Stereoselective Degradation Kinetics of Tebuconazole in Rabbits

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ABSTRACT Tebuconazole\[(RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol\] is a potent triazole fungicide and consists of a pair of enantiomers. The enantioselective degradation kinetics of tebuconazole was investigated in rabbits by intravenous (iv) injection. The concentrations of \((-)-(R)-\)tebuconazole and \((+)-(S)-\)tebuconazole in plasma and tissues were determined by HPLC with a cellulose tris(3,5-dimethylphenylcarbamate)-based chiral stationary phase. Enantioselective analysis methods for this fungicide in plasma and tissues were developed and validated. Good linearities were obtained over the concentration range of 0.25–25 mg/l for both enantiomers. The degradation followed pseudo-first-order kinetics and the degradation of the \((+)-(S)-\)tebuconazole was much faster than that of the \((-)-(R)-\)tebuconazole in plasma after administration of racemic tebuconazole. This study also indicated that environmental assessment of enantiomeric degradation may be needed to fully evaluate risks of tebuconazole use. Chirality 19.141–147, 2007. VV 2006 Wiley-Liss, Inc.

KEY WORDS: enantioselective; chiral analysis; degradation kinetics; tebuconazole; rabbit

INTRODUCTION

A large number of organic agrochemicals are chiral molecules and consist of mixtures of stereoisomers. The biological and physiological properties of the enantiomers are often different, also the biological transformation of chiral compounds can be stereoselective, and the uptake, metabolism, excretion of enantiomers thus may be very different.\(^1,2\) Therefore, the enantiomeric composition of chiral compounds may be changed in these processes. Investigations that treat racemates as though they were single entities that can produce inaccurate and misleading results.\(^3,4\) So chiral analysis is required for a full understanding of the biological behavior of such compounds.\(^5\) Chiral separation methods that are highly efficient and stereoselective are badly needed to detect the various enantiomeric pesticides in environmental matrices and to understand the enantiomeric discrimination in environment.\(^6\) Information from stereoselective analysis of chiral pesticides will help to improve our understanding of the pesticide’s safety to humans, animals, and the environment.

Tebuconazole\[(RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol\] is used for disease control on fruit, nut, cereal, and vegetable crops worldwide. Tebuconazole is a systemic fungicide that acts by inhibiting the synthesis of ergosterol to prevent fungal mycelium development, tebuconazole has a stereogenic center in the alcohol moiety (Fig. 1) and consists of a pair of enantiomers. Rial-Otero et al.\(^7,8\) studied residual levels and degradation rates of tebuconazole in lettuce plants grown in a greenhouse under agricultural conditions typical of northwestern Spain and described a sensitive method for the simultaneous quantification of tebuconazole and other commonly used grapevine fungicides in vineyard soils. Garland et al.\(^9\) used a benchtop quadrupole mass spectrometer in the selected ion monitoring (SIM) mode and a high-resolution mass spectrometer in the SIM mode coupled with gas chromatography to quantify both tebuconazole and propiconazole in boronia leaf material and oil. Timothy et al.\(^10\) conducted a 63-day laboratory incubation to evaluate tebuconazole’s dissipation kinetics and impact on soil microbial activity in Tifton loamy sand. But enantioselective degradation have not been reported yet. Thus, this research was conducted to determine the stereoselectivity of two tebuconazole enantiomers in rabbits. The main purpose of this study, was to better understand the stereoselective biodegradation of tebuconazole isomers in animals. The results may have some implication for the environmental and ecological risks assessment for chiral pesticides.

MATERIALS AND METHODS

Chemicals and Reagents

rac-tebuconazole (\(>99\%\) purity) was provided by the China Ministry of Agriculture’s Institute for Control of...
Agrochemicals. (−)-(R)-tebuconazole and (+)-(S)-tebuconazole were prepared on an Agilent HPLC with a preparative chiral column (CDMPC-CSP, provided by the Department of Applied Chemistry, China Agricultural University, Beijing). Water was purified by a Milli-Q system. Ethyl acetate, n-hexane, and 2-propanol (HPLC grade) were from Fisher Scientific (Fair Lawn, NJ), and ethanol, ethyl acetate (analytical grade) was from VAS (Beijing, China).

Racemic tebuconazole was prepared in ethanol. The solutions were stored in capped test tubes at −20°C.

**HPLC-DAD Analysis**

Chromatography was performed using an Agilent 1100 series HPLC (Agilent Technology, U.S.A.) equipped with a G1311A pump, G1322A degasser, G1328A injector, a 20-U l aliquot was injected into the HPLC. Enantiomers were separated on CDMPC-CSP (provided by the Department of Applied Chemistry, China Agricultural University, Beijing) using a mobile phase of hexane by the Department of Applied Chemistry, China Agricultural University, Beijing) using a mobile phase of hexane and a modifier of 2-propanol with a flow rate of 1.0 ml/ min. The CSP was prepared according to the procedure described in the literature.\(^\text{11,12}\) CSP was packed into a 250 mm × 4.6 mm (i.d.) stainless steel column. Chromatographic separation was conducted at 20°C and DAD detection at 220 nm.

**Sample Preparations**

Analysis of tebuconazole was performed using the methods as follows: (1 ml) of the rabbit plasma or 1 g of homogenized tissue matrix was weighed into a 50 ml polypropylene centrifuge tube. About 10 ml of Ethyl acetate was added, and the sample was vortexed for 5 min. After centrifugation at 4000 rpm for 5 min, the clear solution was decanted into a test tube. The extraction and centrifuge steps were repeated with another 10 ml of ethyl acetate. The organic phase was combined and dried over anhydrous Na\(_2\)SO\(_4\) (2 g) then transferred to a 50-ml round-bottom flask and concentrated to dryness at 40°C in a rotary evaporator. The resulting residue was redissolved in 0.25 ml 2-propanol, and this solution was vortexed, a 20-μl aliquot was injected into the HPLC.

**Calibration Curves and Assay Validation**

Plasma (1 ml) obtained from untreated rabbits was spiked with working standard rac-tebuconazole solutions to generate calibration samples ranging from 0.25 to 25 μg/ml for both (−)- and (+)-tebuconazole. Calibration samples were prepared as described earlier. Calibration curves were generated by plotting the concentration of each enantiomer in the spiked solutions versus the peak area of each enantiomer. Linear regression analysis was performed using Microsoft Excel. The precision and accuracy of the method were obtained by comparing the predicted concentration (obtained from the calibration curve) to the found concentration of each enantiomer spiked in blank plasma. The standard deviation (SD) and the coefficient of variation (CV = SD/mean) were calculated over the entire calibration range. The within-day precision was determined in six replicates at concentration of 0.5, 7.5, and 15 μg/ml on the same day. The between-day precision was evaluated in six replicates at the earlier concentrations on six different days. The recoveries of each enantiomer of tebuconazole from plasma and tissue samples were determined analyzing quality control samples at three different concentrations. The peak-area ratios of six extracted samples at each concentration were compared with those of six injections of standard solutions to derive a percent recovery. The limit of quantification (LOQ) was defined as the lowest concentration in the calibration curve with acceptable precision and accuracy (The acceptance criteria for the LOQ were that the precision and accuracy for extracted samples were under 20% variability).

**Degradation Studies**

Male Japanese white rabbits weighing 2–2.25 kg (provided by the Experimental Animal Research Institute of China Agriculture University) were housed under a 12-h light/dark cycle at 22°C. Twelve hours before experiments, the rabbits were fasted, but had free access to water. Racemic tebuconazole was dissolved in ethanol and administered at 30 mg/kg body weight (bd wt) by iv injection in the ear vein. Blood samples were collected in heparinized tubes at 0 (blank), 5, 15, 30, 60, 120, 240, and 480 min after treatment, and one time point corresponds to one animal. Four milliliter of blood was centrifuged at 4000 rpm for 5 min, and the plasma was transferred to a new tube. The heart, kidney, liver, lung, fat, muscle, spleen, and brain of each rabbit were excised and weighed separately. Plasma and tissue samples were stored at −20°C for later analysis.

![Chemical structures of (+)-(S)-tebuconazole and (−)-(R)-Tebuconazole.](image-url)
Fig. 2. Representative HPLC chromatograms of (A) extract from untreated rabbit plasma, (B) extract from untreated rabbit plasma spiked with rac-tebuconazole (10 μg/ml), (C) extract from a plasma sample collected from a rabbit 15 min, (D) extract from a liver sample collected from a rabbit 30 min, (E) extract from a kidney sample collected from a rabbit 15 min, (F) extract from a fat sample collected from a rabbit 120 min after iv treatment with rac-tebuconazole at 30 mg/kg bd wt, [n-hexane/2-propanol = 85:15 (plasma), 92:8 (tissues), flow rate = 1.0 ml/min].
The enantiomer fraction (EF) was used as a measure of the enantioselectivity of the tebuconazole enantiomers in rabbits. EF is defined by eq. 2.

\[
EF = \frac{\text{peak area of } (-)}{\text{peak area of } (+)} \tag{2}
\]

A racemic EF = 0.500, whereas preferential degradation of the (-) or (+) yields EF < 0.500 and > 0.500, respectively. The peak area corresponding with the limit of detection will be used if the enantiomer is not found.

### Degradation Kinetics Analysis

The degradation of both tebuconazole enantiomers appeared to follow a pseudo-first-order kinetic reaction, and the degradation rate constants were derived from "ln(C0/C) versus t" plots by regression analysis for experiment (Excel 2000, Microsoft). The 5 min point was the maximum value of the concentration. The half-life \( T_{1/2} \) of \( \frac{C_0}{2} \) was estimated from eq. 1.

\[
T_{1/2} = \frac{0.693}{k} \tag{1}
\]

The enantiomer fraction (EF) was used as a measure of the enantioselectivity of the tebuconazole enantiomers in rabbits. 

### Kinetic Degradation in Plasma

Plasma concentration-time curves of (-)-(R)- and (+)-(S)-tebuconazole after iv treatment of rabbits with rac-tebuconazole at 30 mg/kg bd wt are shown in (Fig. 3A). The (+)-(S)-tebuconazole degraded faster than (-)-(R)-enantiomer. The EFs not only exceeded 0.50, but also increased with time in the plasma (Fig. 3B). The \( T_{1/2} \) of (-)-(R)-enantiomer and (+)-(S)-tebuconazole were 123 and 88 min, respectively.

### Kinetic Degradation in Tissues

Both enantiomers increased initially and reached the maximum at 20–60 min, then decreased with time in the liver, kidney, spleen, heart, brain, fat, and muscle (Fig. 4), but they were the maximum at initial 15min in the lung and decreased with time. (+)-(S)-tebuconazole degraded faster than the (-)-(R)-enantiomer in the liver, kidney, lung, muscle, spleen, and fat but slower in heart and brain. The (-)-(R)-enantiomer concentrations were in the following order at 240 min: fat > liver > brain > liver > spleen > kidney > muscle. For the (+)-(S)-enantiomer, the distribution pattern was the following: fat > liver > brain > liver > spleen > heart > kidney > muscle. The higher EFs were measured in plasma, fat, and heart. These results indicated there was substantial stereoselectivity on degradation of tebuconazole enantiomers in rabbits.

### RESULTS

#### Chiroptical Detection

The optical pure enantiomer was detected on automatic polarimeter WZZ-1S(made in China SPOIF), on CDMPC chiral stationary phase the levorotatory enantiomer in the n-hexane/2-propanol mobile phase elutes first. So the first eluted enantiomer was confirmed as (-)-tebuconazole, while the second one was (+)-tebuconazole in our study.

#### Assay Validation

There were no endogenous interference peaks eluted at retention times equal to (-)-(11 min) and (+)-tebuconazole (13 min) in blank plasma and in tissue samples the retention times were changed into (-)-(19 min) and (+)-tebuconazole (25 min). Representative HPLC chromatograms of extracts from untreated rabbit plasma spiked with rac-tebuconazole (10 μg/ml), treated plasma, liver, kidney, and fat sample are shown in Figure 2. As shown, (-) and (+)-tebuconazole were baseline separated.

### Discussion

In previous reports, the racemate of tebuconazole yielded a good resolution on a CDMPC-CSP by normal-
TABLE 2. Summary of method recovery data for both Tebuconazole enantiomers from fortified rabbit plasma and some tissues (n = 6)*

<table>
<thead>
<tr>
<th>Matrix</th>
<th>rac-Tebuconazole fortification</th>
<th>Recovery</th>
<th>(-)-Tebuconazole</th>
<th>(+)-Tebuconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>30 (μg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>85.22 ± 12.78</td>
<td>85.83 ± 12.88</td>
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<tr>
<td></td>
<td>1</td>
<td>91.64 ± 6.87</td>
<td>92.31 ± 6.92</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>100 (μg/g)</td>
<td>86.42 ± 2.27</td>
<td>86.07 ± 2.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>86.26 ± 2.04</td>
<td>90.41 ± 5.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100.57 ± 11.61</td>
<td>110.80 ± 11.12</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>100</td>
<td>83.14 ± 5.85</td>
<td>82.86 ± 5.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>81.74 ± 5.15</td>
<td>80.86 ± 5.25</td>
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<td>10</td>
<td>90.48 ± 7.88</td>
<td>90.91 ± 7.86</td>
<td></td>
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<tr>
<td>Kidney</td>
<td>100</td>
<td>86.18 ± 5.31</td>
<td>87.53 ± 4.09</td>
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<tr>
<td></td>
<td>50</td>
<td>84.14 ± 6.85</td>
<td>84.66 ± 5.95</td>
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<td>1</td>
<td>88.73 ± 12.29</td>
<td>82.33 ± 11.72</td>
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<td>Heart</td>
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<td>88.99 ± 2.78</td>
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<td>5</td>
<td>99.07 ± 4.55</td>
<td>98.33 ± 4.76</td>
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</tr>
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<td>1</td>
<td>88.01 ± 5.74</td>
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<tr>
<td>Spleen</td>
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<td>81.35 ± 7.59</td>
<td>80.69 ± 7.66</td>
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<tr>
<td></td>
<td>10</td>
<td>80.43 ± 3.88</td>
<td>81.44 ± 3.95</td>
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<td>1</td>
<td>84.38 ± 11.12</td>
<td>86.15 ± 12.55</td>
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<tr>
<td>Fat</td>
<td>60</td>
<td>98.32 ± 3.85</td>
<td>96.84 ± 3.99</td>
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<tr>
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<td>5</td>
<td>82.45 ± 8.92</td>
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<td>Muscle</td>
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<td>90.35 ± 5.85</td>
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<td>85.26 ± 3.75</td>
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<tr>
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<td>0.5</td>
<td>91.14 ± 8.54</td>
<td>90.78 ± 7.73</td>
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</table>

*Values represent the means ± SD.

phase HPLC. The selectivity factor increased when the percentage of 2-propanol in the mobile phase (2-propanol + n-hexane) decreased. In the present work, the ratio of the mobile phase was changed when separated enantiomers mixture of tebuconazole in plasma and tissue in order to avoid the endogenous interference. To confirm the elution order of the two enantiomers on CDMPC-CSP, the optical pure enantiomer was prepared on CDMPC-CSP and detected on automatic polarimeter WZZ-1S, the first eluted enantiomer was levorotatory. Shapovalova et al. confirmed the absolute configuration of tebuconazole on Chiracel OD–H column with the same mobile phase, and the CSP of CDMPC is the same to Chiracel OD–H, so the first enantiomer was confirmed as (-)-(R)-tebuconazole, the second was (+)-(S)-tebuconazole in our study.

Many pesticides degrade enantioselectively in the environment. (+)-hexaconazole decreased more rapidly than the (-)-hexaconazole in plasma, liver, and kidney after intravenous (iv) administration of racemic hexaconazole in the rabbit.10 The (+)-(S)-metalaxyl levels in plasma, liver, and kidney decreased more rapidly than the (-)-(R)-metalaxyl after iv administration of racemic metalaxyl in the rabbit. Wang et al.18 reported that enantioselective degradation and chiral conversion of θ-cypermethrin (TCYM) in rats via iv injection. The degradation of the (+)-TCYM was much faster than that of the (-)-TCYM in plasma, heart, liver, kidney, and fat after administration of racemic TCYM. In the present study, we found that an acute administration of rac-tebuconazole resulted in stereoselective disposition of the (-)-and (+)-enantiomers of tebuconazole. Concentration of the (+)-enantiomer in plasma decreased more rapidly than that of the (-)-enantiomer. This finding was evidenced by the plasma EF values, which increased with time.

Plasma protein binding may contribute to these differences. Stereoselective plasma protein binding seems likely in the present study because initial levels of the separate enantiomers were different from each other, and chiral conversion of tebuconazole in plasma may also play a role in these differences.

In the present study, stereoselective degradation of rac-tebuconazole enantiomers in some tissues was observed. There were several possible factors involved. The first factor was chiral inversion of the two enantiomers in plasma. The second factor was likely stereoselective distribution of (+)- and (-)-tebuconazole in tissues. The drug was easily stockpiled by the lung (lung first-pass effect).19 In this study, the concentration of tebuconazole was very high in the lung and decreased with the time, when tebuconazole distributed to the other tissues, the stereoselective distribution may be happened.

Fig. 3. Plasma concentration-time curves (A) and EF(B) of tebuconazole enantiomers in rabbits following rac-tebuconazole administration at 30 ng/kg bd wt (□ = (+)-tebuconazole, ◆ = (-)-tebuconazole).

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Stereoselective metabolism and excretion would also result in stereoselective behavior of the chiral chemicals in animals. In this study, the EFs in the liver deviated from 0.5 suggested that the metabolism of the two enantiomers in rabbit liver was stereoselective. In rabbit kidney, the concentration of the (+)-tebuconazole was lower than its antipode and EF of two enantiomers in kidney increased with time. Thus renal excretion of rac-tebuconazole may have been stereoselective, with (+)-tebuconazole excreted more rapidly than its antipode.

The detection of two enantiomers in brain tissue revealed that both enantiomers could penetrate the blood-brain barrier. High enantiomeric ratio of α-hexachlorocyclohexane had been observed in the brains of neonatal fur seals, sheep, harbor seals and double-crested cormorants. In this study, the EF ratio in rabbit brain

Fig. 4. Tissues concentration-time curves of tebuconazole enantiomers in rabbits following rac-tebuconazole administration at 30 mg/kg bd wt (■ = (+)-tebuconazole, ◆ = (−)-tebuconazole).
tissue was similar to that in plasma. The stereoselective disposition of the tebuconazole enantiomers in plasma might be at least part of the explanation.

The high concentration of the two enantiomers in fat suggested the lipophilic nature of tebuconazole.

CONCLUSIONS

A stereoselective analytical method of tebuconazole enantiomers in vivo was built, racemic tebuconazole showed stereoselective degradation kinetics in rabbits. The degradation of the two enantiomers of racemic tebuconazole occurred at different rates.

LITERATURE CITED