Enantioselective bioaccumulation and metabolization of diniconazole in earthworms (*Eiseniafetida*) in an artificial soil

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ARTICLE INFO

Article history:

Received 8 May 2013

Received in revised form

12 October 2013

Accepted 16 October 2013

Available online 6 November 2013

Keywords:

Diniconazole

Earthworm

Bioaccumulation

Metabolization

Enantioselectivity

ABSTRACT

Degradation and enantioselective bioaccumulation of diniconazole in earthworms (*Eiseniafetida*) in artificial soil was investigated using liquid chromatography–tandem mass spectrometry (LC–MS/MS) method under laboratory condition. Three exposure concentrations (1 mg/kg, 10 mg/kg and 25 mg/kg) of diniconazole in soil (dry weight) to earthworms were used. The uptake kinetics fitted the first-order kinetics well. The bioaccumulation factors (BAF) of R, S isomers were 6.6046 and 8.5115 in 25 mg/kg dose exposure, 2.6409 and 2.9835 in 10 mg/kg dose exposure, 1.7784 and 2.0437 in 1 mg/kg dose exposure, respectively. Bioaccumulation of diniconazole in earthworm tissues was enantioselective with a preferential accumulation of S-diniconazole and the enantiomer fractions were about 0.45–0.50 in all three level dose exposures. In addition, it was obvious that both R-diniconazole and S-diniconazole had bioaccumulation effect in earthworm. Diniconazole was metabolized to 1,2,4-triazole, (E)-3-(1H-1,2,4-triazol-1-yl) acrylaldehyde, (E, S)-4-(2, 4-dichlorophenyl)-2, 2-dimethyl-5-(1H-1,2,4-triazol-1-yl)pent-4-ene-1,3-diol, and (E)-4-(2, 4-dichlorophenyl)-3-hydroxy-2,2-dimethyl-5-(1H-1,2,4-triazol-1-yl) pent-4-enoic acid in earthworms; the metabolites of 1,2,4-triazole and (E)-3-(1H-1,2,4-triazol-1-yl)acrylaldehyde could be detected in soil as well.

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1. Introduction

Earthworm was a kind of creature living in topsoil, which was usually exposed to a variety of agricultural chemicals. Thus, earthworm was recognized as effective indicators for soil pollution. They were excellent models for evaluating the bioaccumulative potential of commercial chemicals (Armitage and Gobas, 2007; Connell and Markwell, 1990). US Environmental Protection Agency (EPA), Ministry of Agriculture (China) and Organization for Economic Cooperation and Development (OECD) had used earthworms (*Eiseniafetida*) as model organisms for assessing the potential biological effect of the chemicals released into the environment. Enantioselective toxicity and bioaccumulation of pesticides in earthworms had got considerable attention in recent years (Chojnacka, 2010). The enantioselective bio-accumulation of Alpha-cypermethrin (Diao et al., 2011; Ma et al., 2012), fipronil (Liu et al., 2012), metalaxyl (Xu et al., 2011), and benalaxyl (Xu et al., 2009) were found in earthworm.

Diniconazole[(E)-(RS)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pent-1-en-3-ol] was a systemic chiral fungicide with two enantiomers as shown in Scheme 1. It was used to

control fungi particularly ascomycetes and basidiomycetes. This fungicide has been widely used in China for many years. The properties data from Pesticide Properties Data Base (PPDB) displayed that it was hydrophobic with log K_{OW} 4.3, indicating a great bioaccumulation possibility (Kelly et al., 2007). What was more serious was that the accumulation would cause severe hepatotoxicity and acute renal failure (Koc et al., 2012). It was found that R-(–)-diniconazole strongly inhibited α -sterol demethylase demethylation (P-450 DM), while S isomer was a weaker inhibitor (Yoshida et al., 1986). The biological properties of diniconazole enantiomers were significantly different in later investigations (Furuta and Doi, 1994; Wang et al., 2009). We also found the bioaccumulation of diniconazole in *Tenebrio molitor* Larvae was enantioselective with preferential accumulation of S-enantiomer in previous work (Liu et al., 2013). Hence, the future study on enantioselective bioaccumulation of diniconazole in different organisms and food web was important and interesting.

High performance liquid chromatography (HPLC) was the most important techniques (Jiang et al., 2001; Ye et al., 2009) in chiral analysis. In the recently years, HPLC coupled with tandem mass (HPLC-MS) has been introduced in enantiomers analysis for the advantages in complex bio-sample matrix analysis (Li et al., 2012). In this study, reversed phase chiral LC–MS/MS was developed to investigate the enantioselective degradation and accumulation of diniconazole in earthworm (*Eiseniafetida*) in an artificial soil under laboratory condition.

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Scheme 1. Chemical structures of two enantiomers from diniconazole.

2. Materials and methods

2.1. Chemicals and reagents

Diniconazole standard (purity > 99.5%) was purchased from Dikma (Beijing, China). Diniconazole technical material was obtained from Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA). Standard solution of diniconazole was prepared in acetonitrile at 1000 mg/L and kept in darkness at 4 °C.

Methanol, acetonitrile, cyclohexane and ethyl acetate were HPLC grade, and were purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Dikma (Beijing, China). Milli-Q quality water (Millipore, Bedford, MA) was used during the whole analysis. All the other chemicals were of analytical reagent grade from Beijing Chemical Factory Co (Beijing, China).

2.2. Earthworms and soil

Earthworms (*Eiseniafetida*) bred in a wooden breeding box containing a mixture of soil and cattle manure. The earthworms were healthy and active before introduced to the experiment.

Since natural soils from a particular source may not be available throughout the year, and indigenous organisms as well as the presence of micro-pollutants can influence the test, an artificial soil was used in this study. The artificial soil was made up by 10% of Sphagnum-peat, 70% of industrial quartz sand and 20% kaolinite clay. The dry constituents of the soil are mixed thoroughly one week before starting this experiment. The dry constituents of the artificial soil were stored at room temperature until use.

Adult earthworms with clitellum of similar weight (250–600 mg), from the same source were separated from the acclimatization chamber. The earthworms were rinsed with water, and excess water was removed from the earthworms by gently blotting them dry on moist filter paper using soft steel forceps. The earthworms were weighed to the nearest 1 mg and were distributed randomly to pre-weighed test vessels; each test vessel typically contained one earthworm.

2.3. Experimental arrangements

During the period of experiment, replicated groups of earthworms were exposed to soil which had been spiked with diniconazole. In addition to the test animals, groups of control earthworms were held under identical conditions without diniconazole.

The soils were spiked with diniconazole for three concentrations: 1, 10 and 25 mg/kg (dry weight). 500 g of dry soil was spiked with 100 mL racemic solutions of 5 mg/L, 50 mg/L and 125 mg/L diniconazole respectively and mixed thoroughly. After solvent evaporation, adjust the moisture content to 40–60% of maximum water holding capacity by adding deionized water. Well treated soil was put into a 1 L glass breaker and incubated for four days to reach equilibrium. 48 replicates were conducted for each concentration, respectively. Three controls were prepared. Deionized water was added to moisturize the soil constituents. Ten earthworms with similar weight (0.3–0.6 g) were transferred into each breaker. The entire studies were carried out under a controlled 16/8 h light/dark cycle in man-made climate equipment. The temperature was set at 20 ± 2 °C. Add food ration and control soil moisture by re-weighing the test vessels and compensate evaporated water every seven days. Three replicates of soil and earthworms samples were taken after 0.25, 1, 2, 4, 7, 11, 14, and 21 days.

The sampled earthworms were rinsed by deionized water and placed on moist filter paper to depurate most of their gut contents for about 1 h. Then, earthworms were dried with clear filter paper, and then both earthworms and soil were weighted for future analysis.

2.4. Sample cleanup

Soil (1 g) or homogenized earthworms (1 g) were weighted into a breaker flask. These samples were extracted by twenty milliliter acetonitrile, and the mixtures were exposed to ultrasonic for ten minutes, then centrifuged at 3000 r/min for five minutes. The organic layer filtered through anhydrous sodium sulfate (10 g) and evaporated to dryness on a vacuumed rotary at 30 °C. Dissolve the residuary by 10 mL mixture of cyclohexane and ethyl acetate (50:50, V/V) for further cleanup.

The cleanup procedure was taken out on an AccuPrep MPS & AccuVap Inline Gel Permeation Chromatography (GPC) cleanup system (J2 SCIENTIFIC, Columbia, USA) coupled with a CO785 cleanup column. Mobile phase were cyclohexane and ethyl acetate (50:50, V/V), and the flow rate was set at 4.7 mL/min. The cleanup method was set to three fractions. The first fraction was eluted between 0 and 7.5 min, which was discarded. The second fraction eluted between 7 min and 20 min was collected for further concentration. The third fraction eluted between 20 min and 22 min was used to clean system.

The AccuVap Inline concentrates the GPC collect fraction(s) as it elutes from the column. At the endpoint, the dryness portion was dissolved in 2 mL. The samples were then diluted 10 to 100 times for the injection of HPLC-MS/MS to reduce the matrix effect.

2.5. Analysis conditions

LC was performed on a Waters Acquity UPLC system (Waters Corp., Mildford, MA) equipped with a column of Chiralcel-OD-3R (Daicel, Shanghai, China), a binary solvent manager system and a sampler manager. The separation was carried out isocratically using solvent A (0.05% formic acid in ultrapure water) and solvent B (acetonitrile) in a 40:60 V/V ratio and 0.25 mL/min flow rate for 35 min. The column was kept at 30 °C and the temperature in the sample manager was kept at 10 °C. Mass spectrometry was performed on a Waters Quattro Premier XE Mass Spectrometer (Waters, Milford, MA, USA). The desolvation gas (nitrogen) flow was set to 600 L/h at a temperature of 350 °C; the cone gas (nitrogen) was set to 50 L/h, and the electrospray ionization (ESI) source temperature to 120 °C. The capillary and cone voltages were set to 1.7 kV and 30 V, respectively. The Masslynx NT v.4.1 (Waters) software was used to control the instrument, and analyze the data obtained. The Electrospray ionization (ESI) source was operated in positive. MS analyses were performed in the multiple reaction monitoring (MRM) modes, and the cone voltage was set at 35 V. Transitions m/z 326.17 > 70.19 and m/z 326.17 > 159.13 were used for quantification and confirmation when the collision energies (CE) were set at 24 eV and 36 eV, respectively.

2.6. Calculation

The degradation of chemicals in soil was commonly obeyed the first order equation (Guo et al., 2010).

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

where C_0 and C are the concentration at times zero and t respectively, and k is the rate constant.

$T_{1/2}$ presents the time taken for 50% transformation of an isomer when the transformation described by first-order kinetics

$$T_{1/2} = \frac{\ln(2)}{k} \quad (2)$$

The enantiomer fraction (EF) (Harner et al., 1999; Diao et al., 2011,) was used to evaluate the enantioselectivity of the bioaccumulation of enantiomers in soil and

earthworm tissue. EF was expressed as follows:

$$EF = \frac{C_R}{C_S + C_R} \quad (3)$$

where C_S was the concentration of S enantiomer, and C_R was the concentration of R enantiomer.

Kinetic calculations were based on the concentrations of diniconazole in earthworm and soil, respectively. The determination of kinetic parameters of uptake, and of the uptake curve was performed using the following model equation:

$$C_a = \frac{k_s}{k_e} \times C_s \times (1 - e^{-k_e t}) (0 < t < t_c) \quad (4)$$

where C_a was the concentration of diniconazole in earthworm, and C_s was the concentration of diniconazole in the soil, k_e was the elimination rate constant, k_s was the uptake rate constant, and t_c was the time in days at the end of the uptake phase.

When steady state was reached during the uptake phase (i.e. $t = \infty$), Eq. (4) may be reduced to

$$C_a = \frac{k_s}{k_e} \times C_s \quad (5)$$

The uptake curve of the test fungicide was obtained by plotting the average bioaccumulation factor (BAF) for each specific sampling date during the uptake phase against time on arithmetic scales. The BAF was calculated from

$$BAF = \frac{C_a}{C_s} \quad (6)$$

The kinetic bioaccumulation factor (BAF_k) was defined as the ratio of the soil uptake rate constant k_s and the elimination rate constant k_e

$$BAF_k = k_s/k_e \quad (7)$$

when steady state was reached, according to Eq. (5)

$$BAF_k = \frac{C_a}{C_s} = \frac{k_s}{k_e} \quad (8)$$

According Eqs. (5)–(8), (4) was transferred to

$$BAF = BAF_k \times (1 - e^{-k_e t}) (0 < t < t_c) \quad (9)$$

The uptake rate constant k_s were then calculated given k_e and BAF_k which was derived from the uptake curve

$$k_s = BAF_k \times k_e \quad (10)$$

3. Results and discussion

3.1. Method validation

According to Qiu's et al. (2011), the first peak eluted was S-(–)-diniconazole and the second was R-(+)-diniconazole as shown in Fig. 1. The peak was it also wrote to be S-enantiomers. In this study, an analytical method was developed to quantify R-diniconazole and S-diniconazole. Samples were quantified using external standards, with six standards of varying concentrations between 0.005 mg/kg and 2.0 mg/kg for calibration curves (S-isomer: $y = 868222x - 358.81$, $r^2 = 0.99999$, R-isomer: $y = 864978x - 945.23$, $r^2 = 0.99998$). The limit of detection (LOD) was 0.001 mg/kg at a signal-to-noise (S/N) ratio of 3. The limit of quantification (LOQ) was established at 0.003 mg/kg, which yielded a signal-to-noise ratio of 10. The typical chromatogram

Fig. 1. Optical detection of two enantiomers of diniconazole. Flow rate=0.25 mL/min, 0.05% formic acid in ultrapure water: acetonitrile=40:60 (V/V). The flow orders were S-diniconazole and R-diniconazole.

was shown in Fig. 1. R-diniconazole and S-diniconazole was baseline separated, and no endogenous interference peaks eluted at retention times 18.48 min (S-diniconazole) and 19.66 min (R-diniconazole). Recoveries were determined by spiking racemic mixture in blank sample at three fortification levels (0.01 mg/kg, 0.1 mg/kg and 2.0 mg/kg). The mean recoveries (five replicates each level) were in the range of 94.3–103.5% in soil, and 88.6–98.7% in earthworm. The relative standard deviations (RSDs) were ranged from 1.2 to 4.6% in soil, and 2.1–4.9% in earthworm.

3.2. Enantioselective degradation in soil

Concentrations and EFs of R-diniconazole and S-diniconazole in soil during the period of 21 days of incubation were calculated by equation (Balcioğlu et al., 2005) and (2). The results were shown in Table 1, Figs. 2 and 3. The sum of two enantiomers decreased about 6.77%, 6.82%, and 9.14% at 1 mg/kg, 10 mg/kg and 25 mg/kg dose, respectively, at the end of 21 days exposure. The decrease may result from the degradation and biotransformation (Bending et al., 2007).

The EFs (Fig. 3) increased a little in three dosage treatment soil during the experiment. Although the fitting according first order equation might be of large error since that less than 10% diniconazole degraded in soil, the half-lives of R-diniconazole and S-diniconazole were still calculated to evaluate the enantioselective difference in soil. The half-life ($T_{1/2}$) of R-diniconazole in soil, according Table 1, was ranged 198.61 days to 210.04 days, and it was 141.17 days to 172.85 days for S-diniconazole. It proved the enantioselective degradation of diniconazole in soil and R-diniconazole enantiomer was more persistent than S-diniconazole in three dosages treatments in soil.

3.3. Enantioselective metabolites and bioaccumulation in earthworms

3.3.1. Enantioselective metabolites in earthworms

We checked the metabolites in soil samples and earthworm samples, four main metabolites were found in the study. The chromatography and the ESI mass spectrums of the metabolites were showed in Fig. 4. Compound II and Compound III were found both in soil and earthworm. The two compounds were found until the second day in soil, while they were found after 6 h for the first time in earthworm. The compound IV and compound V were detected in earthworm only.

Compound I had the same retention times with standard diniconazole sample. The ESI mass spectrum exhibited the molecular ions at $m/z = 326.39$, 328.36 and 330.36 , meanwhile the abundance ratio was about 9:6:1. Therefore, it could be inferred the compound contained two chlorine atoms. All information indicated that compound I was diniconazole.

The retention time of compound II was at 6.78 min. The ESI mass spectrum of compound II exhibited a molecular ion at $m/z = 69.02$ in a positive ion mode, which was equal to the molecular weight of 1,2,4-triazole. Hence, the compound II was inferred to be the metabolite by breaking of the chemical bond between 10# and 11# atom of diniconazole.

Table 1

The transformation equation and half-life of diniconazole isomers in soil.

Dose (mg/kg dw)	S-diniconazole	$T_{1/2}$	R-diniconazole	$T_{1/2}$
1	$C_S = 0.6952 e^{-0.00470t}$	147.47	$C_R = 0.68814 e^{-0.00347t}$	203.87
10	$C_S = 6.81569 e^{-0.00491t}$	141.17	$C_R = 6.81264 e^{-0.00349t}$	198.61
25	$C_S = 16.24691 e^{-0.00401t}$	172.85	$C_R = 15.79056 e^{-0.00330t}$	210.04

Fig. 2. Concentration of diniconazole enantiomers in soil samples and earthworm samples. (A) Was 1 mg/kg treatments, (B) was 10 mg/kg treatments, (C) was 25 mg/kg d_{wt} treatments.

The retention time of compound III was at 7.15 min. The ESI mass spectrum of compound III exhibited a molecular ion at $m/z=123.64$ in a positive ion mode. It can be inferred that the compound did not contain chlorine isotope from Fig. 4. Hence, the compound out to be come from the part which did not contain chlorine atoms by breaking of the bond between 3# and 8# atom of diniconazole. But the compound III was 57 aum lighter than the part without chlorine atoms, which means a loss of t-butyl. According to the analysis of above, the compound III was inferred to be (E)-3-(1H-1,2,4-triazol-1-yl) acrylaldehyde.

The retention time of compound IV was 10.61 min. The ESI spectrum of the compound IV ($m/z=342.38, 344.33, 346.33$) showed that it was 16 aum weight than diniconazole. Hence, it ought to be the oxidation product of diniconazole. The fragment $-C_4H_9O$ ($m/z=73$) was very clear in spectrum of compound IV.

Fig. 3. The EFs of diniconazole enantiomers in soil samples and earthworm samples. (A) Was 1 mg/kg treatments; (B) was 10 mg/kg treatments, (C) was 25 mg/kg treatments.

This indicated that the oxidation reaction occurred on the 17#, 20#, and 21# atom of diniconazole. Considering that if the oxidation reaction occurred on the t-butyl group, the chiral center of diniconazole did not change, the compound would have two isomers. But, from this study, the compound had four peaks (Fig. 4), which implied that they were E,S isomers besides R,S-isomers. So, the compound IV ($m/z=342.38, 344.33, 346.33$) might be (E,S)-4-(2, 4-dichlorophenyl)-2,2-dimethyl-5-(1H-1,2,4-triazol-1-yl)pent-4-ene-1,3-diol (Isobe et al., 1991).

The retention time of compound V was 9.17 min and 10.99 min. The ESI spectrum of the compound V ($m/z=356.34, 358.33, 360.303$) showed that it was 16 aum weight than compound IV.

Fig. 4. The chromatography and mass spectra of diniconazole and metabolites in earthworm.

And the extraction chromatography of compound V showed that it had two peaks. Though, it was inferred that the compound V was the oxidation product of compound IV. The abundance of fragment ($m/z=271.33$), which was came from the loss of hydroxyl group and 1,2,4-triazole group of compound V. Hence, it was confirmed that compound V might be (E)-4-(2,4-dichlorophenyl)-3-hydroxy-2, 2-dimethyl-5-(1H-1,2,4-triazol-1-yl) pent-4-enoic acid.

The metabolism path way of diniconazole was showed in Fig. 5. Two kinds of metabolism mechanism were found in the progress, qthe breakdown and oxidation metabolism mechanism. Diniconazole was metabolized to 1,2,4-triazole, (E)-3-(1H-1,2,4-triazol-1-yl) acrylaldehyde, (E,S)-4-(2,4-dichlorophenyl)-2,2-dimethyl-5-(1H-1,2,4-triazol-1-yl) pent-4-ene-1,3-diol, and (E)-4-(2,4-dichlorophenyl)-3-hydroxy-2,2-dimethyl-5-(1H-1,2,4-triazol-1-yl)pent-4-enoic acid.

3.3.2. Enantioselective bioaccumulation in earthworms

According to Kelly's et al. (2007) study, the chemicals with a log K_{OW} between 2 and 5 showed a bioaccumulation potential. Since log K_{OW} of diniconazole is 4.3, the bioaccumulation of the pesticide in earthworm should be evaluated with the bioaccumulation factor (BAF).

Concentrations of diniconazole enantiomers in exposed earthworm were shown in Fig. 3A–C. The concentration of diniconazole increased staidly in earthworms, while they were exposed to the diniconazole spiked soil. The concentration in earthworms was even higher than that in soil at the first sampling after 6 h exposure in the 10 mg/kg exposure section. The concentration of diniconazole isomers accessed the soil concentration after 7 days in the three levels. The two enantiomers of the 1 mg/kg dosage treatment rapidly increased to 0.9026 mg/kg for R-diniconazole and 0.9389 mg/kg for S-diniconazole in the initial six hours, then slowly increased to 1.233 mg/kg for R-diniconazole and 1.500 mg/kg for S-diniconazole in the remaining experiment period; The two enantiomers of 10 mg/kg dosage treatment increased to 10.255 mg/kg for R-diniconazole and 10.574 mg/kg for S-diniconazole in the first six hours, and reached steady state at 17.649 mg/kg for R-diniconazole and 18.831 mg/kg for S-diniconazole after 14 days; The two enantiomers of the 25 mg/kg dose treatment increased to 15.195 mg/kg for R-diniconazole and 14.841 mg/kg for S-diniconazole in the first six hours, and reached at 80.237 mg/kg for R-diniconazole and 91.895 mg/kg for S-diniconazole in the end of incubation.

By fitting the BAF and time into Eq. (9), a nonlinear dynamic fitting gave the estimated value of BAF_k and k_e . k_s was calculated with

Fig. 5. The metabolize path way of diniconazole in earthworm. (I) diniconazole, (Tsukatani et al. (2008) 1H-1,2,4-triazole, (III) (E)-3-(1H-1,2,4-triazol-1-yl)acrylaldehyde, (IV) (E)-4-(2,4-dichlorophenyl)-2,2-dimethyl-5-(1H-1,2,4-triazol-1-yl)pent-4-ene-1,3-diol, (V) (E)-4-(2,4-dichlorophenyl)-3-hydroxy-2,2-dimethyl-5-(1H-1,2,4-triazol-1-yl)pent-4-enoic acid.

Table 2

Calculated uptake rate coefficient (k_s), elimination rate coefficient (k_e), BAF_k of diniconazole enantiomers in 1 mg/kg, 10 mg/kg and 25 mg/kg dose exposure.

Dose (mg/kg dw)	Exposuregroup	BAF_k	k_e	k_s	R^2
1	S-diniconazole	2.0437	0.5026	1.0272	0.1565
1	R-diniconazole	1.7784	0.8102	1.4409	0.2149
10	S-diniconazole	2.9835	0.2153	0.6423	0.6335
10	R-diniconazole	2.6409	0.2490	0.6576	0.5334
25	S-diniconazole	8.5115	0.0601	0.5112	0.9510
25	R-diniconazole	6.6046	0.0803	0.5302	0.9395

Eq. (10). The result was showed in Table 2. The BAF_k values also varied from the exposed concentration. From the results, the 25 mg/kg treatment had the highest BAF of 8.5115 for S-isomer and 6.6046 for R-isomer. The 1 mg/kg treatment had the lowest BAF_k of 2.0437 for S-isomer and 1.7784 for R-isomer. The BAF_k s were in direct proportion to the exposure concentration. The results could be explained from the k_e and k_s . In the 1 mg/kg exposure treatment, the k_s were 0.2062 for S-isomer and 0.1313 for R-isomer; meanwhile the k_e were 1.9862 for S-isomer and 1.1382 for R-isomer in 25 mg/kg exposure treatment. This indicated that the uptake rate coefficient of diniconazole enantiomers were in direct proportion to the exposure concentration. However, k_e was in inverse proportion to the exposure concentration.

In 1 mg/kg exposure treatment, the elimination ratio was very high, which result in the lower of the BAF_k . Many factors like

dermal elimination, respiratory outflow, metabolism, growth and urine (Gobas and Gobas, 2003) may effect this result. In the 10 mg/kg and 25 mg/kg exposure treatment, dermal elimination may be the dominant factor of elimination; the effect of the other factors can be ignored. In the 1 mg/kg exposure treatment, the dermal elimination may be not the dominant factor; the effect of the other factors must be considered. Hence, the 1 mg/kg exposure treatment took more time to reach the steady state.

The enantioselective bioaccumulations in earthworms were also observed in the experiment. The uptake rate coefficients (k_s) of S-diniconazole isomer were all high than the R-diniconazole isomer. And the elimination rate coefficient (k_e) of R-diniconazole isomer were high than the S-hexaconazole isomer. The change of concentration EFs were showed in Fig. 4. In the end of 21 days exposure, the concentrations of S-isomer and R-isomer were obviously different, and the EFs were ranged from 0.45 to 0.50.

4. Conclusions

The results of this study indicated that diniconazole enantiomers were very persistent in soil. The sum of R-diniconazole and S-diniconazole decreased below 10% of spiked in the 21days experiment duration. The S-diniconazole degraded slightly fast than the R-diniconazole in the soil. The kinetics of diniconazole enantiomers fitted the first-order kinetics in the bioaccumulation

of diniconazole in earthworm tissues. Both R-diniconazole and S-diniconazole had bioaccumulation effect ($BAF_k > 1$) in this experiment. The BAF_k was in direct proportion to the exposure concentration. And the accumulation was enantioselective with a preferential accumulation of S-diniconazole. The different metabolites of diniconazole in soil were obtained.

Acknowledgments

This work was supported by the National High Technology Research and Development Program (863) of China (No. 2012AA06A302) and National Natural Science Foundation of China (No. 21277163).

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