Abstract

Traditional toxicity tests in the field of ecotoxicology may suffer from insufficient sensitivity limited by the concentration range they are covering and do provide limited insight in the changes that occur in cells or organisms upon exposure to chemical stressors. Therefore, there is a need for new concepts for toxicity testing to improve environmental risk assessment. Metabolomics is an excellent tool since it can mimic realistic environmental exposure scenarios. In addition, since metabolites are often highly conserved across different species, predictions can be made with regard to interspecies shared endpoints. To evaluate pesticide-induced toxicity the acetylcholine esterase (AChE) inhibition assay is commonly applied. The use of this assay addressing only a single endpoint is, however, restricted to carbamates and organophosphate pesticides, which induce a direct enzyme inhibition. Among pesticides, the neonicotinoids have a different mode of action for which it is not available a bioassay. Therefore a testing strategy is needed to study the toxic effects of these compounds in detail.

In this thesis, a new analytical strategy based on interaction hydrophilic liquid chromatography (HILIC) and mass spectrometry (MS) was developed and validated to quantify the main neurotransmitters, their precursors and their metabolites in order to study the neuronal metabolism disruption. In exposed L. stagnalis species and zebrafish larvae, the neuronal metabolism was perturbed at concentration levels which did not show effects in the traditional toxicity testing. In parallel to the targeted approach a cross-platform metabolomic strategy based on the application of complementary analytical techniques was applied in order not to overlook other metabolic pathways that might be altered by toxicant exposure. Therefore, to further increase metabolome coverage different modes of liquid chromatography (LC) with electrospray ionization (ESI) and gas chromatography with atmospheric pressure chemical ionization (GC-APCI) were coupled to high resolution Time of flight mass spectrometry (ToF MS). In Chapter 5, the effects of the neonicotinoid imidacloprid on the CNS of the invertebrate species L. stagnalis were studied using metabolomics. In this study, the snails were exposed to environmentally relevant concentrations of imidacloprid. Metabolomics was more sensitive than traditional toxicity testing and revealed differences between control and exposed groups. The levels of many identified metabolites were found to be significantly altered and several pathways were significantly affected by realistic environmental exposures to imidacloprid. Results indicated that imidacloprid may cause inflammation and neuron cell injury. Moreover, since an enhancement of the ratio between choline and acetylcholine was observed, a possible increase in cholinergic gene expression was suggested as adaptive response to the binding of imidacloprid to the nAChRs. The integration of the combined targeted and non-targeted metabolomics strategy in an effect directed analysis (EDA) study was explored in Chapter 6. The toxicological effects of surface water extracts were related to its most abundant contaminants by EDA, using the AChE bioassay and metabolomics on the CNS of L. stagnalis. The neutral extract disturbed more metabolic pathways than the three most abundant chemicals, indicating the contribution of other chemicals. The use of metabolomics for the evaluation of toxicological responses in organisms exposed to environmental extracts is a very promising approach since it showed to be more sensitive compared to traditional toxicity testing and able to focus on multiple endpoints simultaneously.