# Stereoselective Metabolism of 1,2,4-Triazole Fungicides in Hepatic Microsomes and Implications for Risk Assessment

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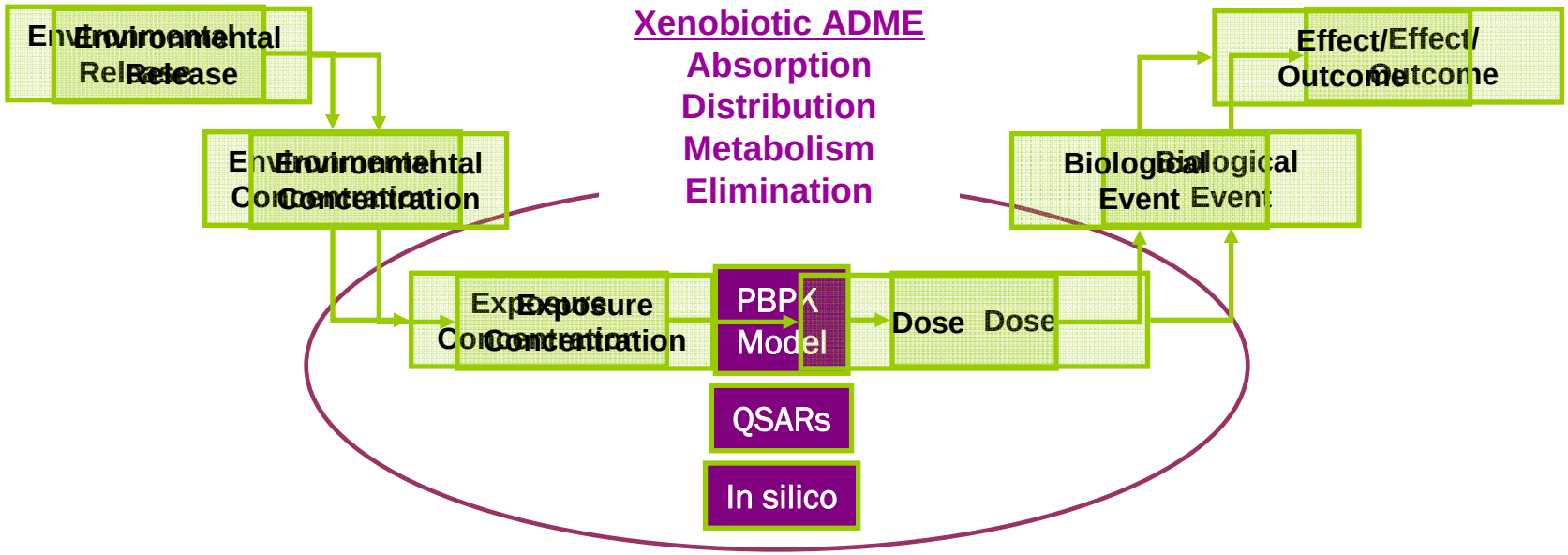
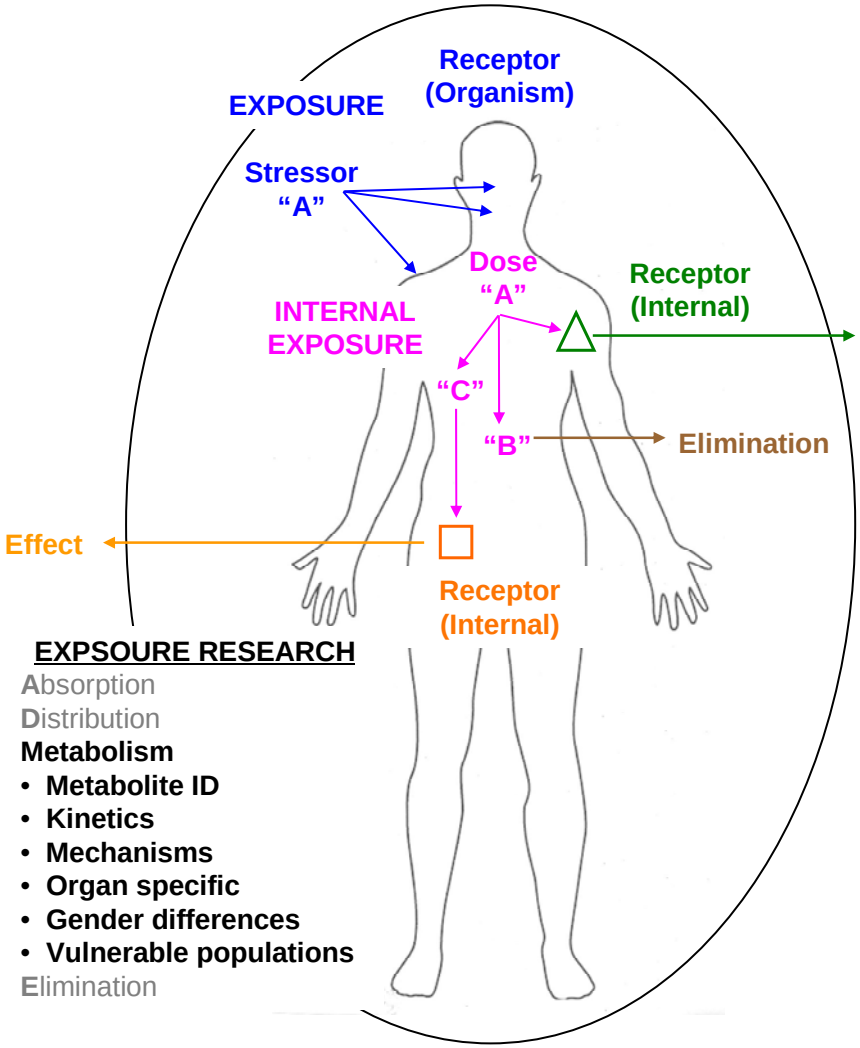
# Source-to-Outcome Continuum



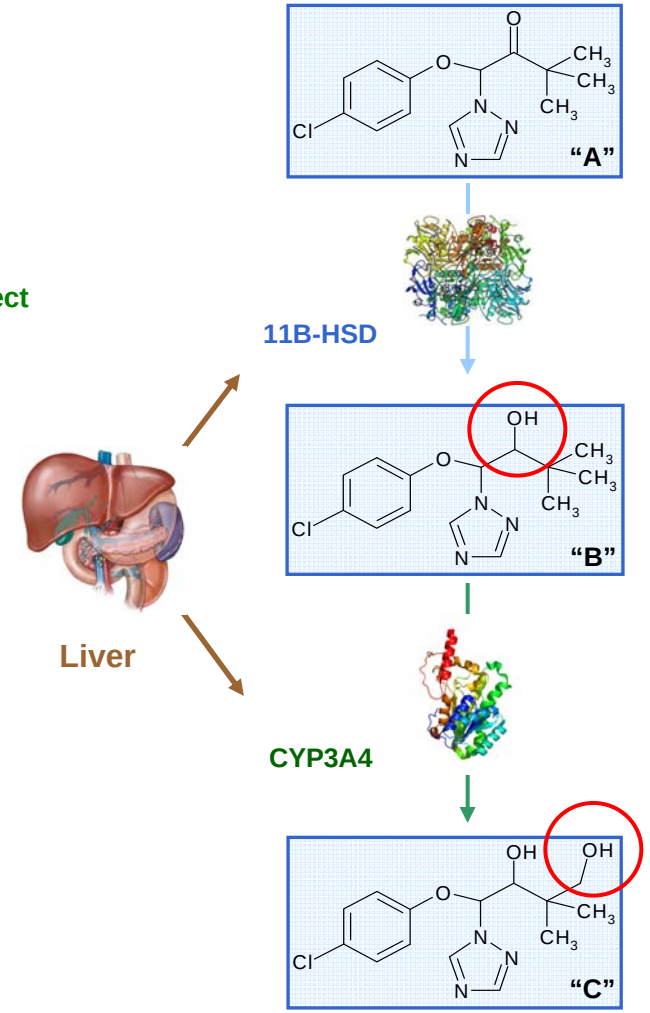
# Metabolism: Pathways, Kinetics, and Mechanisms




**EXPSOURE RESEARCH**

Absorption
Distribution
**Metabolism**
- **Metabolite ID**
- **Kinetics**
- **Mechanisms**
- **Organ specific**
- **Gender differences**
- **Vulnerable populations**

Elimination



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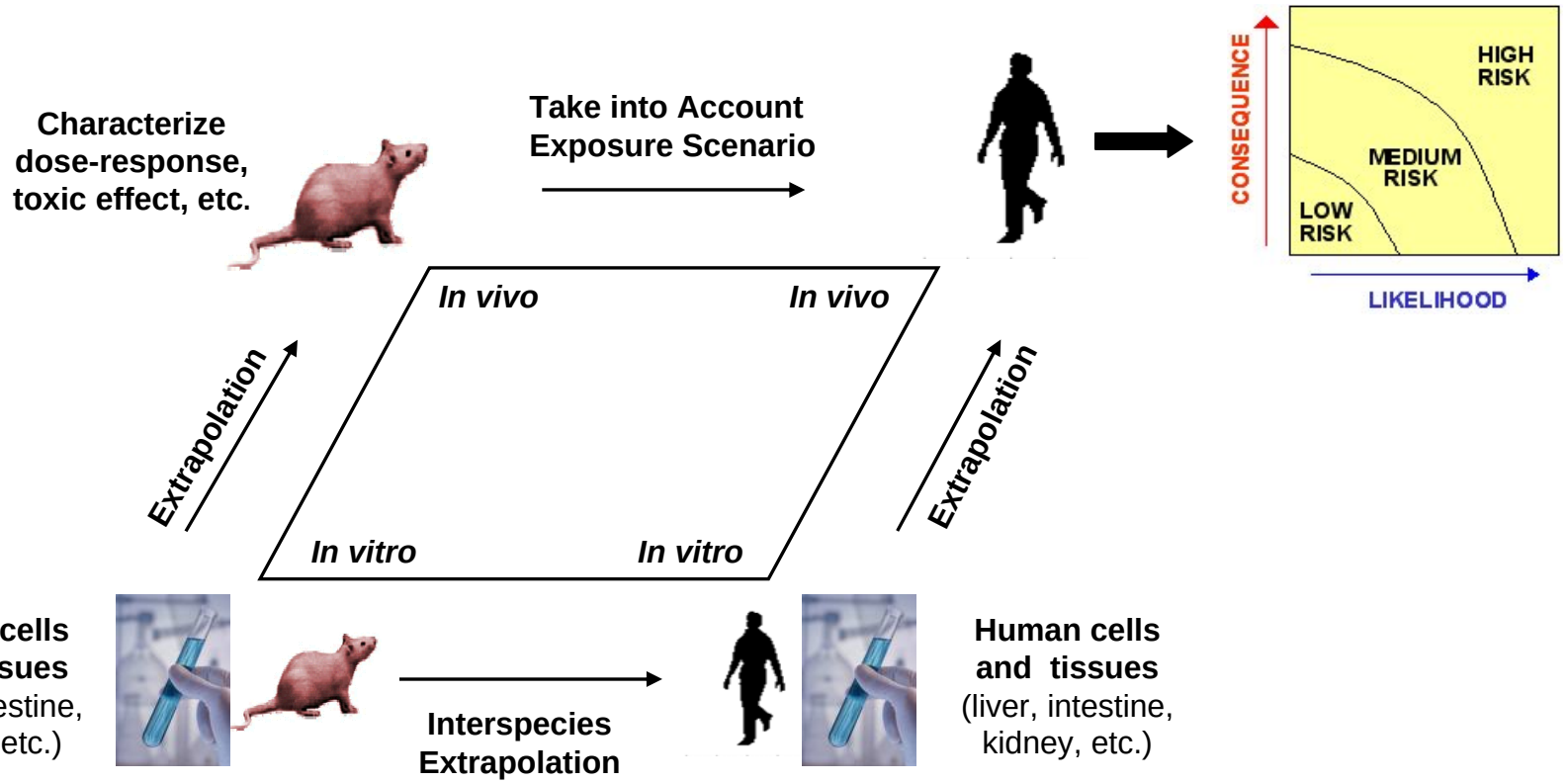
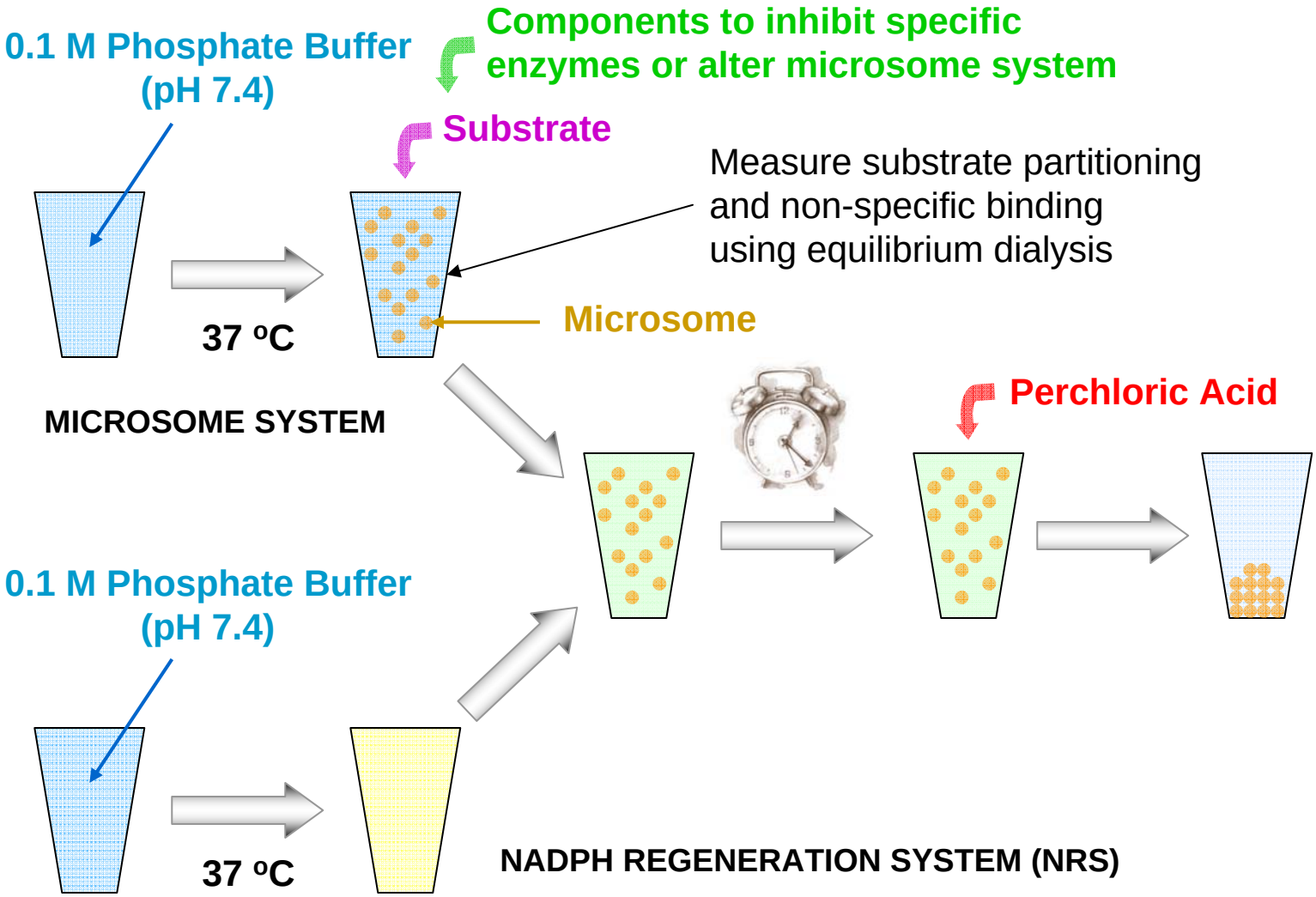
# Parallelogram Model for Risk Assessment

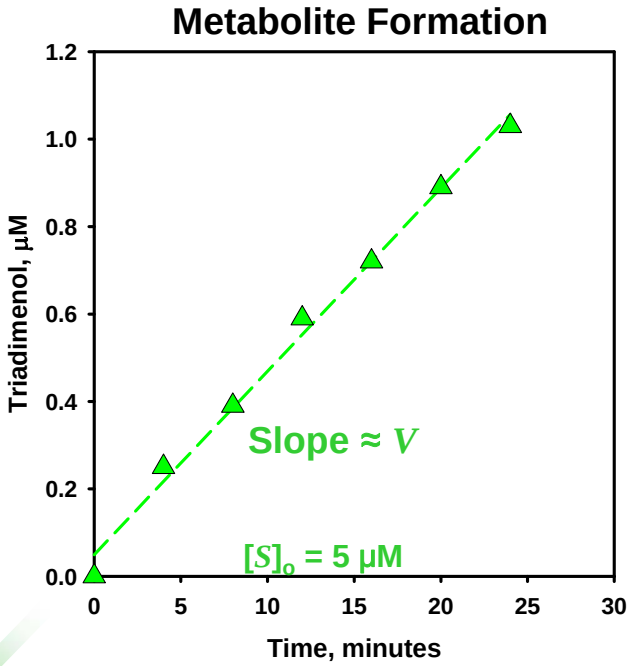


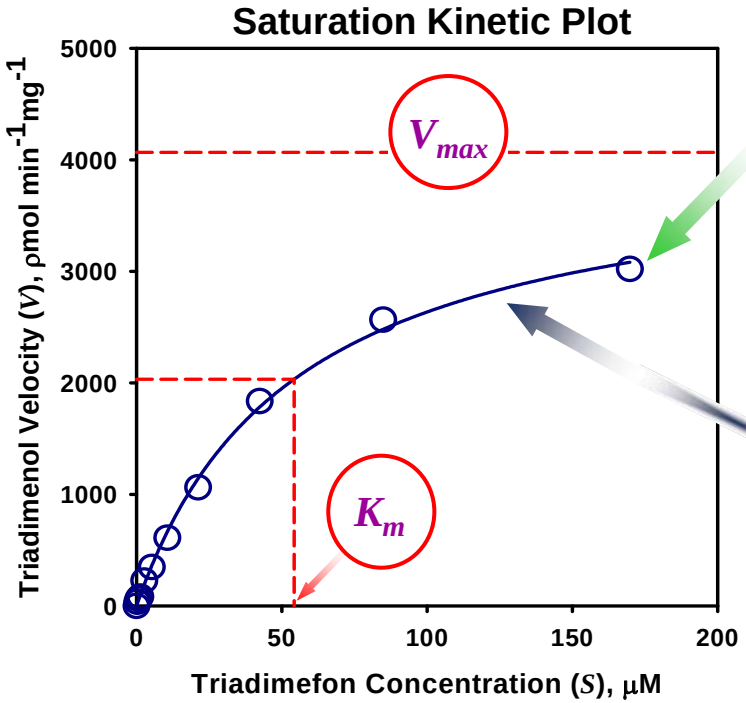
# In Vitro Metabolism Assay

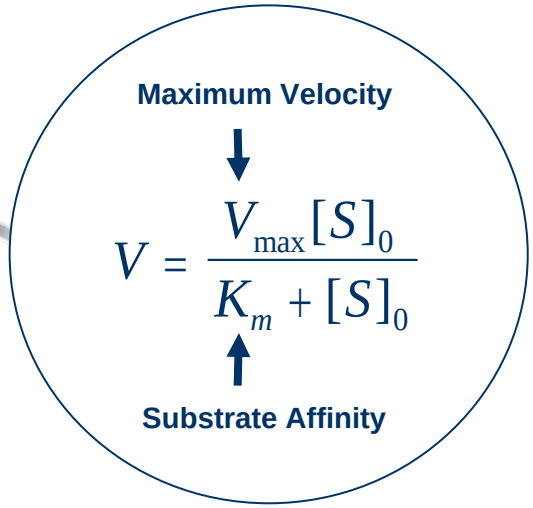


# Determining Kinetic Parameters

**The process for determining the kinetic parameters of Metabolite Formation is illustrated here; the process for Substrate Depletion is analogous**



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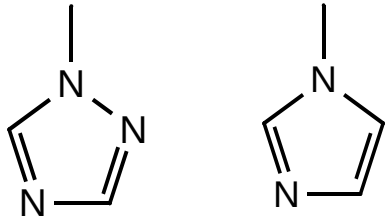
# Conazole Fungicides



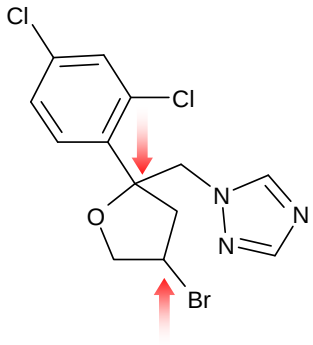

## Background

- 1,2,4-triazoles and imidazoles
- Inhibit steroid demethylation in fungi
- Used for over 30 years
- Approximately 25% of all fungicides sold
- Agricultural and medicinal uses

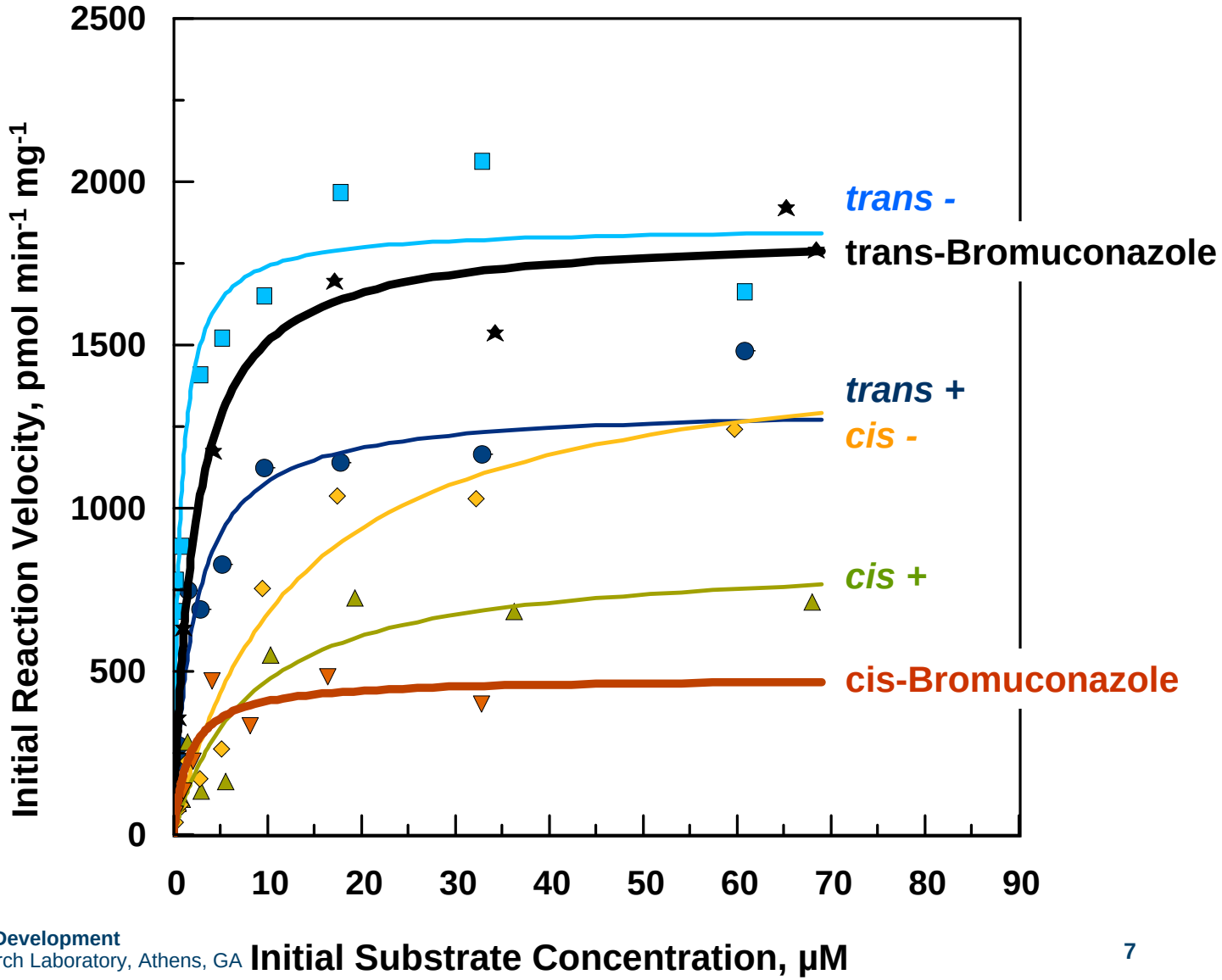
## Issues

- Potent cytochrome P450 inducers and inhibitors that can disrupt steroid and hormone biosynthesis in mammals
- Tumorigenesis in rodents
- Common mode of action and cumulative risk assessment has been proposed for human health risk assessment
- Majority are chiral

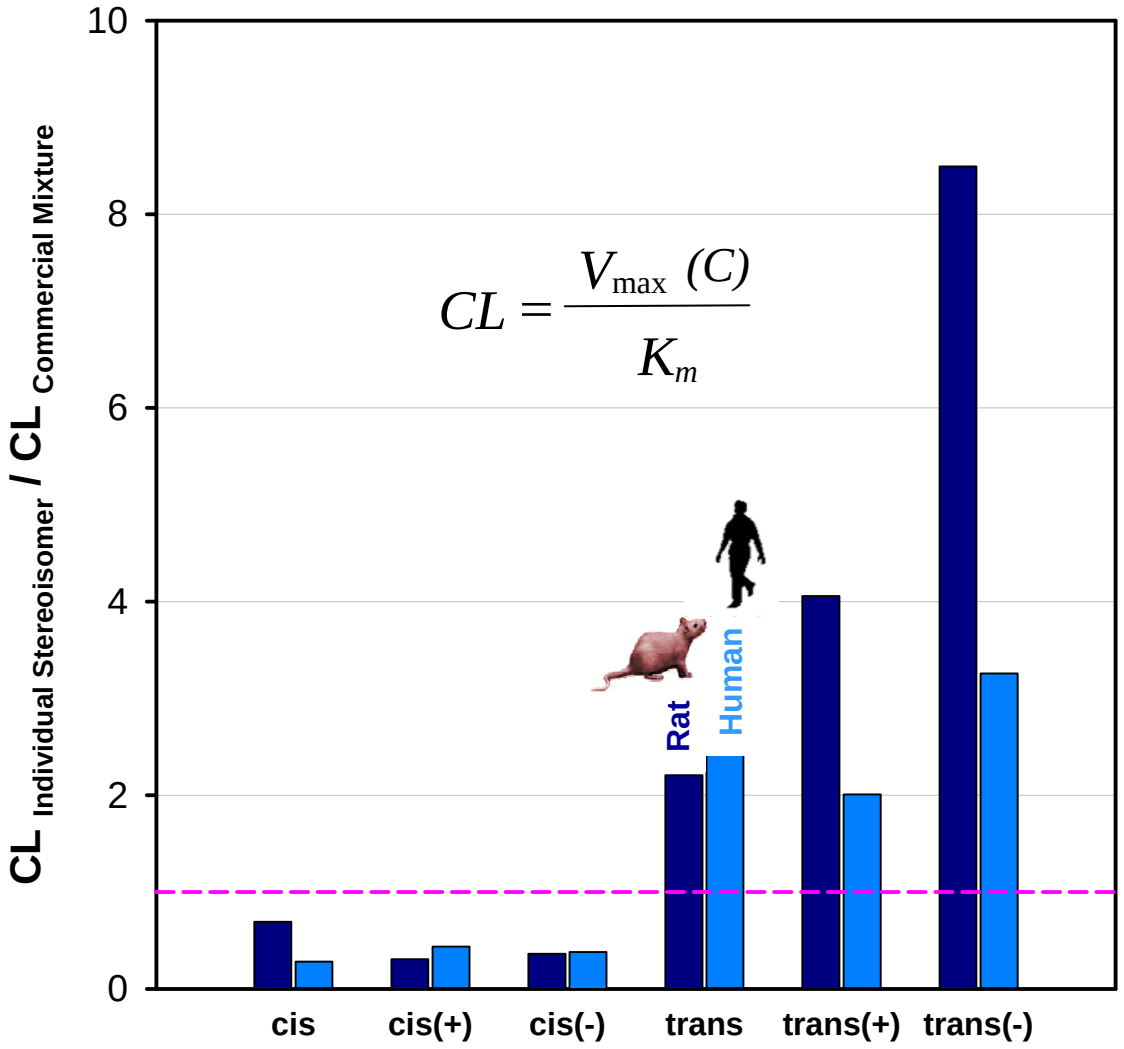
# Bromuconazole Metabolism




Chiral Center

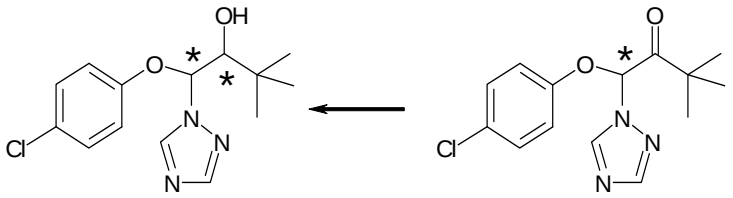


# Stereoselective Bromuconazole Clearance



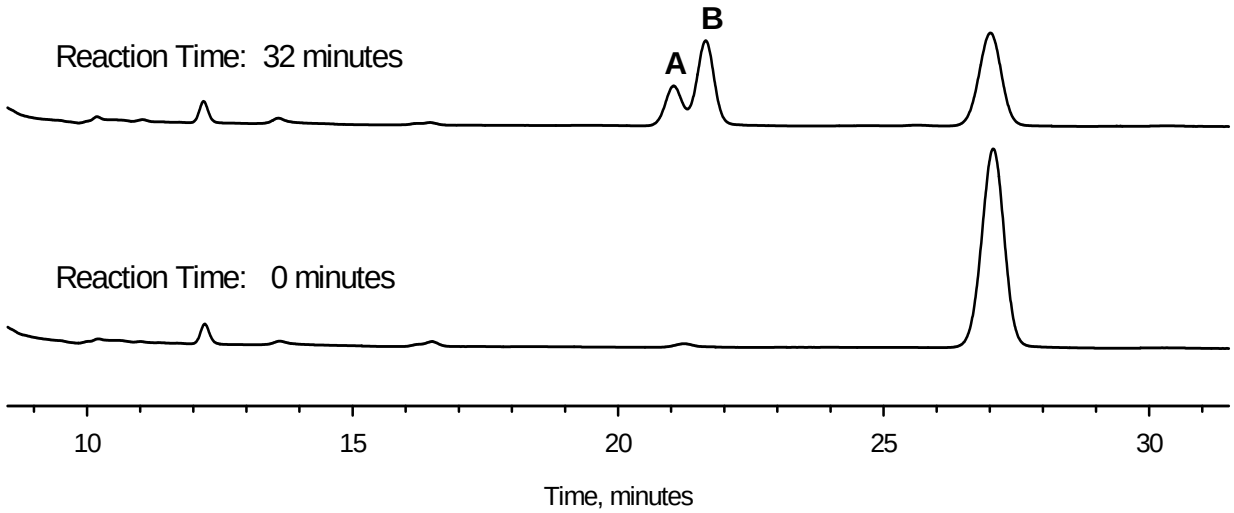
# Triadimefon Metabolism

**Carbonyl Reduction**




**Triadimenol** **Triadimefon**

**HPLC Analysis**



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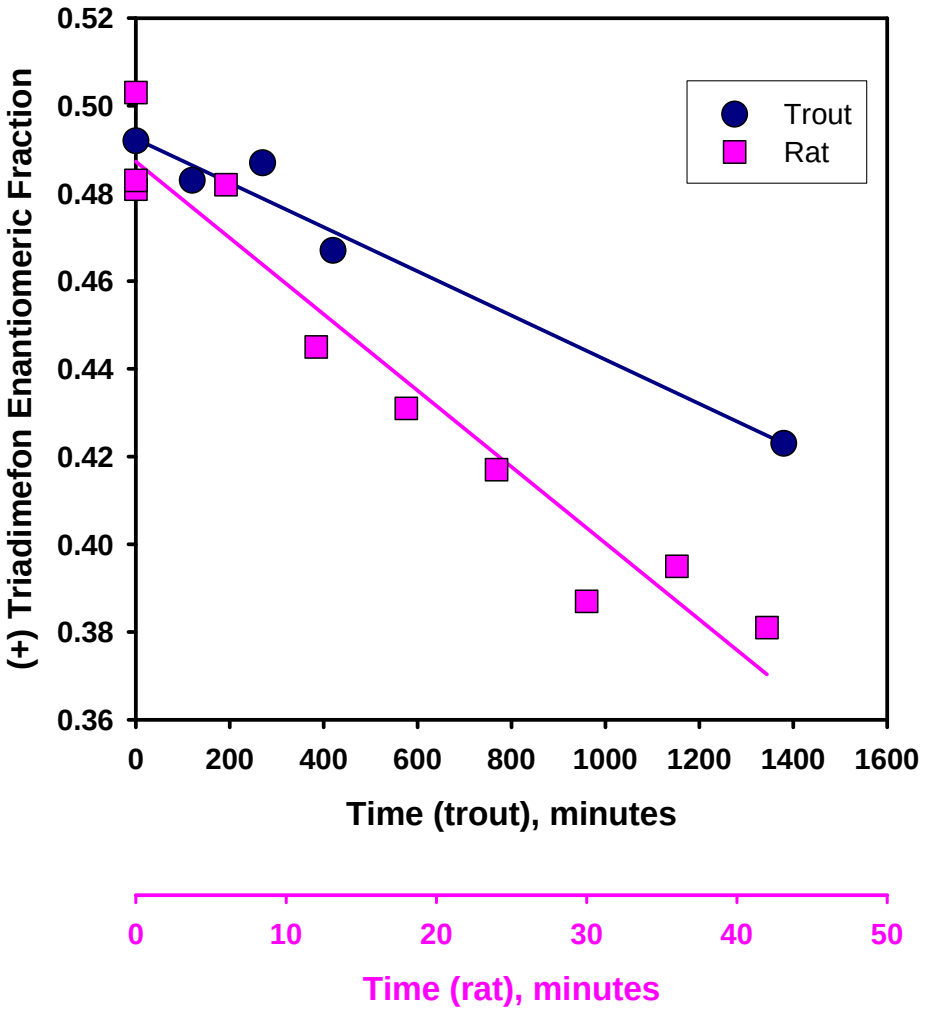
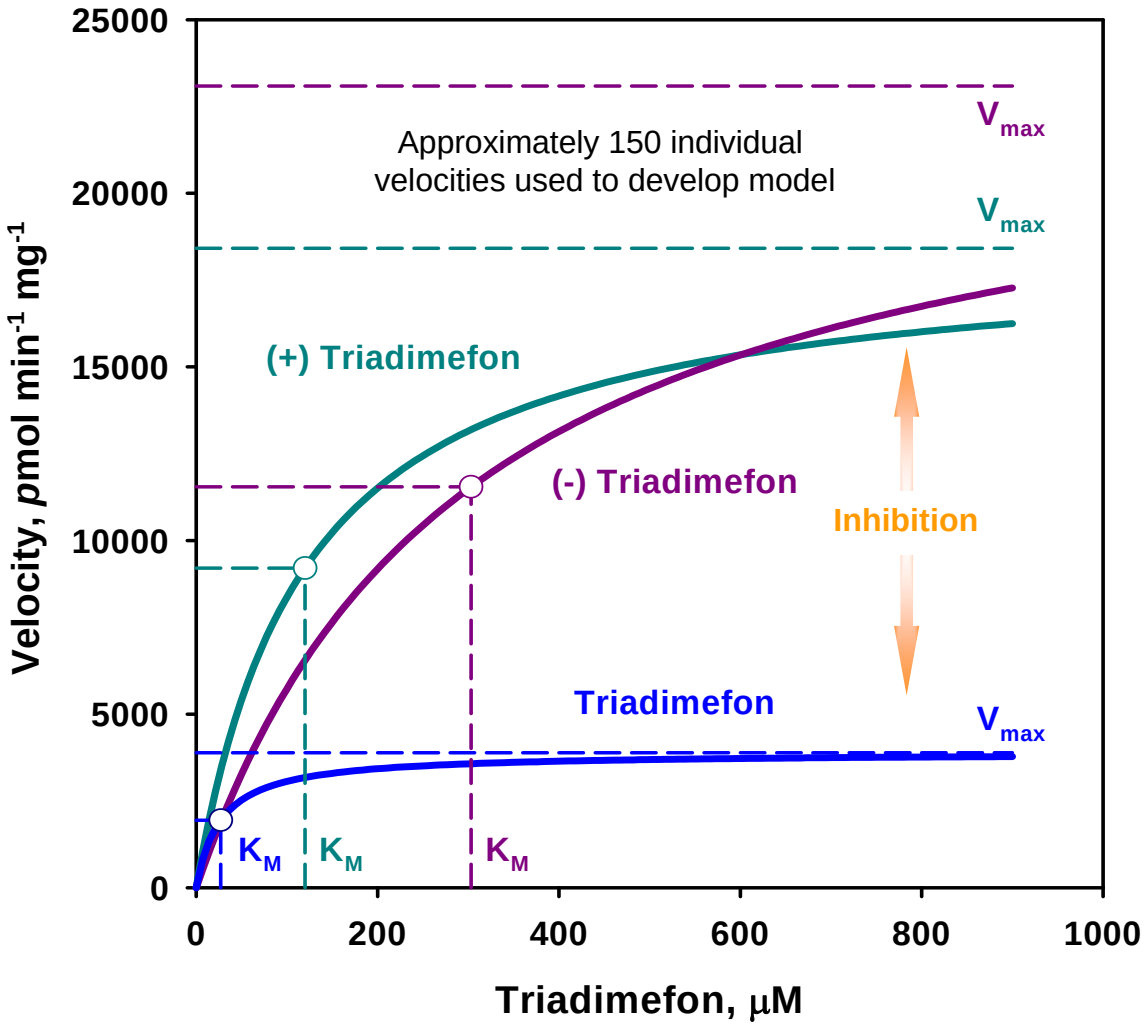
# Enantioselective Triadimefon Depletion Chiral GC/MS

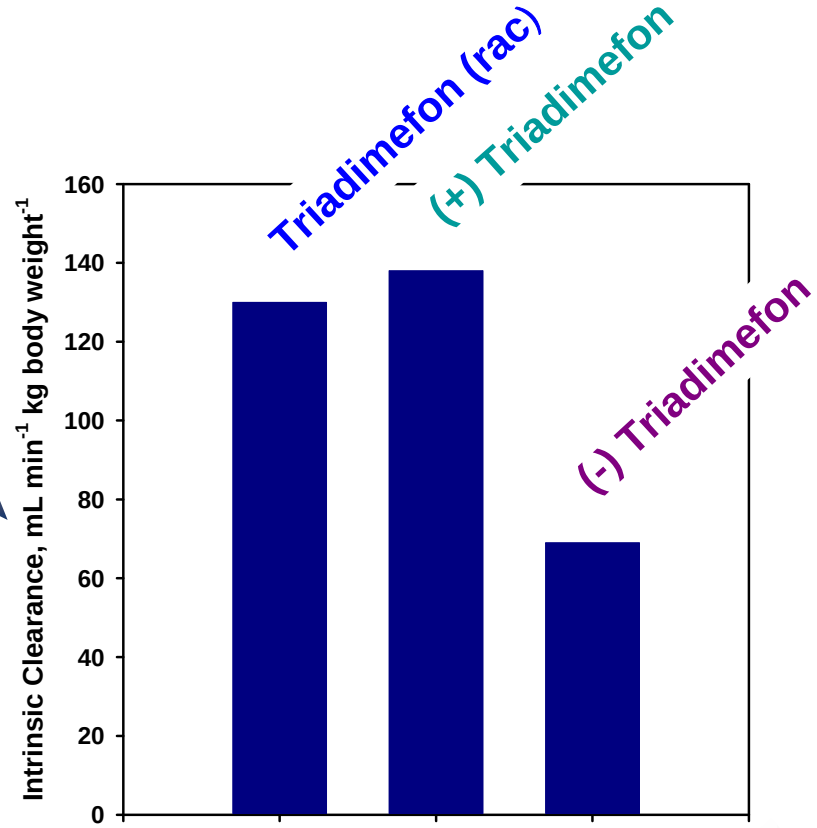


# "Mixtures Effects" for Triadimefon Metabolism



# Stereochemistry and Intrinsic Clearance

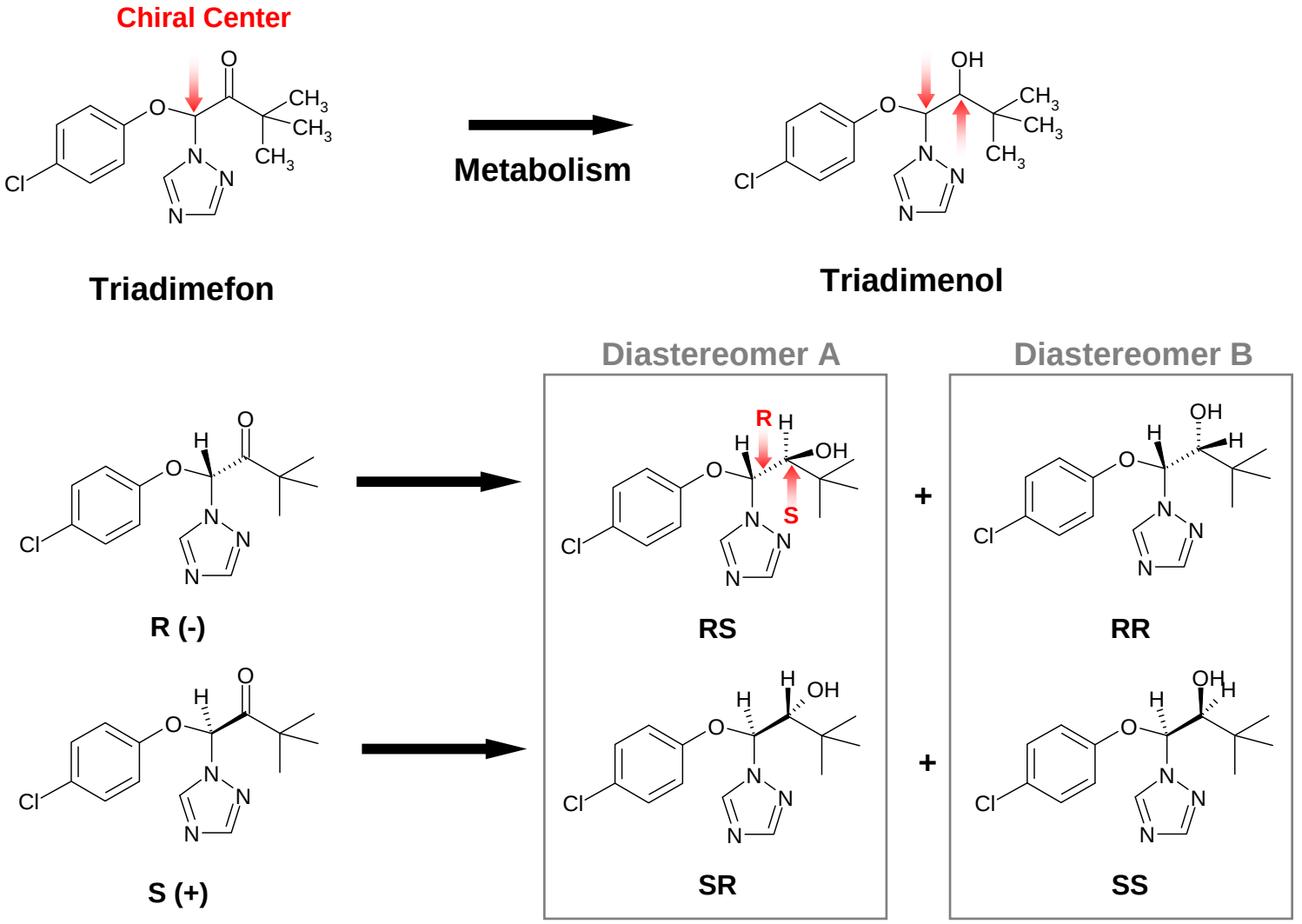
$$CL = \frac{V_{max}\ (C)}{K_m}$$




| | | | |
|---|---|---|---|
| $V_{max}$, pmol/min mg: | 3891 | 18,414 | 23,088 |
| $K_M$, µM: | 27 | 120 | 303 |
| CL, mL/min kg wt: | 130 | 138 | 69 |

Predict Bioaccumulation

# Stereoselective Triadimenol Formation



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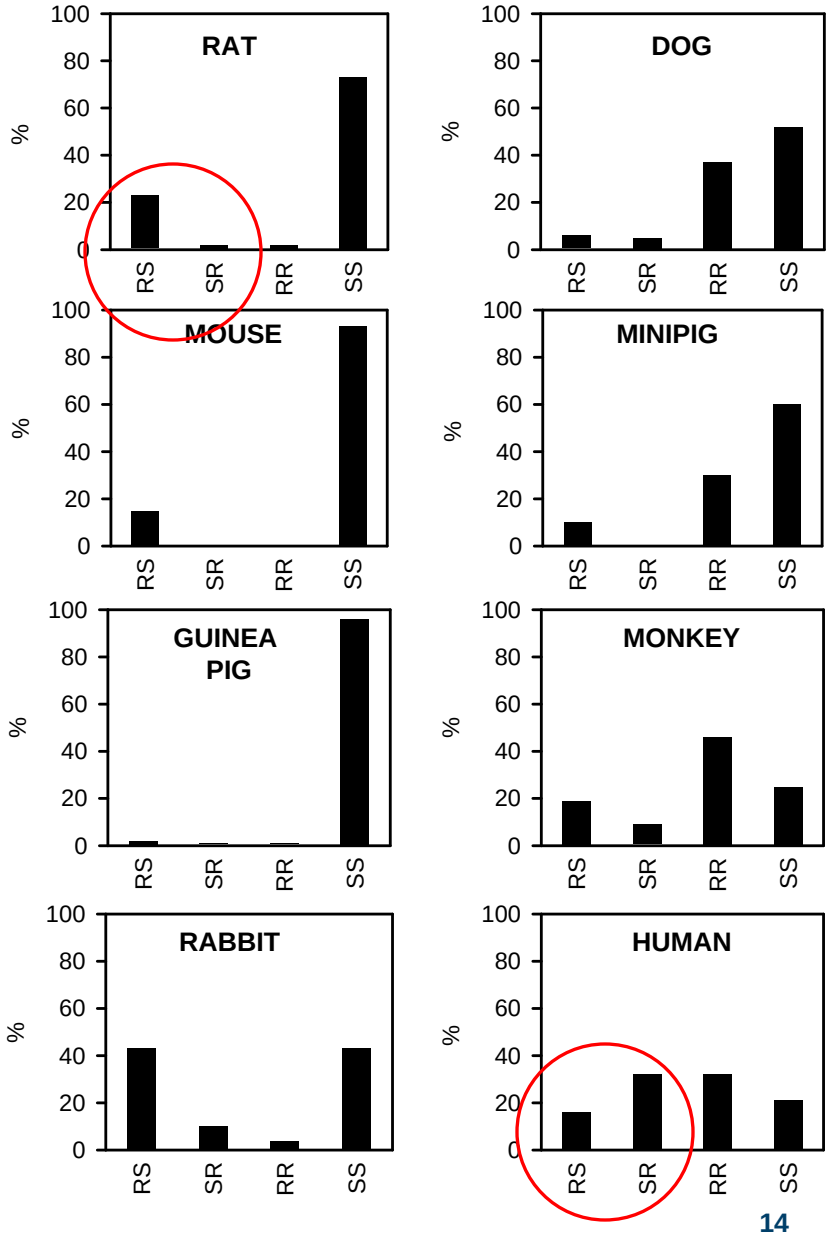
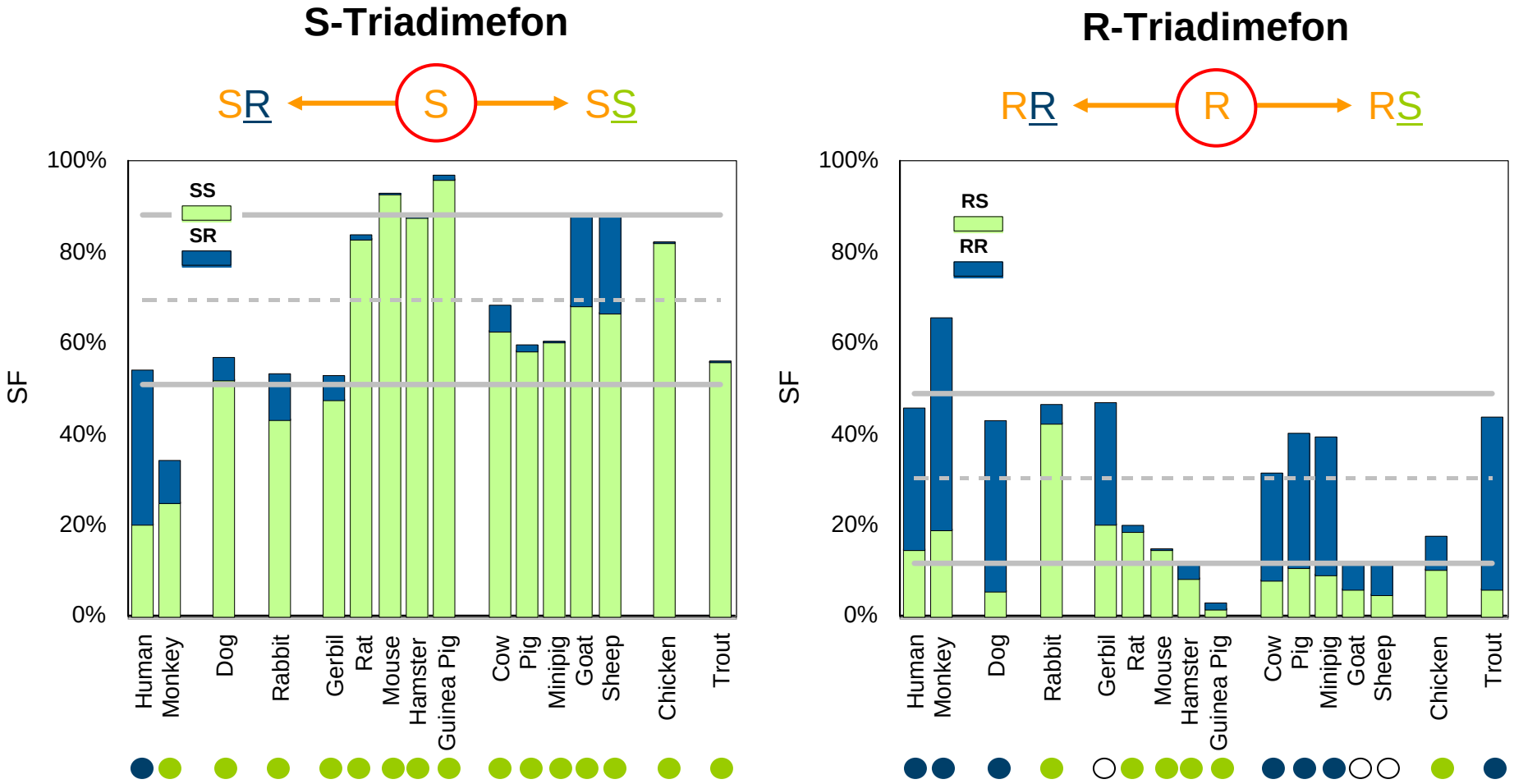
# Species Dependent Triadimenol Formation and Resulting Internal Exposures

- All in vitro assays exposed to only triadimefon
- Metabolism results in mixture of RS, SR, RR and SS triadimenol
- (RS + SR) is 10X more toxic than (SS + RR)
- SR inhibits cholesterol biosynthesis 100X more than the other stereoisomers



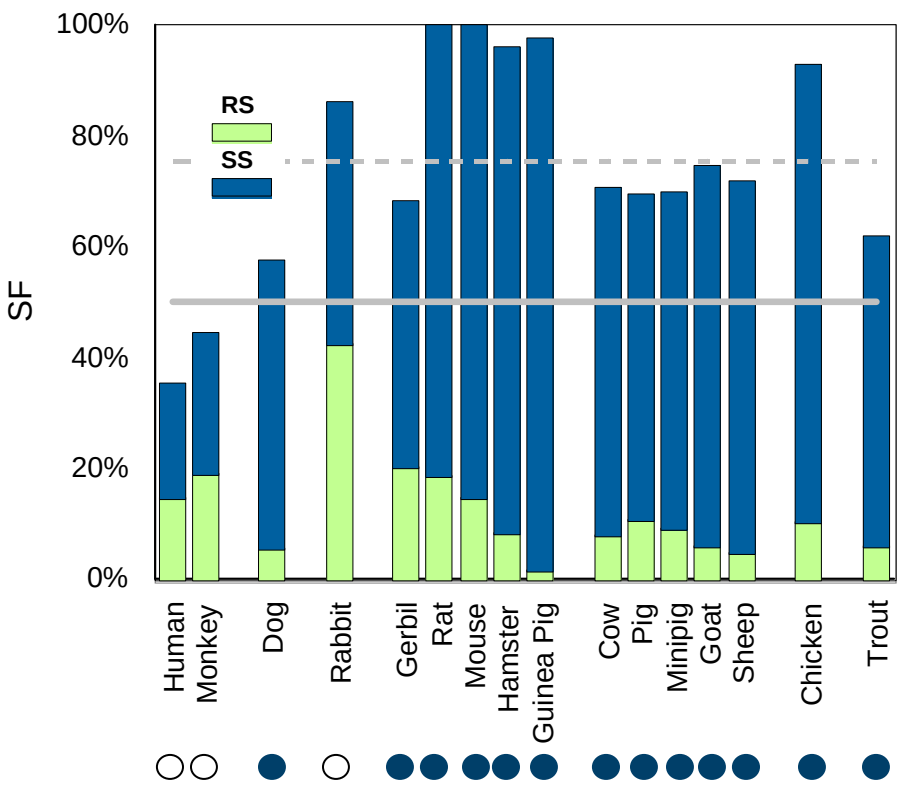
# Enantioselective Metabolism of Triadimefon



# Stereoselective Carbonyl Reduction

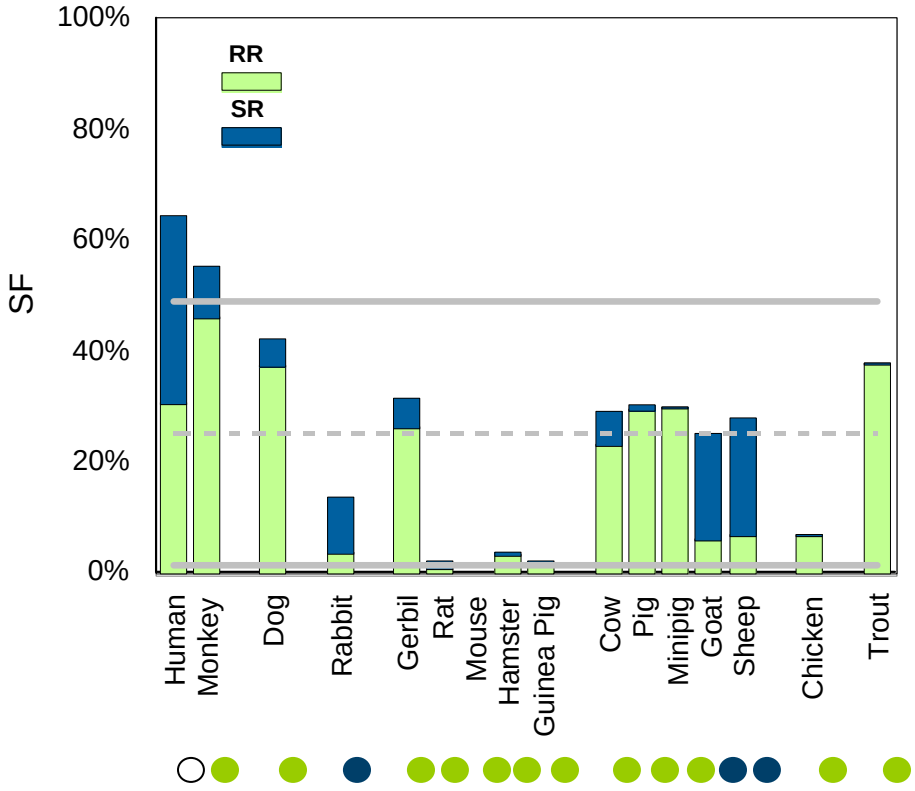
## S-Alcohol Formation

S ⟶ SS  R ⟶ RS




## R-Alcohol Formation

S ⟶ SR  R ⟶ RR



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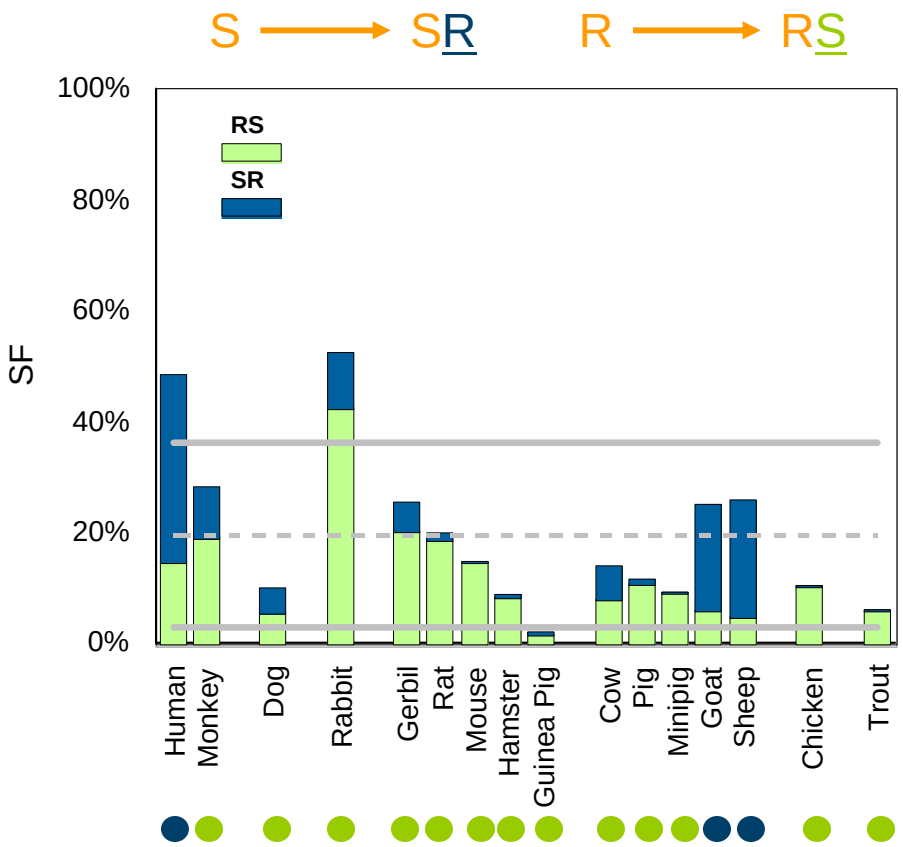
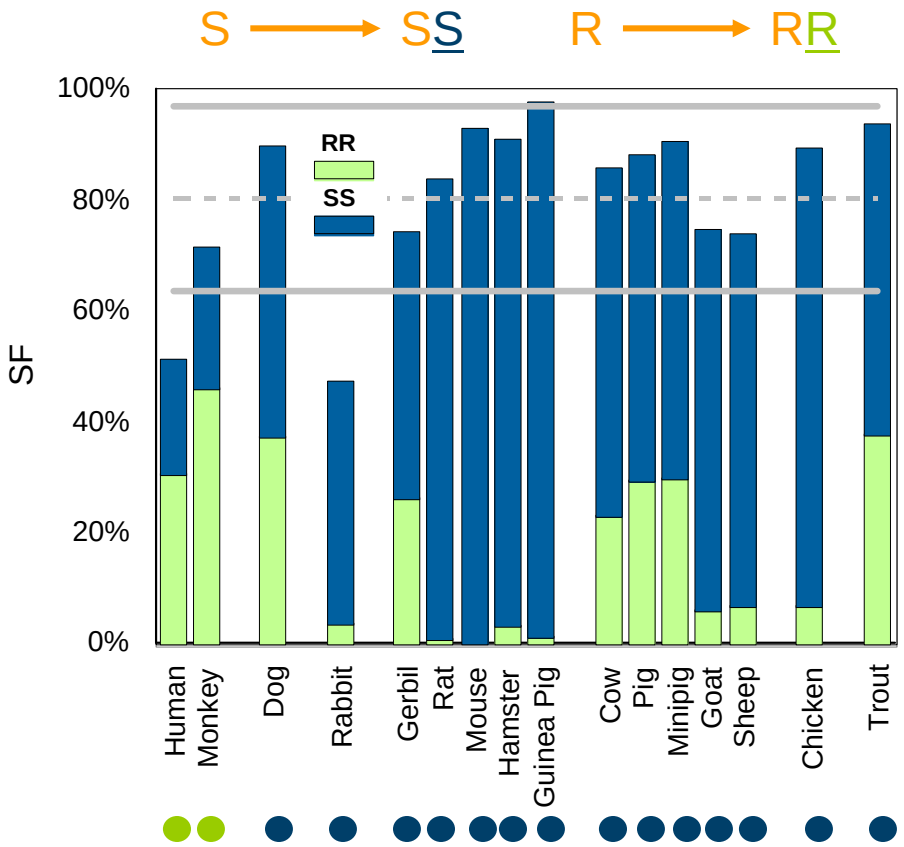
# Stereoselective Diastereomer Formation

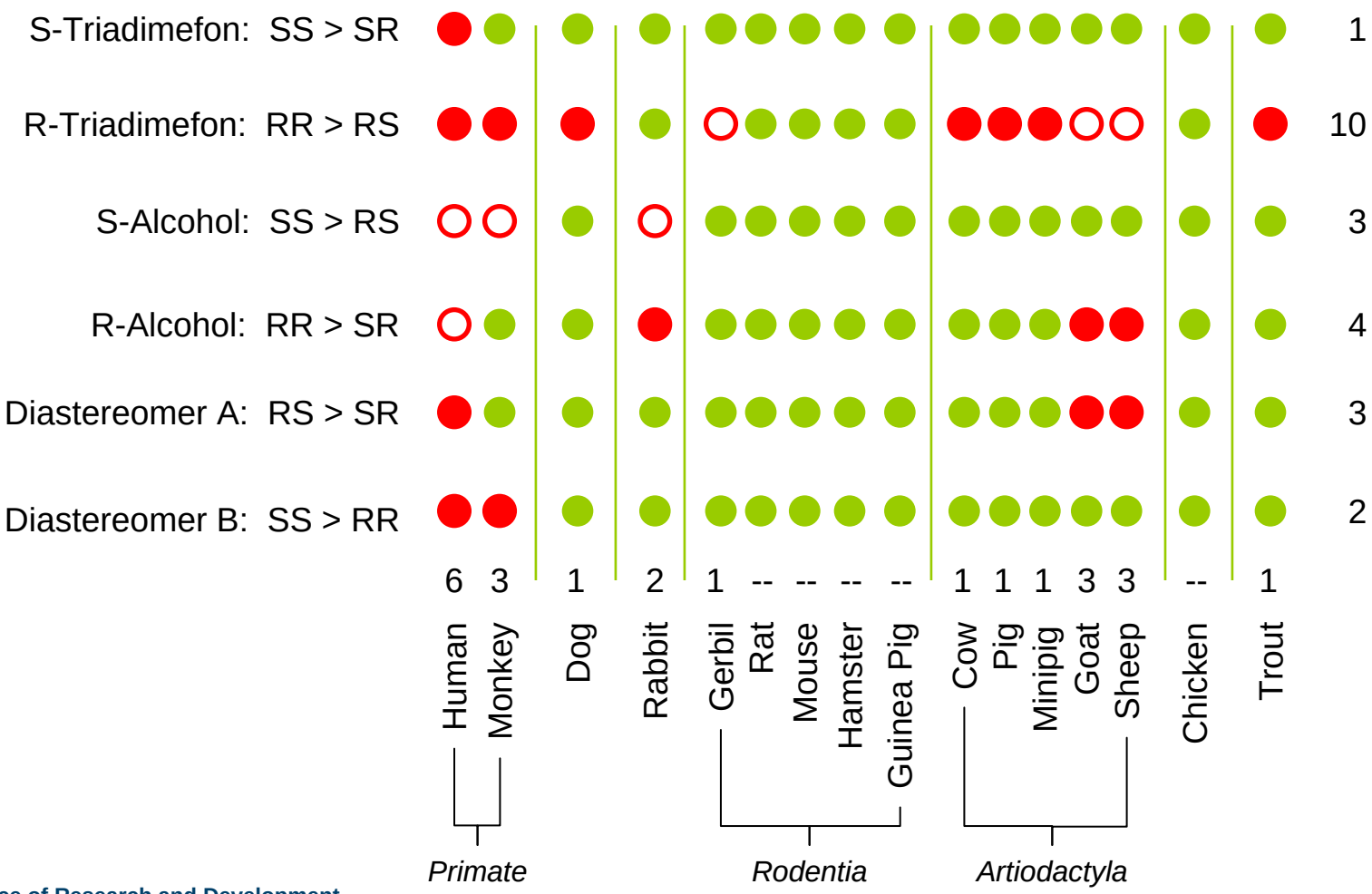
## Diastereomer A Formation



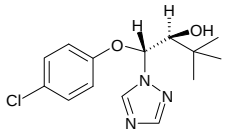

## Diastereomer B Formation

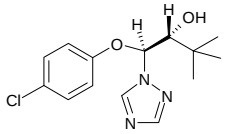


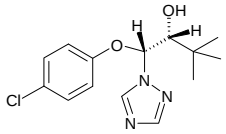
# Summary of Stereoselective Triadimenol Formation

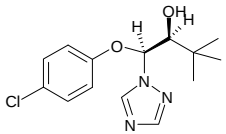


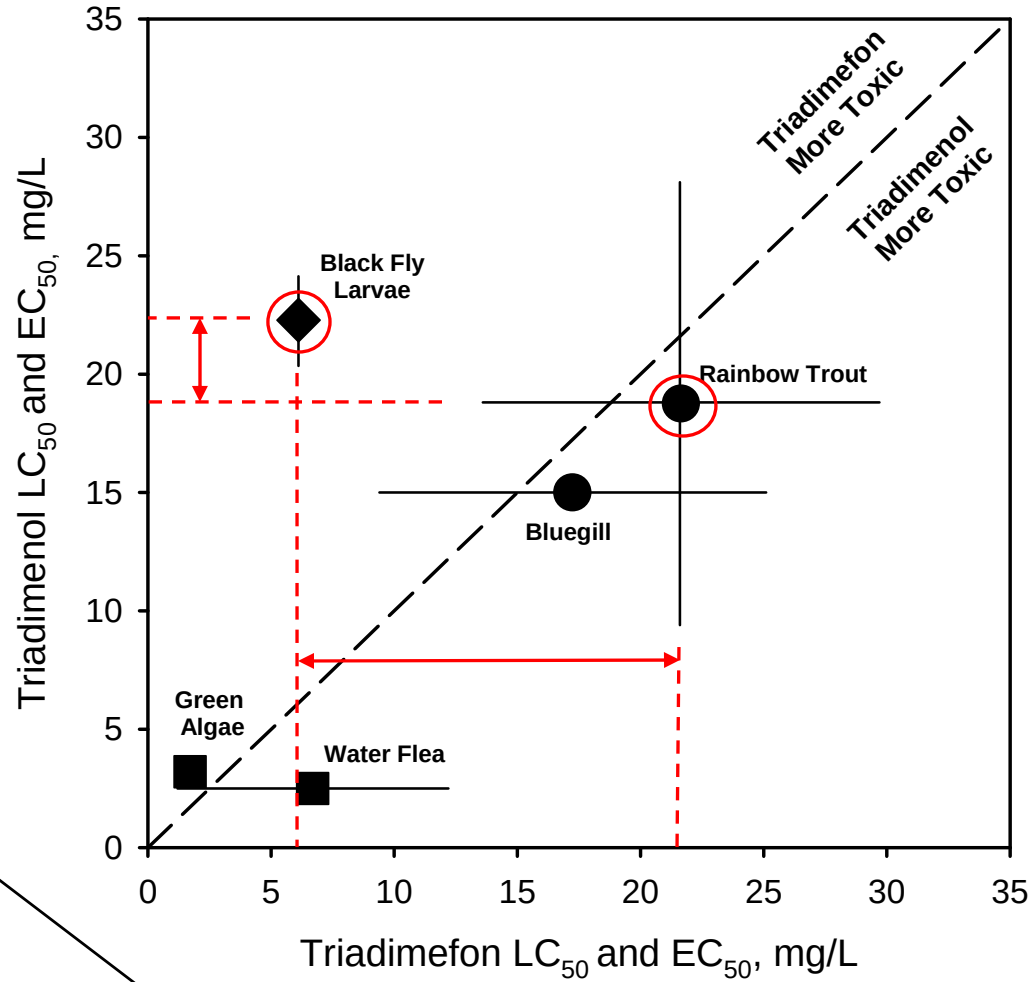
# Classic Toxicity Data

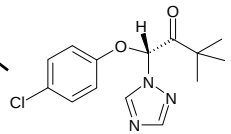


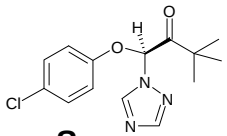



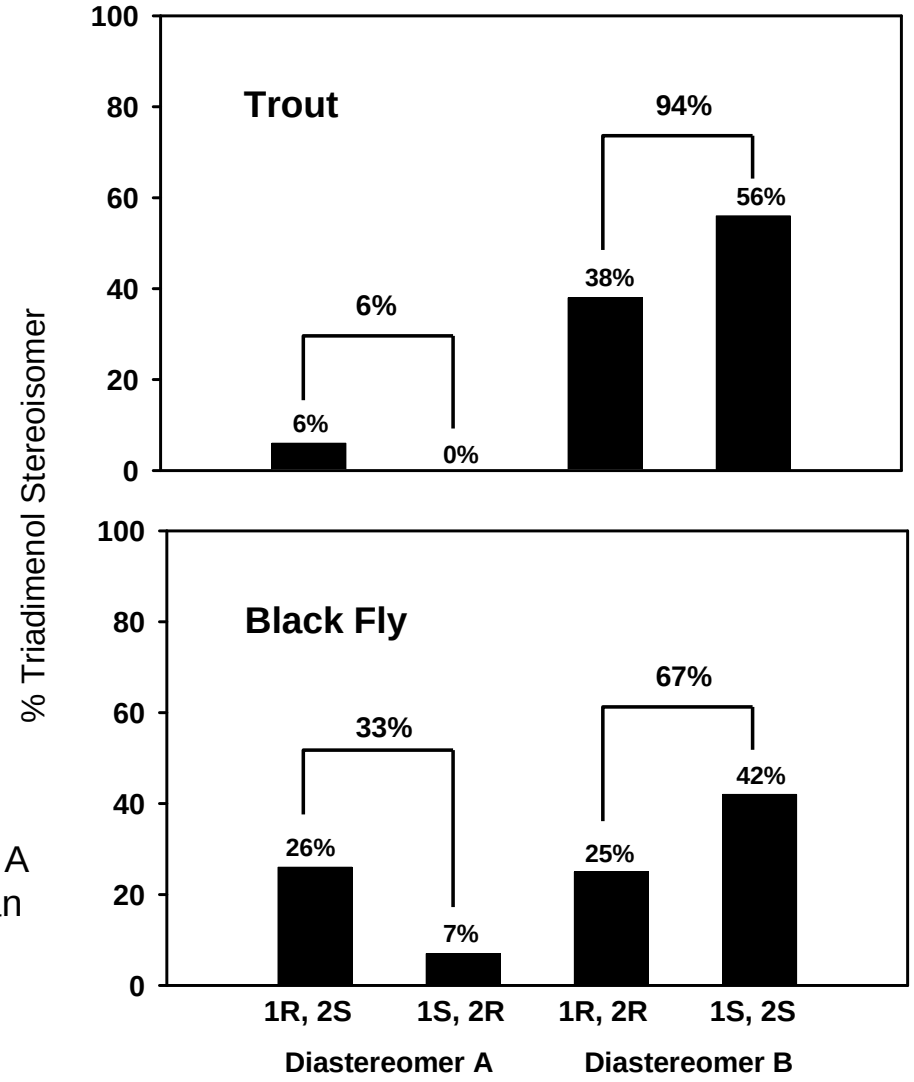



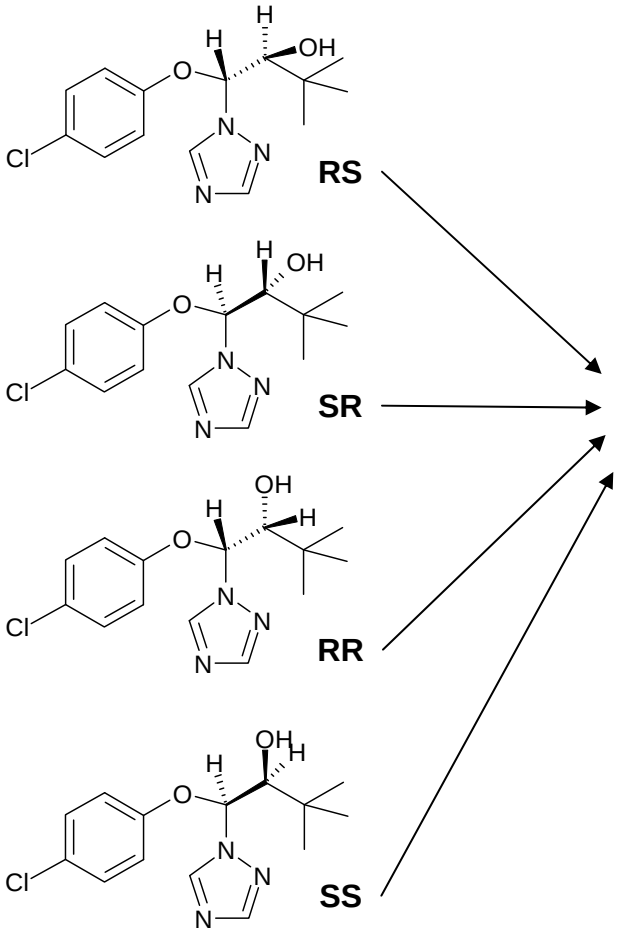



# Mechanistic Based Approach to Understanding Toxicity: Metabolism of Triadimefon to Triadimenol



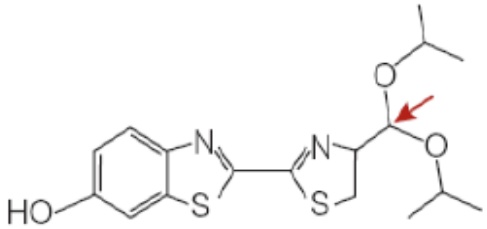

Triadimenol diastereomer A is 10 times more toxic than diastereomer B in rat

# In vitro CYP Inhibition Assay




**Cytochrome P450**

**1A1, 1A2**
**1B1**
**2C8, 2C9, 2C19**
**2D6**
**3A4, 3A7**



**CYP Assay Probe**

**Product**

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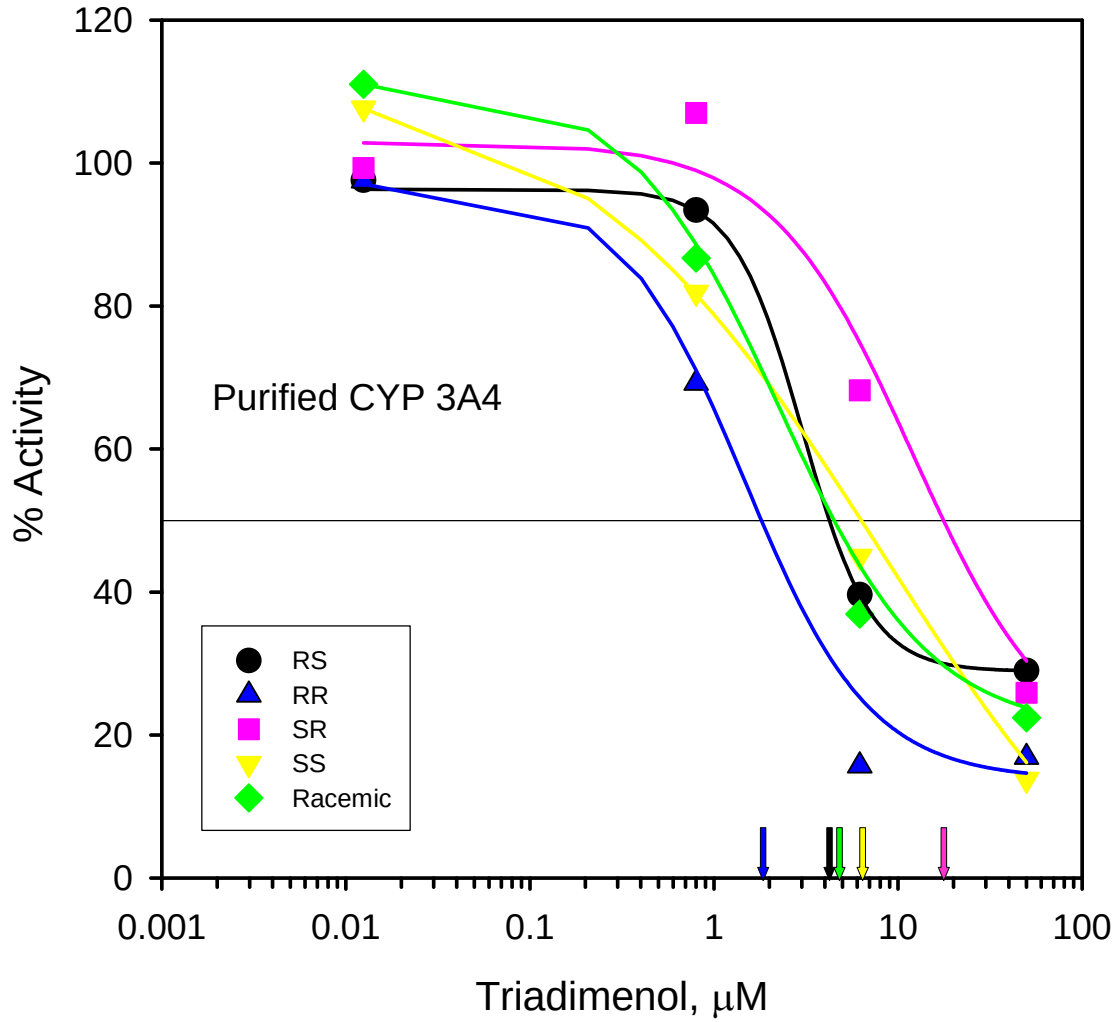
# Stereoselective CYP Inhibition



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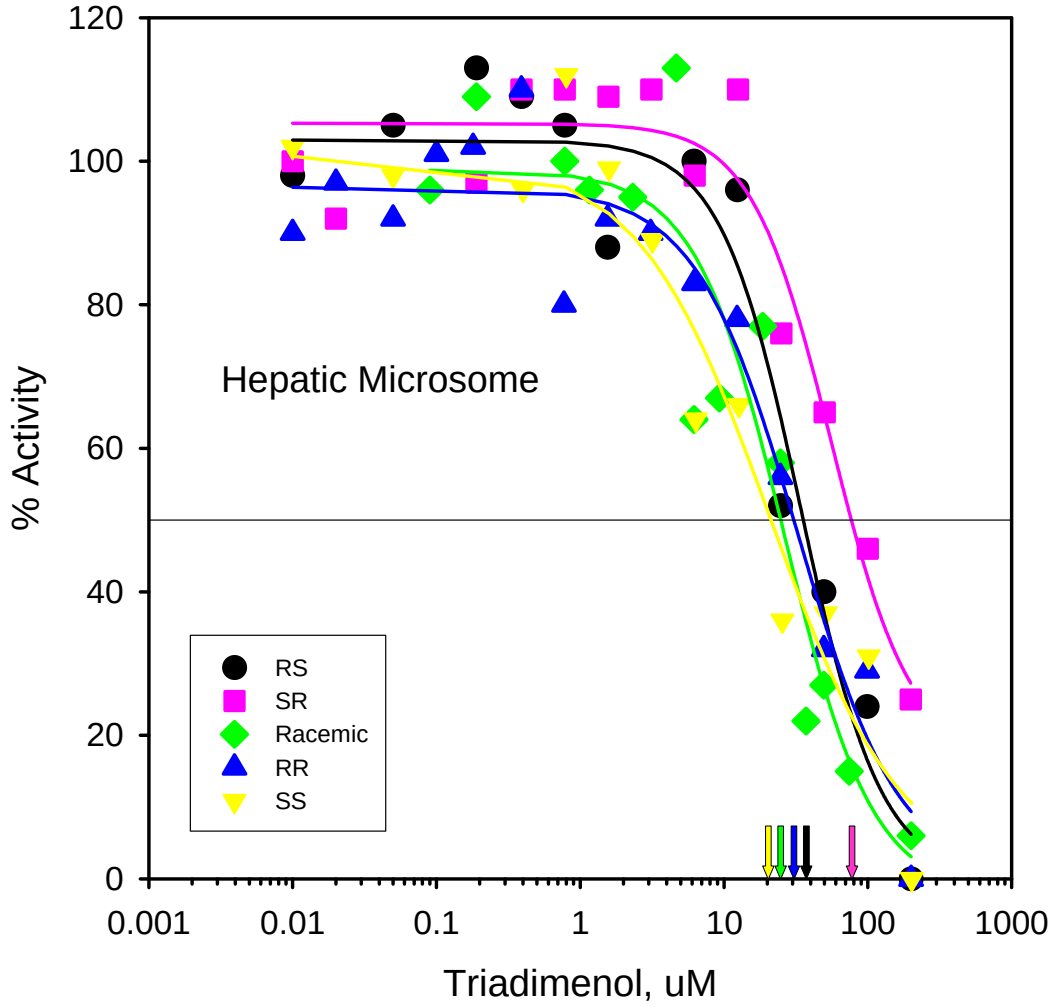
# Stereoselective Inhibition of Propiconazole Metabolism



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# Implications for Risk Assessment

- **Approach chiral chemicals as potential mixtures:** Stereoisomers of the “same” chemical can have different physical and chemical properties, resulting in different exposure scenarios, pharmacokinetics, pharmacodynamics and biological outcomes.
- **The composition of these mixtures can vary with time:** Enantioselective metabolism is the rule rather than the exception. It can disproportionally alter the relative concentration of stereoisomers as well as lead to new stereoisomers via the transformation of prochiral centers.
- **Decreased effectiveness of safety factors:** Increased uncertainty in understanding ”mixtures” issues for chiral compounds can be further amplified in species extrapolations, and may unknowingly increase risks to human health and the environment.