Towards the identification and quantification of candidate metabolites of tebuconazole fungicide

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)

Results

Results – 1
Detection and quantification of candidates based on literature
- Triazole
- Triazyl acetate
- p-chlorophenol
- p-chlorobenzonic acid

Detection and quantification of candidate based on empirically observed peaks
- Ethane, 1-(4-chlorophenyl): Detected and quantified (GC-MS/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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