Biotransformation of azole fungicides in the aquatic invertebrate *Gammarus pulex*

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Biotransformation by enzymes in organisms can greatly modify internal concentrations of xenobiotics and influences their toxicity potential. Thus for reliable risk assessments, there is a need to understand the mechanisms responsible for xenobiotic biotransformation. Yet, so far limited data exists about biotransformation of xenobiotics in non-target organisms. Azole compounds - including triazoles and imidazoles - are an important class of fungicides, that are used in both, agriculture and pharmacology and they are frequently detected in surface waters. The freshwater invertebrate *Gammarus pulex* (*G .pulex)* was chosen as test organism due to its great importance in aquatic food webs.
In the present study biotransformation of azole fungicides has been investigated for the first time in *G. pulex*. In addition to the identification of biotransformation products (BTPs), we were interested in how biotransformation mechanisms are linked to the molecular structure, i.e. if the structural similarity of different azole fungicides leads to related biotransformation reactions in *G. pulex.* To this end, organisms were exposed to five triazole (cyproconazole, epoxiconazole, fluconazole, propiconazole, tebuconazole) and two imidazole fungicides (ketoconazole, prochloraz) at concentrations of 200 µg L^{-1} for 24 h. Chemical analysis was performed using automated on-line solid phase extraction coupled to liquid chromatography high resolution tandem mass spectrometry. Identification of suspected BTPs was carried out by isotopic pattern analysis and the interpretation of MS/MS spectra.
In total 21 BTPs were identified, 1-6 per compound. Overall, the measured BTP concentrations inside the organism were low compared to the internal concentrations of the parent compound. For fluconazole, which differs from the other compounds in terms of its much higher polarity, no evidence for biotransformation was found at all. In most cases biotransformation resulted in more polar compounds. BTPs were mainly formed through oxidation and conjugation reactions, as well as in combination of the two. The most common reaction was aliphatic or azole ring hydroxylation. The largest number of BTPs, including sulfate conjugates and cysteine products, was observed for the parent compounds epoxiconazole and prochloraz, which showed the highest bioaccumulation. For these two compounds and their BTPs kinetic rates of uptake, biotransformation and elimination were determined based on a time-course experiment.
Our results indicate that imidazole and triazole based compounds behaved differently in terms of reactions occurring at the azole functional moiety. Cleavage of the azole functional moiety was only observed for the imidazole compounds. Once the azole ring is broken, the ability to act as fungicide is compromised. Yet, if the azole moiety remains intact, the fungicidal activity may be conserved.