Chiral pesticide residue analysis and food safety

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Outline

Ⅰ. Chirality and chiral pesticide

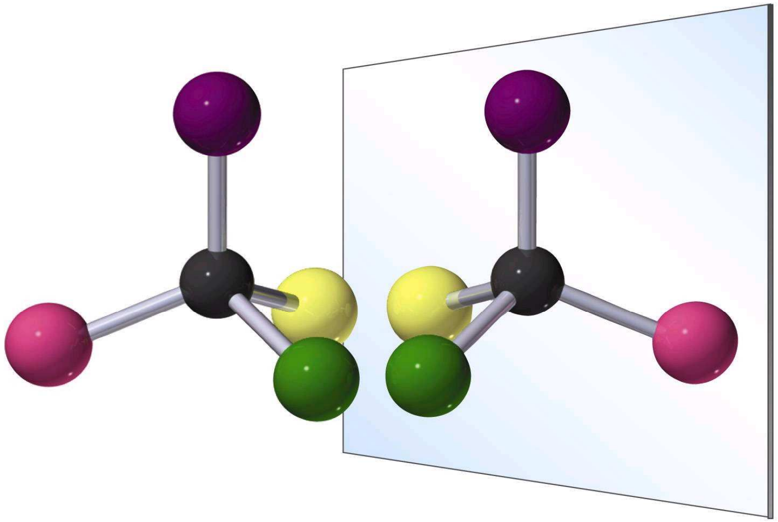
Ⅱ. Advance in chiral pesticide separation

Ⅲ. Advance in chiral pesticide behavior

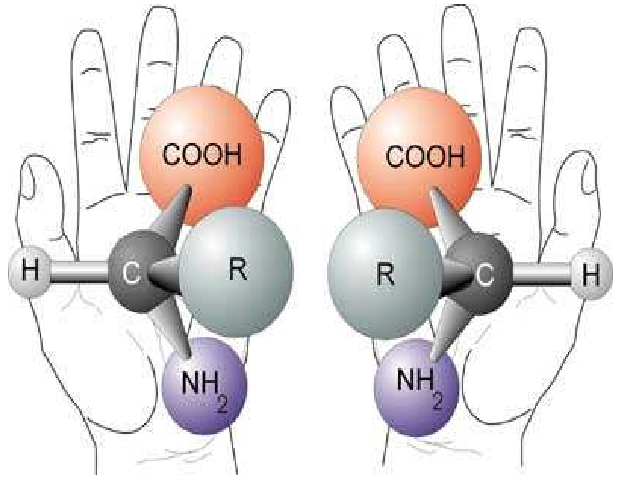
Ⅳ. The future of chiral pesticide

Chirality and chiral pesticide

- Chirality: Stereochemistry concepts, lacks an internal plane of symmetry, The geometric property of a molecule being **non-superimposable** on its mirror image; like the human hands.

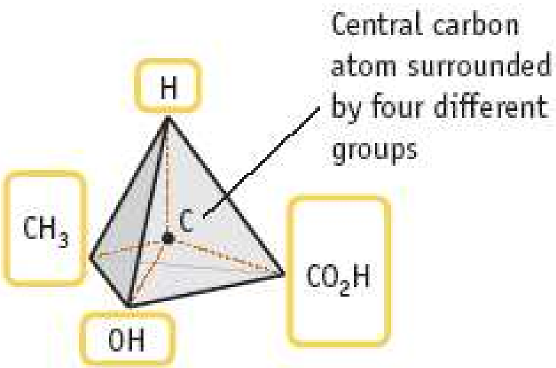


nonsuperimposable
mirror images

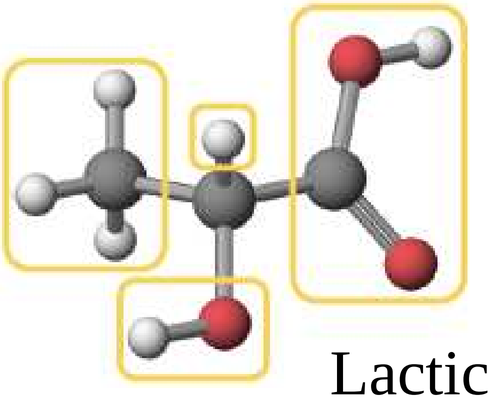


Part Ⅰ Chirality and chiral pesticide

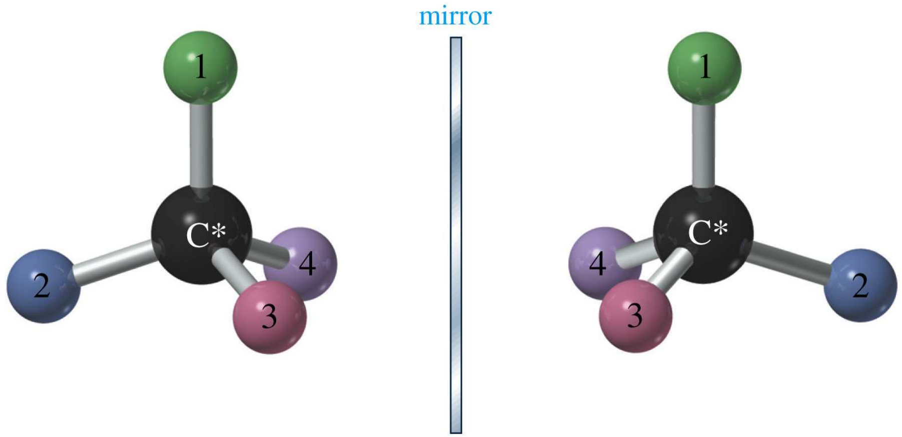
- Chirality center： When an atom has four non-equivalent atoms or groups attached to it, this is termed as the ***chirality center***.



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

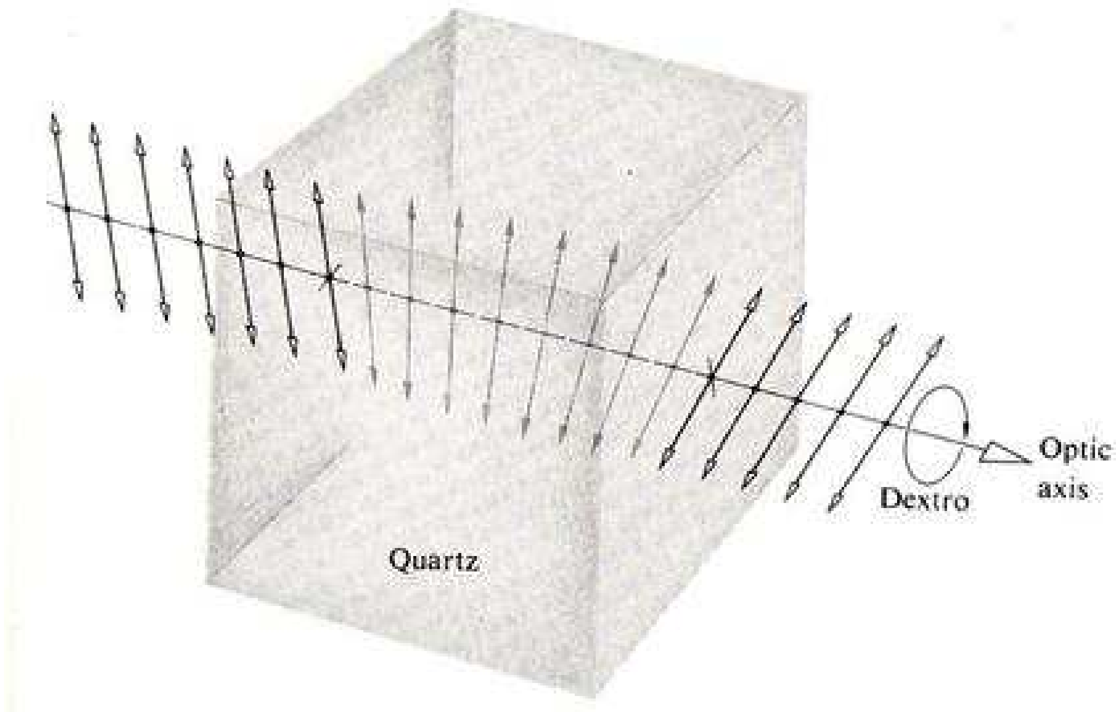


Lactic acid



Chirality and chiral pesticide

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



Part Ⅰ

Chirality and chiral pesticide

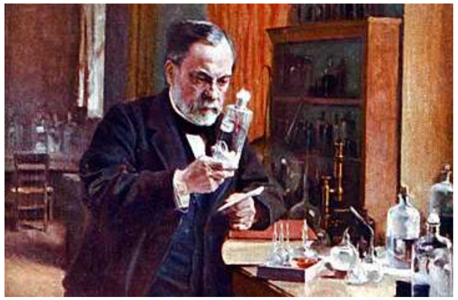
Archimedes of Syracuse (250 B.C.), The design of the Archimedean water screw and the study of the spiral structure

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.

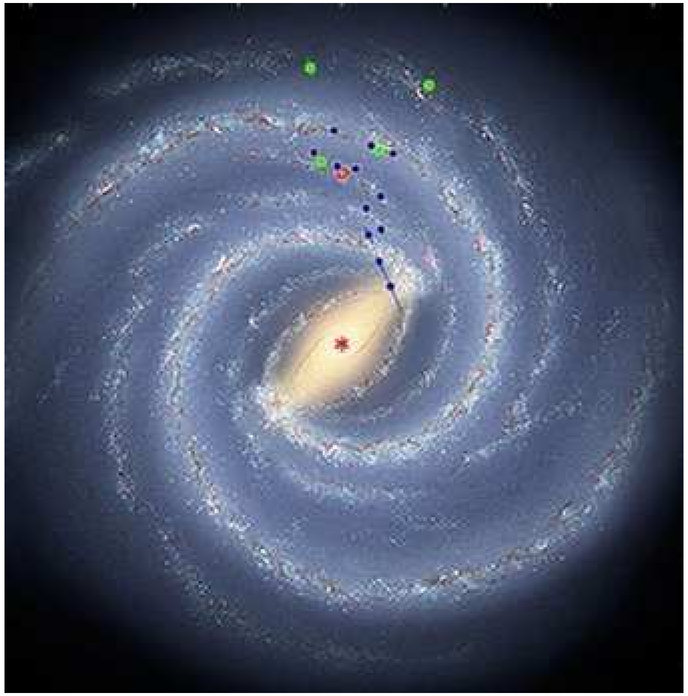
"I am inclined to think that life, as manifested to us, must be a function of the dissymmetry of the universe and of the consequences it produces…… Life is dominated by dissymmetrical actions. I can even foresee that all living species are primordially, in their structure, in their external forms, functions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

——Louis Pasteur （1848，Paratartaric acid is identified as the stereoisomer of tartaric acid. Pasteur postulates that nature has a chiral asymmetry ）



Part Ⅰ

Chirality and chiral pesticide



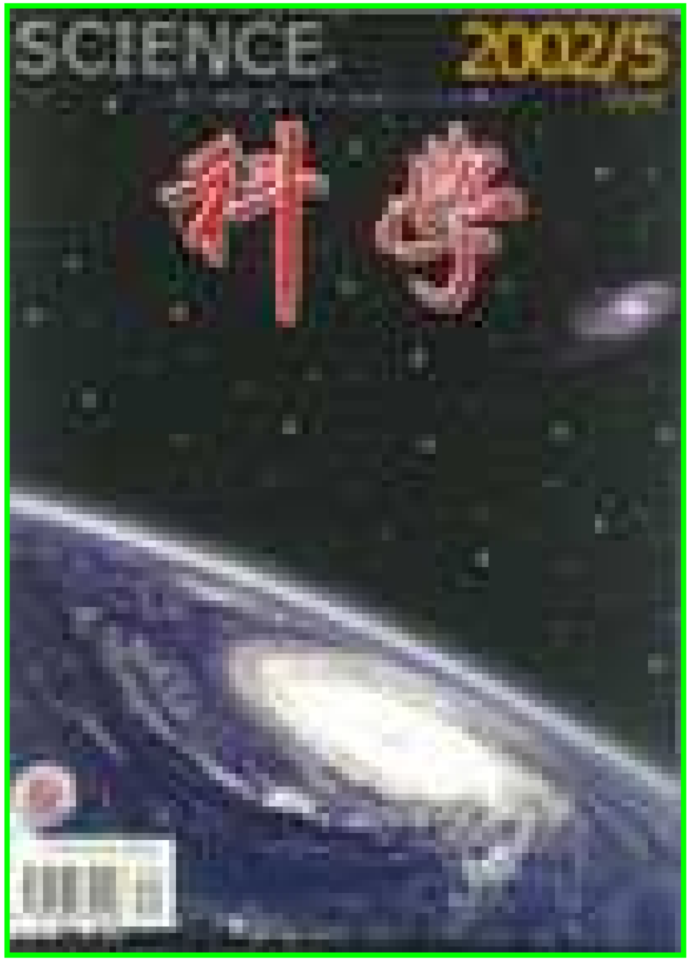
Milky Way galaxy is a dextrorotation object.

Spiral in right-hand rule.

Chirality and chiral pesticide

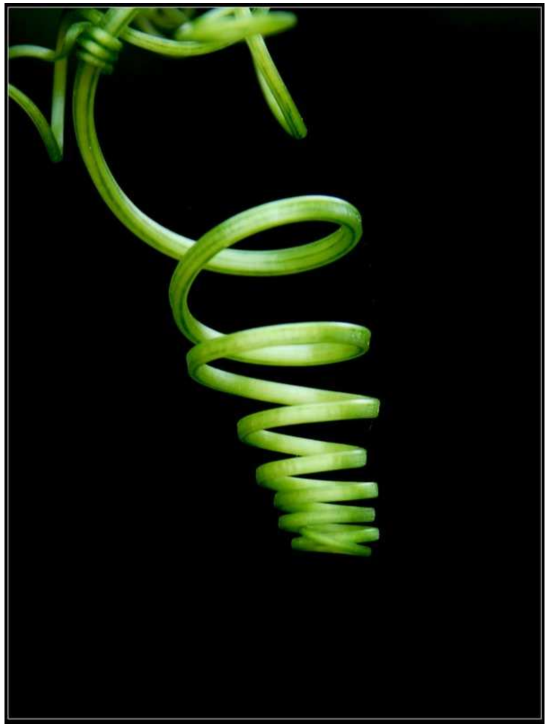
Hurricane Alberto (2000, August) in Atlantic Ocean

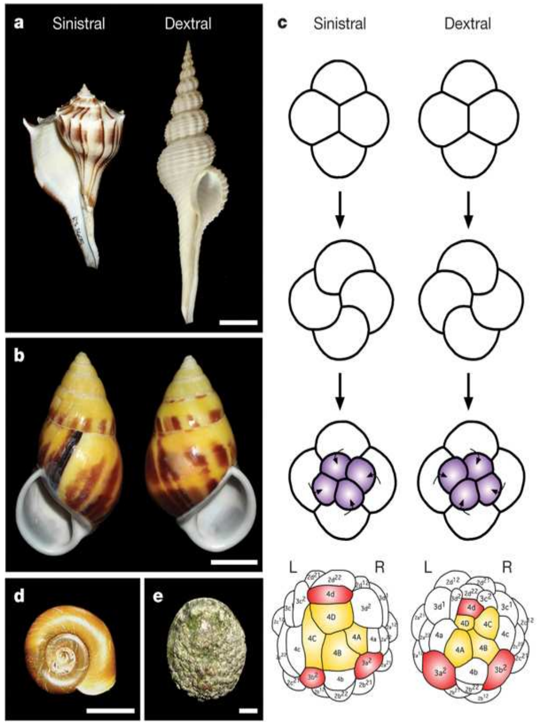
Hurricane Alberto is a levorotation object.



Part Ⅰ

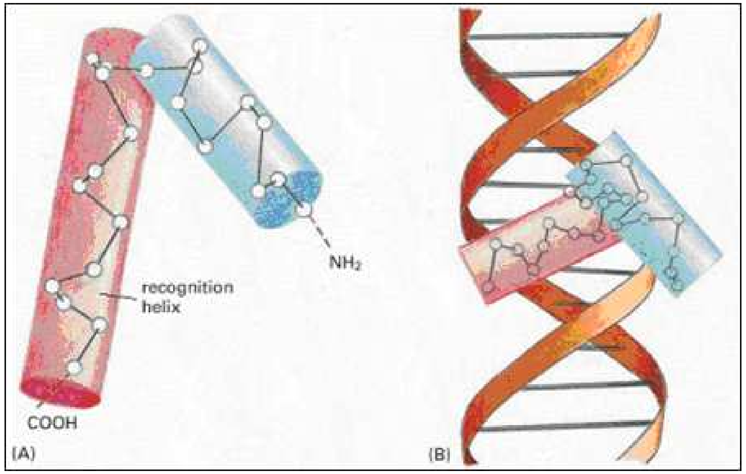
Chirality and chiral pesticide

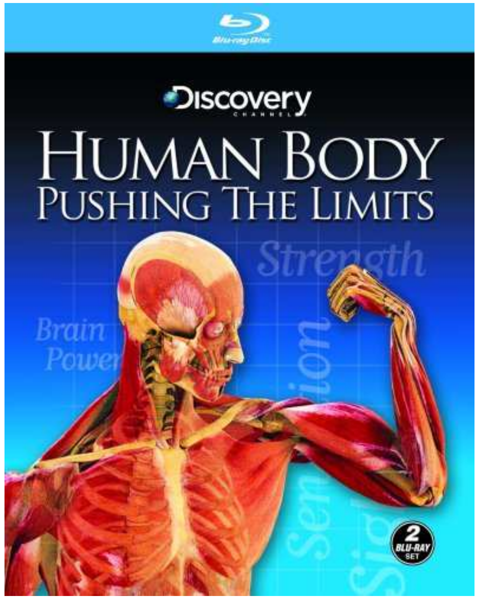




C Grande & NH Patel . *Nature.* 2009, 457:1007-1011

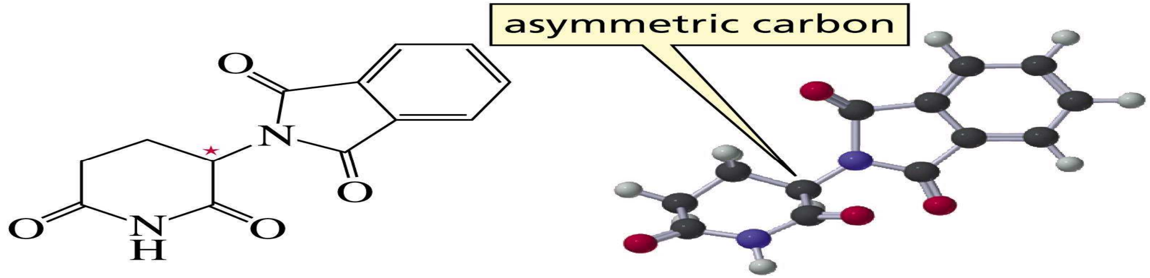
Part Ⅰ Chirality and chiral pesticide



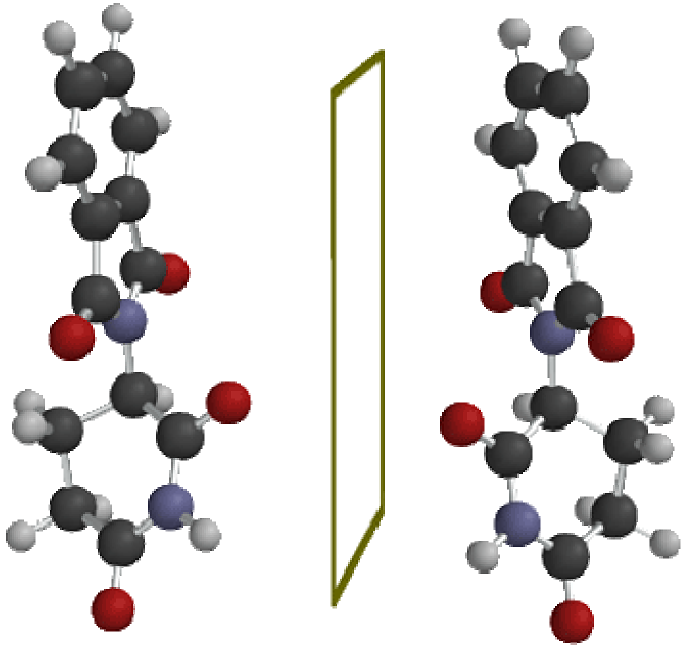


most amino acids are L, buildup the left-hand human body.

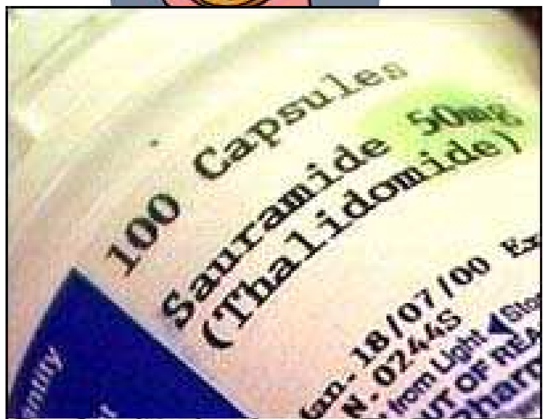
Part Ⅰ Chirality and chiral pesticide



thalidomide

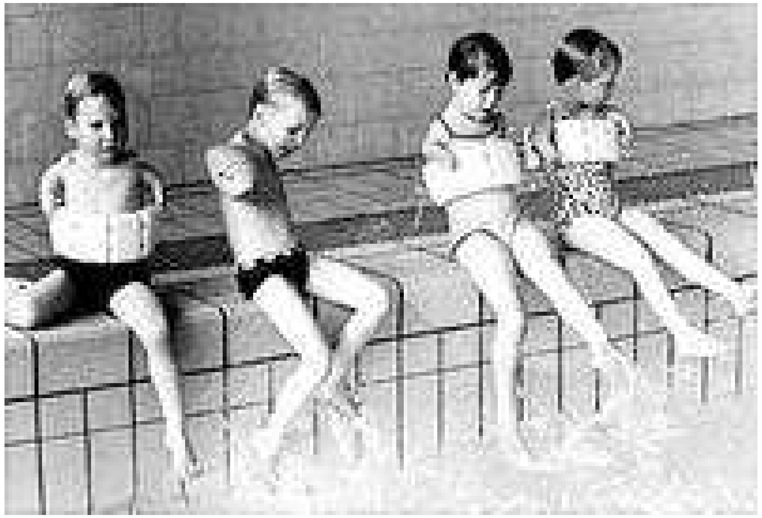


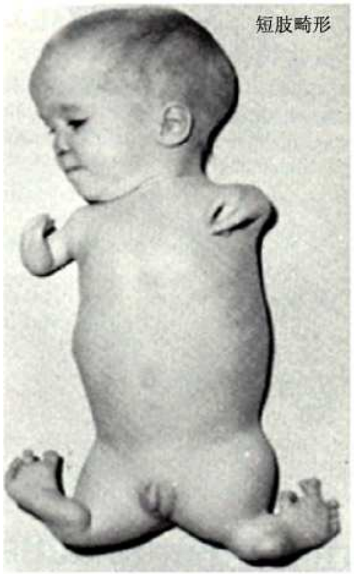
(R)-(+)-Thalidomide (S)-(-)-Thalidomide



- R(+)Thalidomide S(-)Thalidomide
- sedative teratogenic

Part Ⅰ Chirality and chiral pesticide



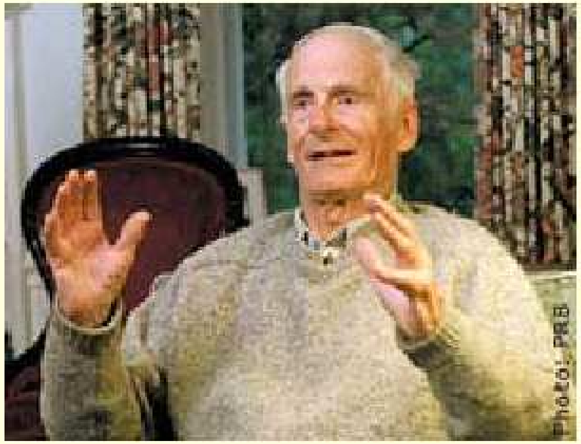


The teratogenic S enantiomer of the thalidomide drug caused a world-shaking tragedy in the 1960s.

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.

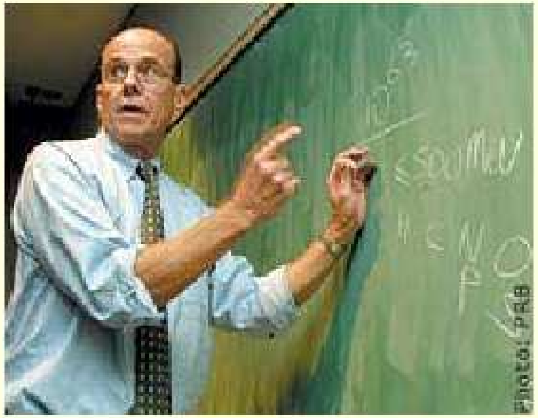
Asymmetric Synthesis

Nobel Prize 2001



诺尔斯
(W. Knowles)

野依良治
(R. Noyori)



夏普莱斯
(K. B. Sharpless)

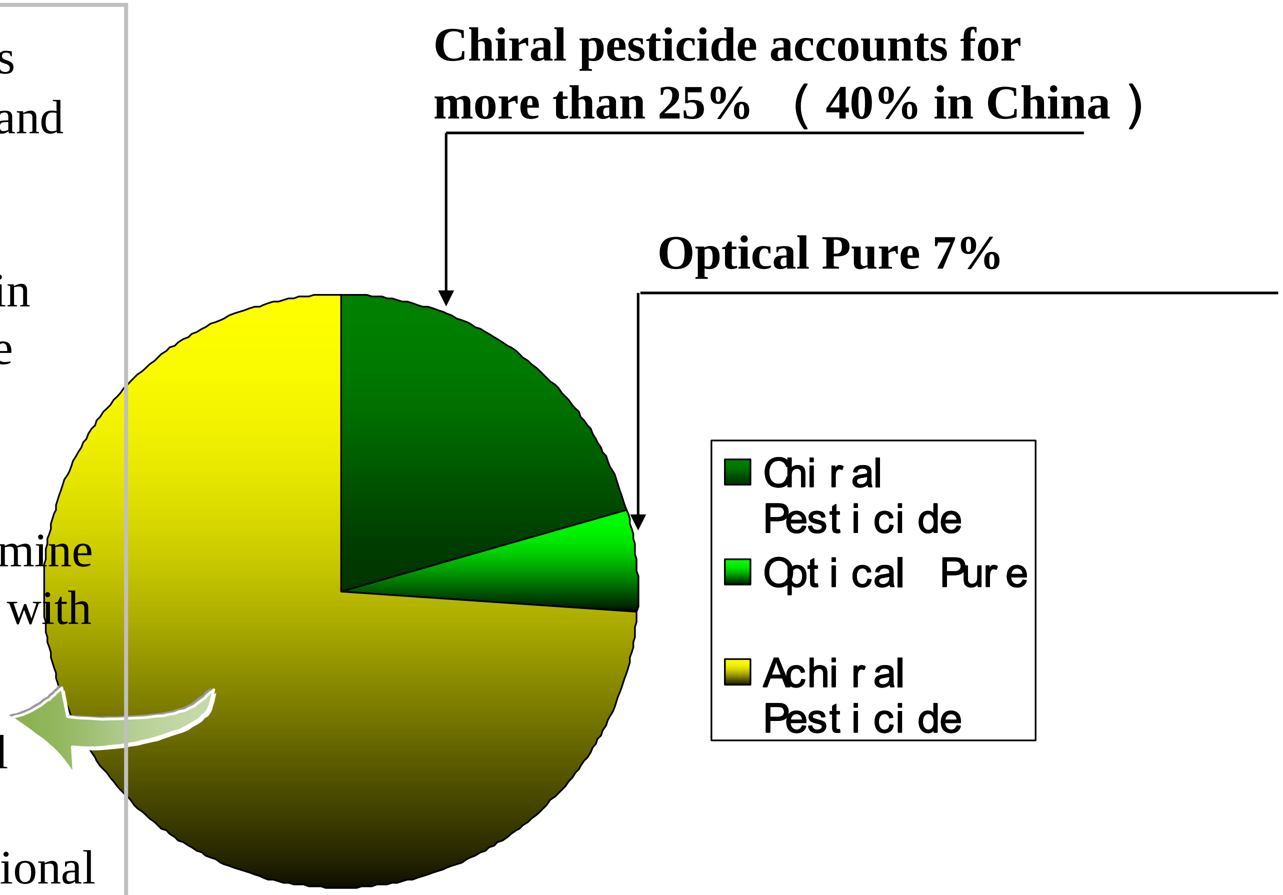
Part Ⅰ Chirality and chiral pesticide

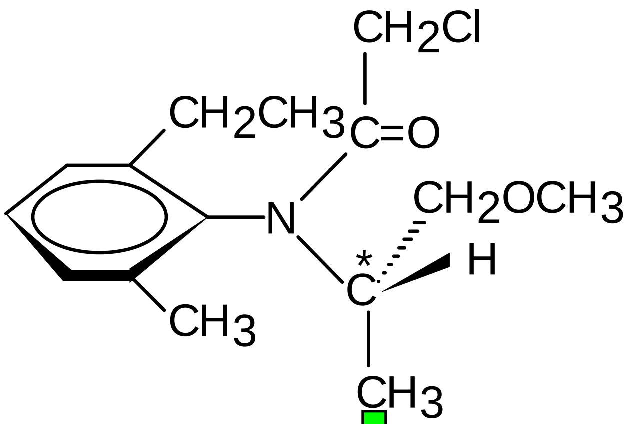
It's of great importance to conduct the enantioselective research on chiral pesticides

- Enantiomers usually possess different biological activities and enantioselective toxicity, the processes of absorption, distribution, and degradation in organism and environment are often enantioselective

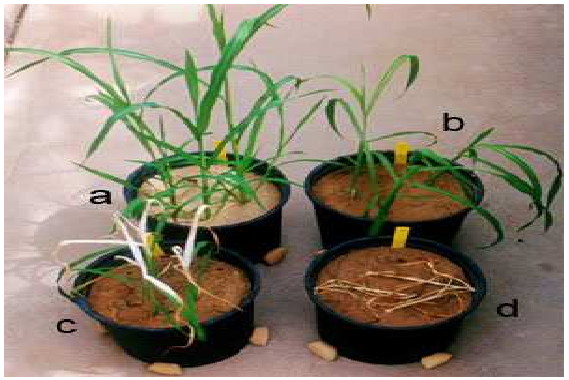
- Environmental studies have historically neglected to determine the adverse effects associated with particular enantiomers

- The risk evaluations of chiral pesticides residue in food and environmental based on traditional methods are not reliable

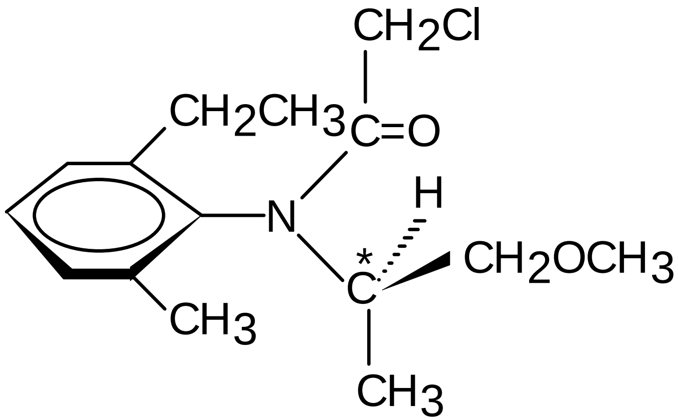




S-metolachlor

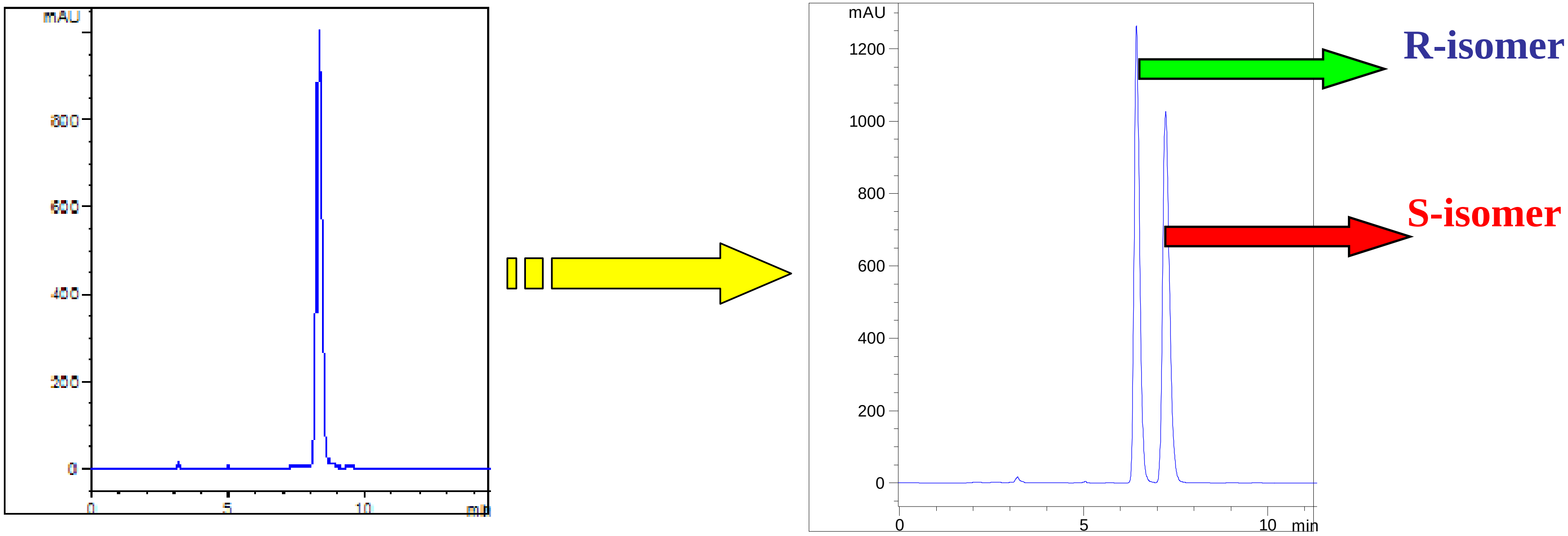


High Herbcidal activity



R-metolachlor

cancerogenic potential



Traditional achiral analytical level

chiral analytical level

- 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.

Part Ⅱ Advance in chiral pesticide separation

- 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.

- *Anal. Chem.*, ***2010***, ***82*** *(12), pp 4712–4722*

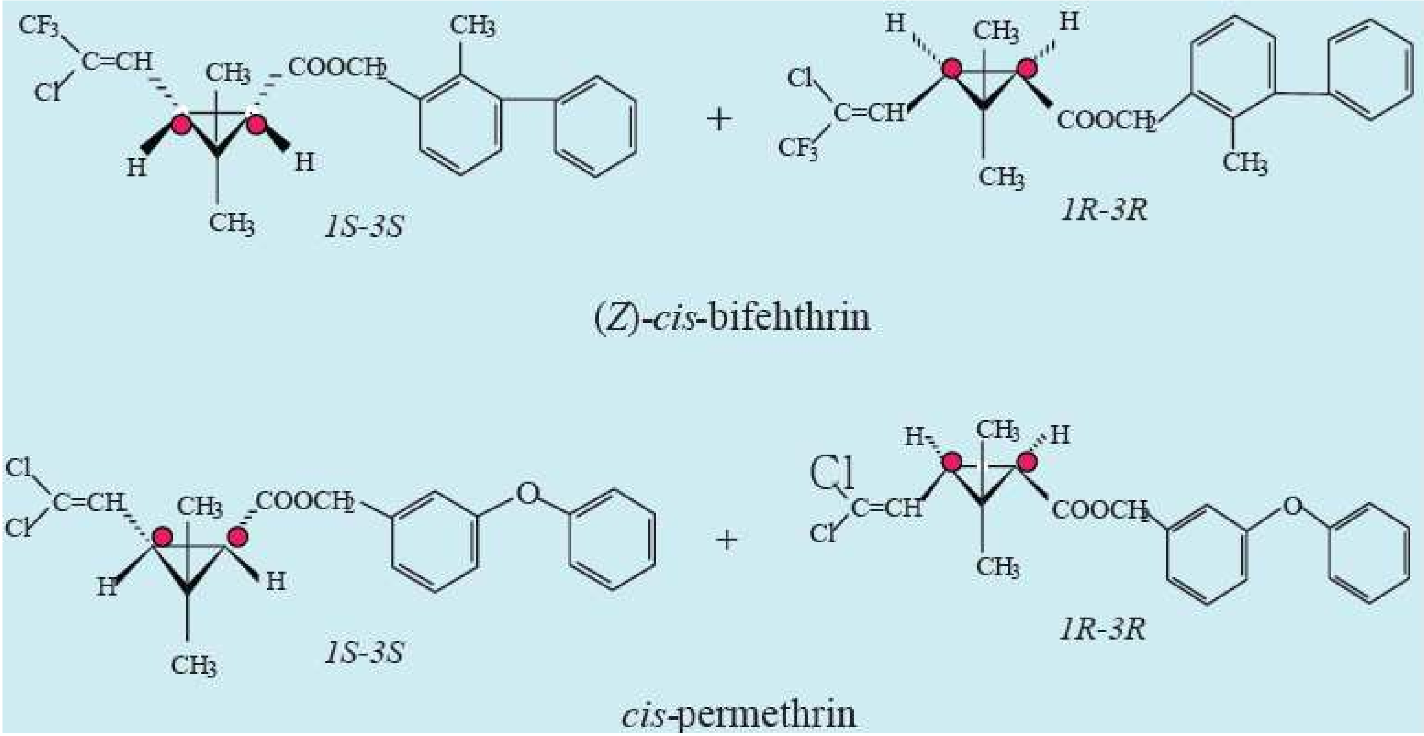
Part Ⅱ Advance in chiral pesticide separation

1. Synthetic pyrethroids

2 and 3 chiral centers (n=2-3)

□2 and 4 diastereomers[$(2-1)^n$]

2, 4, or 8 enantiomers(2^n)



Cis:

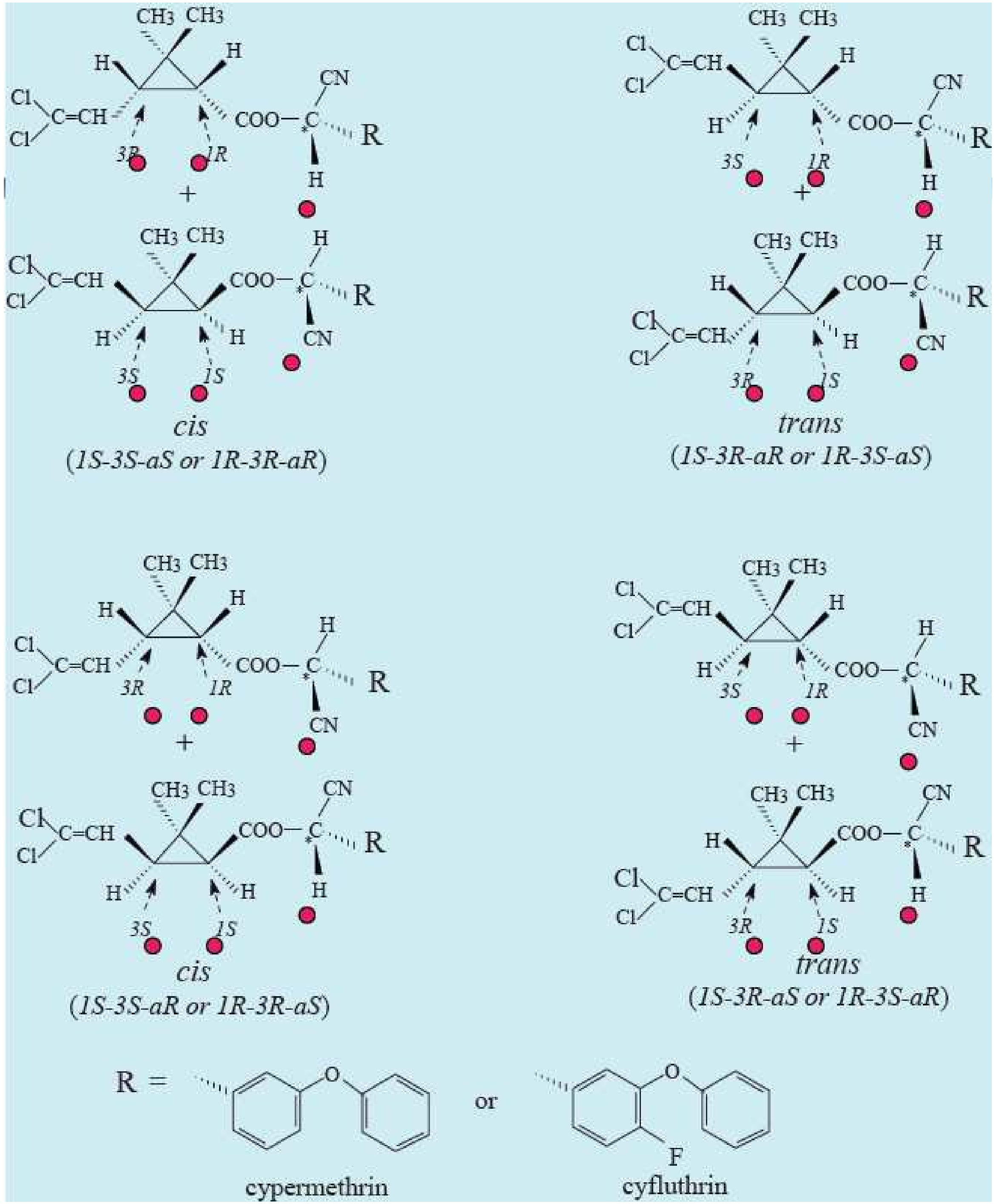
1S-3S

1R-3R

Trans:

S-3R

1R-3S



Cis:

1S-3S-αS

1R-3R-αR

1S-3S-αR

1R-3R-αS

Trans:

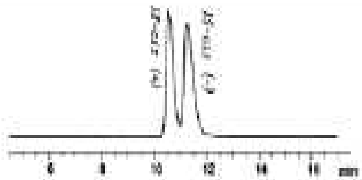
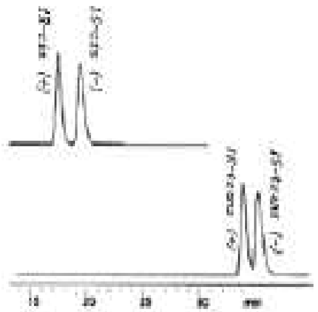
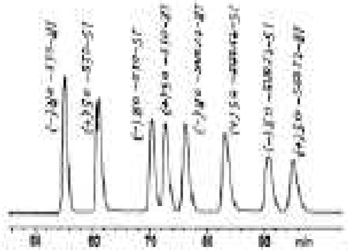
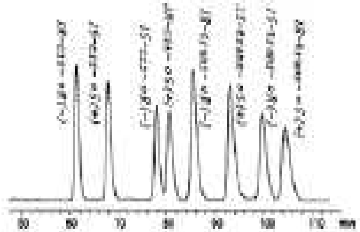
1S-3R-αR

1R-3S-αS

1S-3R-αS

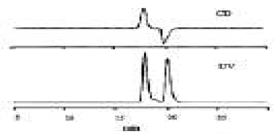
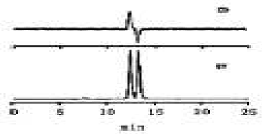
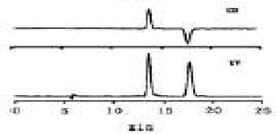
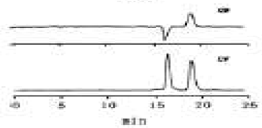
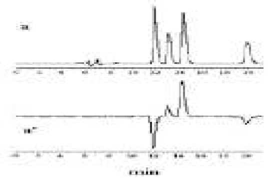
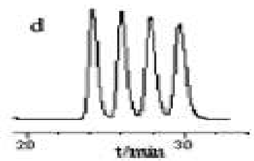
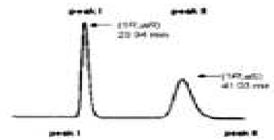
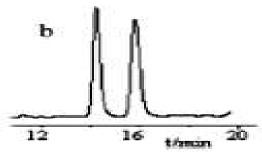
1R-3S-αR

Table 1. Enantioselective separation of synthetic pyrethroids (SPs) by HPLC (I)

Entry	Commercial names	Separation conditions	Chromatograms[a]	Ref.
1	*cis*-Bifenthrin (BF, Fig. 1, 1)	Sumichiral OA-2500I Hexane/1,2-Dichloroethane = 500/1 or 99.5/0.5, room temperature, UV = 230 nm		[1,15,21]
2	*cis*-Permethrin *trans*-Permethrin (PM, Fig. 1, 2)	Sumichiral OA-2500I Hexane/1,2-Dichloroethane = 500/1 or 99.5/0.5, room temperature, UV = 230 nm		[1,15]
3	Cypermethrin (CP, Fig. 3, 3)	Tandem Chirex 00G-3019-OD Hexane/1,2-Dichloroethane/Ethanol = 500/30/0.15, room temperature, UV = 230 nm		[15]
4	Cyfluthrin (CF, Fig. 3, 4)	Tandem Chirex 00G-3019-OD Hexane/1,2-Dichloroethane/Ethanol = 500/30/0.15, room temperature, UV = 230 nm		[15]

[a] A complete separation was defined as when the Rs exceeded 1.5.

Table 2. Enantioselective separation of synthetic pyrethroids (SPs) by HPLC (II)

Entry	Commercial names	Separation conditions	Chromatograms[a]	Ref.
1	Lambda-cyhalothrin (LCT, Fig. 3, 5)	Chiralpak AS Hexane/Ethanol = 95/5 25°C, 0.4 mL/min, UV = 236 nm		[23]
		Chiralpak AD Hexane/Ethanol = 98/2 25°C, 0.4 mL/min, UV = 236 nm		[23]
		Chiralecl OD Hexane/2-Propanol = 95/5, 25°C, 0.5 mL/min, UV = 236 nm		[23]
		Chiralecl OJ Hexane/Ethanol = 95/5, 25°C, 0.6 mL/min, UV = 236 nm		[23]
		Chiralecl OJ Hexane/2-Propanol = 90/10, 25°C, 0.4 mL/min, UV = 236 nm		[23]
2	Fenvalerate (Fig. 3, 8)	(*R*)-1-phenyl-2-(4-methylphenyl)ethylamine amide derivative of (*S*)-valine to aminopropyl silica gel through a 2-amino-3,5-dinitro-1-carboxamido-benzene unit Hexane/1,2-dichloromethane/ethanol = 98.45/1.2/0.35, Room temperature, 1 mL/min, UV = 230 nm		[26]
3	Cycloprothrin (Fig. 3, 10)	Chiralcel OJ-H Hexane/isopropanol = 70/30, 35°C, 1 mL/min, UV = 254 nm		[25]
		Chiralcel OD-H Hexane/isopropanol = 90/10, 35°C, 1 mL/min, UV = 254 nm		
4	Fenpropathrin (Fig. 3, 11)	(*R*)-1-phenyl-2-(4-methylphenyl)ethylamine amide derivative of (*S*)-valine to aminopropyl silica gel through a 2-amino-3,5-dinitro-1-carboxamido-benzene unit Hexane/1,2-dichloromethane = 94/6, Room temperature, 1 mL/min, UV = 230 nm		[26]

[a] A complete separation was defined as when the Rs exceeded 1.5.

Part Ⅱ Advance in chiral pesticide separation

- 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:

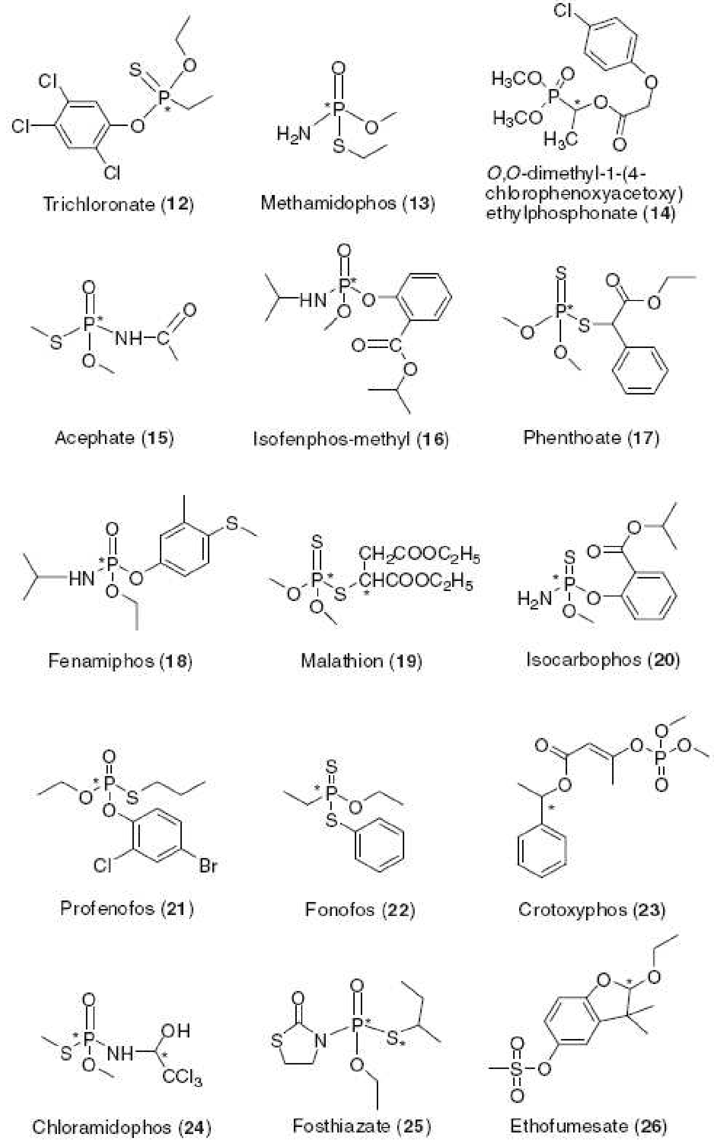
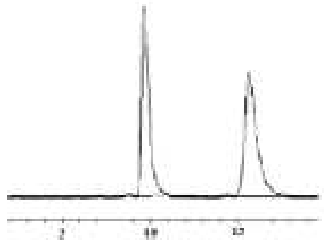
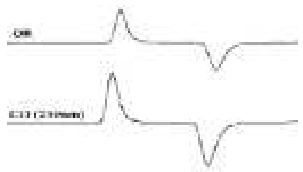
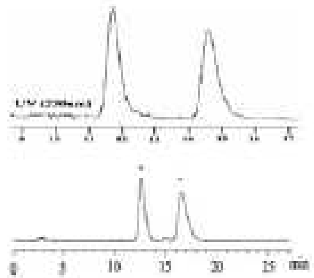
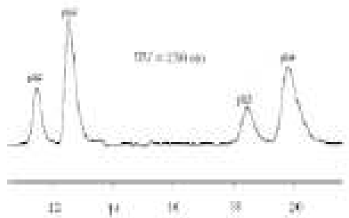


Figure 4. Chemical structures of organophosphorous pesticides (OPs).

Table 3. Enantioselective separation of organophosphates (OPs) by HPLC

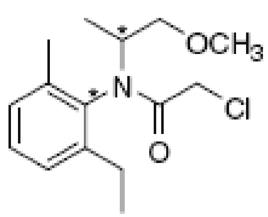
Entry	Commercial names	Separation conditions	Chromatograms[a]	Ref.
1	Fenamiphos (Fig. 4, **18**)	Whelk-OJ Hexane/2-Propanol = 95/5, room temperature, UV = 210 nm,		[39]
2	Methamidophos (Fig. 4, **13**)	Chiracel OD Hexane/2-Propanol = 80/20, 25°C, 0.5 mL/min, UV = 230 nm		[31]
3	Trichloronate (Fig. 4, **12**)	Chiralcel OJ Hexane/Heptane/EtOH = 90/5/5, 21°C, 1.0 mL/min, UV = 300 nm		[13]
4	Chloramidophos (Fig. 4, **24**)	CHIRALPAK AD Hexane/EtOH = 90/10, 25°C, 1.0 mL/min, UV = 230 nm		[43]
5	Fosthiazate (Fig. 4, **25**)	CHIRALPAK AD Hexane/EtOH = 95/5, Room temperature, 1.0 mL/min, UV = 230 nm		[44]

[a] A complete separation was defined as when the Rs exceeded 1.5.

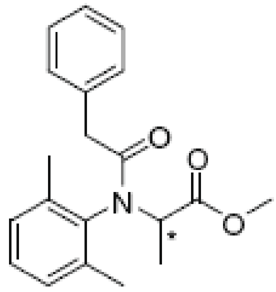
Part Ⅱ Advance in chiral pesticide separation

- The above studies showed that the OPs having two pairs of enantiomers can normally be separated on Chiralpak AD.

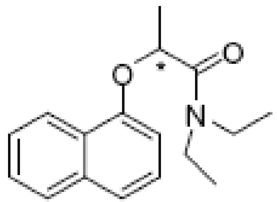
3. **Acylanilides**



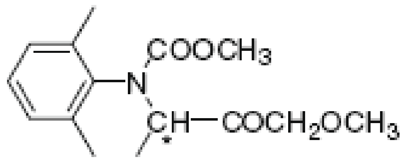
Metolachlor (27)



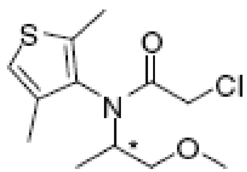
Benalaxyl (28)



Napropamide (29)

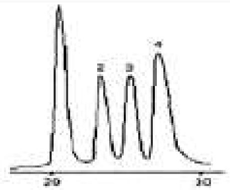
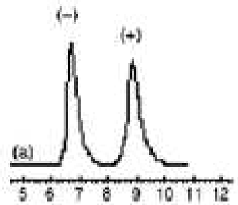
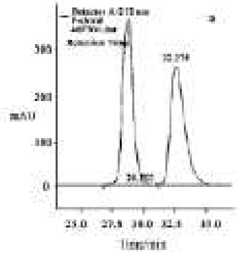
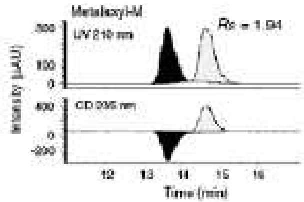
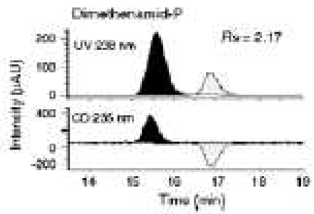


Metalaxyl (30)



Dimethenamid (31)

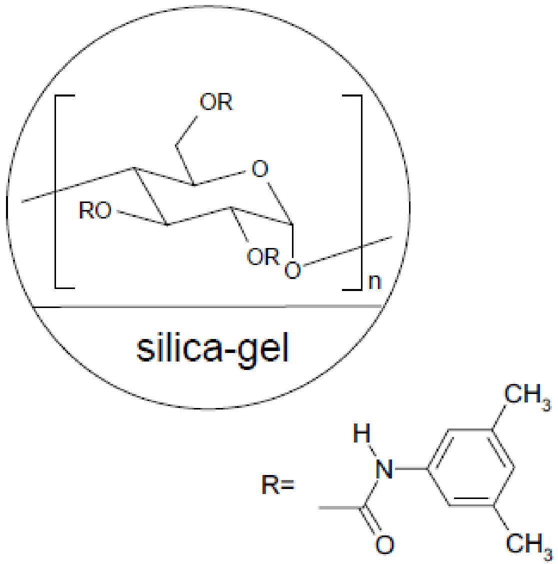
Figure 5. Chemical structures of acylanilides.

Table 4. Enantioselective separation of acylanilide herbicides by HPLC				
Entry	**Commercial names**	**Separation conditions**	**Chromatograms**[a]	**Ref.**
1	Metolachlor (Fig. 5, **27**)	Chiralcel OD-H Hexane/Diethyl ether = 91/9, 20°C, 0.8 mL/min, UV = 230 nm		[11]
2	Benalaxyl (Fig. 5, **28**)	(*R,R*) Whelk-O 1 Hexane/isopropanol = 70/30, 20°C, 1.0 mL/min, UV = 220 nm		[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		[51]
4	Metalaxyl (Fig. 5, **30**)	Chiralpak AD-H Hexane/ethanol = 60/40, 0°C, 0.5 mL/min, UV = 210 nm		[53]
5	Dimethenamid (Fig. 5, **31**)	Chiralpak AD-H Hexane/ethanol = 90/10, 0°C, 0.5 mL/min, UV = 238 nm		[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 .



Part Ⅱ Advance in chiral pesticide separation

4. Imidazolinones

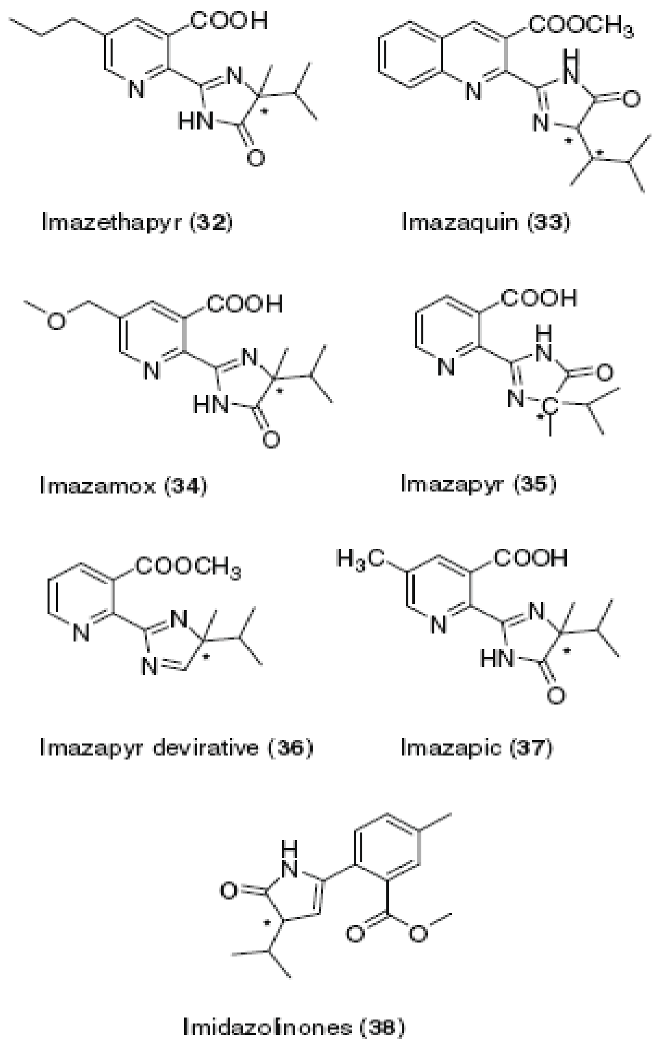
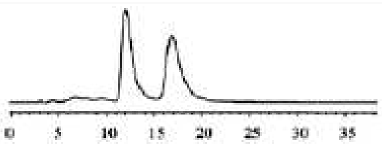
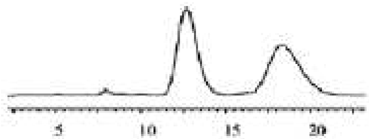
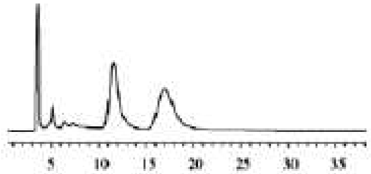
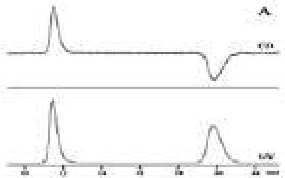
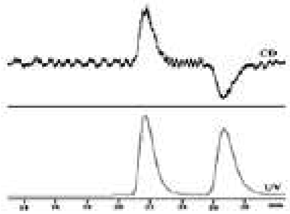
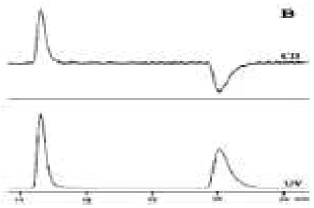


Figure 6. Chemical structures of imidazolinones.

Table 5. Enantioselective separation of imidazolinone herbicides by HPLC

Entry	Commercial names	Separation conditions	Chromatograms	Ref.
1	Imazamox (Fig. 6, **34**)	Chiralcel OJ Hexane/Alcohol/TFA[a] = 75/25/0.1, room temperature, 1.0 mL/min, UV = 254 nm		[12]
2	Imazapyr derivative (Fig. 6, **36**)	Chiralcel OJ Hexane/Alcohol/TFA = 90/10/0.1, room temperature, 1.0 mL/min, UV = 254 nm		[12]
3	Imazapic (Fig. 6, **37**)	Chiralcel OJ Hexane/Alcohol/TFA = 75/25/0.1, room temperature, 1.0 mL/min, UV = 254 nm		[12]
4	Imazethapyr (Fig. 6, **32**)	Chiralcel OJ Hexane/2-Propanol/Acetic acid = 84.6/15.4/0.1, 25°C, 0.8 mL/min, UV = 275 nm		[56]
5	Imazaquin (Fig. 6, **33**)	Chiralcel OJ-H Hexane/2-Propanol/Acetic acid = 84.6/15.4/0.1, 25°C, 0.8 mL/min, UV = 275 nm		[56]
6	Imazapyr (Fig. 6, **35**)	Chiralcel OJ Hexane/2-Propanol/Acetic acid = 84.6/15.4/0.1, 25°C, 0.8 mL/min, UV = 275 nm		[56]

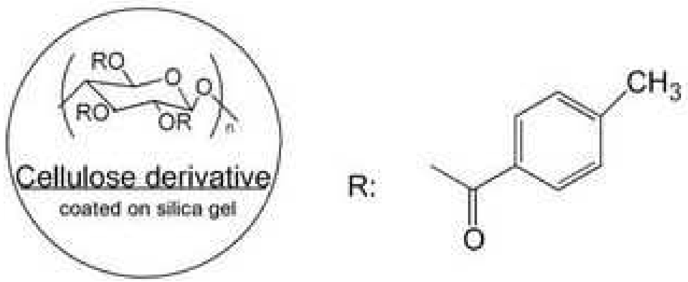
[a] TFA: trifluroacetic acid.

Advance in chiral pesticide separation

- As for Imidazolinones herbicides, OJ column is the best choice for their HPLC resolution.

CHIRALCEL®OJ-H

150 x 4.6 mm ID
250 x 4.6 mm ID
250 x 10 mm ID
250 x 20 mm ID



Part Ⅱ Advance in chiral pesticide separation

5. Triazole fungicides

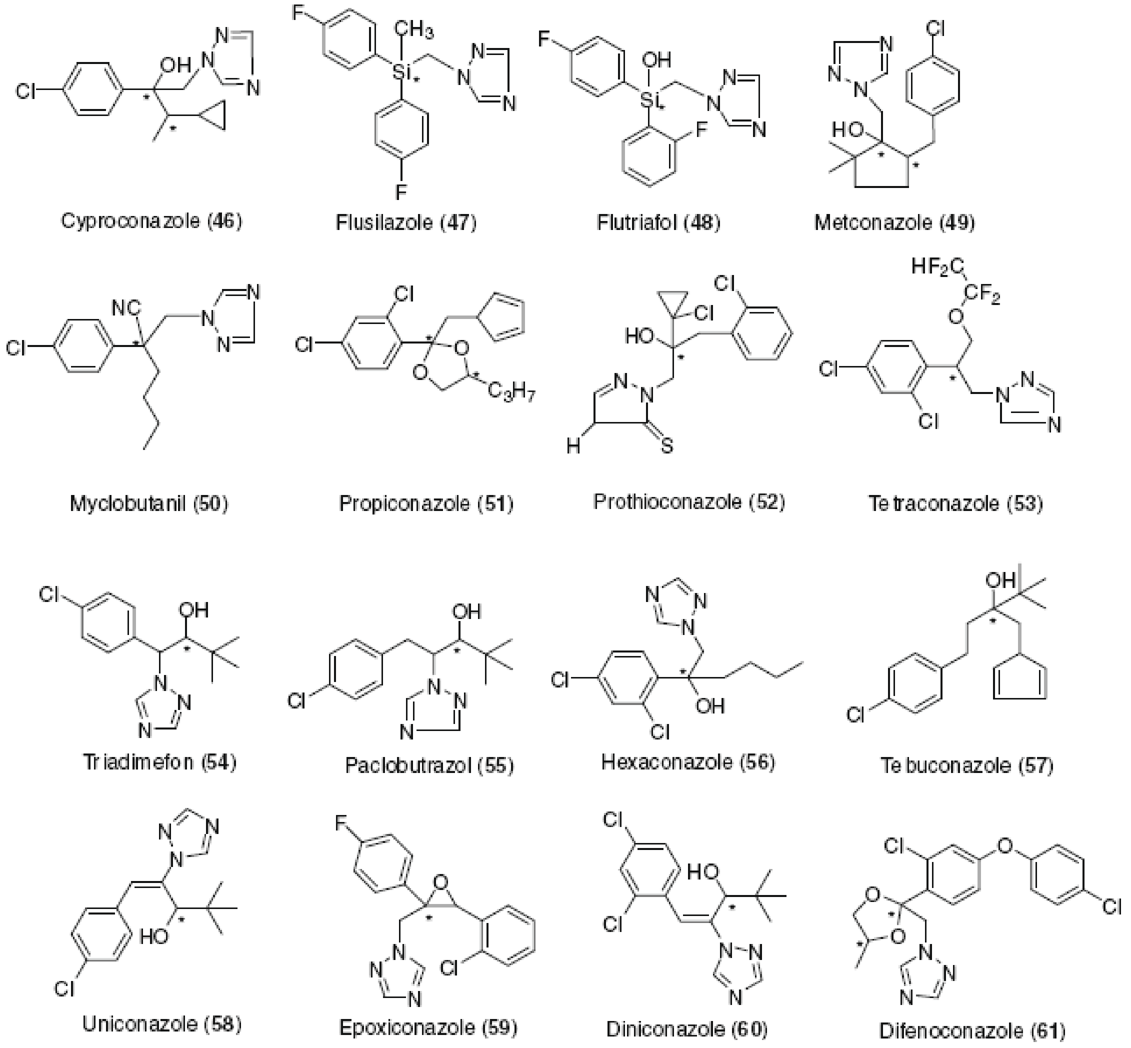
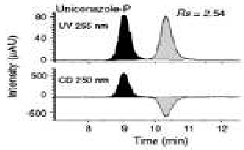
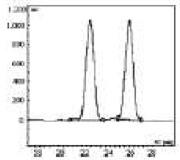
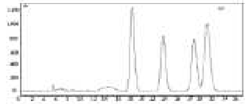
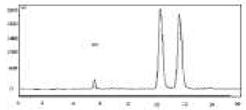
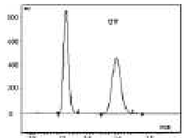
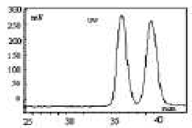
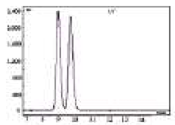
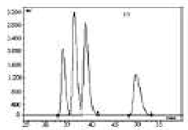


Figure 8. Chemical structures of triazole-related fungicides.

Table 7. Enantioselective separation of triazole fungicides and related chiral pesticides by HPLC

Entry	Commercial names	Separation conditions	Chromatograms[a]	Ref.
1	Paclobutrazol (Fig. 8, 55)	Tris-(*S*)-1-phenylethylcarbamate CSP Hexane/2-Propanol = 95/5, 20°C, 1.0 mL/min, UV = 230 nm		[62]
2	Uniconazole (Fig. 8, 58)	Chiralpak AD-H Hexane/isopropanol = 80/20, 30°C, 0.5 mL/min, UV = 255 nm		[53]
3	Flutriafol (Fig. 8, 48)	Chiralcel OD-H Hexane/Ethanol = 90/10, 15°C, 1.0 mL/min, UV = 230 nm		[68]
4	Propiconazole (Fig. 8, 51)	Chiralcel OD-H Hexane/2-Propanol = 90/10, 15°C, 1.0 mL/min, UV = 230 nm		[68]
5	Triadimefon (Fig. 8, 54)	Chiralcel OD-H Hexane/2-Propanol = 90/10, 25° C, 1.0 mL/min, UV = 228 nm		[68]
6	Hexaconazole (Fig. 8, 56)	Chiralcel OD-H Hexane/2-Propanol = 90/10, 25°C, 1.0 mL/min, UV = 230 nm		[68]
7	Tebuconazole (Fig. 8, 57)	Chiralcel OD-H Hexane/2- Propanol = 90/10, 25°C, 1.0 mL/min, UV = 227 nm		[68]
8	Diniconazole (Fig. 8, 60)	Chiralcel OD-H Hexane/2-Propanol = 90/10, 25°C, 1.0 mL/min, UV = 253 nm		[68]
9	Difenoconazole (Fig. 8, 61)	Chiralcel OJ-H Hexane/Ethanol = 90/10, 25°C, 1.0 mL/min, UV = 230 nm		[68]

[a] A complete separation was defined as when the Rs exceeded 1.5.

Part Ⅱ Advance in chiral pesticide separation

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

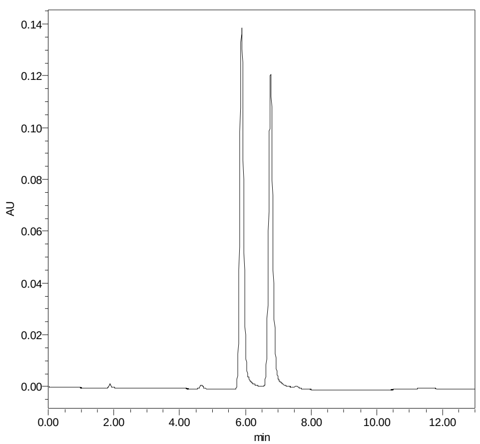
Part Ⅱ Advance in chiral pesticide separation

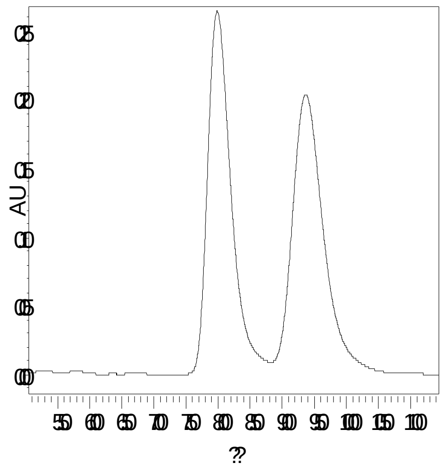
- With the development of chiral separation materials, nowadays, reversed CSP with more and more frequently used for resolution of chiral pesticides.

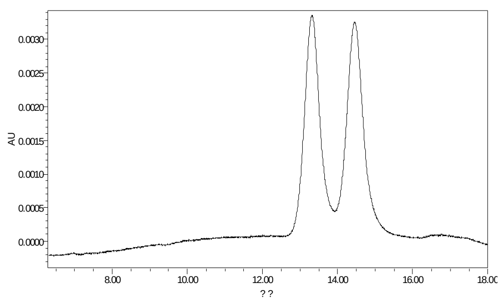
- *CAU* laboratory have successfully separated more than 20 chiral pesticides under reversed phase condition such as :
- paclobutrazol, myclobutanil *(Anal. Methods, 2010, 2, 617–622)*
- Epoxiconazole, terallethrin, benalaxyl, pyriproxyfen and diclofopmethyl *(J. Sep. Sci. 2007, 30, 310 – 321)*
- hexaconazole, flutriafol, diniconazole, tebuconazole, uniconazle 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*).

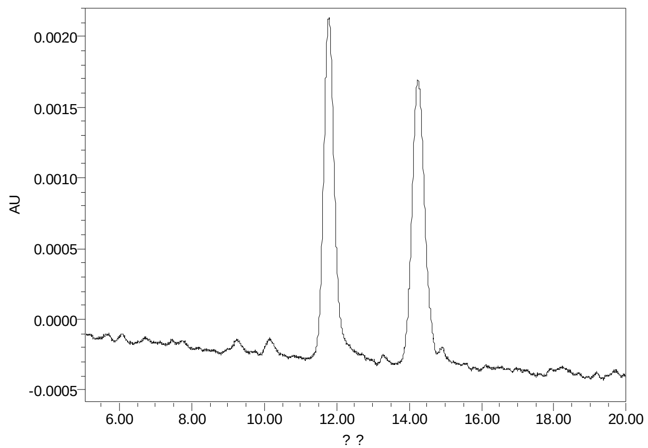
Part Ⅱ Advance in chiral pesticide separation

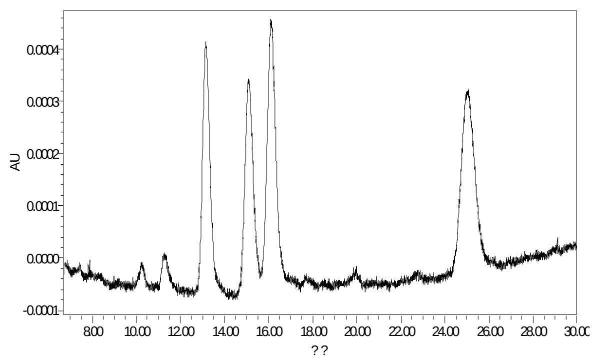
- some chiral pesticides under reversed phase condition :myclobutanil, simeconazole, tebuconazle , fenbuconazole and its metabolize in our lab.











RP chiral column

Chiral detector

LC/MS/MS System

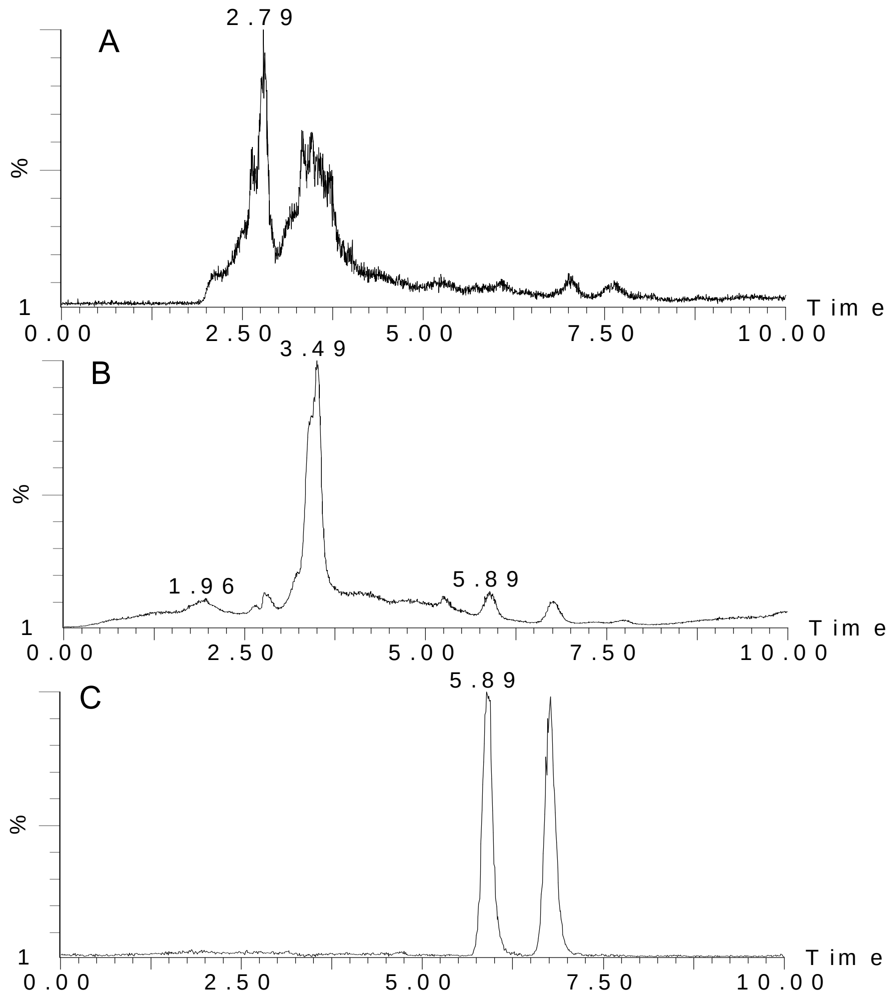
RP HPLC System

Part Ⅱ Advance in chiral pesticide separation

The virtue of RP HPLC

- (1)RP HPLC can be easily to combine with mass sepctormetry; it is helpful to compound qualification.
- (2) establish more sensitive and efficient multi-enantiomer analytical method

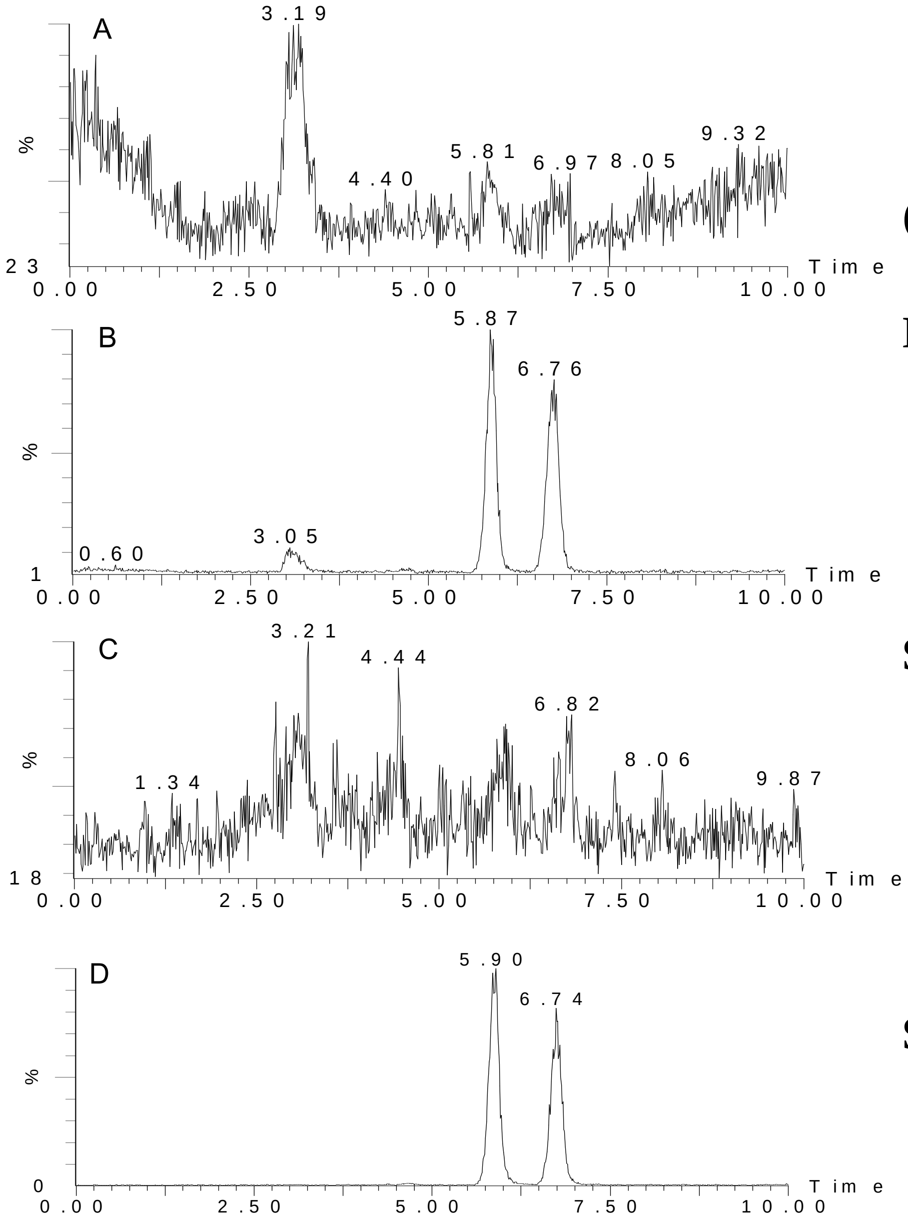
Part Ⅱ Advance in chiral pesticide separation



Chromatograms of myclobutanil enantiomers in cucumber sample

A: Full Scan mode B: SIM mode, C: MS/MS mode

Part Ⅱ Advance in chiral pesticide separation



Chromatograms of myclobutanil:

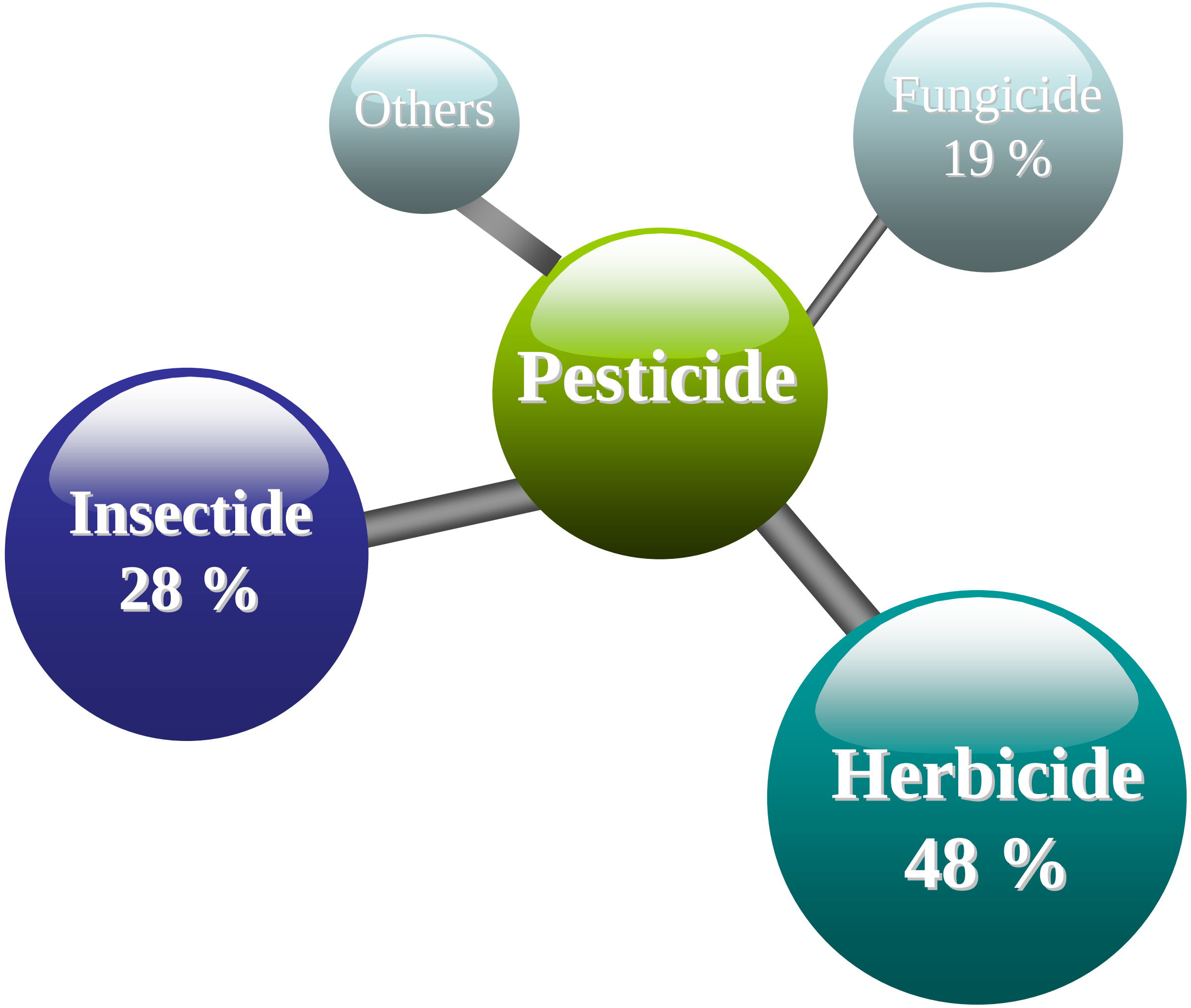
(A) black cucumber sample,

(B) Spiked cucumbert sample (0.01 mg/kg),

（C）black soil sample，

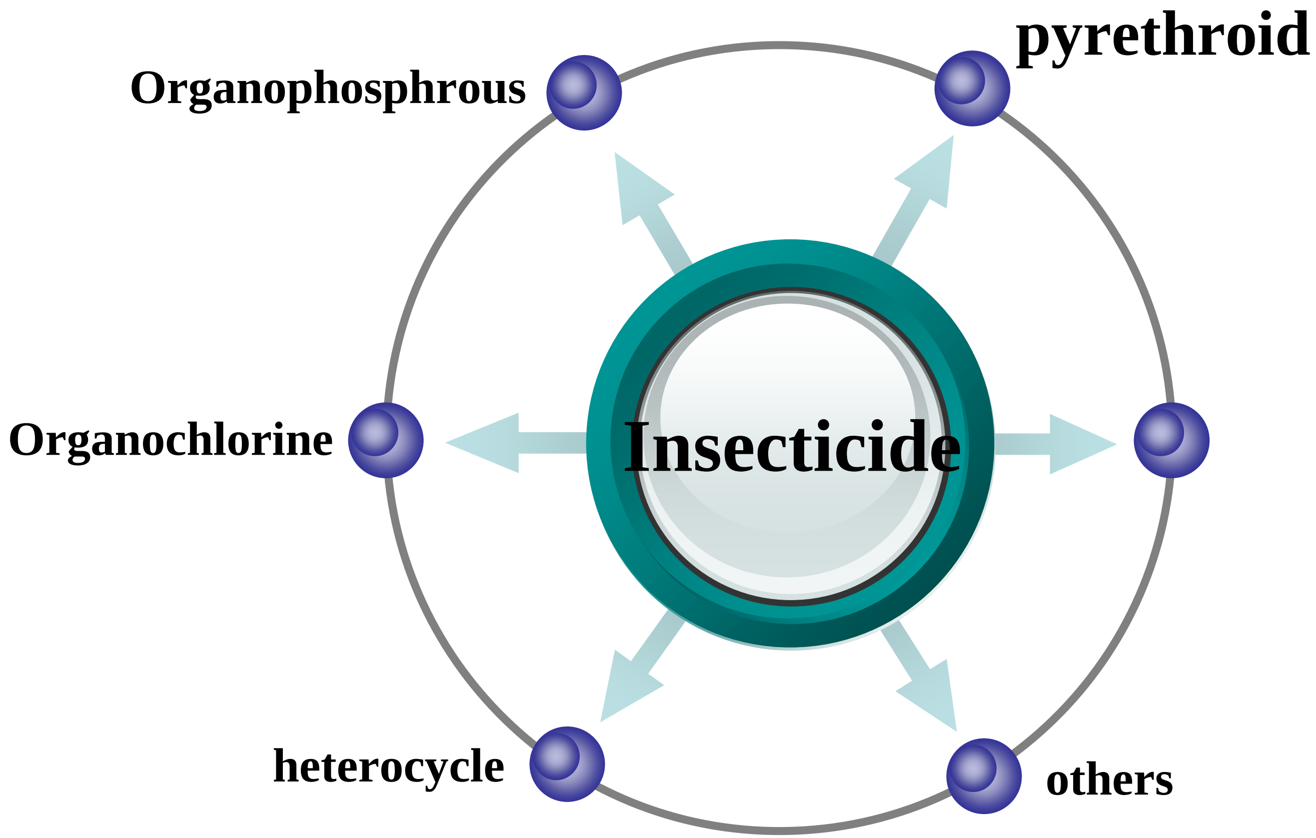
（D）Spiked soil sample(0.01 mg/kg ）

Advance in chiral pesticide behavior



Reference： USEPA. Pesticide industry sales and usage [EB/OL]. (2004) http://www.epa.gov/oppbeadl/pestsales/

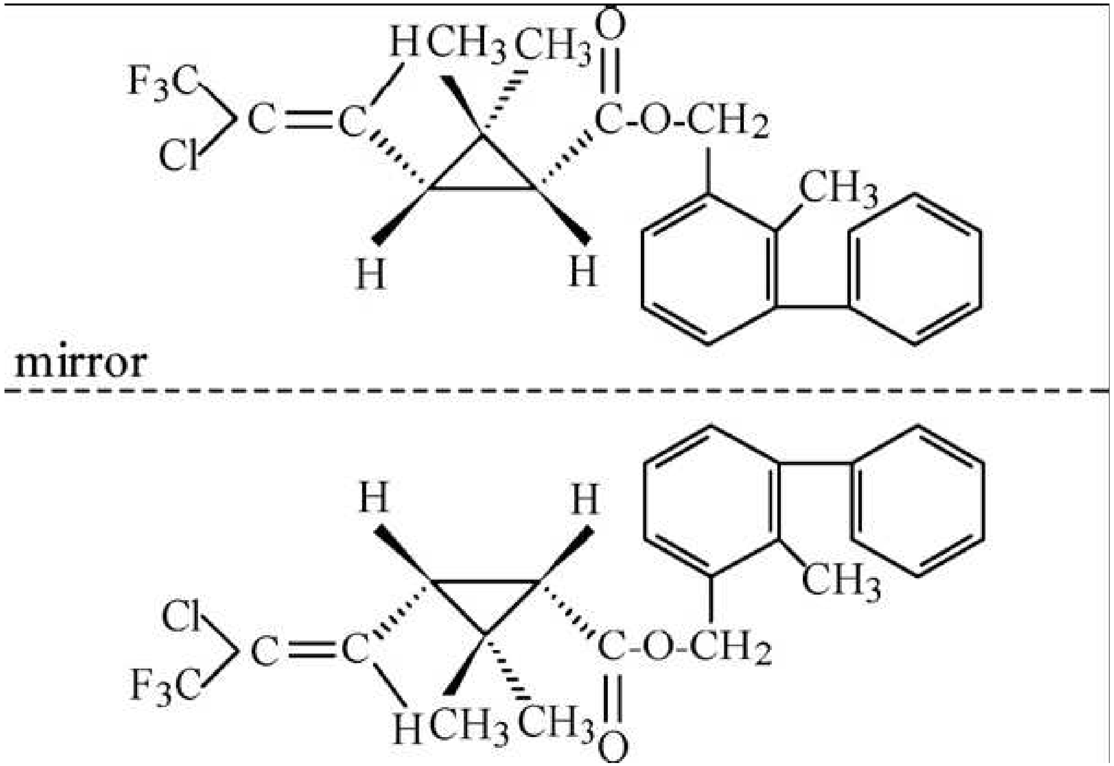
Part III Advance in chiral pesticide behavior



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



Part III

Advance in chiral pesticide behavior

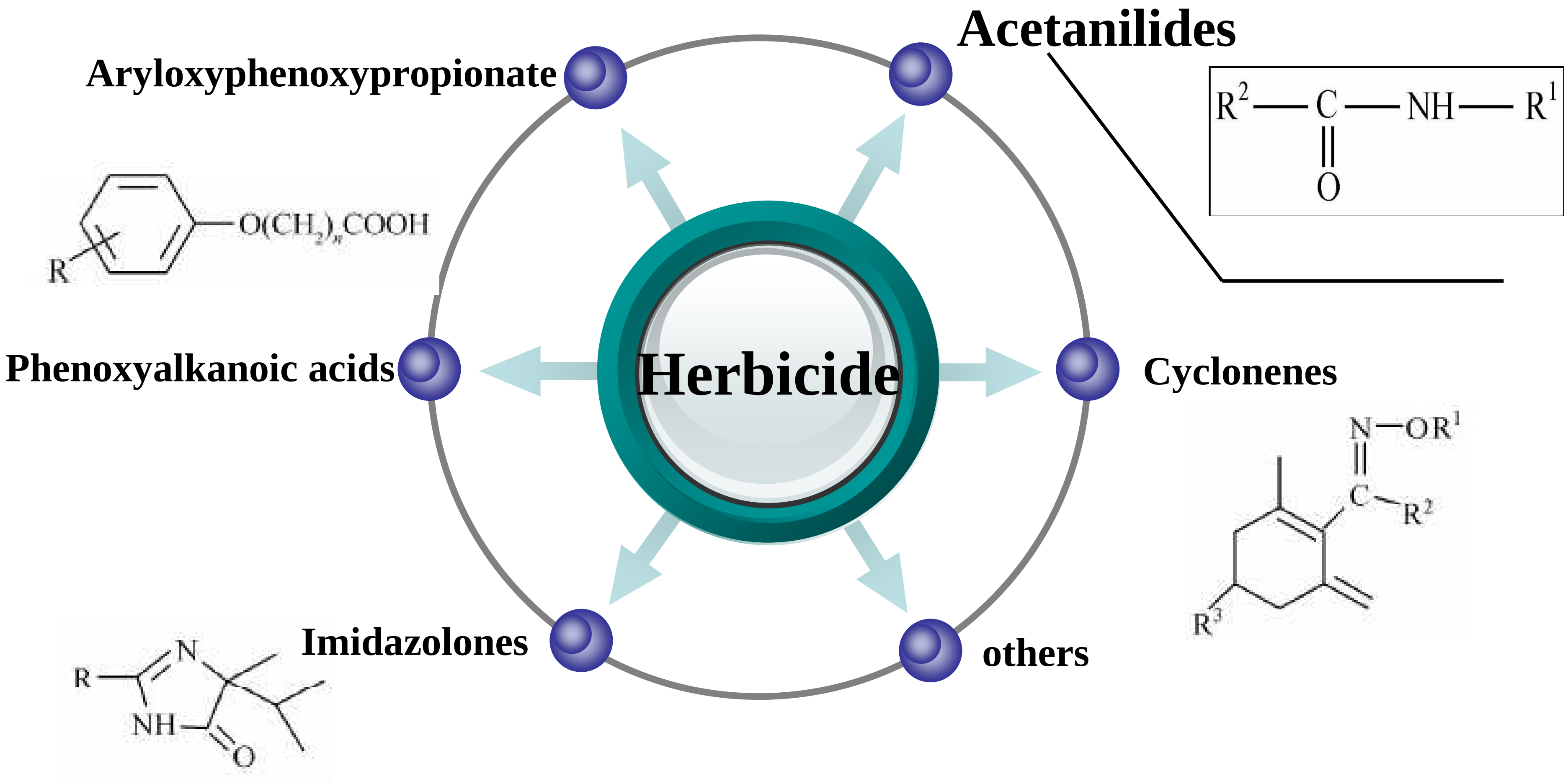
Enantioselective cytotoxicity in human amnion epithelial (FL)cells -Conclusion

- (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-*cis*-BF and 1S-*cis*-BF was more than 300-fold. This results are in good agreement with some other studies which reported that 1R-*cis*-BF is markedly active on the target organism (*Liu et al.*, *chirality* , *2005*, *17*, *127*-*133*).

Part III Advance in chiral pesticide behavior

- (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.

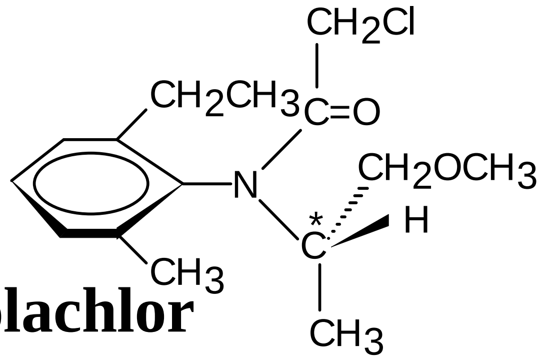
Part Ⅲ Advance in chiral pesticide behavior



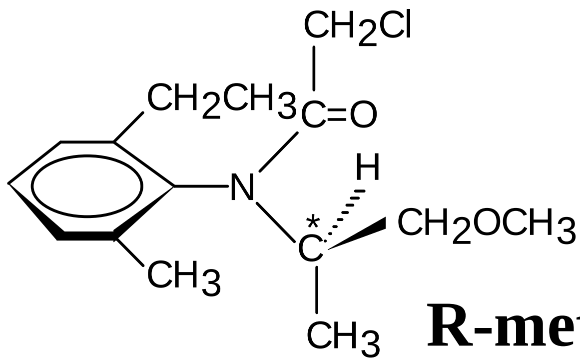
Part III

Advance in chiral pesticide behavior

Enantioselectivity of the bioactivity   **Acetanilides herbicides**

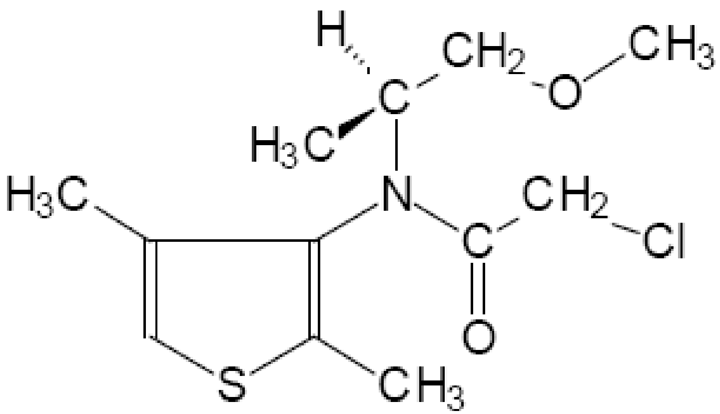


S-metolachlor



R-metolachlor

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



dimethenamid-p

The S-dimethenamid has much higher herbicidal activity than the R-dimethenamid

Part III Advance in chiral pesticide behavior

Enantioselectivity of the bioactivity **Imidazolones herbicides**

The enantiomers of Imidazolones herbicides possess different herbicidal activity, gerenally, 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.

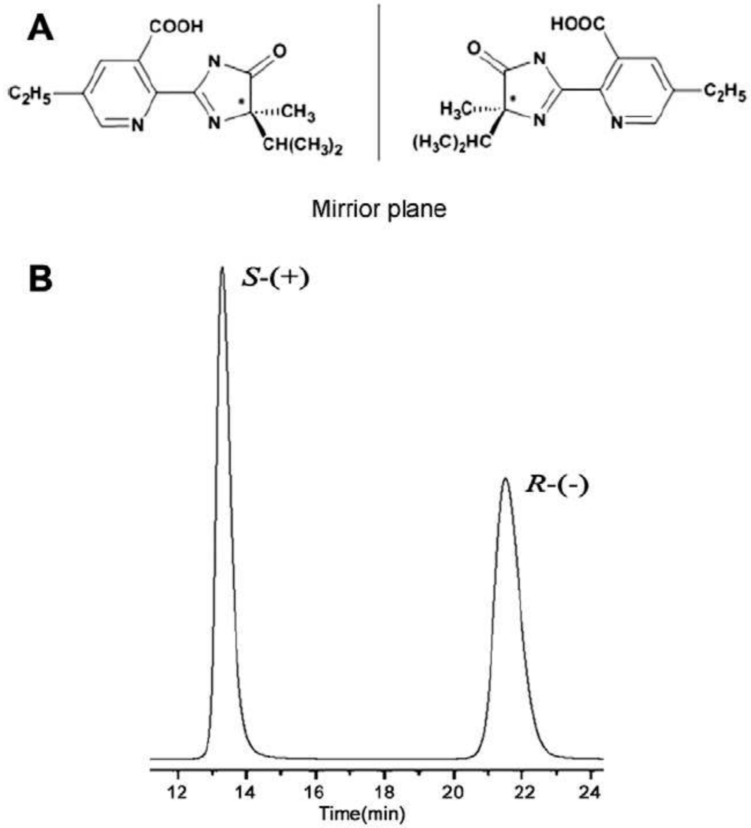


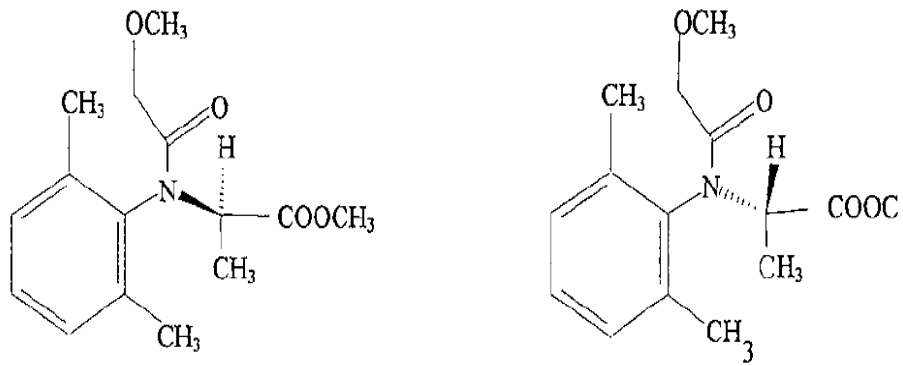
Fig. 1. (A) Chemical structures of Imazethapyr (IM) with "*" indicate the asymmetric position. (B) The HPLC chromatogram shows enantiomeric separation of imazethapyr (IM) on a CHIRALCEL OJ-H column.

Part III

Advance in chiral pesticide behavior

Enantioselectivity of the environmental behavior **Acetanilides herbicides**

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.



(-)-(R)-Metalaxyl (+)-(S)-Metalaxyl

Fig. 1. Structures of the two enantiomers of Metalaxyl.

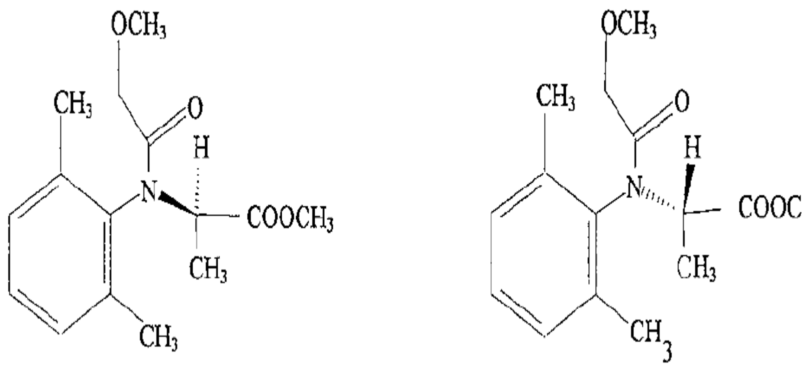
Metalaxyl

Advance in chiral pesticide behavior

Enantioselectivity of the environmental behavior **Acetanilides herbicides**

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 (kR > kS), leading to residues with a composition [S] > [R]. However, in aerobic soils with pH 4−5, both enantiomers were degraded at similar rates (kR ≈ kS), and in aerobic soils with pH < 4 and in most anaerobic soils, the enantioselectivity was reversed (kR < kS)

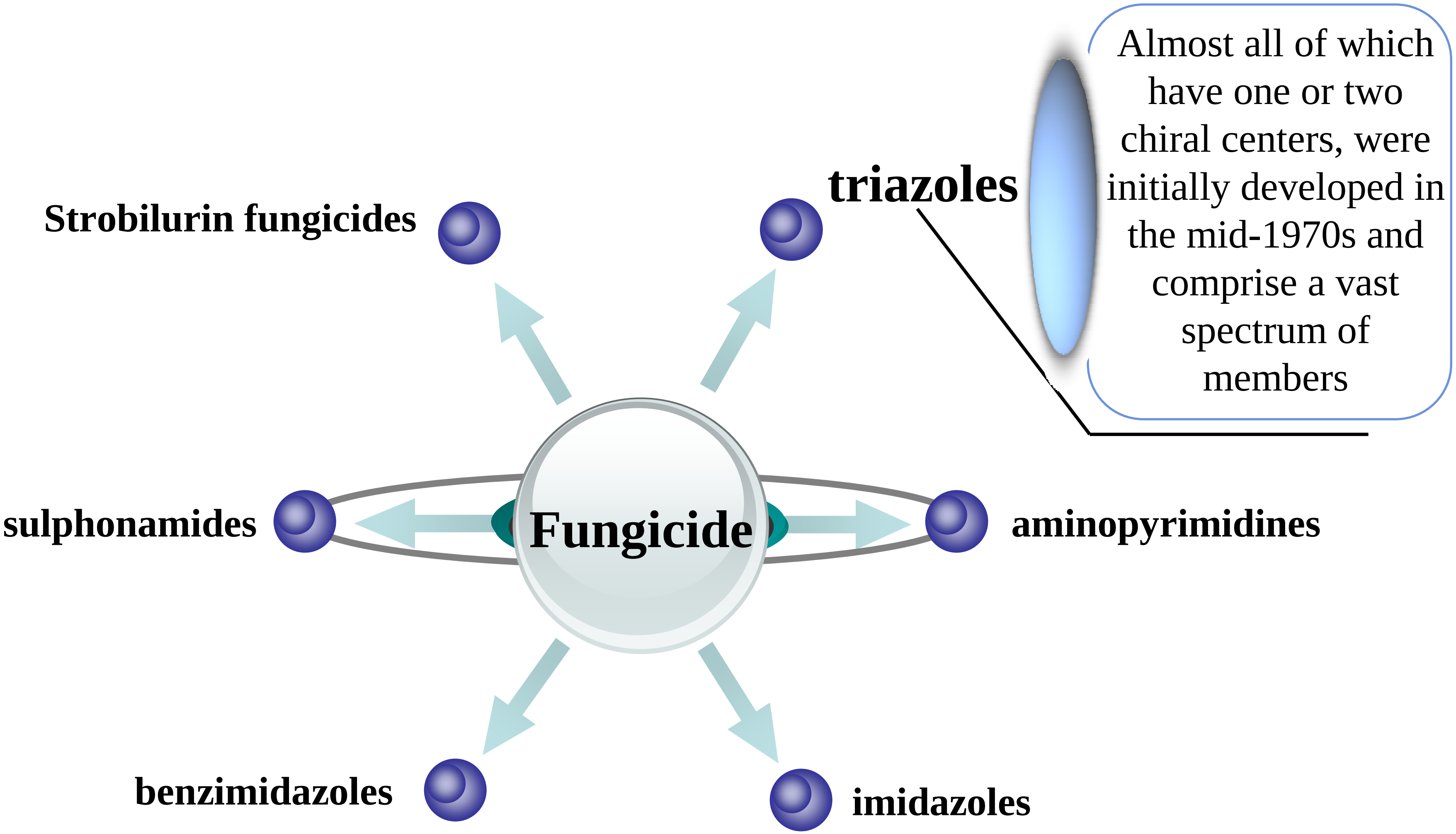


(-)-(R)-Metalaxyl (+)-(S)-Metalaxyl

Fig. 1. Structures of the two enantiomers of Metalaxyl.

Metalaxyl

Part III Advance in chiral pesticide behavior



Advance in chiral pesticide behavior

Table 1. bioactivity and ecotoxicity

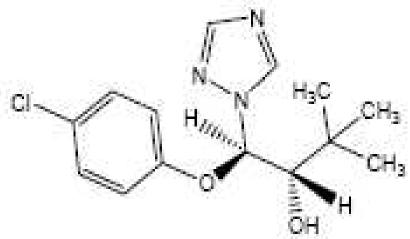
compound	enantiomer Bioactivity	enantiomer ecotoxicity	Racemate ecotoxicity
triadimefon	R isomer=S isomer	No date	reproductive defects(*,**)
triadimenol	RS 1000 times	No date	Low toxicity
tebuconazole	(-) isomer>(+) isomer	No date	Rat Liver defects，possible human carcinogen(**)
Myclobutanil	No data	No date	Potential reproductive defects*
difenoconazole	No data	No date	Fish high toxicity(**)
simeconazole	No data	No date	Low toxicity
propiconazole	No data	No date	Rat Liver defects，possible human carcinogen(**)

注：(*Goetz，2007, 2009; **Nesheim, O.N. 2002.)

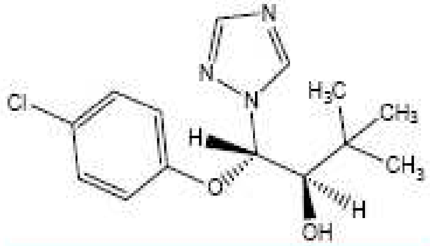
Part Ⅲ Advance in chiral pesticide behavior

Biological activities of triazole fungicides enantiomers differ greatly

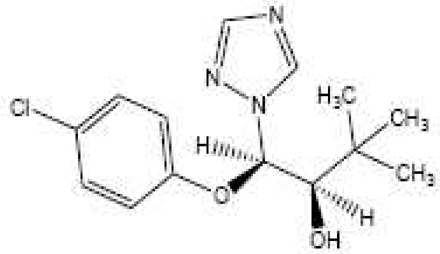
- Triadimenol contains two chiral center, of which has 4 enantiomers(RS、SR、RR and SS)；The biological activities of SR was 1000 times higher than the other 3 enantiomers



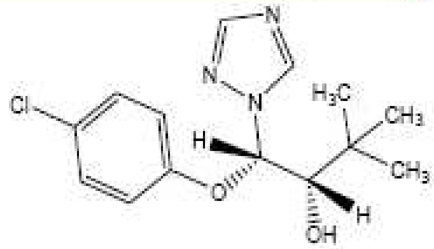
RS-triadimenol enantiomer (low activity)



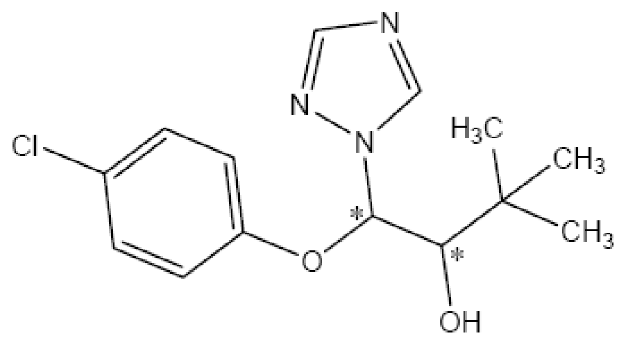
SR-triadimenol enantiomer (high activity)



RR-triadimenol enantiomer (low activity)



SS-triadimenol enantiomer (low activity)



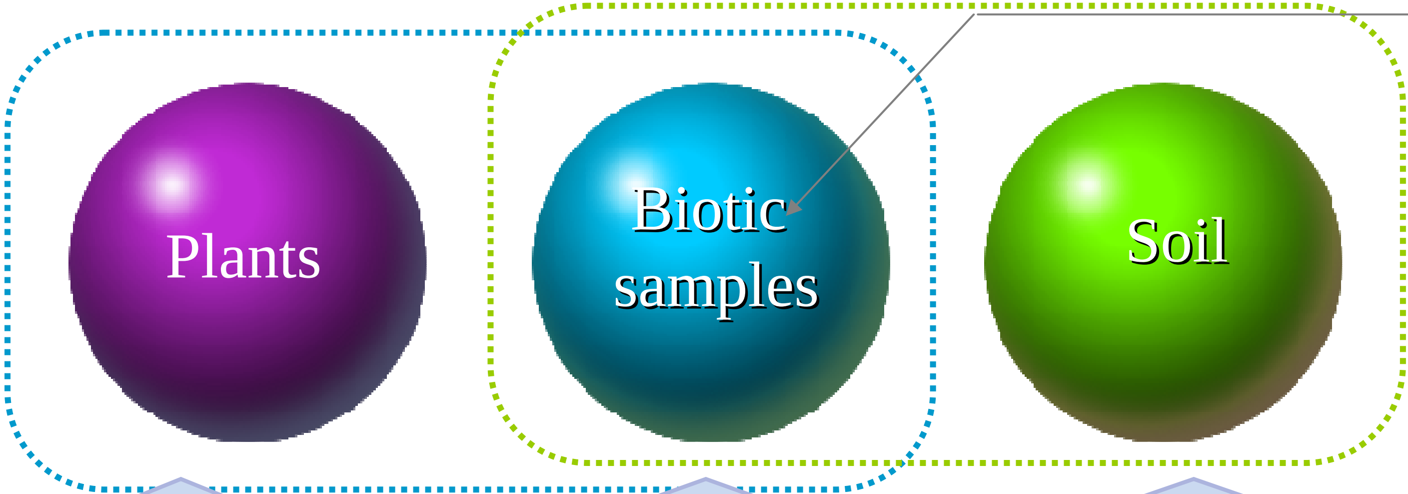
Triadimenol

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



Enantioselective degradation of triadimenol in cucumber

Professor **Yongquan Zheng**

Research team

Reaearch team of Prof.**Zhou zhiqiang**

Keneke *et al*

Clark-**triadimenol**

Buerge-**flusilazole**

Part Ⅲ Advance in chiral pesticide behavior

Enantioselective degradation in biotic samples

Biotic samples

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 toxicties was inclined to be accumulated.

Part III

Advance in chiral pesticide behavior

Enantioselective degradation in soil

soil

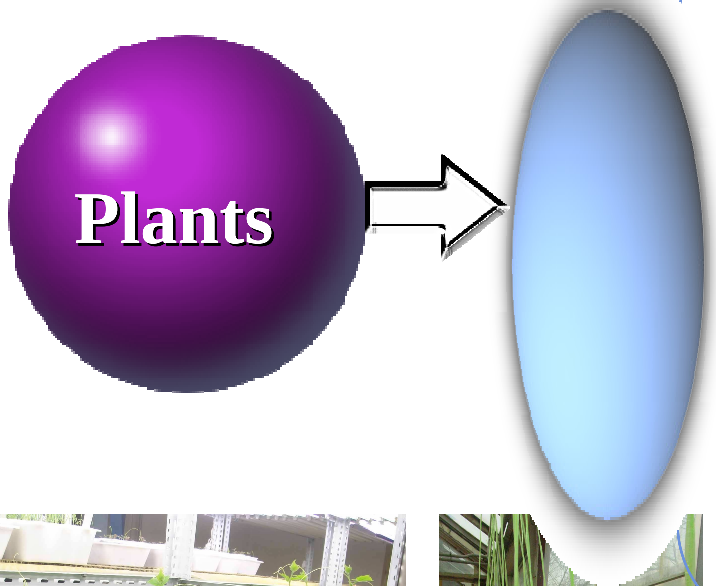
Triadimenol enantiomers were applied as barley seed-coating agent by Clark, 49 days later, found that the 44% RS was transformed into RR, 24%SR was transformed into SS（17%）amd RR（7%））

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 enantiometrs of cyproconazole were degraded at different rate

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.！

Part III Advance in chiral pesticide behavior

Enantioselective degradation in Plants

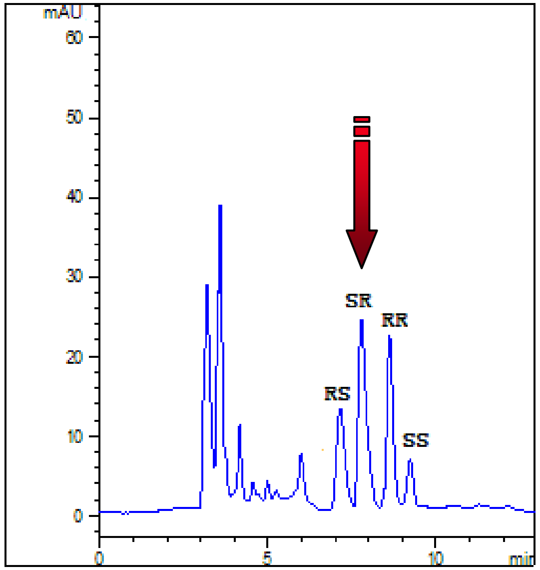


The stereoselective degradation of triadimenol in cucumber tissues has been investigated. It is the first report on the enantioselective environmental behavior of triadimenol on plant.

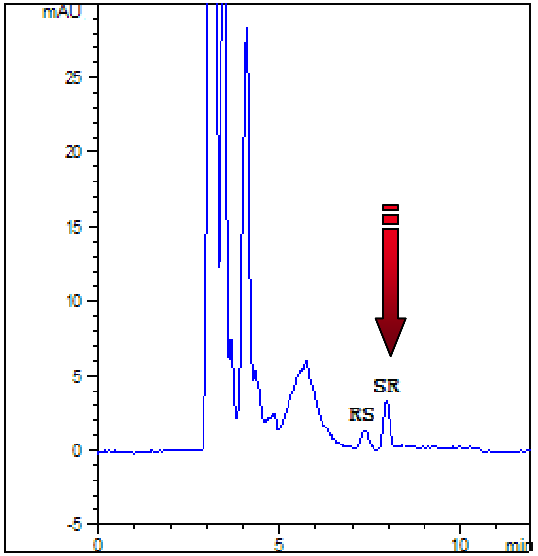
We found that: the degradation of triadimenol in cucumber plants was stereoselective under field conditions. The results indicated that RS enantiomer was degraded faster than SR enantiomer, and SS enantiomer was degraded faster than RR enantiomer, which resulted in plants enriched with SR and RR enantiomers.

Part III Advance in chiral pesticide behavior

Enantioselective degradation in Plants



leaves



Cucumber

friuts

Stereoselective Degradation of Fungicide Triadimenol in Cucumber Plants

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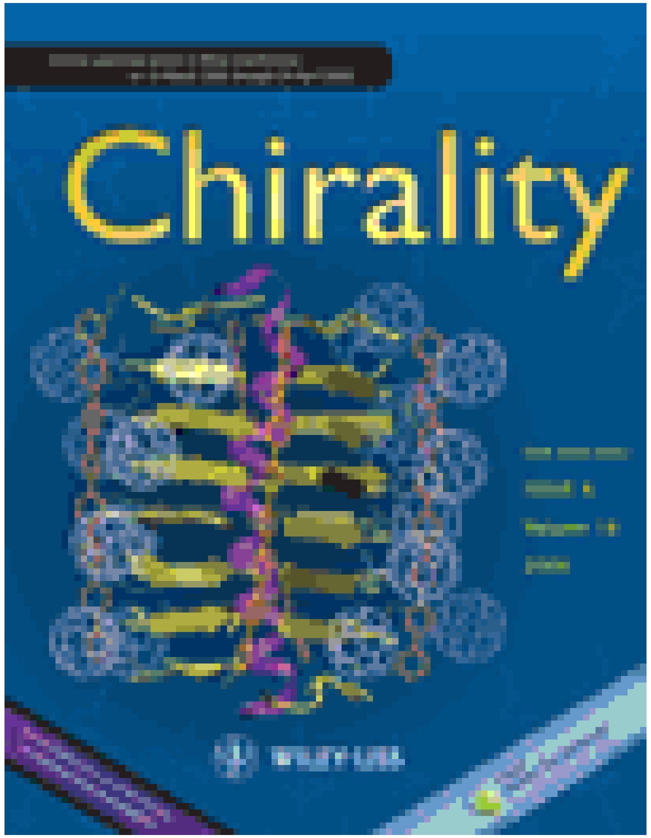

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Stereoselective Degradation of Fungicide Triadimenol in Cucumber Plants

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ABSTRACT The stereoselective degradation of triadimenol in different cucumber plant tissues (root, stem, leaf, and fruit) has been investigated. *Rac*-triadimenol was applied to cucumber plants by root irrigation mode under field conditions. The degradation kinetics and the enantiomer fraction were determined by normal-phase high performance liquid chromatography with diode array detector and online optical rotatory dispersion detector on Chiralpak® AS-H column. It has been shown 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. Furthermore, it was found that leaf was the dominating location for triadimenol enantiomer accumulation and stereoselective degradation, comparing with the root, stem, and fruit tissue. *Chirality 22:292–298, 2010.* © 2009 Wiley-Liss, Inc.

KEY WORDS: triadimenol; degradation; stereoselectivity; cucumber plants

INTRODUCTION

At present, many organic pesticides, about 25% of the total,[1] are chiral compounds and consist of two or more enantiomers or stereoisomers, which have identical physicochemical properties. Most of them are released into the environment as racemates or a mixture of stereoisomers. However, enantiomers or stereoisomers sometimes show differences in bioactivity, toxicity, metabolism, excretion, and environmental manner.[2–4]

Triadimenol, (1RS, 2RS; 1RS, 2SR)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl) butan-2-ol, is a broad-spectrum systemic fungicide used to control many plant diseases. Particularly, it displays an outstanding curative and protective efficacy against powdery mildew of cereal and vegetables.[5] As triadimenol possesses two chiral centers at C-1 and C-2, it can exist in the four stereoisomeric forms RS, SR, RR, and SS (Fig. 1). The fungicidal activities of these against some fungi have been reported[6,7] to differ significantly, the SR form being the most fungitoxic (up to 1000-fold more active than the other three). In view of the differences in antifungal activity of the stereoisomers, it was considered important to investigate their fate individually under field conditions. The metabolism of the triadimenol enantiomers in barley plants following uptake after seed treatment has been reported[8] and differences were found to exist between the enantiomers in the number and type of metabolites produced. But stereoselective degradation of triadimenol in the cucumber has not been reported even if it was widely used to control vegetables diseases. Therefore, this research was conducted to determine the stereoselective degradation of the four stereoisomers by HPLC-CSP (High Performance Liquid Chromatogram-Chiral Solid Phase) in cucumber plants. The results may improve our understanding of the triadimenol fate in vegetable plants.

MATERIALS AND METHODS

Chemicals and Reagents

The analytical standard of triadimenol A isomer (racemate of RS enantiomer and SR enantiomer, 99.9% purity) and B isomer (racemate of RR enantiomer and SS enantiomer, 99.9% purity) were provided by Bayer CropScience, Germany. And four stereoisomers of triadimenol (RS, SR, RR, SS) were prepared by HPLC with Chiralpak® AS-H column. The commercial product triadimenol WP (15% of triadimenol) was obtained from the Yancheng Limin Chemical Company (Jiangsu, China). Stock solution of *rac*-triadimenol was prepared in hexane and stored at −20°C. Working standard solutions were obtained by dilutions of the stock solution in isopropanol. Ethanol and hex-

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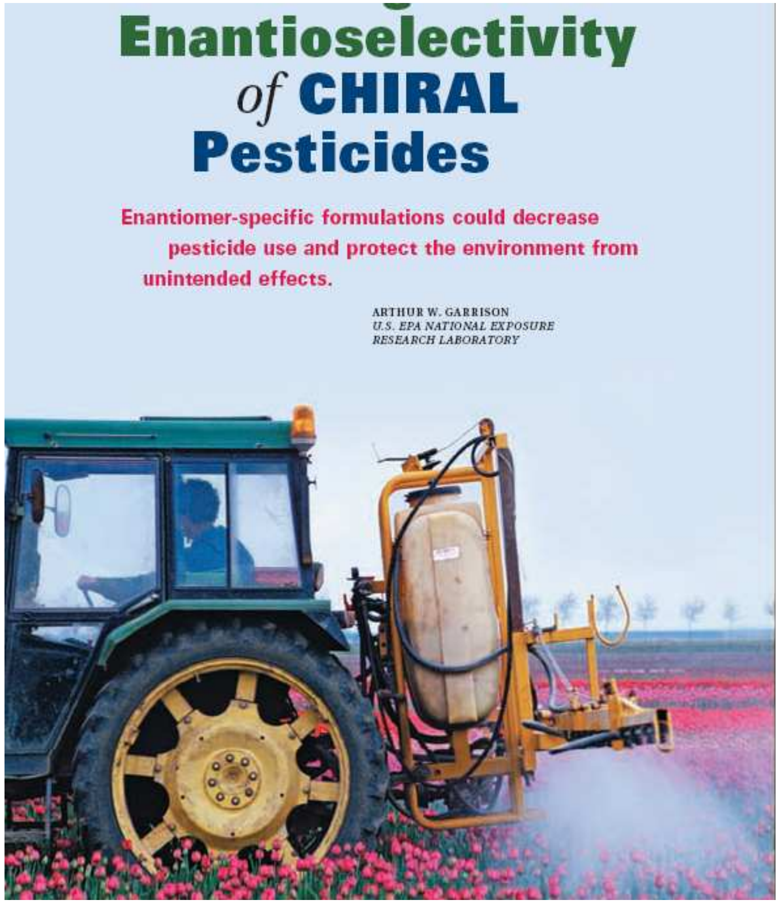
Part Ⅳ

The future of chiral pesticide

1. To ensure the food safety, the study of chiral pesticide must be carried out, will always be a great task in future.

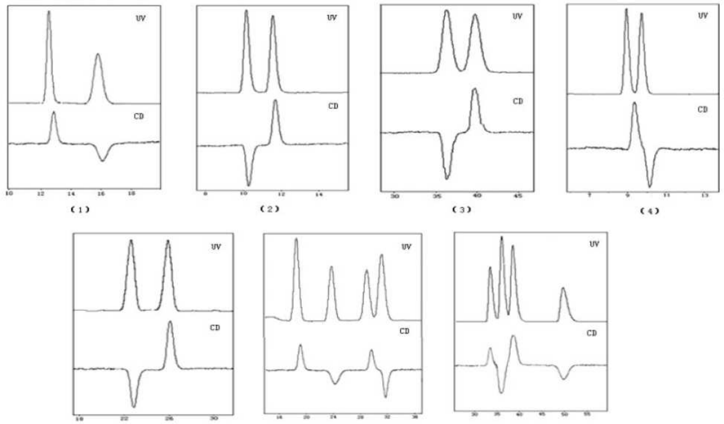
Regulatory authorities should be provided with data on both the fate and effects of separate enantiomers so that they can make the best possible risk assessments for single- or enriched-enantiomer pesticides that may be submitted for registration. These additional data would allow risk assessors to consider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



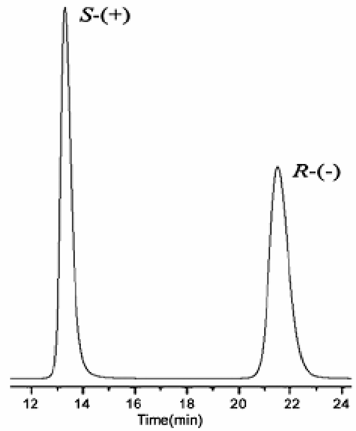
Part III Advance in chiral pesticide behavior

2.The study on separation of chiral pesticide will still be the hot topic.

The enantiomers of Imidazolones herbicides possess different herbicidal activity, gerenally, 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.



Part Ⅳ

The future of chiral pesticide

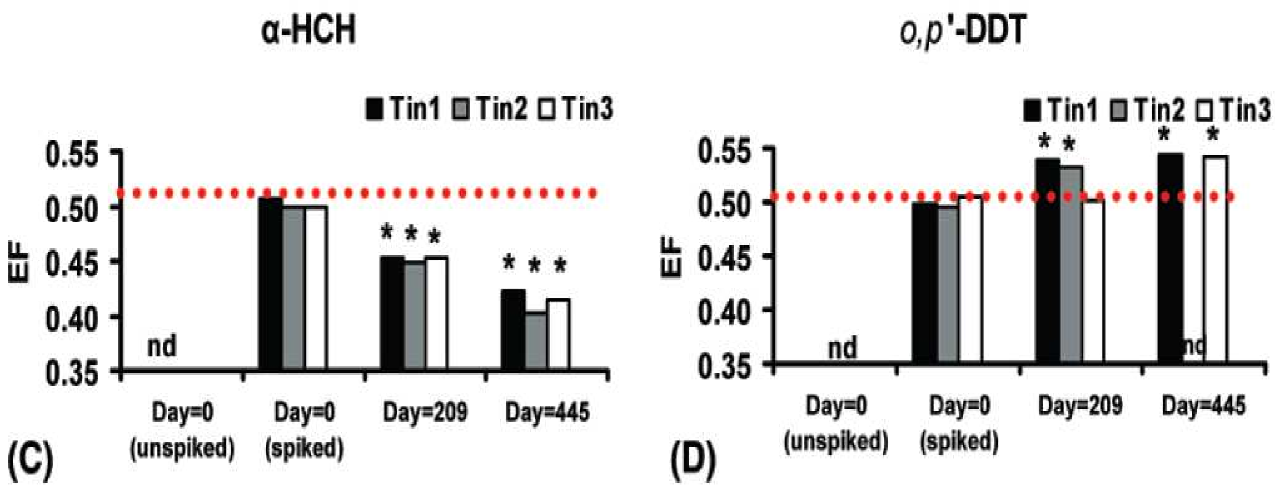
3.The study on the fate of chiral pesticide will still be the hot study field.

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.

Part Ⅳ

The future of chiral pesticide

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



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

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


Thanks for your attention