BIOTRANSFORMATION OF TRIADIMEFON TO TRIADIMENOL: ENANTIOMER ANALYSIS AND ENANTIOSELECTIVITY IN SOIL SLURRIES

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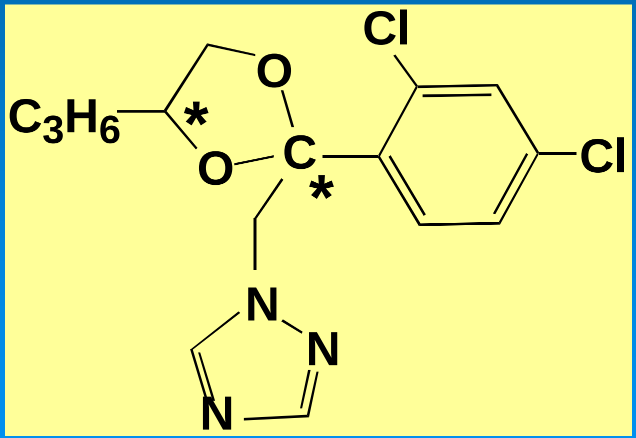
DISCLAIMER: *Although this work was reviewed by EPA, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

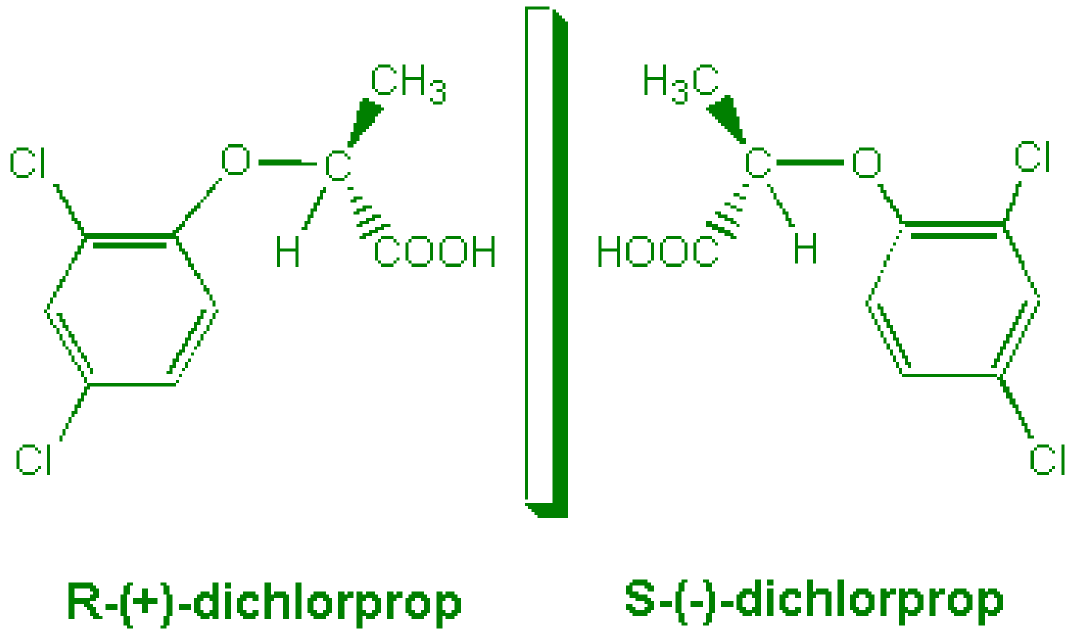
INTRODUCTION

- CONAZOLES ARE IMPORTANT FUNGICIDES – ABOUT 60 IN USE
- USED AS BOTH AGRICULTURAL AND MEDICINAL FUNGICIDES
- INHIBIT STEROL C-14 DEMETHYLATION THROUGH INTERFERENCE WITH A CYTOCHROME P-450 MONOOXYGENASE ENZYME
- MOSTLY 1,2,4-TRIAZOLES, e.g.: PROPICONAZOLE – ALL ARE CHIRAL



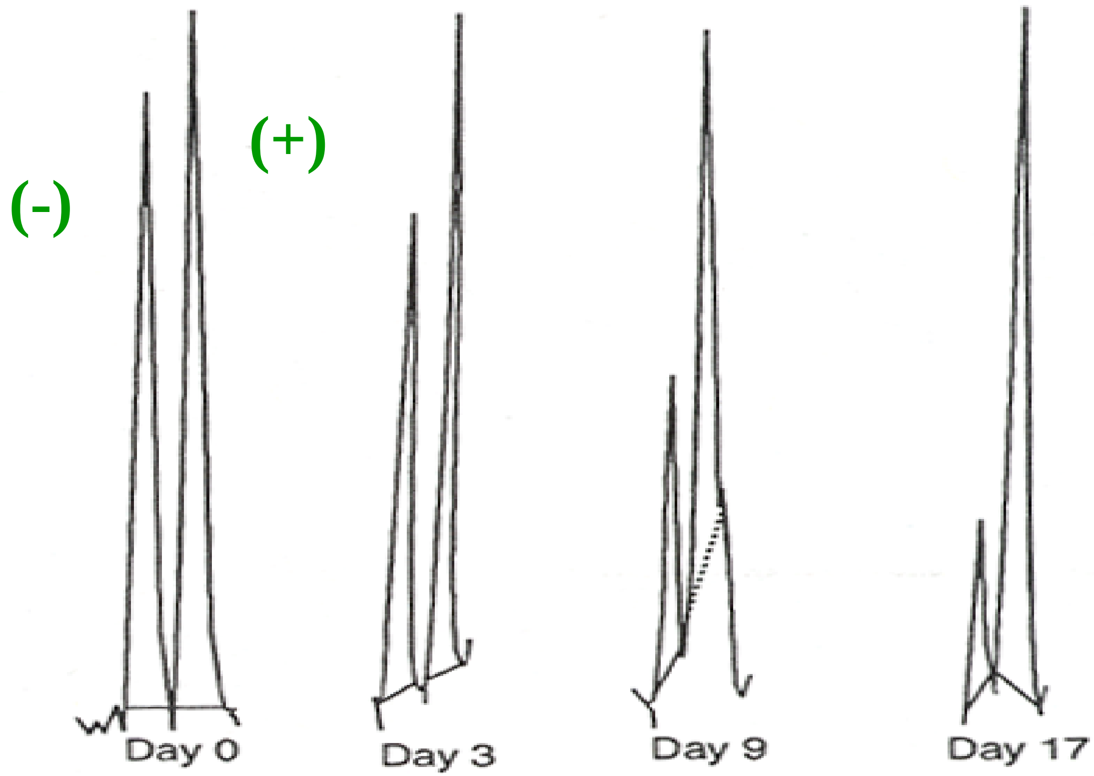
- CONAZOLES OCCUR AS ENVIRONMENTAL CONTAMINANTS:

Kahle M, Buerge, IJ, Hauser, A, Muller, MD, Poiger, T. Environ.Sci.Technol. 2008 (ASAP)



Enantiomers of the Chiral Herbicide Dichlorprop

CHIRALITY AND ENANTIOSELECTIVITY



Degradation of dichlorprop acid

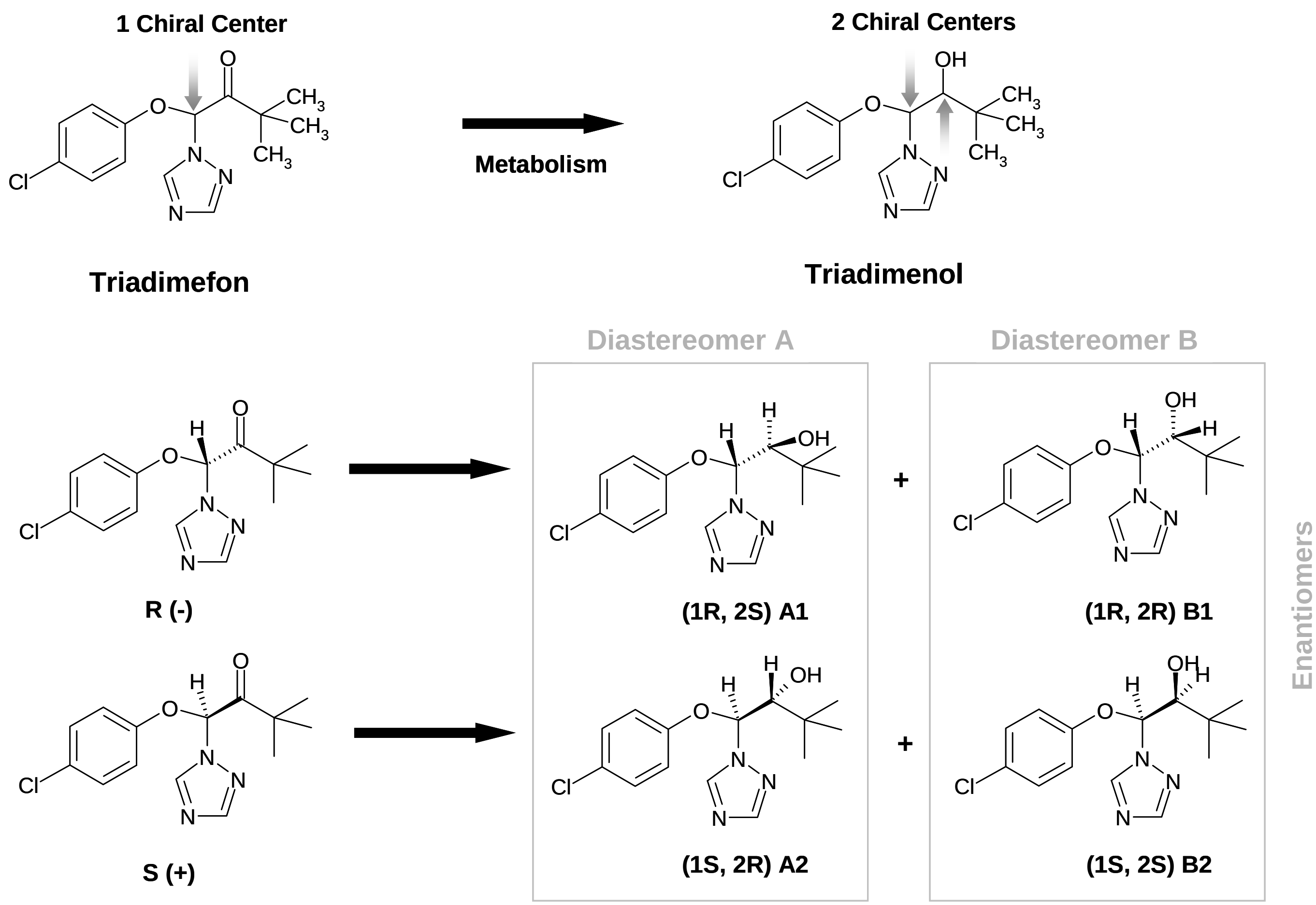
The (-)-enantiomer degrades twice as fast as the (+)

RESEARCH IN ENVIRONMENTAL CHIRAL CHEMISTRY

- **Analysis of enantiomers**
  - **- GC, HPLC, CE**
- **Occurrences of chiral chemicals and their enantiomers**
  - **- soil, sediment, water, biota, food**
- **Bioaccumulation**
  - **- fish, plants, other organisms**
- **Biotransformation of chiral chemicals**
  - **- enantioselectivity, kinetics, metabolism**
- **Effects**
  - **- separation of enantiomers for testing with various end points**

HYPOTHESIS

TRIADIMEFON AND PROPICONAZOLE, IMPORTANT REPRESENTATIVE CONAZOLES, WILL BE TRANSFORMED ENANTIOSELECTIVELY BY SOIL MICROBES



METABOLOMIC TRANSFORMATION OF TRIADIMEFON TO TRIADIMENOL. THE REDUCTION OF A CARBONYL GROUP TO AN ALCOHOL YIELDS A SECOND CHIRAL CENTER AND 4 STEREOISOMERS.

(slide courtesy of John Kenneke)

APPROACH / METHODS

- SELECT 3 SOILS WITH DIFFERENT PROPERTIES
  PREPARE 20mL OF SLURRY, 1 PART SOIL
  5 PARTS STERILE WATER, IN TRIPLICATE
- SPIKE SOIL SLURRY MICROCOSMS WITH
  TRIADIMEFON OR PROPICONAZOLE, 50 mg/L
- AUTOCLAVE CORRESPONDING SPIKED
  SLURRIES AS CONTROLS, IN TRIPLICATE
- SHAKE CONTINUOUSLY IN THE DARK

APPROACH / METHODS, CONTINUED

- AT SELECTED TIMES, REMOVE 1mL ALIQUOTS OF EACH SLURRY
- ANALYZE BOTH WATER AND SOIL PHASES BY GC-MS, SOME BY CE, USING CHIRAL ANALYSIS TECHNIQUES
- CALCULATE KINETIC VALUES AND MEASURE ENANTIOSELECTIVITY

$$EF = \frac{[\text{first eluting enantiomer}]}{[\text{both enantiomers}]}$$

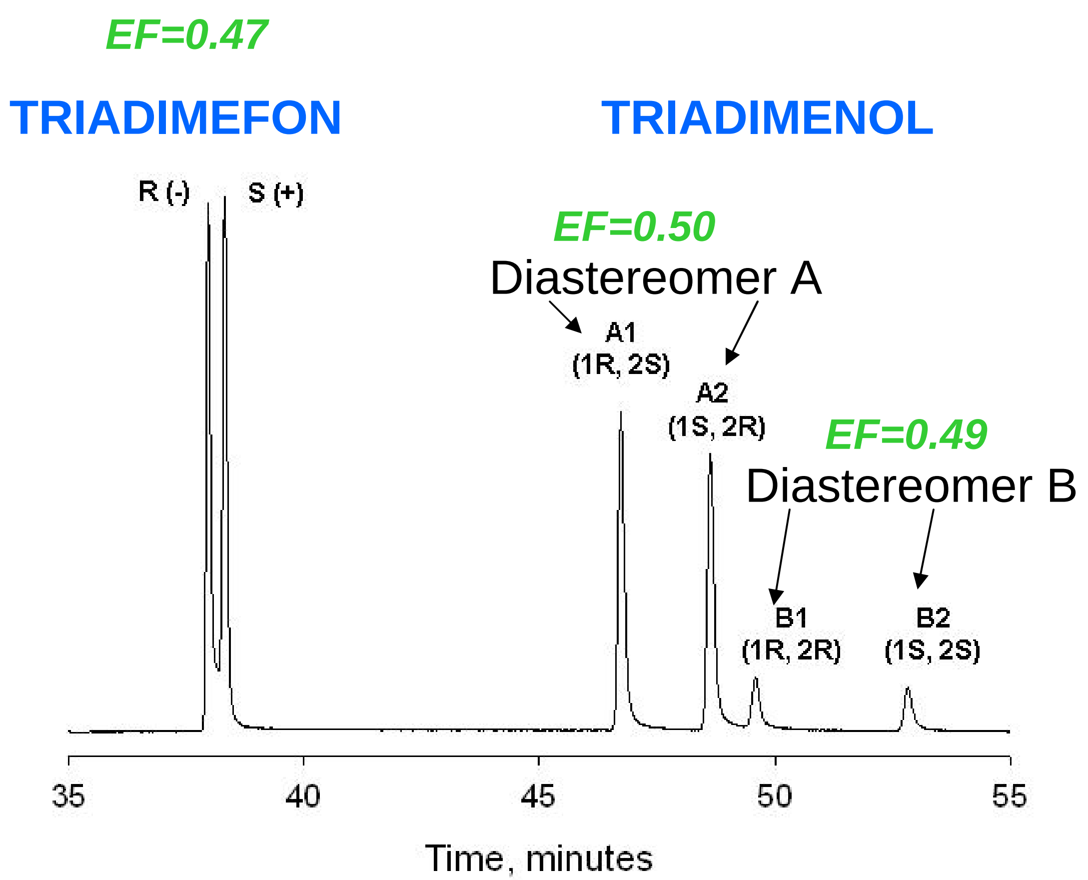
GC-MS

H-P 5973 MSD interfaced with a 6980 GC

BGB 172 chiral column with 20% chiral phase (*tert*-butyldimethylsilylated-B- cyclodextrin) (BGB Analytik AG, Switzerland)

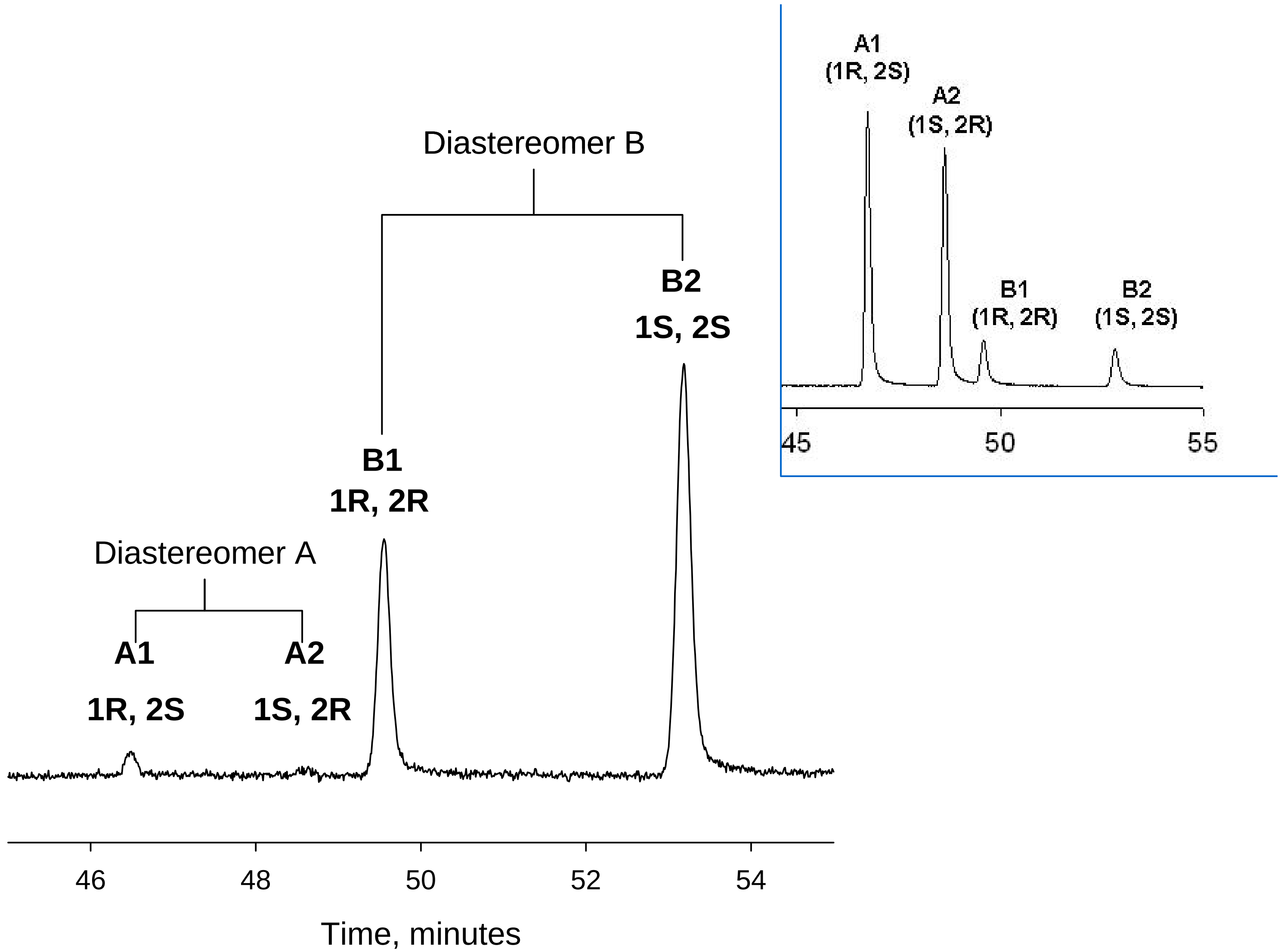
Inj. Temp: 275^o; Temp. program: 150-220^o @ 2^o/min, hold for 60 min; helium flow: 1.5mL/min.

Detection by selected ion monitoring.



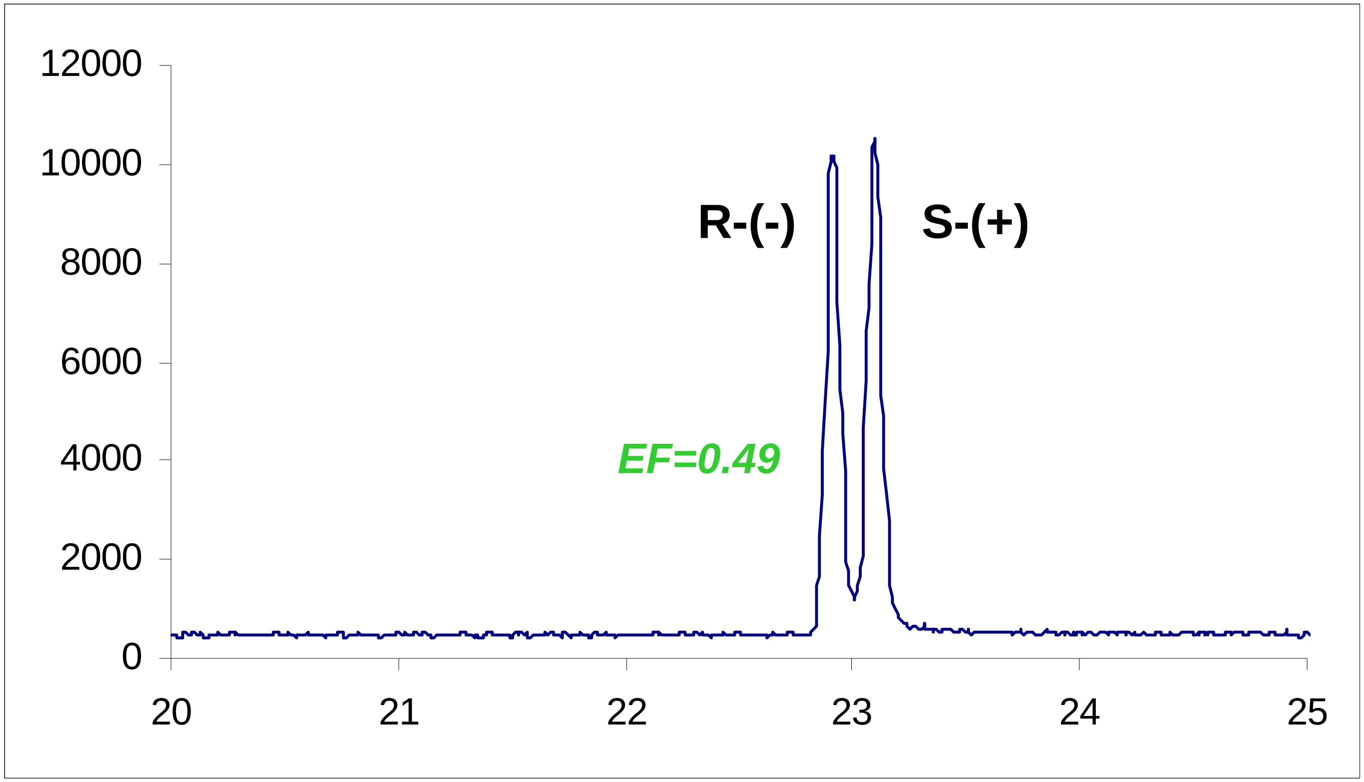
GC-MS (SIM) OF TRIADIMEFON AND TRIADIMENOL MIXED COMMERCIAL STANDARDS. ALL ENANTIOMERS SEPARATE ON THE BGB-172 CHIRAL GC PHASE.

EF=[1st enantiomer]/[1st +2nd enanantiomers]

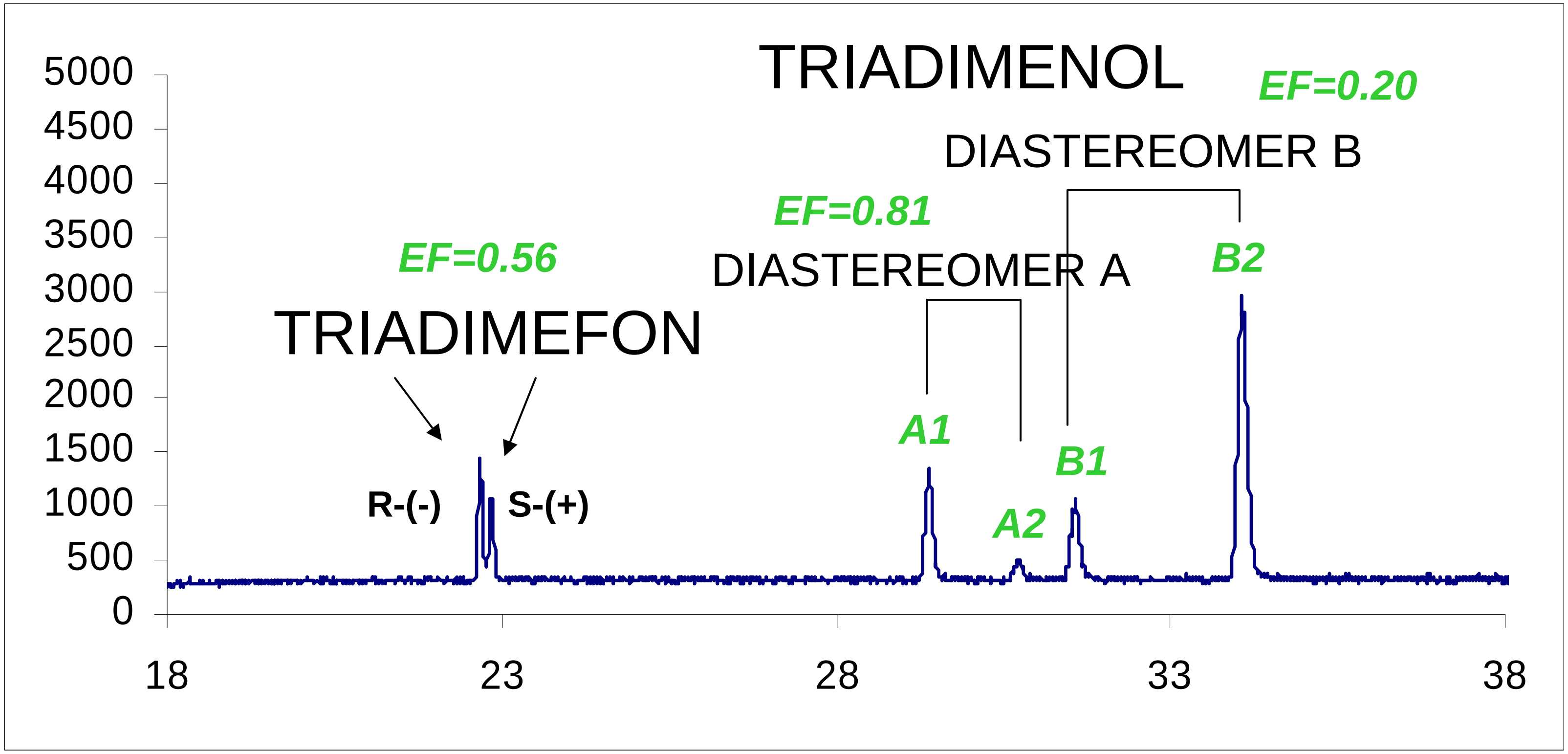


GC-MS (SIM) OF TRIADIMENOL STEREOISOMERS PRODUCED BY REDUCTION OF TRIADIMEFON AFTER EXPOSURE TO RAINBOW TROUT MICROSOMES FOR 480 MIN. (Courtesy of John Kenneke)

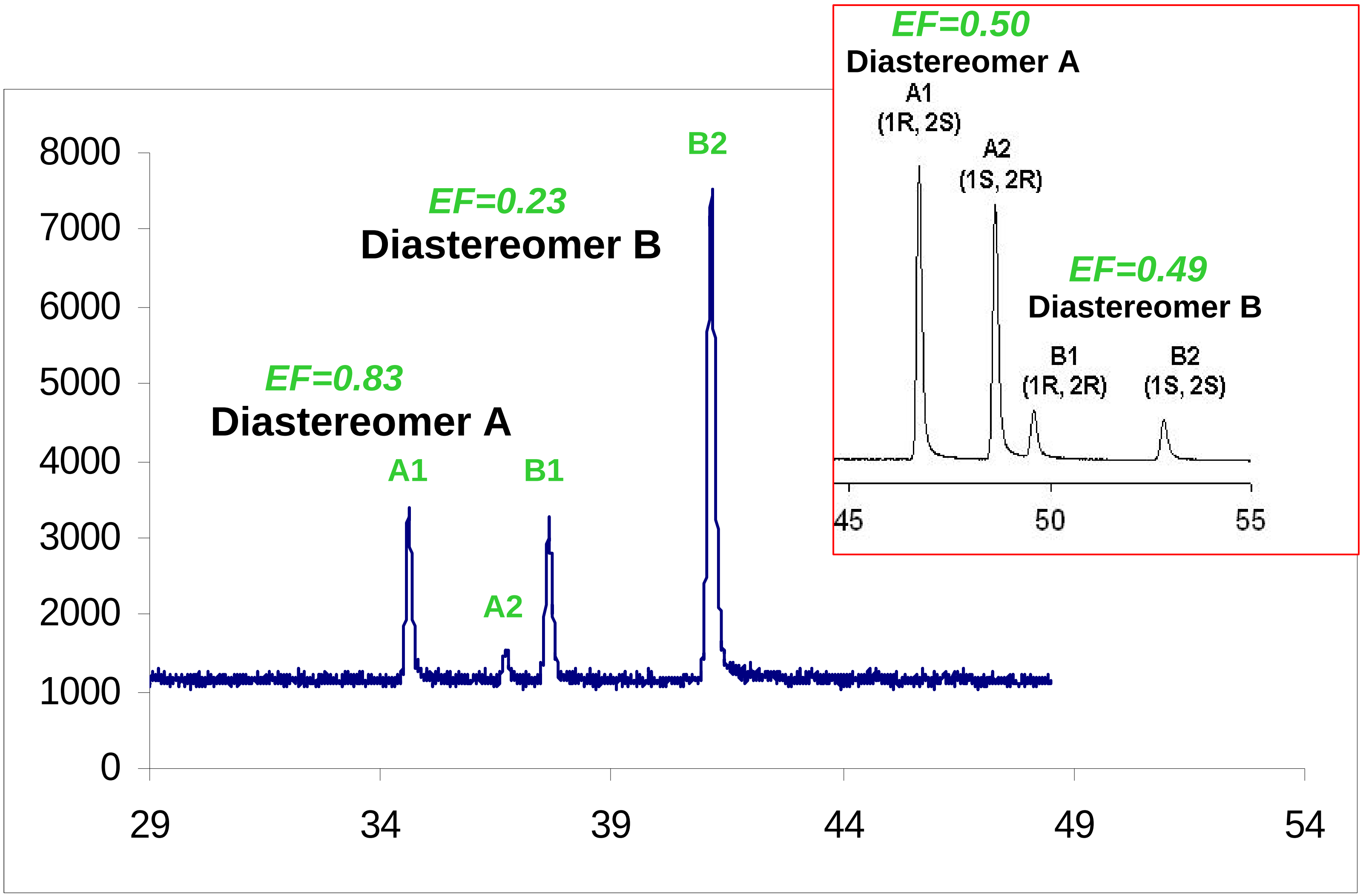
INSET: GC-MS OF COMMERCIAL TRIADIMEN0L STANDARD.



Triadimefon, after 2 hours (t_0), in water phase of UGA soil-water slurry spiked at 50 mg/L. GC-MS

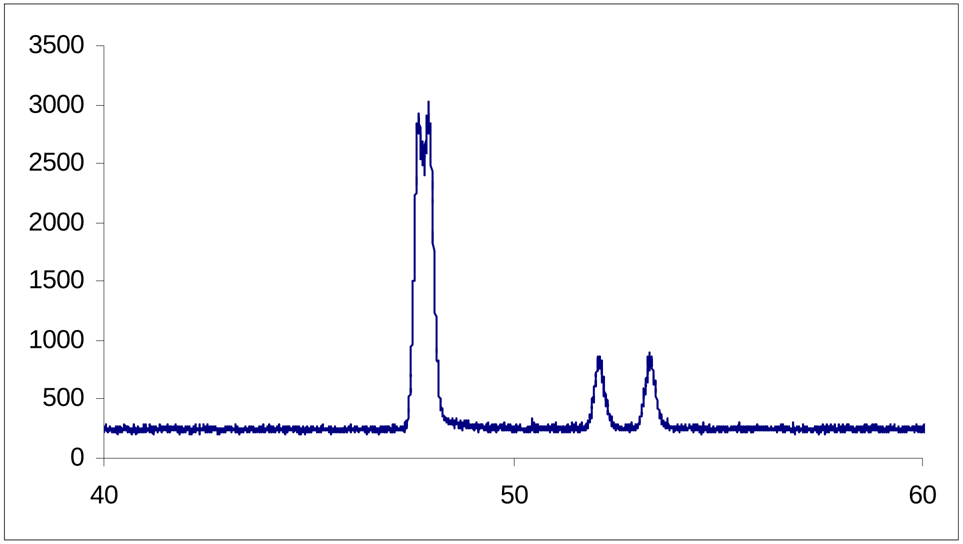


Triadimefon being biotransformed to triadimenol, after 19 days, in water phase of UGA soil-water slurry. GC-MS

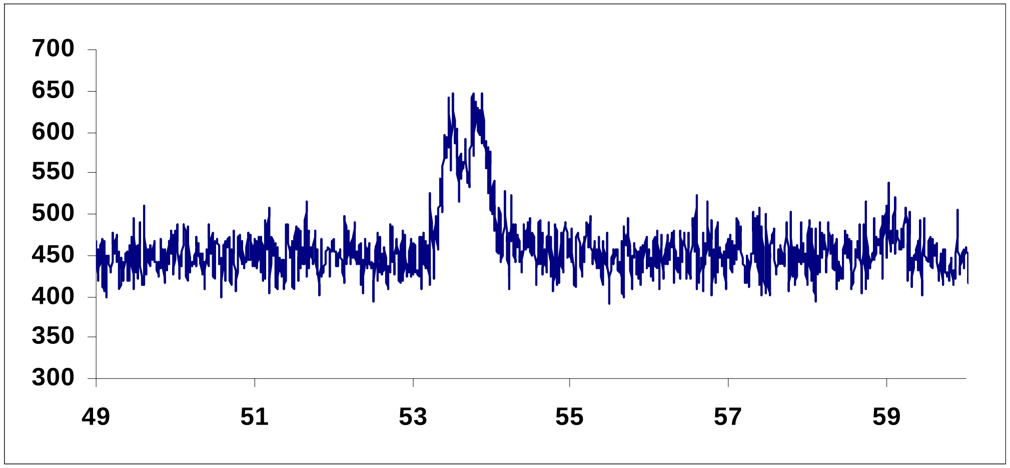


Triadimefon completely biotransformed to triadimenol after 96 days, water phase of UGA soil-water slurry; GC-MS. Inset: GC-MS of commercial triadimenol standard.

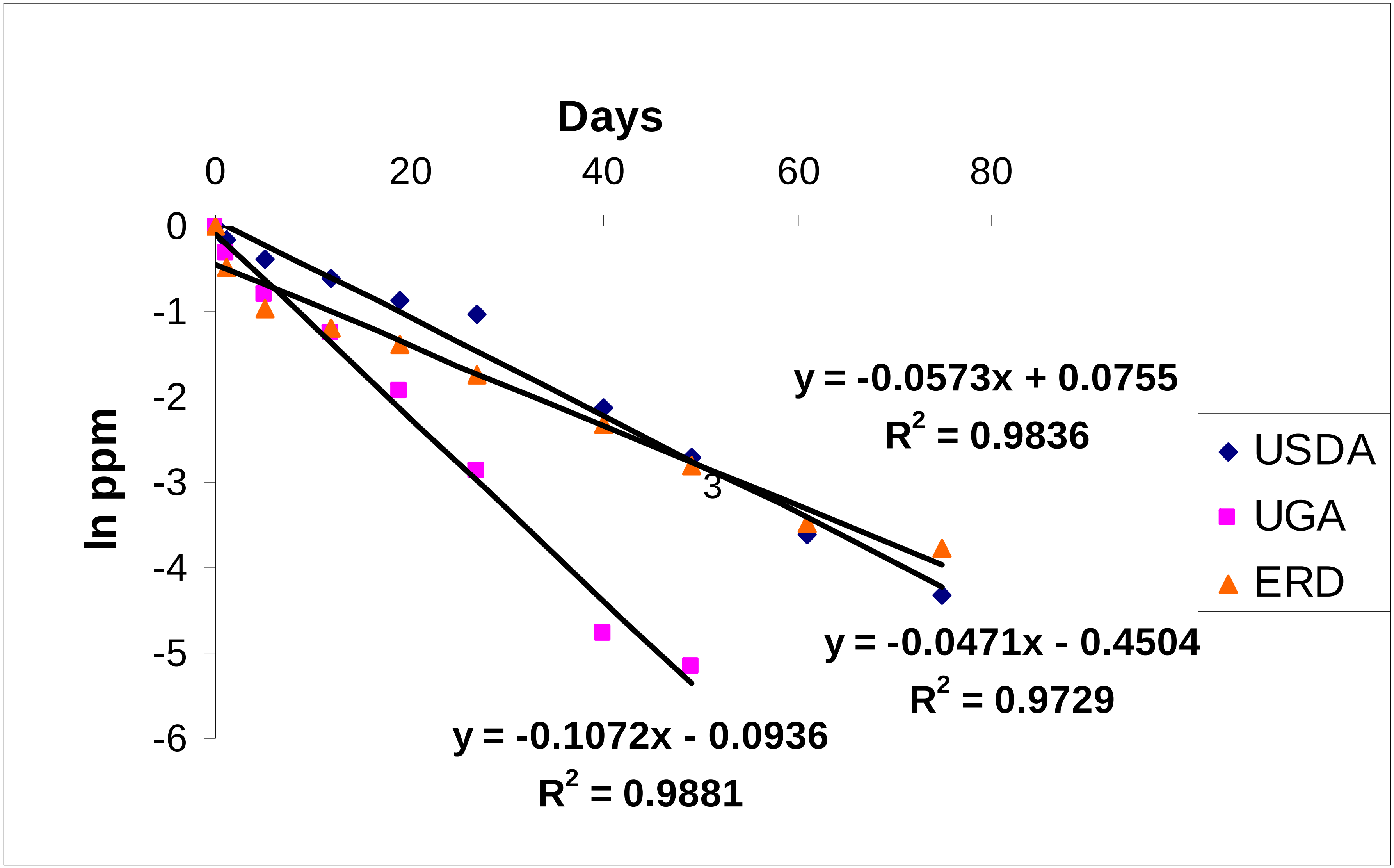
t_0 (2 hr)
9.0 mg/L



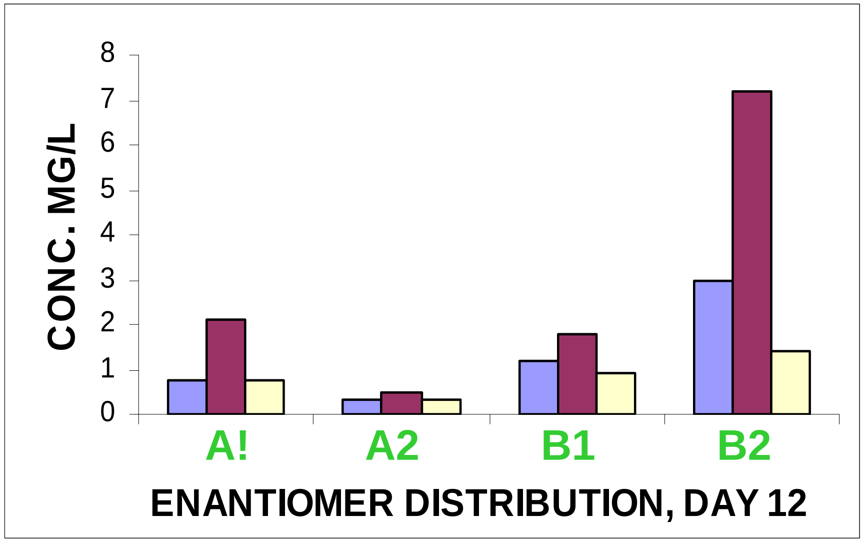
151days
3.8 mg/L

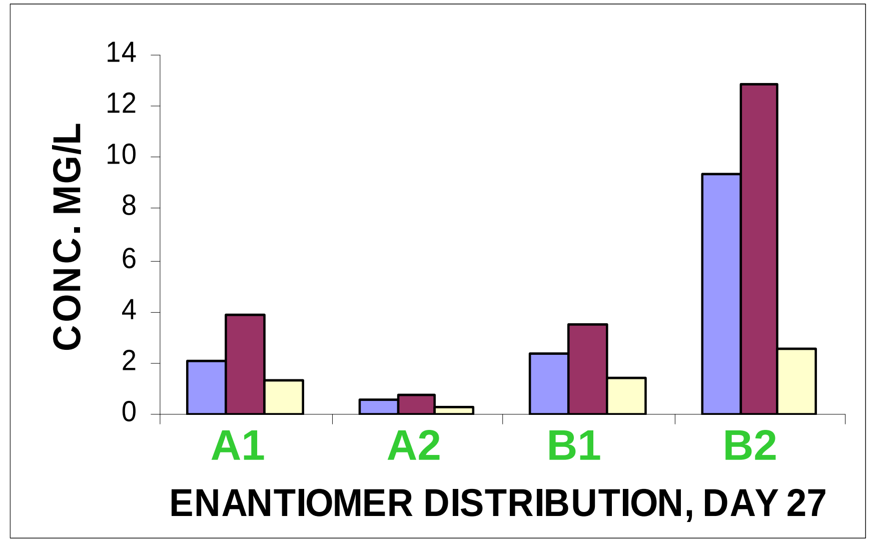


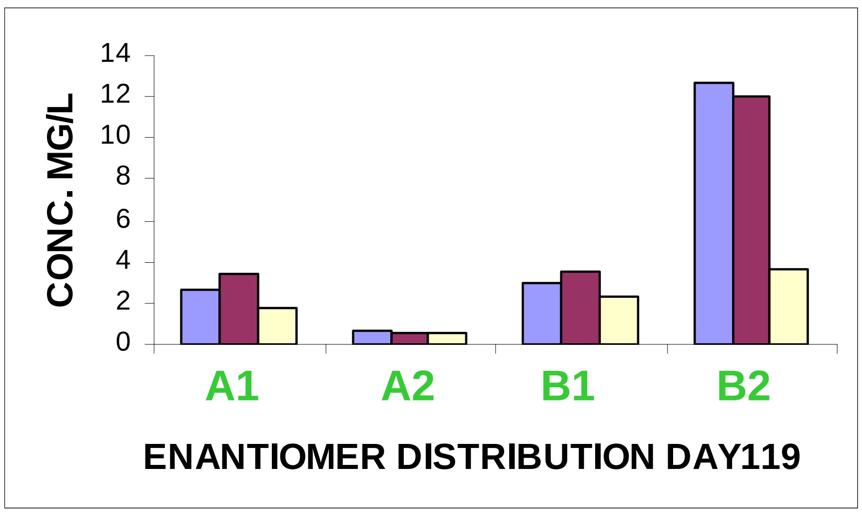
Propiconazole in water phase of UGA soil-water slurry, spiked at 50 mg/L. GC-MS shows reduction in concentration with time, but no enantioselectivity.



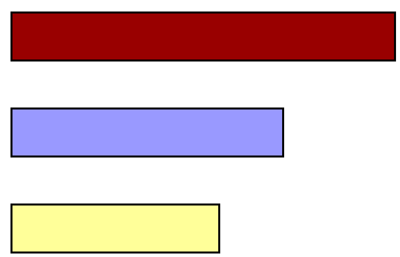
Degradation of triadimefon in 3 soil-water slurries - measurements made in water phase







DISTRIBUTION OF ENANTIOMERS DURING BIOTRANSFORMATION OF TRIADIMEFON TO TRIADIMENOL IN 3 SOIL-WATER SLURRIES

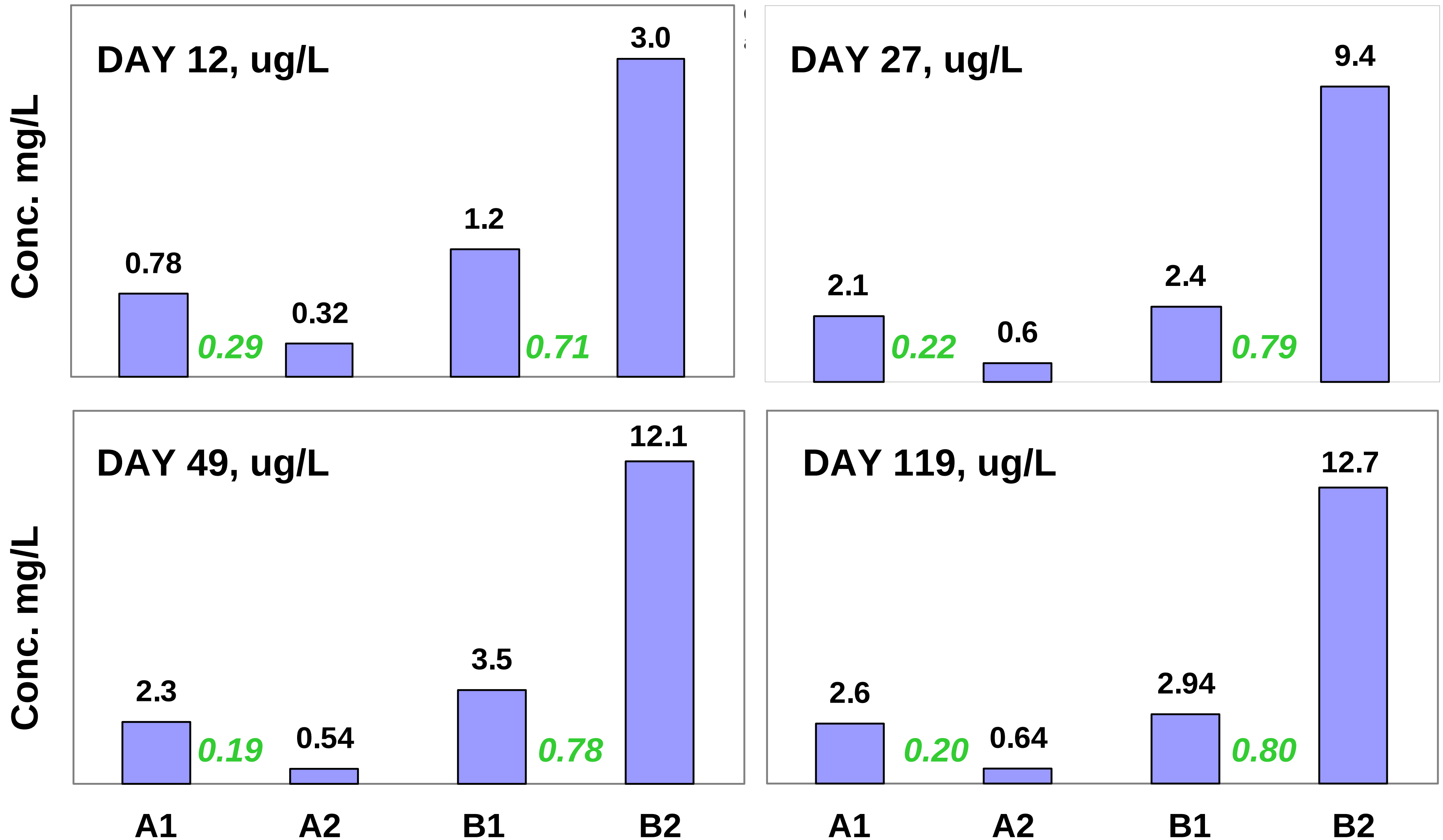


UGA 0.107 d^{-1}

USDA 0.057 d^{-1}

ERD 0.047 d^{-1}

Enantiomer patterns are similar at each time and for all soils

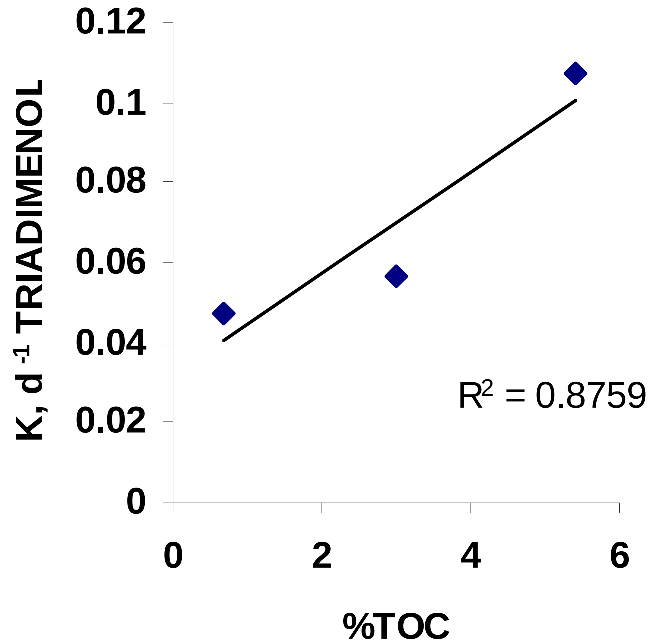


DISTRIBUTION OF TRIADIMENOL ENANTIOMERS DURING BIOTRANSFORMATION OF TRIADIMEFON IN USDA SOIL

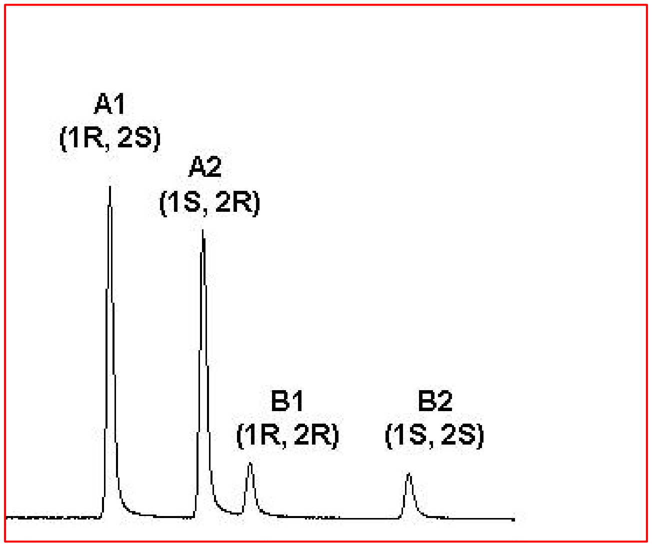
Concentrations of all enantiomers increase with time, but enantiomer ratios are similar with time

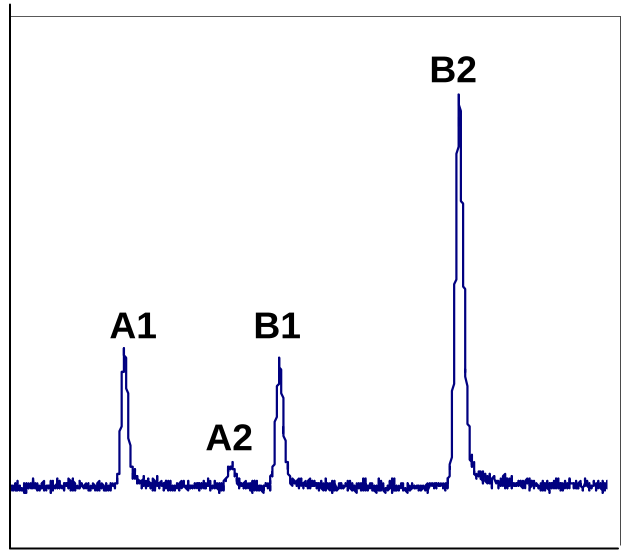
SOIL CHARACTERIZATION

SOIL	pH	CEC meq/100g	SAND %	SILT %	CLAY %	TOC %	k d^{-1}
USDA	6.2	8.9	70	19	11	3.0	0.057
UGA	6.3	13.9	72	18	10	5.4	0.107
ERD	4.8	11.3	58	20	22	0.7	0.047



Correlation of Soil %TOC with Triadimefon Biotransformation Rates





Triadimenol Standard **Triadimenol in Soil (after 96 days)**

<u>Concluding Question</u>: How would one assess the risk of Triadimenol in Soil?

CONCLUSIONS

- **Concentrations of the individual enantiomers of Triadimenol *as a metabolite* do not match those of triadimenol in its *commercial standard***
- **Enantiomers may have different toxicities**
- **Stereochemistry must be considered for accurate risk assessment of chiral xenobiotics *and their metabolites***