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Enantioselective Degradation and Chiral Stability of Phenthoate in Soil

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Many pesticides are chiral and the individual enantiomers often show different biological activities. The environmental behavior of individual enantiomers may also be different and several studies have revealed different enantiomeric composition of chiral compounds in the environment, indicating that the enantiomers must be treated as different compounds. However, such a difference has not been extensively examined and only limited chiral pesticides have so far been investigated for enantioselectivity in their environmental fate (Buerge et al., 2003; Garrison et al., 2000; Lewis et al., 1999; Müller and Kohler, 2004; Romero et al., 2001). Many other species, such as organophosphorus pesticides (OPs) and synthetic pyrethroid insecticides, have received little attention (Lewis et al., 1999; Liu et al., 2004). Although these compounds are in wide use, the occurrence of enantioselectivity in their environmental behavior is poorly understood. In this study, we describe the enantioselective degradation of phenthoate [*O*,*O*-dimethyl *S*-(α-(carboethoxy)benzyl) phosphorodithioate], a common organophosphorus insecticide, in two soils. Due to the presence of one asymmetric carbon atom, phenthoate is chiral and consists of a pair of enantiomers (Fig. 1), in which (+)-phenthoate has more insecticidal activity to most organisms (Ohkawa et al., 1976). To date, phenthoate is mainly applied as the racemic form and no information is available on the enantioselectivity of this pesticide in the environment.

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On the other hand, knowledge on enantioselectivity must include chiral stability of individual enantiomers, since some chiral pesticides are configurationally unstable and may undergo enantiomerization (Buser and Müller, 1998; Müller and Buser, 1997; Romero et al., 2001). Such enantiomerization is of great importance, because it serves as an alternative route for the occurrence of enantioselectivity, and may also influence the pesticide efficacy as well as side effects. Therefore, we also investigated the chiral stability of phenthoate in this work by the incubation of enantiopure (+)-phenthoate. Findings from this study may be used to better understand the chiral profiles of phenthoate as well as relevant configurational analogues in the environment.

Materials and Methods

Racemic phenthoate (98%) was purchased from Bayer Company (Germany).

Enantiopure (+)-phenthoate was prepared in this study via enantiomeric resolution of the racemic phenthoate by chiral HPLC. In this step, a phenthoate racemate of known quantity was injected into the chiral HPLC system hundreds of times and a mobile phase fraction corresponding to (+)-phenthoate was collected manually by observing its UV signal. The collected sample was then gently concentrated to dryness under a vacuum evaporator and used as enantiomer standard. The enantiopurity of the prepared (+)-phenthoate, checked with chiral HPLC using the same column system, was >99%. Stock solutions were made up at a concentration of 1 mg/mL in hexane and preserved at 4°C. *n*-Hexane, ethyl acetate and isopropanol were all of analytical grade, redistilled and filtered through a 0.45 μm

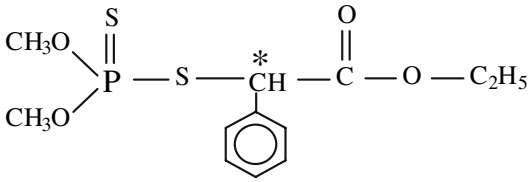

Fig. 1 Chemical structure of phenthoate

Table 1 Characteristics of the two soils

	Classification	Organic matter (%)	pH	Sand (%)	Silt (%)	Clay (%)
Tianjin soil	Sandy loam	0.717	8.2	34	40	26
Hubei soil	Light clay loam	1.367	5.4	26	36	38

filter before use. Other solvents and chemicals were of analytical or pesticide residue grade.

A liquid chromatography apparatus consisting of a Varian model 2010 pump (Varian, Northeast Florham Park, NJ, USA), a Rheodyne model 7125 injector with a 20 μL loop, a Varian model 2050 UV detector set at 230 nm and an Anastar chemical analytical work station (version 5.2) was used. The HPLC columns used were an achiral silica gel column (Dalian Elite Co. Ltd., China, 250 × 4.6 mm i.d., 5 μm particle size) with a guard cartridge of the same phase (30 × 4.6 mm i.d.), and a Chiralcel OD column (Daicel Chemical Industries Ltd., Japan, 250 × 4.6 mm i.d., 10 μm particle size) protected with a guard cartridge of the same phase (30 × 4.6 mm i.d.).

Garden soil from Tianjin City in North China and agricultural soil from Wuxue city in Hubei Province, South China, were used in this study. The soils were typical alkaline and acidic loams from China with pH values of 8.2 and 5.4, respectively (Table 1). Blank determination of the soils prior to fortification revealed no phenthoate present (detection limit < 0.02 μg/g). When sterilized experiments were performed, the soils, water as well as glassware used were all autoclaved (120°C) for four hours for two days consecutively.

Separate experiments were carried out with the racemate and enantiopure (+)-phenthoate in the two soils. Portions of 100 g soil were transferred into 250 mL conical flasks and 15 mL water were added. After preincubation in the dark at 25°C for a week, racemic phenthoate was fortified into the Tianjin soil (experiment S1) and Hubei soil (experiment S2) at a concentration of 2.5 μg/g of dry soil. Similarly, (+)-phenthoate was also applied in Tianjin (experiment S3) and Hubei soil (experiment S4) at a concentration of 1.25 μg/g, which corresponded to the halved amount of the racemic mixture. In addition, sterilized control experiments S5, S6, S7 and S8 were performed and compared to corresponding nonsterilized experiments S1, S2, S3 and S4, respectively. After thorough mixing, 15 mL more water were added to give final 23% moisture content. The flasks were then sealed with cotton-wool plugs and stored at 25°C in the dark. During preincubation and incubation processes, distilled water was added at 3–4 day intervals to maintain the initial moisture. Soil samples equivalent to 6 g dry soil weight were removed for analysis at different time intervals and frozen at –20°C until they could be analyzed.

The extraction of phenthoate from soil was carried out by a matrix solid-phase dispersion (MSPD) method (Li et al., 2002). In brief, the soil samples collected were placed into a mortar (50 mL capacity), then 9 g Florisil adsorbent and 2 mL distilled water were added and the complex was gently blended for ten minutes to obtain a homogeneous mixture. This mixture was introduced into a 30 × 1.5 cm i.d. glass chromatographic column containing a coarse frit and 2 cm of anhydrous sodium sulfate. Finally a 1.0 cm layer of anhydrous sodium sulfate was placed on the top of the column and a mixture of *n*-hexane–ethyl acetate (7:1/v:v) was added to the column and allowed to elute dropwise by gravity. The initial 15 mL eluent was collected into a graduated conical tube and then carefully blown to dryness with a mild nitrogen stream in a water bath of 50°C. The final residue was dissolved in an achiral HPLC mobile phase (*n*-hexane–isopropanol, 100:0.1 v:v). After filtration through a 0.45 μm filter, the sample solution was subjected to an achiral HPLC column and quantified using an external standard method. When fortified at 2.0 and 0.2 μg/g, average recoveries ($n = 3$) of phenthoate from the MSPD extraction process were 86.7 ± 3.9%, 85.1 ± 2.6% and 81.2 ± 4.5%, 76.3 ± 6.7% for Tianjin soil and Hubei soil, respectively.

The enantiomer-specific analysis of phenthoate was achieved by a combination of achiral and chiral HPLC. After filtration, the MPSD extract was directly subjected to the silica-gel column for the total quantitation of phenthoate. The mobile phase, operating at 1.0 mL/min, consisted of an isocratic mixture of *n*-hexane–isopropanol (100:0.1 v:v). Under this condition, phenthoate eluted at 7.8 min. As for chiral separation, another clean-up procedure was necessary. The remainder samples after quantitation were concentrated and injected into the silica-gel column. A mobile phase fraction containing phenthoate was collected manually by observing its UV signal. After gently being blown to dryness and dissolved in the chiral HPLC mobile phase, the samples were analyzed on the Chiralcel OD column for enantiomeric ratio (ER) determination. The mobile phase was 100:0.8 hexane–isopropanol (v/v) and the flow rate was set at 1.0 mL/min. Under this condition, the two enantiomers eluted at 10.5 and 11.5 minutes. In a previous paper, the first and later eluted enantiomers were assigned as (+)- and (–)-phenthoate,

respectively, by polarimetric measurement (Li et al., 2002). Preliminary experiments showed no interconversion between phenthoate enantiomers obtained during extraction and HPLC separation processes.

As a measure of enantioselectivity, the enantiomeric ratio (ER) was defined as ER = C_+/C_-, where C_+ and C_- are the concentrations of the earlier eluted (+)- and later eluted (-)-enantiomer, respectively. The ERs were determined by their peak areas, assuming the same response factor for enantiomers originating from the same compound. Based on the total concentration of phenthoate and corresponding ER value, the concentration of each enantiomer was then calculated. One replicate sample per three samples was analyzed, and the precision of the enantiomer concentrations as well as ER values was satisfactory with a percent difference lower than 10%.

Finally, two separate experiments were carried out to evaluate the chiral stability of (+)-phenthoate in water. Buffers with pH 8.4 (buffer 1) and 5.2 (buffer 2), which were approximate to the two soil pH values, were prepared by dissolving different proportions of KH_2PO_4 and Na_2HPO_4 in 1000 mL sterilized distilled water. 0.25 mg (+)-phenthoate in 0.5 mL hexane were pipetted into a 500 mL flask. After evaporation to dryness under a stream of nitrogen, 250 mL of buffer 1 (experiment S9) or buffer 2 (experiment S10) were added separately to give a final concentration of 1.0 mg/L for (+)-phenthoate. The solutions were incubated in the dark at 25°C for 12 hours. Then, three × 80 mL hexane were added to extract the phenthoate. The combined hexane layers were dried with 20 g anhydrous sodium sulfate and after concentration, an aliquot was analyzed by chiral HPLC.

Results and Discussion

Under nonsterilized conditions, phenthoate was degraded readily in the two soils to level <15% after 13 days' incubation (experiments S1 and S2). In contrast, in experiments S5 and S6 with the sterilized soils, the degradation was much slower (only ≈15% and 3% disappeared for experiments S5 and S6, respectively) and the ER values also remained close to 1.0, indicating the degradation of phenthoate in experiments S1 and S2 was mainly biologically mediated. In Fig. 2, we plotted degradation curves of individual enantiomers from experiments S1 and S2. By assuming an exponential equation of first-order reaction, the kinetic data (degradation rate constant k and half-life $t_{1/2}$) of the two enantiomers as well as overall phenthoate (sum of (+) and (–)-phenthoate) were also calculated and listed in Table 2. From Fig. 2 and Table 2, we can see that phenthoate degraded faster in Tianjin alkaline soil than in Hubei acidic soil. At the same time, the occurrence of enantioselectivity was also observed in both soils with the insecticidally more active (+)-enantiomer degrading faster than its antipode (–)-phenthoate. Thus, the degradation would lead to residues enriched with the less active (–)-phenthoate. In Table 3, we give the changes in ER values from the two soils as a function of time. In the case of Tianjin soil, the ER changed from an initial 1.0 to 0.68 after 96 hours incubation. As for the Hubei soil, however, the ER decreased only to 0.93 after 312 hours incubation, suggesting that there is low enantioselective activity in this type of soil. The difference can be attributed to different microorganisms or enzymes contained in different soils (Lewis et al., 1999; Monkiedje et al., 2003; Romero et al., 2001).

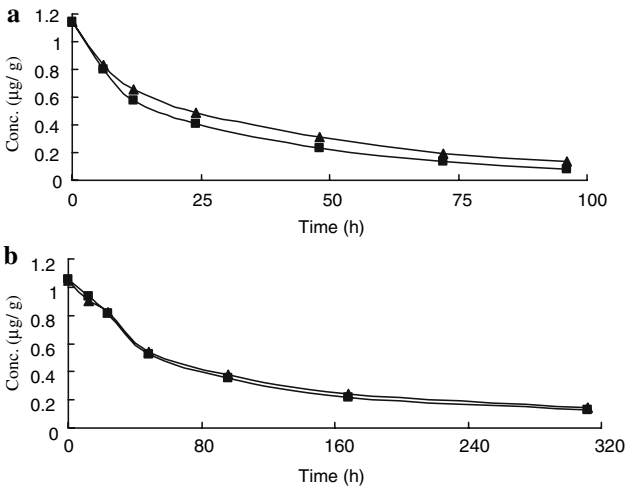

Fig. 2 Degradation curves of phenthoate enantiomers in (a). Tianjin soil (experiment S1) and (b) Hubei soil (experiment S2). ■: (+)-phenthoate, ▲: (–)-phenthoate

Table 2 Kinetic data for phenthoate degradation in the two soils

Experiment	Soil	Detected compound	$k \times 10^3$ (h^{-1})	$t_{1/2}$ (h)	r^2
S1	Tianjin soil	(+), (–)-Phenthoate	27.5	25.2	0.9540
		(+)-Phenthoate	30.0	23.1	0.9505
		(–)-Phenthoate	25.4	27.3	0.9594
S2	Hubei soil	(+), (–)-Phenthoate	6.6	105.0	0.9933
		(+)-Phenthoate	6.8	101.9	0.9930
		(–)-Phenthoate	6.5	106.6	0.9933

Table 3 ER values for phenthoate degradation in experiments S1 and S2

Experiment	Incubation time (h)								
	0	6	12	24	48	72	96	168	312
S1	1.00	0.96	0.88	0.83	0.76	0.70	0.68	—	—
S2	1.01	—	1.03	0.99	0.97	—	0.94	0.92	0.93

—: no sample was taken

As for enantiopure (+)-phenthoate incubation experiments, it was interesting that opposite results of chiral stability was obtained for the two soils. In Hubei soil (experiments S4 and S8), chiral analysis showed that there was only one peak of (+)-phenthoate all through the incubation time, indicating that no enantiomerization occurred in this soil. In the case of Tianjin soil (experiments S3 and S7), however, chiral analysis showed the appearance of (–)-phenthoate, indicating that significant enantiomerization of (+)-phenthoate to (–)-phenthoate occurred during the incubation process. Moreover, the enantiomerization was obviously chemically induced, since it took place in both nonsterilized and sterilized soils.

In order to demonstrate the enantiomerization more clearly, we plotted changes in concentrations of the two enantiomers as a function of time from experiments S3 and S7 (Fig. 3). In nonsterilized experiment S3 (Fig. 3a), the data showed a continuous decrease of the concentration of (+)-phenthoate. At the same time, there was a quick but small inversion reaction of (+)-enantiomer to (–)-enantiomer from the beginning. After about 12 hours, the concentration of (–)-phenthoate increased to a maximum of 0.17 μg/g. After that, (–)-phenthoate concentration gradually decreased, suggesting subsequent dissipation occurred for this enantiomer. In the case of sterilized experiment S7 (Fig. 3b), the concentration of (–)-phenthoate increased throughout the experiment. At last, its concentration nearly equaled that of (+)-phenthoate. Therefore, (+)-phenthoate showed a higher inversion tendency in sterilized soil than in normal soil. That is to say, the microorganisms in soil would somewhat inhibit the enantiomerization.

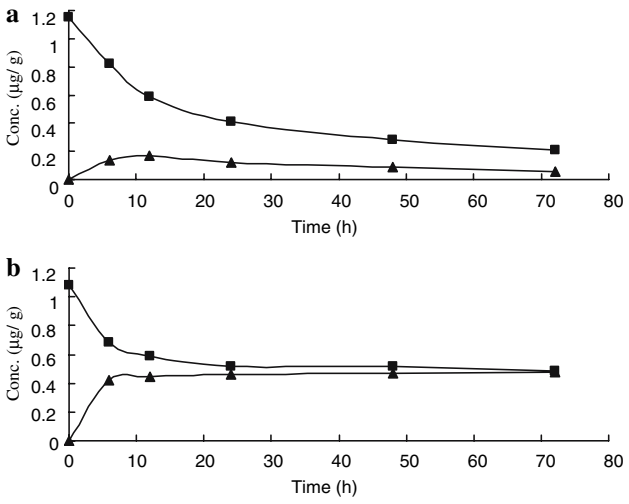

Fig. 3 Degradation of (+)-phenthoate and formation of (–)-phenthoate from incubation of (+)-phenthoate in Tianjin soil. (a) Nonsterilized experiment S3; (b) sterilized experiment S7. ■ : (+)-phenthoate, ▲: (–)-phenthoate

Because there are reports that some chiral pesticides, such as fenvalerate and cypermethrin, could undergo enantiomerization in water as well as some polar solvents (Bradbury et al., 1987; Maguire, 1990; Liu et al., 2005), we conjecture that the chemically induced enantiomerization of phenthoate observed in this study may be subject to a similar process. In the final part of this study, we carried out two water incubation experiments (experiments S9 and S10) fortified with (+)-phenthoate. In comparison with the soil experiments, the water pH values adjusted very similarly to those of the two soils. As a result, significant chiral inversion was observed in experiment S9 with pH 8.4. After 12 hours, almost 45.1% of (+)-phenthoate was inversed to (–)-phenthoate, resulting in an ER value of 1.22. On the contrary, almost no enantiomerization was observed in experiment S10 with pH 5.2. We can see that the pH effect was very consistent with that of soil incubation experiments. Due to the moderate water solubility of phenthoate (10 mg/L at 25?), it is reasonable to conclude that the chemically induced enantiomerization in Tianjin alkaline soil originated mainly from water catalysis. The water-induced enantiomerization was generally ascribed to a ground substitution reaction, in which the active proton at the chiral center position (i.e. chiral-H) exchanged with the solvent (Liu et al., 2005). Since there is also an active chiral-H in phenthoate (Fig. 1), the enantiomerization of (+)-phenthoate seemed to proceed via a similar process. Moreover, the enantiomerization was pH-dependent, as documented in this work, and took place more easily in alkaline media.

Consequently we can conclude that the enantioselectivity in phenthoate in Tianjin soil was attributed to microbial degradation as well as a chemically induced enantiomerization process. It is obvious that enantiomerization would more or less reduce the efficacy of the biologically active enantiomer. Therefore, much more attention should be paid if enantiopure (+)-phenthoate as well as relevant configurational analogues are applied in alkaline soil. The chemically induced enantiomerization increases the complexity of chiral profiles of chiral com-

pounds and shows some new and relevant aspects for the assessment of behavior of chiral pesticides in the environment.

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