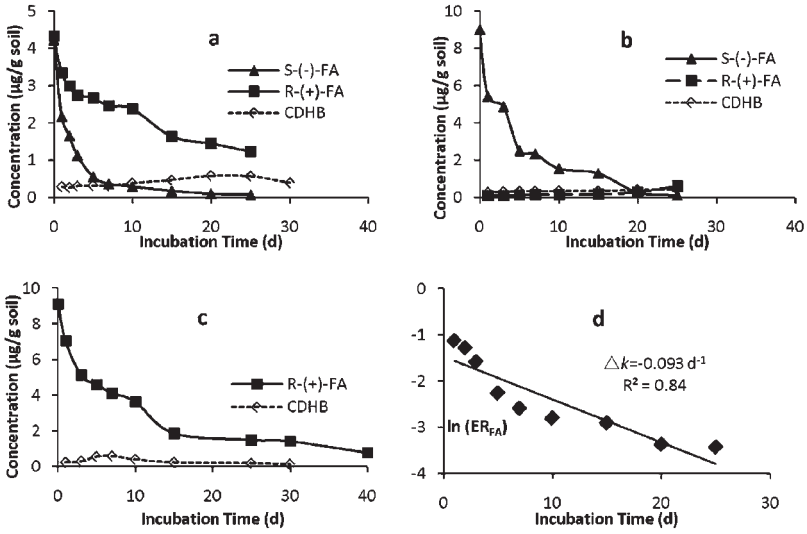

Figure 5. Degradation of FA in soil (expt SC4, SC5, and SC6 with rac-, *S*-(−)-, *R*-(+)-FA, respectively. Note the concurrent formation and degradation of CDHB arising from FA): (**a**) degradation of rac-FA (expt SC4); (**b**) degradation of *S*-(−)-FA (expt SC5); (**c**) degradation of *R*-(+)-FA (expt SC6); (**d**) the plot of ln(ER) from experiment SC4 (incubation of rac-FA) versus incubation time *t* showing a linear relationship.

sterilized soil samples, ER values were comparable to the ratio found for racemate. Thus, the degradation of FA in native soil samples was preponderantly mediated by biological processes.

Inversion of FA in Soil. Inversion was studied by separate incubations of the two enantiomers of FA under native conditions. We found *R*-(+)-FA and *S*-(−)-FA during the incubation of *S*-(−)-FE as mentioned above meaning there was inversion of *S*-(−)-FA to *R*-(+)-FA during the incubation of *S*-(−)-FA in Shangdong soil and Neimenggu soil but not in Jiangxi soil. Some studies suggested that soil properties such as soil pH, organic carbon, and soil texture have a major impact on the activity of the soil microbial community, and then could influence chiral signatures including enantiomerization in soils (*8*, *24*, *25*).

No indication for inversion was found in Jiangxi soil from the incubation of the pure *S*-(−)- and *R*-(+)-FA (expts SN5 and SN6, respectively), revealing no conversion of *S*-(−)- to *R*-(+)-FA during incubation and vice versa. *S*-(−)- and *R*-(+)-FA were both configurationally stable in Jiangxi soil.

The data from experiment SY5 and SC5 (incubation of the *S*-(−)-FA in Shandong soil and Neimenggu soil, respectively) showed a continuous decrease of the concentration of *S*-(−)-FA; meanwhile, the concentrations of *R*-(+)-FA in the two soils increased from initial values of 0 to 0.22 and 0.61 μg/g after 25 days of incubation, respectively (**Figure 5b**). The results illustrated conversion of the *S*-(−)-FA into *R*-(+)-FA. However, from experiment SY6 and SC6 (incubation of the *R*-(+)-FA in Shandong soil and Neimenggu soil, respectively), *R*-(+)-FA was configurationally stable (**Figure 5c**), and inversion was thus unimportant compared to degradation.

In this study, the stereochemistries of FE and its primary chiral product, FA, were investigated in soils using the racemic and the enantiopure compounds in combination with enantioselective analyses. The enantioselective degradation of FE occurred and the two enantiomers were configurationally stable in soils. Enantioselective degradation of FA was observed in the three soils, while there was inversion of *S*-(−)-FA to *R*-(+)-FA during the incubation of *S*-(−)-FA only in two soils. Moreover, enantioselectivity was attributed to microbial processes under native conditions. These results for major differences in the degradation of the enantiomers may have some implications for better environmental and ecological risk assessment for chiral pesticides.

LITERATURE CITED

(1) Müller, M. D.; Buser, H. R. Environmental behavior of acetamide pesticide stereoisomers. 2. Stereo- and enantioselective degradation in sewage sludge and soil. *Environ. Sci. Technol.* **1995**, *29*, 2031−2037.

(2) Monkiedje, A.; Spiteller, M.; Bester, K. Degradation of racemic and enantiopure metalaxyl in tropical and temperate soils. *Environ. Sci. Technol.* **2003**, *37*, 707−712.

(3) Liu, W. P.; Gan, J. Y.; Schlenk, D.; Jury, W. A. Enantioselectivity in environmental safety of current chiral insecticides. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (3), 701−706.

(4) Diao, J. L.; Xu, P.; Wang, P.; Lu, D. H.; Lu, Y. L.; Zhou, Z. Q. Enantioselective degradation in sediment and aquatic toxicity to Daphnia magna of the herbicide lactofen enantiomers. *J. Agric. Food Chem.* **2010**, *58*, 2439−2445.

(5) Buser, H. R.; Müller, M. D.; Poiger, T.; Balmer, M. E. Environmental behavior of the chiral acetamide pesticide metalaxyl: enantioselective degradation and chiral stability in soil. *Environ. Sci. Technol.* **2002**, *36*, 221−226.

(6) Bewick, D. W. Stereochemistry of fluazifop-butyl transformations in soil. *Pestic. Sci.* **1986**, *17*, 349−356.

(7) Hegeman, W. J. M.; Laane, R. W. P. M. Enantiomeric enrichment of chiral pesticides in the environment. *Rev. Environ. Contam. Toxicol.* **2002**, *173*, 85−116.

(8) Lewis, D. L.; Garrison, A. W.; Wommack, K. E.; Whittemore, A.; Steudler, P.; Melillo, J. Influence of environmental changes on degradation of chiral pollutants in soils. *Nature* **1999**, *401*, 898−901.

(9) Bieringer, H. H.; Hörlein, G.; Langelueddeke, P.; Handte, R. HOE 33171-a new selective herbicide for the control of annual and perennial warm climate grass weeds in broadleaf crops. *Proc. Brit. Crop Prot. Conf. Weeds* **1982**, *1*, 11−17.

(10) Tal, A.; Romano, M. L.; Stephenson, G. R.; Schwan, A. L.; Hall, J. C. Glutathione conjugation: A detoxification pathway for fenoxaprop-ethyl in barley, crabgrass, oat, and wheat. *Pestic. Biochem. Physiol.* **1993**, *46*, 190−199.

(11) Lee, W. M.; Elliott, J. E.; Brownsey, R. W. Inhibition of acetyl-CoA carboxylase isoforms by pyridoxal phosphate. *J. Biol. Chem.* **2005**, *280*, 41385−41843.

(12) Lin, J.; Chen, J. W.; Cai, X. Y.; Qiao, X. L.; Huang, L. P.; Wang, D. G.; Wang, Z. Evolution of toxicity upon hydrolysis of fenoxaprop-*p*-ethyl. *J. Agric. Food Chem.* **2007**, *55* (18), 7626−7629.

(13) Smith, A. E.; Aubin, A. J. Degradation studies with ^{14}C-fenoxaprop in prairie soils. *Can. J. Soil Sci.* **1990**, *70*, 343−350.

(14) Lin, J.; Chen, J. W.; Wang, Y.; Cai, X. Y.; Wei, X. X.; Qiao, X. L. More toxic and photoresistant products from photodegradation of fenoxaprop-*p*-ethyl. *J. Agric. Food Chem.* **2008**, *56* (17), 8226−8230.

(15) Hoagland, R. E.; Zablotowicz, R. M. Biotransformations of fenoxaprop-ethyl by fluorescent Pseudomonas strains. *J. Agric. Food Chem.* **1998**, *46* (11), 4759−4765.

(16) Kurihara, N.; Miyamoto, J.; Paulson, G. D.; Zeeh, B.; Skidmore, M. W.; Hollingworth, R. M.; Kuiper, H. A. Chirality in synthetic agrochemicals: bioactivity and safety consideration. *Pure Appl. Chem.* **1997**, *69* (9), 2007−2025.

(17) Wiberg, K.; Letcher, R. J.; Sandau, C. D.; Norstrom, R. J.; Tyskind, M.; Bidleman, T. F. The enantioselective bioaccumulation of chiral chlordane and α-HCH contaminants in the polar bear food chain. *Environ. Sci. Technol.* **2000**, *34*, 2668−2674.

(18) Müller, M. D.; Poiger, T.; Buser, H. R. Isolation and identification of the metolachlor stereoisomers using high-performance liquid chromatography, polarimetric measurements, and enantioselective gas chromatography. *J. Agric. Food Chem.* **2001**, *49*, 42−49.

(19) Diao, J. L.; Lv, C. G.; Wang, X. Q.; Dang, Z. H.; Zhu, W. T.; Zhou, Z. Q. Influence of soil properties on the enantioselective degradation of the herbicide lactofen in soils. *J. Agric. Food Chem.* **2009**, *57*, 5865−5871.

(20) Müller, M. D.; Buser, H. R. Conversion reactions of various phenoxyalkanoic acid herbicides in soil. 1. Enantiomerization and enantioselective degradation of the chiral 2- phenoxyal-kanoic acid herbicides. *Environ. Sci. Technol.* **1997**, *31*, 1953−1959.

(21) Wink, O.; Luley, U. Enantioselective transformation of the herbicides diclofop-methyl and fenoxaprop-ethyl in soil. *Pestic. Sci.* **1988**, *22* (1), 31−40.

(22) Garrison, A. W. Probing the enantioselectivity of chiral pesticides. *Environ. Sci. Technol.* **2006**, *40*, 16−23.

(23) Moon, J. K.; Keum, Y. S.; Hwang, E. C.; Park, B. S.; Chang, H. R.; Li, Q. X.; Kim, J. H. Hapten syntheses and antibody generation for a new herbicide, metamifop. *J. Agric. Food Chem.* **2007**, *55* (14), 5416−5422.

(24) Buerge, I. J.; Poigner, T.; Müller, M. D.; Buser, H. R. Influence of pH on the stereoselective degradation of the fungicides epoxiconazole and cyproconazole in soils. *Environ. Sci. Technol.* **2006**, *40*, 5443−5450.

(25) Jones, W. J.; Mazur, C. S.; Kenneke, J. F.; Garrison, A. W. Enantioselective microbial transformation of the phenylpyrazole insecticide fipronil in anoxic sediments. *Environ. Sci. Technol.* **2007**, *41*, 8301−8307.

Received for review September 15, 2010. Revised manuscript received November 11, 2010. Accepted November 15, 2010. This work was supported by the National Natural Science Foundation of China (Contract Grant No. 20707038).