The Enantioselective Pharmacokinetics Metabolism of Diniconazole in Quail (Coturnix coturnix japonica)

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ABSTRACT The pharmacokinetics of diniconazole enantiomers in quail (Coturnix coturnix japonica) were investigated by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Quails were exposed to racemic diniconazole in capsule by oral at dose of 10 mg/kg (body weight). The maximal concentrations observed in blood, heart, liver, and kidney were 3.18, 11.35, 12.32, 15.03 μg/g for S-diniconazole, and 1.13, 3.70, 6.00, 2.60 μg/g for R-diniconazole. The elimination of enantiomers all met the one-compartment model in blood, heart, liver, and kidney well. The elimination half-lives (T1/2) of S-diniconazole were 2.87, 3.85, 5.29, and 4.42 h in blood, heart, liver, and kidney, respectively; the T1/2 of R-diniconazole were 2.44, 3.42, 146.23, and 74.02 h in blood, heart, liver, and kidney, respectively. The enantiomer fractions (EFs) steadily increased from 0.50 to 0.92 in blood samples and 0.91 in heart samples. Meanwhile, the values increased to 0.70 and 0.80 in liver and kidney initially, and then decreased to 0.33 and 0.44 at the end of the experiment. Metabolism was examined as well and it was found that diniconazole was metabolized to 1, 2, 4-triazole, (E)-3-(1H-1, 2, 4-triazol-1-yl) acrylaldehyde, (E, S)-(R, S)-4-(2, 4-dichlorophenyl)-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-ene-1, 3-diol, (E)-4-(2, 4-dichlorophenyl)-3-hydroxy-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl)pent-4-enolic acid, and 1, 3-dichlorobenzen in all samples of quail.

KEY WORDS: diniconazole; quail; pharmacokinetics; metabolism; enantioselectivity

INTRODUCTION

The Japanese quail (Coturnix coturnix japonica) is a species of Old World quail found in East Asia. It is a migratory species, breeding in Manchuria, southeastern Siberia, northern Japan, and the Korean Peninsula, and wintering in the south of Japan and southern China. Because its lifespan is relatively short and its physiology is comparable to that of human, the adult quail is often used in aging and disease investigations. Furthermore, it has also been introduced into the risk assessment of pesticides, biocides, and chemicals. Because of good practices in teratogenicity and toxicity studies, countries and organizations, such as the Ministry of Agriculture (China), the EPA (Environmental Protection Agency), and the Organization for Economic Cooperation and Development (OECD), have used Coturnix coturnix japonica to assess the potential biological effects of the chemicals if released into the environment.

Diniconazole is a systemic fungicide used for the control of fungi, particularly ascomycetes and basidiomycetes. It has been widely used for years. Like most triazoles, diniconazole is a systemic fungicide with two enantiomers as shown in Figure 1. The enantiomers are known to be similar in physicochemical properties, but differ significantly in their biological properties. The R-isomer is more fungitoxic than the S-isomer,11 Furthermore, S-diniconazole decreased faster than the R-diniconazole, and enantiomer fractions (EFs) increased with time after administration of racemic diniconazole in rabbit blood.12 Another study reported that diniconazole causes severe hepatotoxicity and acute renal failure.13 But which enantiomers caused the hepatotoxicity and acute renal failure has not been studied. Thus, it was necessary to investigate the pharmacokinetics and metabolism of diniconazole enantiomers.

In recently years, high-performance liquid chromatography (HPLC) was used mostly in chiral analysis. For the advantage of sensitive detection and less matrix interference, HPLC coupled with tandem mass spectroscopy (MS/MS) has been introduced for determining the enantiomers. In the present work, an effective method using LC-MS/MS was developed for diniconazole enantiomers analysis in a bio-sample matrix. The pharmacokinetics of diniconazole enantiomers in Coturnix coturnix japonica blood and organs were investigated. The enantioselective elimination and metabolism were examined as well.

MATERIALS AND METHODS

Chemicals and Reagents

Diniconazole standard (purity ≥99.5%) was purchased from Dikma (Lake Forest, CA). Standard solutions of diniconazole were prepared in acetonitrile at 1000 mg/L and kept in darkness at 4°C. Stock solutions of diniconazole standard were prepared in methanol at 100 mg/L and kept in darkness at 4°C.

Methanol, acetonitrile, cyclohexane, and ethyl acetate were of HPLC grade, 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 (Beijing, China).

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 Coturnix coturnix japonica Exposure

This study was designed to assess the enantioselective metabolism of diniconazole in quail (Coturnix coturnix japonica). The quails (1 month old) were bred in the ecotoxicological laboratory, Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), Beijing, China. The birds were weighed individually shortly before the experiment began and were allocated to the groups by a randomization plan on the basis of body weight. The birds were marked individually by numbered leg brands. The quails were offered a commercial diet throughout maintenance before the study and during the experiment. Relative humidity was 30%–75%, temperature 18°C–22°C and illumination on an 8/16 light/dark cycle under natural intensity. One week before dosing, to allow acclimatization, the birds were selected by body weight about 90–110 g and assigned to individual cages with 10 birds (8 birds as the control); each cage contained a drinker and food hopper.

The birds received a single dose of diniconazole in capsule by oral. Birds were assigned to a control group (not treated) and groups exposed to racemic diniconazole at a dose of 10 mg/kg according to body weight. The observation period lasted 24 hours. The birds were sacrificed at 1, 2, 3.5, 4.5, 5.5, 7.5, 12, 15, 18, 24 hours after treatment. Blood, heart, liver, and kidney samples were weighed into a breaker and food hopper.

Sample Preparation

One-gram samples of blood or one gram of homogenized heart, liver, or kidney samples were weighed into a breaker flask. The sample with 10 g anhydrous sodium sulfate was extracted with 20 mL acetonitrile, and the mixtures were exposed to ultrasonic treatment for 20 minutes and homogenized with an IKA T25 homogenizer (Germany) at 13,000 r/min, then centrifuged at 3000 r/min for 5 minutes. The organic layer was filtered through anhydrous sodium sulfate (10 g) and evaporated to dryness in a rotary vacuum at 30°C. The residue was dissolved in a 10-mL mixture of cyclohexane and ethyl acetate (50:50, V/V) for further cleanup.

The cleanup procedure was performed with an AccuPrep MPS & AccuVap Inline Gel Permeation Chromatography (GPC) cleanup system (2 Scientific, Columbia, MO) coupled with a CO785 cleanup column (Bio-beads S-X3). Mobile phases 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 set to 0–7.5 min and the eluent was discarded. The second fraction was set to 7–20 min; the eluent was collected for further concentration. The third fraction was set to 20–22 min, which was for the cleaning system.

AccuVap Inline concentrated the GPC collection fraction as it was eluted from the column. At the endpoint, the dryness portion was dissolved in 2 mL acetonitrile for analysis.

Analysis Conditions

LC was performed on a Waters Acquity UPLC system (Waters, Milford, MA) equipped with a column of Chiralcel OD-3R (Daicel, Japan), a binary solvent manager system, and a sampler manager. The separation was carried out isocratically using solvent A (0.05% formal acid in ultrapure water) and solvent B (acetonitrile) in a 40:60 V/V ratio at 0.25 mL/min flow rate for 25 min. The column was kept at 30°C, and the sample manager was set to 10°C. Mass spectrometry was performed on a Waters Quattro Premier XE Mass Spectrometer. 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 3 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 ESI source was operated in positive mode. 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. For identification of the structure of metabolic products of diniconazole, the UPLC-MS/MS instrument was operated in full-scan and daughter-scan mode. Mass range was set at 50 to 450 Da. The daughter-scan model was used to analyze the structure of the parent ion.

RESULTS AND DISCUSSION

Method Development

For this study, analytical methods were developed for quantitative analysis of R-diniconazole and S-diniconazole. Samples were quantified using external standards, with six standards of varying concentrations between 0.005 mg/L and 2.0 mg/L for calibration curves (S-diniconazole: y = 868222.7x - 358.8, r² = 0.99999; R-diniconazole: y = 864978.5x - 945.2, r² = 0.99999). The limit of detection18 was 0.001 mg/kg at a signal-to-noise ratio (S/N) of 3. The limit of quantification (LOQ) was established at 0.003 mg/kg, which yielded an S/N of 10. A typical chromatogram is shown in Figure 2, 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 a racemic mixture in control samples at three fortification levels (0.01 mg/kg, 0.1 mg/kg, and 2.0 mg/kg). The mean recoveries of diniconazole enantomers (five replicates at each level) were in the range of 92.1%–102.3% in heart, liver, kidney, and blood. The relative standard deviations (RSDs) ranged from 1.4% to 4.3%.

Diniconazole Enantiomers in Coturnix coturnix japonica

Figure 3 shows the concentration of diniconazole enantiomers in blood, heart, liver, and kidney versus time profiles exposed for 24 h. After a single oral administration of racemic diniconazole, the concentration of diniconazole in blood reached a maximal concentration of 3.18 μg/g for S-enantiomer, and 1.13 μg/g for R-enantiomer at 2 h. Then, the concentration of S-enantiomer and R-enantiomer in blood declined rapidly and approached 0.17 and 0.32 μg/g at 24 h after administration. The concentrations in heart reached the maximal concentration of 11.35 μg/g for S-enantiomer, and 5.5 μg/g for R-enantiomer. The concentration of S-enantiomer and R-enantiomer in liver and kidney samples were below the LOQ throughout the experiment.

Fig. 1. Chemical structures of two enantiomers from diniconazole.

Fig. 2. Optical detection of two enantiomers of diniconazole. Flow rate = 0.25 mL/min, 0.05% formal acid in ultrapure water: acetonitrile = 40:60 (V/V). The flow orders were S-diniconazole and R-diniconazole.

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and 3.70 μg/g for R-enantiomer after 4.5 h, and then the concentrations declined steadily postexposure.

Remarkably higher concentrations of diniconazole enantiomers than in blood were measured in liver and kidney. The concentrations of S-enantiomer in liver reached a maximal concentration of 12.32 μg/g at 7.5 h and then declined steadily postexposure. The concentrations of R-enantiomers reached a steady-state condition after 7.5 h, and kept around 6.00 μg/g for the remaining observation time. The concentrations of S-enantiomer in kidney reached the maximum of 15.03 μg/g at 4.5 h, and then declined steadily. For the R-enantiomers, the concentrations reached a steady-state condition of 2.60 μg/g at 2.5 h, and stayed for the remaining observation time.

This result was very important. If the accumulation of diniconazole in liver and kidney caused severe hepatotoxicity and acute renal failure, then it could be inferred that the concentration of S-diniconazole was much higher than R-diniconazole. When the differences of virulence were little, S-diniconazole was the main cause of hepatotoxicity and acute renal failure.

**Pharmacokinetics of Diniconazole Enantiomers**

The concentration–time curves were fitted by a one-compartment model:

\[ C_t = \frac{A e^{k_a t}}{V (k_e - k_a)} \]  

Where \( C_t \) is the concentration in heart, liver, kidney, and blood at time t, \( F \) is the bioavailability fraction, \( D \) is the dose; \( V \) is the volume of distribution; \( k_e \) is the elimination rate constant, and \( k_a \) the absorption rate constant.

\[ A = \frac{F D k_a}{V (k_e - k_a)} \]  

The half-life (\( T_{1/2} \)) is calculated by equation (3).

\[ T_{1/2} = \frac{\ln 2}{k_e} \]  

The results are shown in Table 1. The \( k_a \) values were all greater than the \( k_e \), which implied that the absorption rate were greater than the elimination rate. For the enantiomers, the \( k_a \) values of S-diniconazole in blood, heart, and kidney were greater than that of R-diniconazole, obviously. Moreover, the \( k_a \) values of S-diniconazole in liver were almost equal to the values of R-diniconazole. Great differences of the elimination rate of diniconazole enantiomers were observed in liver and kidney. The \( k_e \) of S-diniconazole in liver was 28 times as that of R-diniconazole. The difference between the two enantiomers in kidney was not so great as that in liver, but also exceeded 16 times. In the blood and heart samples the \( k_e \) of the two enantiomers were almost equal.

The elimination half-lives of S-enantiomer were 3.85, 5.29, 4.42, and 2.87 h in heart, liver, kidney, and blood. The data of R-enantiomer were 3.42, 146.23, 74.02, and 2.44 h in heart, liver, kidney, and blood. The elimination half-lives

<table>
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<tr>
<th>Enantiomers</th>
<th>Parameter</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Blood</th>
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<tr>
<td>S-</td>
<td>( k_a )</td>
<td>0.52</td>
<td>0.34</td>
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<td></td>
<td>( A )</td>
<td>36.24</td>
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<tr>
<td>diniconazole</td>
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<td>0.18</td>
<td>0.13</td>
<td>0.16</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>( T_{1/2} ) (h)</td>
<td>3.85</td>
<td>5.29</td>
<td>4.42</td>
<td>2.87</td>
</tr>
<tr>
<td>R-</td>
<td>( k_a )</td>
<td>0.68</td>
<td>0.30</td>
<td>0.91</td>
<td>0.68</td>
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<tr>
<td></td>
<td>( A )</td>
<td>6.07</td>
<td>6.34</td>
<td>2.82</td>
<td>4.03</td>
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<tr>
<td></td>
<td>( k_e )</td>
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<td>0.0047</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>( T_{1/2b} ) (h)</td>
<td>3.42</td>
<td>146.23</td>
<td>74.02</td>
<td>2.44</td>
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</table>

**Fig. 3.** Concentrations of diniconazole enantiomers in heart, kidney, liver and blood samples. (--) represent S-diniconazole, (---) represent R-diniconazole.
of S-enantiomer were a little longer than R-enantiomer in heart and blood. Contrarily, the half-lives of R-enantiomer in liver and kidney were far longer than S-enantiomer, which exceeded 3 days. This means the R-enantiomer would cause harm in the long run.

**EF Variety in Quail**

The enantiomer fraction (EF)\(^{20}\) was used as a measure of the enantioselectivity in animal kinetics of the two isomers in the study. The EFs of two enantiomers in heart, liver, kidney, and blood during a period of 24 h were calculated by equation (4):

\[
EF = \frac{C_s}{C_s + C_R}
\]

(4)

Where \(C_s\) is the concentration of S-enantiomer, and \(C_R\) is the concentration of R-enantiomer.

The EFs (Fig. 4) increased steadily in heart, liver, kidney, and blood samples in the first hours, and then the differences evened up. The EFs kept increasing in heart and blood...
samples, while they began to decrease in liver and kidney samples. At end of the experiment, the EFs reached 0.91 in heart samples and 0.92 in blood samples. Meanwhile, the EFs in liver samples and kidney sample were 0.33 and 0.44, respectively.

It was clear that the enantioselectivity existed in all the processes of absorption, distribution metabolism, and elimination. The absorption rate of S-enantiomer was greater than that of R-enantiomer in the distribution phase. The elimination rate of S-enantiomer was greater than that of R-enantiomer in the elimination phase in liver and kidney. The elimination rate of S-enantiomer was greater than that of R-enantiomer in the elimination phase in heart and blood.

Metabolites of Diniconazole in Quail

The metabolites of diniconazole in heart, kidney, liver, and blood were identified by mass spectrometry. There were four main metabolites found in all four samples. The chromatography and the ESI mass spectrums are shown in Figure 5.

Compound I was diniconazole. The retention times were at 18.46 min (S-diniconazole) and 19.68 min (R-diniconazole). The ESI mass spectrum exhibited molecular ions at m/z = 326.39, 328.36 and 330.36, and the abundance ratio was about 9:6:1. This is characteristic of two chlorine atoms in diniconazole.

Compound II was inferred to be 1, 2, 4-triazole. The retention time was 6.78 min. The mass spectrum 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, compound II was inferred to be the metabolite by breaking of the chemical bond between #10 and #11 atoms of diniconazole. This compound could be found in all the samples of liver, kidney, heart, and blood. The concentration declined by time in kidney, heart, and blood. However, the concentration increased first in liver, and reached the highest at the 7.5 h, then declined to a steady state (Fig. 6A).

Compound III was inferred to be (E)-3-(1H-1, 2, 4-triazol-1-yl) acrylaldehyde. The retention time was 7.15 min. The mass spectrum exhibited a molecular ion at m/z = 123.64 in a positive mode. It could be inferred that the compound did not contain the chlorine isotope from Figure 5. Hence, the compound ought to be the part of diniconazole which did not contain chlorine atoms (breaking the bond between #3 and #8 atoms). But compound III was 57 amu lighter than the part without chlorine atoms, which means a loss of t-butyl. According to the analysis above, compound III was inferred to be (E)-3-(1H-1, 2, 4-triazol-1-yl) acrylaldehyde. This compound could be found in liver and kidney, heart and blood. The concentration declined with time (Fig. 6B).

Compound IV was (E)-(R, S)-4-(2, 4-dichlorophenyl)-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-ene-1, 3-diol. The retention time was 10.61 min. The mass spectrum (m/z = 342.38, 344.33, 346.33) showed that it was 16 amu weight than diniconazole. Hence, it ought to be the oxidation product of both diniconazole enantiomers. The fragment of -C4H9O (m/z = 73) was very clear in the spectrum of compound IV. This indicated that the oxidation reaction occurred on the #17, #20, and #21 atoms of diniconazole enantiomers. 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, as shown in Figure 5, which implied that there existed (S)-(R, S)-isomers besides (E)-(R, S)-isomers. So compound IV might be (E, S)-(R, S)-4-(2, 4-dichlorophenyl)-2, 2-dimethyl-5-
(1H-1, 2, 4-triazol-1-yl)pent-4-ene-1, 3-diol. This compound was found in all the samples of liver, kidney, heart, and blood. The sum of the concentrations of compound IV enantiomers increased first and reached the highest at 15 h, then declined (Fig. 6C). Thus, this compound was an important intermediate in the progress of metabolism. The ratios of the enantiomers were different in the four kinds of samples, and were different at different times (Table 2).

Compound V was (E)-4-(2, 4-dichlorophenyl)-3-hydroxy-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-enoic acid. The retention time was 9.17 and 10.99 min. The mass spectrum (m/z = 356.34, 358.33, 360.303) showed that it was 16 aum weight than compound IV. The extraction chromatography of compound V showed that it had two peaks (the ratios were V-1:V-2 = 5:1 in liver at 3.5 h) as shown in Figure 5. It was inferred that compound V was the oxidation product of compound IV. But, if compound V was from compound IV, there would be four enantiomers: where were the other two enantiomers? Considering the steric hindrance effect of the structure, the S-isomer was hard to form, and the S-isomer of the compound was very limited, so only the E-isomer of compound V was found in the study. The abundance of fragment (m/z = 271.33) 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. This compound could be found in the samples of liver, kidney, heart, and blood. The ratios of the enantiomers were similar in the four kinds of samples at the same time, and the enantiomer fractions were steady at 5:1 from the 1st hour to the 24th hour in the experiment. The sum concentration of enantiomers increased first, and then declined (Fig. 6D). Thus, like compound IV, this compound was also an important intermediate in the progress of metabolism.

From the above results, there was a problem that if compound I broke to compound III, where has the other part gone? With this confusion, we examined the extraction of samples by a GC/MS system (Trace DSQ, Thermo, Pittsburgh, PA). We found that there was a compound (Compound VI) with fragments 146, 148, 150, 111, and 113 (Fig. 7), which was not determined in the LC-MS/MS spectrum. By searching in the National Institute of Standards and Technology (NIST) database, it was confirmed to be 1, 3-dichlorobenzen. This compound was only found in liver and kidney samples after 3.5 h.

The metabolism pathways of diniconazole are deduced in Figure 8. Two kinds of metabolism mechanisms were found in the progress, the breakdown and oxidation metabolism mechanism. Diniconazole was metabolized to 1, 2, 4-triazole, (E)-3-(1H-1,2,4-triazol-1-yl) acrylaldehyde, (E, R)-(R, S)-4-(2, 4-dichlorophenyl)-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-enoic acid, (E)-4-(2, 4-dichlorophenyl)-3-hydroxy-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl)pent-4-enoic acid and 1, 3-dichlorobenzen.

CONCLUSIONS

In this study, a GPC cleanup following a chiral LC-MS-MS method was developed for the analysis of diniconazole enantiomers in quail. The method was applied to the study of the enantioselective pharmacokinetics and metabolism of diniconazole in quail. The results showed that the maximal concentrations observed in blood, heart, liver, and kidney were 3.18, 11.35, 12.32, 15.03 μg/g for S-enantiomer, and 1.13, 3.70, 6.00, 2.60 μg/g for R-enantiomer. The elimination half-lives of S-enantiomer were 2.87, 3.85, 5.29, and 4.42 h in blood, heart, liver, and kidney samples after 3.5 h.
blood, heart, liver, and kidney; the half-lives of Renantionti were 2.44, 3.42, 146.23, and 74.02 h in blood, heart, liver, and kidney. The EFs steadily increased to 0.92 in blood samples and 0.91 in heart samples from 0.50. Meanwhile, the values increased to 0.70 and 0.80 in liver and kidney first, and then decreased to 0.33 and 0.44. The metabolic products were identified by mass spectrometry. The R-diniconazole was eliminated faster than the S-diniconazole in heart and blood. However the S-diniconazole was eliminated faster than the R-diniconazole in liver and kidney. The pesticide was extensively metabolized in the liver and kidney. It was found that diniconazole was metabolized to 1, 2, 4-triazole, (E)-3-(1H-1,2,4-triazol-1-yl) acrylaldehyde, (E, S)-(R, S)-4-(2, 4-dichlorophenyl)-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-ene-1, 3-diol, (E)-4-(2, 4-dichlorophenyl)-3-hydroxy-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-enoic acid and 1, 3-dichlorobenzenes in all samples of blood, heart, liver, and kidney.

Diniconazole can alter normal thyroid hormone levels in humans, but the toxicological significance in humans is extremely low and it is unlikely that diniconazole would increase thyroid tumor in humans.22 But as mentioned, diniconazole would cause severe hepatotoxicity and acute renal failure.13 From this study it was shown that S-diniconazole accumulated very fast and sharply in liver and kidney. So it would be the main factor of diniconazole poisoning. Considering that the S-diniconazole did not display strong fungitoxicity, the R-diniconazole ought to replace the racemic diniconazole in the market to reduce the risk of diniconazole poisoning. Considering in the long run that R-diniconazole is a little persistent in quail, which would cause accumulation in the food chain, using R-diniconazole must be strictly controlled.

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