Enantioselective Degradation of Indoxacarb in Cabbage and Soil under Field Conditions

DALI SUN,1,3† JING QIU,2† YIJUN WU,1 HONGWU LIANG,2 CHENGLAN LIU3 AND LI LI1,*

1State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
2Institute of Quality Standards and Testing Technology for Agro-Products, Key Laboratory of Agro-product Quality and Safety, Chinese Academy of Agricultural Sciences, Beijing 100081, China
3Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, Laboratory of Insect Toxicology, South China Agricultural University, Guangzhou 510642, China

ABSTRACT The enantioselective degradation of indoxacarb in cabbage and soil has been investigated in Beijing and Anhui under open conditions. Indoxacarb enantiomers in samples were extracted with acetonitrile, cleaned up by florisil SPE column, separated on high performance liquid chromatography with a cellulose-tris-(3, 5-dimethylphenylcarbamate)-based chiral stationary phase (CDMPC-CSP), and determined by a photodiode array detector. The validation of the developed method by fortification rac-indoxacarb in cabbage and soil showed good accuracy and precision. The results of field trials indicated that the dissipation of indoxacarb enantiomers followed pseudo-first-order kinetics or first-order kinetics in cabbage and soil at two locations. The half-lives of two enantiomers in cabbage ranged from 2.8 to 4.6 d which were shorter than those in soil ranging from 23 to 35 d. The changes of enantiomeric fraction values proved that enantioselective degradation of indoxacarb happened in cabbage and soil. The (+)-indoxacarb showed faster degradation in the Beijing cabbage, whereas in the Anhui cabbage, (+)-indoxacarb preferentially degraded. In soil, preferential degradation of (+)-indoxacarb was observed at two locations. Chirality 00:000–000, 2012. © 2012 Wiley Periodicals, Inc.

KEY WORDS: indoxacarb; degradation; enantioselective; cabbage; soil

INTRODUCTION Chiral pesticides currently constitute about 30% of all pesticides used, and the ratio is increasing as compounds with more complex structures are introduced into use. They are primarily consisted of the mixtures of enantiomers or racemates which have identical physical-chemical properties.1,2 Nevertheless, many enantiomers or stereoisomers have different bioactivity, toxicity, metabolism, excretion, and degradation behaviors in the environment.3,4 The biological activity of the chiral pesticides comes from both enantiomers or only one of them (the other one may have little or no activity and toxic effects against nontarget organisms).5 Therefore, the evaluation of chiral pesticide residues in the environment, on the basis of the data obtained by racemates, is not reliable. Enantiomers have been known to selectively interact with biological systems that are usually enantioselective and have drastically different behaviors in the environment.6 Therefore, it is essential and urgent to investigate the enantioselective degradation kinetics of chiral pesticides in organisms or the environment to understand enantiomeric safety.

Indoxacarb, methyl-7-chloro-2,5-dihydro-2-((methoxycarbonyl)(4-(trifluoromethoxy)phenyl)-amino)carbonyl)-indenol,1,2-[3,4]-oxadiazine-4a(3H)-carboxylic acid methyl ester (Fig. 1), is an effective oxadiazine insecticide against a broad spectrum of insect pests.7 It has been chosen as the replacement of the synthetic organophosphate insecticide and used on a range of crops, including fruits, vegetables, soybeans, alfalfa, and peanuts, to control many insects such as Heliothis armigera, Pieris rapae, Plutella xylostella, Prodenia litura, Laphygma exigua Hubner, and so on, but is harmless to numerous nontarget insects.8,9 It works by blocking the sodium channel of the target’s nerve cells and is easily metabolized in the midgut of lepidopteran larvae to the decarbomethoxylated form.10–12 Indoxacarb has a chiral carbon and consists of two enantiomers. The activity of this insecticide is mainly attributed to the (+)-indoxacarb.13 There are mainly three series of products including DPX-JW062, DPX-MP062, and DPX-KN128 composed of the ratio 1:1, 1:3, and 0:1, respectively, of (+)-indoxacarb and (−)-indoxacarb.14

At present, limited methods have been reported on enantioselective separation and residual determination of indoxacarb. Liu investigated the effects of mobile phase composition, flow rate, and column temperature on enantioselective separation of indoxacarb through high performance liquid chromatography (HPLC).15 Dong investigated the effects of the alcohol modifier types and concentrations on the separation on amylose-based CSP.16 Cheng studied the determination of indoxacarb enantiomer residues in vegetables, fruits, and soil by HPLC.8 Dong reported indoxacarb residues in cotton and soil, and their results showed that the half-lives of indoxacarb in cotton and soil were 6.9–7.4 d and 7.9–10.3 d.17 However, to our knowledge, there were no reports on the behaviors of indoxacarb enantiomers in organisms or the environment, which were essential to evaluate its environmental risk.

1Dali Sun and Jing Qiu contributed equally to this work.
Contract grant sponsor: National Natural Science Foundation of China. 21177156.
Contract grant sponsor: National Key Technology R&D Program. 2009BADB7B03.
*Correspondence to: Li Li, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China. E-mail: lili2008@izoz.ac.cn
Received for publication 16 October 2011; Accepted 23 February 2012
DOI: 10.1002/chir.22047
Published online in Wiley Online Library (wileyonlinelibrary.com).
In this study, a chiral HPLC with cellulosed-CSP was used to separate and determine two enantiomers of indoxacarb. The developed method was then successfully applied to study the enantioselective degradation of indoxacarb in cabbage and soil in Beijing and Anhui under field conditions.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

The analytical standard of rac-indoxacarb (purity at 99.5%) was purchased from Dr. Ehrenstorfer GmbH (Germany). The formulation product of 20% indoxacarb-WDG (water dispersible granule) was obtained from a commercial source and composed of (−)-indoxacarb and (+)-indoxacarb with a ratio of 1:3. A stock solution of rac-indoxacarb was prepared in hexane and stored at −20°C. Working standard solutions were obtained by diluting the appropriate amount of the stock solution in hexane. Water was purified by a Milli-Q system. Acetonitrile, acetone, and sodium chloride were all of analytical grade purchased from Beijing Chemical Works (Beijing, China). Isopropanol and hexane were of HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA). Cleanert florisol solid phase extraction (SPE) cartridges (1000 mg/6 ml) were purchased from Agela Technologies Inc (Tianjin, China).

**Field Application on Soil and Cabbage**

The indoxacarb formulation was dissolved in water and sprayed at the dosage of 27 g/ha for foliar application and at the dosage of 337.5 g/ha for soil application. No indoxacarb or similar structural pesticides were applied to the treated plots before. The control cabbage and soil samples were collected randomly on the day in the plots without treatment with the indoxacarb formulation. Samples were collected after treatment at 0 (2h), 1, 2, 3, 5, 10, 14, 21, 30, and 45 days after treatment (DAT). The soil was sampled to a depth of 0–10 cm in each plot, using a tube auger. Stones and plant debris were manually removed. All samples were put into polyethylene bags and transported to the laboratory where they were kept at −20°C until analysis.

**Extraction and Purification Procedures**

Ten grams of cabbage or soil samples were weighed in a 50-ml polypropylene centrifuge tube, followed by an addition of 20 ml acetonitrile (soil samples were added with 5 ml distilled water first), then shaken vigorously by hand. The tube was then put in an ultrasonic bath for 15 min. Five grams of sodium chloride was added into the extract, shaken vigorously for 1 min, and centrifuged at 3000 rpm for 5 min. An aliquot of supernatant was dissolved in 2 ml of hexane for the next clean-up. The sample extract was decanted to a 50-ml PTFE centrifuge tube and then transferred to the cartridge. The all the mentioned solutions were discarded. The mixture of hexane/acetone (9/1, v/v) was added into the cartridge (5 ml × 3 times) to elute the analyte and was concentrated to dryness on an evaporator under pressure at 30°C. The residue was finally reconstituted with 2.5 ml of hexane. A 20-μl aliquot was injected into the HPLC.

**Apparatus and Chromatographic Conditions**

The chromatographic determination was performed using Waters HPLC system equipped with a 2695 separations module and a 2998 photodiode array detector (PAD). The enantiomers of indoxacarb were separated on a Phenomenex Lux cellulose-1 column filled with CSP of cellulose-tris-(3, 5-dimethylphenylcarbamate) (CDMPC) (250 × 4.6 mm, 5 μm). The mobile phase was a mixture of hexane/isopropanol (85/15, v/v). The flow rate was 0.8 ml/min. PAD detection was conducted at 310 nm at room temperature.

The enantiomeric elution orders were determined on an Agilent 1200 series HPLC equipped with G1322A degasser, G1311A quattumpump, G1315C diode array detector, and G1329A autosampler (Wilmington, DE, USA). The left and right rotation enantiomers of indoxacarb were confirmed by CHIRALYSE-MP optical rotation detector, produced by the IBZ MESS TECHNIK Company (Germany) and provided by Beijing Separation Science and Technology Development Co., Ltd (Beijing, China). The optical signals were first transformed with an Agilent 359000E A/D converter and processed by an Agilent Chemstation.

**Calibration Curves and Assay Validation**

A series of matrix-match rac-indoxacarb standard solutions with concentrations of 0.1, 0.5, 1, 5, and 10 mg/l were prepared with a blank extract. Peak area of each enantiomer was measured and plotted against its concentration. The standard deviation (SD) and the relative standard deviation (RSD) were calculated at the entire calibration range. A series of blank samples fortified with rac-indoxacarb at 0.05, 1, and 5 mg/kg were prepared for method validation and determined as described previously. Recovery was estimated by comparing the peak area of the extracted analyte to that of an equivalent amount of the matrix-matched standard. The limit of detection (LOD) was considered to be the concentration that produced a signal to noise (S/N) ratio of 3, and the limit of quantification (LOQ) was defined as the lowest concentration in the calibration curve with acceptable accuracy and precision.

It was assumed that the degradation of the enantiomers in the plant samples followed first-order kinetics in the Anhui cabbage and in the soil of (+)-indoxacarb; the rest of the enantiomers followed pseudo-first-order kinetics. From the linear range of semilogarithmic and logarithmic plots, corresponding rate constants \( \dot{c} \) for the (−)-indoxacarb and (+)-indoxacarb were determined, (−) and (+) versus time \( x \), respectively.

\[
C = C_0 e^{-\dot{c}x}
\]

\[
T_{1/2} = \ln 2/\dot{c} = 0.693/\dot{c}
\]

The enantiomeric fraction (EF) was used to measure the enantioselective degradation of indoxacarb enantiomers in cabbage and soil samples. This descriptor was proposed as a more meaningful representation of graphical data than the enantiomeric ratio (ER) and was more easily employed in mathematical fate expressions. EF was defined as the following equation:

\[
EF = \frac{\text{peak areas of the (+)}}{\left(\frac{\text{peak areas of the (+)}}{\text{peak areas of the (+)}} \right) + \text{peak areas of the (+)}}
\]

The EF values range from 0 to 1, and the formulation represents 0.75.

**RESULTS AND DISCUSSION**

**Identification of Enantiomeric Elution Orders**

On CDMPC-CSP, the first eluted enantiomer on hexane/isopropanol showed a negative optical signal. Correspondingly,
the first and second eluted enantiomers were (−)-indoxacarb and (+)-indoxacarb, respectively (Fig. 2).

**Calibration and Method Validation**

Typical chromatograms of blank and fortified samples of the cabbage and soil are shown in Figure 3. Two enantiomers were separated completely, and there were no interference peaks at their retention times. Linear calibration curves were obtained over each indoxacarb enantiomer concentration ranging from 0.1 to 10 mg/l. The summary of calibration data in Table 1 showed good linearity for analysis of two enantiomers.

The mean recoveries of two enantiomers in cabbage and soil were determined at three fortification levels of 0.05, 1, and 5 mg/kg (Table 2). Recoveries of both indoxacarb enantiomers ranged from 95% ± 7.4% to 108% ± 5.2% in cabbage and from 85% ± 4.3% to 99% ± 10.1% in soil. The LOQ for two enantiomers were found to be 0.05 mg/kg. The LOD was 0.01 mg/kg both in the cabbage and the soil.

**Degradation of Indoxacarb in Cabbage**

Under field conditions, the degradation of both indoxacarb enantiomers in the Beijing cabbage followed pseudo-first-order kinetics. The degradation regressive metabolizing functions and half-lives of the two enantiomers were shown in Table 3.

**Fig. 2.** Elution orders of indoxacarb enantiomers separation on CD and UV chromatograms at 310 nm with hexane/isopropanol (85/15, v/v) at a flow rate of 0.8 ml/min.

**Fig. 3.** Representative HPLC chromatograms of the (A) extract from untreated cabbage, (B) extract from cabbage fortified with rac-indoxacarb (2 mg/kg), (C) extract from the cabbage sample treated with indoxacarb formulation (5 d), (D) extract from untreated soil, (E) extract from soil fortified with rac-indoxacarb (2 mg/kg), and (F) extract from the soil sample treated with indoxacarb formulation (21 d) (hexane/isopropanol = 85/15, flow rate = 0.8 ml/min).

*Chirality* DOI 10.1002/chir
In Anhui cabbage, (-)-indoxacarb followed the pseudo-first-order kinetics, whereas its antipode followed the first-order kinetics. The enantiomeric concentration of (-)-indoxacarb and (+)-indoxacarb increased from 5.08 to 12.03 mg/kg in the first 2 days because of the dominant absorption process and then declined to 0.1 and 0.06 mg/kg in 30 days for the Beijing cabbage. In the Anhui cabbage, the (-)-indoxacarb increased from 2.20 to 3.71 mg/kg within 2 days and then decreased with time, whereas the (+)-indoxacarb degraded from 4.67 to 0.16 mg/kg with time. These two enantiomers were undetected after 21 days of application (Fig. 4). The degradation half-lives of (-)-indoxacarb and (+)-indoxacarb were 4.6 and 3.5 d in Beijing cabbage, whereas they were 2.8 and 3.1 d in the Anhui cabbage, respectively. The data of the half-lives showed that (+)-indoxacarb degraded faster than its antipode at the Beijing location, whereas it was slower at the Anhui location.

The EF values of two enantiomers for the cabbages in Beijing and Anhui were shown in Figure 4. The EFs in the cabbage decreased from 0.71 to 0.38 in Beijing in 30 days and from 0.68 to 0 (the (+)-indoxacarb was undetected, remaining (-)-indoxacarb) after 21 days in Anhui. There is no significant change of EF values in the cabbage within 10 days after the application. The EF values were always below 0.75, indicating that preferential degradation of (+)-indoxacarb was observed in Beijing, which resulted in the enrichment with (-)-indoxacarb in the cabbage. The decrease of (-)-indoxacarb could be a consequence of the chiral inversion which might be caused by...

**TABLE 1.** The linear calibration curves of rac-indoxacarb ranging from 0.1–10 mg/l

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Enantiomers</th>
<th>Linear calibration curves</th>
<th>Related coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijing cabbage</td>
<td>(-)-indoxacarb</td>
<td>y = 27135x + 1.76</td>
<td>R² = 0.997</td>
</tr>
<tr>
<td></td>
<td>(+)-indoxacarb</td>
<td>y = 26647x + 205.53</td>
<td>R² = 0.9952</td>
</tr>
<tr>
<td>Anhui cabbage</td>
<td>(-)-indoxacarb</td>
<td>y = 26216x + 4016.2</td>
<td>R² = 0.9971</td>
</tr>
<tr>
<td></td>
<td>(+)-indoxacarb</td>
<td>y = 25144x + 5236.2</td>
<td>R² = 0.9926</td>
</tr>
<tr>
<td>Beijing soil</td>
<td>(-)-indoxacarb</td>
<td>y = 25367x + 5001.7</td>
<td>R² = 0.9914</td>
</tr>
<tr>
<td></td>
<td>(+)-indoxacarb</td>
<td>y = 25369x + 2002.2</td>
<td>R² = 0.9982</td>
</tr>
<tr>
<td>Anhui soil</td>
<td>(-)-indoxacarb</td>
<td>y = 25315x + 1599.3</td>
<td>R² = 0.9987</td>
</tr>
<tr>
<td></td>
<td>(+)-indoxacarb</td>
<td>y = 25315x + 1599.3</td>
<td>R² = 0.9987</td>
</tr>
</tbody>
</table>

**TABLE 2.** Recoveries of each enantiomer in cabbage and soil at three levels

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Fortification level (mg/kg)</th>
<th>Recovery (% ±SD) (n = 5)</th>
<th>(-)-indoxacarb</th>
<th>(+)-indoxacarb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>0.05</td>
<td>108 ± 5.2</td>
<td>95 ± 7.4</td>
<td>92 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>99 ± 0.6</td>
<td>99 ± 0.8</td>
<td>98 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>98 ± 2.5</td>
<td>100 ± 2.6</td>
<td>99 ± 1.4</td>
</tr>
<tr>
<td>Soil</td>
<td>0.05</td>
<td>99 ± 10.1</td>
<td>91 ± 11.0</td>
<td>94 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>85 ± 4.3</td>
<td>88 ± 7.3</td>
<td>90 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>94 ± 5.6</td>
<td>94 ± 4.2</td>
<td>95 ± 5.6</td>
</tr>
</tbody>
</table>

**TABLE 3.** Regressive functions of enantiomers in cabbage and soil

<table>
<thead>
<tr>
<th>Test material</th>
<th>Enantiomers</th>
<th>Regressive functions</th>
<th>Related coefficient</th>
<th>Half-lives (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijing cabbage</td>
<td>(-)</td>
<td>y = 3.9945e-0.140x</td>
<td>R² = 0.9971</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>y = 11.725e-0.0204x</td>
<td>R² = 0.9941</td>
<td>3.5</td>
</tr>
<tr>
<td>Anhui cabbage</td>
<td>(-)</td>
<td>y = 3.5473e-0.0231x</td>
<td>R² = 0.9929</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>y = 5.781e-0.0248x</td>
<td>R² = 0.9942</td>
<td>3.1</td>
</tr>
<tr>
<td>Anhui soil</td>
<td>(-)</td>
<td>y = 1.7067e-0.0237x</td>
<td>R² = 0.7742</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>y = 4.0552e-0.0322x</td>
<td>R² = 0.8388</td>
<td>23.0</td>
</tr>
</tbody>
</table>
ENANTIOSELECTIVE DEGRADATION OF INDOXACARB IN CABBAGE AND SOIL UNDER FIELD CONDITIONS

by the biotransformation process. However, there is no chromatographic evidence to prove this decrease. Whereas, in the Anhui cabbage, (−)-indoxacarb degraded faster than its antipode. It is possible that the corresponding enzyme system was restrained by (+)-indoxacarb (high activity) in the cabbage. The enrichment of higher activity enantiomer in plants has been reported by previous research.

The similar half-lives in cabbage at two geographically separated experimental fields suggested the similar dissipation rates at two locations. The degradation of indoxacarb enantiomers in cabbage might be partly explained by the plant growth inhibition activity. Enzyme systems and metabolic processes in cabbage might also play an important role in the degradation at the two locations. Further research should be carried out to clarify whether there are underlying processes of enantiomeric transformation in cabbage.

Degradation of Indoxacarb in Soil

The experimental results showed that both enantiomers of indoxacarb degraded much slower in soil than in cabbage. The initial concentration of (−)-indoxacarb in Beijing soil was 0.51 mg/kg and decreased to 0.37 mg/kg in 30 days. The degradation rate was 73%, whereas the initial concentration of its antipode was 1.71 mg/kg which decreased to 0.68 mg/kg in 30 days. A conspicuous degradation was found. The degradation of (+)-indoxacarb in the Anhui soil followed the first-order kinetics. The concentration of (+)-indoxacarb degraded from 4.68 to 0.70 mg/kg within 45 days. Whereas, the initial concentration of (−)-indoxacarb was 1.63 mg/kg, increased to 1.7 mg/kg in 2 days, and then decreased with time. The half-lives of (−)-indoxacarb and (+)-indoxacarb, respectively, were 32 and 21 d in the Anhui soil. These results suggested a great difference in the degradation rates between two enantiomers.

Different enantioselectivities in these two locations were discovered (Fig. 5). The EF values in the Beijing soil declined from 0.73 to 0.69 within 5 days, increased to 0.72, and then decreased with time to 0.66. In the Anhui soil, the EF values declined from 0.72 to 0.66. The changes of EF values indicated that the degradation of indoxacarb in soil was enantioselective with preferential degradation of (+)-indoxacarb.

Microbial decomposition played an important role in the enantioselective metabolism of many chiral chemicals in soil. Different types of microorganisms in soil probably resulted in different degradation behaviors of the indoxacarb enantiomers. Meanwhile, different characteristics of soil, such as organic matter, particle size, and pH, could also influence the enantioselective behaviors in soil. Therefore, further studies should be carried out to understand the relationships between soil characteristics and enantioselective degradation.

CONCLUSION

This study investigated the enantioselective degradation of indoxacarb in cabbage and soil from Beijing and Anhui, under open conditions with a developed and validated enantioselective method. The degradation process of the indoxacarb enantiomers followed the pseudo-first-order or first-order kinetics in cabbage and soil. The half-lives of indoxacarb in cabbage were much shorter than in soil, indicating a fast degradation rate in cabbage. The enantioselective degradation of the two enantiomers was observed in cabbage and soil at two locations with (+)-indoxacarb decreasing quicker, but the enantioselectivity was more apparent in soil than in cabbage.

Different enzyme systems and microbial decomposition metabolism might be involved in the different degradation behaviors of the two enantiomers in cabbage and soil, respectively.

LITERATURE CITED


Chirality DOI 10.1002/chir


