Chiral pesticide residue analysis and food safety

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2010.09.28
# Outline

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Part I  Chirality and chiral pesticide

- **Chirality**: Stereochemistry concepts, lacks an internal plane of symmetry, The geometric property of a molecule being **nonsuperimposable** on its mirror image; like the human hands.
Part I  Chirality and chiral pesticide

- **Chirality center**: When an atom has four non-equivalent atoms or groups attached to it, this is termed as the *chirality center*.

**Chirality generally occurs when a C atom has 4 different groups attached.**

[Lactic acid diagram]
Part I  Chirality and chiral pesticide

- The chiral compound have optical rotation activity, could make the light change spreading direction.
Part I  

**Chirality and chiral pesticide**

Archimedes of Syracuse (250 B.C.), The design of the Archimedean water screw and the study of the spiral structure

Dominque F.J. Arago (1811) Discovery of the rotation of the polarization of light in quartz crystals

Jean-Baptiste Biot (1835) Discovery of the rotation of the polarization of light in sugar solution.

“I am inclined to think that life, as manifested to us, must be a function of the dissymmetry of the universe and of the consequences it produces…… Life is dominated by dissymmetrical actions. I can even foresee that all living species are primordially, in their structure, in their external forms, functions of cosmic dissymmetry.”

——Louis Pasteur  (1848, Paratartaric acid is identified as the stereoisomer of tartaric acid. Pasteur postulates that nature has a chiral asymmetry )
Milky Way galaxy is a dextrorotation object.
Spiral in right-hand rule.
Hurricane Alberto (2000, August) in Atlantic Ocean
Hurricane Alberto is a levorotation object.
most amino acids are L, buildup the left-hand human body.
Part I  
Chirality and chiral pesticide

- R(+)Thalidomide
- S(-)Thalidomide
- sedative
- teratogenic
The teratogenic S enantiomer of the thalidomide drug caused a world-shaking tragedy in the 1960s.

During 4 years, 12000 seal abnormal baby was born, they all have no arm and with a short natural life. Alarm bell was stroke to recognize the chirality compound.
Asymmetric Synthesis
Nobel Prize 2001

诺尔斯 (W. Knowles)
野依良治 (R. Noyori)
夏普莱斯 (K. B. Sharpless)
It’s of great importance to conduct the enantioselective research on chiral pesticides. Chiral pesticide accounts for more than 25% (40% in China). Environmental studies have historically neglected to determine the adverse effects associated with particular enantiomers. The risk evaluations of chiral pesticides residue in food and environmental based on traditional methods are not reliable.
S-metolachlor

High herbicidal activity

R-metolachlor
cancerogenic potential
• In most cases, it is more difficult to develop the pesticide residue analytical method in chiral level.
Part II

Advance in chiral pesticide separation

Chiral separation technique

- high performance liquid chromatography (HPLC)
- gas chromatography (GC)
- capillary electrophoresis (CE)
- supercritical fluid chromatography (SFC)
- micellar electrokinetic chromatography (MEKC)
- microemulsion electrokinetic chromatography (MEEKC)
- capillary electrochromatography (CEC)
- microchip CE
Part II  Advance in chiral pesticide separation

HPLC Chiral Stationary Phases (CSP)

• Polysaccharide CSPs.(dominant): Chiralpak IA, IB, IC, AD, AS; chiralcelOJ, OD, OG.
• Cyclodextrin CSPs and Mobile Phase Additives.
• Macrocyclic Antibiotic CSPs.
• Crown Ether CSPs.
• Protein CSPs.
• Brush/Pirkle-Type CSPs.
• Ligand-Exchange and Ion-Exchange CSPs: chiral acid, bases, amino acid.
• Molecularly Imprinted Polymer (MIP) CSPs.
• From the number of papers published over the past two years, high performance liquid chromatography (HPLC) continue to be one of the most heavily utilized techniques. HPLC is by far the dominantly used method, with a conservative estimate of over 1000 publications appearing using chiral HPLC in numerous journals and languages.

•  

Part II  Advance in chiral pesticide separation

1. Synthetic pyrethroids

   2 and 3 chiral centers (n=2-3)
   □ 2 and 4 diastereomers[(2-1)ⁿ]
   2, 4, or 8 enantiomers(2ⁿ)

   **Cis:**
   1S-3S
   1R-3R

   **Trans:**
   S-3R
   1R-3S

\[ (Z)-cis\text{-bifenthrin} \]
\[ cis\text{-permethrin} \]
Cis:
1S-3S-αS
1R-3R-αR
1S-3R-αS
1R-3S-αR

Trans:
1S-3R-αR
1R-3S-αS
1S-3R-αS
1R-3S-αR

R = cypermethrin or cyfluthrin
<table>
<thead>
<tr>
<th>Entry</th>
<th>Commercial names</th>
<th>Separation conditions</th>
<th>Chromatograms</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>cis</em>-Bifenthrin (BF, Fig. 1, 1)</td>
<td><em>cis</em>-Bifenthrin Sumichiral OA-2500H, Hexane/1,2-Dichloroethane = 500/1 or 99.5/0.5, room temperature, UV = 230 nm</td>
<td><img src="image1" alt="Chromatogram" /></td>
<td>[1,15,21]</td>
</tr>
<tr>
<td>2</td>
<td><em>cis</em>-Permethrin <em>trans</em>-Permethrin (PM, Fig. 1, 2)</td>
<td><em>cis</em>-Permethrin Sumichiral OA-2500H, Hexane/1,2-Dichloroethane = 500/1 or 99.5/0.5, room temperature, UV = 230 nm</td>
<td><img src="image2" alt="Chromatogram" /></td>
<td>[1,15]</td>
</tr>
<tr>
<td>3</td>
<td>Cypermethrin (CP, Fig. 3, 3)</td>
<td>Cypermethrin Tandem Chirex 00G-3019-OD, Hexane/1,2-Dichloroethane/Ethanol = 500/30/0.15, room temperature, UV = 230 nm</td>
<td><img src="image3" alt="Chromatogram" /></td>
<td>[15]</td>
</tr>
<tr>
<td>4</td>
<td>Cyfluthrin (CF, Fig. 3, 4)</td>
<td>Cyfluthrin Tandem Chirex 00G-3019-OD, Hexane/1,2-Dichloroethane/Ethanol = 500/30/0.15, room temperature, UV = 230 nm</td>
<td><img src="image4" alt="Chromatogram" /></td>
<td>[15]</td>
</tr>
</tbody>
</table>

*a* A complete separation was defined as when the Rs exceeded 1.5.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Commercial names</th>
<th>Separation conditions</th>
<th>Chromatograms&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lambda-cyhalothrin (LCT, Fig. 3, 5)</td>
<td>Chiralpak AS Hexane/Ethanol = 95/5 25°C, 0.4 mL/min, UV = 236 nm</td>
<td><img src="image" alt="Chiralpak AS Chromatogram" /></td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chiralpak AD Hexane/Ethanol = 98/2 25°C, 0.4 mL/min, UV = 236 nm</td>
<td><img src="image" alt="Chiralpak AD Chromatogram" /></td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chiralcel OD Hexane/2-Propanol = 95/5, 25°C, 0.5 mL/min, UV = 236 nm</td>
<td><img src="image" alt="Chiralcel OD Chromatogram" /></td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chiralcel OJ Hexane/Ethanol = 95/5, 25°C, 0.6 mL/min, UV = 236 nm</td>
<td><img src="image" alt="Chiralcel OJ Chromatogram" /></td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chiralcel OJ Hexane/2-Propanol = 90/10, 25°C, 0.4 mL/min, UV = 236 nm</td>
<td><img src="image" alt="Chiralcel OJ Chromatogram" /></td>
<td>[23]</td>
</tr>
<tr>
<td>2</td>
<td>Fenvalerate (Fig. 3, 8)</td>
<td>(R)-1-phenyl-2-(4-methylphenyl)ethylamine amide derivative of (S)-valine to aminopropyl silica gel through a 2-amino-3,5-dinitro-1-carboxamidobenzene unit Hexane/1,2-dichloromethane/ethanol = 98.45/1.2/0.35, Room temperature, 1 mL/min, UV = 230 nm</td>
<td><img src="image" alt="Fenvalerate Chromatogram" /></td>
<td>[26]</td>
</tr>
<tr>
<td>3</td>
<td>Cycloprothrin (Fig. 3, 10)</td>
<td>Chiralcel OJ+H Hexane/isopropanol = 70/30, 35°C, 1 mL/min, UV = 254 nm</td>
<td><img src="image" alt="Cycloprothrin Chromatogram" /></td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chiralcel OD+H Hexane/isopropanol = 90/10, 35°C, 1 mL/min, UV = 254 nm</td>
<td><img src="image" alt="Chiralcel OD+H Chromatogram" /></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Fenpropatrin (Fig. 3, 11)</td>
<td>(R)-1-phenyl-2-(4-methylphenyl)ethylamine amide derivative of (S)-valine to aminopropyl silica gel through a 2-amino-3,5-dinitro-1-carboxamidobenzene unit Hexane/1,2-dichloromethane = 94/6, Room temperature, 1 mL/min, UV = 230 nm</td>
<td><img src="image" alt="Fenpropatrin Chromatogram" /></td>
<td>[26]</td>
</tr>
</tbody>
</table>

<sup>a</sup> A complete separation was defined as when the Rs exceeded 1.5.
Chiralcel OD is desirable for the separation of SPs with one chiral center.

As for SPs with three chiral centers (e.g., CP and CF), two tandem Chirex 00G-3019-OD columns are the best choice for their HPLC resolution.
Part II  Advance in chiral pesticide separation

2. Organophosphates:

![Chemical structures of organophosphorus pesticides (OPs).](image-url)
<table>
<thead>
<tr>
<th>Entry</th>
<th>Commercial names</th>
<th>Separation conditions</th>
<th>Chromatograms (^a)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fenamiphos (Fig. 4, 18)</td>
<td>Whelk-OJ</td>
<td>Hexane/2-Propanol = 95/5, room temperature, UV = 210 nm,</td>
<td>[39]</td>
</tr>
<tr>
<td>2</td>
<td>Methamidophos (Fig. 4, 13)</td>
<td>Chiracel OD</td>
<td>Hexane/2-Propanol = 80/20, 25°C, 0.5 mL/min, UV = 230 nm</td>
<td>[31]</td>
</tr>
<tr>
<td>3</td>
<td>Trichloronate (Fig. 4, 12)</td>
<td>Chiralcel OJ</td>
<td>Hexane/Heptane/EtOH = 90/5/5, 21°C, 1.0 mL/min, UV = 300 nm</td>
<td>[13]</td>
</tr>
<tr>
<td>4</td>
<td>Chloramidophos (Fig. 4, 24)</td>
<td>CHIRALPAK AD</td>
<td>Hexane/EtOH = 90/10, 25°C, 1.0 mL/min, UV = 230 nm</td>
<td>[43]</td>
</tr>
<tr>
<td>5</td>
<td>Fosthiazate (Fig. 4, 25)</td>
<td>CHIRALPAK AD Hexane/EtOH = 95/5, Room temperature, 1.0 mL/min, UV = 230 nm</td>
<td></td>
<td>[44]</td>
</tr>
</tbody>
</table>

\(^a\) A complete separation was defined as when the Rs exceeded 1.5.
The above studies showed that the OPs having two pairs of enantiomers can normally be separated on Chiralpak AD.
Part II  Advance in chiral pesticide separation

3. Acylanilides

![Chemical structures of acylanilides](image)

*Figure 5. Chemical structures of acylanilides.*
Table 4. Enantioselective separation of acylanilide herbicides by HPLC

<table>
<thead>
<tr>
<th>Entry</th>
<th>Commercial names</th>
<th>Separation conditions</th>
<th>Chromatograms*</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| 1     | Metolachlor (Fig. 5, 27) | Chiralcel OD-H  
Hexane/Diethyl ether = 91/9, 20°C, 0.8 mL/min,  
UV = 230 nm | ![Chromatogram 1](image1) | [11] |
| 2     | Benalaxyl (Fig. 5, 28) | (R,R) Whelk-O 1  
Hexane/isopropanol = 70/30, 20°C, 1.0 mL/min,  
UV = 220 nm | ![Chromatogram 2](image2) | [49] |
| 3     | Napropamide (Fig. 5, 29) | Chiralcel AD-RH  
Chiralcel OD-RH  
Acetonitrile/Water = 50/50, 10-35°C, 0.5 mL/min,  
UV = 212 nm | ![Chromatogram 3](image3) | [51] |
| 4     | Metalaxyl (Fig. 5, 30) | Chiralpak AD-H  
Hexane/ethanol = 60/40, 0°C, 0.5 mL/min,  
UV = 210 nm | ![Chromatogram 4](image4) | [53] |
| 5     | Dimethenamid (Fig. 5, 31) | Chiralpak AD-H  
Hexane/ethanol = 90/10, 0°C, 0.5 mL/min,  
UV = 238 nm | ![Chromatogram 5](image5) | [53] |

*a A complete separation was defined as when the Rs exceeded 1.5.
Part II  Advance in chiral pesticide separation

- Chiralcel AD is desirable for the separation of Acylanilides.
4. Imidazolinones

Figure 6. Chemical structures of imidazolinones.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Commercial names</th>
<th>Separation conditions</th>
<th>Chromatograms</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Imazamox (Fig. 6, 34)</td>
<td><strong>Chiralcel OJ</strong>&lt;br&gt; Hexane:Alcohol/TFA = 75/25/0.1, room temperature, 1.0 mL/min, UV = 254 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[12]</td>
</tr>
<tr>
<td>2</td>
<td>Imazapyr derivative (Fig. 6, 36)</td>
<td><strong>Chiralcel OJ</strong>&lt;br&gt; Hexane:Alcohol/TFA = 90/10/0.1, room temperature, 1.0 mL/min, UV = 254 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[12]</td>
</tr>
<tr>
<td>3</td>
<td>Imazapic (Fig. 6, 37)</td>
<td><strong>Chiralcel OJ</strong>&lt;br&gt; Hexane:Alcohol/TFA = 75/25/0.1, room temperature, 1.0 mL/min, UV = 254 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[12]</td>
</tr>
<tr>
<td>4</td>
<td>Imazethapyr (Fig. 6, 32)</td>
<td><strong>Chiralcel OJ</strong>&lt;br&gt; Hexane:2-Propanol/Acetic acid = 84.6/15.4/0.1, 25°C, 0.8 mL/min, UV = 275 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[56]</td>
</tr>
<tr>
<td>5</td>
<td>Imazaquin (Fig. 6, 33)</td>
<td><strong>Chiralcel OJ-H</strong>&lt;br&gt; Hexane:2-Propanol/Acetic acid = 84.6/15.4/0.1, 25°C, 0.8 mL/min, UV = 275 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[56]</td>
</tr>
<tr>
<td>6</td>
<td>Imazapyr (Fig. 6, 35)</td>
<td><strong>Chiralcel OJ</strong>&lt;br&gt; Hexane:2-Propanol/Acetic acid = 84.6/15.4/0.1, 25°C, 0.8 mL/min, UV = 275 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[56]</td>
</tr>
</tbody>
</table>

*a* TFA: trifluoroacetic acid.
Part II  Advance in chiral pesticide separation

- As for Imidazolinones herbicides, OJ column is the best choice for their HPLC resolution.
Part II  Advance in chiral pesticide separation

5. Triazole fungicides

Figure 8. Chemical structures of triazole-related fungicides.
<table>
<thead>
<tr>
<th>Entry</th>
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<th>Separation conditions</th>
<th>Chromatograms*</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paclitaxel (Fig. 8, 55)</td>
<td>Tris-(S)-1-phenylethylcarbamate CSP Hexane:2-Propanol = 95:5, 20°C, 1.0 mL/min, UV = 230 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[62]</td>
</tr>
<tr>
<td>2</td>
<td>Uniconazole (Fig. 8, 56)</td>
<td>Chiralpak AD-H Hexane:2-Propanol = 80:20, 30°C, 0.5 mL/min, UV = 255 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[53]</td>
</tr>
<tr>
<td>3</td>
<td>Fluridil (Fig. 8, 48)</td>
<td>Chiralcel OD-H Hexane:Ethanol = 90:10, 15°C, 1.0 mL/min, UV = 230 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[68]</td>
</tr>
<tr>
<td>4</td>
<td>Propiconazole (Fig. 8, 51)</td>
<td>Chiralcel OD-H Hexane:2-Propanol = 90:10, 15°C, 1.0 mL/min, UV = 230 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[68]</td>
</tr>
<tr>
<td>5</td>
<td>Triadimefon (Fig. 8, 54)</td>
<td>Chiralcel OD-H Hexane:2-Propanol = 90:10, 25°C, 1.0 mL/min, UV = 228 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[68]</td>
</tr>
<tr>
<td>6</td>
<td>Hexaconazole (Fig. 8, 56)</td>
<td>Chiralcel OD-H Hexane:2-Propanol = 90:10, 25°C, 1.0 mL/min, UV = 230 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[68]</td>
</tr>
<tr>
<td>7</td>
<td>Tebuconazole (Fig. 8, 57)</td>
<td>Chiralcel OD-H Hexane:2-Propanol = 90:10, 25°C, 1.0 mL/min, UV = 227 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[68]</td>
</tr>
<tr>
<td>8</td>
<td>Diniconazole (Fig. 8, 60)</td>
<td>Chiralcel OD-H Hexane:2-Propanol = 90:10, 25°C, 1.0 mL/min, UV = 253 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[68]</td>
</tr>
<tr>
<td>9</td>
<td>Difenoconazole (Fig. 8, 61)</td>
<td>Chiralcel OJ-H Hexane:Ethanol = 90:10, 25°C, 1.0 mL/min, UV = 230 nm</td>
<td><img src="image" alt="Chromatogram" /></td>
<td>[68]</td>
</tr>
</tbody>
</table>

* A complete separation was defined as when the Rs exceeded 1.5.
Disadvantages of Normal HPLC

- Although normal HPLC with CSP can well separated mostly chiral pesticide, but still have some defects:
  - (1) limit of mobile phase selection
  - (2) difficult to coupled with MS technique,
  - (3) Bad sensitivity and selectivity
Part II  Advance in chiral pesticide separation

• With the development of chiral separation materials, nowadays, reversed CSP with more and more frequently used for resolution of chiral pesticides.

• CAU laboratory have successfully separated more than 20 chiral pesticides under reversed phase condition such as:
  • paclobutrazol, myclobutanil (Anal. Methods, 2010, 2, 617–622)
  • Epoxiconazole, terallethrin, benalaxyl, pyriproxyfen and diclofopmethyl (J. Sep. Sci. 2007, 30, 310 – 321)
  • hexaconazole, flutriafol, diniconazole, tebuconazole, uniconazole and triadimefon (Chinese Journal of Analytical Chemistry, 2010, 5, 688-692)
  • fenamiphos, terallethrin, fenoxaprop-ethyl, benalaxyl, lactofen quizalofop-ethyl, fluroxypyr-meptyl, napropamide, metalaxyl and 2,4-D-ethylhexyl (chromatographia, 2010, 71, 855-865).
Part II  Advance in chiral pesticide separation

- some chiral pesticides under reversed phase condition: myclobutanil, simeconazole, tebuconazole, fenbuconazole and its metabolize in our lab.
RP chiral column

Chiral detector

LC/MS/MS System

RP HPLC System
Part II  Advance in chiral pesticide separation

The virtue of RP HPLC

- (1) RP HPLC can be easily to combine with mass spectrometry; it is helpful to compound qualification.
- (2) establish more sensitive and efficient multi-enantiomer analytical method
Part II  Advance in chiral pesticide separation

Chromatograms of myclobutanil enantiomers in cucumber sample
A: Full Scan mode  B: SIM mode,  C: MS/MS mode
Chromatograms of myclobutanil:

(A) black cucumber sample,
(B) Spiked cucumber sample (0.01 mg/kg),
(C) black soil sample,
(D) Spiked soil sample (0.01 mg/kg)
Part III  Advance in chiral pesticide behavior

Part III

Advance in chiral pesticide behavior

Insecticide

- Pyrethroid
- Organophosphorous
- Organochlorine
- Heterocycle
- Others
Part III  Advance in chiral pesticide behavior

- Bifenthrin (BF) has two chiral centers and therefore 2 pairs of enantiomers. At present, the commercial formulations of BF is the cis form and has one pair of enantiomers: 1R-cis-BF and 1S-cis-BF.
(1) the enantioselective toxicity of BF between the non-target organism (such as human cells) and the target organism (*P. rapae* L.) was reversed, and the difference between the 1R-\textit{cis}-BF and 1S-\textit{cis}-BF was more than 300-fold. This results are in good agreement with some other studies which reported that 1R-\textit{cis}-BF is markedly active on the target organism (*Liu et al.*, *chirality*, 2005, 17, 127-133).
(2) the results indicated that 1S-cis enantiomer was stronger (123 times) than the 1R enantiomer in estrogenic activity (Environ. Sci. Technol. 2007, 41, 6124-6128). And in this study, we found that 1S-cis-BF presented greater toxicity than 1R-cis-BF and cis-BF in FL cells. According to the result of the target and nontarget organism, the 1R-cis-BF isomer is a more effective and safe insecticide enantiomer.
Part III  Advance in chiral pesticide behavior

Herbicide

- Acetanilides
- Phenoxyalkanoic acids
- Aryloxyphenoxypropionate
- Cyclonenes
- Imidazolones
- Others
Enantioselectivity of the bioactivity  

**Acetanilides herbicides**

Bioactivity of S-Metolachlor is 10 times higher than R-Metolachlor;

The S-dimethenamid has much higher herbicidal activity than the R-dimethenamid.
Enantioselectivity of the bioactivity

The enantiomers of Imidazolones herbicides possess different herbicidal activity, generally, the herbicidal activity of R-Imidazolones was 8-10 times higher than the S-Imidazolones.

Haifeng Qian et al. selected Japonica rice variety Xiushui 63 seedlings to evaluate the enantioselectivity of imazethapyr (IM). Significant differences in rice seedling morphology, antioxidant enzyme, oxidant marker and gene transcription were observed between the two IM enantiomers.

Fig. 1. (A) Chemical structures of Imazethapyr (IM) with “*” indicate the asymmetric position. (B) The HPLC chromatogram shows enantiomeric separation of imazethapyr (IM) on a CHIRALCEL OJ-H column.
Marucchini C. et al, have investigated a possible enantioselective degradation in soil and plants of the fungicide rac-Metalaxyl and (-)-(R)-Metalaxyl. The degradation of the two steroisomers of Metalaxyl proved to be enantioselective and dependent on the media: the (+)-(S)-enantiomer showed a faster degradation in plants, while the (-)-(R)-Metalaxyl showed a faster degradation in soil.

Fig. 1. Structures of the two enantiomers of Metalaxyl.
Ignaz J. Buerge have conducted an investigation on the enantioselective degradation in 20 different soils covering a wide range of soil properties. *Found that:*

In aerobic soils with pH > 5, the fungicidally active R-enantiomer was degraded faster than the S-enantiomer ($k_R > k_S$), leading to residues with a composition $[S] > [R]$. However, in aerobic soils with pH 4–5, both enantiomers were degraded at similar rates ($k_R \approx k_S$), and in aerobic soils with pH < 4 and in most anaerobic soils, the enantioselectivity was reversed ($k_R < k_S$).
Part III  Advance in chiral pesticide behavior

Almost all of which have one or two chiral centers, were initially developed in the mid-1970s and comprise a vast spectrum of members.
### Table 1. Bioactivity and ecotoxicity

<table>
<thead>
<tr>
<th>compound</th>
<th>enantiomer Bioactivity</th>
<th>enantiomer ecotoxicity</th>
<th>Racemate ecotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>triadimefon</td>
<td>R isomer=S isomer</td>
<td>No date</td>
<td>Reproductive defects(∗, ∗∗)</td>
</tr>
<tr>
<td>triadimenol</td>
<td>RS 1000 times</td>
<td>No date</td>
<td>Low toxicity</td>
</tr>
<tr>
<td>tebuconazole</td>
<td>(-) isomer&gt;(+ ) isomer</td>
<td>No date</td>
<td>Rat Liver defects, possible human carcinogen(∗∗)</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>No data</td>
<td>No date</td>
<td>Potential reproductive defects∗</td>
</tr>
<tr>
<td>difenoconazole</td>
<td>No data</td>
<td>No date</td>
<td>Fish high toxicity(∗∗)</td>
</tr>
<tr>
<td>simeconazole</td>
<td>No data</td>
<td>No date</td>
<td>Low toxicity</td>
</tr>
<tr>
<td>propiconazole</td>
<td>No data</td>
<td>No date</td>
<td>Rat Liver defects, possible human carcinogen(∗∗)</td>
</tr>
</tbody>
</table>

注：(*Goetz, 2007, 2009; **Nesheim, O.N. 2002.*)
Biological activities of triazole fungicides enantiomers differ greatly

- Triadimenol contains two chiral center, of which has 4 enantiomers (RS, SR, RR and SS); The biological activities of SR was 1000 times higher than the other 3 enantiomers
Part III  Advance in chiral pesticide behavior

Current research status on chiral triazole fungicide

Research of enantioselective behavior in biotic samples were conducted extensively in recent year

Plants

Biotic samples

Soil

Enantioselective degradation of triadimenol in cucumber

Professor Yongquan Zheng

Research team

Research team of Prof. Zhou zhiqiang

Keneke et al

Clark-triadimenol

Buerge-flusilazole
**Part III**  
**Advance in chiral pesticide behavior**

Enantioselective degradation in biotic samples

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Stereoselective degradation kinetics of Tebuconazole and Hexaconazole in rabbits and rats were conducted by Zhou zhiqiang *et al*. *Found that:* the degradation of enantiomer with lower bioactivities was faster than the ones with higher bioactivities.

Stereoselective degradation kinetics of Triadimefon in Rainbow trout were conducted by Kenneke *et al*. *Found that:* Its metabolites Triadimenol was detected, SR enantiomers, of which has the highest bioactivities was degraded slowly.

As for triazole fungicide, the processes of its absorption, distribution, and degradation in organism are often enantioselective, the enantiomer with higher bioactivities and toxicities was inclined to be accumulated.
Enantioselective degradation in soil

Triadimenol enantiomers were applied as barley seed-coating agent by Clark, 49 days later, found that the 44% RS was transformed into RR, 24%SR was transformed into SS (17%) and RR (7%).

Stereoselective degradation kinetics of epoxiconazole and cyproconazole in different kinds of soil were conducted by Buerge et al. Found that: enantioselective degradation of epoxiconazole has been found in alkaline soil, the four enantiometers of cyproconazole were degraded at different rates.

It is of great significance to make an intensive investigation on enantioselective degradation of chiral triazole pesticides in soil to supply more accurate data for evaluating the environments risks and food safety.
The stereoselective degradation of triadimenol in cucumber tissues has been investigated. It is the first report on the enantioselective environmental behavior of triadimenol on plant.

We found that: the degradation of triadimenol in cucumber plants was stereoselective under field conditions. The results indicated that RS enantiomer was degraded faster than SR enantiomer, and SS enantiomer was degraded faster than RR enantiomer, which resulted in plants enriched with SR and RR enantiomers.
Part III

Advance in chiral pesticide behavior

Enantioselective degradation in Plants

leaves

Cucumber

fruits
Stereoselective Degradation of Fungicide Triadimenol in Cucumber Plants

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ABSTRACT: The stereoselective degradation of triadimenol in different cucumber plant tissues (root, stem, leaf, and fruit) has been investigated. After triadimenol was applied to cucumber plants by root application under field conditions, the regioselective degradations and the marfey headers were detected and determined by high-performance liquid chromatography coupled with mass spectrometric and nuclear magnetic resonance techniques. After treatment, triadimenol was detected in all the plant samples. The major identified degradation products in cucumber plants was not stereoselective under field conditions. The results indicated that both enantiomers were degraded faster than 20% faster, and 91 enantiomer was degraded faster than 85% faster, which resulted in the racemic mixture with 18% and 82% as enantiomers. Furthermore, it was found that no newly discovered toxicity for the stereoisomers or any chemical changes with respect to the starting material, triadimenol, was detected in cucumber plants. The results suggest an understanding of the stereoselectivity is not applicable in vegetable plants.

INTRODUCTION

Triadimenol (2,3-dimethyl-1,4-dioxane-2,3-dicarboxylic acid) is a broad-spectrum systemic fungicide widely used in many plant diseases. Nevertheless, it damages non-targeted species and poses potential risks to non-targeted organisms. An experimental test for the development of a chiral soil biodegradation model for triadimenol was described in this study. The key findings were that the 20% fast-degradation product was identified by derivatization of the racemic mixture. The difference in the half-life of the triadimenol treatment was quantitatively determined with the help of derivatization.

MATERIALS AND METHODS

The experimental study of triadimenol metabolism in the soil and in the plant was performed by Derivation of Chemical Company, China. Detailed studies of the triadimenol were conducted and included the determination of the stereoselectivity of the four stereoisomers.

RESULTS

The results showed that the stereoselectivity of the four stereoisomers was better than 20% for the four stereoisomers. The results suggest that the stereoselectivity is not applicable in vegetable plants.
1. To ensure the food safety, the study of chiral pesticide must be carried out, will always be a great task in future.

Regulatory authorities should be provided with data on both the fate and effects of separate enantiomers so that they can make the best possible risk assessments for single- or enriched-enantiomer pesticides that may be submitted for registration. These additional data would allow risk assessors to consider each enantiomer as an individual compound with its own set of biological properties and would provide a sound scientific base for regulatory decisions.
2. The study on separation of chiral pesticide will still be the hot topic.

The enantiomers of Imidazolones herbicides possess different herbicidal activity, generally, the herbicidal activity of R-Imidazolones was 8-10 times higher than the S-Imidazolones.

Haifeng Qian et al. selected Japonica rice variety Xiushui 63 seedlings to evaluate the enantioselectivity of imazethapyr (IM). Significant differences in rice seedling morphology, antioxidant enzyme, oxidant marker and gene transcription were observed between the two IM enantiomers.
3. The study on the fate of chiral pesticide will still be the hot study field.

The traditional risk evaluations of chiral pesticides residue are not reliable if enantioselective behaviors happened. Consequently, it is of great significance to develop enantiomeric analysis methods of chiral pesticides and investigate the different environmental behavior of the individual enantiomer to supply more accurate data for evaluating the environmental risk and food safety.
The future of chiral pesticide

4. The study on stereoselective mechanism of chiral pesticide in organism and environment would still be a challenge.

Future research should elucidate whether different microbial populations or enzymes selectively degrade certain enantiomers of chiral pesticide or whether certain species or tissues selectively accumulate one enantiomer but not the other.

The different enantiomeric ratios for α-HCH or in different seas, species, and tissues within species are curious.
Thanks for your attention