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# Kinetics, Mechanisms and Stereoselective Metabolism of 1,2,4-Triazole Fungicides and the Implications for Human Health and Ecological Risk Assessment

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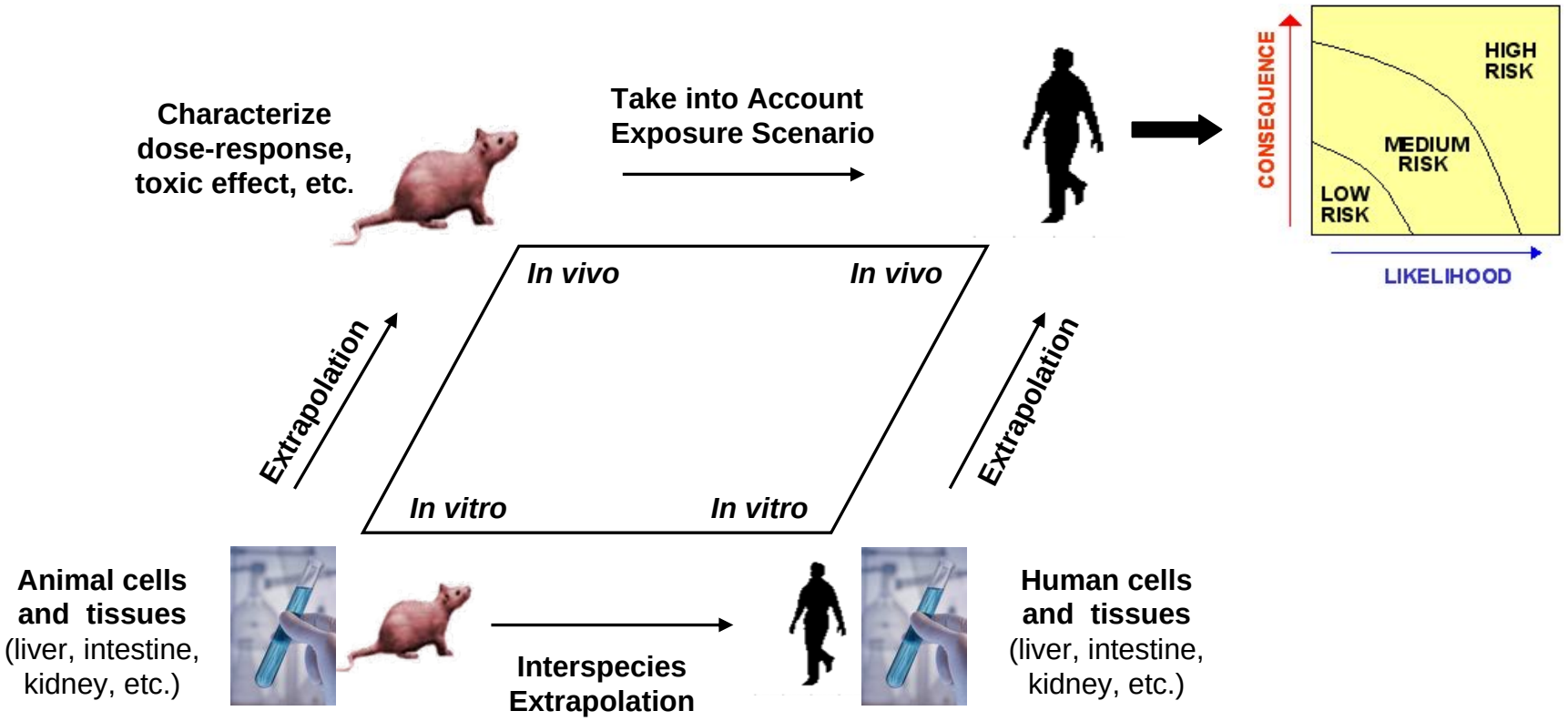
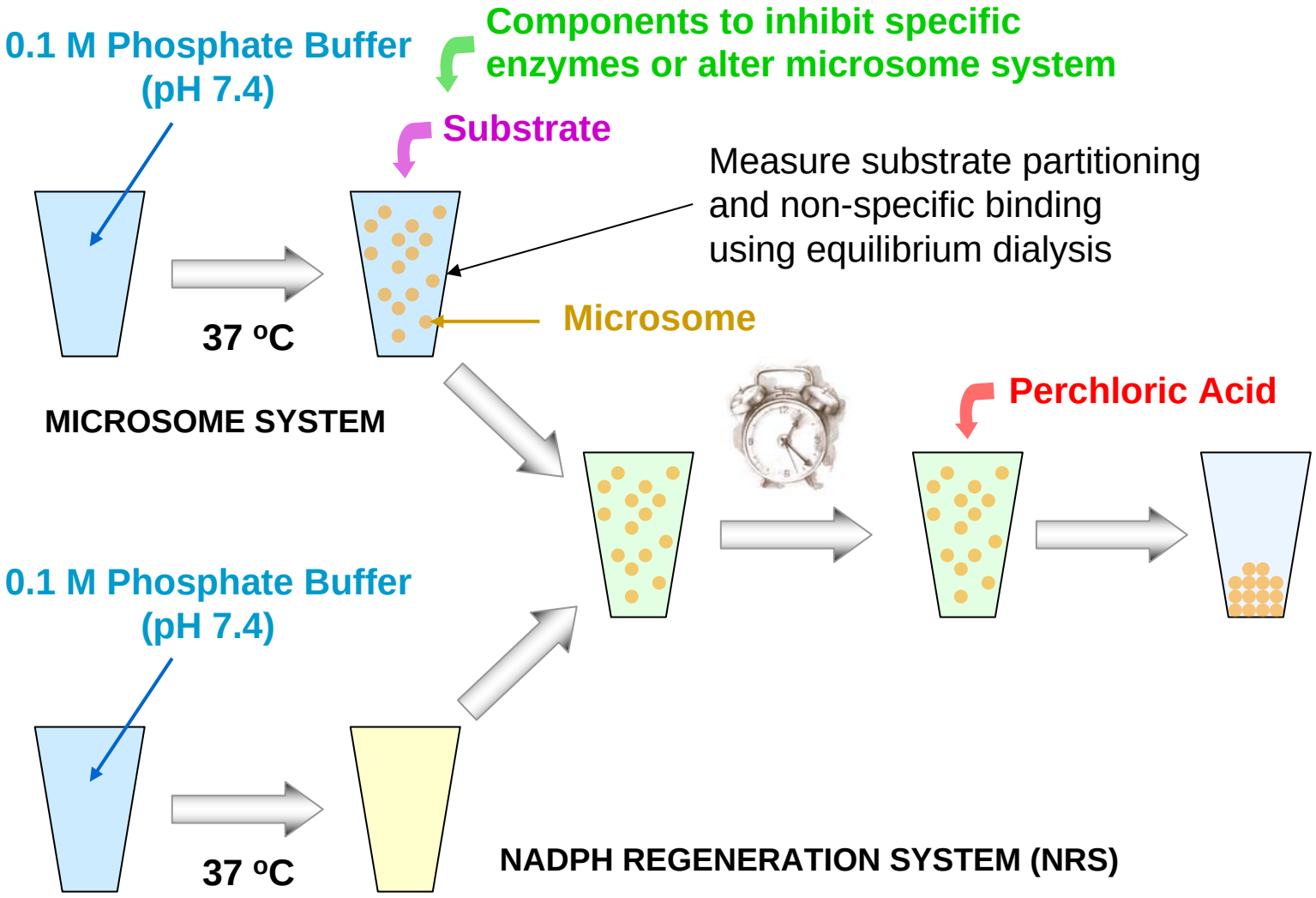
# Parallelogram Model for Risk Assessment

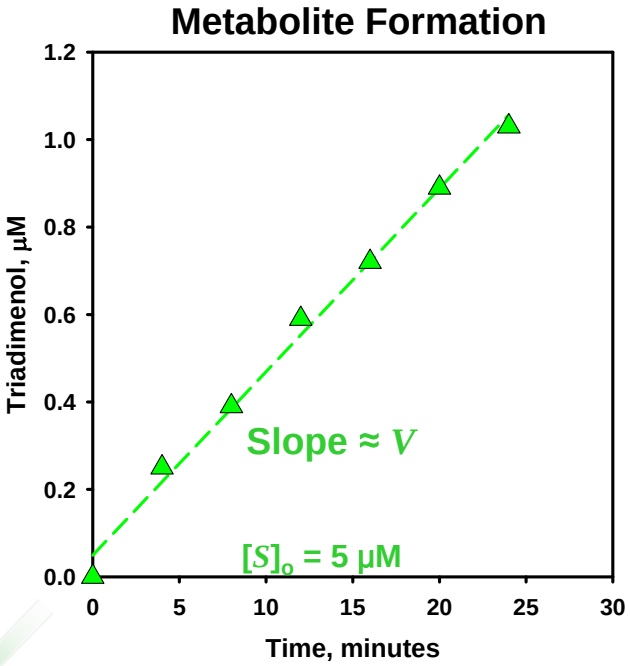


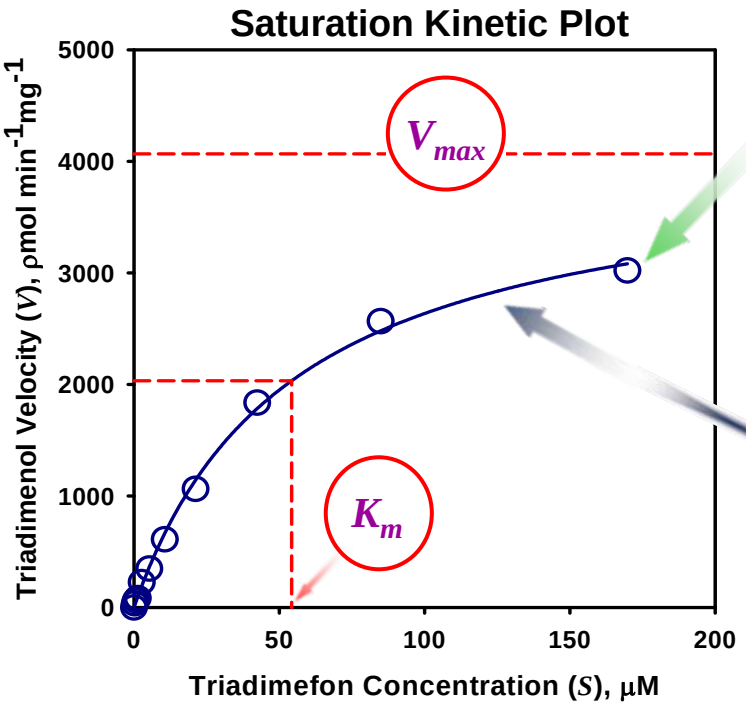
# In Vitro Metabolism Assay

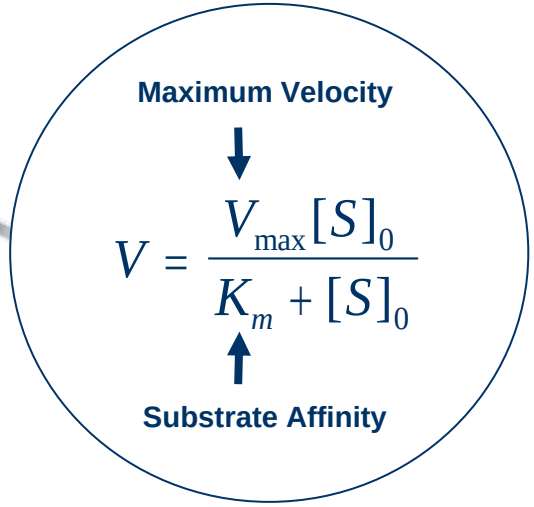


# Determining Kinetic Parameters

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



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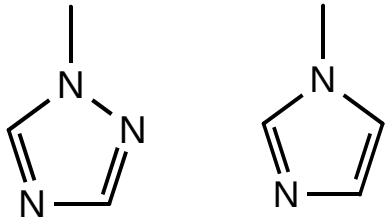
# Conazole Fungicides




## Background

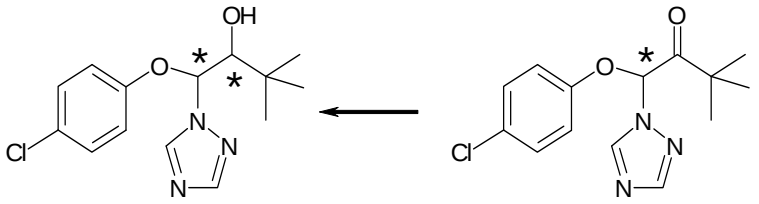
- 1,2,4-triazoles and imidazoles
- Inhibit steroid demethylation in fungi
- Used for over 30 years
- Approximately 25% of all fungicides sold
- Agricultural and medicinal uses

## 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

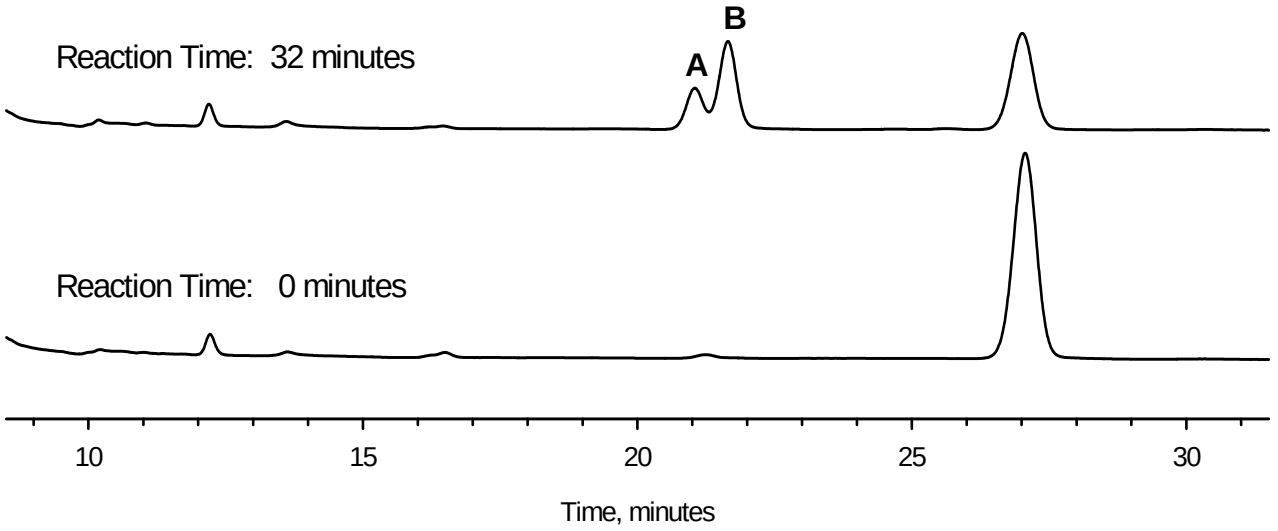
# Triadimefon Metabolism

**Carbonyl Reduction**

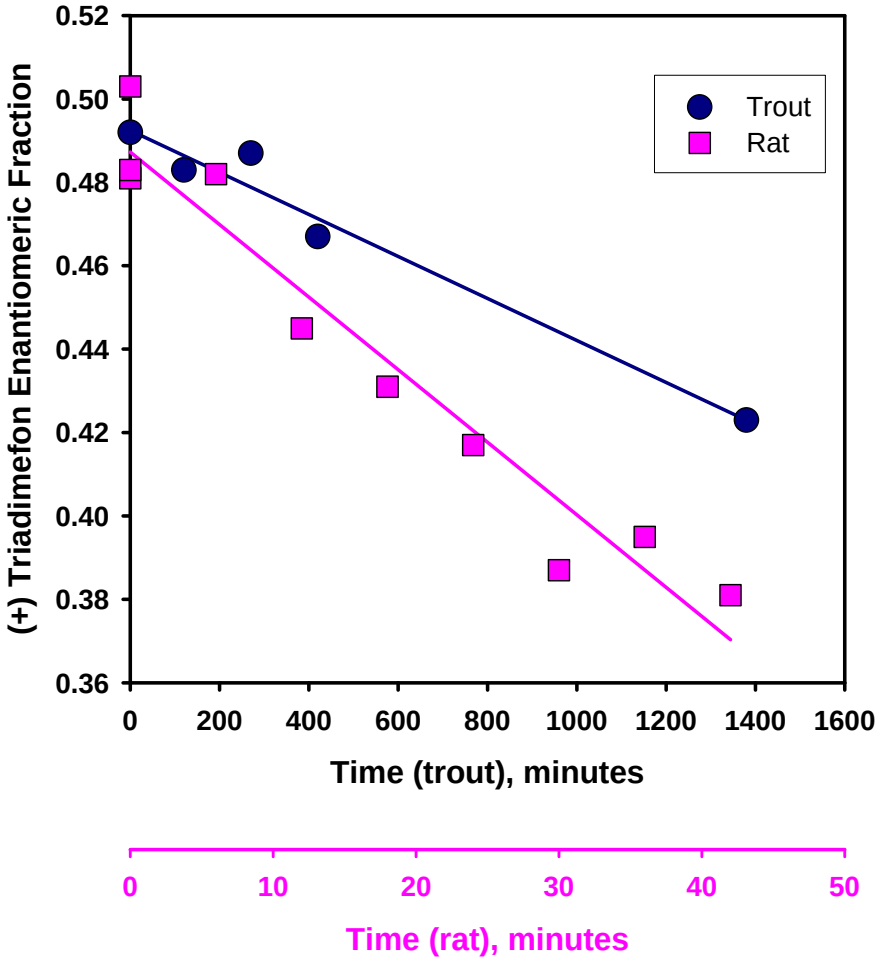



**Triadimenol** **Triadimefon**

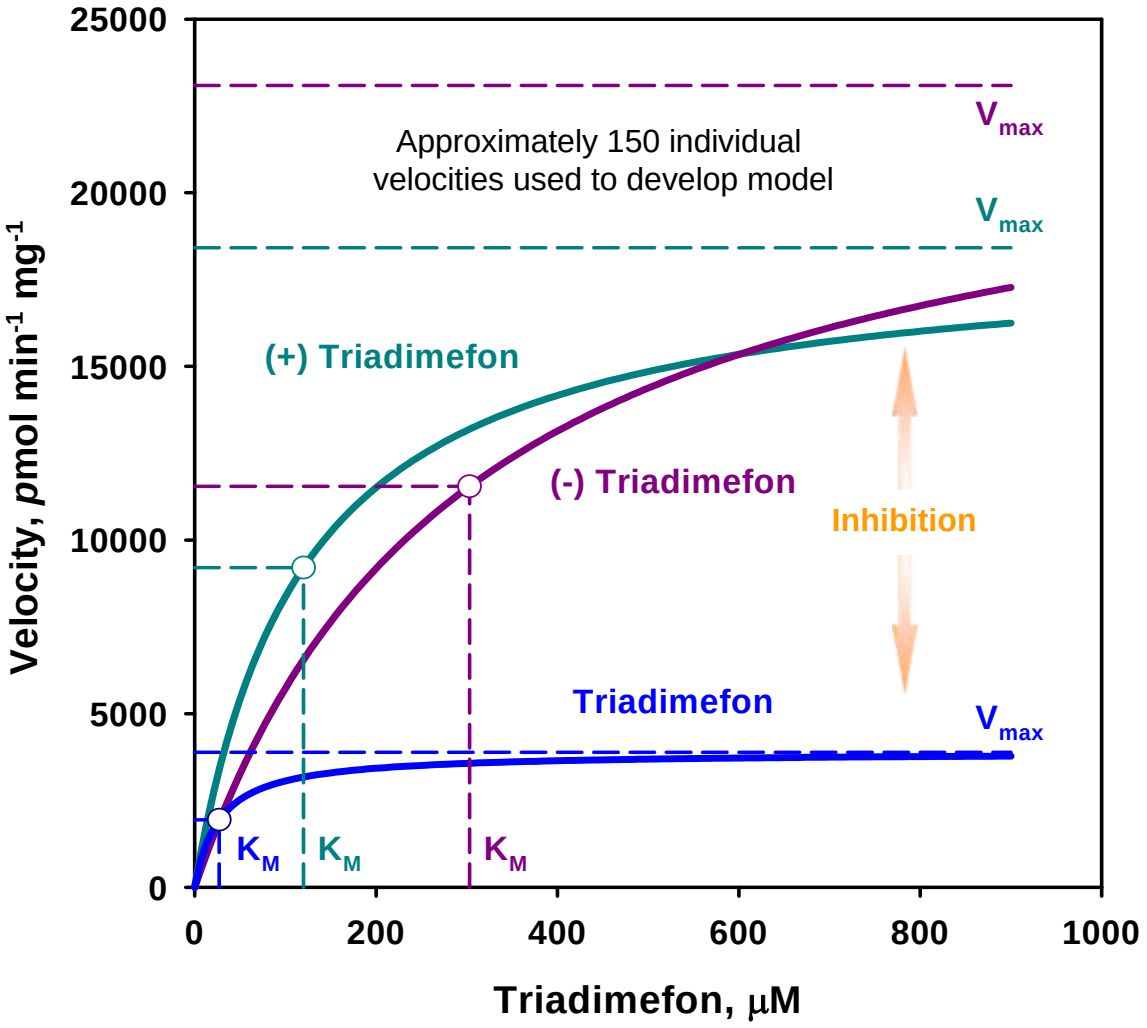
**HPLC Analysis**



# Stereoselective Triadimefon Depletion Chiral GC/MS

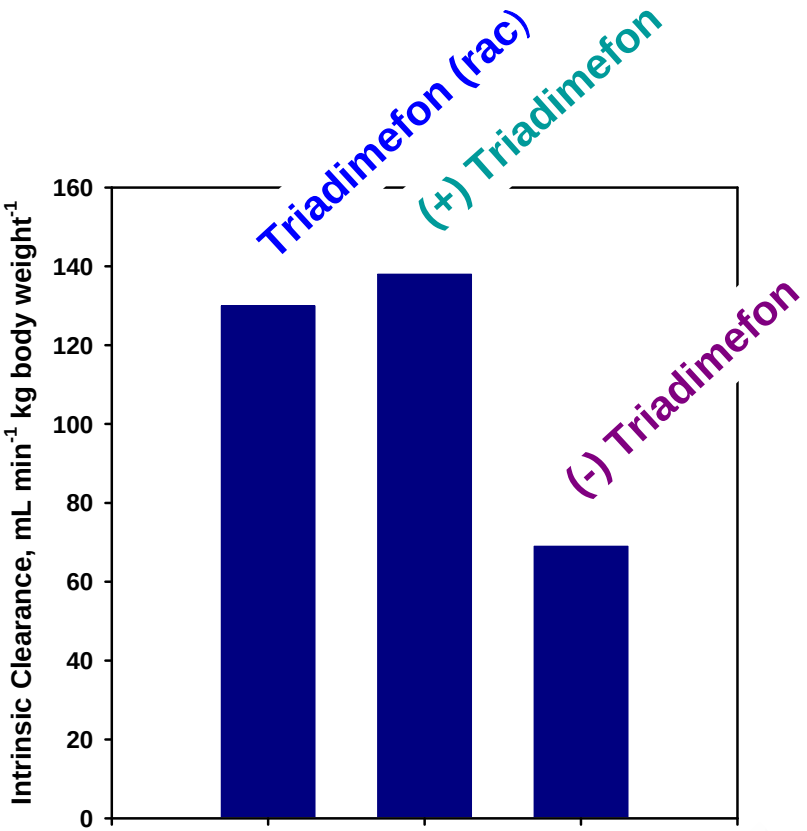


# “Mixtures Effects” for Triadimefon Metabolism



# Stereochemistry and Intrinsic Clearance

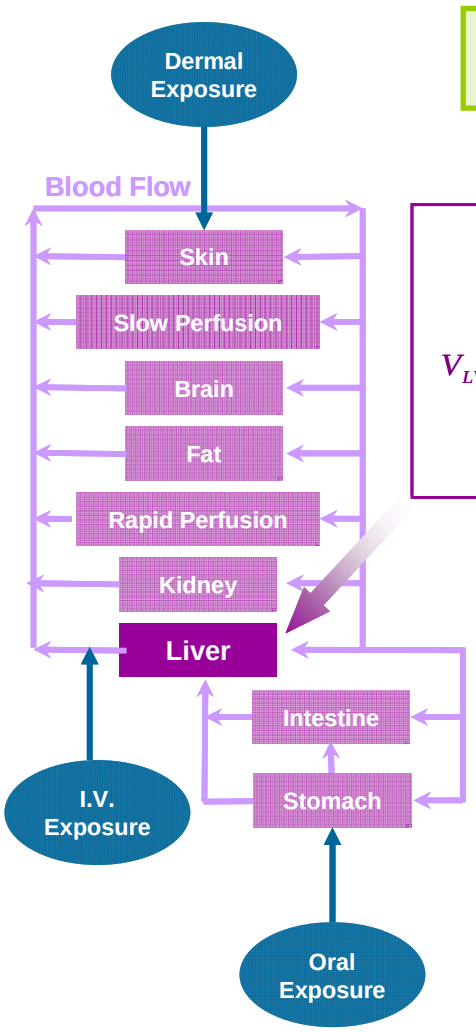
$$CL = \frac{V_{max}\ (C)}{K_m}$$




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

Predict Bioaccumulation

# Physiological Based Pharmacokinetic (PBPK) Model for Triadimefon in Rat



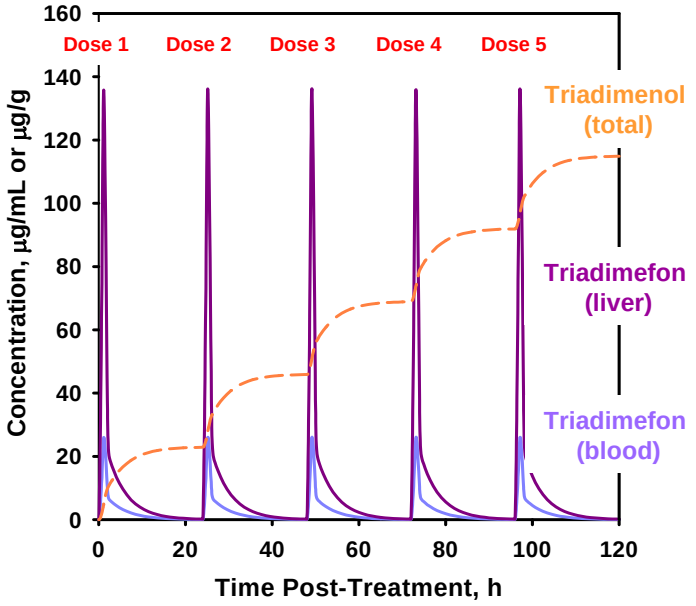


**Mathematical Expression for Liver Compartment**

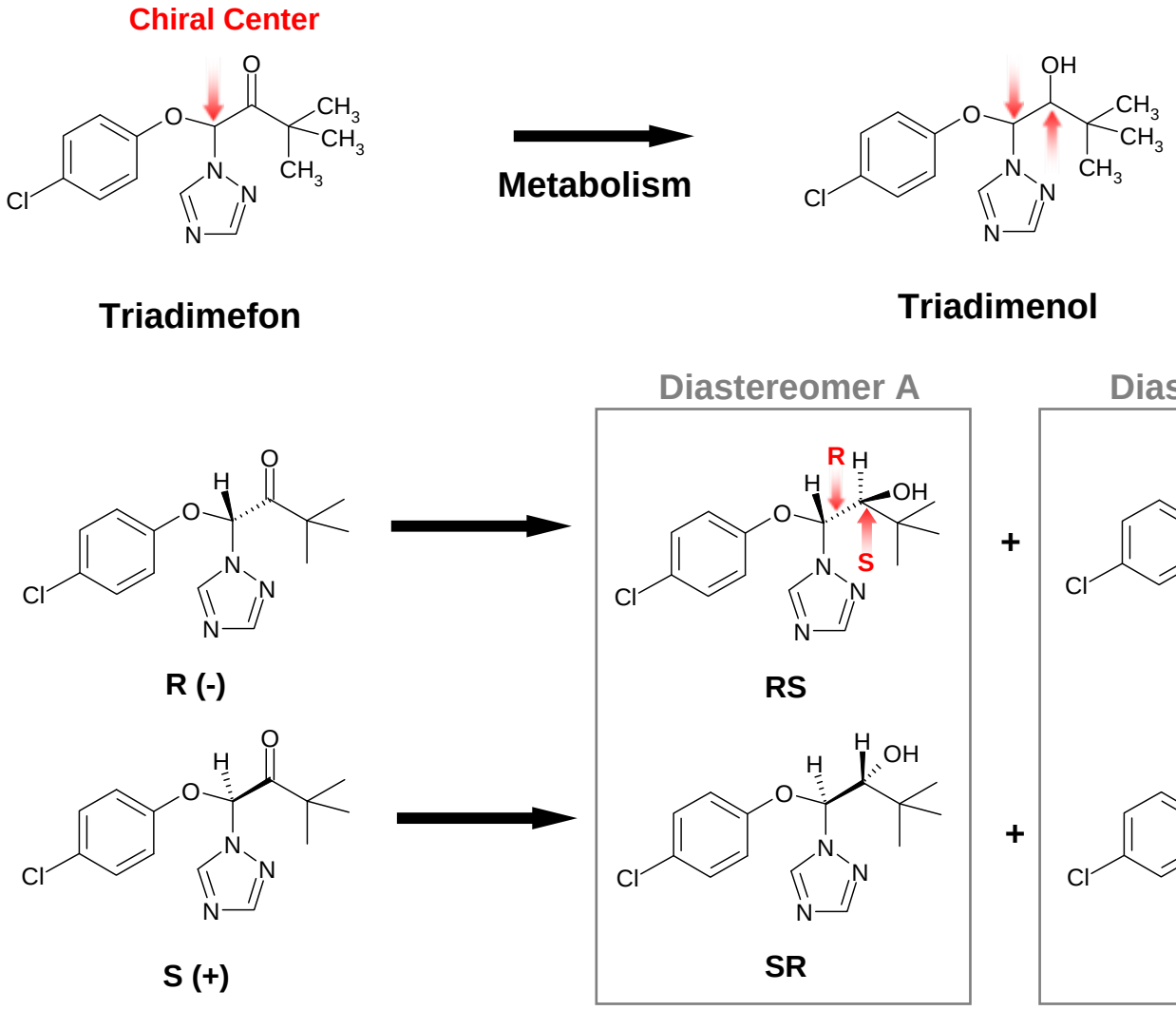
$$V_{LV}\frac{dC_{LV_i}}{dt} = Q_{B,LV}C_{LV_i} - Q_{B,LV}\frac{C_{LV_i}}{R_{LV,VB_i}} - \sum_j \frac{V_{\max,LV,i,j}C_{LV,i}}{K_{m,LV,i,j} + C_{LV,i}} + \sum_k \frac{V_{\max,LV,k,i}C_{LV,k}}{K_{m,LV,k,i} + C_{LV,k}}$$

***Kinetic Values Measured In Vitro***

***Validate Model In Vivo***



# Stereoselective Triadimenol Formation



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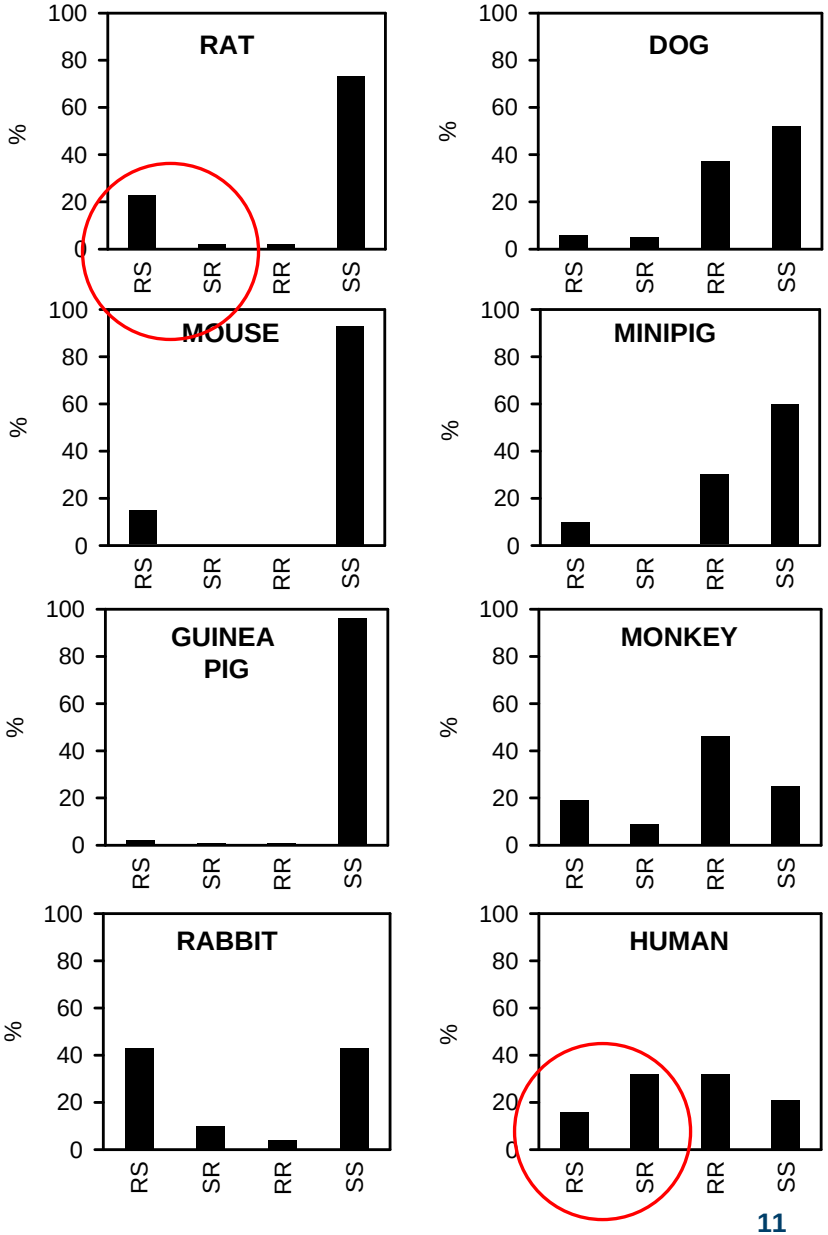
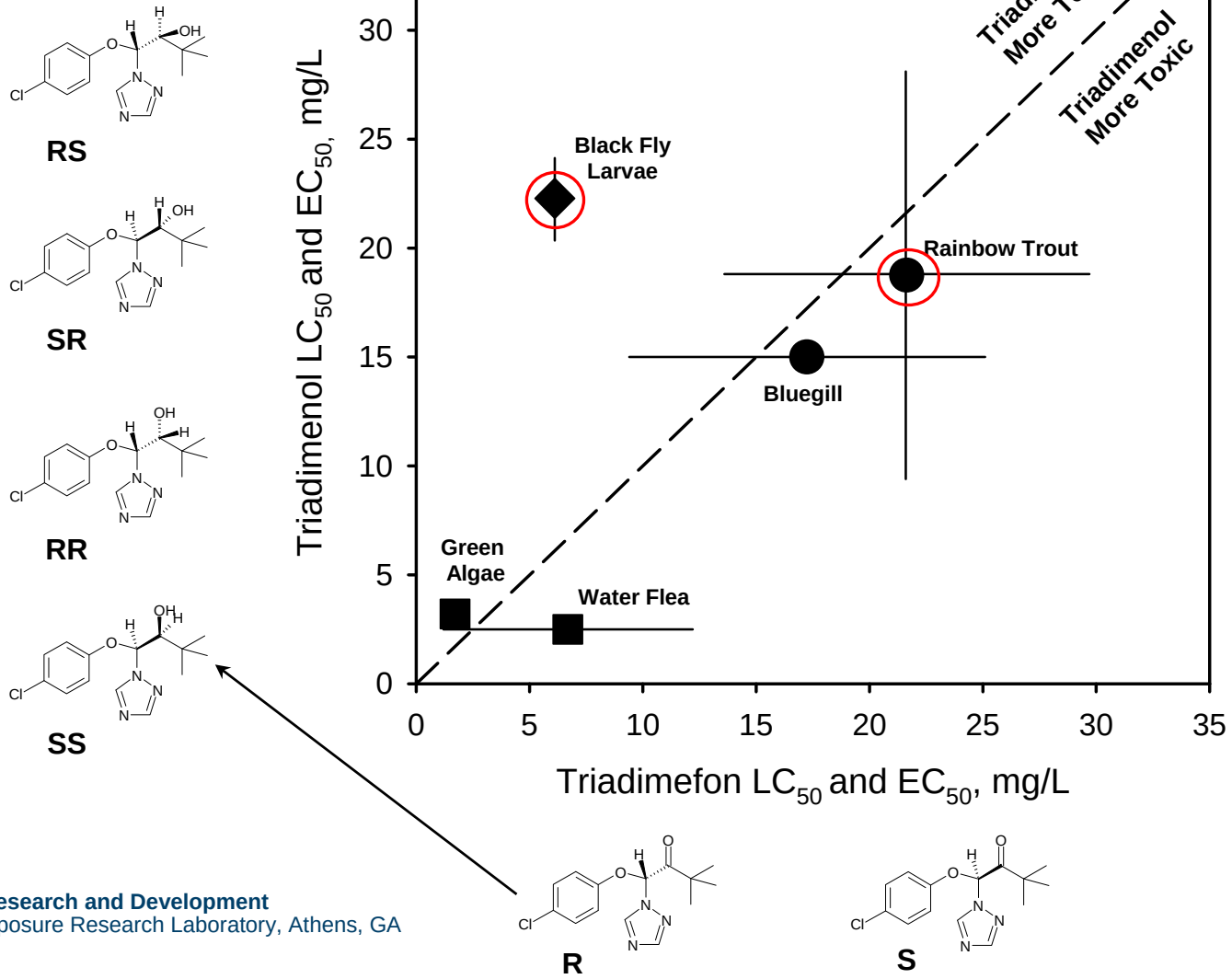
# Species Dependent Triadimenol Formation and Resulting Internal Exposures

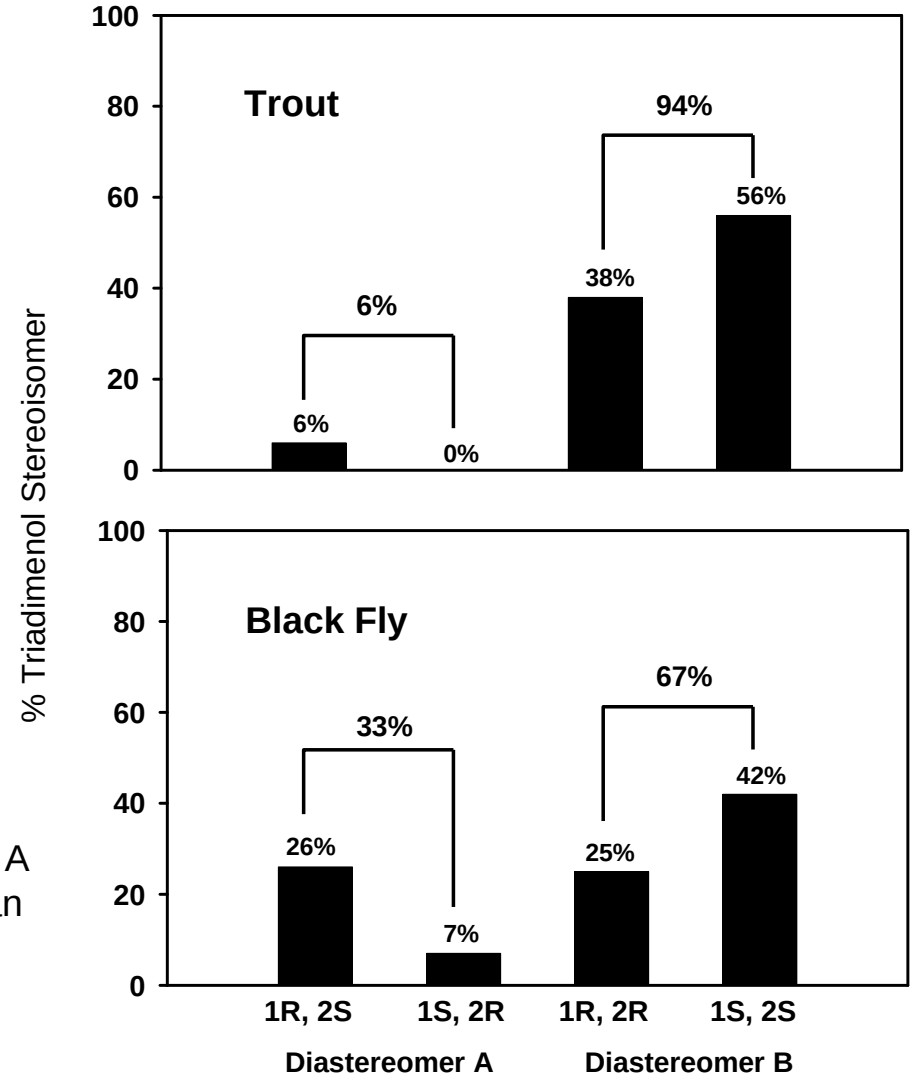
- All in vitro assays exposed to only triadimefon
- Metabolism results in mixture of RS, SR, RR and SS triadimenol
- (RS + SR) is 10X more toxic than (SS + RR)
- SR inhibits cholesterol biosynthesis 100X more than the other stereoisomers



# Classic Toxicity Data

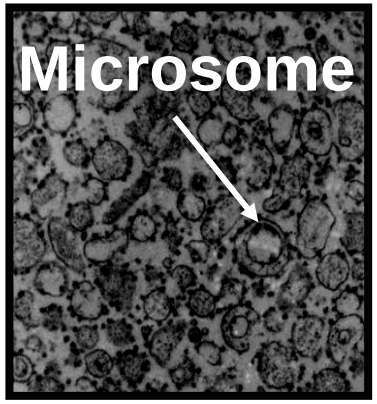


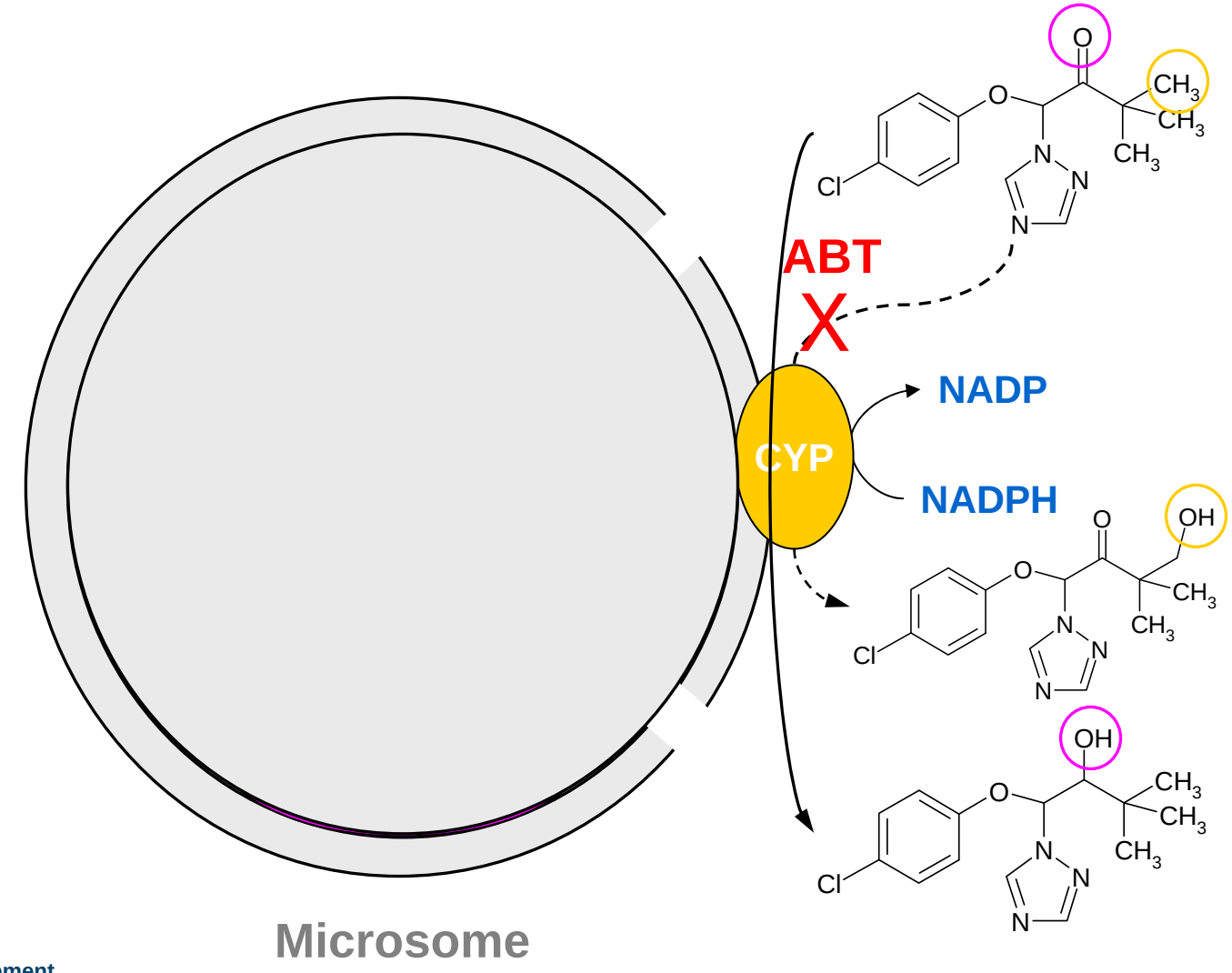
# Mechanistic Based Approach to Understanding Toxicity: Metabolism of Triadimefon to Triadimenol



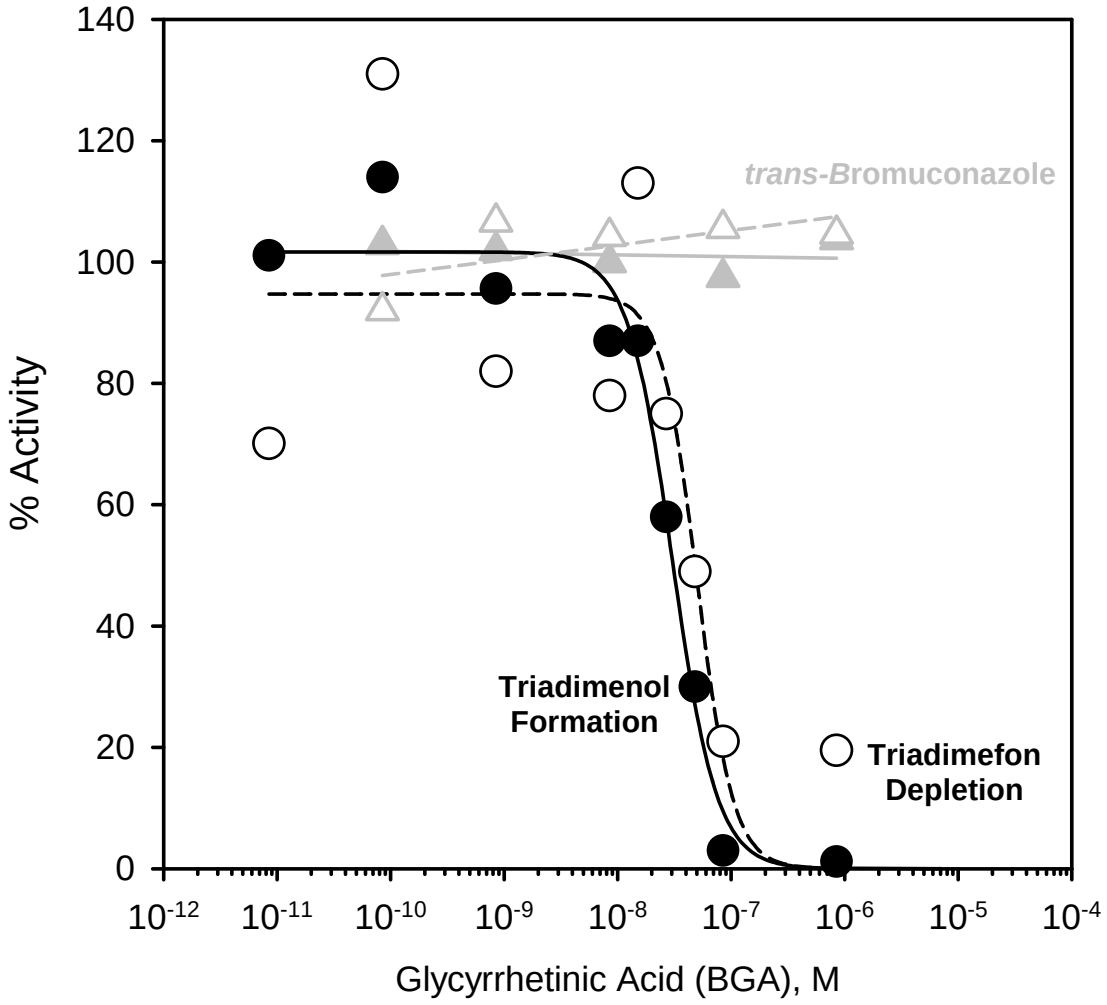

Triadimenol diastereomer A is 10 times more toxic than diastereomer B in rat

# Mechanism of Triadimefon Metabolism in Mammals

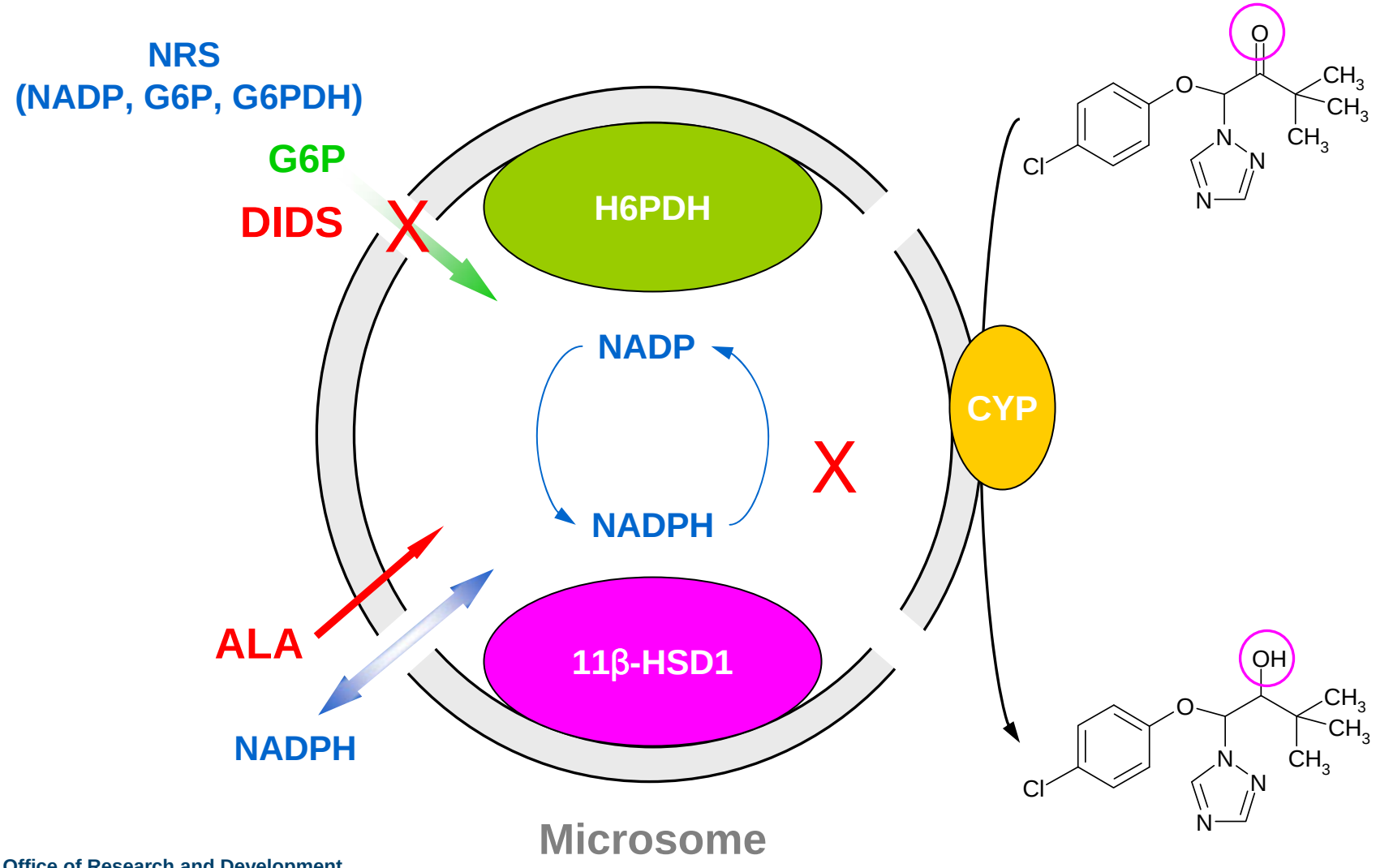


# 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1) Inhibition with BGA



# Mechanism of Triadimefon Metabolism in Mammals



# Enzyme Docking: Directing Laboratory Studies and Developing In Silico Screening Tools