Toxicometabolomics and biotransformation product screening in single zebrafish embryos

Anton Ribbenstedt

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Abstract

Over the last decade environmental agencies worldwide have escalated their work to phase out animal testing for the purposes of chemical regulation. Meanwhile the number of commercially available chemicals and the requirements for hazard assessments have both increased, creating a large need for substitution of traditional in vivo assays with in vitro tests. One example of this is the replacement of the OECD acute toxicity test of adult fish (test guideline [TG] 203) with zebrafish embryos (TG 236). With new insights into the toxicological properties of chemicals, the demand on these replacement tests is also changing character with a shifted focus towards mechanistic understanding of toxicity. The omics sciences encompass a group of analytical methods which have proven to be very powerful for unveiling of mechanistic information of biochemical processes. Metabolomics is one of the younger members of this family and entails the large-scale analysis of endogenous metabolites and their perturbation in living organisms. The overall objective of this thesis was to develop modifications to the TG236 OECD assay to obtain omic data suitable for use in chemical hazard assessment. To achieve this goal, we started by developing a targeted and non-targeted metabolomics workflow and evaluated the performance of the two types of analysis (**Paper I**). We also evaluated the efficiency of three signal drift correction approaches, which is an important step in data quality improvement for non-targeted analysis, and reported previously unlisted biochemicals present in NIST reference material. In **Paper II** we applied the workflow in **Paper I** to a newly developed, in-plate extraction method for single zebrafish embryos which were exposed to the pharmaceutical and environmental pollutant propranolol. Data processing workflows were developed to overcome challenges arising from the occurrence of the exposure compound and its biotransformation products (or in-source fragments of these) in the final multivariate statistical models, obscuring their outputs and prediction capabilities. Once developed, the workflow allowed us to detect several probable modes-of-action of propranolol in zebrafish, and link them to apical endpoints in the embryos, which were then confirmed through thorough literature searches. The final output from the models was ultimately used to determine a benchmarking dose based on metabolomics endpoints for the first time. In **Paper III**, the data processing workflow from **Paper II** was modified to capture propranolol biotransformation products. A total of 7 structures were identified, of which 4 were confirmed with authentic standards, all from the datasets generated in **Paper II**. In **Paper IV** we combined the workflows from **Papers I**, **II** and **III** and applied them to the pharmaceutical carbamazepine, which occurs at high concentrations in wastewater treatment plant effluents. Through this approach we determined several modes-of-action for carbamazepine in zebrafish embryos and measured biotransformation products in both embryos and exposure water. Overall, this thesis demonstrated the possibilities of high-throughput chemical mode-of-action determination in single zebrafish embryos using targeted and non-targeted liquid chromatography mass spectrometry, data filtering scripts and multivariate statistics while simultaneously screening for biotransformation products.

Keywords: *Non-target, REACH, Orbitrap, single embryo, zebrafish embryo, metabolism, high throughput, hydroxylation, glucuronidation, metabolite screening, phase I, phase II, pharmaceutical, structure elucidation, conjugation, propranolol, beta blocker, Non-targeted, toxicometabolomics, Orbitrap, single embryo, 3R, metabolomics, MoA, mode of action, zebrafish embryo, carbamazepine, biotransformation.*

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Department of Environmental Science

Stockholm University, 106 91 Stockholm