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

John F. Kenneke, Christopher S. Mazur, A. Wayne Garrison, Rebecca D. Miller, Thomas J. Sack, Cather C. Brown, and Jimmy K. Avants
Source-to-Outcome Continuum

Xenobiotic ADME
Absorption
Distribution
Metabolism
Elimination

Environmental Release
Environmental Concentration
Exposure Concentration
PBPK Model
QSARs
In silico

Biological Event

Effect/Outcome

Exposure Concentration
Dose

Office of Research and Development
National Exposure Research Laboratory, Athens, GA
Metabolism: Pathways, Kinetics, and Mechanisms

EXPOSURE RESEARCH
Absorption
Distribution
Metabolism
  • Metabolite ID
  • Kinetics
  • Mechanisms
  • Organ specific
  • Gender differences
  • Vulnerable populations
Elimination

Effect

Liver

CYP3A4

11B-HSD

“B”

“C”

“A”

Office of Research and Development
National Exposure Research Laboratory, Athens, GA
Parallelogram Model for Risk Assessment

Characterize dose-response, toxic effect, etc.

In vivo

Take into Account Exposure Scenario

Extrapolation

In vitro

Extrapolation

Animal cells and tissues (liver, intestine, kidney, etc.)

Interspecies Extrapolation

Human cells and tissues (liver, intestine, kidney, etc.)
In Vitro Metabolism Assay

Components to inhibit specific enzymes or alter microsome system

Substrate

Measure substrate partitioning and non-specific binding using equilibrium dialysis

MICROSOME SYSTEM

NADPH REGENERATION SYSTEM (NRS)

0.1 M Phosphate Buffer (pH 7.4)

37 °C

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

Initial Reaction Velocity, pmol min⁻¹ mg⁻¹

Initial Substrate Concentration, µM

trans -
trans-Bromuconazole

trans +
cis -
cis +
cis-Bromuconazole

Chiral Center
Stereoselective Bromuconazole Clearance

\[ CL = \frac{V_{\text{max}}(C)}{K_m} \]
Triadimefon Metabolism

Carbonyl Reduction

HPLC Analysis

Reaction Time:  32 minutes

Reaction Time:   0 minutes
Enantioselective Triadimefon Depletion
Chiral GC/MS

Time (trout), minutes

Time (rat), minutes
“Mixtures Effects” for Triadimefon Metabolism

Approximately 150 individual velocities used to develop model.

Vmax

Vmax

Inhibition

Triadimefon

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Stereochemistry and Intrinsic Clearance

\[ CL = \frac{V_{\text{max}} (C)}{K_m} \]

- **Triadimefon (rac)**
  - \( V_{\text{max}} \): 3891 pmol/min mg
  - \( K_m \): 27 μM
  - \( CL \): 130 mL/min kg weight

- **(+) Triadimefon**
  - \( V_{\text{max}} \): 18,414 pmol/min mg
  - \( K_m \): 120 μM
  - \( CL \): 138 mL/min kg weight

- **(-) Triadimefon**
  - \( V_{\text{max}} \): 23,088 pmol/min mg
  - \( K_m \): 303 μM
  - \( CL \): 69 mL/min kg weight

**Predict Bioaccumulation**
Stereoselective Triadimenol Formation

Triadimefon

Metabolism

Triadimenol

Diastereomer A

Diastereomer B

R (-)

S (+)
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

S-Triadimefon

R-Triadimefon
Stereoselective Carbonyl Reduction

S-Alcohol Formation

S → SS  R → RS

R-Alcohol Formation

S → SR  R → RR

SF

0% 20% 40% 60% 80% 100%

RR  SR

SF

0% 20% 40% 60% 80% 100%

RR  SR

Human  Monkey  Dog  Rabbit  Gerbil  Rat  Mouse  Hamster  Guinea Pig  Cow  Pig  Minipig  Goat  Sheep  Chicken  Trout

Human  Monkey  Dog  Rabbit  Gerbil  Rat  Mouse  Hamster  Guinea Pig  Cow  Pig  Minipig  Goat  Sheep  Chicken  Trout
Stereoselective Diastereomer Formation

**Diastereomer A Formation**

- **S** → **SR**
- **R** → **RS**

**Diastereomer B Formation**

- **S** → **SS**
- **R** → **RR**

The diagrams show the percentage of diastereomer formation across various species:

- Human
- Monkey
- Dog
- Rabbit
- Gerbil
- Rat
- Mouse
- Hamster
- Guinea Pig
- Cow
- Pig
- Minipig
- Goat
- Sheep
- Chicken
- Trout

Specific percentages are indicated for each species, illustrating the stereoselectivity in diastereomer formation.
### Summary of Stereoselective Triadimenol Formation

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<td><strong>Amount</strong></td>
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Classic Toxicity Data

Triadimefon LC\textsubscript{50} and EC\textsubscript{50}, mg/L

Triadimenol LC\textsubscript{50} and EC\textsubscript{50}, mg/L

Black Fly Larvae

Rainbow Trout

Bluegill

Green Algae

Water Flea

Triadimefon More Toxic

Triadimenol More Toxic

RS

SR

RR

SS
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
Stereoselective CYP Inhibition

Purified CYP 3A4

Triadimenol, μM

% Activity

RS
RR
SR
SS
Racemic
Stereoselective Inhibition of Propiconazole Metabolism
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.