Enantiomer-Specific Fate and Effects of Modern Chiral Pesticides

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This presentation has been reviewed and approved by the U.S.EPA, but does not necessarily reflect official Agency policy.
RESEARCH AREAS

Analytical separation of enantiomers
  - GC, HPLC, CE, SFC

Environmental occurrence of enantiomers
  - soil, sediment, water, biota, food

Transformation
  - rates, enantioselectivity

Bioaccumulation

Effects
  - preparative separation of enantiomers
  - enantioselectivity
  - metabonomics
ENANTIOSELECTIVITY IN TRANSFORMATION AND OCCURRENCE

• 25% OF PESTICIDES ARE CHIRAL – 2 OR MORE ENANTIOMERS

• MICROBIAL TRANSFORMATION CAN BE ENANTIOMER-SELECTIVE, LEADING TO SELECTIVE PERSISTENCE

• EXAMPLES
Enantiomers of the Chiral Herbicide Dichlorprop
The (-)-enantiomer degrades twice as fast as the (+)
ChirasilDex CB column

Half-life of Racemate = 0.7 days

Thanks to Alton Whittemore, EPA, Athens
Conditions:
30mM borate. 100mM SDS
15% acetonitrile
40mM gamma-CD
Enantiospecific Transformation of Metalaxyl in Ohio Soil

Thanks to Jessica Jarman and Jack Jones, EPA, Athens
METALAXYL DEGRADATION RATES AND ENANTIOSELECTIVITY ARE HIGHLY VARIABLE IN SOIL

<table>
<thead>
<tr>
<th>Soil</th>
<th>R-(+), t ½ d</th>
<th>S-(-), t ½ d</th>
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</thead>
<tbody>
<tr>
<td>Ohio</td>
<td>11</td>
<td>63</td>
</tr>
<tr>
<td>USDA</td>
<td>42</td>
<td>85</td>
</tr>
<tr>
<td>HSB</td>
<td>223</td>
<td>863</td>
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<tr>
<td>Swiss</td>
<td>12</td>
<td>46</td>
</tr>
</tbody>
</table>

\(^a\) Buser, et al., ES&T, 36, 221(2002)
Exposure of fonofos to soil-water slurry for 8 weeks.

CE-MEKC buffer: 20mM borate, pH 8.5, 100mM SDS, 25mM gamma-CD, 15% AcN

ENANTIOMER PEAKS

Water, EF=0.46

Soil, EF=0.47
NON-SELECTIVE DEGRADATION: 30 mg/L IMAZAQUN IN ATHENS SOIL SLURRY

CE CONDITIONS:
50mM acetate, pH 4.5
15mM dimethyl beta-CD

\[
\text{COOH} \quad \text{HN} \quad \text{N} \quad \text{HN} \quad \text{CE CONDITIONS:} \quad 50\text{mM acetate, pH 4.5} \quad 15\text{mM dimethyl beta-CD}
\]

\[
0.002 \quad 0.007 \quad 0.012 \quad 0.017 \quad 0.022
\]

\[
9.01 \quad 9.46 \quad 9.91 \quad 10.36 \quad 10.81 \quad 11.26
\]

\[
0.002 \quad 0.007 \quad 0.012 \quad 0.017 \quad 0.022
\]

\[
9.31 \quad 9.76 \quad 10.21 \quad 10.66 \quad 11.11 \quad 11.56
\]
BCAA enantiomers before and after enantioselective microbial degradation

50 mM tetraborate, pH 8.5
40 mM trimethyl-β-cyclodextrin as chiral selector

Standard
ER = 1.00

After microbial degradation in Oconee River water for 84 days
ER = 0.26
Six river waters and one STP effluent showed enantioselective BCAA biotransformation, with considerable variation in rates. At a 6-months later sampling, selectivity is not observed in one river water and is reversed in another. Differences in BCAA degradation suggest there are several populations that enantioselectively degrade BCAA at different rates and are active at different times.

Cooperation with Charles Wong, Jack Jones, Lorrie Howell and Jimmy Avants, EPA, Athens
PYRETHROID INSECTICIDES
- several enantiomers -

Permethrin

Bifenthrin
PYRETHROID ENANTIOSELECTIVITY

Selectivity was observed for cis-bifenthrin in both toxicity and microbial degradation:

(+)-enantiomer is ~20X more toxic (LC50) than the (-)-enantiomer to both Ceriodaphnia dubia and C. magna

(+)-enantiomer was also more persistent in an aged field sample

CAVEATS FOR ENANTIOSELECTIVITY RESEARCH ON POLLUTANTS IN ENVIRONMENTAL MEDIA

1. CHANGES IN MICROBIAL POPULATION CAN CHANGE SELECTIVITY

2. SOME MICROBIAL PROCESSES ARE NOT SELECTIVE

3. SHORT ENANTIOMER HALF-LIVES MAY MAKE SELECTIVITY UNIMPORTANT

4. FASTER ABIOTIC REACTIONS MAY NEGATE ANY SELECTIVITY OF BIOTIC PROCESSES

5. ENANTIOMERIZATION MAY OCCUR

6. ENANTIOSELECTIVE SORPTION?
SELECTIVITY WITHIN THE ORGANISM

BIOACCUMULATION, BIOTRANSFORMATION, AND METABOLITE FORMATION OF FIPRONIL AND CHIRAL LEGACY PESTICIDES IN RAINBOW TROUT

KONWICK, B.J., GARRISON, A.W., BLACK, M.C. AVANTS, J.K. AND FISK. A.T., ACCEPTED BY ENVIRON.SCI.TECHNOL., MARCH 2006
Objectives

• Bioaccumulation of chiral contaminants
  – Fipronil & Organochlorines

• Biotransformation
  – Changes in enantiomeric fractions (EFs)
  – Log $K_{ow}$ – half life relationships

\[ EF = \frac{(+)}{[(+)+(-)]} \]

EF = 0.5
racemic
Fipronil

- Phenylpyrazole pesticide
- Broad spectrum
  - Rice culture
  - Turfgrass
  - Residential
- GABA disrupter

\[ \log K_{ow} = 4.01 \]
Fipronil Enantiomer Structures

Fipronil R (-)  Fipronil S (+)

Courtesy of Thomas Wiese, College of Pharmacy, Xavier Univ. of LA.
Fipronil Structure Constraints

Fipronil R (-)  Fipronil S (+)

Courtesy of Thomas Wiese, College of Pharmacy, Xavier Univ. of LA.
Fipronil Transformation Pathways

- Reduction
- Hydrolysis
- Oxidation
- Photolysis
- Chemical Reduction (FeS₂)

* Biotic reaction
Bioaccumulation Approach

Fipronil, OCs \textsubscript{FOOD}

\textit{Oncorhynchus mykiss}

Sampling days (3 fish each)
Fipronil & Fipronil Sulfone Standards

**Fipronil**

**Fipronil sulfone**

BGB 172 chiral column on GC-MS
After feeding for 2 days
After feeding for 4 days

PCB65 IS

Fipronil sulfone

Fipronil

Time, min

Abundance
Fipronil & Sulfone Metabolite

Fipronil
BMF = 0.05
Half-life 0.58 d

Fipronil Sulfone
BMF = 4.8
Half-life 2.36 d

Concentration (μg/g lipid)

Day

uptake depuration
**Fipronil**

![Graph showing Fipronil uptake and depuration with concentration (µg/g lipid) and enantiomeric fraction (EF) over time (days 2-48).](image-url)

**Key Points:**
- Concentration (µg/g lipid) along the y-axis, ranging from 0.05 to 8.
- Days 2 to 48 on the x-axis.
- Fipronil Concentration shown by black circles.
- Fipronil EF shown by square markers.
- Enantiomeric Fraction (EF) ranging from 0.40 to 0.60.
- ND (Not Detected) indicated at specific time points.

**Legend:**
- ● Fipronil Concentration
- □ Fipronil EF

**Note:** The graph illustrates the uptake and depuration of Fipronil over time, highlighting the concentration and enantiomeric fraction changes.
ENANTIOSELECTIVITY IN EFFECTS

SEPARATE ENANTIOMERS ARE REQUIRED TO STUDY ENANTIOSELECTIVITY OF EFFECTS

• PREPARATIVE SEPARATION BY HPLC
• FIPRONIL ENANTIOMER TOXICITY
• ENDOCRINE DISRUPTER EFFECTS
• VINCLOZOLIN ENANTIOMER TOXICITY
• CONAZOLE EFFECTS
• BROMUCONAZOLE METABOLISM
• TRIADIMEFON METABOLOMICS
PREPARATIVE SEPARATION OF ENANTIOMERS

Pilot separation by analytical size chiral column

Preparative separation of enantiomers from 1-2 g of racemate; typically 2 X 25 cm chiral column

Recovery typically 85-95%

Typically >98% pure by HPLC/UV

Optical rotation measured in the analytical mobile phase using in-line PDR chiral detector

Chiral Technologies, Inc., Exton, PA
<table>
<thead>
<tr>
<th></th>
<th>IN LIGHT</th>
<th>IN DARK</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+) ENANTIOMER</td>
<td>13.62</td>
<td>12.09</td>
</tr>
<tr>
<td>(-) ENANTIOMER</td>
<td>36.17</td>
<td>32.29</td>
</tr>
<tr>
<td>RACEMATE</td>
<td>18.58</td>
<td>19.39</td>
</tr>
</tbody>
</table>

**ACUTE ENANTIOSELECTIVE TOXICITY OF FIPRONIL AND ITS DESULFINYL PHOTOPRODUCT TO CERIODAPHNIA DUBIA; KONWICK, BJ, FISK, AT, GARRISON, AW, AVANTS, JK AND BLACK MC. ENVIRON.TOXICOL.CHEM. 24 (2005) 2350-2355**
FIPRONIL CRONIC TOXICITY TO DAPNIA

Number of Offspring, 8-day trial (LOEC)

(+)-enantiomer  2ug/L
(+)-enantiomer  15ug/L
(-)-enantiomer  30ug/L

Toxicity, Neonates born during experiment (LC50$_{48}$)

(+)-enantiomer  8ug/L
(+)-enantiomer  32ug/L
(-)-enantiomer  50ug/L

ENDOCRINE DISRUPTER EFFECTS

• THE ENANTIOMERS OF CHIRAL PESTICIDES ARE EXPECTED TO DIFFER IN THEIR ENDOCRINE DISRUPTER EFFECTS

• ENANTOMERS OF 11 PESTICIDES HAVE BEEN SEPARATED AND SCREENED FOR ED ACTIVITY BY THOMAS WIESE, XAVIER UNIV. OF NEW ORLEANS

• THE ED ACTIVITY IS USUALLY, BUT NOT ALWAYS, ENANTIOSELECTIVE; O,P’-DDT IS A STRIKING EXAMPLE
Enantiospecific Estrogen Activity of o,p’-DDT

- Rainbow Trout Cell Hepatoma Cells
- Human Breast Cancer Cells

Wiese TE, Nehls S (2001) Enantiomer Selective Estrogen and Antiandrogen Activity of Chiral Pesticides
VINCLOZOLIN

A FUNGICIDE AND ENDOCRINE DISRUPTER
VINCLOZOLIN EFFECTS ON MEDAKA

• Expose separate enantiomers to medaka for 72 hours – change water every 24 hours

• Excise livers and prepare microsomal proteins for proteomic assay

• Separate fluorescent-labeled proteins by gel electrophoresis, quantitate expression levels, pick induced proteins robotically

• Analyze/classify peptide fractions by MALDI-MS for protein identification

Chris Mazur, Emily Rogers and Drew Ekman with Tracy Andacht and Richard Winn, Univ. of Georgia
CONAZOLES

Fungicides and pharmaceuticals

Test compounds selected by the EPA for the new Computational Toxicity Program

Bioaccumulation in rainbow trout – enantioselective?

Metabolism *in vitro* by enzymes – enantioselective?

Metabolomics by NMR – endogenous metabolite patterns to help determine toxicity mechanisms

*Aaron Fisk and Brad Konwick, UGA, with Drew Ekman, John Kenneke and Jimmy Avants, EPA*
Enzymatic reactions in rat microsomal material, followed by chiral HPLC with UV detection (202nm). Under the physiological conditions of a rat, the trans isomer reacts faster than the cis.

Thanks to Chris Mazur, John Kenneke and John Evans
Bromuconazole 47 (trans)

Enantiomeric Ratio

Time, minutes

26 ppm, 37°C
Rat microsome
Assessing Triazole Toxicity Using NMR-based Metabolomics

Drew Ekman, Tim Collette, Wayne Garrison, John Kenneke, Chris Mazur

U.S. EPA
National Exposure Research Laboratory (NERL)
Athens, GA

U.S. Triazole Task Force meeting, March 2006
‘omics in toxicology: genomics/proteomics/metabolomics

- Metabolomics measures responses to chemicals through analyses of endogenous metabolite levels (e.g. glucose, lactate, etc.).
- Metabolomics can provide a connection between genomics/proteomics and histopathology.
- Genomics and proteomics measure responses to chemicals on the genetic and cellular protein level, respectively.
- Information-rich spectroscopic techniques (primarily NMR and MS) are used for metabolomics.
Metabolomics

A Common Approach in Toxicology:

Test animals (e.g. mice) are dosed with toxicants whose modes of action are known.

Levels of endogenous metabolites (e.g., in mice urine) are measured as a function of time with NMR spectroscopy.

Chemometric tools, like Principal Component Analysis (PCA), are used to identify changes in NMR spectra that are associated with toxicity and mode of action.

Databases of NMR patterns associated with major toxic modes of action are constructed.

New chemicals (whose effects are unknown) can then be classified according to toxicity and mode of action based on NMR patterns observed after dosing experiments.
$^1$H NMR Spectra of Urine Samples From Mice Treated With Various Toxins

600 MHz $^1$H NMR spectra showing the effect of tissue-specific toxins on the metabolic profile of urine

Changes reflect the site and/or mechanism of toxicity

Lindon, et al., 2002
Investigating the Enantioselective Toxicity of Triazole Fungicides In Rainbow Trout Through the Use of NMR-based Metabolomics

Partners: Aaron Fisk and Brad Konwick, University of Georgia, Athens
TRIADIMEFON

ENANTIOMER 1

S-(-)

ENANTIOMER 2

R- (+)
Enantiospecific Toxicity Study Design

- Juvenile rainbow trout were exposed (via gavage) to either one of the enantiomers or the racemate.
- Two dose levels and two time points were employed.
  - High dose: 720 mg/kg/day; 24 and 48 hours
  - Low dose: 144 mg/kg/day; 24 and 48 hours
- Livers were collected and extracted (perchloric acid) for NMR analysis.
- Principal Components Analysis (PCA) performed to investigate differences in control vs. dosed classes.
NMR spectrum of trout liver extract

Portion of the spectrum of a control liver extract (polar fraction) with several metabolites labeled.
PCA Scores Plot of 24-hour exposed (high dose) fish

At 24 hours, fish exposed to the (-) enantiomer or the racemate display stronger responses than those exposed to the (+) enantiomer.
PCA Scores Plot of 48-hour exposed (high dose) fish

- ■ = control
- ■ = (-) enantiomer
- + = (+) enantiomer
- ● = racemate

By 48 hours the (+) enantiomer exposed fish are displaying a response to treatment.
Potential Benefits of Using Metabolomics In Risk Assessments

- Increased ability to determine toxic mode-of-action
- Metabolite changes often reflect toxicity
- Rapid analysis
  - Approximately 300 samples/day can be analyzed with current equipment.
- Amenable to conducting cross-species comparisons
  - Status of genome sequencing not a factor.
- Offers unique advantages for human studies (biofluids)
  - Non-invasive
  - Whole organism endpoints.
Reduced number of animals required to assess toxicity
Metolachlor

• 4 enantiomers
• the two S-enantiomers are herbicidally active
• Syngenta is now marketing a 90% S-enriched formulation
CONCLUSIONS

- Many “modern” pesticides are chiral
- CE is useful for laboratory studies of pesticide transformation
- Microbial transformation is usually observed to be enantioselective, but it is not possible at this time to predict the direction or extent of selectivity in a different environment; e.g., metalaxyl
- Enantiomers usually differ in their effects, including toxicity and ED activity, but little definitive work has been done with pesticides
Metabonomics, proteomics and other modern molecular biochemistry techniques are applicable to investigate toxicity mechanisms.

Accurate risk assessment requires investigation of the fate and effects of each enantiomer of a chiral pesticide.

The ultimate goal of this research is to show whether the manufacture and use of single-enantiomer pesticides is of benefit to the environment.