Enantioselective Toxicity and Biotransformation of Fipronil in the Fathead Minnow (*Pimephales promelas)*

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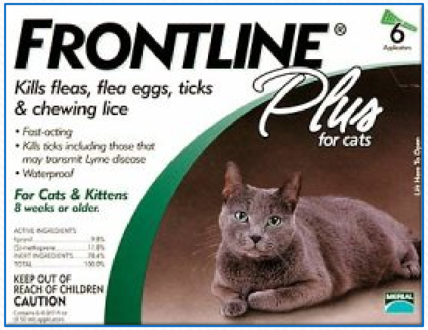
A little background info…

- Application increasing
- Variety of uses



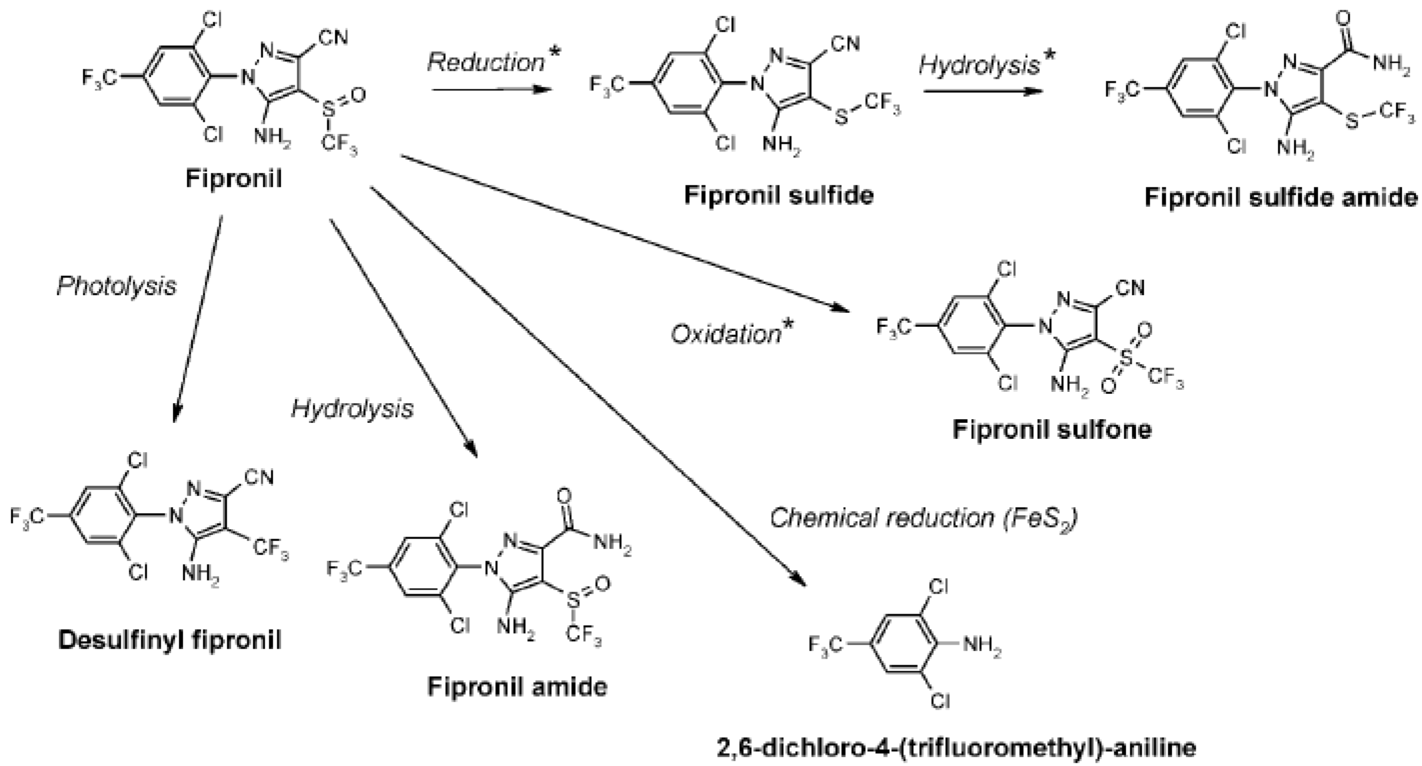
- Received attention for nontarget organism toxicity

Behavior in Aquatic Environment

- Hydrophobic : log Kow = 4.0 (Roberts et al. 1999)
- Sorbs to sediment organics: log Koc = 2.9 (USGS 2003)
- Degradates more persistent (Lin et al. 2008, Walse et al. 2004)

Rice agriculture in Mermentau Basin, LA

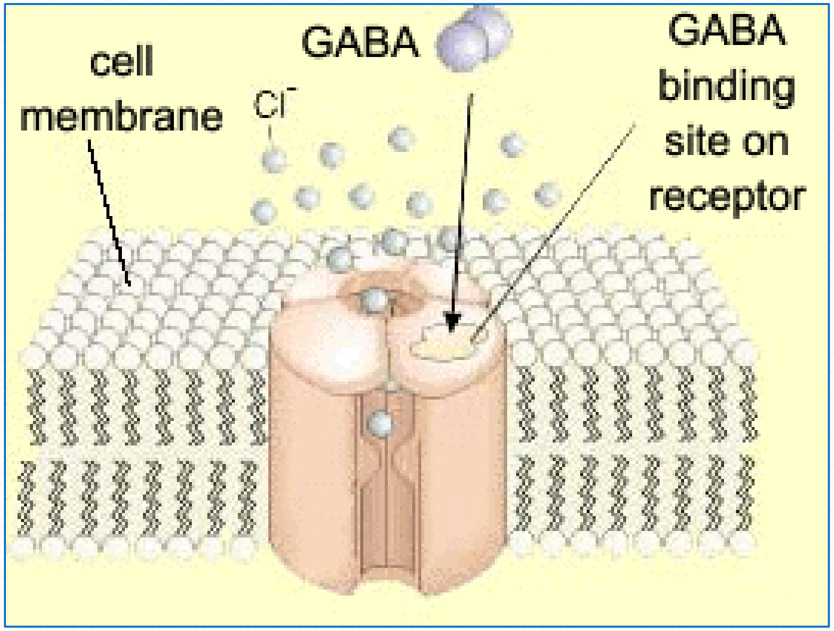
Fipronil Degradation



From Jones et al. 2007

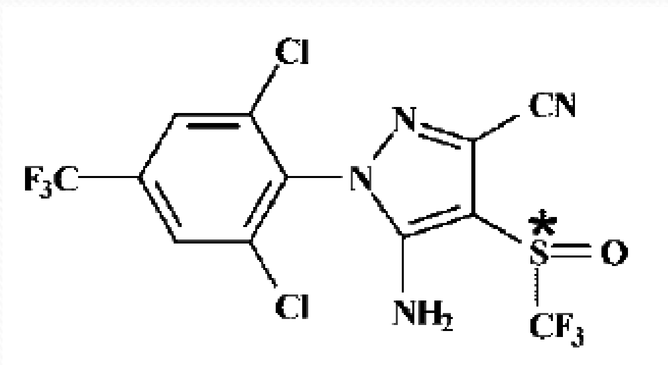
Fipronil MOA

- GABA receptor in invertebrates (Cole at al. 1993)
- Glycine receptor suggested as possible binding site in zebrafish (Stehr et al. 2006)

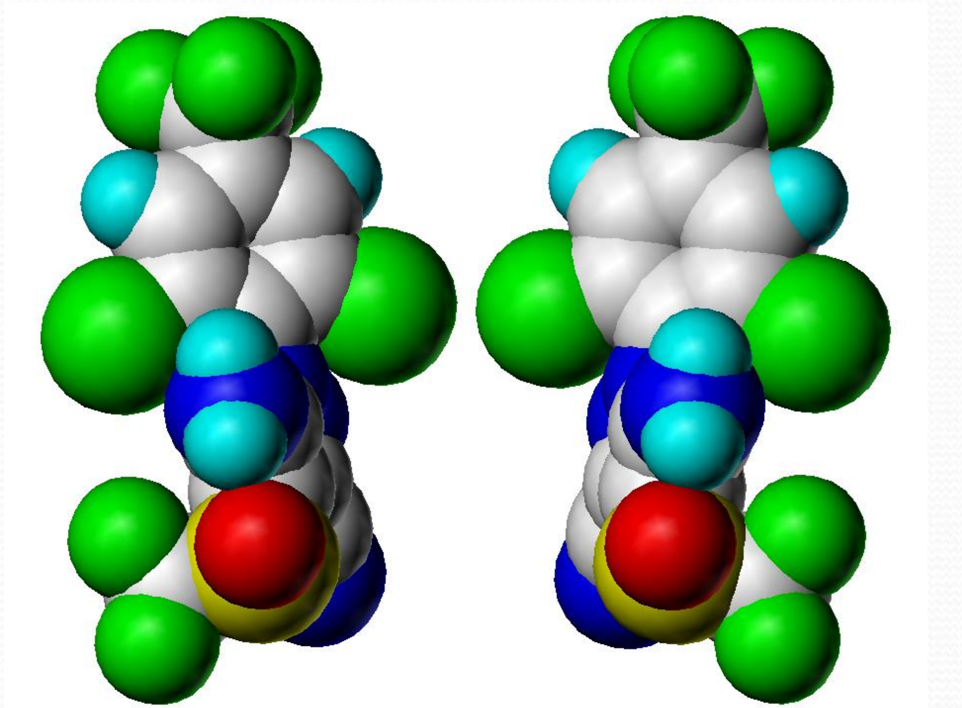


GABA and Glycine are structurally similar

Chirality of Fipronil



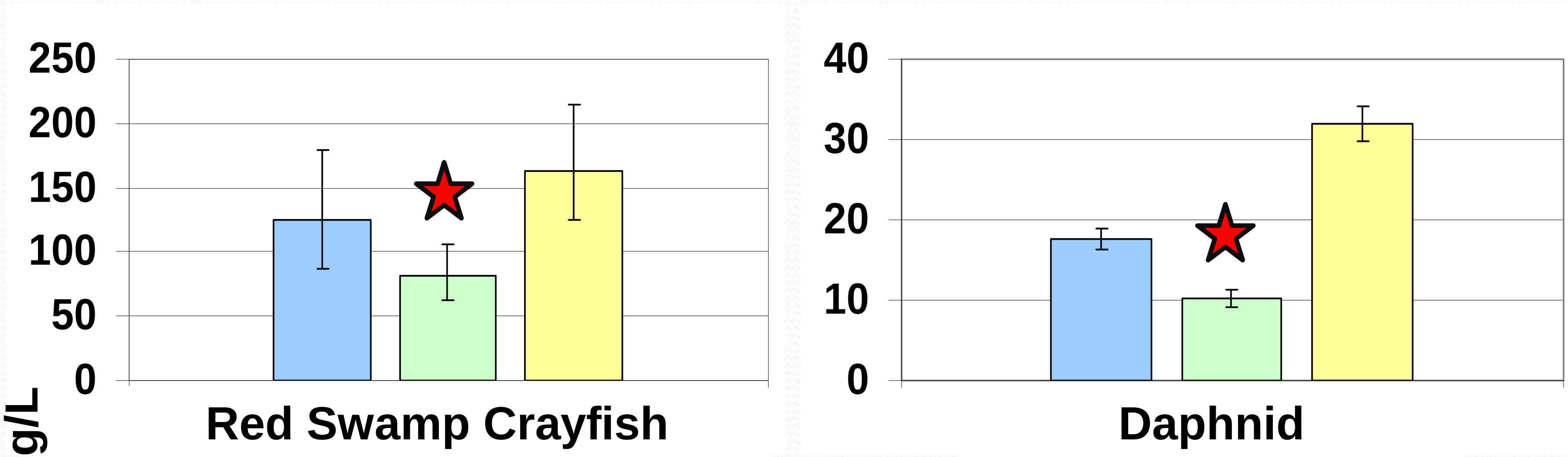
* Chiral Center

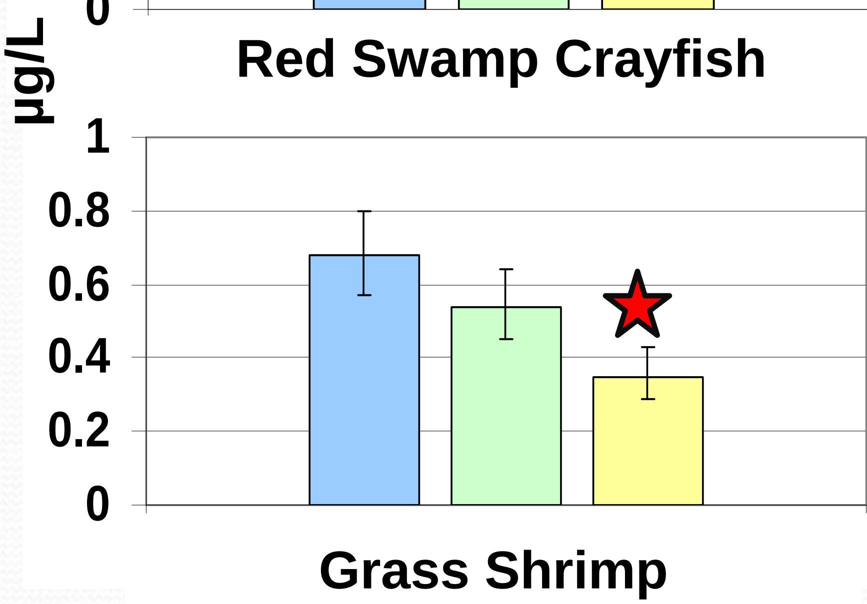


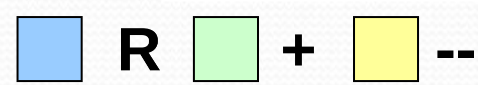
(R,-) (S,+)

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

Acute Enantioselective Toxicity - Invertebrates

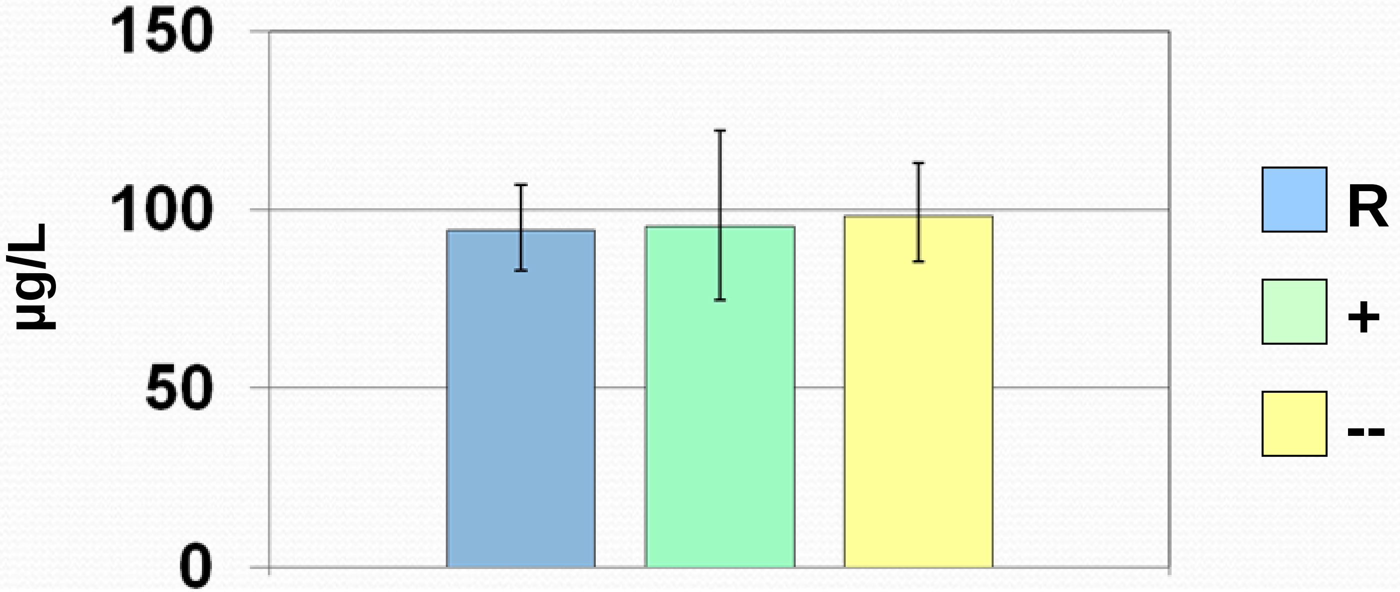






- Crayfish, Shrimp: Mean LC50 (95% CI) (Overmyer et al. 2007)
- Daphnid: Mean LC50 (SE) (Konwick et al. 2005)

Acute Enantioselective Toxicity - Fish

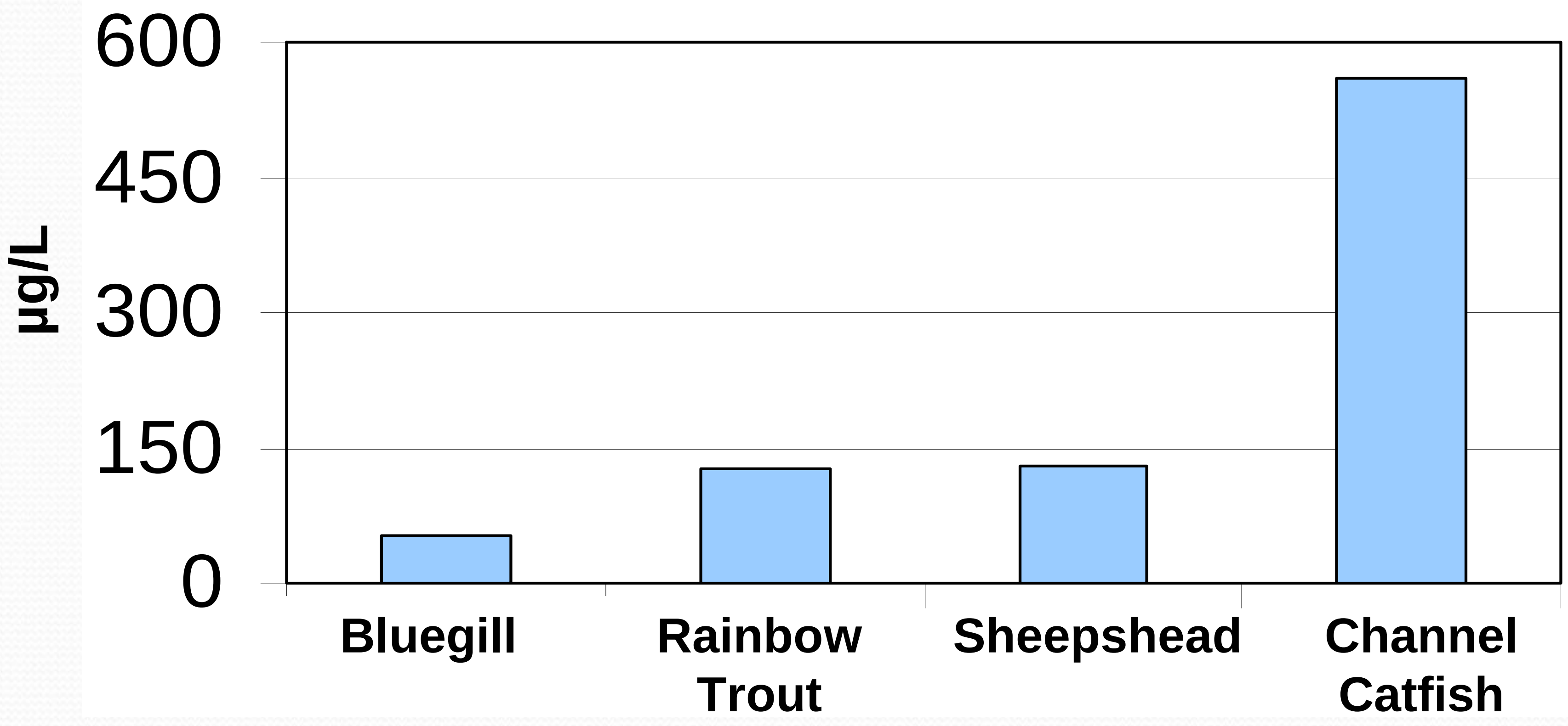


Japanese medaka

Mean LC50 (95% CI)

(Nillos et al. 2005)

Acute Toxicity - Fish

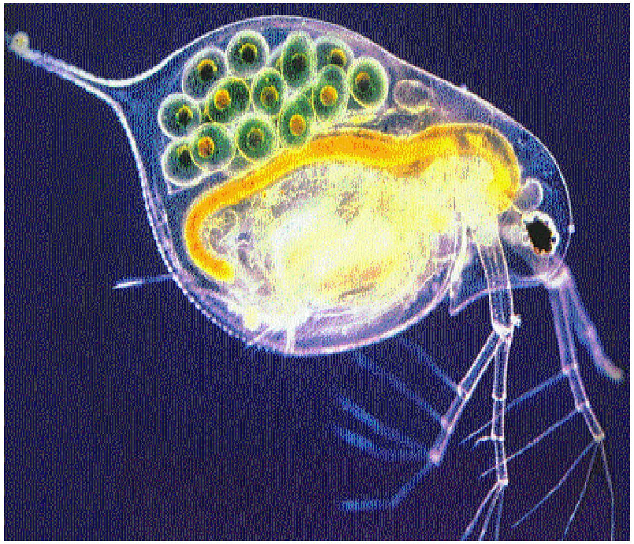


Mean LC50 of racemic fipronil

(USEPA Ecotox Database)

Chronic Enantioselective Toxicity

Daphnid: (+) enantiomer reduced reproduction at lower concentrations than racemate and (-) enantiomer
(Wilson et al. 2007)

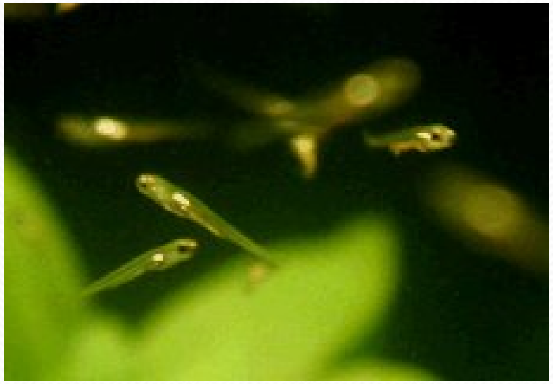


Objectives

- Quantify enantioselective toxicity of fipronil to fathead minnow
  - 96 hr acute, 7 day subchronic toxicity
- Measure behavior of fipronil in a sediment-water-fish system
  - 42 day exposure to fipronil spiked sediment
  - Accumulation/biotransformation of fipronil in fish
  - Degradation products in sediment and water

Pimephales promelas

- EPA small fish model
- Regulatory and research applications
- Many characteristics conducive to toxicology research



Acute and Chronic Toxicity

- 7 day test with larval fathead minnows
  - Racemate
  - (+) Enantiomer
  - (-) Enantiomer
- Measure acute toxicity after 4 days
- Measure subchronic toxicity via growth and survival
- Compare toxicity of racemate and enantiomers

Subchronic Lethality

Chemical	µg/L	
	7-day LC50*	95% Confidence Interval
Racemate	208[a]	192-225
(+) Enantiomer	228[a]	201-244
(-) Enantiomer	365[b]	333-398

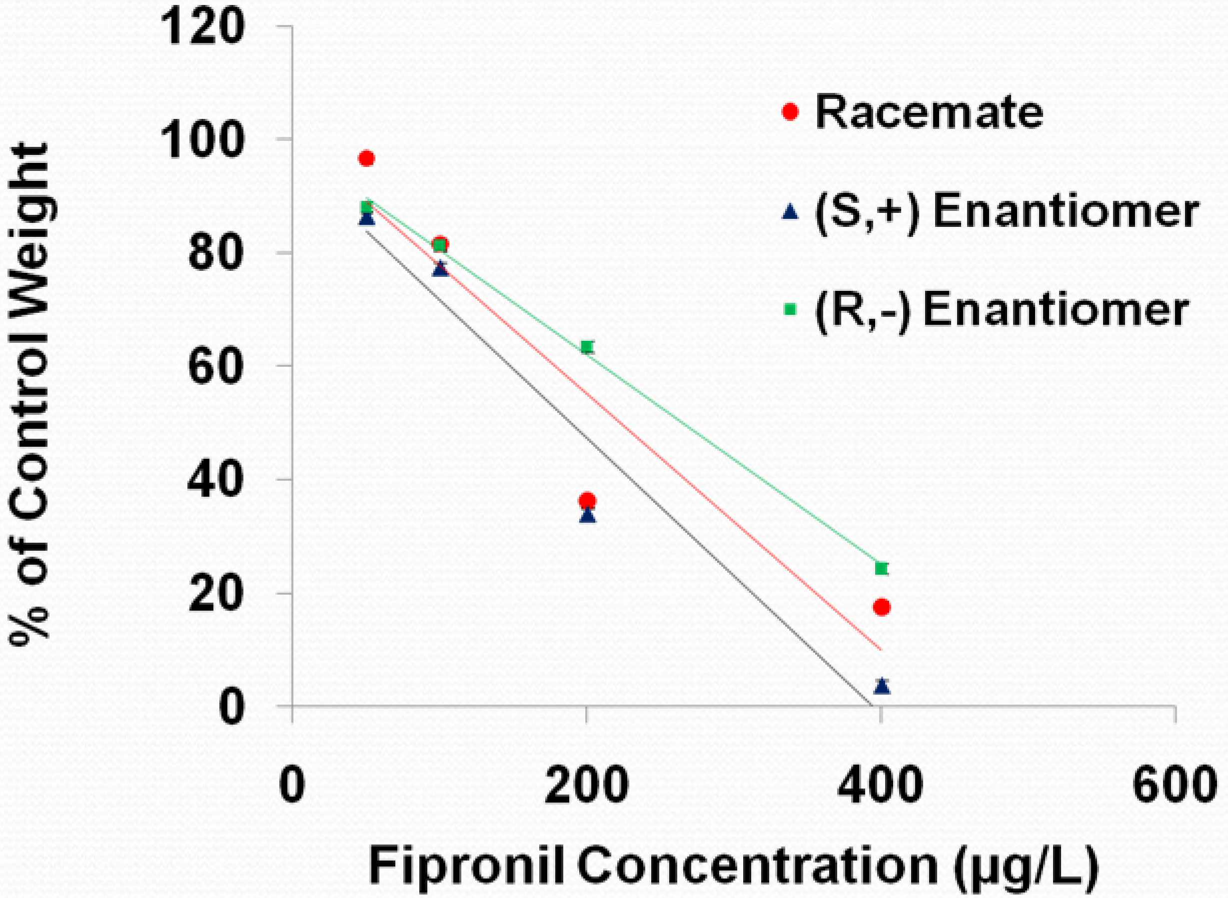
* Different letters indicate significantly different values

Subchronic Effect on Growth

Chemical	Slope*	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Racemate	-0.00273[a]	-0.00322	-0.00223
(+) Enantiomer	-0.00288[a]	-0.00329	-0.00246
(-) Enantiomer	-0.00196[b]	-0.00216	-0.00176

* Different letters indicate significantly different values

Subchronic Effect on Growth



Results

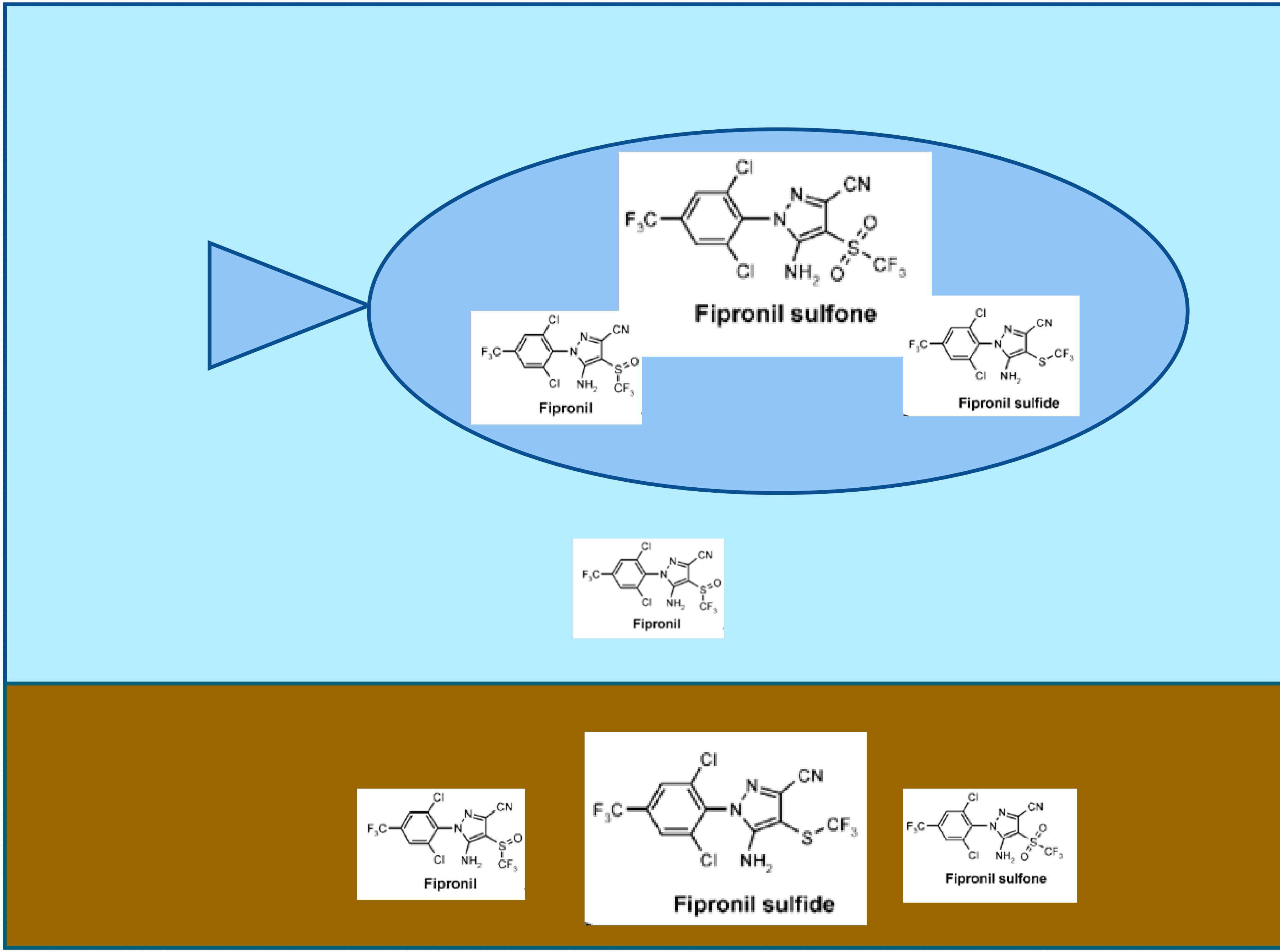
- Racemate and (+) enantiomer significantly more toxic than (-) enantiomer

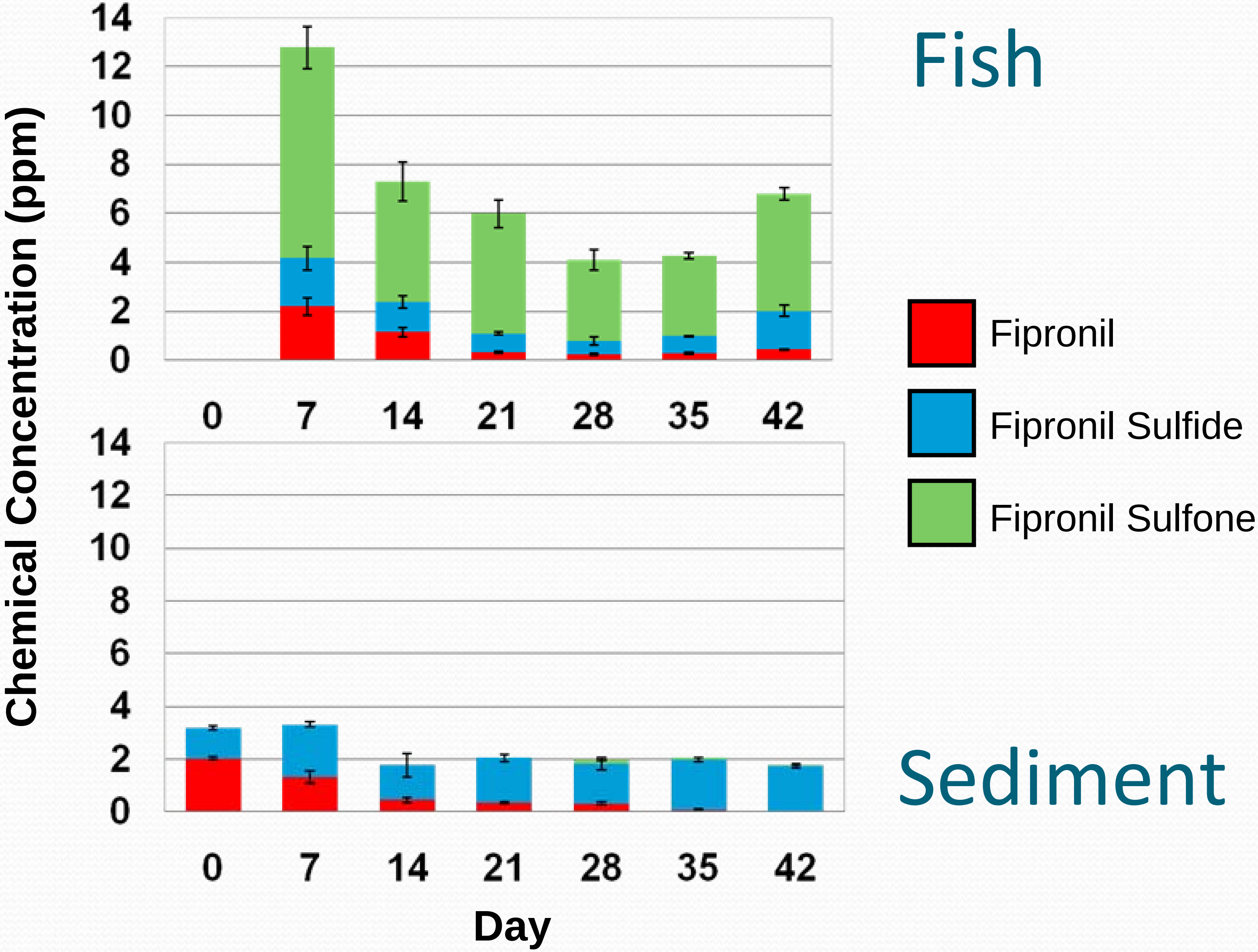
- Curious trend…Would expect racemate toxicity to be intermediate compared to enantiomers

Behavior of Fipronil in a Sediment-Water-Fish System

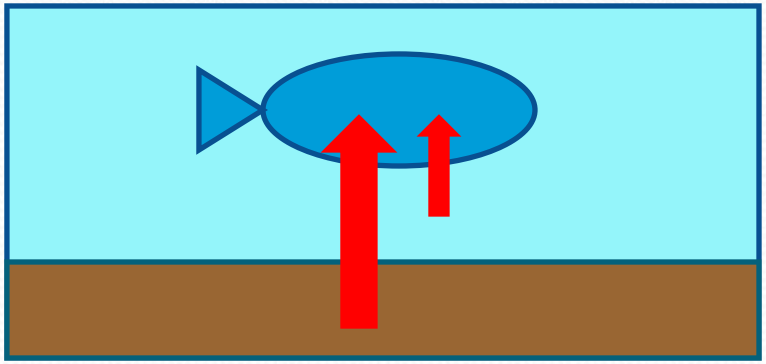
1. Collect sediment from USDA site
2. Spike sediment with racemate
3. Age for 6 months under anoxic conditions
4. Load sediments into test vessels
5. Add overlying water
6. Add airlines and fish
7. Sample fish, sediment, water weekly for 6 weeks
8. Analyze samples

Fish age 101-120 days


Fipronil sulfone
Fipronil
Fipronil sulfide
Fipronil
Fipronil
Fipronil sulfide
Fipronil sulfone


Fish
Sediment
Chemical Concentration (ppm)
Day
0
2
4
6
8
10
12
14
0
7
14
21
28
35
42
Fipronil
Fipronil Sulfide
Fipronil Sulfone

Bioaccumulation Factor



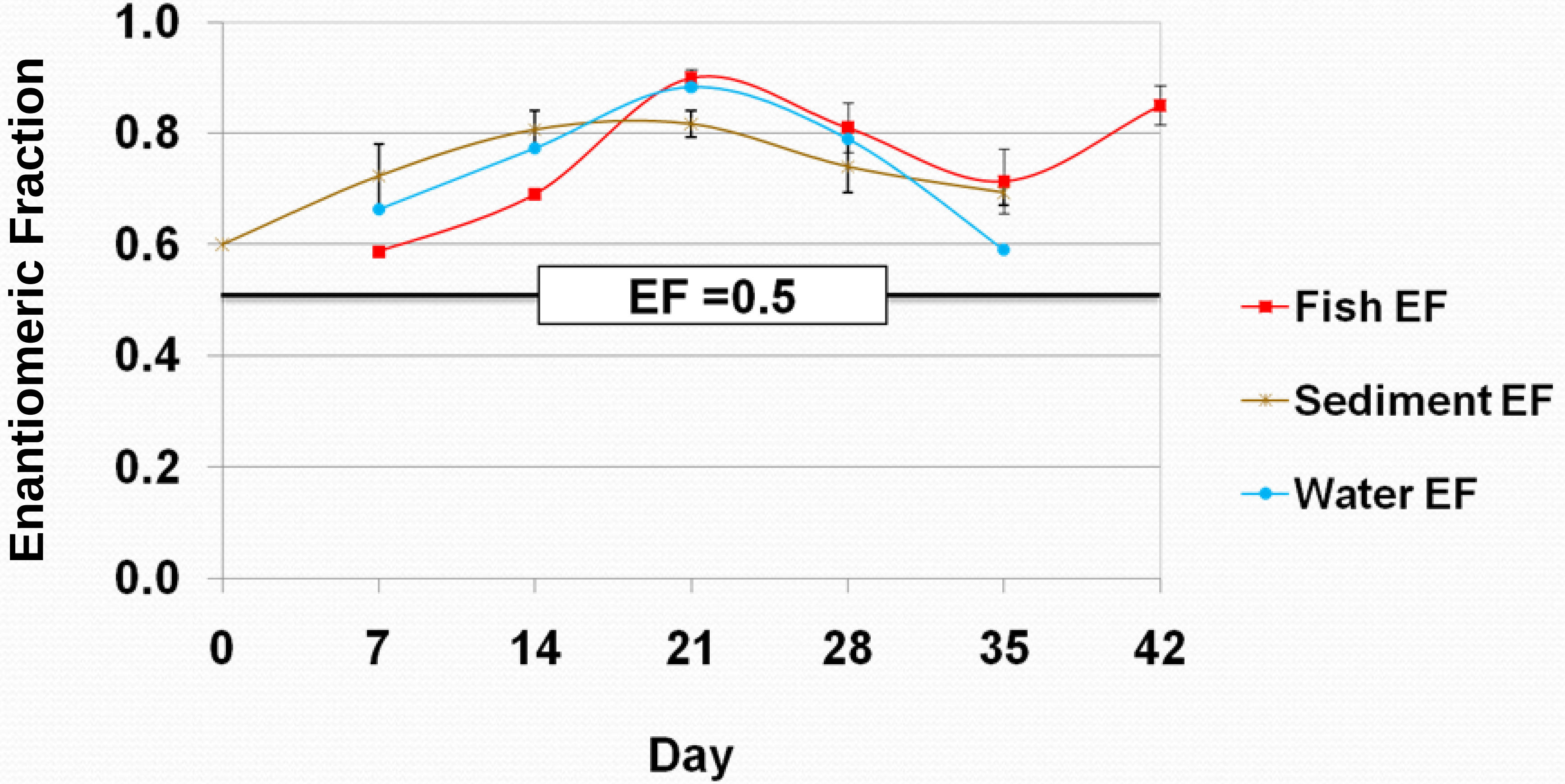
Bioaccumulation Factor =

$$\frac{[\text{All Forms in Fish}]}{[\text{All Forms in Sediment + Water}]}$$

Day	BAF
7	3.90
14	5.12
21	3.02
28	2.14
35	2.08
42	3.83

Enantiomeric Fraction

$$EF = E_{+}/(E_{+}+E_{-})$$

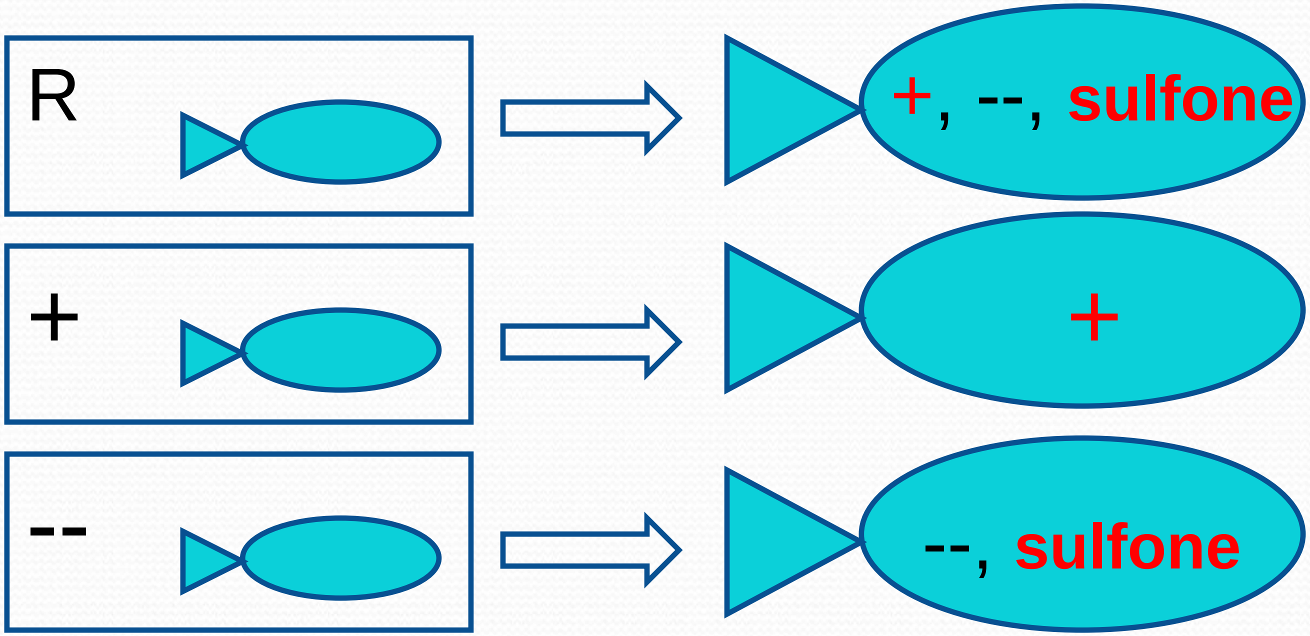


Summary of Results

- Primary form in sediment: fipronil sulfide
- Fish bioaccumulate fipronil and transform to fipronil sulfone
- Fish and Sediment EF>0.5 throughout study
- Racemate and (+) more toxic than (-) enantiomer

Enantioselective Toxicity

- Assume loss of (-) enantiomer to sulfone metabolite (from bioaccumulation study)
- Assume increased toxicity of sulfone (based on trout and bluegill data) and (+) enantiomer (from tox study)



Red = toxic

Risk Assessment of Fipronil

- Need to consider more than just fipronil
  - In sediments, fipronil sulfide likely source of toxicity
  - Aquatic orgorganisms may be exposed primarily to metabolites, exposure not limited to forms in sediment
  - Changes in EF also important factor
  - Role of metabolic activation

Acknowledgments

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- SETAC-Travel Award
- EPA

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www.etfrc.com/benzos1.htm (Slide 5)

http://www.noaanews.noaa.gov/stories2006/ (Slide 10, 12)

http://www.state.sd.us/DENR/DES/Surfacewater/ (Slide 12)

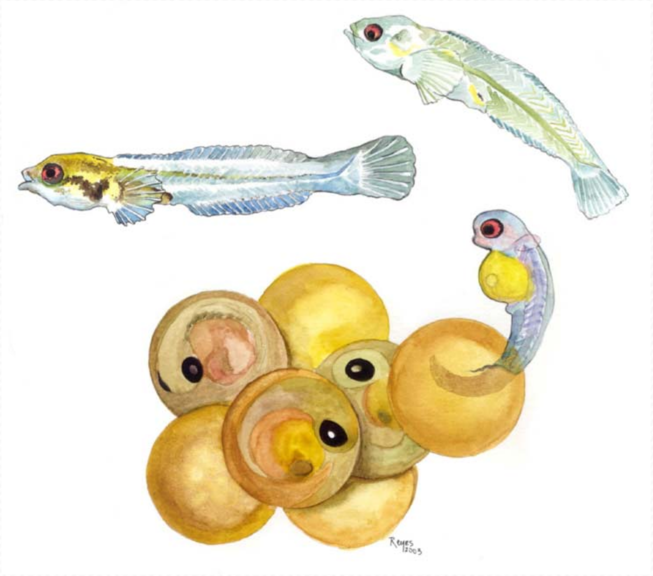
USGS (Slide 2, 3)

Questions?

"Sure it costs more. We have to squash bugs by hand."

Toxicity of Degradation Products

- Oxidation degradation product fipronil sulfone more lethal to *O. mykiss* and *L. macrochirus*
- Desulfynil photoproduct more lethal to *C. dubia,* but less lethal in *P. clarkii*



Chronic Toxicity

- No decrease in egg production in female *P. palaemonetes,* but alters sex ratio
- Decrease development in *Amphiascus tenuiremis* and decrease female egg extrusion
- Inhibits growth of *O. mykiss* and *Americamysis bahia*