1. Optimal experimental design for KE discovery

Chemical analysis, modelling and preliminary experimental data used to determine optimal exposure conditions for omics investigations

**Multi-omics investigations**

- Transcriptome sequencing
- Metabolome/ lipidome analysis

**Adverse outcome measures**

- Survival, fitness, growth
- Reproduction

**Measure the association of molecular and phenotypic changes**

Model test organisms: *Daphnia magna* and *Chlamydomonas reinhardtii*

Utilising organisms that span two trophic levels will enhance the predictive capability of the discovered KE(s) both within and across species

2. Quantification of KEs and prediction of AOs

Following KE discovery, targeted studies used to quantify metabolites or genes comprising molecular KEs as a function of AO

**AOP Framework**

- Environmental contamination
- Genes/Metabolite differential analysis
- Genes/metabolite correlation network analysis
- Metabolic active module identification

**Integrating omics into Environmental Risk Assessment**

E.g. The metabolic profile of *D. magna* exposed to cadmium is highly associated with a decrease in reproductive fitness. Modeling the metabolomic data against the reproductive data produces a model that is highly predictive of reproductive dysfunction (unpublished data)

**Multi-omics integration and network analysis**

- Analysis of gene expression and metabolomics datasets to identify differentially expressed genes and metabolites as biomarkers of toxicity.
- Network models will be constructed based on the omics datasets and prior biological knowledge.
- Subsequent analysis of the network models will identify subnetworks of connected genes or metabolic reactions that are perturbed by toxicity.

3. Sequence alignment and phylotoxicoLOGY

**For AOP development, chemical selection is prioritised using those toxicants which have targets conserved across many species**

**Omic approaches will aid in discovering molecular key events that are predictive of observed adverse outcomes**

**The genes/metabolites involved in the discovered KE can be mapped onto phylogenetic trees to infer their origins and functional conservation among related species**

**Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS): Sequence based approach to predict relative intrinsic susceptibility**

4. Computational analysis

- Build computational models that predict change in phenotype (AO) based on molecular KEs

**Example of a sulphated lipid: C_{40}H_{72}O_8S**

- Predictive network(s) of molecular or phenotypic events
- The association of molecular and phenotypic effects. This can help accelerate the discovery of more effective and relevant molecular signatures, regarded as molecular key events (KEs). Within AOPs, KEs are toxicological events that are predictive of adverse outcomes

- Omics technologies offer new possibilities for assessing the molecular and biochemical responses of organisms to chemicals, and subsequently, the discovery of KEs

- Complex modelling techniques are able to select combinations of molecular events, linked phenotypic responses that are predictive of adverse outcomes

- Once identified the KEs can be mapped onto phylogenetic trees to infer their origins and functional conservation among related species

- Here we propose to use omics as part of an integrated (weight of evidence) approach in combination with available in silico and in vitro data to support the categorisation of chemicals by their key events

**Rationale**

- Adverse Outcome Pathways (AOPs) utilise the identification of potential pathways underlying harmful environmental effects. This can help accelerate the discovery of more effective and relevant molecular signatures, regarded as molecular key events (KEs). Within AOPs, KEs are toxicological events that are predictive of adverse outcomes

- Omics technologies offer new possibilities for assessing the molecular and biochemical responses of organisms to chemicals, and subsequently, the discovery of KEs

- Complex modelling techniques are able to select combinations of molecular events, linked phenotypic responses that are predictive of adverse outcomes

- Once identified the KEs can be mapped onto phylogenetic trees to infer their origins and functional conservation among related species

- Here we propose to use omics as part of an integrated (weight of evidence) approach in combination with available in silico and in vitro data to support the categorisation of chemicals by their key events