JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Enantioselective Analysis and Degradation Studies of Isocarbophos in Soils by Chiral Liquid Chromatography–Tandem Mass Spectrometry

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ABSTRACT: An enantioselective method is presented for the determination of isocarbophos in soil by liquid chromatography coupled with tandem mass spectrometry. The pesticide residues in soil samples were extracted with acetonitrile, and complete enantioseparation was obtained on an amylose tris(3,5-dimethylphenylcarbamate) chiral column using acetonitrile/2 mM ammonium acetate solution containing 0.1% formic acid (60:40, v/v) as the mobile phase. The absolute configuration of isocarbophos enantiomers was determined by the combination of experimental and calculated electronic circular dichroism spectra. The method was utilized to investigate the degradation of isocarbophos in soils (Changchun, Hangzhou, and Zhengzhou) under sterilized or native conditions. Isocarbophos enantiomers were configurationally stable in the selected soils, and the pesticide degradation was not enantioselective in the sterilized condition. The degradation behavior of rac-isocarbophos was different under native conditions, with no enantioselectivity in the Changchun soil and with the *S*-(+)-isocarbophos enriched in the Hangzhou and Zhengzhou soils.

KEYWORDS: *isocarbophos, absolute configuration, enantioselectivity degradation, soil*

INTRODUCTION

Organophosphorus pesticides (OPs) have been widely used in modern agriculture since they were introduced in the 1950s because of their high effectiveness and relatively low cost.[1−3] Many OPs have at least one chiral center such as carbon, sulfur, or phosphorus.[1,4,5] Enantiomers of such chiral OPs have identical physicochemical properties but may have different bioactivities and toxicities, and the environmental fate of each is often enantioselective.[5−8] For example, both ruelene enantiomers disappeared at the same rate for the first 2 months during the transformation of 50 mg/L of racemic ruelene in the slurry of a Norway soil, and (+)-ruelene was preferentially lost after almost 2 months.[9] Similarly, (+)-phenthoate degraded more rapdily than the (−)-enantiomer in both Tianjin and Hubei soils.[10] The degradation of malathion in environmental samples showed that the *S*-(−)-enantiomer degraded more rapidly than the *R*-(+)-enantiomer.[11] In our study,[12] pyraclofos showed dramatically different enantioselective degradation behaviors in three soils under native condition, with no enantioselectivity in the Zhengzhou soil and with *R*-(−)-pyraclofos enriched in the Nanchang soil and *S*-(+)-antipode enriched in the Hangzhou soil. Therefore, there has been an increasing interest in studying the enantioselective environmental behaviors of different chiral OPs.

Isocarbophos [(*RS*)-(*O*-2-isopropoxycarbonylphenyl *O*-methyl phosphoramidothioate] (Figure 1) is an organophosphorous insecticide and acaricide used to control chewing and sucking insects and spider mites on many crops.[13−15] Isocarbophos was originally synthesized and evaluated by Bayer AG. Isocarbophos is presently produced by Chinese manufacturers such as Hubei Xianlong Chemical Industry Co., Ltd., and Hebei Veyong Biochemical Co., Ltd. The acute oral LD_{50} and the acute percutaneous LD_{50} of isocarbophos for rats are 28.5 and 447 mg/kg, respectively.[16] Due to its high toxicity, the use of isocarbophos on vegetables and fruits in China has been banned, but it is still widely used on cotton and rice in China. Isocarbophos contains an asymmetrical phosphorus center and consists of two enantiomers with

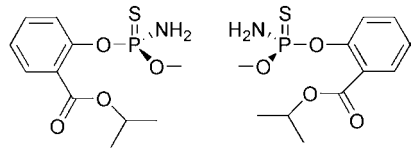

Figure 1. Chemical structures of isocarbophos. This molecule consists of a pair of enantiomers with a chiral center at the phosphorus atom.

Received: June 17, 2012
Revised: September 25, 2012
Accepted: September 26, 2012
Published: September 26, 2012

dx.doi.org/10.1021/jf302620s | *J. Agric. Food Chem.* 2012, 60, 10188−10195

different toxicities. Lin et al.[14] investigated the enantioselective toxicity of isocarbophos, and the result showed that the median lethal concentrations (LC_{50}) of the (+)- and (−)-enantiomers of isocarbophos toward *Daphnia magna* were 7.08 and 353 μg/L, respectively, after 48 h of static exposure, displaying a 50-fold difference between the enantiomers. Liu et al.[17] reported that (−)-isocarbophos was about 2 times more toxic than (+)-isocarbophos in Hep G2 cells. Recently, Lu et al.[18] performed a comprehensive investigation of the growth and physiological effects of isocarbophos and its enantiomers to *Scenedesmus obliquus*, and enantioselectivity was observed in the dose response. However, information related to the degradation of isocarbophos enantiomers in soils is limited. To evaluate the enantioselective behavior of two isocarbophos enantiomers in the environment, it is useful to establish the method for separation and determination of isocarbophos residues in soils and provide information concerning enantiomer degradation behavior in different cultivated soils.

High-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) has been used extensively in pesticide residue analysis in food and environmental samples for high selectivity, high sensitivity, and simple preliminary treatment. In recent years, HPLC-MS/MS has been also applied for enantioselective analysis of chiral pesticides.[19−23] However, to our knowledge, no study has been reported about enantioselective determination of isocarbophos by HPLC-MS/MS until now. In this study, a sensitive analytical method was developed for the determination of isocarbophos enantiomers in soil through HPLC-MS/MS. The method was also conducted to investigate the possible enantioselective residue behavior of isocarbophos in soils from three geographically different locations representing different physicochemical characteristics in China under native as well as sterilized conditions. Moreover, the assignment of absolute configurations is an important aspect for understanding the biochemical processing of chiral compounds.[24−26] The absolute configurations of isocarbophos enantiomers were first determined according to a comparison of experimental and calculated electronic circular dichroism (ECD) spectra.

MATERIALS AND METHODS

Chemicals and Materials. HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Purified water was prepared by using a Milli-Q water purification system (Millipore Corp., Billerica, MA, USA). Two enantiomers of isocarbphos with purity ≥98.0% were obtained from Daicel (Shanghai, China). Formic acid (≥96% purity) was purchased from TEDIA (Fairfield, OH, USA). Sodium chloride (NaCl) and anhydrous magnesium sulfate ($MgSO_4$) were supplied by Huadong Medicine (Hangzhou, China). The chiral analytical column amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD-RH) was purchased from Daicel (Tokyo, Japan), and the column was sized 150 mm × 2.1 mm i.d. packed with 3 μm particles.

ECD Calculations. The geometry of two isocarbophos enantiomers was optimized by Gaussian 09 using density functional theory at the level of B3LYP/6-31+G*. The calculations of ECD of two isocarbophos enantiomers were carried out in the Gaussian 09 software package using time-dependent density functional theory (TDDFT) methods at the level of B3LYP/6-31+G*.[12,25]

Circular Dichroism Spectroscopy. The ECD spectroscopy was performed on a Jasco-J815 circular dichroism spectropolarimeter (Jasco, Japan) at room temperature. The spectra was collected from 200 to 400 nm with a 50 nm/min scan speed. A quartz cell with a path length of 0.1 cm was used for the scanning, and the average of three scans was reported.

Soil Samples. Three soil samples with different physicochemical properties were collected at a 0−15 cm plow layer from geographically distinct agriculture regions of China (Changchun City, Jilin Province, northeastern China; Hangzhou City, Zhejiang Province, southeastern China; and Zhengzhou City, Henan Province, central China). No isocarbophos was found at detectable levels in these soil samples. All samples were air-dried at room temperature, homogenized, passed through a 2 mm sieve, and stored in the dark for a few days until use. More details on specific physicochemical characteristics (particle size, texture, pH, and organic matter content) of the soil samples are listed in Table 1.

Table 1. Characteristics of the Selected Soils Used in the Degradation Experiment

	particle size					
soil site	sand (%)	silt (%)	clay (%)	soil texture	pH[a]	organic matter[b](%)
Changchun	26.5	67.4	6.1	silt loam	7.45	2.25
Hangzhou	8.2	82.6	9.2	silt	6.68	1.30
Zhengzhou	42.9	48.9	8.2	loam	8.32	1.28

[a]Suspension of soil in water, 1:2.5 (w/w). [b]Following the potassium dichromate volumetric method.

Incubation in Soils under Native Conditions. Separate incubation experiments were conducted with the pure *R*-(−) and *S*-(+) and the racemic compounds using 50 mL polypropylene centrifuge tubes covered with aluminum foil. Before pesticide treatment, the soils were preincubated in the dark at 30 ± 1 °C for 7 days to activate the soil microorganisms. The soil sample of each centrifuge tube was approximately 5.0 g (dry weight equivalent) and was treated with 25 μg (50 μL of a 500 μg/mL stock solution in acetone) of *R*-(−)-, or *S*-(+)- isocarbophos or 50 μg of racemic isocarbophos (*R*/*S* = 1:1), and they were set to air-dry for 5 min before being homogenized thoroughly. Then the soil samples were rehydrated by the addition of 1.2 mL of purified water [about 60% of the field holding capacity (w/w)] and incubated at 25 °C in the dark; the samples were weighed regularly, and purified water was added to the samples to compensate for water loss during the whole experiments. Three replicate samples were removed from each treatment at different time intervals (0, 0.25, 0.75, 1.25, 2, 3, 4, 5, 6, and 7 days) and immediately transferred into a freezer (−40 °C).

Incubation in Soils under Sterilized Conditions. To determine if the enantioselective degradation was a result of microbially mediated transformations, portions of 5.0 g of soil set in 50 mL polypropylene centrifuge tubes were 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. The sterilized samples were treated with 50 μg of racemic isocarbophos (*R*/*S* = 1:1, spike level, 10 μg/g), and sterile water was added in a biological clean workbench. The samples were then covered with glass paper to maintain sterile conditions.

Extraction of Soil Samples. Samples were thawed at room temperature, and 10 mL of water was added to each polypropylene centrifuge tube containing 5.0 g of soil. To extract the isocarbophos residues, 25 mL of acetonitrile, 1.5 g of NaCl, and 6 g of anhydrous $MgSO_4$ were added to the samples. Then the tube was stirred on a vortex shaker for 3 min, ultrasonically extracted for 10 min, and centrifuged at 6000 rpm for 5 min. A portion of 5 mL of organic layer was transferred and evaporated to 3 mL under a gentle stream of nitorgen in a water bath at 40 °C, and then water was added to 5 mL. The resulting solution was filtered through a 0.22 μm Teflon filter for analysis.

Enantioselective HPLC-MS/MS Analysis. HPLC-MS/MS analysis was performed on a TSQ Discovery triple-quadrupole mass spectrometer and a Surveyor liquid chromatograph (Thermo Fisher Scientific, Waltham, MA, USA). Xcalibur 2.0.7 (Thermo Fisher Scientific) software was used to process the quantitative data obtained from calibration standards and samples. Enantioselective chromato-

graphic separation was performed on a Daicel column Chiralpak AD-RH. The column temperature was set at 30 °C. The mobile phase consisted of 60% (v/v) (A) acetonitrile and 40% (v/v) (B) 2 mM ammonium acetate aqueous solution containing 0.1% formic acid. The mobile phase was delivered at a flow rate of 0.3 mL/min in isocratic mode, and the injection volume was 10 μL. Electron spray ionization (ESI) was operated in the positive ion mode. Spray voltage was set at 4.2 kV. The capillary temperature was set to 350 °C. Auxiliary gas and sheath gas were normal nitrogen. Collision gas was high pure argon with pressure at 1.5×10^{-3} Torr in the collision cell. Transition m/z 307 → 121 was used for quantification, and m/z 307 → 131 was used for confirmation.

Q-TOF MS Analysis. Accurate MS/MS analysis was performed on a micrOTOF QII (Q-TOF) mass spectrometer (Bruker Co., USA). The target molecule was selected by the first quadrupole mass spectrometer and then fragmented in the collision cell by collisional activation. The produced fragment ions were analyzed and recorded by the second time-of-flight mass spectrometer. The capillary voltage was 3500 V. The collision energy ranged from 5 to 30 V. An ESI flow rate of 180 μL/h was used, the temperature was at 180 °C, and the collision gas was argon at a pressure of 2.5 bar.

Method Validation. The solvent calibration curves were obtained by plotting peak areas of quantification ion transition against analyte concentrations from 5 to 500 μg/L with regression analysis. To evaluate the matrix effect, the matrix-matched calibration curves in the range from 5 to 500 μg/L were also constructed for the three soils, respectively. The linearity was expressed as correlation coefficient. The limits of detection (LODs) were determined as 3 times the signal-to-noise ratio of the quantitative ion transition by the analyses of spiked sample containing isocarbophos at low concentration levels with five replicate extractions. The limit of quantification (LOQ) was defined as the lowest spiking level of each enantiomer on acceptable recovery.[27]

Method accuracy and precision were evaluated by recovery studies using spiked samples at three concentration levels (0.05, 0.5, and 5.0 μg/g for each isocarbophos enantiomer based on five replicates). For method recovery studies, samples without residue (5.0 g) were spiked prior to extraction by the addition of appropriate volumes of the pesticide standard solution in acetone. The treated samples were analyzed following the described procedure, and the recoveries were calculated. The precision of the method was determined by the repeatability and expressed as the relative standard deviation (RSD).

RESULTS AND DISCUSSION

Absolute Configuration Assignment. Chiroptical methods such as vibrational circular dichroism and ECD have become lately popular for the unambiguous determination of the absolute configurations of chiral molecules with the rapid development of computational methods.[24,28] We applied TDDFT methodology to calculate the ECD spectra of isocarbophos in this work. The absolute configuration of isocarbophos enantiomers was finally established by comparing the signs of the experimental ECD to the signs of the spectra calculated. The overall curves of computed ECD (Figure 2A) obtained by TDDFT calculations and experimental ECD (Figure 2B) are similar. Generally, the individual enantiomer of a chiral compound corresponded to a specific CD signal at a given wavelength, and the first eluted enantiomer was confirmed as (−)-enantiomer, whereas the second one was the (+)-enantiomer from 200 to 275 nm in our study (Figure 2B). Through the combination of computed and experimental ECD spectra, we can correctly assign the configurations of isocarbophos enantiomers eluted from the columns. The enantiomers (peaks 1 and 2 in Figure 4) are assigned as *R*-(−)-isocarbophos and *S*-(+)-isocarbophos, respectively.

Optimization and Fragmentation Pathway of MS/MS. Isocarbophos was analyzed by HPLC-MS/MS using ESI positive mode to obtain the optimal MS/MS conditions. A

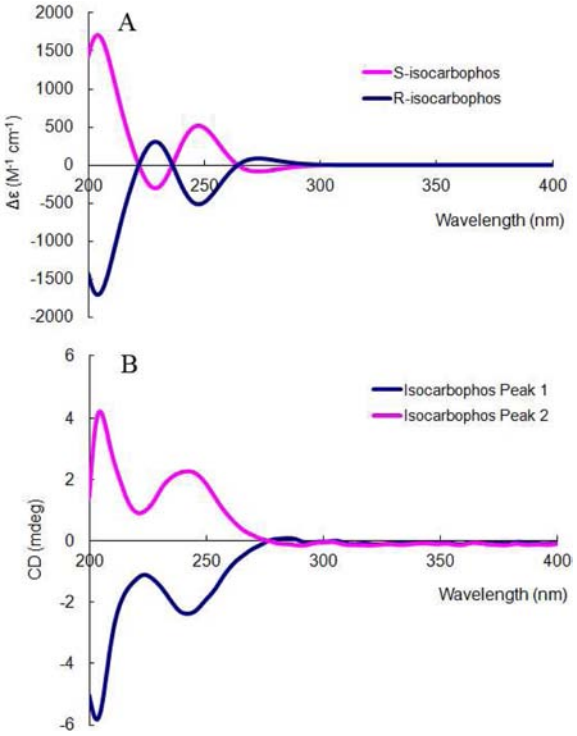

Figure 2. (A) Calculated ECD; (B) experimentally measured ECD spectra of isocarbophos enantiomers in acetonitrile (20 mg/L).

syringe pump was used to provide an analyte infusion (5 mg/L, in the range of 5 μL/min) into the LC eluent via a T-connection to obtain a constant signal. For full spectrum scan, isocarbophos shows the base peak at $[M + NH_4]^+$, so ammonium acetate was used in the mobile phase. Mass fragments were produced from the ion at $[M + NH_4]^+$ by collision-induced dissociation (CID) using argon at 1.5 mTorr. For isocarbophos, major fragment ions including m/z 273, 231, 137, and 121 produced from m/z 307 were detected. To provide more information on isocarbophos fragmentation pathway, pseudo-MS/MS/MS as well as in-source CID combined with MS/MS was performed. In-source CID was used as the first quasi-MS/MS stage to generate first-generation fragment ions from the ammonium adduct molecular ion. Then, in the second MS/MS step, the ion m/z 273 was isolated in Q1 and made to undergo CID in the collision quadrupole (Q2). Fragment ions including m/z 231, 137, and 121 produced from m/z 273 were detected in Q3. In addition, accurate ion mass (307.0872, 273.0328, 230.9856, 137.0023, 121.0257) was measured by Q-TOF MS, and the mass measurement error of the TOF-MS was <3.3 mDa. The tentative fragmentation pathway of isocarbophos is displayed in Figure 3. For isocarbophos, transitions m/z 307 → 231 and 307 → 121 were selected for quantification and confirmation, respectively, for their high response and low background interference.

Assay Validation. Using m/z 307 → 231 as the quantification transition, the solvent and matrix-matched calibration curves were linear from 5 to 500 μg/L for each isocarbophos enantiomer, with the correlation coefficients >0.994. The matrix effect was calculated by comparing the slope of matrix-matched standard curve with the slope of the standard calibration curve.[29] Signal suppressions for isocarbophos enantiomers were observed in the three soil matrix

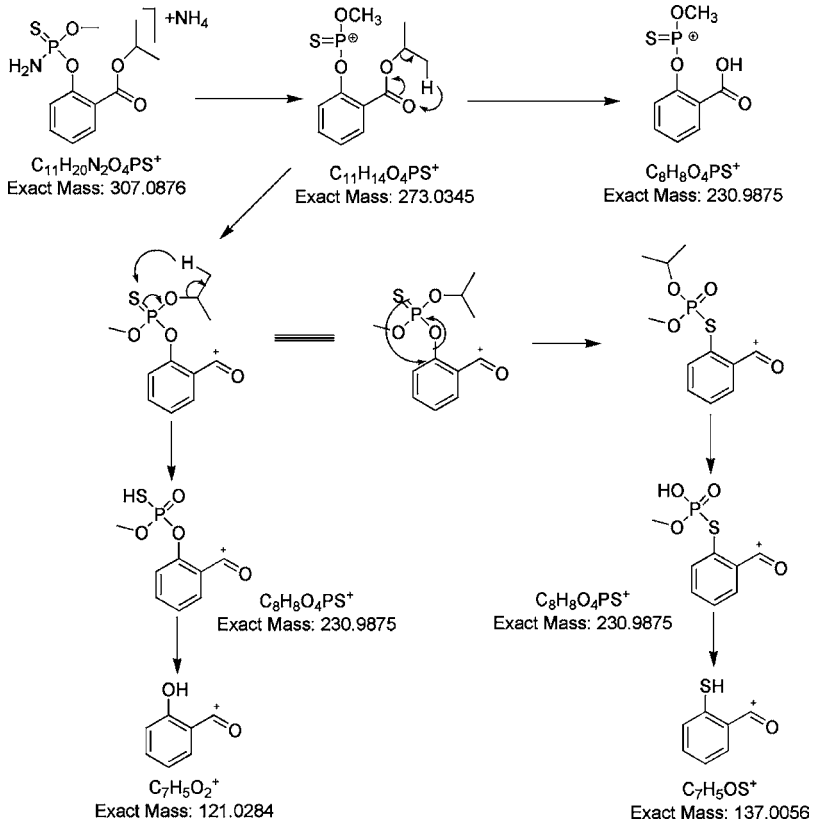

Figure 3. Fragmentation pathway of isocarbophos by MS/MS.

extracts as the slope ratios were in the range of 0.815−0.902. Matrix-matched calibration standards were utilized to eliminate the matrix effect and to obtain more realistic results in the soil. For isocarbophos enantiomers, the recovery ranges in low, intermediate, and high spiked levels were 89.2−92.1, 91.9−94.7, and 92.5−97.1%, respectively, and the repeatability RSD ranged from 2.1 to 8.8%. Recovery and precision results listed in Table 2 illustrate that the method was efficient and reliable.

Table 2. Mean Recovery and RSD Values Obtained for Isocarbophos Enantiomers in Soils at Three Spiked Levels (n = 5)

	mean recovery (%), RSD (%)					
	Changchun soil		Hangzhou soil		Zhengzhou soil	
fortified level (μg/g)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)
0.05	90.5, 8.4	89.2, 5.7	91.6, 8.8	90.3, 7.3	89.7, 7.1	92.1, 7.8
0.5	91.9, 7.0	93.1, 7.9	94.3, 3.4	92.8, 5.7	93.8, 4.5	94.7, 5.9
5.0	94.6, 6.0	92.7, 5.5	92.5, 5.6	93.0, 6.4	97.1, 3.2	96.2, 2.1

The LODs were estimated to be 0.005 μg/g for each enantiomer by the analyses of spiked sample containing isocarbophos at low concentration levels with five replicate extractions. The LOQs were 0.05 μg/g for each enantiomer.

Chiral Stability of Isocarbophos in Soil. Li et al.[30] reported that some chiral pesticides, such as phenthoate and triadimefon, could undergo enantiomerization in soils. In this work we investigated the chiral stability of isocarbophos by separate incubations of the enantiopure enantiomers under native conditions. No enantiomerization of *R*-(−)- to *S*-(+)-isocarbophos and vice versa was detected during the degradation of enantiopure *R*-(−)- or *S*-(+)-enantiomers in soils (Figure 4), and it was demonstrated that the isocarbophos enantiomers were configurationally stable in the three soils.

Data Analysis. The kinetic study of isocarbophos in soil was performed by plotting residue concentration against time, and the degradation rate constant (k) of an isocarbophos enantiomer was calculated using the first-order kinetic eq 1 by regression analysis; the half-life ($t_{1/2}$, days) was estimated from eq 2:

$$C = C_0\, e^{-kt} \quad (1)$$

$$t_{1/2} = \ln 2/k = 0.693/k \quad (2)$$

C and C_0 are the concentrations of the *S*-(+)- or *R*-(−)-enantiomer at time t and time 0, respectively.

The enantiomer fraction (EF) was used as a measure of the enantioselectivity of the degradation of enantiomers in soils. The use of EF to represent the enantioselectivity was more meaningful than the conventional enantiomeric ratio (ER).[31] EF is defined by eq 3. The EF values ranged from 0 to 1, with EF = 0.5 representing the racemic mixture.

$$\text{EF} = \text{peak area of the (−)-enantiomer} / [\text{(+)-enantiomer} + \text{(−)-enantiomer}] \quad (3)$$

Degradation of Racemic Isocarbophos in Soils. Racemic isocarbophos degradation was monitored in the three soils for 7 days under sterilized and native conditions. The concentrations of the two enantiomers of isocarbophos in soils were determined. The degradation of each enantiomer in

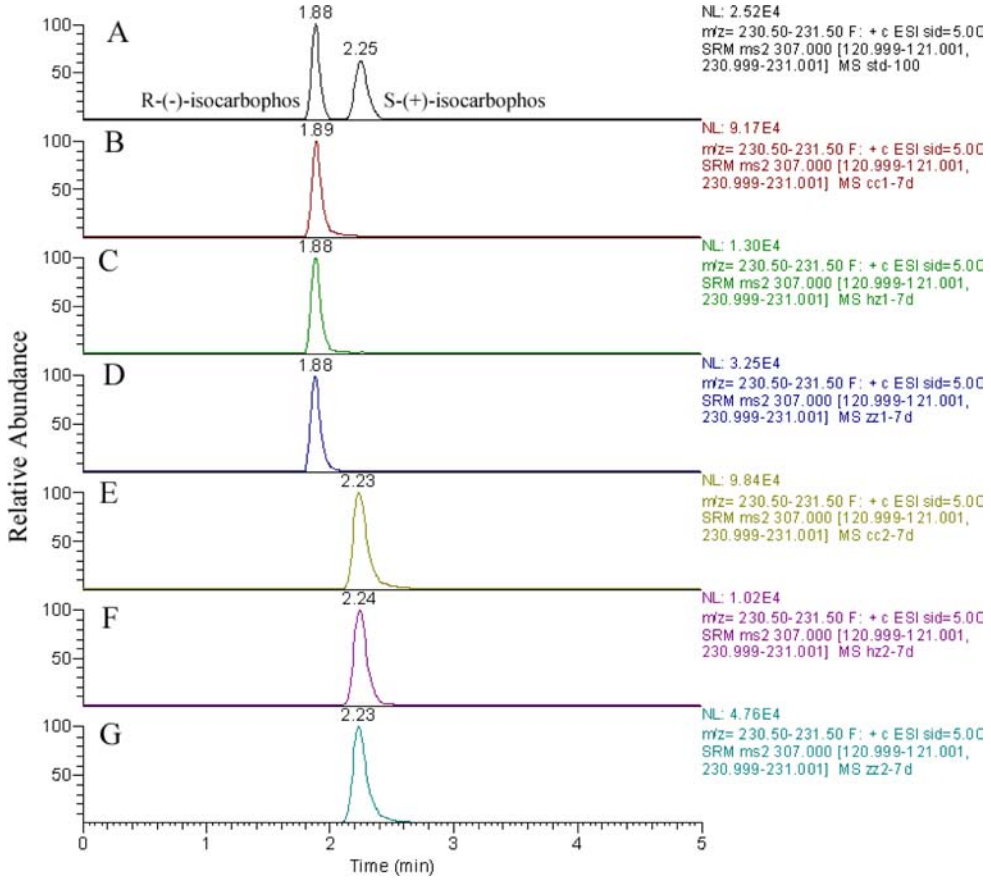

Figure 4. Chromatograms of the *R*-(−)- and *S*-(+) isocarbophos enantiomers in the standard solution (A) and from the degradation experiments with the *R*-(−)-isomer for soils (B) Changchun, (C) Hangzhou, and (D) Zhengzhou and with the *S*-(+)-isomer for soils (E) Changchun, (F) Hangzhou, and (G) Zhengzhou after 7 days of incubation under native condition.

soils generally fit a first-order kinetics decay model. Rate constants (k), correlation coefficients (R^2), and half-lives ($t_{1/2}$) were calculated (Table 3). The soil pH was an important factor during pesticide degradation in soils. The half-lives ($t_{1/2}$) of isocarbophos in the three sterile soils (Changchun, Hangzhou, and Zhengzhou) were about 9.2, 13, and 4.5 days, respectively.

Table 3. First-Order Rate Constant (k), Half-Life ($t_{1/2}$), and Correlation Coefficient (R^2) Values for the Degradation of Racemic Isocarbophos in Soil Samples

condition	isocarbophos enantiomer	k (day^{-1})	$t_{1/2}$[a] (days)	R^2
		Changchun Soil		
sterilized	*R*-(−)	0.0754	9.19 ± 0.38	0.916
	S-(+)	0.0756	9.17 ± 0.46	0.922
native	*R*-(−)	0.264	2.62 ± 0.12	0.953
	S-(+)	0.256	2.71 ± 0.10	0.938
		Hangzhou Soil		
sterilized	*R*-(−)	0.0541	12.81 ± 0.63	0.925
	S-(+)	0.0522	13.28 ± 0.49	0.919
native	*R*-(−)	0.587	1.18 ± 0.02	0.977
	S-(+)	0.529	1.31 ± 0.03	0.941
		Zhengzhou Soil		
sterilized	*R*-(−)	0.155	4.48 ± 0.17	0.953
	S-(+)	0.153	4.53 ± 0.20	0.965
native	*R*-(−)	0.362	1.92 ± 0.02	0.947
	S-(+)	0.248	2.80 ± 0.05	0.954

[a]Values represent the mean ± SD (n = 3).

The degradation $t_{1/2}$ in Zhengzhou soil having a pH of 8.32 was 4.5 days and relatively shorter than those in the other two soil samples (Changchun and Hangzhou) having pH values of 7.45 and 6.68, which may prove isocarbophos is easier to degrade in an alkaline matrix. This is in accordance with the other studies,[11,12] in which degradation of OPs proceeded at higher rates under alkaline conditions. Soil textural composition is viewed as an important parameter in the degradation processes as well. The Zhengzhou soil was the loam soil in the experiments, and the other two soils were a silt loam soil and a silt soil, respectively, and so the Zhengzhou soil contained more sand than other two soils. The higher the sand fraction, the less the compound is adsorbed and the more available it is for degradation; the dissipation rate of isocarbophos might be quicker in the soil that contains more sand. Therefore, the shortest degradation $t_{1/2}$ occurred in the Zhengzhou alkaline soil in this work.

With regard to the degradation behavior of isocarbophos in each selected soil, degradation under sterilized condition was much slower than degradation under native condition. It was shown that besides the abiotic degradation, a main part of the degradation of isocarbophos was attributed to the transformation promoted by the microbial community in agricultural soils. Some studies suggested that soil properties such as texture, pH, and organic matter content could have major impacts on the activity of the soil microbial community and then could influence chiral signatures in soils.[10−12] Microorganisms in soil may play an important role in the

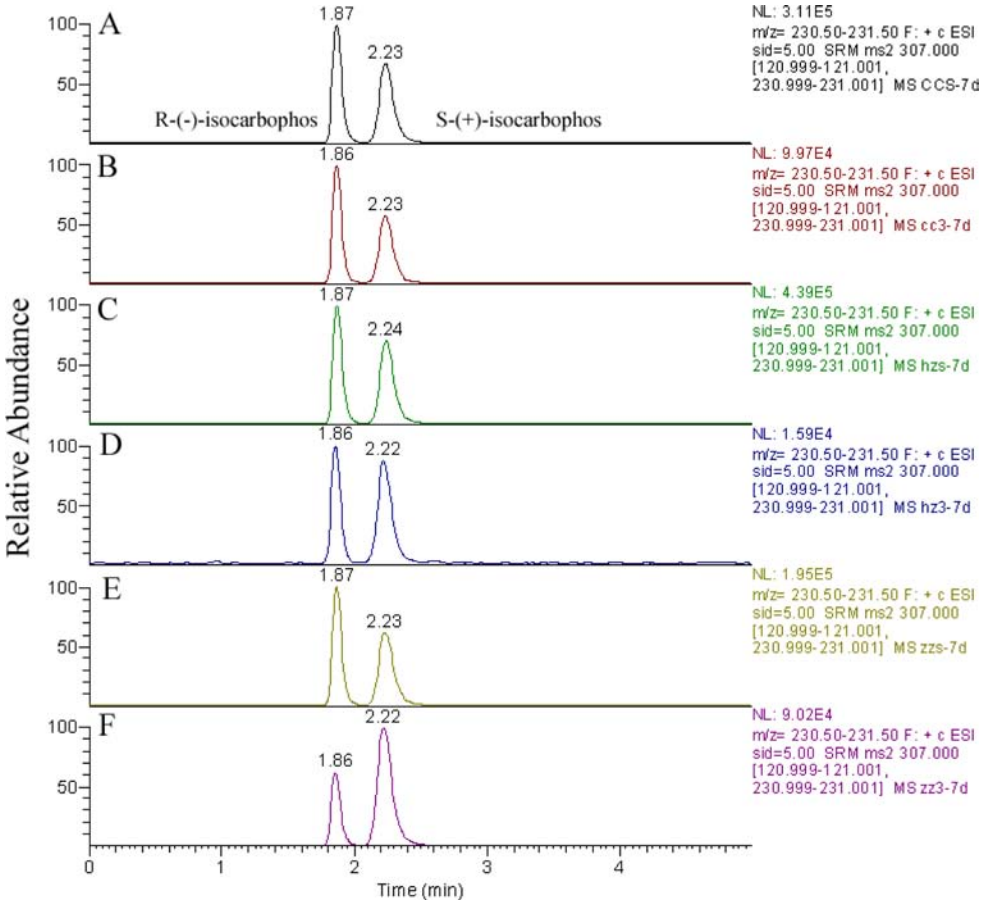

Figure 5. Extracted ion chromatograms of isocarbophos enantiomers from the degradation experiments with racemic isocarbophos for soils after 7 days of incubation: (A) Changchun sterilized soil; (B) Changchun native soil; (C) Hangzhou sterilized soil; (D) Hangzhou native soil; (E) Zhengzhou sterilized soil; (F) Zhengzhou native soil.

Table 4. Enantiomer Fraction (EF) of rac-Isocarbophos in Experimental Soil Samples under Sterilized and Native Conditions (n = 3)

	EF[a]					
	sterilized condition			native condition		
time (days)	Changchun	Hangzhou	Zhengzhou	Changchun	Hangzhou	Zhengzhou
0	0.505 ± 0.004	0.501 ± 0.005	0.499 ± 0.003	0.495 ± 0.006	0.496 ± 0.007	0.498 ± 0.005
0.25	0.501 ± 0.008	0.502 ± 0.003	0.496 ± 0.005	0.499 ± 0.007	0.487 ± 0.009	0.473 ± 0.007
0.75	0.497 ± 0.003	0.499 ± 0.005	0.493 ± 0.005	0.496 ± 0.009	0.478 ± 0.006	0.427 ± 0.005
1.25	0.493 ± 0.005	0.495 ± 0.009	0.496 ± 0.004	0.495 ± 0.011	0.463 ± 0.003	0.404 ± 0.002
2	0.502 ± 0.003	0.506 ± 0.006	0.494 ± 0.008	0.501 ± 0.003	0.454 ± 0.011	0.373 ± 0.007
3	0.506 ± 0.006	0.500 ± 0.004	0.498 ± 0.005	0.499 ± 0.009	0.440 ± 0.005	0.338 ± 0.013
4	0.498 ± 0.005	0.502 ± 0.003	0.495 ± 0.003	0.498 ± 0.006	0.435 ± 0.008	0.322 ± 0.006
5	0.495 ± 0.003	0.496 ± 0.002	0.493 ± 0.006	0.503 ± 0.010	0.416 ± 0.007	0.309 ± 0.010
6	0.502 ± 0.007	0.493 ± 0.003	0.484 ± 0.005	0.495 ± 0.003	0.403 ± 0.009	0.289 ± 0.003
7	0.493 ± 0.005	0.495 ± 0.004	0.490 ± 0.007	0.507 ± 0.004	0.382 ± 0.006	0.252 ± 0.008

[a]Values represent the mean ± SD (n = 3).

enantioselective metabolism of many chiral compounds. The enantioselective degradation behavior could be conducted by comparing the $t_{1/2}$ values of each enantiomer.

After the t test (Excel 2007, Microsoft), there was a difference for isocarbophos enantiomers in the degradation half-lives in Hangzhou soils ($p < 0.01$, Student's paired t test) and in Zhengzhou soils ($p < 0.001$, Student's paired t test), and all of them indicated that the degradation of isocarbophos was highly enantioselective in the two soils (Hangzhou and Zhengzhou), whereas no obvious enantioselectivity existed in Changchun soils. Typical chromatograms of three soils after 7 days of incubation were displayed in Figure 5. No significant difference between peak areas of *R*-(-)- and *S*-(+)-enantiomers in the three soils under sterilized condition was shown, which was greatly different from the results of higher *S*-(+)- than *R*-(−)-enantiomers in the Zhengzhou soil samples after incubation for 7 days under native condition (shown in Figure 5F).

The enantioselective degradation behavior could be also conducted by EFs. The EF values from the racemic isocarbophos degradation in the three soils under native condition and sterilized condition are listed in Table 4. The EF values of isocarbophos in all of the tested sterilized soils were approximately 0.5 after treatment. No significant enantioselectivity for isocarbophos degradation in the three soils under sterilized condition was found. The EFs from the incubation of rac-isocarbophos in the Changchun soil were also close to 0.5, and no significant enantioselectivity was shown. The EFs from the incubation of racemic isocarbophos in Hangzhou soils showed a continuous decrease with time from an initial value of 0.496 to 0.382 (concentration $R < S$), and the EFs from the incubation of racemic isocarbophos in Zhengzhou soils also showed a continuous decrease with time from an initial value of 0.498 to 0.252 (concentration $R < S$), so enantioselectivity happened in Hangzhou soils and Zhengzhou soils.

In conclusion, an enantioselective HPLC-MS/MS method for the determination of isocarbophos in soil samples was presented, and the absolute configuration of isocarbophos enantiomers was first determined according to a combination of experimental and calculated ECD spectra. The degradation behavior of rac-isocarbophos was different in the three soils under native conditions, with no enantioselectivity in the Changchun soil and with the *S*-(+)-isocarbophos enriched in the Hangzhou and Zhengzhou soils. The enantioselectivity for the degradation behavior of chiral isocarbophos in the Hangzhou and Zhengzhou soils demonstrated that the activity of microorganisms responsible for the degradation of the *R*-(−)-isocarbophos enantiomer prevailed over the degradation of the *S*-(+)-enatiomer. The difference in different cultivating soils could be attributed to different microbial communities and could provide more information to evaluate environmental and ecological risks of chiral organophosphorus pesticides.

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Funding

This work was supported by the National Natural Science Foundation of China (No. 21107094), the National High Technology R&D Program of China (the 863 Program, No. 2011AA100806), and the Fundamental Research Funds for the Central Universities.

Notes

The authors declare no competing financial interest.

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