An integrative approach for understanding the adverse outcome pathways in algae

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Introduction

- Every chemical produced in, or imported into the EU in substantial amounts needs to pass chemical risk assessment/toxicity evaluation under EU REACH regulations
- Conventional approaches to environmental risk assessment:
  - low-throughput, high resource & time demand
  - black-box approach to mechanism of toxicity
  - phenotypical endpoint-based toxicity tests
  - conservative utilization of hazard information
- This would yield valuable insights into mechanism-based toxicity tests to update and refine existing assessment approaches and increase confidence in risk assessment

Omics technologies = novel approach to profile molecular changes associated with toxicity (=> adverse outcome pathways)

Methods and framework of experimental design

- Tiered experimental design for toxicological biomarker discovery
  - Phase I: Screening for low-throughput biomarkers and molecular changes
  - Phase II: Higher replicated investigation of biomarkers and molecular changes
  - Phase III: Targeted quantification of key event response indicators
  - Phase IV: Analysis of key event causality, prevalence, and selectivity

Time course analysis

- RNA and metabolite analysis
  - Time-course dynamics of large molecular changes in omics profiles will be investigated initially
  - Gene expression and metabolite levels will be screened with untargeted metabolomics (direct infusion mass spectrometry; DIMS) and transcriptomics (RNASeq)
- High replication to reproduce findings
- Targeted quantification of molecular relationships

Current status of project: Results of method development

- A 24h toxicity testing format was optimised for biomarker discovery, maximal biomass (barplot right), minimal vialisation, pH drift and optimal growth rate
- Chlorobenzene exposure levels (barplot right) for phase-I analysis were selected on grounds of reproducible growth inhibition, significant, effect size; and 3 dose levels to enable establishment of dose-response criteria
- Metabolite dilution requirements for RNA extractions and metabolite analysis via DIMS (PCA score plot right)
- RNA and metabolite analysis
  - Time-points will evaluate large molecular changes associated with induced toxicity will be analyzed with higher replication to reproduce findings robustly

The adverse outcome pathway concept – organizing toxicological understanding

Images adapted from Zones et al. 2015

The environmental model organism Chlamydomonas reinhardtii will be exposed to a range of chemicals with known and unknown mechanism of toxicity

As a proof-of-concept, the industrial chemical mono-chlorobenzene (mechanism of toxicity undefined) will showcase omics-driven a priori biomarker discovery. Later in the project, specifically acting toxicants will be used, e.g. photosynthesis-inhibitors.