Enantioselective Acute Toxicity Effects and Bioaccumulation of Furalaxyl in the Earthworm (*Eisenia foetida*)

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ABSTRACT The enantioselectivities of individual enantiomers of furalaxyl in acute toxicity and bioaccumulation in the earthworm (*Eisenia foetida*) were studied. The acute toxicity was tested by filter paper contact test. After 48 h of exposure, the calculated LC₅₀ values of the *R*-form, *rac*-form, and *S*-form were 2.27, 2.08, and 1.22 $\mu\text{g cm}^{-2}$, respectively. After 72 h of exposure, the calculated LC₅₀ values were 1.90, 1.54, and 1.00 $\mu\text{g cm}^{-2}$, respectively. Therefore, the acute toxicity of furalaxyl enantiomers was enantioselective. During the bioaccumulation experiment, the enantiomer fraction of furalaxyl in earthworm tissue was observed to deviate from 0.50 and maintained a range of 0.55–0.60; in other words, the bioaccumulation of furalaxyl was enantioselective in earthworm tissue with a preferential accumulation of *S*-furalaxyl. The uptake kinetic of furalaxyl enantiomers fitted the first-order kinetics well and the calculated kinetic parameters were consistent with the low accumulation efficiency. *Chirality* 26:307–312, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: furalaxyl; enantioselectivity; earthworm; acute toxicity; bioaccumulation

INTRODUCTION

Furalaxyl (*R*, *S*)-methyl-*N*-(2, 6-dimethylphenyl)-*N*-(2-furanylcarbonyl)-DL-alaninate, belonging to the acylanilide fungicides, was first introduced in 1977 and is still widely used in the control of plant diseases caused by infection with *Albugo candida*, *Fusarium solani*, *Peronospora parasitica*, *Phytophthora* sp., and *Pythium* sp.^{1,2} It can be absorbed by the roots, stalk, and leaves and can move to all parts of the plant with protective, curative, and eradicator action for vegetables and fruits.³ Furalaxyl is a chiral fungicide and consists of a pair of enantiomers with *R*- and *S*-configuration (Fig. 1). Previous studies have shown that the enantiomers of a chiral pesticide have identical physical and chemical properties but they may perform different in the processes of absorption, accumulation, and degradation when confronted with a chiral environment.^{4,5} This makes it necessary to study the biological activity, toxicity, and environmental behavior of the two enantiomers of furalaxyl individually. Sulimma et al. reported that the *R*-furalaxyl decreased more rapidly than the *S*-isomer by *Brevibacillus brevis*.⁶ Thus, application of commercially available racemic mixtures poses an inevitable biological burden for treated plants and soils.⁶ To further research the effects of chiral pesticides on environmental safety and public health, we checked the uptake and translocation of furalaxyl enantiomers by soil organisms in biological systems. This helps us to address the suitability of replacing pesticide racemic mixtures with single biologically active enantiomers for a more sustainable pesticide use.^{7–10}

Earthworms are widely distributed in topsoil and frequently dominant in soil communities. They are appropriate model organisms for bioavailability as they live in close contact with the soil, have a thin and permeable cuticle, and also consume large amounts of soil. Earthworms can thus be considered model organisms for assessing the potential biological effect of the chemicals released into the environment.^{11–13} Several articles have been reported that earthworms were utilized in both toxicity and bioaccumulation studies,^{14–16} but until now there is no report about the enantioselective transfer or bioaccumulation of furalaxyl from soil to earthworm and the toxicity of furalaxyl on earthworm.

In this study, we selected the commonly used test species, *Eisenia foetida*, for acute toxicity and bioavailability tests under laboratory conditions. The objectives of this study were to (1) evaluate the acute toxicities of *rac*-furalaxyl and its enantiomers to earthworm using a filter paper contact test, (2) investigate the enantioselective bioaccumulation of furalaxyl in earthworm from soil.

MATERIALS AND METHODS

Chemicals and Reagents

Racemic furalaxyl and its two enantiomers were synthesized by our laboratory; the purity of *rac*-enantiomer was 98.0% and both the enantiomeric purities of *R*-enantiomer and *S*-enantiomer were 97.0%. Acetonitrile (high-performance liquid chromatography [HPLC] grade) was obtained from Fisher Scientific (Fair Lawn, NJ). Silica-based sorbents including C₁₈ (40 μm particle size) and primary secondary amine (PSA, Si-(CH₂)₃-NH-(CH₂)₂-NH₂) (40 μm particle size) were obtained from Agela Technologies (Tianjin, China). Water was purified by a Milli-Q (Bedford, MA) system. All other chemicals and solvents were of analytical reagent grade and commercially available.

Synthesis of Racemic and Two Enantiomers of Furalaxyl

The two furalaxyl enantiomers were prepared via chiral synthesis methods,^{17–19} and the synthetic procedure was the same as synthesis of benalaxyl enantiomers we have discussed in detail.¹⁷

The preparation of *R*-furalaxyl with methyl *S*-(-)-lactate (0.77 mL, 8.0 mmol, 1.0 equiv) as a starting material followed the experimental procedure we reported¹⁷ to obtain the light yellow oil *N*-xylyl-D-methyl alaninate (1.38 g). Then sodium bicarbonate (0.26 g, 3.1 mmol, 1.1 equiv) was added to a solution of *N*-xylyl-D-methyl alaninate (0.58 g, 2.8 mmol, 1.0 equiv) in 15 mL of toluene, cooled to a temperature ranging from 5–10°C, and subsequently 2-furoyl chloride (0.40 g, 3.1 mmol, 1.1 equiv)

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Fig. 1. Structures (absolute configurations) of *R*- and *S*-furalaxyl.

were slowly added dropwise. After 4 h at room temperature, the above solution was washed with saturated sodium chloride solution and the organic phase was evaporated in vacuo. The obtained crude product was crystallized with hexane to give 0.57 g of white crystalline solid *R*-furalaxyl with an enantiomeric R/S ratio = 97 (yield 67%).

The preparation of *S*-enantiomer with methyl *R*(+)-lactate as a starting material followed the same experimental procedure, S/R ratio = 97 (yield 67%). The preparation of *rac*-enantiomer with methyl (±)-lactate as a starting material followed the same experimental procedure, R/S ratio = 1 (yield 67%).

Earthworms

Mature earthworms (*Eisenia foetida*), purchased from a northern suburbs farm, Beijing, were maintained in a wooden breeding box (50 × 50 × 20 cm³) containing a mixture of soil and cattle manure. Active, sexually mature worms with body masses between 200 and 300 mg were used in the experiment.

Toxicity Test

According to the OECD guideline 207, a paper contact toxicity assay was used to test the acute toxicity of *rac*-furalaxyl and its two enantiomers to earthworms.²⁰ A range of known concentrations of test substances were prepared with acetone as the solvent. After the depuration period of 24 h on wet filter paper under dark conditions to evacuate the earthworm gut content, earthworms were rinsed in distilled water and cautiously dried by absorbent paper. One milliliter of solution was pipetted and added to the filter paper (5.5 × 11.5 cm²) placed in flat-bottomed glass vial. The concentrations of *S*-furalaxyl on filter papers were 0.63, 0.95, 1.26, 1.58, 1.90, 2.21, and 2.53 μg cm⁻². The concentrations of *rac*-furalaxyl and *R*-furalaxyl on filter papers were 1.26, 1.58, 1.90, 2.21, 2.53, 2.85, and 3.48 μg cm⁻². After drying of the solvent under a stream of compressed air, deionized water (1.0 mL) was added to each vial. Controls were also run in parallel with the carrier solvent alone. Ten 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 the vials were placed in a room at 20 ± 2°C, and mortality of earthworms was assessed after incubation for 48 and 72 h, respectively.

Soil Collection and Earthworm Exposures

The site to collect soil was a farm of Changping District, Beijing, China. 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 a dark, dry place. Physicochemical properties of the soil were as follows: sand loam, organic matter, 3.22 ± 0.12 %; clay, 4.23 ± 0.11 %; silt, 38.04 ± 1.14 %; sand, 55.12 ± 1.26 %; and pH (water, ratio 1:2.5), 7.1 ± 0.2.

To ensure that 250 g of medium was spiked homogeneously with *rac*-furalaxyl, *S*-enantiomer, *R*-enantiomer, the procedure was as follows. First, the chemicals were dissolved respectively in acetone (10 mL), and then the acetone solution was slowly added to dry soil (50 g). The spiked soil was left in a fume cupboard overnight to remove acetone. Next, the contaminated dry soil (50 g) was mixed thoroughly with 200 g of uncontaminated medium. The final concentrations of *rac*-furalaxyl in soil were at 20 and 50 mg kg⁻¹ soil. *S*-form and *R*-form were at 10 mg kg⁻¹ soil. Then water (90 g) was added to each jar to restore the 36% water content.

Earthworms used in the bioaccumulation test weighed between 200 and 300 mg and were acclimated to the test soils for 1 week prior to the start of the test. We used three replicates per concentration and 10

vehicle control replicates. Earthworms were exposed to each jar containing 340 g contaminated soil after evacuating their gut contents on moist filter paper for 3 h at 20°C. The jars containing contaminated soil and worms were weighed and the loss of water by evaporation was compensated by addition of water every 2 d. All of the vessels were placed in an incubator and maintained at 20 ± 2°C with a photoperiod of 16 h light and 8 h dark. Worms were collected after exposure periods (0.25, 0.5, 1, 2, 3, 5, 7, 10, 14, 21, and 31 d), rinsed with distilled water, and allowed to depurate most of their gut contents. Water on the surface of the worms was dried by absorbent paper cautiously, and then the worms were weighed and frozen at -20°C. Soil samples (6.8 g wet weight) from each jar were also stored at -20°C.

Sample Extraction and Purification Procedure

All the samples were thawed for about 15 min at room temperature. For soil samples (6.8 g per sample), they were weighed into a 30-mL polypropylene centrifuge tube, followed by addition of 10 mL acetonitrile containing 1% of acetic acid. After the mixture was vortexed for 1 min and exposed to ultrasonic vibration for 20 min, anhydrous MgSO₄ (2.0 g) and CH₃COONa (1.0 g) were added. Then the tube was shaken vigorously for 1 min using a vortex mixer, exposed to ultrasonic vibration for 20 min, and centrifuged at 5000 rpm for 3 min. 1.0 mL aliquot of the acetonitrile extracts was transferred into a 2-mL centrifuge tube containing PSA (50 mg), C₁₈ (50 mg), and anhydrous MgSO₄ (100 mg) for cleanup. After shaking and centrifugation, the resulting solution was filtered through a 0.22-μm filter. Then 0.1 mL solution was taken and diluted to 10 mL with acetonitrile for HPLC-dual mass spectrometry (MS/MS) analysis.

For analysis of the earthworms, the samples (2.0 g per sample) were blended with 8 mL of acetonitrile containing 1% of acetic acid. The mixture was homogenized with an Ultra-Turrax T18 homogenizer for 30 s, vortex-mixed for 1 min, exposed to ultrasonic vibration for 20 min, and then anhydrous MgSO₄ (1.6 g) and CH₃COONa (0.8 g) were added. Then the purification procedure was the same as the above soil sample. Finally, 0.05 mL solution was taken and diluted to 1 mL with acetonitrile for HPLC-MS/MS analysis.

Chemical Analysis and Method Validation

HPLC-MS/MS analyses were performed on a TSQ QUANTUM ACCESS MAX triple quadrupole MS and an Accela 600 pump/autosampler HPLC (Thermo Electron, Hopkinson, MA). The system was controlled and data were collected and analyzed by the Thermo Fisher LC Quan software package (v. 2.7). Enantiomers were separated on a Chiralpak IC column (4.6 × 250 mm i.d., Daicel, Japan) with 5 μm particle size. The mobile phase was a mixture of 80% acetonitrile and 20% water with a flow rate of 0.35 mL min⁻¹. Chromatographic separation was conducted at 20°C and the injection volume was 10 μL.

The electrospray ionization (ESI)-MS interface was operated in the positive ion mode with selected reaction monitoring (SRM). The ESI source conditions were as follows: spray voltage, 3200 V; sheath gas pressure, 40 Arb; auxiliary gas pressure, 5 Arb; collision gas (Ar): 1.5 mTorr; vaporizer temperature, 250°C; capillary temperature, 350°C. For furalaxyl, *m/z* 302 > 242 was used for quantification, *m/z* 302 > 95 was used for confirmation, and collision energies were 30 eV and 16 eV, respectively. Racemic furalaxyl was ideally baseline separated and no enantiomerization was observed for furalaxyl under this analytical condition (Fig. 2). The first eluted enantiomer was *S*-form (12.83 min) and the second one was *R*-form (14.11 min). Good linear calibration curves were obtained over the concentration range of 1–500 ng mL⁻¹ for *S*-furalaxyl and *R*-furalaxyl. The calibration curves of matrix-matched standard solutions (1–500 ng mL⁻¹) by adding blank earthworm and soil sample extracts were also obtained. Comparing the slope of solvent standard curve with matrix-matched standard curve, the slope ratios ranged from 0.86–0.98, so there was no significant matrix-induced ion signal suppression / enhancement (SSE).

Recovery of furalaxyl enantiomers was determined in blank soil and earthworm samples by being fortified at different concentration levels (0.01, 0.1, 1.0, and 10 mg kg⁻¹ based on five replicates). The samples were left for 1 h to ensure that the spiked pesticides were evenly distributed

Fig. 2. Simultaneous stereoselective separation of furalaxyl. (a) rac-furalaxyl (b) S-furalaxyl (c) R-furalaxyl.

and then extracted and determined as previously described. The recoveries were calculated by comparing the measured concentrations to the fortified concentrations and the relative standard deviation (RSD) revealed the method precision. The average recoveries for furalaxyl enantiomers ranged between 88 and 97% in soil, between 80 and 95% in earthworm tissue with RSD below 16%. There was no significant matrix effect for furalaxyl enantiomers determination with the HPLC-MS/MS method. In this study, the limit of detection (LOD) and limit of quantitation (LOQ) were estimated to be $0.07 \mu\text{g kg}^{-1}$ and $0.22 \mu\text{g kg}^{-1}$ for each enantiomer in earthworm tissue and soil sample. Compared with the newly prepared solutions, the stability test of furalaxyl enantiomers was also evaluated in the stock solutions and matrices, which were prepared 2 months previously and stored at -20°C . Both enantiomers were stable under the stock solutions and the spiked earthworm and soil samples.

Equilibrium Partitioning Model and Data Analysis

Equilibrium partitioning theory model was introduced to describe the uptake process of furalaxyl enantiomers.^{21–23} The uptake kinetic parameters and the uptake curve were performed by using the following model equation:

$$dC_{\text{Worm}}/dt = k_1 C_{\text{Soil}} - k_2 C_{\text{Worm}} \quad (1)$$

$$C_{\text{Worm}} = \frac{k_1 C_{\text{Soil}}}{k_2} [1 - \exp(-k_2 t)] = K C_{\text{Soil}} [1 - \exp(-k_2 t)] \quad (2)$$

$$K = \frac{k_1}{k_2} \quad (3)$$

Where t was the exposure time, C_{Worm} (mg kg^{-1}) was the concentration of furalaxyl enantiomers in earthworm tissues, C_{Soil} (mg kg^{-1}) was the concentration in soil, k_1 (d^{-1}) denoted the uptake rate constant from soil,

and k_2 (d^{-1}) denoted the rate constant characterizing the sum of elimination, growth dilution, and metabolic transformation of the two enantiomers in the earthworm, K was the constant ratio of k_1 and k_2 .

The bioaccumulation factors (BAF) was used to express the relative bioaccumulation capacities of pesticides in the organisms versus the surrounding environmental and was calculated as:

$$\text{BAF}(\text{kg dry kg}^{-1} \text{ wet weight}) = \frac{C_{\text{worm}}}{C_{\text{soil}}} \quad (4)$$

When the uptake and elimination reached steady state during the accumulation phase (i.e., $t = \infty$),¹³ Eq. (2) may be reduced to:

$$C_{\text{worm}} = K C_{\text{soil}} \quad (5)$$

$$K = \frac{C_{\text{worm}}}{C_{\text{soil}}} \quad (6)$$

when steady state was reached, according to Eqs. (3), (4), and (6):

$$\text{BAF} = K = \frac{k_1}{k_2} \quad (7)$$

The enantiomer fraction (EF) was used as measure the enantioselective behaviors of furalaxyl during the experiment. The EF values range from 0 to 1, with EF = 0.5 representing the racemic mixture. It was defined as:

$$\text{EF} = \frac{(S)}{((S) + (R))} \quad (8)$$

where (S) was the concentration of S-furalaxyl and (R) was that of R-furalaxyl. A paired t -test was carried out to compare the means of the EF values in earthworm and soil samples with EF = 0.5.

RESULTS AND DISCUSSION

Acute Toxicity Assay

The acute toxicity for furalaxyl individual enantiomers and racemate was measured by a filter paper contact test and used earthworms (*Eisenia foetida*) as the test organism. Earthworms take up organic compounds through their skin as well as from feeding on soil particles in gut. Hydrophobic chemicals with a log K_{ow} above 5 mainly were absorbed from the gut contents, and chemicals with a log K_{ow} below 5 mainly were absorbed through the skin in the earthworms.^{11,24} As the log K_{ow} for furalaxyl is 2.70, skin absorption is the main route for furalaxyl in earthworm tissue. So the filter paper contact test is sufficient for assay of the acute toxicity of furalaxyl enantiomers to earthworm.

In the filter paper contact test, the LC_{50} values calculated are shown in Table 1. From these results, the LC_{50} values generally decreased over exposure time, and the calculated LC_{50} values at both 48 and 72 h exposure of R-furalaxyl, rac-furalaxyl, and S-furalaxyl were 2.27, 2.08, and $1.22 \mu\text{g cm}^{-2}$ (48 h); 1.90, 1.54 and $1.00 \mu\text{g cm}^{-2}$ (72 h), respectively. These results indicated that the acute toxicity of furalaxyl enantiomers was enantioselective to earthworm, and R-furalaxyl was the least toxic compared with racemate and S-form. The order of toxicity potency at both 48 and 72 h was S-form racemate > R-form. However, for benalaxyl and metalaxyl, which have a similar structure with furalaxyl, the fungicidally active R-benalaxyl was more toxic to earthworm than S-benalaxyl, and rac-metalaxyl exhibited more than two times the toxicity than R-metalaxyl.^{14,24} After 48 h of exposure, the calculated LC_{50} values of R-benalaxyl, rac-benalaxyl and S-benalaxyl were 4.99, 5.08, and $6.66 \mu\text{g cm}^{-2}$, and the LC_{50} values of R-metalaxyl and rac-metalaxyl were 52 and $22 \mu\text{g cm}^{-2}$, respectively.^{14,24} Thus, the order of acute toxicity potency of

TABLE 1. Calculated LC₅₀ values for enantiomers of furalaxyl

Chemicals	Exposure time (48 h)			Exposure time (72 h)		
	LC ₅₀ (μg cm ⁻²)	95% Confidence intervals	R ²	LC ₅₀ (μg cm ⁻²)	95% Confidence intervals	R ²
<i>R</i> -furalaxyl	2.27	1.87-2.74	0.965	1.90	1.54-2.19	0.933
<i>rac</i> -furalaxyl	2.08	1.72-2.40	0.959	1.54	1.06-1.82	0.960
<i>S</i> -furalaxyl	1.22	0.55-1.76	0.952	1.00	0.69-1.23	0.954

R² represents the correlation coefficient.

acylanilide fungicides to worms was *S*-furalaxyl > *rac*-furalaxyl > *R*-furalaxyl > *R*-benalaxyl > *rac*-benalaxyl > *S*-benalaxyl > *rac*-metalaxyl > *R*-metalaxyl. The change of acyl side chains in acylanilide fungicides may result in the chemicals' toxicology difference, and furanylcarbonyl toxicity potency may be higher than phenylacetyl and methoxyacetyl.

Bioaccumulation and Elimination in Earthworm

The concentrations of the two furalaxyl enantiomers in earthworm tissue and soil were determined. The concentrations in soil samples appeared to decrease 20–30% after 31 days exposure at different concentrations (Fig. 3). The chemicals' degradation and biotransformation may be the main reasons for the loss of soil concentrations. Residue of the main metabolite, furalaxyl acid, was lower in soil. The concentrations of furalaxyl enantiomers were almost the same in spiked soil (20 and 50 mg kg⁻¹) and the EF values were 0.5 at the beginning and remained so with almost no change during the course of the experiment ($P > 0.050$).

The concentrations of furalaxyl individual enantiomers were observed in earthworm tissue at the same sample point during the bioaccumulation period. The concentrations of *S*-form were higher than that of *R*-form in spiked soils (Fig. 3).

The EF values in earthworm tissue were observed to deviate from 0.50 and maintained a range of 0.55–0.60, as shown in Figure 4, and these deviations were significant ($P < 0.001$). The results implied that the bioaccumulation of furalaxyl in earthworm tissue was enantioselective, with preferential accumulation of *S*-enantiomer. The possible metabolite of furalaxyl acid was not found in any dose of earthworm tissue. According to Figure 3a,b, a higher rate of application of furalaxyl resulted in a higher concentration in earthworm tissue, and the concentration of both enantiomers reached the highest levels on the 2–3rd day. After a short-time decrease, the concentrations increased gradually and obtained the maximum values again on the 10th day. Thereafter, the concentrations in earthworm tissue declined and reached steady state as the duration of exposure increased. The peak-shaped accumulation curves were observed in the bioaccumulation of furalaxyl by earthworm (Fig. 3). According to Xu et al.,²⁴ peak-shaped accumulation curves happened while organic compound bioavailability decreased. Although the exact causes are not known yet, sorption and desorption from soil organic matter, or pesticides biodegradation and excretion may all play important roles in the peak-shaped accumulation patterns in our study.^{25–27}

Fig. 3. Average concentrations of *S*-furalaxyl and *R*-furalaxyl in soil and earthworm.

Fig. 4. Enantiomeric fraction (EF) of furalaxyl residues in soil and earthworm.

In the bioaccumulation phase, the concentrations in earthworm tissue showed peak-shaped curves around 2 to 10 d for furalaxyl enantiomers (Fig. 3). At the initial stage, the concentrations of furalaxyl enantiomers in soil were high, and the earthworms responded to these in availability with a high uptake rate. After the uptake of organic pollutants by earthworm increased rapidly in the body at first, it increased slowly or even decreased (Fig. 3), then increased rapidly to reach the highest level. This process was governed by an increase of sorption and slow desorption furalaxyl from soil organic matter, biotransformation, or a decreasing concentration in the soil, or elimination or metabolic transformation of compounds from earthworm induced by enzymes. At the last bioaccumulation stage, the concentrations of furalaxyl enantiomers in earthworm reached steady state, and the uptake-elimination rate approached a steady state smoothly at a body residue consistent with the expected basis of equilibrium. This conclusion was consistent with previous studies.^{24,27} The appearance of peak-shaped accumulation curves in our experiment was affected by the change of bioavailability in earthworms and the chemicals' transportation and fate in the soil.

The calculated BAFs according to Eq. (4) of two enantiomers are shown in Figure 5. The values of BAFs at the low

Fig. 5. Calculated bioaccumulation factors (BAF) for the two enantiomers of furalaxyl by sampling day at different spiked concentration.

concentration group were no different from that at a high concentration level. The BAF values of S-form were larger than that of R-form ($P < 0.001$), indicating that the S-form was preferentially accumulated over the R-form in earthworm tissue. It can be concluded that the accumulated concentration of furalaxyl in earthworm tissue via single skin exposure and gut absorption was enantioselective. The BAF values of furalaxyl were smaller than benalaxyl and larger than metalaxyl. The results should relate to the chemicals hydrophobicity and lipophilicity, since it was already confirmed that the chemicals hydrophobicity and lipophilicity can affect the chemicals transformation and bioaccumulation in earthworm tissue.^{12,28} Although both enantiomers showed low accumulation efficiency in earthworms, furalaxyl was dissipated with DT_{50} 31-65 d in soil²⁹ and was relatively persistent in the environment and earthworms. Therefore, the result of furalaxyl bioaccumulation in earthworms could help provide data for evaluating the environmental risk. Earthworms are widely distributed in topsoil and consumed by predator organisms and so furalaxyl enantiomers may have trophic transfer and/or bioaccumulation and/or harmful effects on organisms through food chains.

In this work, we also investigated whether furalaxyl enantiomers in earthworm tissue were caused by enantiomerization or not by exposing earthworm to the soil contaminated with enantiopure R-furalaxyl and S-furalaxyl, respectively. The results (Fig. 3c,d) showed that furalaxyl was configurationally stable in earthworm tissue and soil during a period of 31 d, showing no interconversion of R- to S-enantiomers, and vice versa. We have reported that¹⁷ benalaxyl was enantiomerization and enantioselective bioaccumulated in *Tenebrio molitor* larvae from wheat bran and the rate of enantiomerization of R-benalaxyl was higher than that of S-benalaxyl. So furalaxyl enantiomers may have enantiomerization in other organisms or higher animals, and it is important to utilize enantiopure enantiomers of chiral pesticides for more precise risk assessment in biological systems.

Kinetic Analysis

We tested if the temporal course of furalaxyl uptake into earthworms from the spiked soil can be described by the equilibrium partitioning model. By fitting the average concentrations in earthworm and time into Eq. (2), a nonlinear regression fitting technique provided by SPSS 16.0 (Chicago, IL), and the regression results are shown in Table 2.

The trends of S-furalaxyl and R-furalaxyl fitted the first-order kinetics under different exposure concentration groups ($r = 0.797-0.877$, $0.819-0.858$, respectively). The results indicated that furalaxyl enantiomers in earthworm tissue were proportional to the concentration in soil, and the accumulation from soil was an equilibrium partition process.³⁰ The rate constants were calculated applying the estimated values obtained from the regression results. The approximations of the rate constants for S-furalaxyl were $0.605-0.934$ (k_1), $1.227-2.079$ (k_2), and $0.449-0.493$ (K), and the rate constants for R-furalaxyl were $0.500-0.893$ (k_1), $1.347-2.372$ (k_2), and $0.342-0.377$ (K). The uptake rate (k_1) of furalaxyl was much lower than the rate of elimination and metabolism (k_2) in earthworm tissue and this estimate was in accord with the low accumulation efficiency (BAF) we have discussed. The K values (Table 2) of furalaxyl enantiomers according to Eq. (2) were consistent with the BAF for earthworms (Fig. 5) when earthworm bioaccumulation reached steady state. A

TABLE 2. Calculated kinetic parameter of furalaxyl enantiomers

Exposure group	Enantiomers	<i>K</i>	<i>k</i> ₁	<i>k</i> ₂	<i>r</i>
50 mg kg ⁻¹ <i>rac</i> -furalaxyl	<i>S</i> -furalaxyl	0.449 ± 0.026	0.934 ± 0.286	2.079 ± 0.676	0.797
	<i>R</i> -furalaxyl	0.377 ± 0.019	0.893 ± 0.238	2.372 ± 0.668	0.819
20 mg kg ⁻¹ <i>rac</i> -furalaxyl	<i>S</i> -furalaxyl	0.493 ± 0.034	0.605 ± 0.192	1.227 ± 0.419	0.846
	<i>R</i> -furalaxyl	0.342 ± 0.017	0.623 ± 0.159	1.820 ± 0.494	0.858
10 mg kg ⁻¹ <i>S</i> -furalaxyl	<i>S</i> -furalaxyl	0.466 ± 0.026	0.635 ± 0.171	1.363 ± 0.394	0.877
10 mg kg ⁻¹ <i>R</i> -furalaxyl	<i>R</i> -furalaxyl	0.371 ± 0.025	0.500 ± 0.162	1.347 ± 0.470	0.844

paired *t*-test showed that there was a significant difference in the *K* values between the two furalaxyl enantiomers (*P* = 0.045). The results also indicated that the accumulation, degradation, and elimination of furalaxyl in earthworm tissue were enantioselective.

CONCLUSION

In this study, the acute toxicity and bioaccumulation of furalaxyl enantiomers to earthworm were assayed. The results of acute toxicity showed that *S*-furalaxyl was the more toxic compared with the racemate and *R*-furalaxyl to the non-target organism, earthworm. The concentrations of two furalaxyl enantiomers in soil and earthworm were also determined and there was a significant trend of enantioselective bioaccumulation in the earthworm with a preferential accumulation of *S*-furalaxyl at four levels of exposure. Thus, we advise that *R*-furalaxyl with fungicidal activity higher than the *S*-enantiomer replace *rac*-furalaxyl for a more sustainable pesticide use for environmental safety and public health.

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