Towards the identification and quantification of candidate metabolites of tebuconazole fungicide

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Interest in identification and quantification of metabolites

- The dissipation of the parent molecule leads to the formation of transformation products (TPs) that may threaten ecosystems and human health
- (Eco-)toxicologically relevant TPs typically emerge only 20 to 30 years after the first pesticide use [Fenner *et al*., 2013]
- More comprehensive analytical methods are needed for the detection of known and unknown TPs to provide better environmental risk assessment and thus prevent from environmental contamination

Materials and Methods

Test substance: Tebuconazole

Tebuconazole (TCZ) belongs to the family of triazole fungicides, effective against various diseases affecting cereals and maize, controls several pathogens in various fruit, nut and vegetable crops (Herrero-Hernández et al., 2011)

Approach: 5 steps

1 – Production of TCZ TPs

- Dissipation of TCZ
- Transformation of TCZ
- Appearance of TCZ TPs

2 – Construction of a library of candidates

- Structure of the molecule
- Literature review
- Suspected reaction
- Metabolic rules

3 – Investigation of unknown TPs

- Analysis by non-target UHPLC-ESI-QTOF MS
- Primary identification by screening MS raw data against TP library (accurate mass, isotope spacing and ratio)
- Selection of compounds with plausible chromatogram peak features and not detected in the controls

4 – Validation of TCZ TPs

Confirmation of unknown metabolites

- Analysis by target UHPLC-ESI-QTOF tandem MS
- Molecular structure correlation through a MSC (Molecular Structure Correlator) program

Confirmation of known TPs (available/synthesizable TPs)

- Quantification against standard curve (GC/LC-MS)

5 – Categorization of TCZ TPs

QSAR-TyPol in silico approach

- Creation of clusters of molecules using 40 molecular descriptors (i.e.: molecular weight, number of atoms, dipole moment, polarizability, total energy,…)
- Estimation of environmental parameters by analogy to reference compounds

Results

Results – 1

Detection and quantification of candidates based on literature

• Triazole	Traces (LC-MS/MS)
• Triazolyl acetate	Traces (LC-MS/MS)
• p-chlorophenol	Not detected (GC-MS/MS)
• p-chlorobenzoic acid	Not detected (GC-MS/MS)

Detection and quantification of candidate based on empirically observed peaks

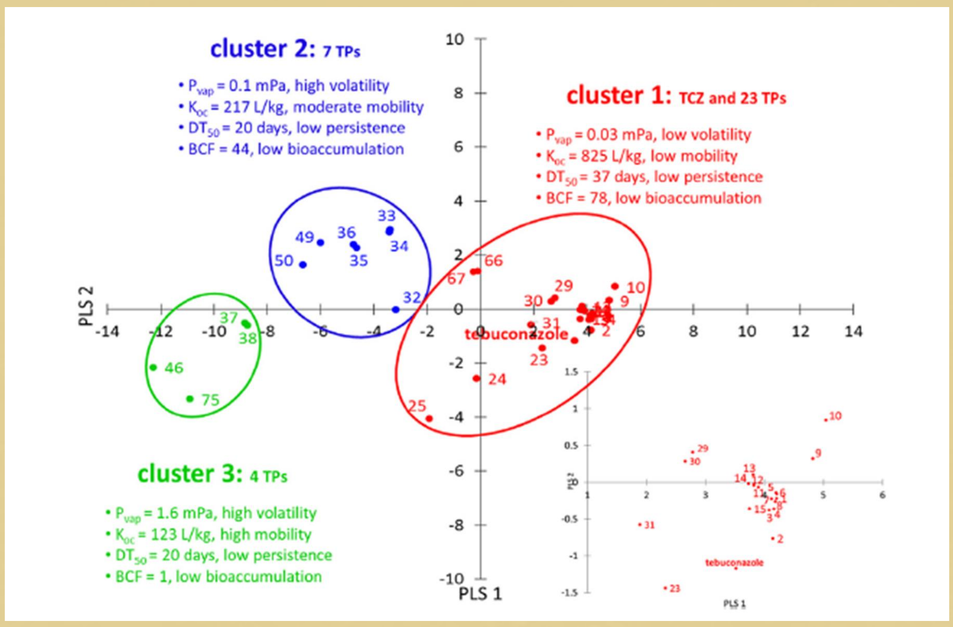
- Ethanone, 1-(4-chlorophenyl) Detected and quantified (GC-MS/MS)

Results – 2

- A library containing 47 empirical and 29 theoretical TCZ TPs was constructed
- QTOF-MS analyses led to detection of 22 empirical and 12 theoretical TPs which were in the library
- 12 TPs passed all identification steps (non-target MS, target tandem MS and MSC)
- 3 TPs passed only the first two steps (non-target MS and target tandem MS)
- 19 TPs were only detected by non target MS

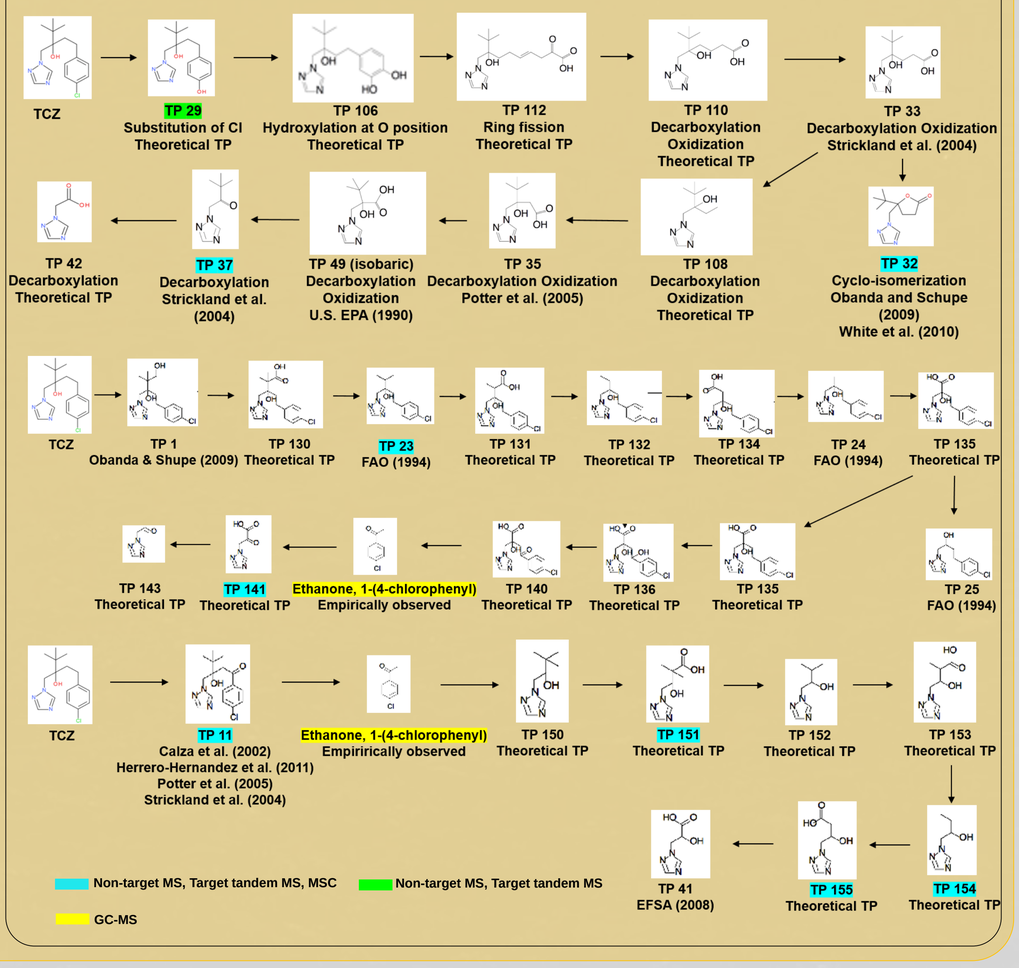
Results – 3

TCZ and 34 TPs were categorized into three different clusters using QSAR - TyPol according to their molecular descriptors including molecular weight, number of atoms, dipole moment, polarizability or total energy (Stork et al. 2016).



Results – 4

Using all the data acquired, 3 possible pathways could be designed



Conclusions

- This approach shows the opportunity we have to be more pertinent and complete in the evaluation of environmental impact of pesticides
- This approach gives a first assessment of the environmental fate of TCZ TPs
- Identification of TPs and prediction of toxicity can be performed as routine in equipped laboratory
- Quantification of TPs and evaluation of their fate will be achieved only after they will be synthesized
- Screening of high number of molecules is possible
- This approach can be extended to other organic pollutants

References

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