Toxicokinetics of tebuconazole following oral and dermal administration

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Background

• Exposure assessment of the fungicide tebuconazole by urine biomonitoring

• For proper interpretation of biomonitoring data:
  • Analysing the correct biomarker(s)
  • Need information on bio-kinetics

• Animal studies suggest diverse metabolites, not necessarily valid for human

• Previous human studies reported tebuconazole-1-hydroxy (TEB-OH), and tebuconazole carboxylic acid (TEB-COOH) as main metabolites¹
  • Occupational exposure event
  • No screening for other potential biomarkers
  • Time course of elimination is unknown

¹Mercadante, R. 2014. Chem Res Toxicol
Aim of the study

Confirm the identity of the human metabolites of TEB in urine after controlled oral and dermal exposure

Determination of the bio-kinetics of tebuconazole and the main metabolite
Study design

1. Human volunteer study
2. Biomarker identification
3. Quantification main metabolite
1. Human volunteer study

• Six healthy non-smoking volunteers (3 male, 3 female)

• Oral and dermal exposure (<ADI) in random order

• Fixed dose:
  • Oral -> 1.5 mg dissolved in 200 mL of tap water
  • Dermal -> 2.5 mg dissolved in 100 µL of acetone applied on 25 cm² of the non-dominant forearm; estimated uptake after 1 h is 19 µg

• Pre-exposure urine sample prior to each exposure event
• Post-exposure urine collection up to 48 h in separate portions

• Refrain from consumption of food items containing relative high residues of TEB (e.g. grapes, stone fruit, tomatoes)
1. Study participants

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<tr>
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<th>Female (mean)</th>
<th>Male (mean)</th>
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<tr>
<td>Age (y)</td>
<td>23</td>
<td>24</td>
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<tr>
<td>Height (cm)</td>
<td>171</td>
<td>186</td>
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<td>Weight (kg)</td>
<td>61</td>
<td>71</td>
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2. Biomarker identification

- 24 h pooled urine sample per person per exposure event
- A pooled pre-exposure sample from all volunteers
- Minimal sample cleanup; with and without deconjugation
- High resolution mass spec analysis; ESI + and ESI -

- Data processing
  - Targeted analysis of the known animal metabolites
  - Metabolite discovery by comparing pre- and post-exposure samples and software-predicted phase I and phase II metabolites

- The compound with highest relative detectability was selected

- After deconjugation TEB-OH showed highest relative detectability
2. Biomarker identification

- Deconjugation
- \( \beta \)-glucuronidase/aryl sulfatase

- UltraFiltration (30 kDa)

- (U)HPLC full scan-HRMS pos&neg ions

- Data processing

- Suspect screening
- Targeted data analysis
  - exact m/z

- Differential analysis
  - Database pesticide phase I/phase II metabolites
  - exposed vs non-exposed differential m/z
  - ‘de-novo’ annotation/identification

- Confirmatory analysis against analytical reference standard

Courtesy: Hans Mol
2. Metabolites (tentatively) identified

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<th>Biomarker (abbreviation)¹</th>
<th>Molar mass</th>
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¹Order of relative detectability
²Metabolites confirmed with analytical standard
³Two isomers found
2. Metabolites in animals
3. TEB-OH quantification

- Addition of internal standard (TEB-OH-D6), buffer and *Helix pomatia*
- Deconjugation at 37 °C for 16 h
- Subzero-temperature liquid-liquid extraction
  - Placing extract with acetonitrile at -20 °C for 20 min.
- Organic layer was analysed with LC-MS/MS
  - LOQ = 0.05 ng/mL
- TEB-OH quantification of each single urine sample
3. TEB-OH excretion over time - Oral
3. TEB-OH excretion over time - Dermal
3. TEB-OH kinetics
Discussion

• Additional metabolites identified were not confirmed with an analytical reference standard

• Some urine samples were quite concentrated or diluted; potential influence on excretion of the metabolite (e.g. water retention)

• Urine collection was limited up to 48 h after exposure; elimination after dermal exposure was not finished yet

• Recovery of TEB-OH is higher than calculated uptake; exposure is not stopped after 1 h; storage compartment in skin
Conclusion

• TEB-OH is the most abundant metabolite after controlled TEB exposure, followed by TEB-COOH

• No differences in retrieved metabolites between oral and dermal exposure

• Similar $K_e$ and $T_{1/2}$ between males and females

• $T_{1/2}$ was twice as high after dermal exposure, presumably due to much slower uptake
Acknowledgements

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