THE ECO‘N’OMICs PROJECT - AN OMICs-BASED SCREENING APPROACH FOR ECOTOXICOLOGICAL RISK PREDICTION

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BACKGROUND
After uptake by environmental organisms ecotoxic compounds induce molecular events, which initiate a cascade of processes across different organizational layers finally resulting in adverse effects at the level of the organism and the population. As an early response to such molecular initiating events, the organism reacts by highly specific changes in gene expression and cell metabolism. That way, the active substance of a pharmaceutical with a given MoA can for example induce gene expression changes in aquatic organisms. This can lead to liver toxicity, neurotoxicity or a reduced reproductive capacity at the organism level and therefore may affect the population. Hence, highly specific changes in gene expression precede every specific adverse environmental effect. These changes can be assessed in order to obtain an early prediction of ecotoxicity. Novel molecular biology and bioinformatics methods, referred to as OMICs analyses, allow for a sensitive and global detection of gene expression changes in a wide range of organisms. Only recently have OMICs started to be used for the identification of environmental side effects of pesticides, biocides and pharmaceuticals, consequently establishing the novel field of “ecotoxicogenomics”. This development opens up new avenues for the detection of early molecular fingerprints and biomarkers in order to identify and differentiate MoAs of ecotoxic substances.

APPROACH
We combine recent transcriptomic and proteomic techniques with ecotoxicological approaches to generate a data base linking substance-specific gene expression signatures with adverse effects on the organism and the population. Therefore, aquatic model organisms such as fish larvae, water flea or algae are exposed to sublethal concentrations of reference substances in an environment based on standard OECD test guidelines. The reference substances are selected to be ecotoxicologically well characterized chemicals with known adverse effects, covering a broad range of MoAs. Our data base covers the substance's MoA, the compound structure, adverse effects on the organism and the population as well as the OMICs-generated molecular fingerprint in aquatic non-target organisms (Figure 1). These data represent the basis for the development of two screening pipelines to rank members of development compound classes based on their ecotoxic potential:
1.) A structure-activity-relationship-based in silico approach to predict MoAs and potential adverse effects
2.) A targeted OMICs approach utilizing the Eco‘n’OMICs molecular fingerprint data base to predict the ecotoxic potential of development compounds.

DATA BASE

FIRST RESULTS

Figure 1: The Eco‘n’OMICs data base links the molecular signature of a substance with its MoA, the compound structure and the adverse effects on the organism and the population.

Figure 2: (A) Test design for the identification of transcriptome changes in D. rerio embryos upon exposure to the aromatase inhibitor fadrozole. (B) Venn diagram of statistically significantly differentially expressed genes (p<0.05) upon exposure to 0.1 µg/L (white), 1 µg/L (grey) and 10 µg/L (black) fadrozole. Hypergeometric distribution p-values for the common subsets are indicated. (C) Scatter plot representation of the gene sets statistically significantly differentially expressed (p<0.05) upon exposure to 1 µg/L (grey) and 10 µg/L (black) fadrozole. Pearson correlation coefficients are indicated for each subset. (D) Concentration response of up-regulated genes of the common subset upon exposure to 1 µg/L and 10 µg/L fadrozole. (E) Concentration response of down-regulated genes of the common subset upon exposure to 1 µg/L and 10 µg/L fadrozole.
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Active substances of pesticides, biocides or pharmaceuticals can display adverse effects in non-target species, which may threaten populations with far-reaching consequences for the ecosystem. Therefore, European legislation requests manufacturers to provide data for environmental risk assessment of active substances in order to become registered. The corresponding OECD tests are time- and cost-consuming and come along with a substantial number of animal tests. Thus, they are performed only in the end of industrial substance development, bearing the risk of a failing registration due to proven adverse environmental effects.

The Eco’n’OMICs project aims at an early ecotoxicological risk prediction for active substance precursors based on substance-induced molecular changes in aquatic model organisms. We combine recent transcriptomics (RNA-Seq) and proteomics (LC-MS/MS) technologies with ecotoxicological approaches to identify gene expression changes induced by a number of ecotoxicologically well characterized reference substances. These substances are selected to be representative for substance classes and to cover a large range of different modes-of-action. The identified substance-specific molecular signatures are linked to a data base with adverse environmental effects and the compound structure. These biomarkers will be used to develop a targeted molecular screening approach for ecotoxicological risk prediction. Together with a structure-based prioritization of substance precursors, the availability of such a molecular screening will enable for a time- and cost-efficient, development of environmentally friendly active substances.