National Institute of Environmental Health Sciences
Division of Translational Toxicology

Risk Assessment of Exposure to Triazole Fungicides by Human Biomonitoring and Mechanistic Data

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Background and Purpose

§ Brazil is currently the world's largest coffee producer, and this commodity has played a significant role in the country's economy and history.

§ However, the nation's high coffee productivity relies on the continued use of pesticides that, while essential for agriculture, may pose risks to human health and the environment due to their non-selective mechanisms of action.

§ Triazole fungicides are widely used in agriculture to combat various fungal diseases. In mammals, these fungicides potently inhibit cytochrome P450 and liver microsomal enzymes, leading to changes in biomarkers upon exposure.

§ High-throughput screening (HTS) data annotated to toxicologically relevant molecular and cellular targets are also available for these compounds from the US federal Tox21 research program.

§ To further foster the interpretation and application of HTS data and computational toxicology workflows, NICEATM has developed the Integrated Chemical Environment (ICE) platform.

§ In risk assessment of pesticide exposure in humans, key factors include analyzing the chemicals' toxicity, understanding dose-response relationships, and evaluating exposure levels. Effect biomarkers are utilized to assess the impact of the exposure.

§ This study assessed exposure to triazole fungicides using human biomonitoring, evaluated potential biomarkers, and compared to mechanistic target data derived from curated HTS assays.

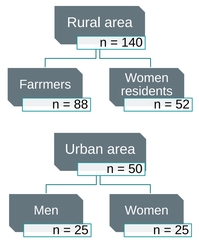
Materials and Methods

Sampling

§ During intensive use of pesticides in the southern region of Minas Gerais, Brazil (Dec 2021 - March 2022)

§ Volunteers with age >18 years

§ 3 triazoles measured



Approved by the Research Ethics Committee of the Federal University of Alfenas (UNIFAL-MG), CAAE: 34644620.2.0000.5142

Biomarkers

§ Oxidative stress biomarkers
- Serum samples
- Spectrophotometer analysis

§ Cytome assay[1]
- Buccal swabs using cytobrush
- Fluorescence microscope

§ Plasma bile acids
- UHPLC-MS/MS

§ Liver enzymes
- Serum samples
- Male volunteers

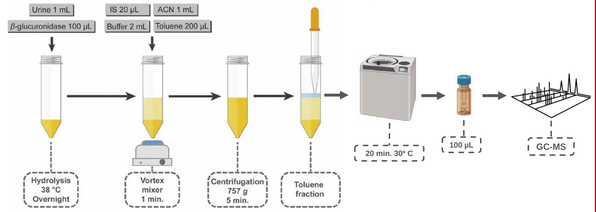
§ Total testosterone

§ Androstenedione
- Male volunteers

Exposure Assessment

§ Questionnaire application to gather exposure conditions

§ Method adapted from Machado et al., (2019)[2]



§ Calculation of Estimated Daily Intake (EDI) in µg/kg-bw/day

§ Hazard Quotient (HQ)

$$EDI = \frac{C \,.\, CE}{bw \,.\, F} \qquad HQ = \frac{EDI}{ADI}$$

C: urinary triazol concentration (µg/g creat.);
CE: reference value for creatinine excretion in urine derived from adults in Brazil (1.22 g creat./day)
bw: average body weight of men and women in the exposed group (kg);
F: triazole urinary excretion factor (0.17 for epoxiconazole, 0.27 for cyproconazole and 0.5 for triadimenol (EFSA);
ADI: Acceptable Daily Intake (µg/kg-bw/day) from Brazil Authority ANVISA (https://www.gov.br/anvisa/pt-br/acessoainformacao/dadosabertos/informacoes-analiticas/monografias-de-agrotoxicos/)

ICE

Integrated Chemical Environment
v.4.0.1

§ Curated Tox21 and ToxCast HTS data, e.g. analytical QC, assay interference, mechanistic targets

§ Filter active assays for triazoles and other pesticide products active ingredients

§ IVIVE tool to calculate Equivalent Administered Dose (EAD)

§ Compare with EDI from human biomonitoring data for triazoles

§ Applied solve_pbtk (oral) and solve_gas_pbtk (inhalation) simulated exposure models

Results

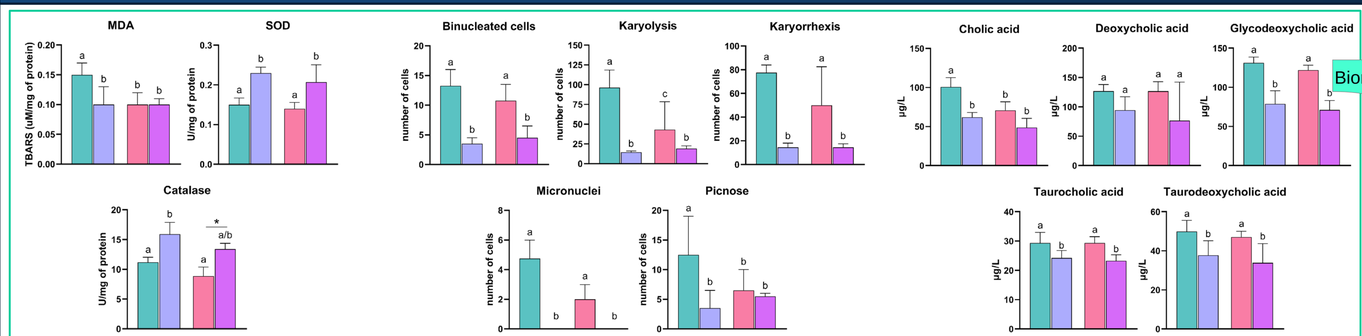


Fig. 1. Oxidative stress biomarkers. Values expressed as median and 95% confidence intervals. Different letters indicate groups that were significantly different ($p < 0.05$) from one another based on Dunn's multiple comparison test as a post hoc, with 5% significance.

Fig. 2. Cytome assay biomarkers. Values expressed as median and 95% confidence intervals. Different letters indicate groups that were significantly different ($p < 0.05$) from one another based on Dunn's multiple comparison test as a post hoc, with 5% significance.

Fig. 3. Plasma bile acid levels. Different letters indicate groups that were significantly different ($p < 0.05$) from one another based on Dunn's multiple comparison test as a post hoc, with 5% significance.

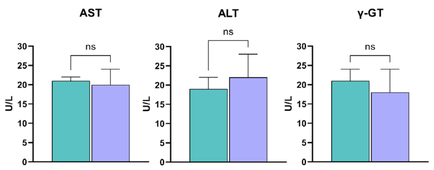


Fig. 4. Liver enzymes. Values expressed as median and 95% confidence interval. *p-value obtained using the Mann-Whitney test, with 5% significance.

Fig. 5. Total testosterone and androstenedione. Values expressed as median and 95% confidence interval. *p-value obtained using the Mann-Whitney test, with 5% significance.

Biomarkers

Exposure Assessment

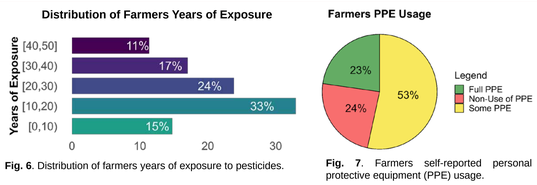


Fig. 6. Distribution of farmers years of exposure to pesticides.

Fig. 7. Farmers self-reported personal protective equipment (PPE) usage.

Table 1. Estimated Daily Intake (EDI, µg/kg-bw/day) of triazole fungicides measured

EDI	<LOQ[a]	P25	Median	P75	Max
Men from the rural area					
Cyproconazole	0.17	0.22	0.24	0.28	0.57
Epoxiconazole	0.53	0.79	1.08	2.96	6.31
Triadimenol	-	-	-	-	1.07
Women from the rural area					
Epoxiconazole	0.66	1.01	1.89	2.54	8.77

[a] Triazole concentration in urine (µg/g creat.) below the limit of quantification (LOQ); LOQ = 10.0 µg L^{-1} for cyproconazole and triadimenol; 30.0 µg L^{-1} for epoxiconazole.

HQ Calculation for Epoxiconazole at the highest quantified value:

EDI = 6.31 µg/kg-bw/day

HQ = 2.1

EDI = 8.77 µg/kg-bw/day

HQ = 2.9

Epoxiconazole ADI = 3.0 µg/kg-bw/day (ANVISA)

ICE

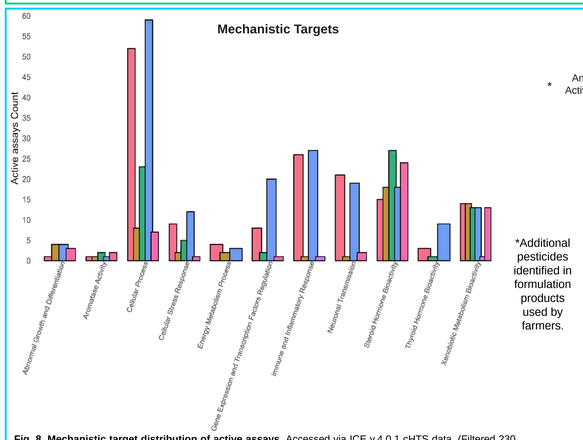


Fig. 8. Mechanistic target distribution of active assays. Accessed via ICE v.4.0.1 cHTS data. (Filtered 230 unannotated active assays: Azoxystrobin = 79; Cyproconazole = 31; Epoxiconazole = 0; Pyraclostrobin = 77; Thiamethoxam = 4; Triadimenol = 39).

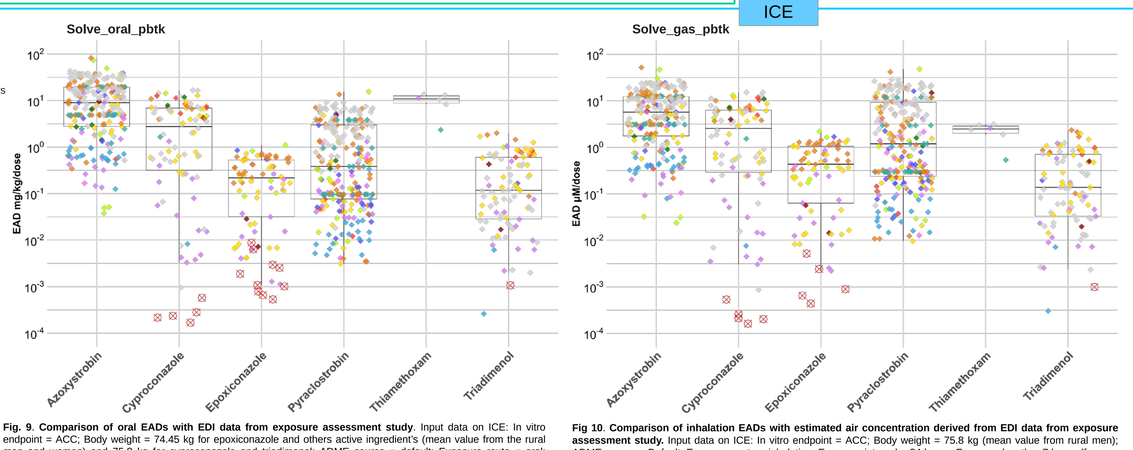


Fig. 9. Comparison of oral EADs with EDI data from exposure assessment study. Input data on ICE: In vitro endpoint = ACC; Body weight = 74.45 kg for epoxiconazole and others active ingredient's (mean value from the rural men and women) and 75.8 kg for cyproconazole and triadimenol; ADME source = default; Exposure route = oral; Exposure interval = 10 hours; Simulation length = 150 days (period of application of fungicides).

Fig 10. Comparison of inhalation EADs with estimated air concentration derived from EDI data from exposure assessment study. Input data on ICE: In vitro endpoint = ACC; Body weight = 75.8 kg (mean value from rural men); ADME source = Default; Exposure route = inhalation; Exposure interval = 24 hours; Exposure length = 7 hours (farmers average time applying pesticides per day); Simulation length = 4 days (number of days actively applying fungicides).

Conclusions

§ This study sheds light on potential biomarkers of effects related to pesticide exposure.

§ Risk assessment for epoxiconazole reveals a potential health risk for both men and women in the exposed group, particularly under the worst-case scenario marked by the highest urinary triazole values, indicative of more intense exposure.

§ HTS data confirmed bioactivities linked to metabolic enzyme pathways, oxidative stress, and cell viability process associated with fungicide exposure.

§ Notably, HTS identified perturbations with androgen, progesterone and estrogen metabolic processes, xenobiotic metabolism, and aromatase activity at environmentally relevant concentrations, consistent with human biomarker data.

§ This work emphasizes the critical role of biomonitoring and mechanistic in vitro data in evaluating pesticide exposure and minimizing the adverse health effects of chronic exposure.

References

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