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Enantioselective Acute Toxicity and Bioaccumulation of Benalaxyl in Earthworm (*Eisenia fedtia*)

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The enantioselectivities of individual enantiomers of benalaxyl in acute toxicity and bioaccumulation in earthworm (*Eisenia fedtia*) were studied. The acute toxicity was tested by paper contact test. After 48 h of exposure, the calculated LC_{50} values of the *R*-(−)-form, *rac*-form, and *S*-(+)-form were 4.99, 5.08, and 6.66 $\mu g/cm^2$, respectively. After 72 h of exposure, the calculated LC_{50} values were 1.23, 1.73, and 2.45 $\mu g/cm^2$, respectively. Therefore, the acute toxicity of benalaxyl enantiomers was enantioselective. A method for determining residues of the two enantiomers of benalaxyl in earthworm tissue by high-performance liquid chromatography based on cellulose tri-(3,5-dimethyl-phenyl-carbamate) chiral stationary phase was developed. During the bioaccumulation experiment, the enantiomer fraction in earthworm tissue was maintained approximately at 0.6, whereas enantiomer fraction in spiked soil was maintained at 0.5; in other words, the bioaccumulation of benalaxyl was enantioselective in earthworm tissue. Peak-shaped accumulation curves were observed for both enantiomers, and the calculated biota to soil accumulations (kg dry kg^{-1} wet weight) at steady state were below 1 for both enantiomers. During the elimination experiment, 79.0% of *R*-(−)-enantiomer and 89.6% of *S*-(+)-enantiomer in earthworm tissue were eliminated within 2 days.

KEYWORDS: Benalaxyl; enantioselectivity; earthworm; acute toxicity; bioaccumulation

INTRODUCTION

It is well-known that organic agrochemicals have played very important roles in agriculture practice for crop protection. Among these agrochemicals, some compounds, which account for more than 25% (*1*), consist of at least two enantiomers or stereoisomers and are called chiral pesticides. Most of the chiral pesticides are manufactured and applied to agro-ecosystems as racemic forms, although the individual enantimoers may show differences in bioactivity, toxicity, metabolism, bioaccumulation, and degradation behaviors (*2*−*4*).

Benalaxyl, meth-N-phenylacetyl-N-2,6-xylyl alaninate, belonging to the acylalanine family, is a xylem-systemic fungicide with protective, curative, and eradicant action. It can be absorbed by the roots, stems, and leaves and apoplastic movement occurs acropetally to all parts of the plant. In agriculture, it is extensively used to control late blights of potatoes and tomatoes and downy mildews of hops, vines, lettuce, onions, soya beans, tobacco, and other crops, caused by fungi of the family Peronosporaceae (*5*−*7*). Benalaxyl molecules consist of a pair of enantiomers (**Figure 1**), *R*-enantiomer with left optical (−) rotation and *S*-enantiomer with right optical (+) rotation, because of the presence of the asymmetric carbon in the alkyl moiety (*8*). Several papers have reported the differences in degradation between the two enantiomers of benalaxyl in tomato, tobacco, sugar beet, capsicum, cucumber, and some kinds of soil (*9*, *10*), so the data obtained from the racemic benalaxyl are not accurate to predict its environment risk on the contaminated sites. Benalaxyl entered the soil ecosystem because of direct spraying on the soil surface during fungicide application in agriculture, dropping from the foliage and stems by washing of rain, and the rotting of plant bodies containing benalaxyl residues in the soil. There is potential for harm in the soil organisms, especially to soil invertebrates, since the half-lives of benalaxyl in silt loam soil are 77 days (*6*). Very little data are available to predict bioaccumulation and toxic effects of individual enantiomers to the soil invertebrates, such as earthworms. Earthworms are important links in transporting environmental contaminants from soil to other organisms in terrestrial food webs (*11*). Earthworms are able to take up organic chemicals through their skin as well as through their gut wall. For chemicals with a log K_{ow} below 5, the skin is probably the most important route, while the gut becomes an important route for chemicals above approximately 5 and dominates above 6 (*12*). *Eisenia fetida* is a standard test species used for the testing of acute and chronic ecotoxicity. According to the OECD guideline 207 (*13*), the paper contact toxicity test is described as an initial screen to indicate the chemicals likely to be toxic to earthworms in soil. It is simple, easy to perform, and gives reproducible results. As the log K_{ow} for benalaxyl is 3.54, skin exposure is a relevant uptake route, which can be assessed by the paper contact test (*14*). The paper contact test is adequate to perform acute toxicity of individual enantiomers of benalaxyl to earthworms. The residue of the two enantiomers of benalaxyl in earthworm tissue and soil is needed to be quantified to assess the bioaccumulation behavior and the biota to soil accumulation factor (BSAF) of individual enantiomers. Methods for stereoselective determination

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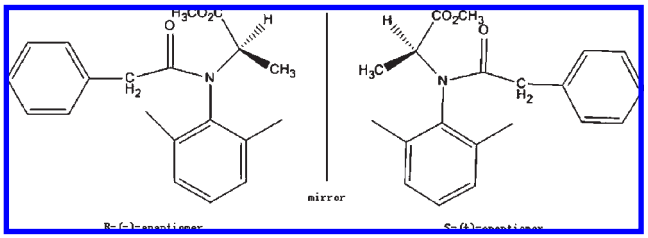

Figure 1. Structures of benlaxyl enantiomers.

of benalaxyl residues in plasma, plants, water, and soil by high-performance liquid chromatography (HPLC) (*3*, *8*−*10*) have been reported, but a method for stereoselective determination of benalaxyl residues in earthworm tissue has not been reported.

The aim of this experiment was to study the differences in acute toxicity to earthworms and bioaccumulation behavior of individual enantiomers of benalaxyl in earthworm tissue. In this paper, we assayed the acute toxicitiy of the *R*-(−)-, *S*-(+)-, and *rac*-forms of benalaxyl using the filter paper contact test and found that the median lethal concentrations (LC_{50}) was different between the two enantiomers. A method for extraction, cleaning, and detection of enantiomers of benalaxyl in earthworm tissue was developed, and we found that the bioaccumulation of benalaxyl was enantioselective during the bioaccumulation stage.

MATERIALS AND METHODS

Chemicals and Regents. The fungicide of *rac*-benalaxyl (>99.0% purity) was provided by the China Ministry of Agriculture Institute for Control of Agrochemicals. The two enantiomers of benalaxyl were prepared by HPLC based on cellulose tri-(3,5-dimethylphenyl-carbamate) chiral stationary phase (CDMPC-CSP) (*15*), and the enantiomeric purities of (*R*)-(−)-enantiomer and (*S*)-(+)-enantiomer were 99.5 and 98.0%, respectively. *n*-Hexane (HPLC grade) and 2-propanol (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ). Ethyl acetate, acetone, and acetonitrile (analytical grade) were purchased from commercial sources.

Earthworms. Mature redworms (Eisenia fetida) purchased from a northern suburbs farm, Beijing, were maintained in a wooden breeding box ($50 \times 50 \times 20\ cm^3$) containing a mixture of soil and cattle manure. The worms were active when introduced in the experiment.

Acute Toxicity Test. According to the OECD guideline 207, a paper contact toxicity assay was used to test the acute toxicity of *rac*-benalaxyl and its two enantiomers to earthworms. A range of known concentrations, 50, 120, 190, 260, 330, 400, and 470 mg/L, of test substances were prepared with acetone as the solvent. After the depuration period of 3 h to evacuate the earthworms' gut contents, they were rinsed in tap water and cautiously dried by absorbent paper. One milliliter of solution was pipetted and added to the filter paper ($5.5 \times 11.5\ cm^2$) and placed in flat-bottomed glass vial (3.6 cm in diameter, 8 cm in length). After the solvent was dried under a stream of compressed air, 1 mL of deionized water was added to each vial. Controls were also run in parallel with the carrier solvent alone. Fifteen replicates for each treatment and each vial containing one worm were done. Each vial was sealed with plastic film with several ventilation holes. After that, all of the vials were placed in a room at 20 ± 2 °C, and mortality was assessed after 48 and 72 h, respectively.

Soil Collection and Earthworm Exposures. The site to collect soil was a grassland area 20 km northwest of Beijing, China, which had not received any pesticide applications for at least 10 years. After the superficial vegetation was removed, the top soil (0−10 cm) was collected. The soils were sieved (2 mm) and air-dried at room temperature and kept in the dark until used within a few days. Physicochemical properties of the soil were as follows: organic matter (OM), 2.13 ± 0.12%; clay, 4.73 ± 0.14%; sand, 54.29 ± 1.38%; silt, 40.98 ± 1.24%; water holding capacity, 44%; and pH, 7.6 ± 0.2.

To ensure that 250 g_{dwt} of medium was spiked homogeneously with *rac*-benalaxyl, we did the procedure in steps (dilution spike). First, the chemical (12.5 mg) was dissolved in 10 mL of acetone, and then, the acetone solution was slowly added to dry soil (50 g) while mixing continued for about 5 min. The spiked soil was left in a fume cupboard overnight after which the acetone had evaporated. Next, the contaminated dry soil (50 g_{dwt}) was mixed thoroughly with 200 g_{dwt} of uncontaminated medium, and then, the contaminated soil (250 g_{dwt}) was transferred to a 500 mL glass jar. Ninety grams of tap water was added to each jar to restore the 36% water content.

In this experiment, the earthworms weighed between 200 and 300 mg. Before the worms were introduced, they were allowed to live in that kind of uncontaminated soil for 1 week to acclimate. After their gut contents were evacuated on moist filter paper for 3 h at 20 °C, 10 g_{wwt} earthworms were exposed to the chemical in each jar containing 340 g_{wwt} of contaminated soil. The jars containing contaminated soil and worms were weighed, and the loss of water by evaporation was compensated by addition of tap water every 2 days. All of the jars were placed in the dark in an environmental chamber controllable to 20 ± 2 °C.

For the bioaccumulation experiment, worms were collected after an exposure period (1, 3, 5, 7, 10, 14, 19, 25, and 32 days), rinsed in tap water, and allowed to depurate most of their gut contents on moist filter paper for 3 h. The depuration period was chosen because the gut retention time was about 2.9 h, and a longer depuration period may cause the chemical to be lost from the tissue and lead to bias (*12*, *16*). Water on the surface of the worms was dried by absorbent paper cautiously, and then, the worms were weighed and frozen at −20 °C (in 50 mL of polypropylene tubes). Soil samples (6.8 g_{wwt}) sampled from each jar were also stored at −20 °C. All of the incubations were carried out in triplicate at each sample point.

For the elimination experiment, after 19 days of exposure in the contaminated soil, worms were recaptured and transferred to 340 g_{wwt} of unspiked medium. During the elimination period, worms were recollected after 0.5, 1, 1.5, and 2 days. After 3 h of depuration and drying of the external water, the worms were stored at −20 °C.

Analysis of Benalaxyl Residues. For the sample pretreatment of soil, ethyl acetate (25 mL) and 4 g of anhydrous sodium sulfate were added to a 50 mL polypropylene centrifuge tube containing 6.8 g_{wwt} of incubated soil sample. Next, the tube was capped, vortex-mixed for 3 min, and centrifuged at 3500 rmp for 5 min, and then, the liquid phase was transferred to a new tube. The remaining part was extracted again following the same extraction step, and the liquid phase was combined. The combined extract was passed through a funnel with about 10 g of anhydrous sodium sulfate to a pear-shaped flask and evaporated to dryness by vacuum rotary evaporator at 45 °C. The dry extract was diluted to 1.0 mL with 2-propanol and passed through a filter membrane (pore size, 0.45 μm).

For analysis of the earthworms, the samples were thawed for about 15 min at room temperature. Twenty-five milliliters of ethyl acetate was added to each tube. Next, the mixture was homogenized with Ultra-Turrax T18 homogenizer for 30 s. The phases were vortex-mixed for 5 min and separated by centrifugation at 3500 rpm for 5 min. The upper organic phase was passed through a funnel with about 10 g of anhydrous sodium sulfate to a pear-shaped flask and evaporated to dryness at 45 °C. For cleanup (fat destruction), 5 mL of acetonitrile was added to dissolve the extract, and then, 3 × 5 mL of *n*-hexane was added for liquid−liquid partition to extract most of the lipid. The upper layer of *n*-hexane was discarded, and the layer of acetonitrile was evaporated to dryness by vacuum rotary evaporator. The Alumina-N-solid-phase extraction (SPE) (500 mg) on a cartridge (6 mL) was used to clean up other interfering substances. The cartridge was preconditioned by rinsing with 5 mL of ethyl acetate followed by 5 mL of *n*-hexane and equilibrated with 10 mL of 1:4 ethyl acetate: *n*-hexane. The sample of dry extract was dissolved in 2 mL of 20% ethyl acetate in *n*-hexane, and then, the solution was passed through the SPE cartridge. The cartridge was eluted with additional 8 mL of 1:4 ethyl acetate:*n*-hexane. The eluates were combined with the loading eluates. The combined 10 mL of eluates was collected in a glass tube, evaporated to dryness under a stream of nitrogen, and diluted to 1.0 mL with 2-propanol.

For HPLC analysis with UV detection, the work was performed on an Agilent 1200 system, equipped with G1322A degasser, G1311A pump, G1314B VWD, and G1329A ALS. AT-930 heater and cooler column attemperator (Titanjin Automatic Science Instrument Co. Ltd., China) was used to control the column temperature. The signal was received and processed by Agilent chemstation software. The enantiomers of benalaxyl were separated on CDMPC-CSP (provided by the Department of Applied

Table 1. Calculated LC_{50} Values for Enanatiomers of Benalaxy1

	R-(−)-enantiomer			*rac*-benalxy1			*S*-(−)-enantiomer		
exposure time (h)	LC_{50} (μg/cm^2)	R^{2a}	P^b	LC_{50} (μg/cm^2)	R^2	P	LC_{50} (μg/cm^2)	R^2	P
48	4.99	0.96	0.003	5.08	0.98	0.006	6.66	0.97	0.018
72	1.23	0.94	0.006	1.73	0.91	0.043	2.45	0.94	0.001

[a] Represents the correlation coefficient. [b] Represents the probability (associated with the *t* test). A *p* value smaller than 0.05 indicates that the correlation of linear equation is significant.

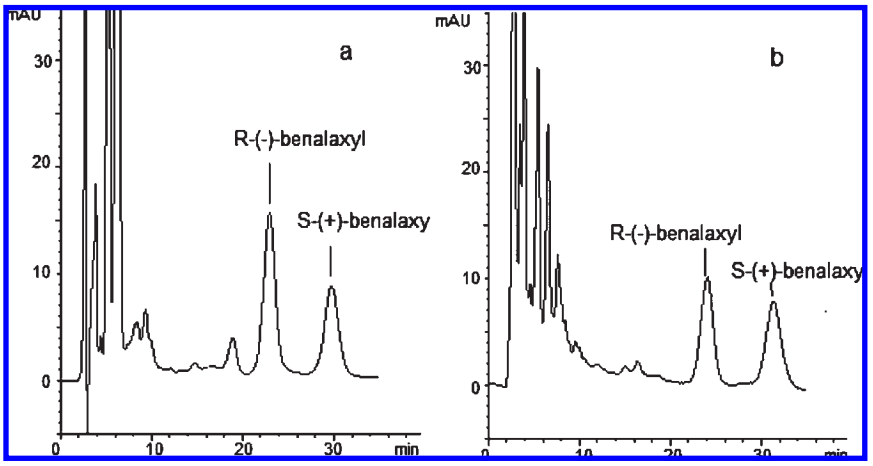

Figure 2. Representative HPLC chromatogram of the expectations of (**a**) earthworm and (**b**) soil after 14 days of exposure (*n*-hexane:2-propanol = 97:3; flow rate, 1.0 mL/min).

Chemistry, China Agricultural University, Beijing). The CSP was prepared according to the procedure described in the literature (*15*). The CSP was packed into a 250 mm × 4.6 mm (I.D.) stainless steel column. In previous reports (*17*), racemic benalaxyl was ideally separated on the CDMPC-CSP. In the present work, the detailed HPLC method was successfully applied to analyze enantiomers of benalaxyl in soils and earthworm samples. A mixture of *n*-hexane and 2-propanol (97:3, v/v) was used as the mobile phase at a rate of 1.0 mL/min. The injection volume was 20 μL, and the UV detection wavelength was 230 nm. The chromatographic separation was conducted at 20 °C. The first eluted enantiomer was *R*-(−)-form, and the second one was *S*-(+)-form according to the previous study (*8*). The average recoveries for both enantiomers at levels between 0.5 and 25 mg/kg ranged between 81 and 95% in earthworm tissue and between 90 and 96% in soil with SD below 10% (n = 3 for each concentration). The limit of detection (LOD) for both enantiomers, defined as the concentration that produced a signal-to-noise ratio of 3, was 0.2 mg/kg both in earthworm tissue and soil.

RESULTS AND DISCUSSION

Acute Toxicity Assay. The acute toxicity was measured by a paper contact test for individual enantiomers and racemate and using *E. fetida* as the test organism. The mortality in relation to concentrations of 50, 120, 190, 260, 330, 400, and 470 mg/L, which were equivalent to 0.791, 1.897, 3.004, 4.111, 5.217, 6.324, and 7.431 μg/cm^2, was assessed and counted during the exposure tenures at 48 and 72 h. The LC_{50} values were determined from the survival data with SPSS (Version 16.0). The mortality of worms at serial concentrations and different exposure tenures and the LC_{50} values calculated are shown in **Table 1**. From these results, the LC_{50} values generally decreased over exposure time, and the order of toxicity potency at both 48 and 72 h was *R*-(−)-enantiomer > racemate > *S*-(+)-enantiomer. The calculated LC_{50} of *S*-(+)-benalaxyl enantiomer was about two times of that for *R*-(−)-benalaxyl after 72 h of exposure.

The enantioselective toxicity assessment of currently used pesticides to nontarget organisms has been limited (*18*, *19*), and currently, the environmental risk of most chiral pesticides is evaluated based on their racemates. In theory, enantiomers often exhibit different toxicities. The one enantiomer of a pesticide may have an adverse effect on some nontarget species, whereas the other enantiomer may not have such an effect. Take the toxicity of isocarbophos to *Daphnia magna* as an example: The predicted acute toxicity (24 h) based on the racemate might be overestimated by 30 times if only the (−)-enantiomer were present in the residue. However, it might cause a 1.7-fold underestimation if only the (+)-enantiomer were present in the residue (*20*). Therefore, the present data available on the toxicity of the racemic mixtures of these chiral pesticides are not reliable, and the study of enantioselective toxicity of chrial pesticides has become very popular for environmental safety issues (*4*, *18*−*20*).

Bioaccumulation and Elimination in Earthworm. The concentrations of the two enantiomers of benalaxyl in earthworm tissue and soil were determined. During the bioaccumulation period, different concentrations of individual enantiomers were observed in earthworm tissue at the same sample point, with concentrations of the *R*-form higher than that of the *S*-form (**Figure 2a**). However, the concentrations of the two enantiomers of benalaxyl were almost the same in soil (**Figure 2b**). The enantiomer fraction (EF) was used to measure the enantioselectivity of the bioaccumulation of enantiomers of benalaxyl in earthworm tissue. The EF values defined range from 0 to 1, with EF = 0.5 representing the racemic mixture. EF was expressed as follows:

$$\mathrm{EF} = \text{peak area of } (-)/[(-)+(+)] \quad (1)$$

A *t* test was carried out to compare the means of the EF values in spiked soil with EF = 0.5 and to compare the means of EF values in earthworm with EF = 0.5. As shown in **Figure 3**, the EF values in earthworm tissue were observed to deviate from 0.5 ($p < 0.001$) in the bioaccumulation experiment, and they were maintained approximately at 0.6, whereas the EF values in spiked soil were 0.5 at the beginning and have remained largely unchanged (p = 0.060). A pair *t* test for the same time points between the EF

values of spiked soil and the EF values of earthworm yielded a p value of 0.00002. These results showed that the bioaccumulation behavior of benalaxyl in earthworm tissue was enantioselective.

Concentrations in earthworm tissue reached the highest level in 7−14 days, but after 14 days of exposure, concentrations decreased and reached steady state for two enantiomers. Therefore, the accumulation curves observed in this work were both peak-shaped for the two enantiomers of benalaxyl (**Figure 4**). In **Figure 4**, the accumulation curve of the *R*-enantiomer is higher than that of the *S*-enantiomer; in other words, the bioaccumulation behavior of benalaxyl in earthworm tissue was enantioselective.

Several authors have reported peak-shaped accumulation curves (*21*−*25*), most often for PAHs, and the suggested explanations for these peak-shaped accumulation curves include induction of active excretion by the worm (*23*), an increase of sorption in soil (*24*), or biodegradation coupled to slow desorption from OM (*25*). In our study, there was no significant biodegradation for both enantiomers by comparing the concentrations in soil in 32 days and that in 1 day, so the possible causes of peak-shaped curves were attributed to an induction of active excretion by worms or an increase sorption in soil, which caused bioavailability to decline. In the present work, mortality for worms occurred during 7 and 14 days, and this was an indication that worms were in poor soil conditions, but mortality did not occurr anymore during 19 and 32 days; in other words, the bioavailability declined as time elapsed. This conclusion is consistent with previous studies, in which organic compounds may undergo a time-dependent sequestration in soil that results in a decline in bioavailability without parallel decline in the concentration of compounds determined by vigorous extraction with organic solvents (*26*). According to the above phenomenon, we can conclude that the decrease of bioavailability was the principal contributor to the appearance of peak-shaped accumulation curves in our experiment.

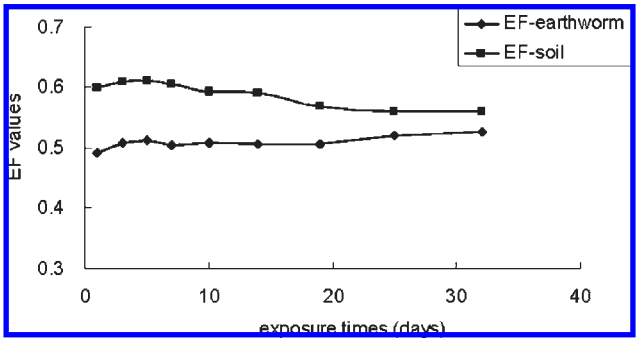

Figure 3. Enantiomeric fraction (EF) of benalaxyl residues in earthworm tissue and soil.

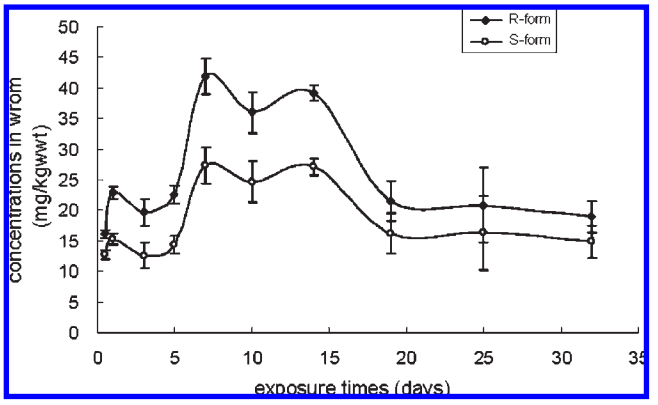

Figure 4. Accumulation curves for benalaxyl enantiomers in earthworm tissue.

In this work, we used BSAF (kg dry kg^{-1} wet weight) to express the bioaccumulation of benalaxyl in earthworm tissue. BSAF is a function of the relative sorptive capacities of the organism versus the surrounding soil, and it was defined as

$$\mathrm{BSAF} = \frac{C_e}{C_s} \quad (2)$$

where C_e (g kg^{-1} wet weight) is the concentration of compound in earthworm tissue and C_s (g kg^{-1} dry) is the concentration of compound in soil. Because the experiment was confined to one species and one type of soil, the BSAF was not normalized to the lipid content of the organism and the organic carbon (OC) of soil (*27*). As such, the BSAF is expressed by kg dry kg^{-1} wet weight not kg OC kg^{-1} lip. The calculated BSAFs of the two enantiomers of benalaxyl are shown in **Table 2**. A pair t test for the same time points between the *S*-enantiomer and the *R*-enantiomer yielded a p value of 0.001. The paired t test showed that there was a significant difference in the BSAFs between the two enantiomers of benalaxyl. The BSAF value of the *S*-enantiomer was smaller than that of the *R*-enantiomer, and this revealed that the *R*-enantiomer was preferentially accumulated over the *S*-enantiomer in earthworm tissue. It can be assumed that the bioaccumulation of benalaxyl was enantioselective.

Additionally, it seems to be a generally held opinion that earthworm taking up organic chemicals was considered as passive diffusion from dissolved phase in soil pore water and gut contents. The final body residue in steady stage is a result of equilibrium between earthworm tissue, soil, and gut contents (*12*). Our experiment results of enantioselective bioaccumulation behaviors of benalaxyl showed that the partition coefficient between OM and earthworm tissue (K_{es} in kg_{OM}/kg_{wwt}) was different for individual enantiomers. K_{es} in this case is a model parameter, and it can be expressed in the follow equation:

$$K_{es} = \frac{C_e}{C_s} = \frac{C_e/C_w}{C_s/C_w} = \frac{\mathrm{BCF}}{K_{sw}} \quad (3)$$

where C_e (g kg^{-1}_{wwt}) is the concentration of compound in earthworm tissue, C_s (g kg^{-1}_{OM}) is the concentration of compound in soil, C_w (g/L) is the concentration of compound in soil pore water, K_{sw} (L/kg_{OM}) is the soil−water partition coefficient, and BCF (L/kg_{wwt}) is the bioconcentration factor with water. Because the OM−worm partition coefficient (K_{es}) was different

Table 2. Calculated BSAFs for Enantiomers of Benalaxy1

	exposure time (days)								
	1	3	5	7	10	14	19	25	32
BSAF[a]-*R*-enantiomer	0.70 ± 0.01	0.49 ± 0.08	0.72 ± 0.11	1.21 ± 0.14	1.05 ± 0.05	1.33 ± 0.03	0.67 ± 0.17	0.58 ± 0.26	0.53 ± 0.05
BSAF[a]-*S*-enantiomer	0.46 ± 0.00	0.33 ± 0.07	0.48 ± 0.06	0.80 ± 0.10	0.74 ± 0.06	0.94 ± 0.05	0.53 ± 0.18	0.49 ± 0.17	0.46 ± 0.09

[a] The unit of BSAF is kg^{-1} wet weigh.

for individual enantiomers, furthermore, the OM−water partition coefficient (K_{sw}) is the same for the two enantiomers of benalaxyl, we can infer that the partition coefficient between water and earthworm tissue was different for individual enantimoers, and the BCF value of the *R*-enantiomer and *S*-enantiomer was different, too.

In elimination experiments, earthworms were transferred to benalaxyl-free soil without contacting and feeding on benalaxyl-spiked soil. The amount of residue of benalaxyl in tissue decreased significantly within 2 days. The same elimination phenomena for avermectin B_{1a}, telodrin, dieldrin, HexaCB, and PCBs in uncontaminated soil were observed (*28*, *29*). The elimination rate is mainly affected by feeding not relying on K_{ow} (*29*).

LITERATURE CITED

(1) Williams, A. Opportunities for chiral agrochemicals. *Pestic. Sci.* **1996**, *46*, 3–9.

(2) Zadra, C.; Marucchini, C.; Zazzerini, A. Behavior of metalaxyl and its pure *R*-enaniomer in sunflower plants (*Helianthus annus*). *J. Agric. Food Chem.* **2002**, *50*, 5373–5377.

(3) Liu, D. H.; Wang, P.; Zhou, W. F.; Gu, X.; Chen, Z. S.; Zhou, Z. Q. Direct chiral resolution and its application to the determination of fungicide benalaxyl in soil and water by high-performance liquid chromatography. *Anal. Chim. Acta* **2006**, *555*, 210–216.

(4) Cai, X. Y.; Liu, W. P.; Sheng, G. Y. Enantioselective degradation and ecotoxicity of the chiral herbicide diclofop in three freshwater alga cultures. *J. Agric. Food Chem.* **2008**, *56*, 2139–2146.

(5) Bradshaw, N. J.; Vaughan, T. B. The effect of phenylamide fungicides on the control of potato late-blight (*Phytophthora infestans*) in England and Wales from 1978 to 1992. *Plant Pathol.* **1996**, *45*, 249–269.

(6) Tomlin, C. *The e-Pesticide Manual, Version 3.0*; British Crop Protection Council: Lyon, France, 2003.

(7) Rosso, I.; Giraudi, G.; Gamberini, R.; Baggiani, C.; Vanni, A. Application of an ELISA to the determination of benalaxyl in red wines. *J. Agric. Food Chem.* **2000**, *48*, 33–36.

(8) Qiu, J.; Wang, Q. X.; Zhu, W. T.; Jia, G. F.; Wang, X. Q.; Zhou, Z. Q. Stereoselective determination of benalaxyl in plasma by chiral high-performance liquid chromatography with diode array detector and application to pharmacokinetic study in rabbits. *Chirality* **2007**, *19*, 51–55.

(9) Gu, X.; Wang, P.; Liu, D. H.; Lv, C. G.; Lu, Y. L.; Zhou, Z. Q. Stereoselective degradation of benalaxyl in tomato, tobacco, sugar beet, capsicum, and soil. *Chirality* **2008**, *20*, 125–129.

(10) Wang, X. Q.; Jia, G. F.; Qiu, J.; Diao, J. L.; Zhu, W. T.; Lv, C. G.; Zhou, Z. Q. Stereoselective degradation of fungicide benalaxyl in soils and cucumber plants. *Chirality* **2007**, *19*, 125–129.

(11) Sellström, U.; De Wit, C. A.; Lundgen, N.; Tysklind, M. Effect of sewage-sludge application on concentrations of higher-brominated diphenyl ethers in soils and earthworms. *Environ. Sci. Technol.* **2005**, *39*, 9064–9070.

(12) Jager, T.; Fleuren, R. H. L.; Hogendoorn, E. A.; De Korte, G. Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta). *Environ. Sci. Technol.* **2003**, *37*, 3399–3404.

(13) OECD. Guidelines for testing of chemicals, earthworm. *Acute Toxicity Tests*; Organization for Economic Cooperation and Development: Paris, 1984; Vol.*207*.

(14) Hackenberger, B. K.; Jarić-Perkušić, D; Stepić, S. Effect of temephos on cholinesterase activity in the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae). *Ecotoxicol. Environ. Saf.* **2008**, *71*, 583–589.

(15) Zhou, Z. Q.; Wang, P.; Jiang, S. R. The preparation of polysaccharide-based chiral stationary phase and the direct separation of five chiral pesticides and related intermediates. *J. Liq. Chromatogr. Related Technol.* **2003**, *26*, 2873–2880.

(16) Jager, T.; Fleuren, R. H. L. J.; Roelofs, W; De Groot, A. C. Feeding activity of the earthworm *Eisenia andrei* in artificial soil. *Soil Biol. Biochem.* **2003**, *35*, 313–322.

(17) Wang, P.; Jiang, S. R.; Liu, D. H.; Zhang, H. J.; Zhou, Z. Q. Enantiomeric resolution of chiral pesticides by high-performance liquid chromatography. *J. Agric. Food Chem.* **2006**, *54*, 1577–1583.

(18) Liu, W.; Gan, J.; 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.

(19) Konwick, B. J.; Fisk, A. T.; Garrison, A. W.; Avants, J. K.; Black, M. C. Acute enantioselective toxicity of fipronil and its desulfiny photoproduct to *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* **2005**, *24* (9), 2350–2355.

(20) Lin, K.; Liu, W.; Li, L.; Gan, J. Single and joint acute toxicity of isocarbophos enantiomers to *Daphnia magna*. *J. Agric. Food Chem.* **2008**, *56*, 4273–4277.

(21) Jager, T.; Baerselman, R.; Dijkman, E.; Groot, A. C.; Hogendoom, E. A.; De Jong, A.; Kruitbosch, J. A. W.; Peijnenburg, W. J. G. M. Available of polycyclic aromatic hydrocarbons to earthworms (*Eisenia andrei*, Oligochaeta) in field-polluted soils and soil-sediment mixtures. *Environ. Toxicol. Chem.* **2003**, *22*, 767–775.

(22) Matscheko, N.; Lundstedt, S.; Svensson, L.; Harju, M.; Tysklind, M. Accumulation and elimination of 16 polycyclic aromatic compounds in the earthworm (*Eisenia fetida*). *Environ. Toxicol. Chem.* **2002**, *21*, 1724–1729.

(23) Reinecke, A. J.; Nash, R. G. Toxicity of 2,3,7,8-TCDD and short-term bioaccumulation by earthworms (Oligochaeta). *Soil Boil. Biochem.* **1984**, *16*, 45–49.

(24) Ma, W. C.; Immerzeel, J.; Bodt, J. Earthworm and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrouscarbons: Studies on phenanthrene and fluoranthene. *Ecotoxicol. Environ. Saf.* **1995**, *32*, 226–232.

(25) Jager, T.; Sanchez, F. A.; Muijs, B.; Van Der Velde, E. G.; Posthuma, L. Toxicokinetics of polycyclic aromatic hydrocarbons in *Eisenia andrei* (oligochaeta) using spiked soil. *Environ. Toxicol. Chem.* **2000**, *19*, 953–961.

(26) Morrison, D.; Morrison, B. K.; Alexander, M. Bioavailability to Earthworms of Aged DDT, DDE, DDD, and dieldrin in soil. *Environ. Sci. Technol.* **2000**, *34*, 709–713.

(27) Gevao, B.; Mordaunt, C.; Semple, K. T.; Piearce, T. G.; Jones, K. C. Bioavailability of nonextractable (bound) pesticide residues to earthworms. *Environ. Sci. Technol.* **2001**, *35*, 501–507.

(28) Sun, Y. J.; Diao, X. P.; Zhang, Q. D.; Shen, J. Z. Bioaccumulation and elimination of avermectin B_{1a} in the earthworm (*Eisenia fetida*). *Chemosphere* **2005**, *60*, 699–704.

(29) Jager, T.; Van Der Wal, L.; Fleuren, R. H. L. J.; Barendregt, A.; Hermens, J. L. M. Bioaccumulation of organic chemicals in contaminated soils: Evaluation of bioassays with earthworms. *Environ. Sci. Technol.* **2005**, *39*, 293–298.

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