RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Enantiomer-Specific Fate and Effects of Modern Chiral Pesticides

Wayne Garrison

U.S. Environmental Protection Agency

National Exposure Research Laboratory

Ecosystems Research Division

Athens, GA

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

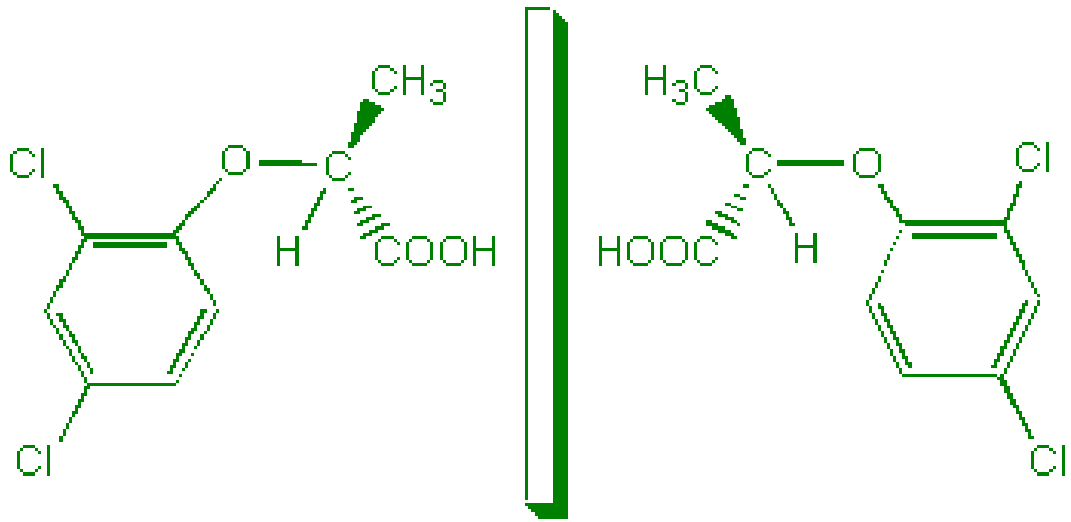
Transformation
- rates, enantioselectivity

Bioaccumulation

Effects
- preparative separation of enantiomers
- enantioselectivity
- metabonomics

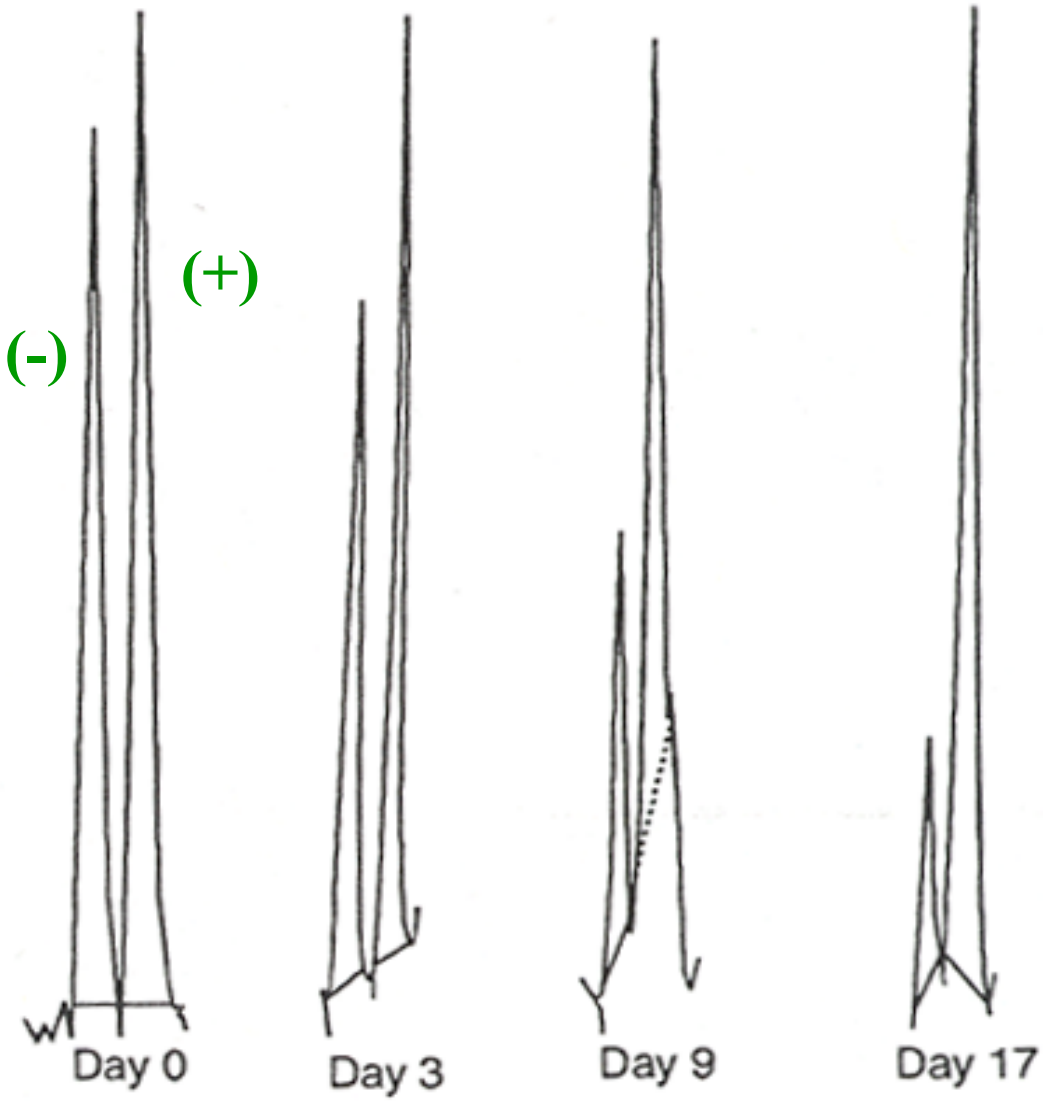
ENANTIOSELECTIVITY IN TRANSFORMATION AND OCCURRENCE

- 25% OF PESTICIDES ARE CHIRAL – 2 OR MORE ENANTIOMERS.
- MICROBIAL TRANSFORMATION CAN BE ENANTIOMER-SELECTIVE, LEADING TO SELECTIVE PERSISTENCE
- EXAMPLES



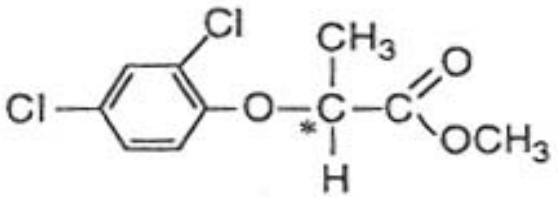
R-(+)-dichlorprop **S-(-)-dichlorprop**

Enantiomers of the Chiral Herbicide Dichlorprop

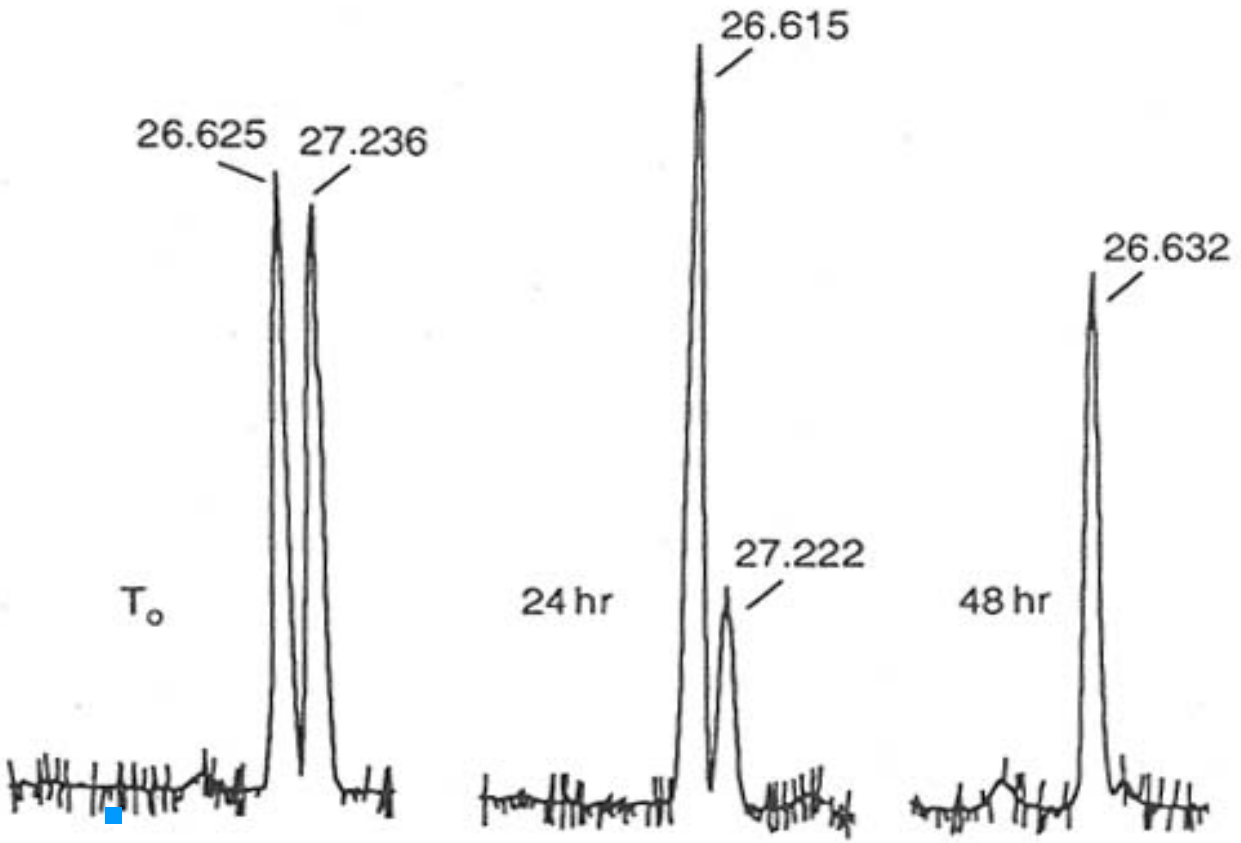


Degradation of dichlorprop acid

The (-)-enantiomer degrades twice as fast as the (+)



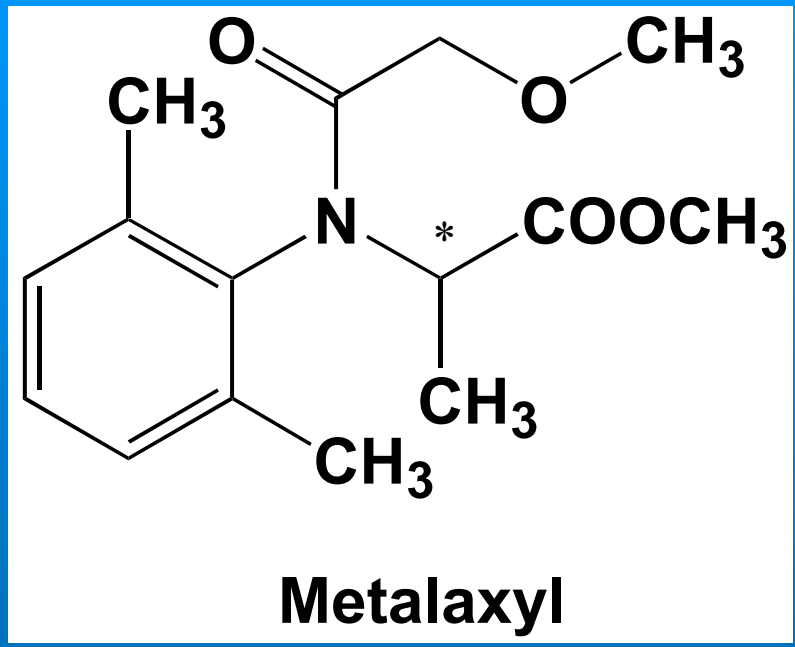
ChirasilDex CB column



(b) Degradation of methyl dichlorprop

Half-life of Racemate = 0.7 days

Thanks to Alton Whittemore, EPA, Athens



Metalaxyl

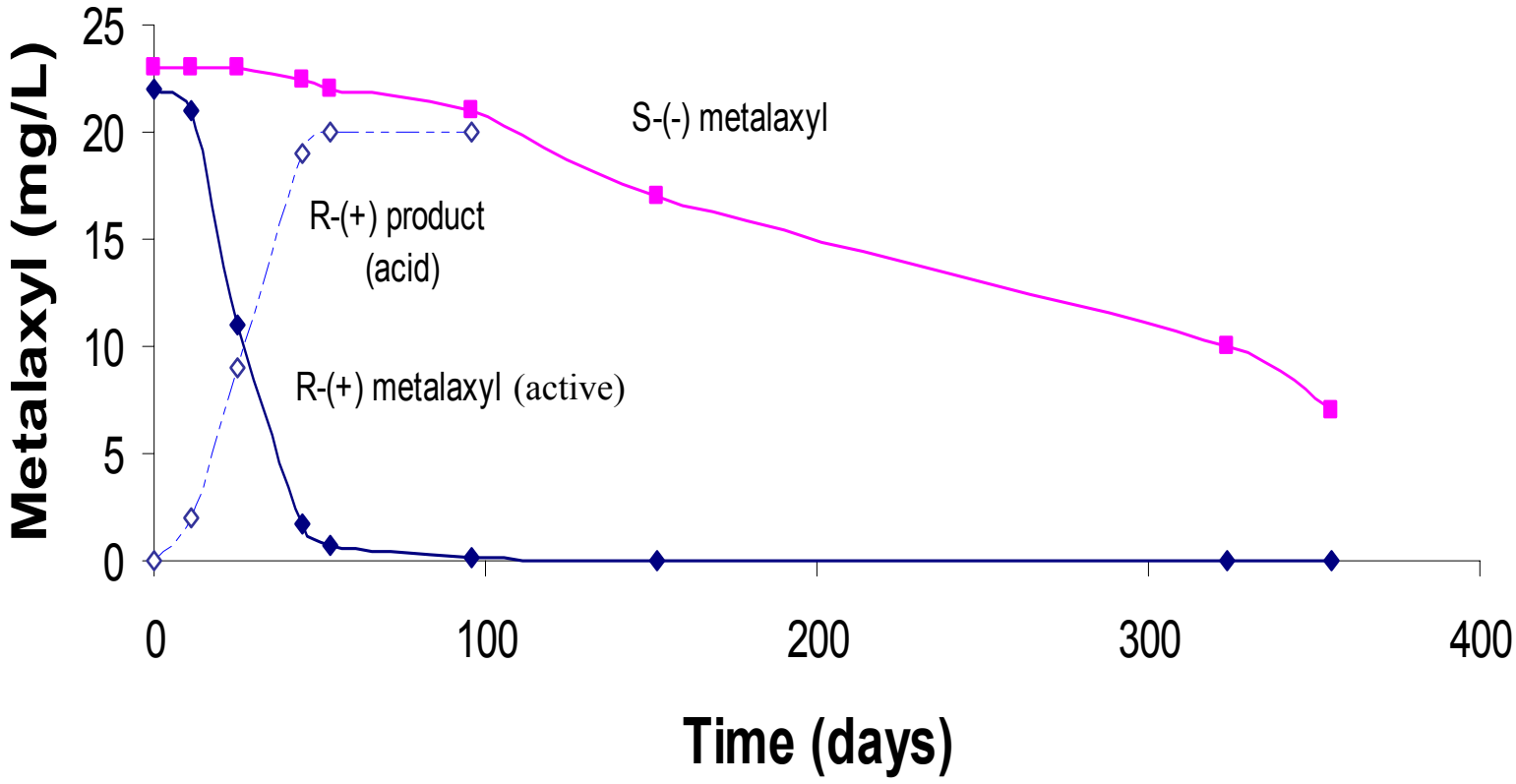
Conditions:

30mM borate. 100mM SDS

15% acetonitrile

40mM gamma-CD

Enantiospecific Transformation of Metalaxyl in Ohio Soil

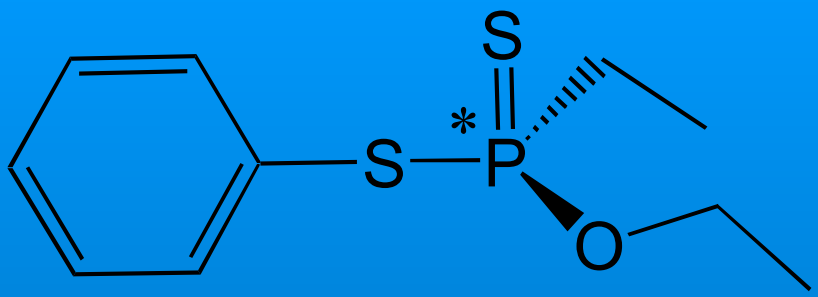


Thanks to Jessica Jarman and Jack Jones,EPA,Athens

METALAXYL DEGRADATION RATES AND ENANTIOSELECTIVITY ARE HIGHLY VARIABLE IN SOIL

Soil	R-(+), t ½ d	S-(-), t ½ d
Ohio	11	63
USDA	42	85
HSB	223	863
Swiss[a]	12	46

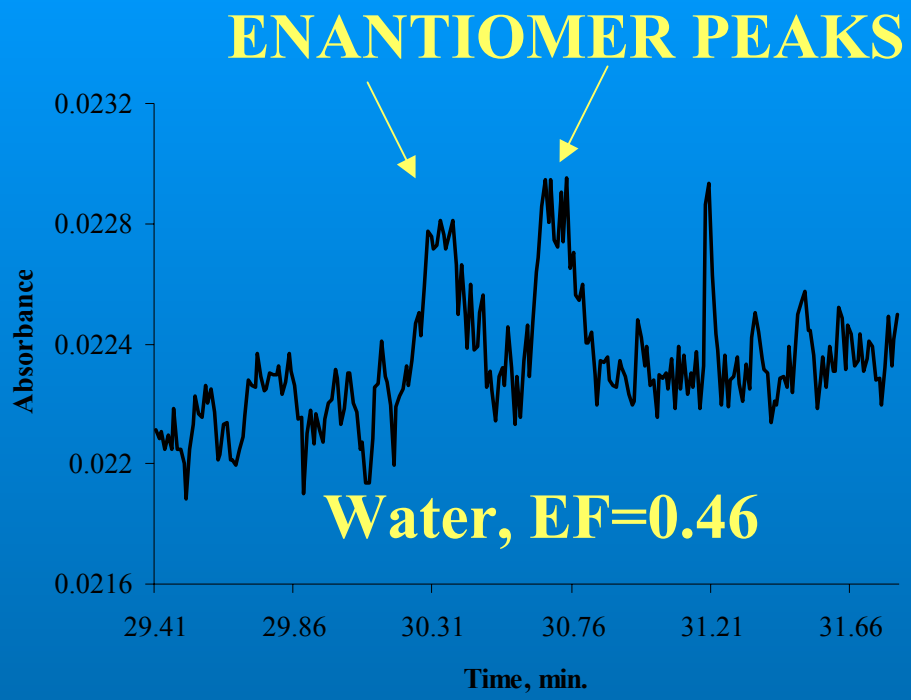
[a] Buser, et al., ES&T, 36, 221(2002)

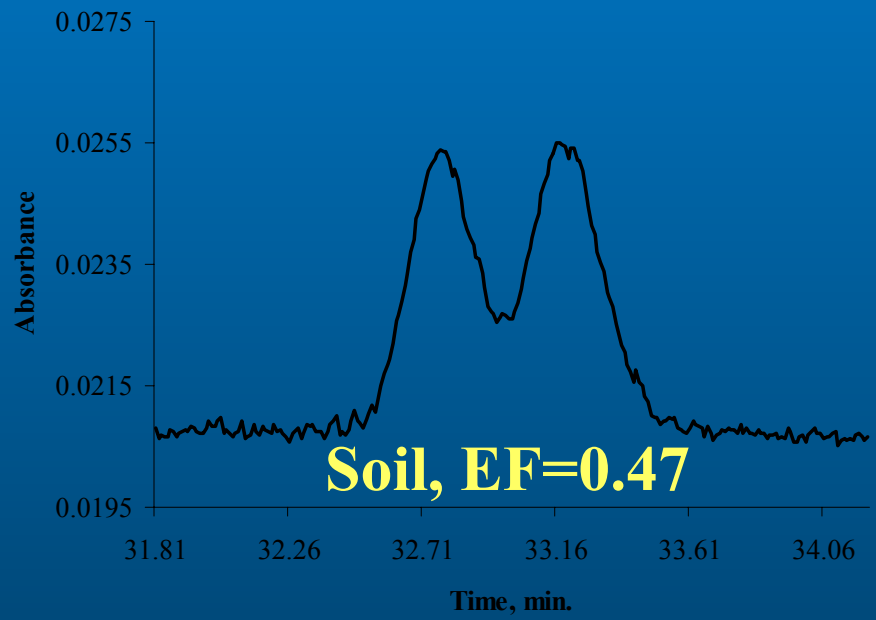


Fonofos

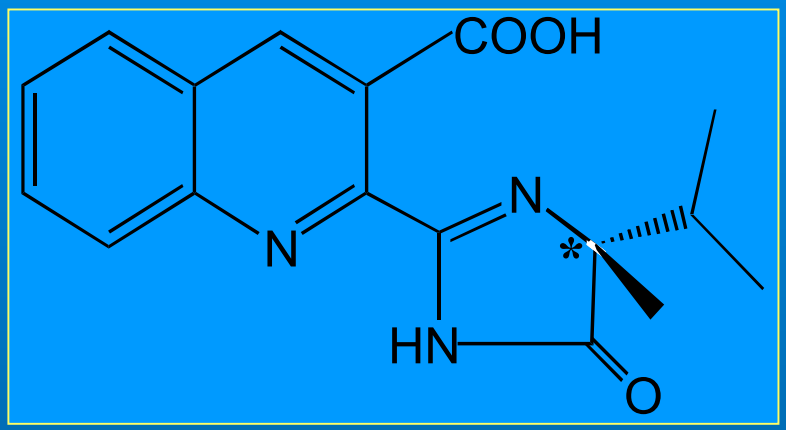
Exposure of fonofos to soil-water slurry for 8 weeks.

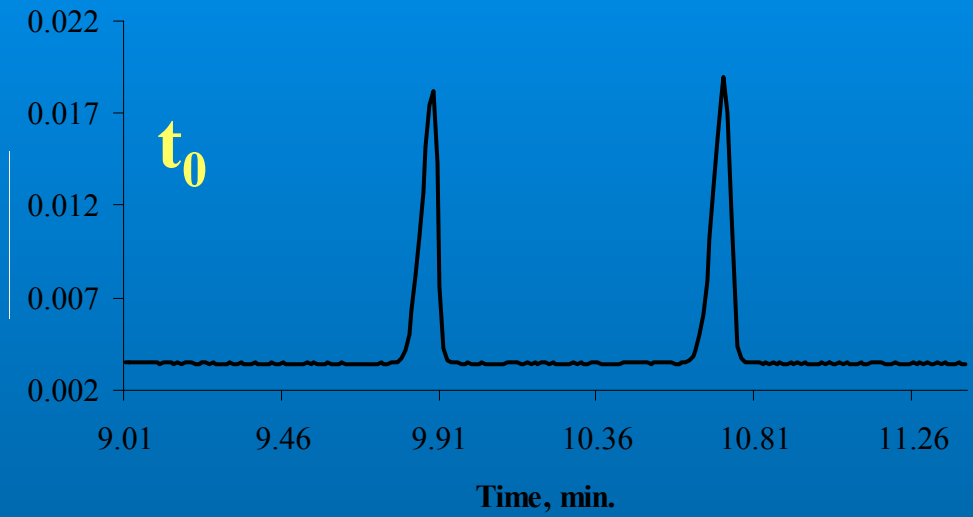
CE-MEKC buffer: 20mM borate, pH 8.5, 100mM SDS, 25mM gamma-CD, 15% AcN





NON-SELECTIVE DEGRADATION: 30 mg/L IMAZAQUIN IN ATHENS SOIL SLURRY

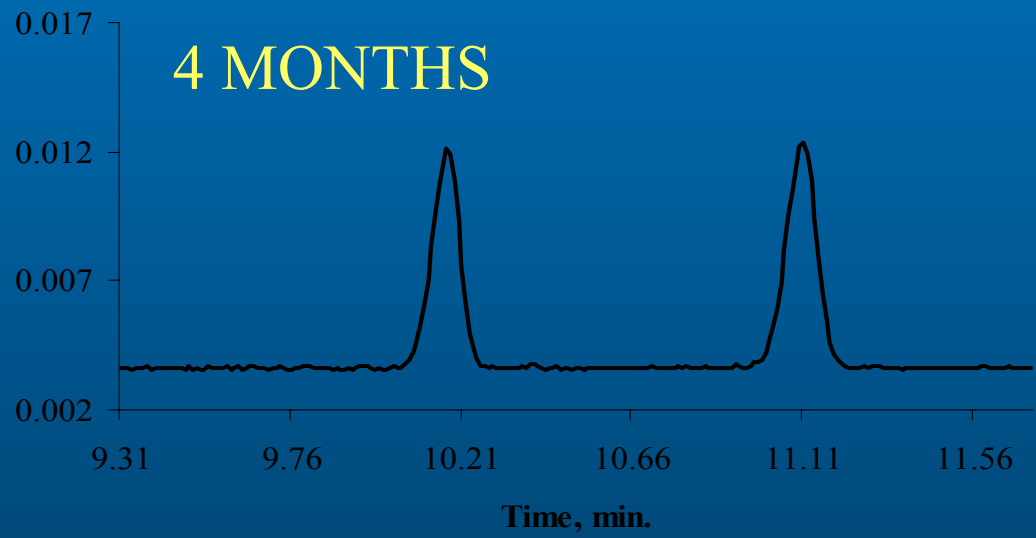




CE CONDITIONS:

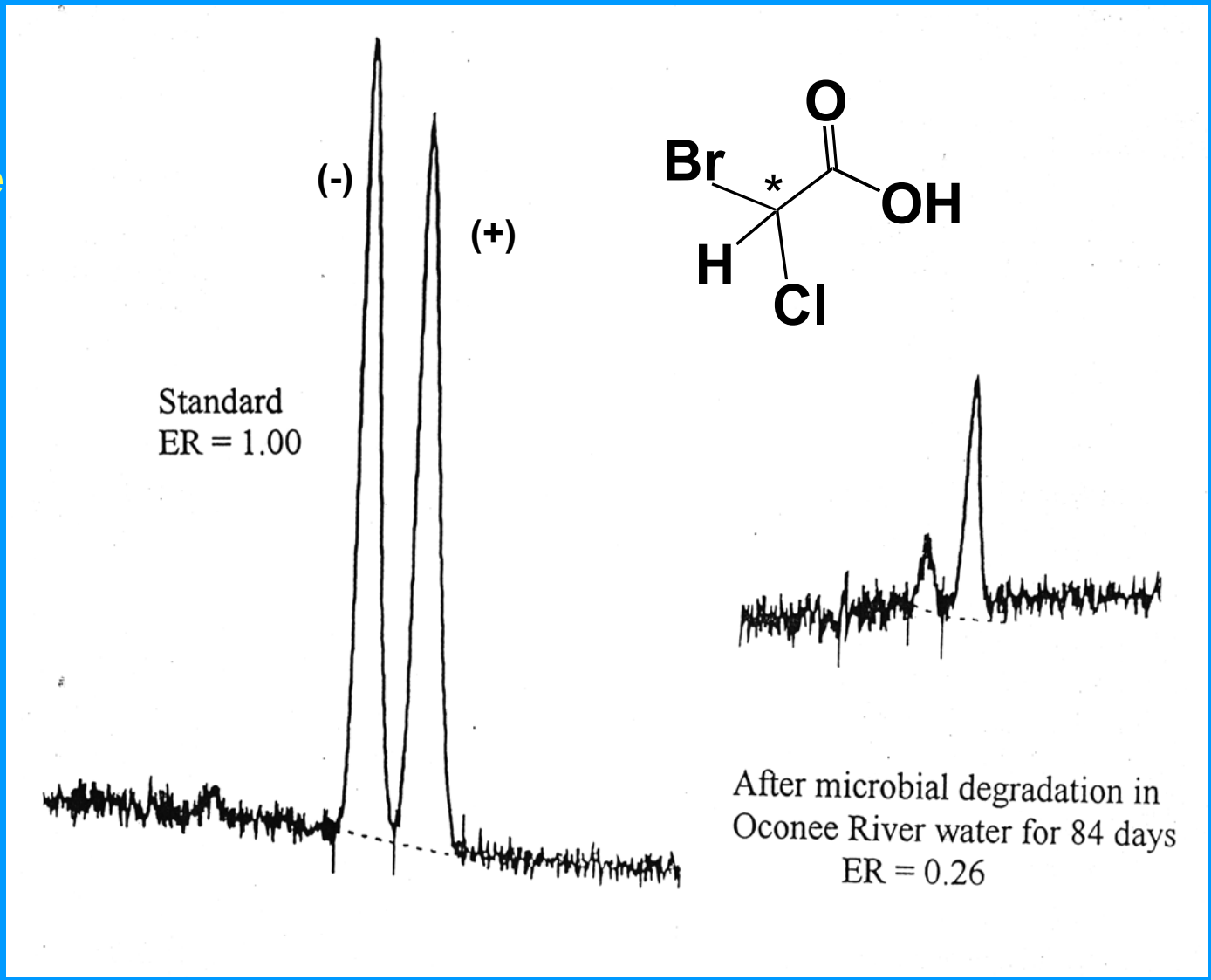
50mM acetate, pH 4.5

15mM dimethyl beta-CD



BCAA enantiomers before and after enantioselective microbial degradation

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



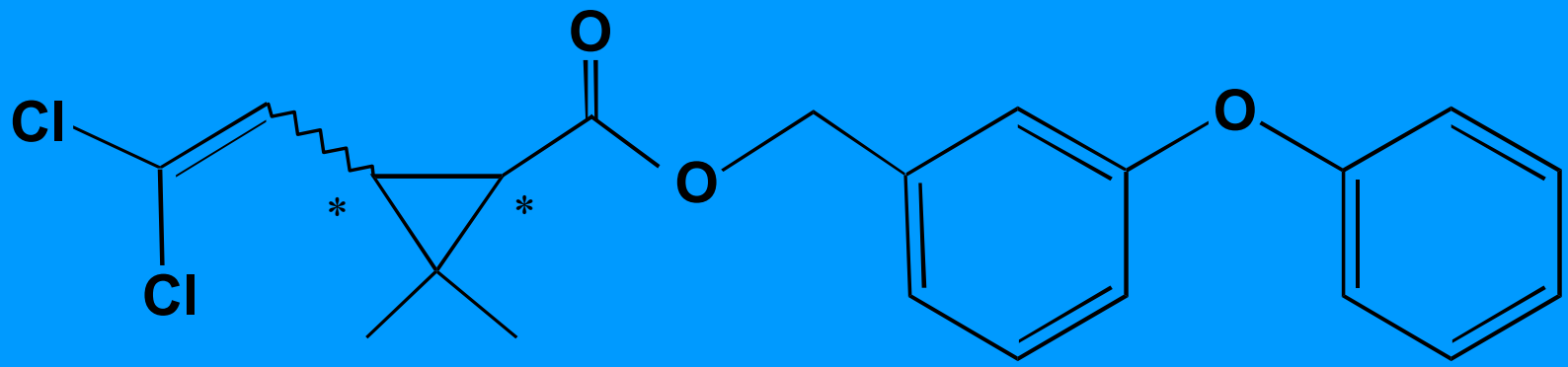
ENANTIOSELECTIVE DEGRADATION OF BCAA

- **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.**

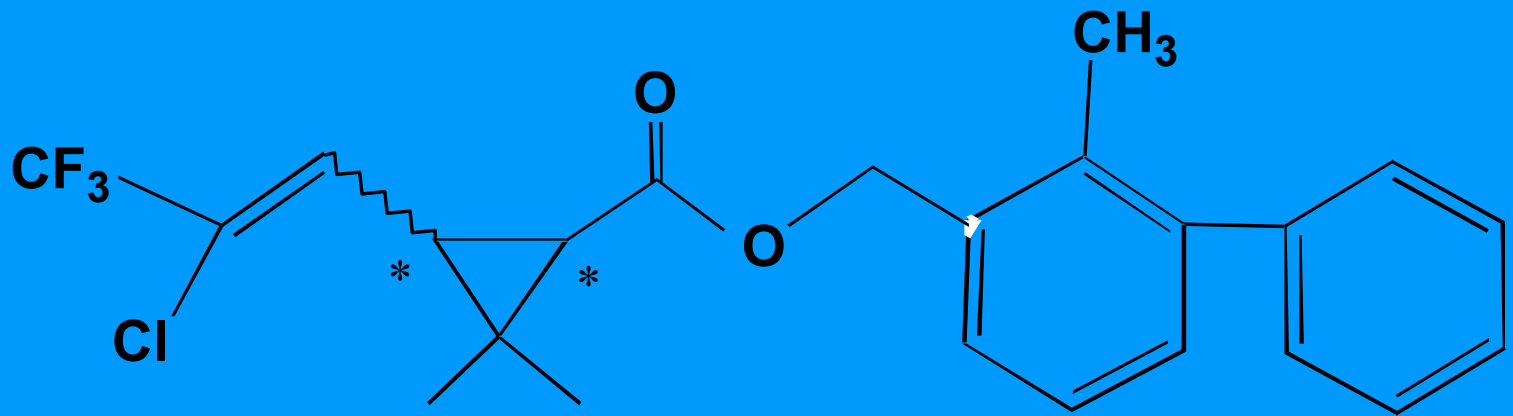
Cooperation with Charles Wong, Jack Jones, Lorrie Howell and Jimmy Avants, EPA, Athens

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

Liu, W., Gan, J. et al. *Proc.Natl.Acad.Sci.USA*, 2005,**102**, 701-706.

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

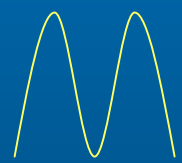
KONWICK, B.J., GARRISON, A.W., BLACK, M.C. AVANTS, J.K. AND FISK. A.T., ACCEPTED BY ENVIRON.SCI.TECHNOL., MARCH 2006

Objectives

- Bioaccumulation of chiral contaminants
  - Fipronil & Organochlorines
- Biotransformation
  - Changes in enantiomeric fractions (EFs)
  - Log K_{ow} – half life relationships

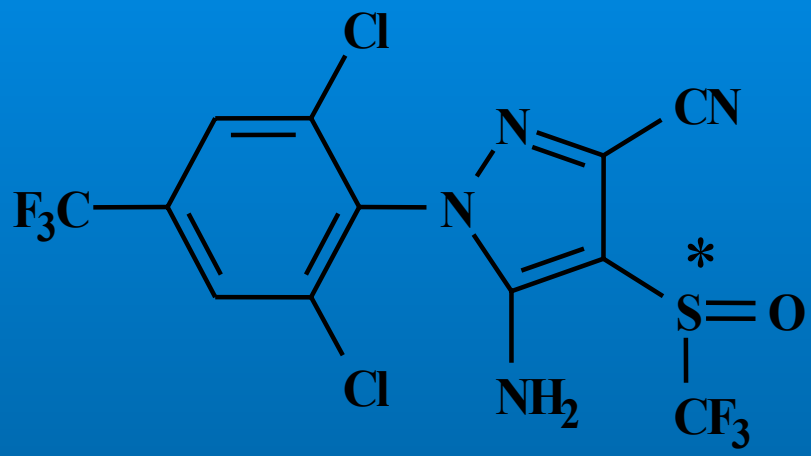
$$EF = (+) / [(+) + (-)]$$

EF = 0.5
racemic



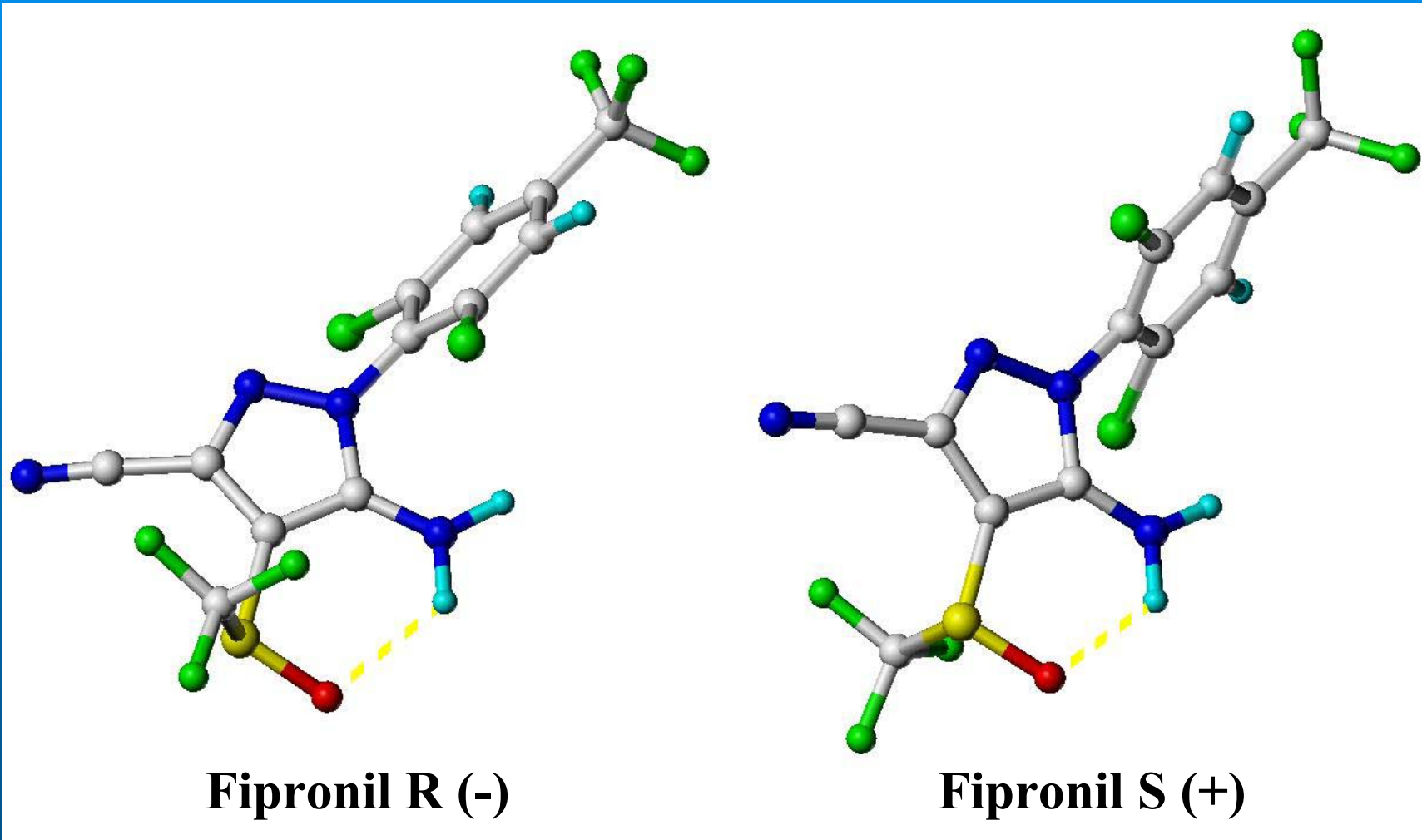
Fipronil

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



log K_{ow} = 4.01

Fipronil Enantiomer Structures

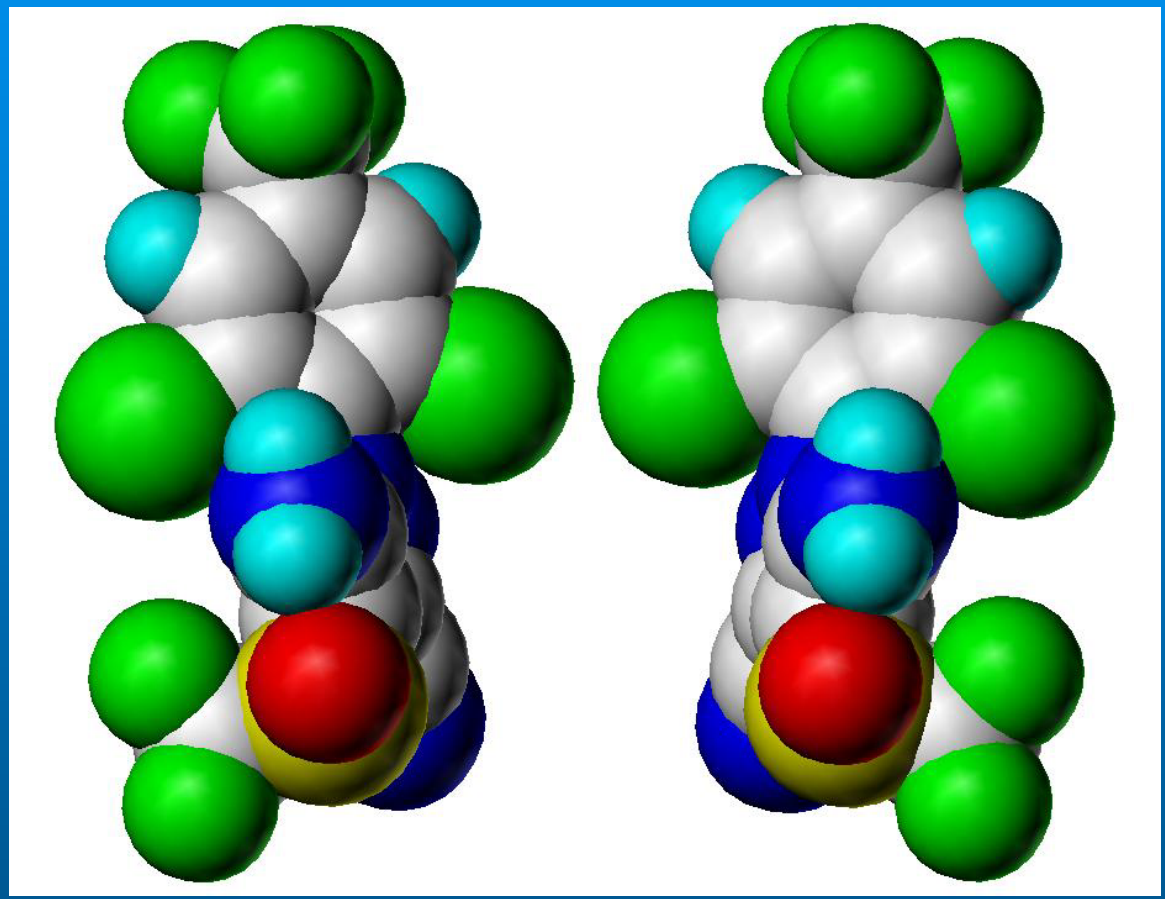


Fipronil R (-)

Fipronil S (+)

Courtesy of Thomas Wiese, College of Pharmacy, Xavier Univ. of LA.

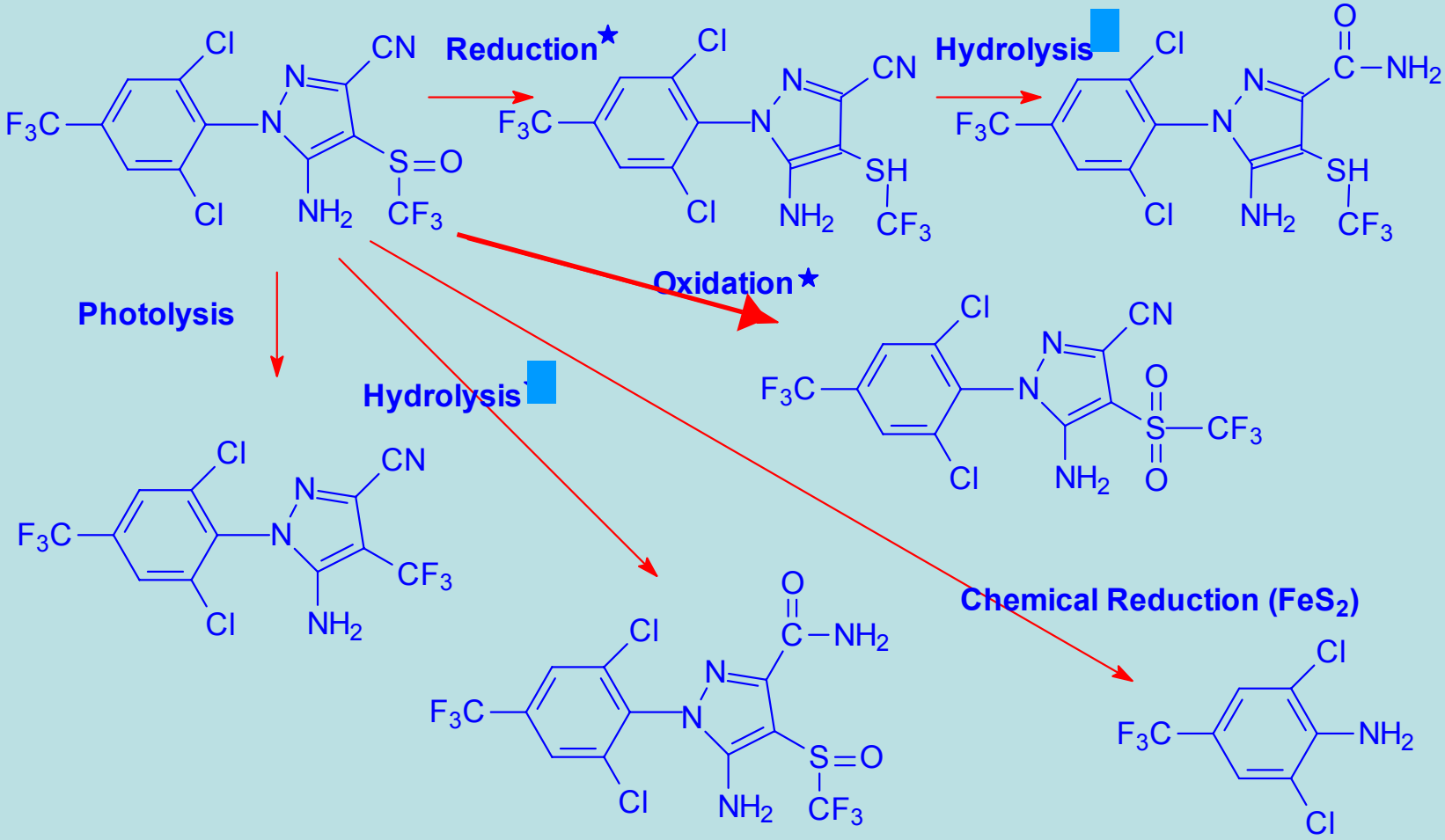
Fipronil Structure Constraints



Fipronil R (-) **Fipronil S (+)**

Courtesy of Thomas Wiese, College of Pharmacy, Xavier Univ. of LA.

Fipronil Transformation Pathways

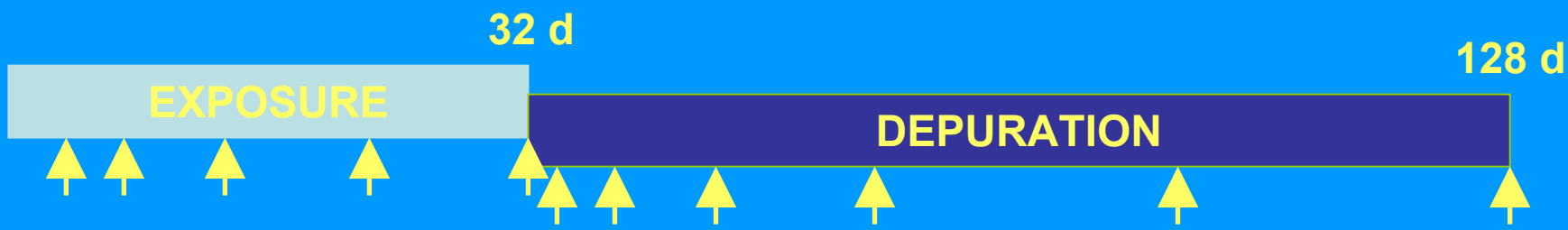


Bioaccumulation Approach

racemic

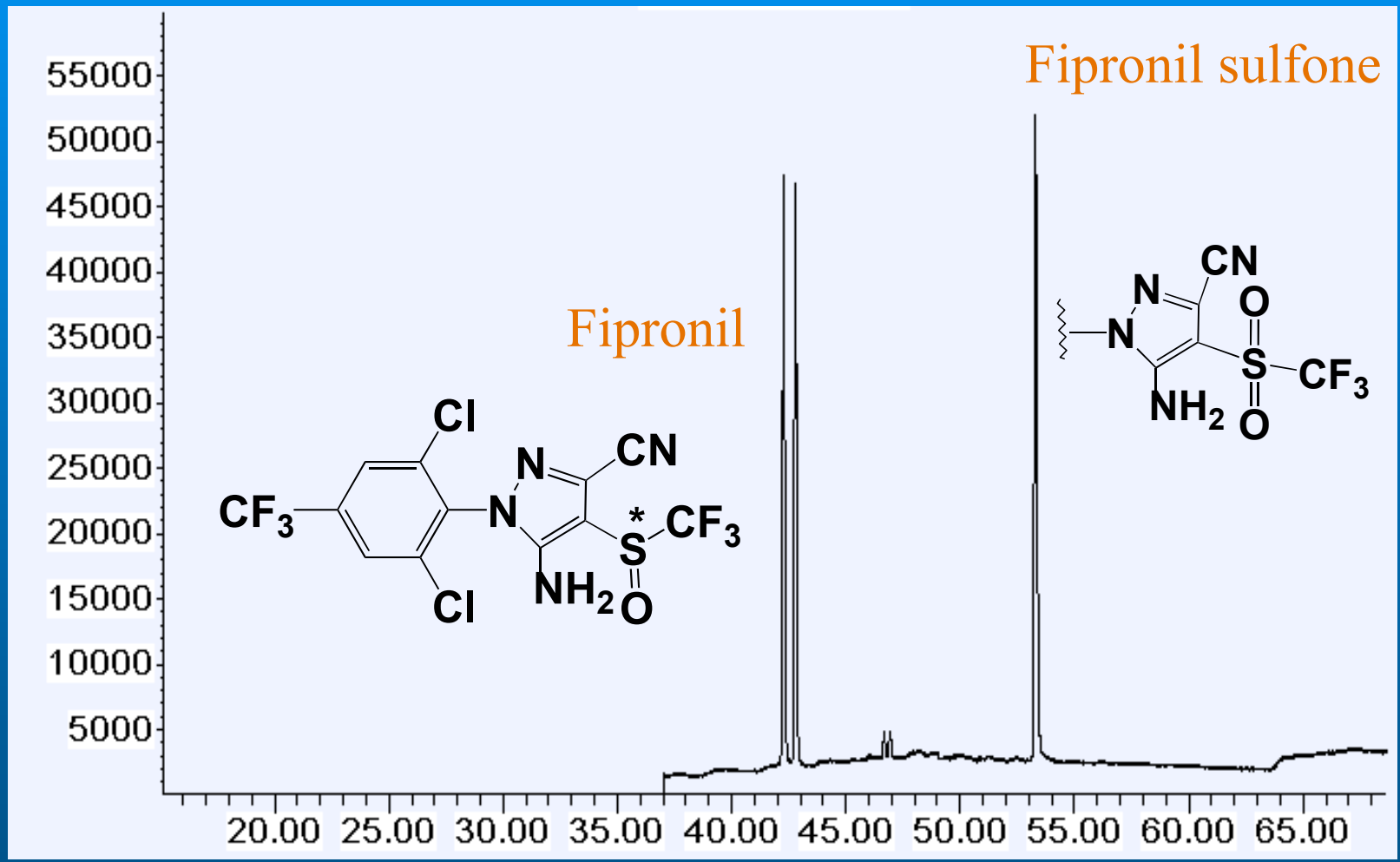
Fipronil,OCs$_{FOOD}$

Oncorhynchus mykiss



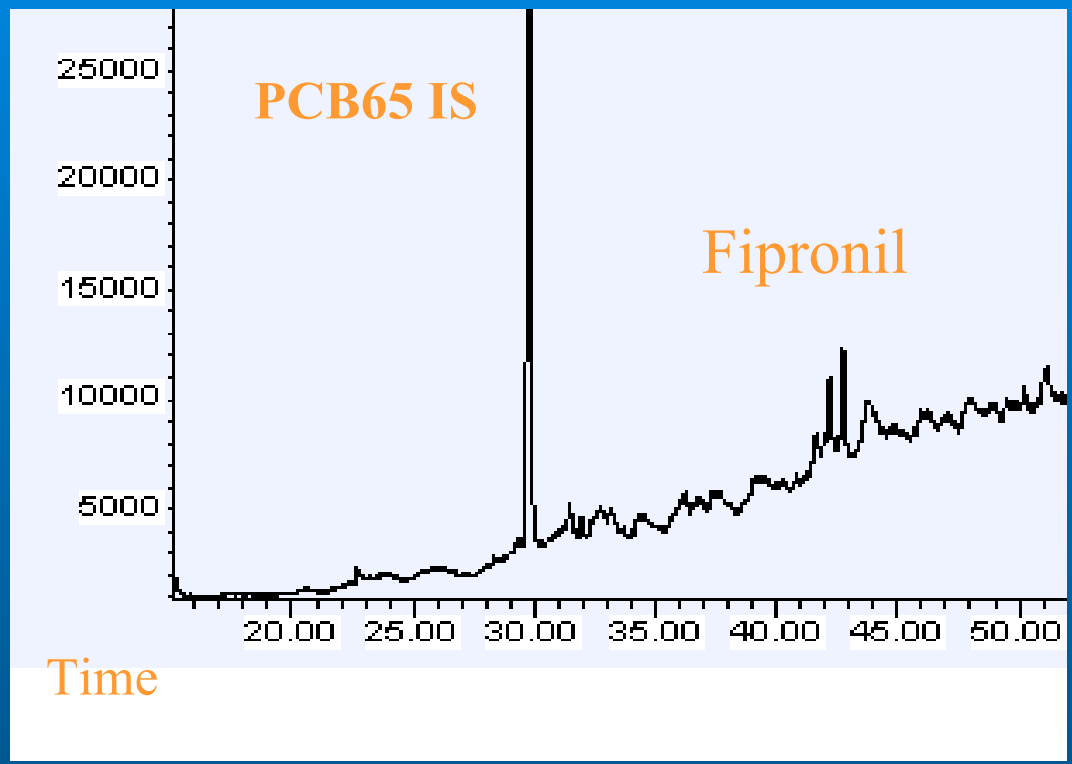
Sampling days (3 fish each)

Fipronil & Fipronil Sulfone Standards

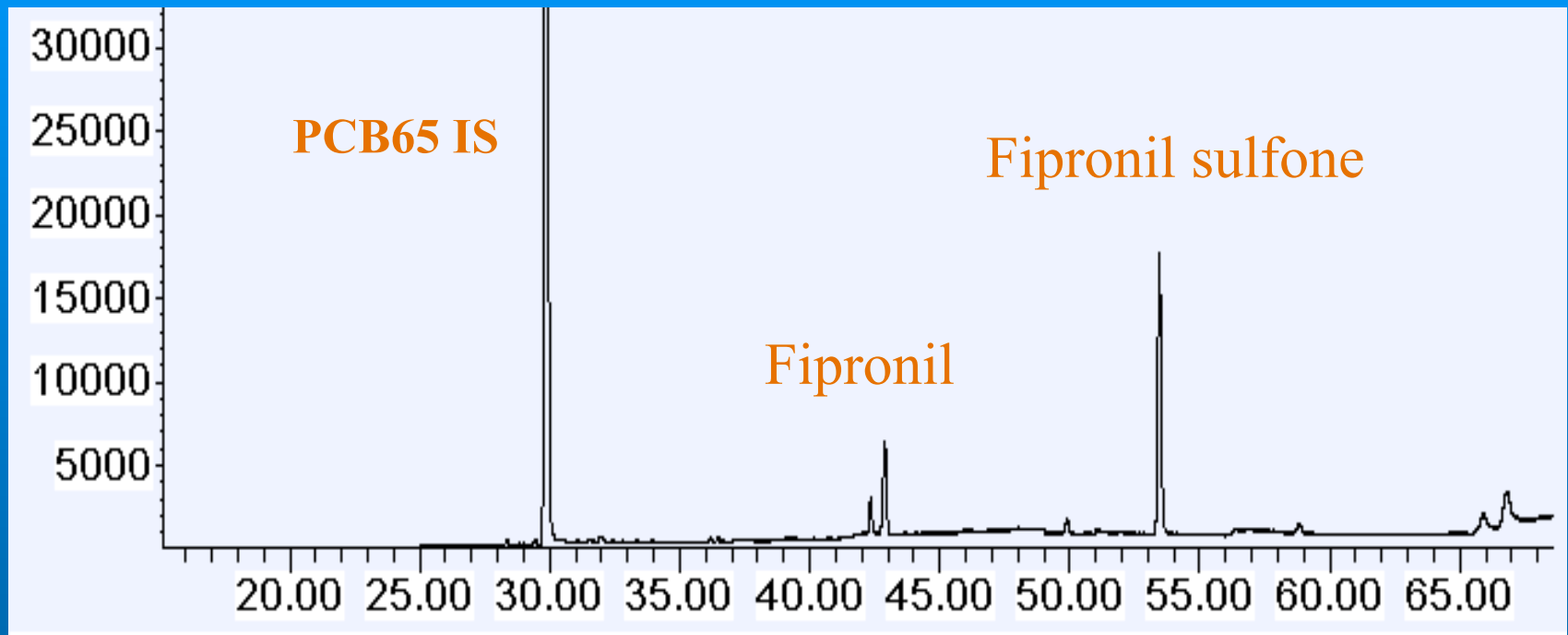


BGB 172 chiral column on GC-MS

Fipronil

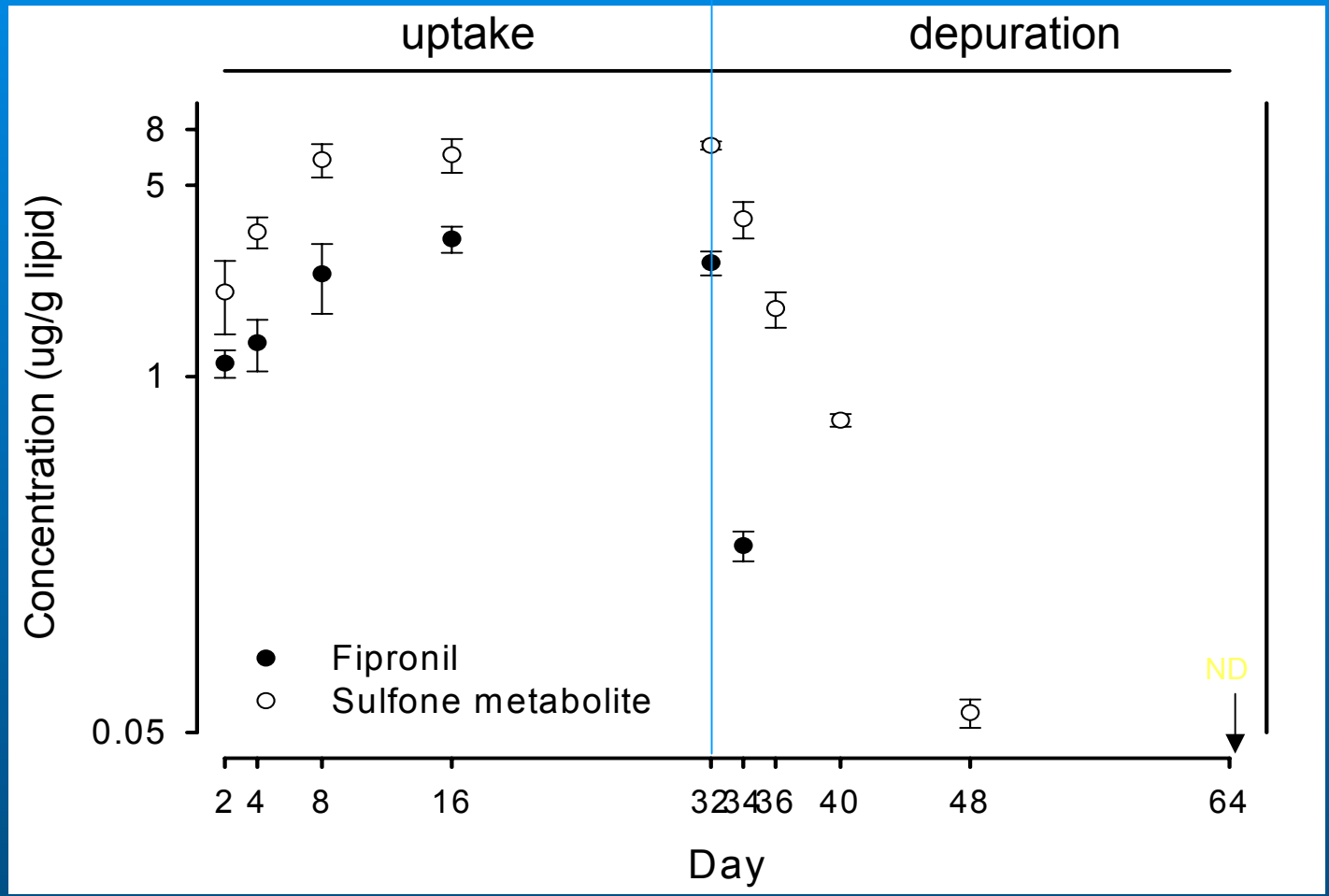


After feeding for 2 days



After feeding for 4 days

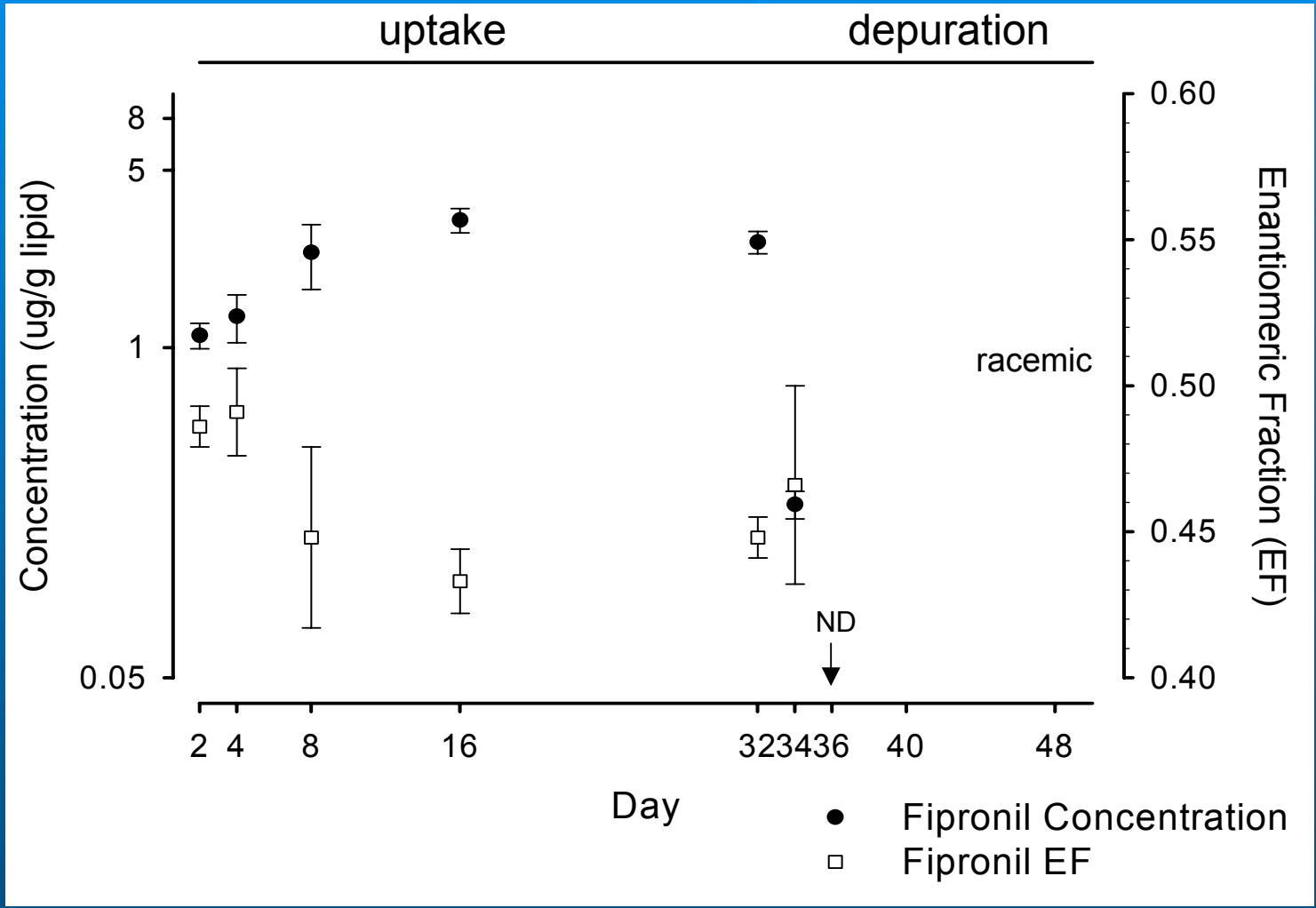
Fipronil & Sulfone Metabolite



Fipronil
BMF = 0.05
Half-life
0.58 d

Fipronil Sulfone
BMF = 4.8
Half-life
2.36 d

Fipronil


uptake
depuration
Concentration (ug/g lipid)
8
5
1
0.05
racemic
ND
Enantiomeric Fraction (EF)
0.60
0.55
0.50
0.45
0.40
2 4 8 16 32 34 36 40 48
Day
Fipronil Concentration
Fipronil EF

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

FIPRONIL ENANTIOSELECTIVE TOXICITY

TO *CERIODAPHNIA DUBIA*, ACUTE (48HR) LC50

	IN LIGHT	IN DARK
(+) ENANTIOMER	13.62	12.09
(-) ENANTIOMER	36.17	32.29
RACEMATE	18.58	19.39

AVERAGE OF 3 TESTS

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

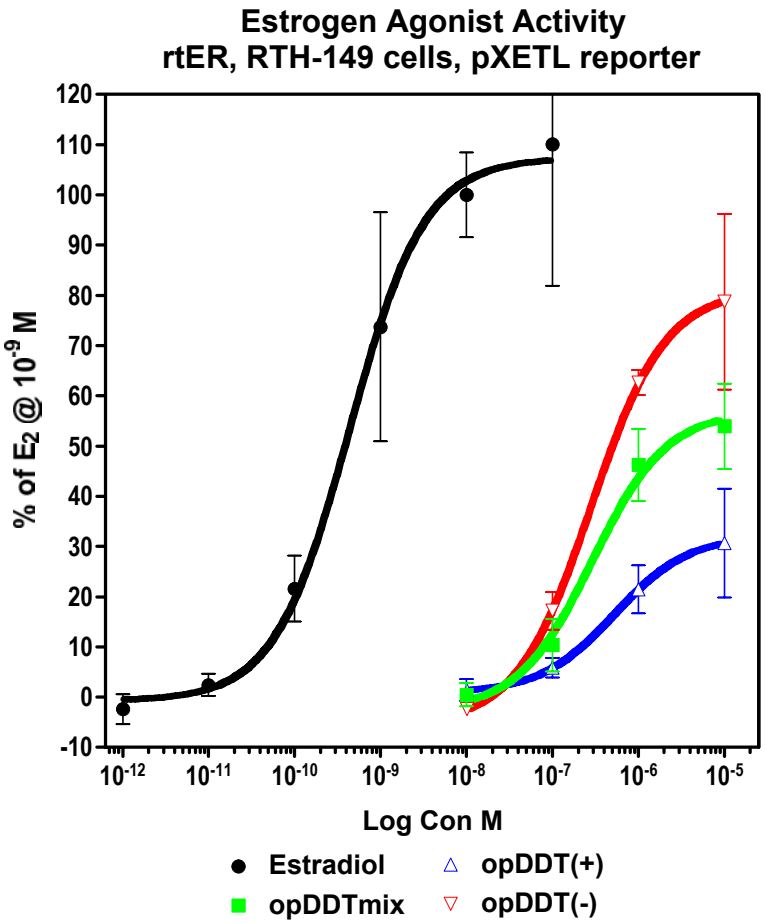
Wilson, W.A., Konwick, B.J., Garrison, A.W., Avants, J.K. and Black, M.C. Prepared for submission to Environ.Toxicol.Chem. March, 2006

ENDOCRINE DISRUPTER EFFECTS

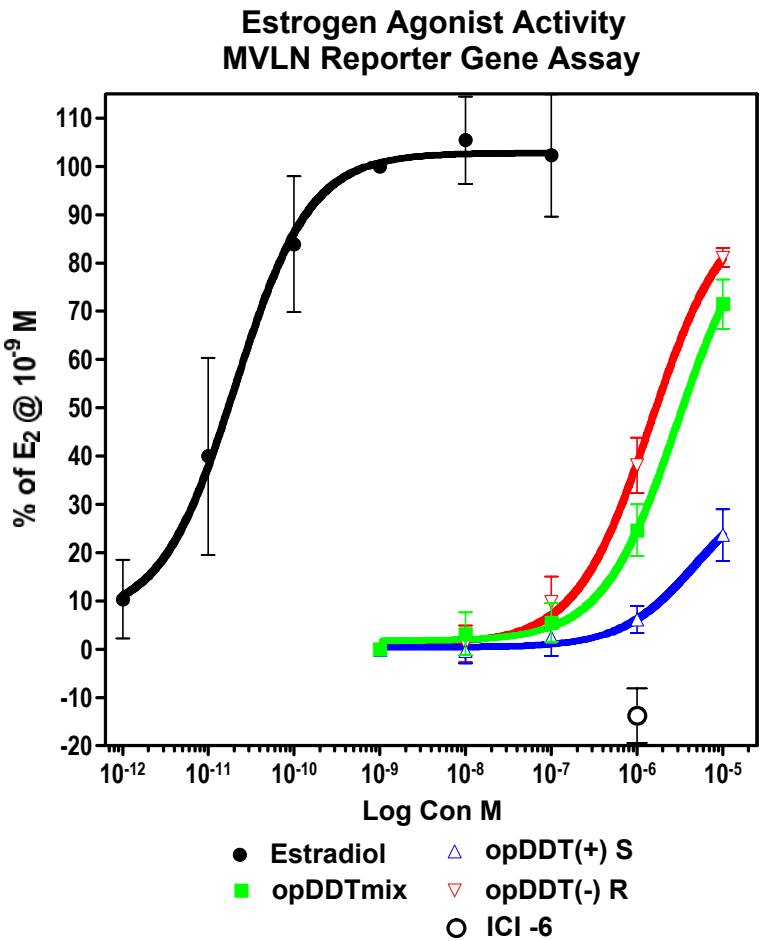
- THE ENANTIOMERS OF CHIRAL PESTICIDES ARE EXPECTED TO DIFFER IN THEIR ENDOCRINE DISRUPTER EFFECTS
- ENANTOMERS OF 11 PESTICIDES HAVE BEEN SEPARATED AND SCREENED FOR ED ACTIVITY BY *THOMAS WIESE, XAVIER UNIV. OF NEW ORLEANS*
- THE ED ACTIVITY IS USUALLY, BUT NOT ALWAYS, ENANTIOSELECTIVE; O,P'-DDT IS A STRIKING EXAMPLE

Enantiospecific Estrogen Activity of o,p'-DDT

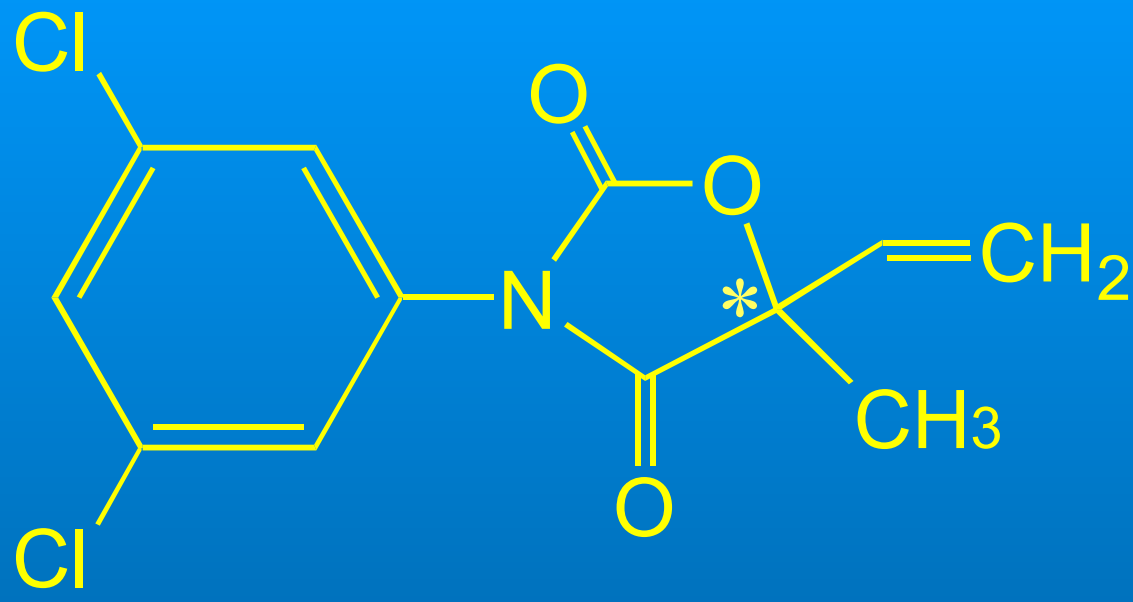
- Rainbow Trout Cell Hepatoma Cells



- Human Breast Cancer Cells



Wiese TE, Nehls S (2001) **Enantiomer Selective Estrogen and Antiandrogen Activity of Chiral Pesticides**
Symposia on Chiral Pollutants: Enantioselectivity and Its Consequences, SETAC National Meeting, Nov 10-15 2001, Baltimore, MD



VINCLOZOLIN

A FUNGICIDE AND ENDOCRINE DISRUPTER

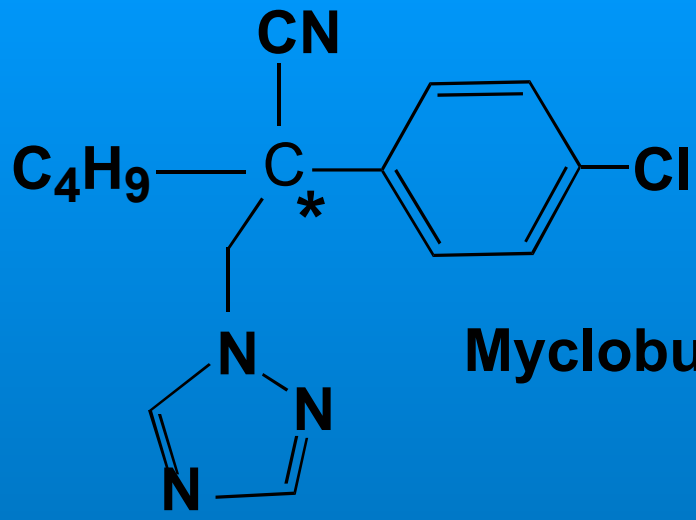
VINCLOZOLIN EFFECTS ON MEDAKA

- 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



Myclobutanil

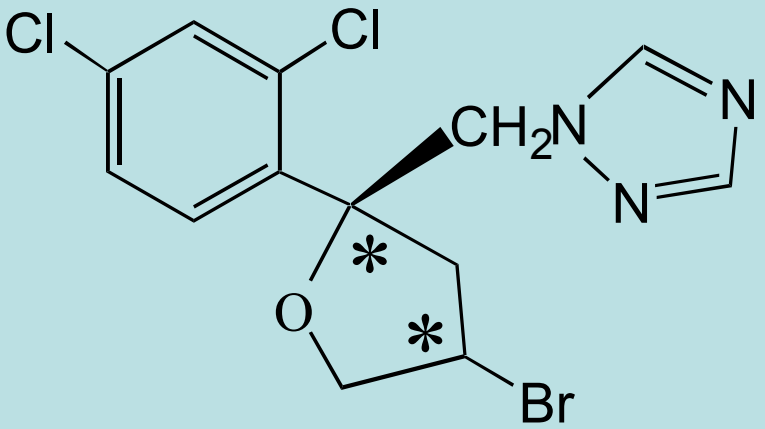
Test compounds selected by the EPA for the new Computational Toxicity Program

Bioaccumulation in rainbow trout – enantioselective?

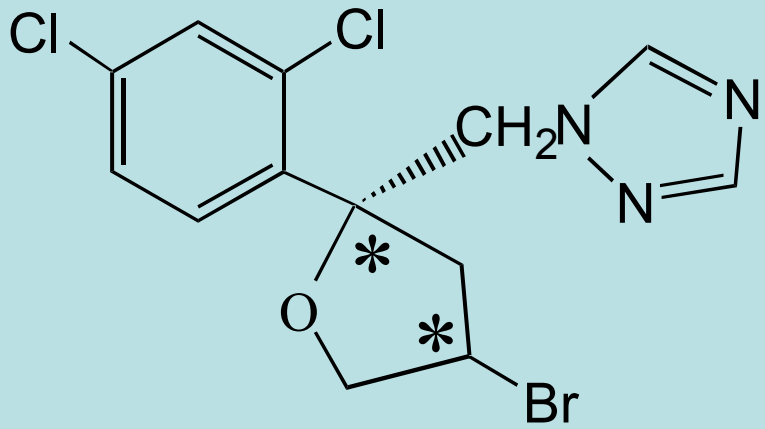
Metabolism *in vitro* by enzymes – enantioselective?

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



CIS (46)



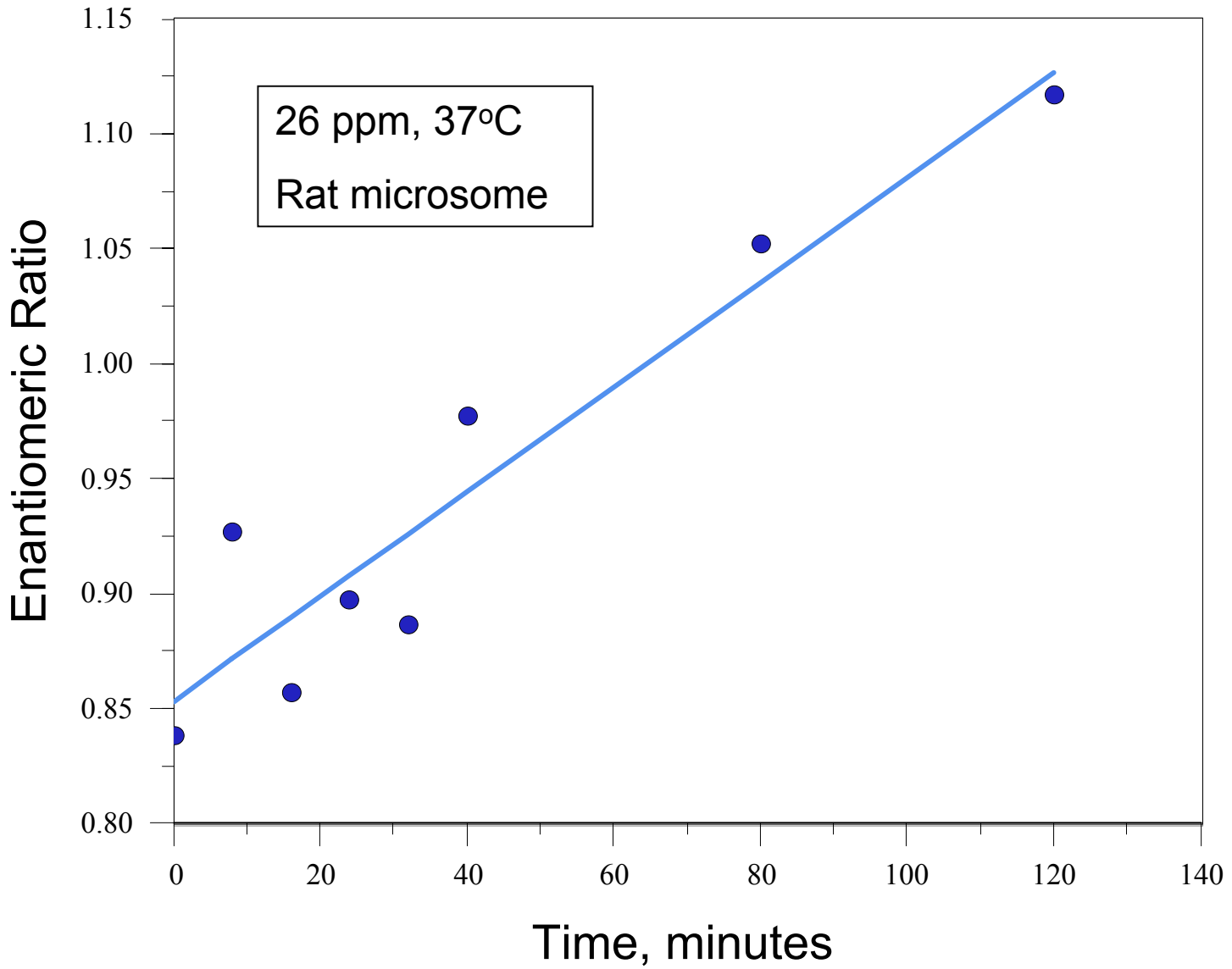
TRANS (47)

BROMUCONAZOLE

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)



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.

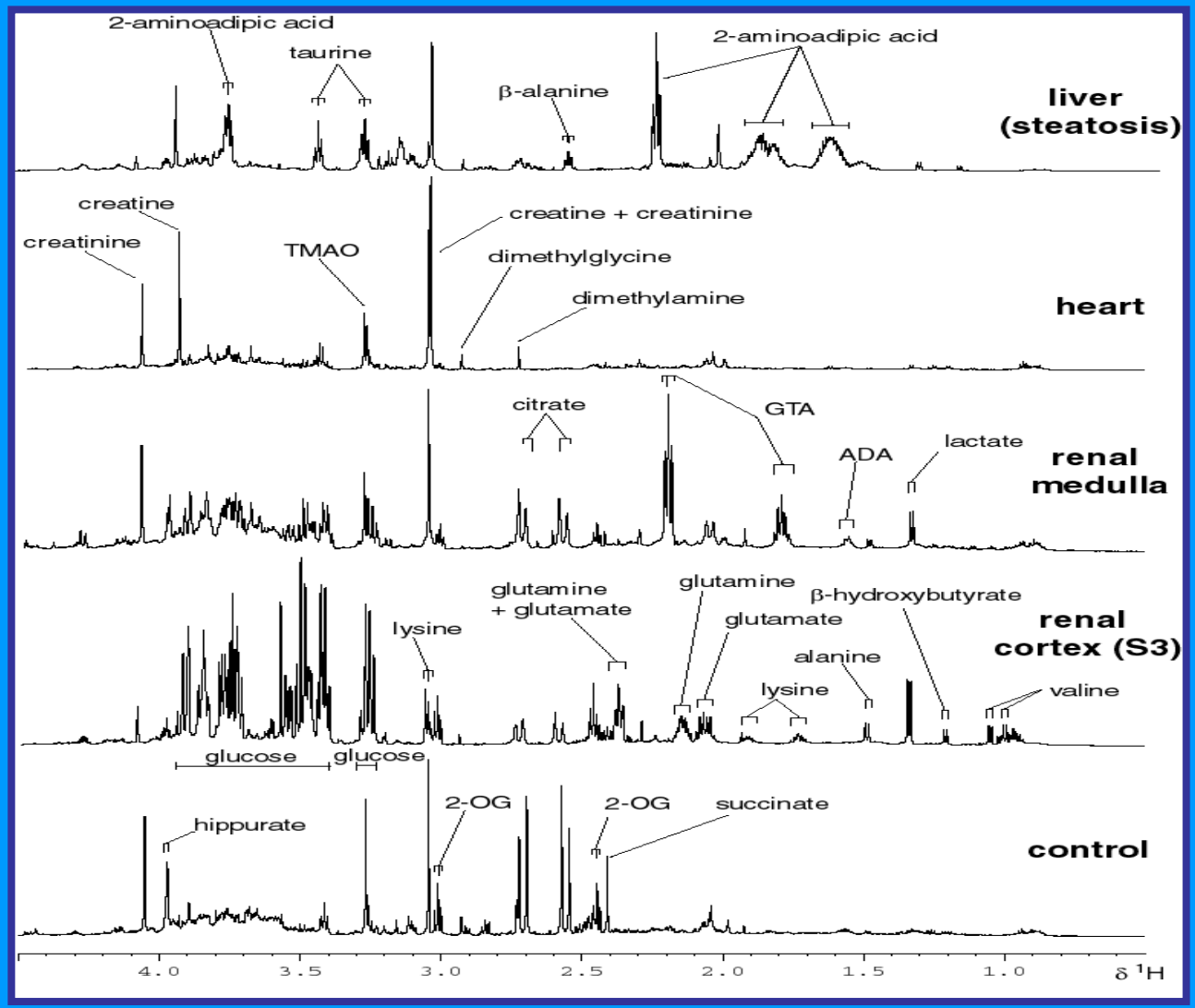
Levels of endogenous metabolites (e.g., in mice urine) are measured as a function of time with NMR spectroscopy.

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



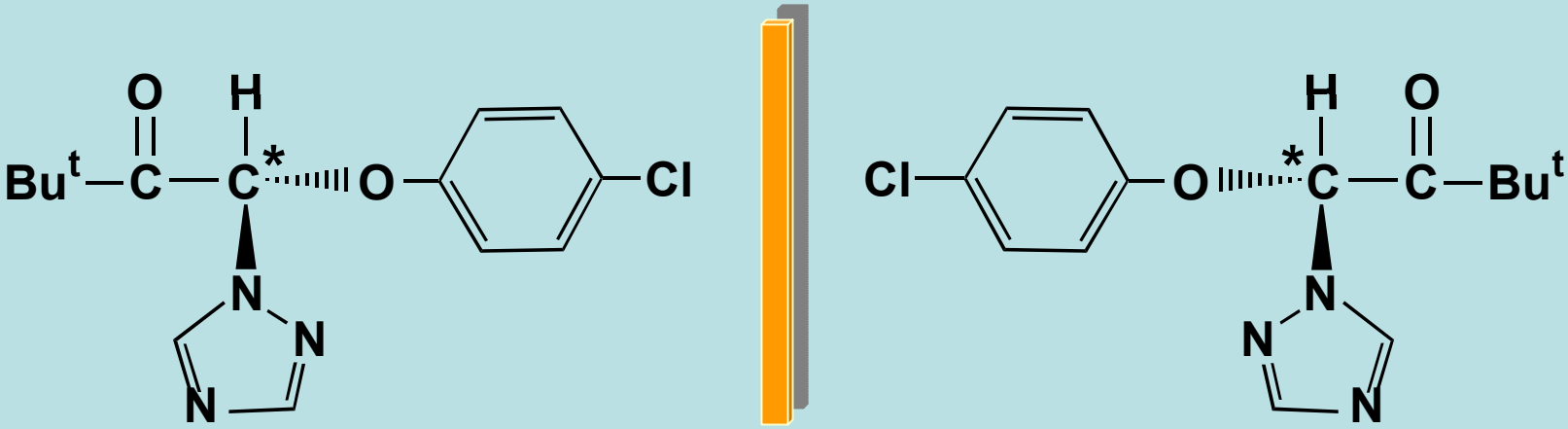
600 MHz ^{1}H NMR spectra showing the effect of tissue-specific toxins on the metabolic profile of urine

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-(-)

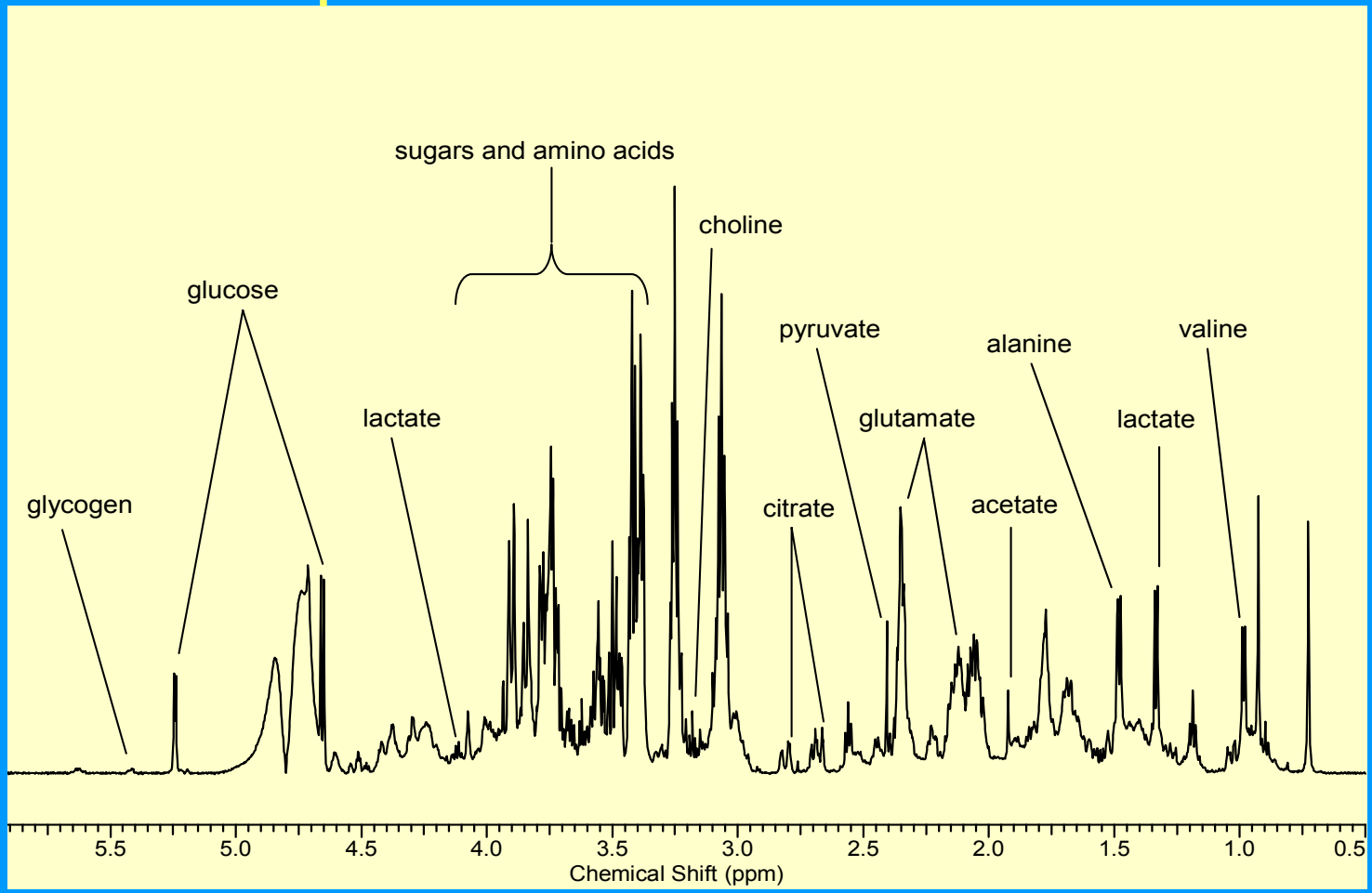
ENANTIOMER 2

R-(+)

Enantiospecific Toxicity Study Design

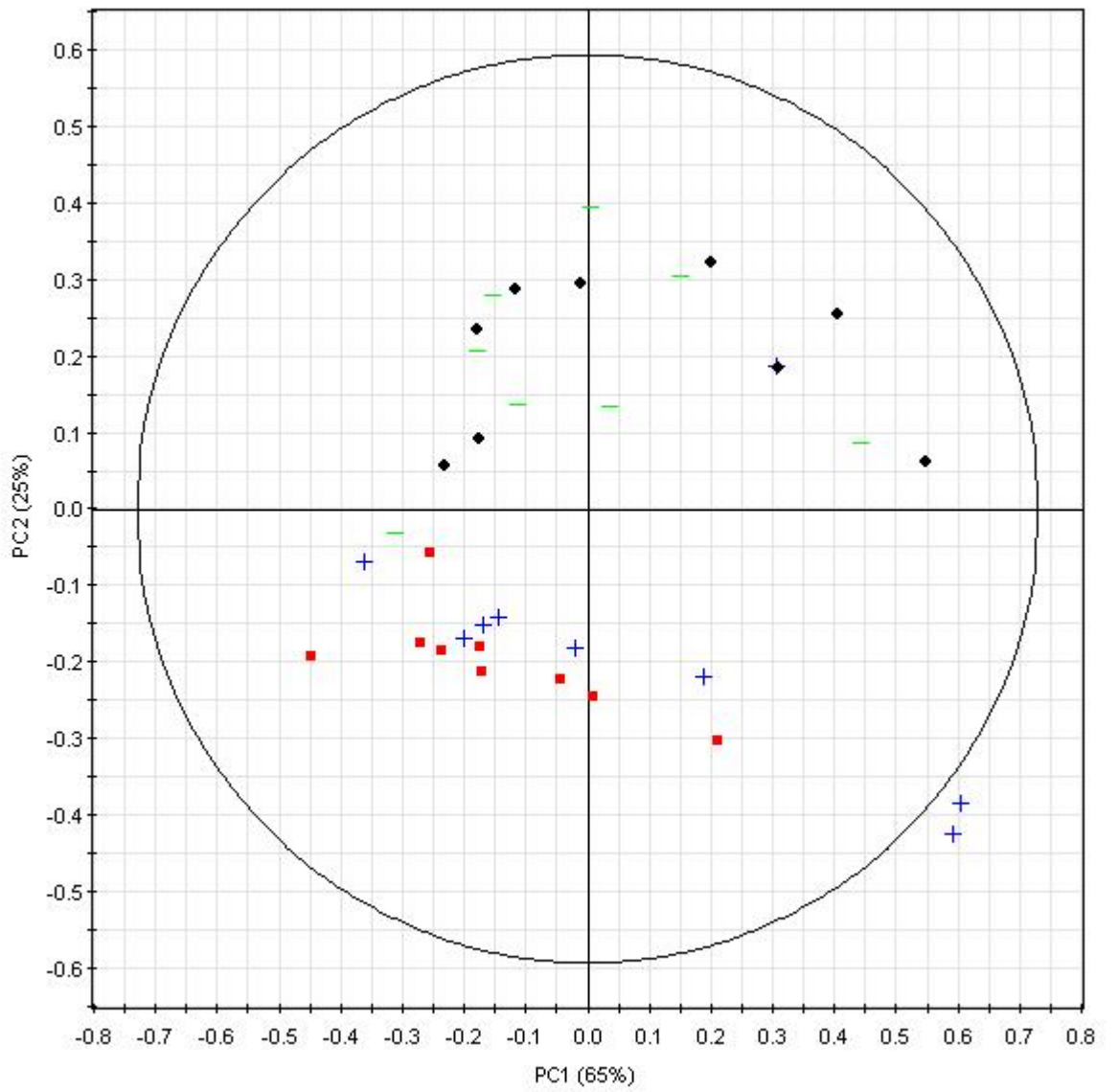
- **Juvenile rainbow trout were exposed (via gavage) to either one of the enantiomers or the racemate.**
- **Two dose levels and two time points were employed.**
- **High dose: 720 mg/kg/day; 24 and 48 hours**
- **Low dose: 144 mg/kg/day; 24 and 48 hours**
- **Livers were collected and extracted (perchloric 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



■ = control

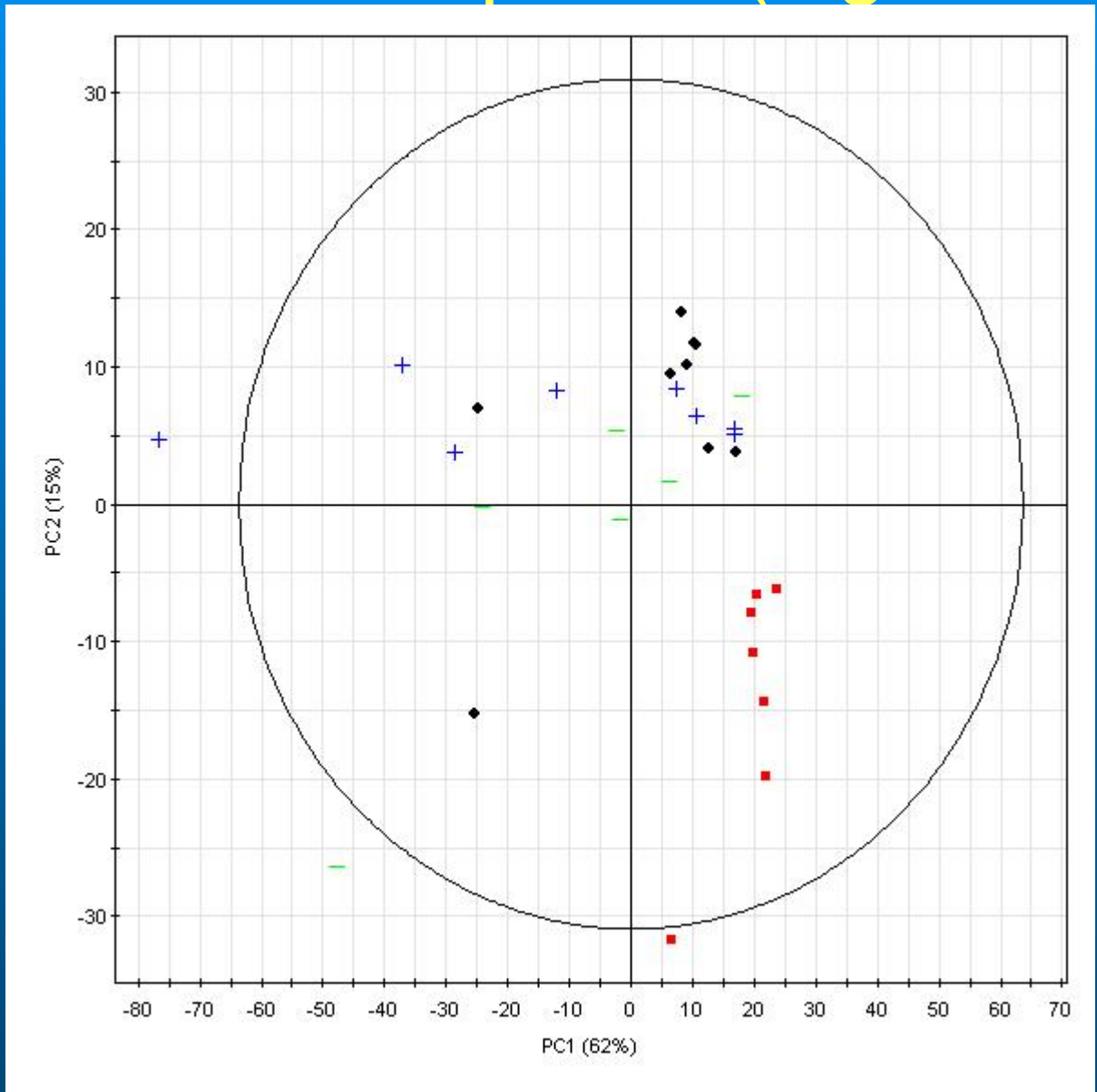
– = (-) enantiomer

\+ = (+) enantiomer

● = racemate

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

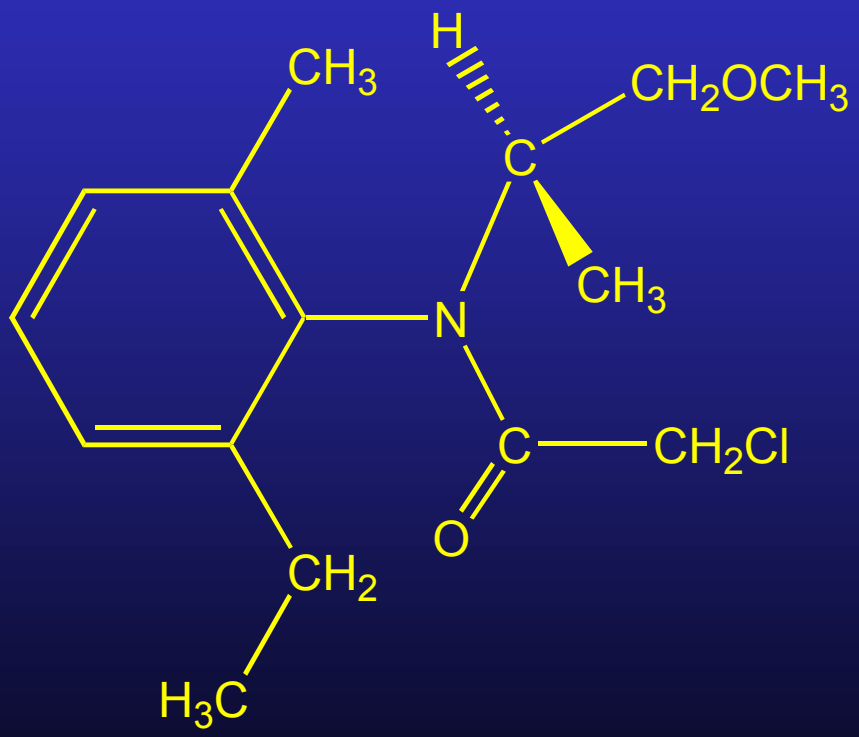
By 48 hours the (+) enantiomer exposed fish are displaying a response to treatment.

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