UNIVERSITY OF BIRMINGHAM

3rd *In Silico* Toxicology Conference 2022

PRECISION TOX

Precision Toxicology

Identifying Molecular Biomarkers of a Chemical Hazard using New Approach Methodologies

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Dr. Xiaojing Li
x.li.12@bham.ac.uk
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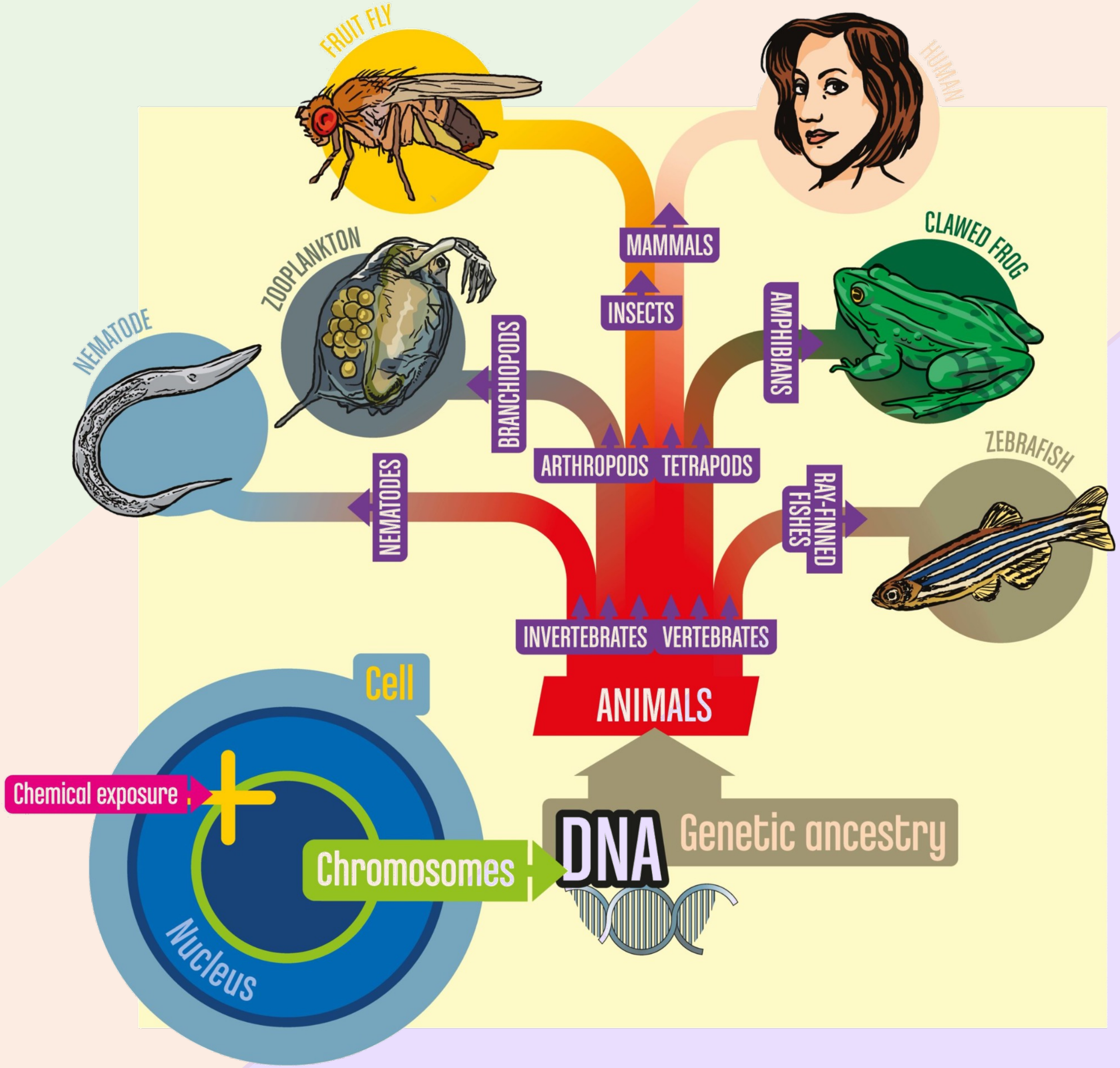
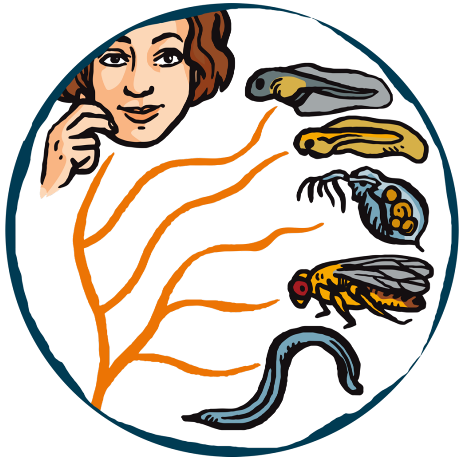
Outline

- Introduction of Precision Toxicology
- Discovering Molecular Biomarkers of Chemical Hazards
- Proof of Concept
- Conclusion



Precision Toxicology

- Aims at **better protecting the health of people and the environment** by establishing new approach methodologies for chemical safety testing
- Three pillars of the project



Phylotoxicology

Variation in Susceptibility

Embedded Translation

- A new regulatory paradigm of detecting toxicity using **molecular biology** for greater certainty at predicting which chemicals cause harm while **avoiding traditional animal testing**.

Replacing mammals by other test species

Non-traditional test species

- ***Daphnia magna* (water flea)**
- *Drosophila melanogaster* (fruit fly)
- *Caenorhabditis elegans* (nematode)
- *Danio rerio* (zebra fish; embryo)
- *Xenopus laevis* (frog; embryo)
- *Homo sapiens* (human; cell-line)

NAMs for chemical toxicity testing

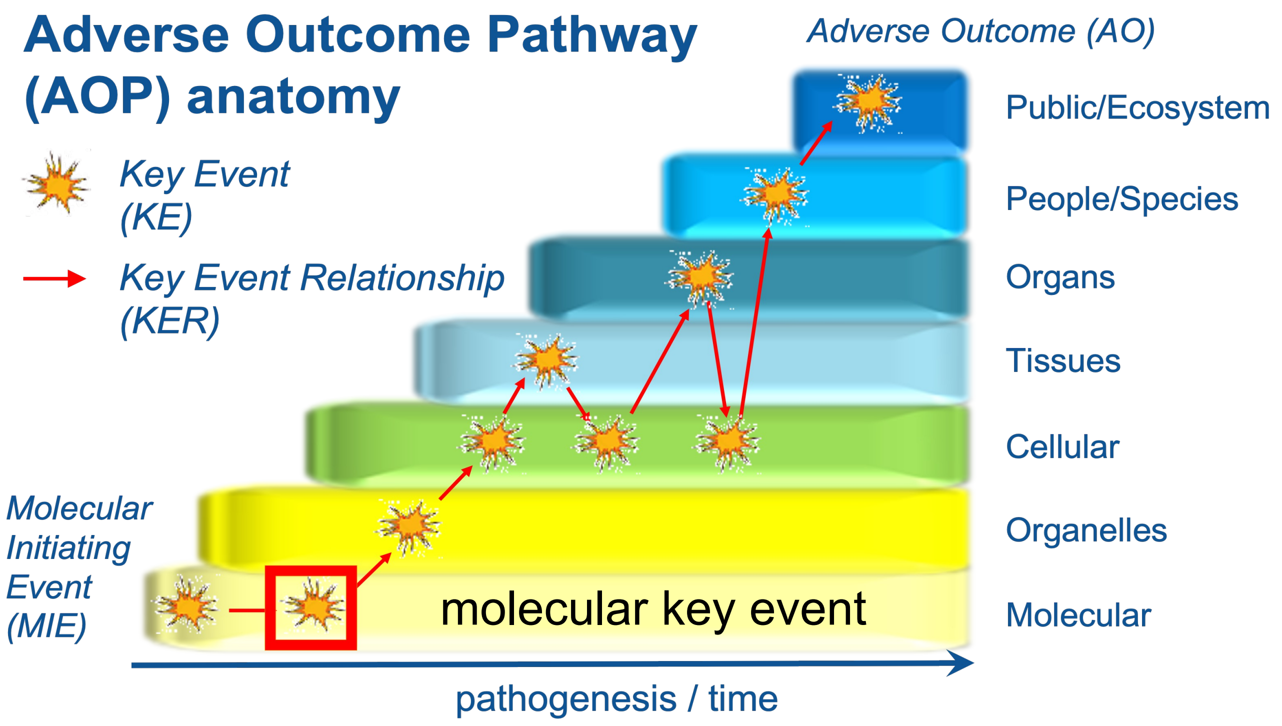
- Computational methods for integrating multi-omics data (transcriptomic and metabolomic)
- Data-driven models for identifying molecular biomarkers

Identifying molecular key event

Omics profiling assists in identification of mKEs

Definition of mKE

- A sparse network of interacting genes and their metabolic products that is a necessary element of the AOP critical to the outcome

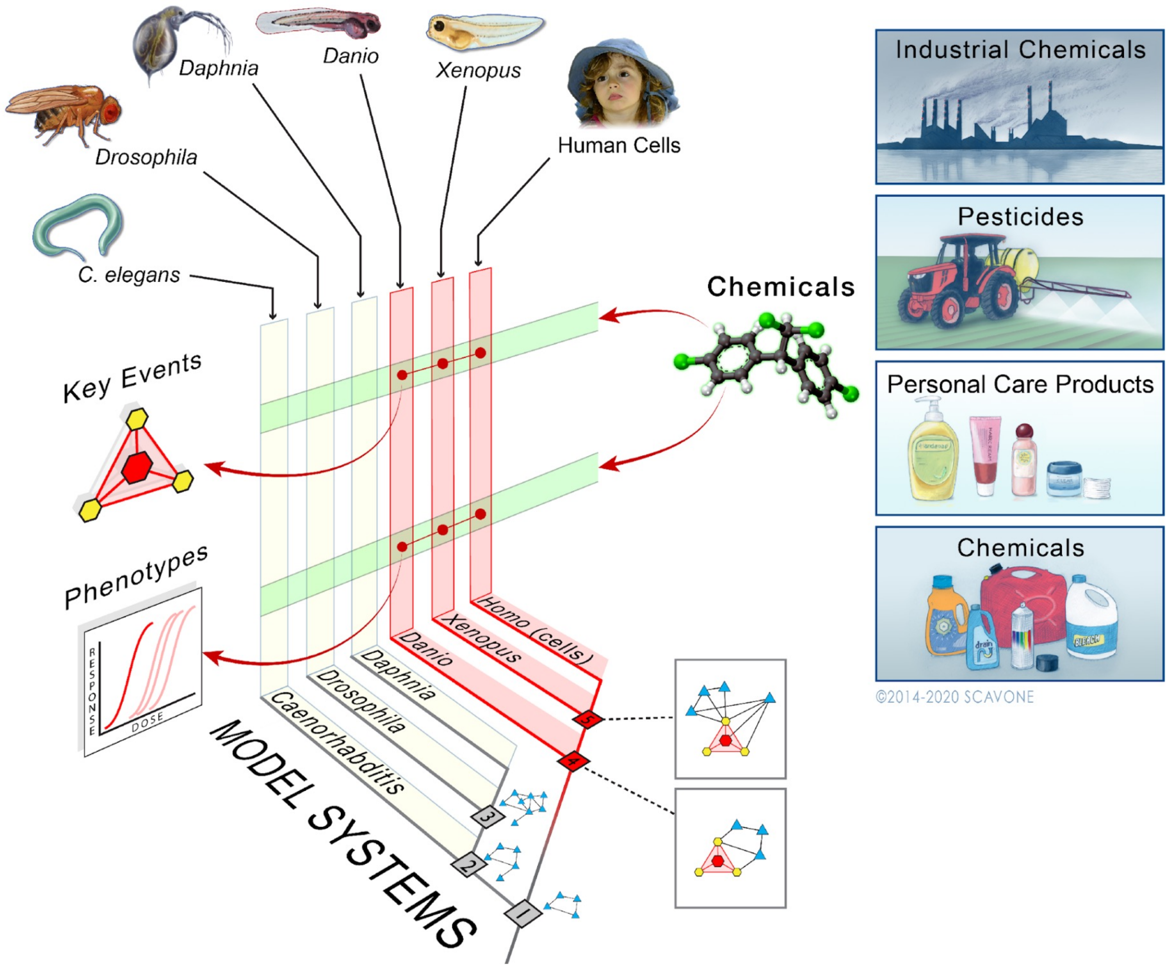


Linking human toxicology and ecotoxicology

Phylotoxicology

- mKE induced by chemicals (chemical responsiveness)
- mKE indicative of similar adversity (hazard relatedness)
- mKE shared by multiple species (evolutionarily conserveness)

→ Molecular biomarkers of a chemical hazard



Discovering molecular biomarker of chemical hazards

- **Step 1** Generating biological profiles
- **Step 2** Constructing biomolecular network
- **Step 3** Association analysis
- **Step 4** Molecular biomarker identification

Step 1: Generating Biological Profiles (multi-omics)

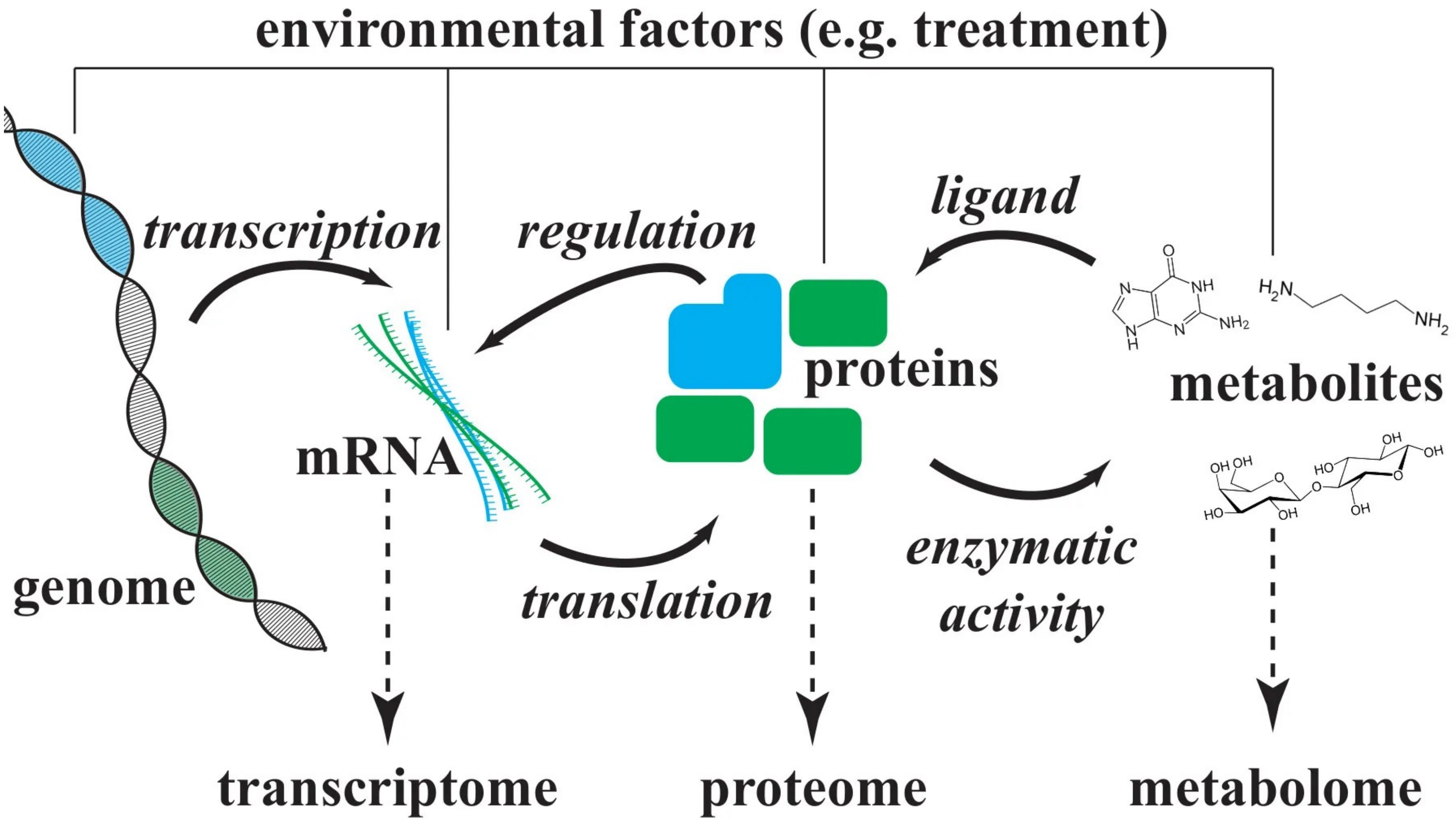
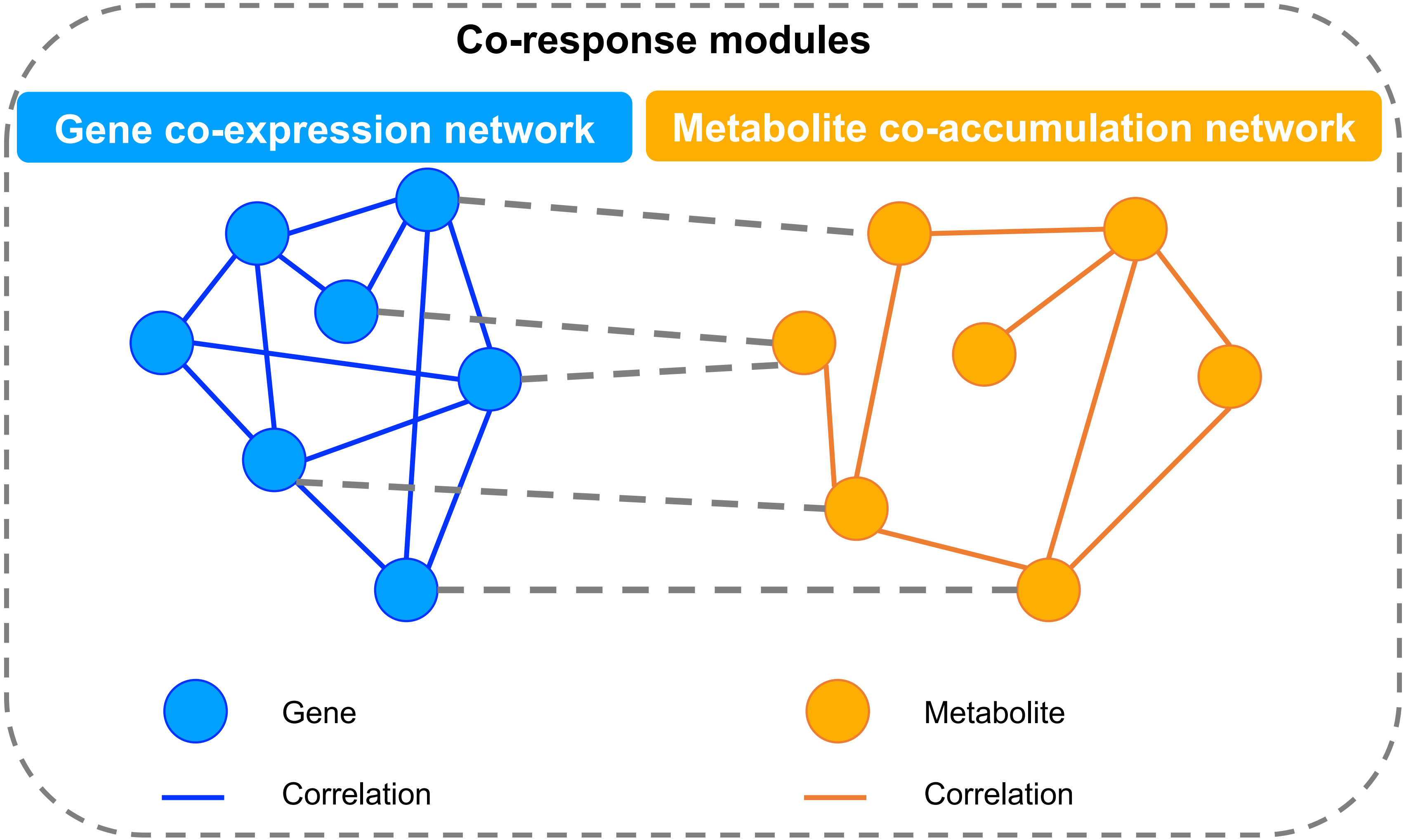


Figure from https://www.blopig.com/blog/2017/03/multiomics-data-analysis/

Step 2: Constructing Biomolecular Network



Step 3: Association analysis

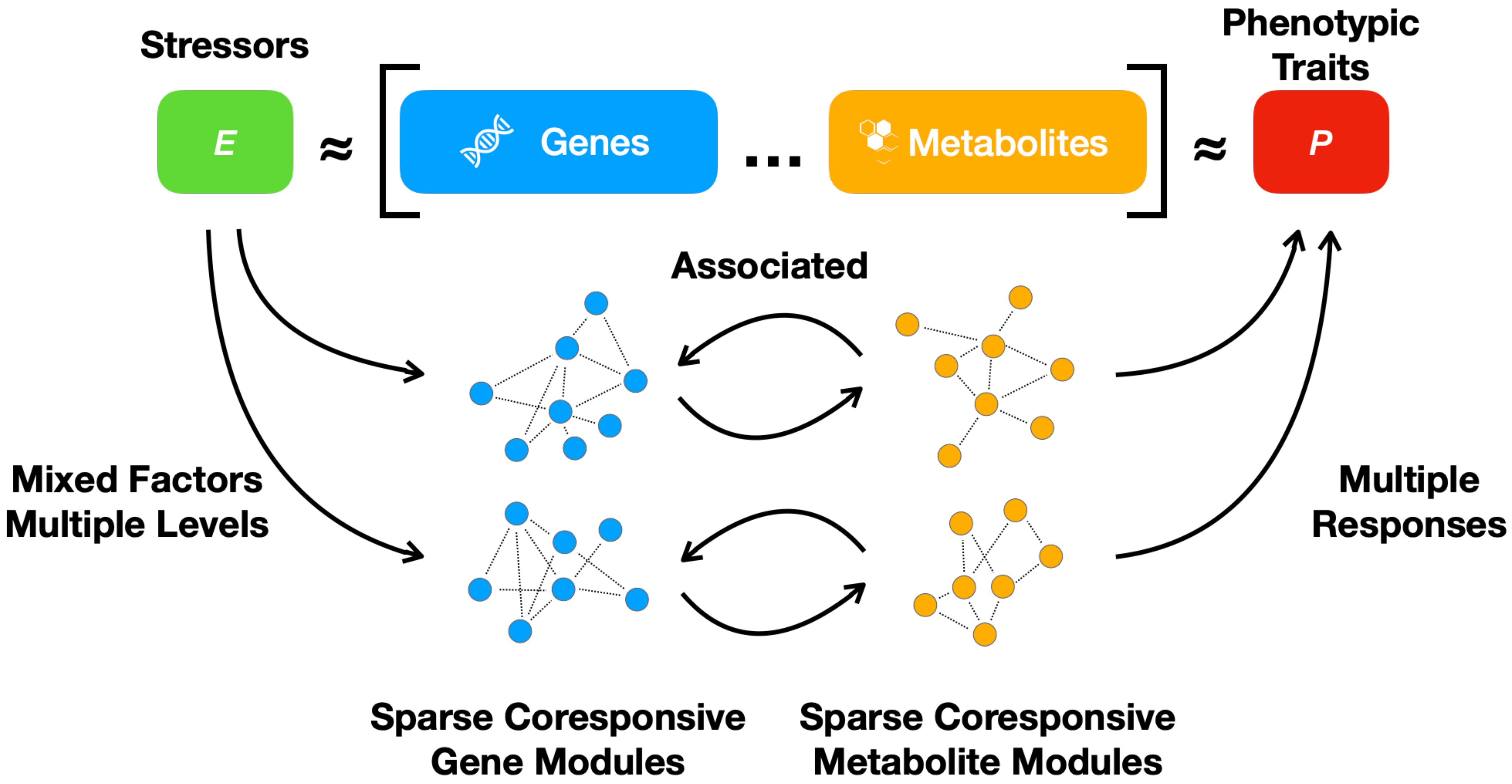
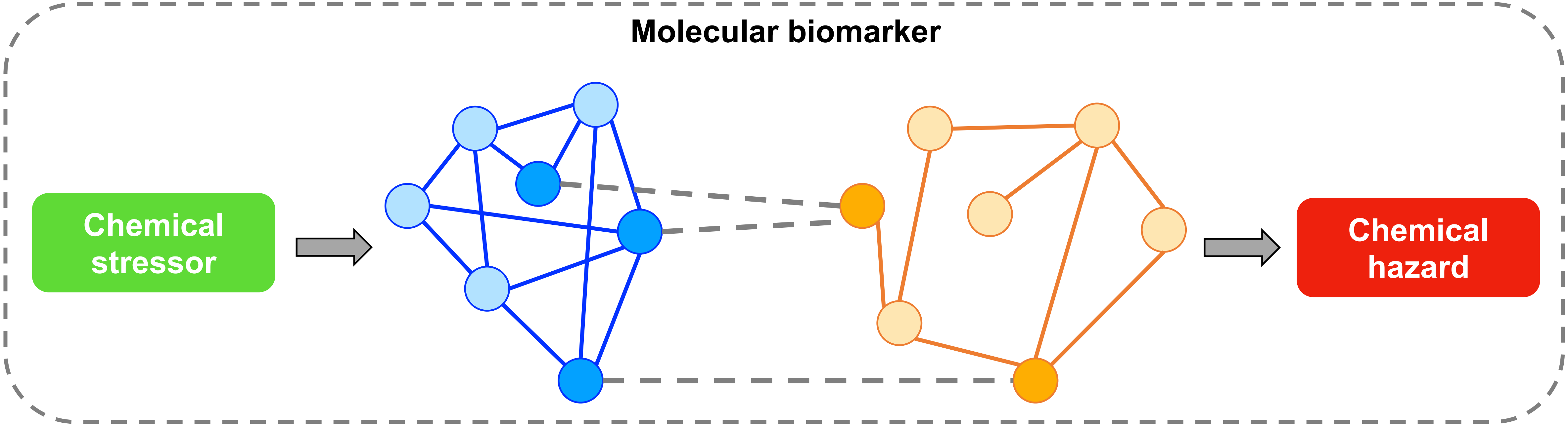


Figure from J Zhou, MOCA, in prep

Step 4: Molecular biomarker identification



Ortholog-based annotation

Classifier construction

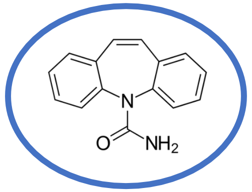
Pathway conservation

Probabilistic modelling

Danube River case

River samples

Danube River



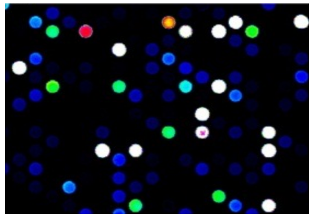
Organic extracts

Exposure

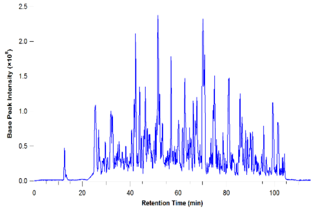
Daphnia magna

(48h exposure)

Bioassay

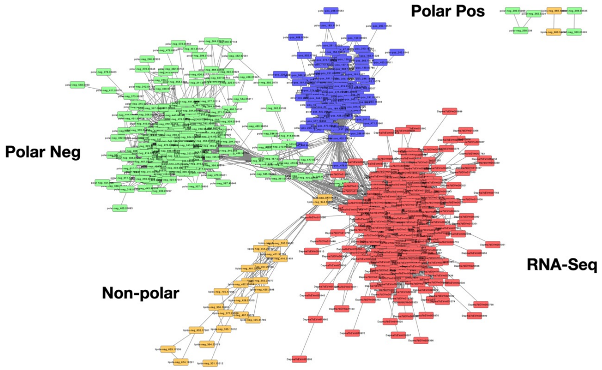


Transcriptome



Metabolome

Analysis



Linking chemicals to multi-omics changes

Water samples

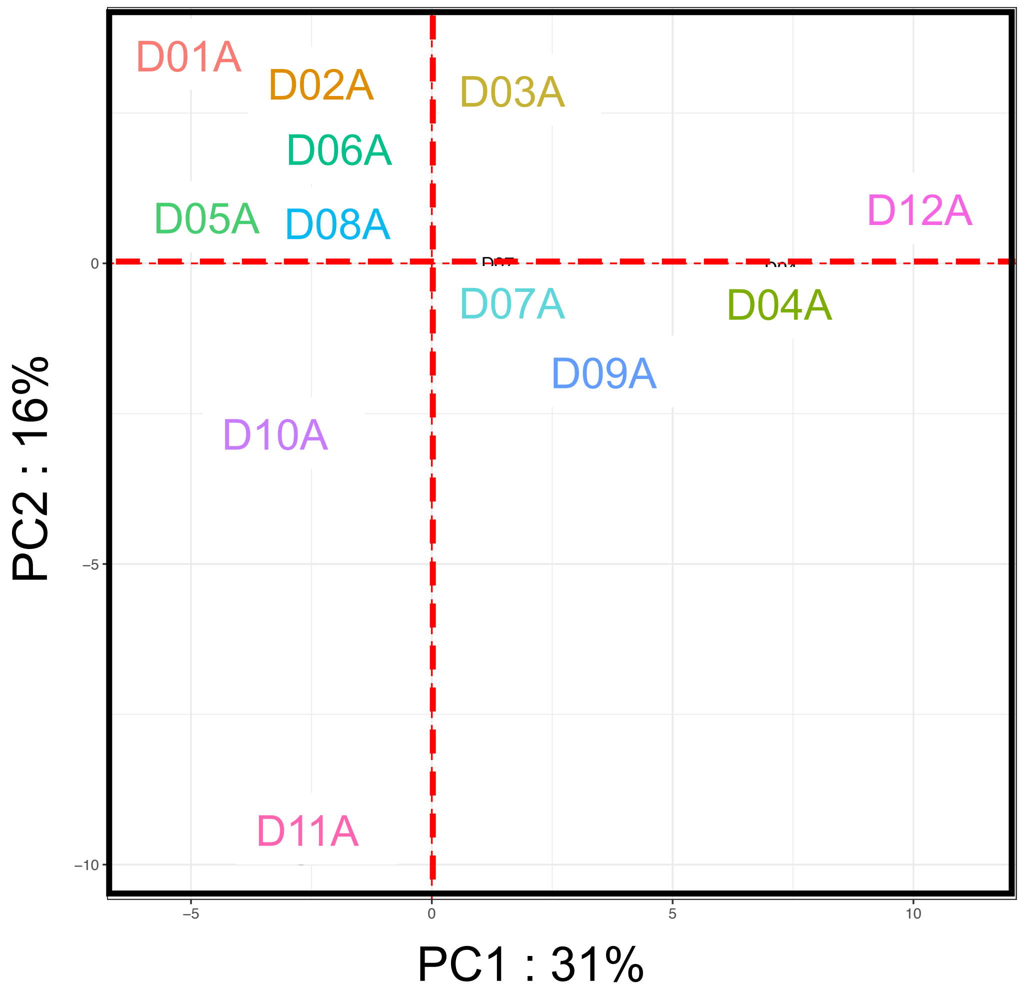
- Water collected from 12 sites in **Danube River**
- Polar organic substances extracted from surface water samples

Chemical targeted screening analysis

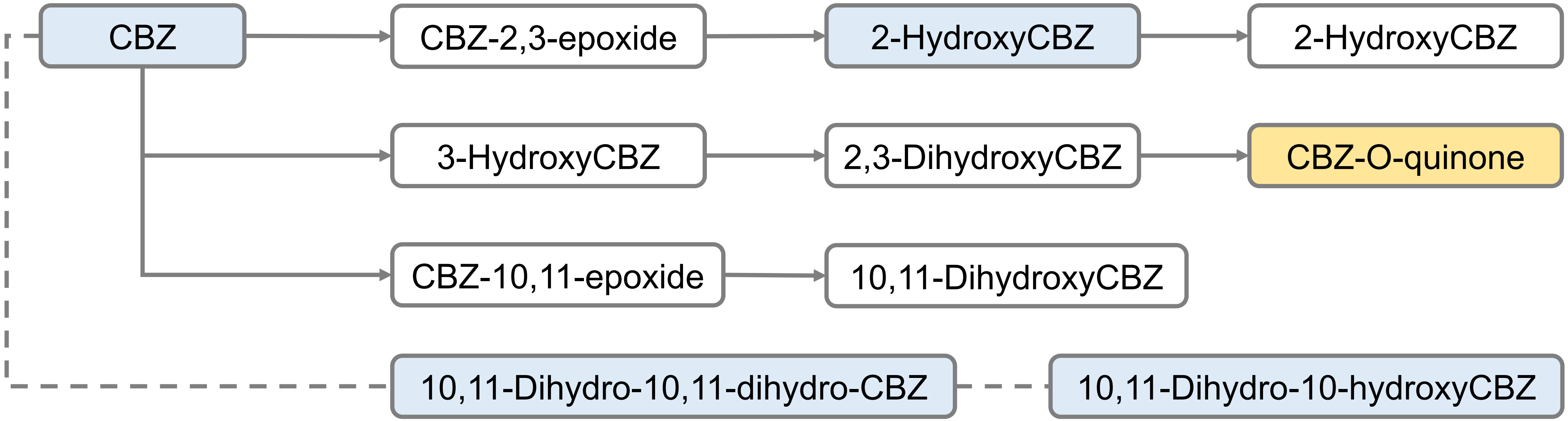
- 457 chemicals with reference
- 91 chemical substances detected and quantified

- Carbamazepine (CBZ) and three transformed products (TPs) are detected in these mixtures
- These four chemicals are widely detected (11 sites) at relatively high concentration (4.9 - 47 μg/L)
- CBZs in site D11: under detection level

PCA plot of Chemical data



Carbamazepine and its transformed products (TPs)

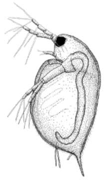


Metabolite — Detected in metabolome

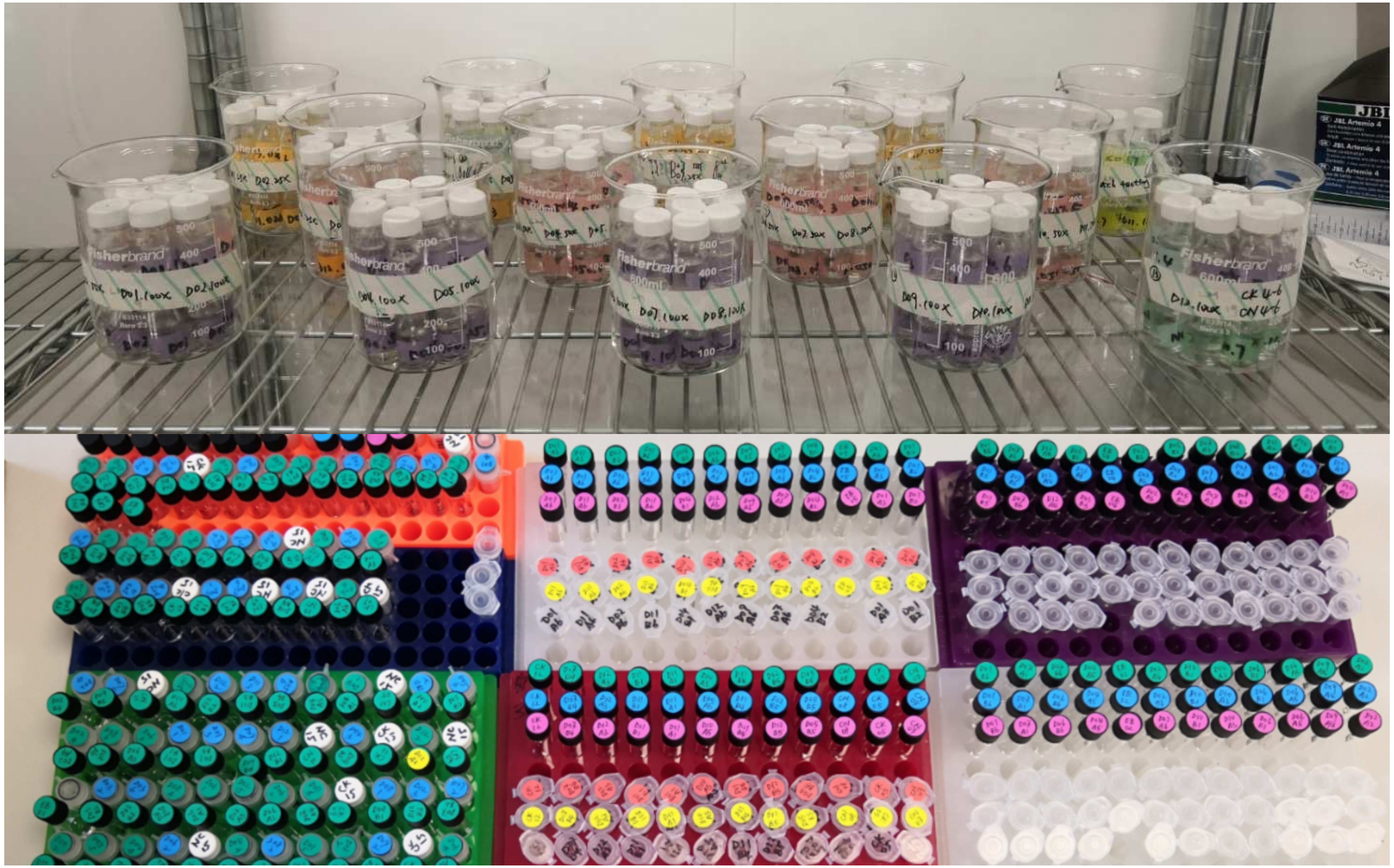
Chemical — Detected in organic mixtures

Figure adapted from KEGG (map00982)

Exposure with *Daphnia magna*



- OECD 202 Acute immobilisation test
- Neonates (< 24 h) were exposed to exposure media for 48 h
- Flash-frozen tissues for multi-omics extraction
- RNAseq for transcriptome
- DIMS for metabolome (Polar metabolites, under positive and negative ion mode)

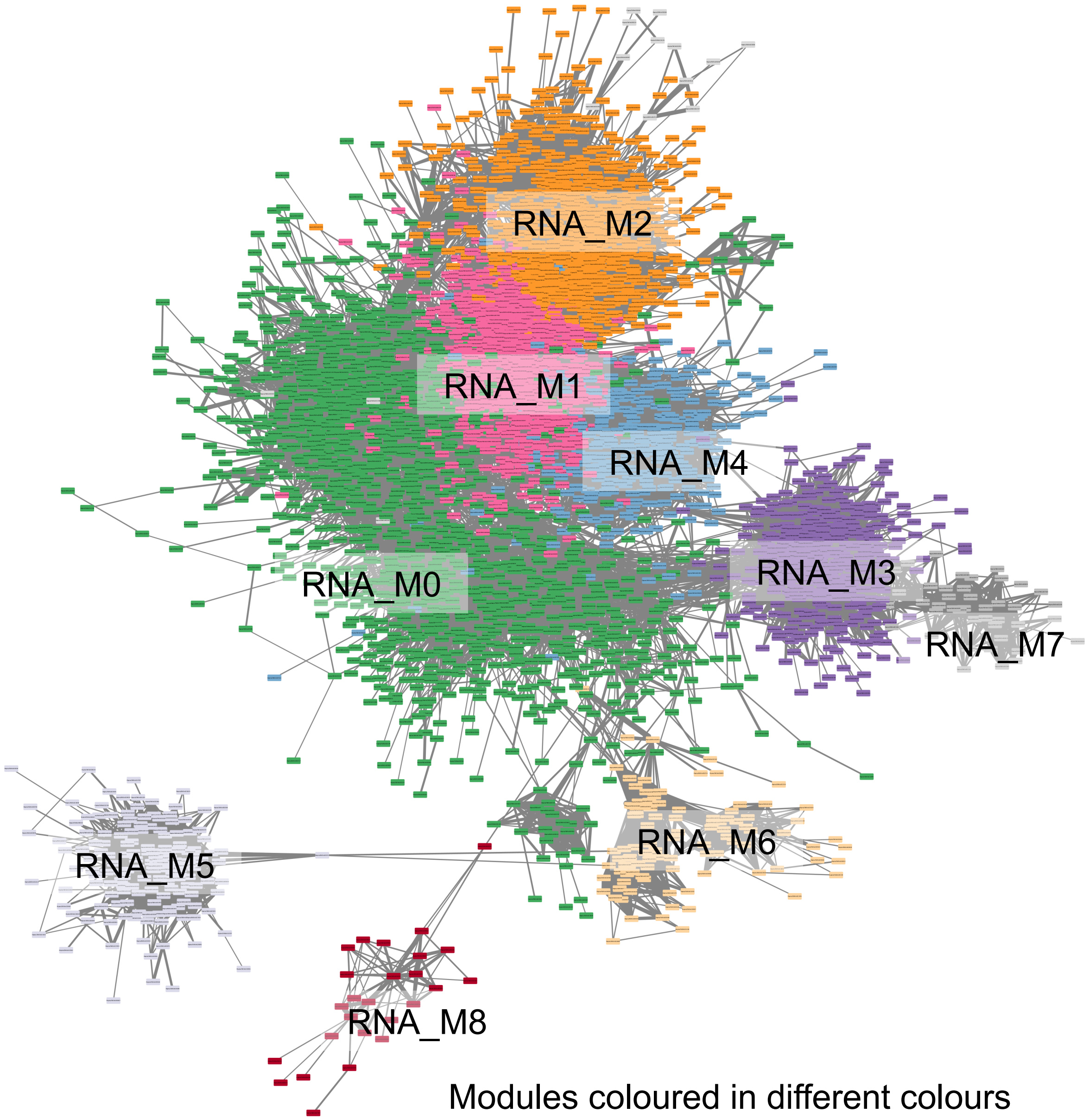


Co-expression network

Summary of co-expression network

Data set	# Feature	# Module	Max size	Min size
RNA	19549	11	1243	20
Polar pos	1285	19	98	10
Polar neg	2331	28	204	11

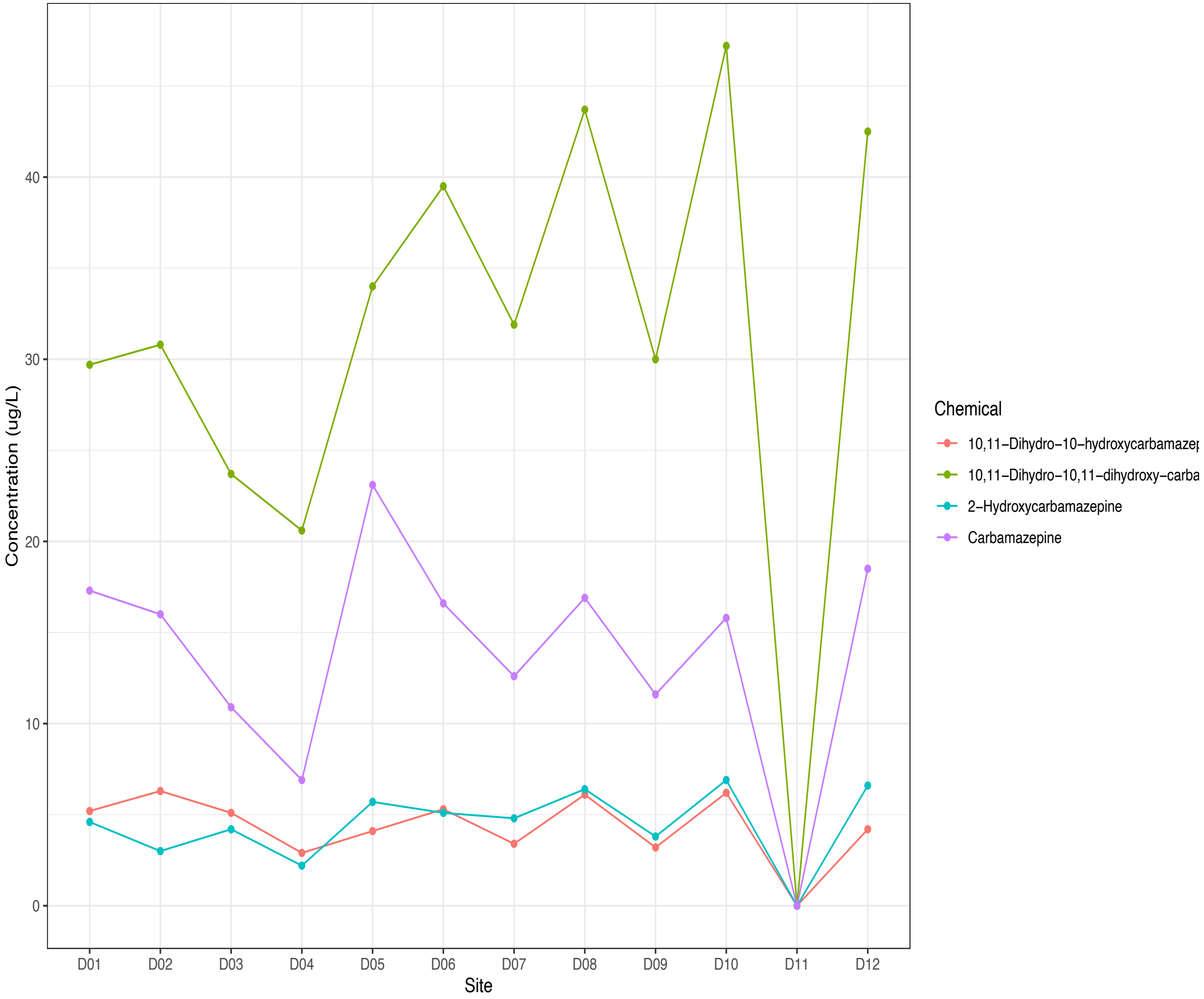
RNA co-expression network



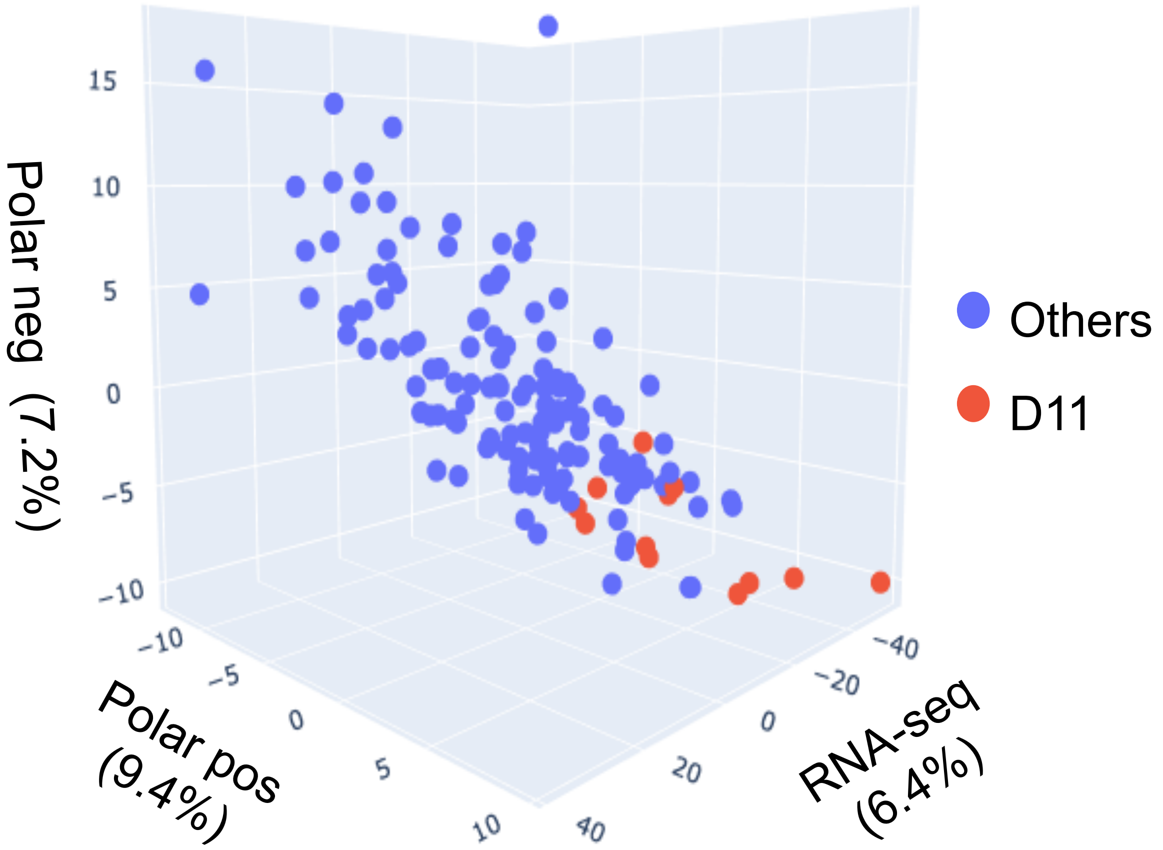
Modules coloured in different colours

Association analysis

CBZ and its TPs distribution



sCCA modelling
(4 chemicals ~ RNA ~ polarpos ~ polarneg)



Modules with significant association with carbamazepine and its TPs

Module ID	FDR q-value	Enriched Pathways
RNA_M1	5.06E-49	Metabolism of xenobiotics by cytochrome P450; Chemical carcinogenesis - DNA adducts; Drug metabolism - cytochrome P450; Nitrogen metabolism; Drug metabolism – other enzymes; PPAR signalling pathway; Glutathione metabolism
RNA_M4	5.79E-05	Chemical carcinogenesis - DNA adducts
RNA_M2	1.35E-43	Metabolism of xenobiotics by cytochrome P450; Chemical carcinogenesis - DNA adducts; Drug metabolism - cytochrome P450; Nitrogen metabolism; Drug metabolism – other enzymes; PPAR signalling pathway; ABC transporter; Chemical carcinogenesis - receptor activation
Polarneg_M10	1.18E-04	CBZ-O-quinone

Pathway conservation

Shared ortholog numbers in selected pathways between *Daphnia magna* and other species

Pathway	***Caenorhabditis elegans***	***Drosophila melanogaster***	***Danio rerio***	***Xenopus laevis***	***Homo sapiens***
Drug metabolism - CYP450 (7)	5 (71 %)	6 (86 %)	6 (86 %)	7 (100 %)	7 (100 %)
Glutathione metabolism (25)	22 (88 %)	21 (84 %)	24 (96 %)	24 (96 %)	24 (96 %)
ABC transporter (21)	12 (57 %)	16 (76 %)	20 (95 %)	20 (95 %)	21 (100 %)

Chemical set

- 10,11-Dihydro-10,11-dihydroxy-carbanazepine
- 10,11-Dihydro-10-hydroxycarbamazepine
- Carbamazepine
- 2-Hydroxycarbamazepine

↔ Association

Co-response modules

- RNA_M1
- RNA_M4
- RNA_M2
- RNA_M6
- Polarpos_M12
- Polarneg_M1
- Polarneg_M10
- Polarneg_M18
- Polarneg_M11
- Polarneg_M13

↔ Interpretation

Pathway profile

- Metabolism of xenobiotics by cytochrome P450
- Chemical carcinogenesis – DNA adducts
- Drug metabolism – cytochrome P450
- Nitrogen metabolism
- Drug metabolism –other enzymes
- PPAR signalling pathway
- ABC transporter
- Chemical carcinogenesis –receptor activation
- Glutathione metabolism

Conclusion

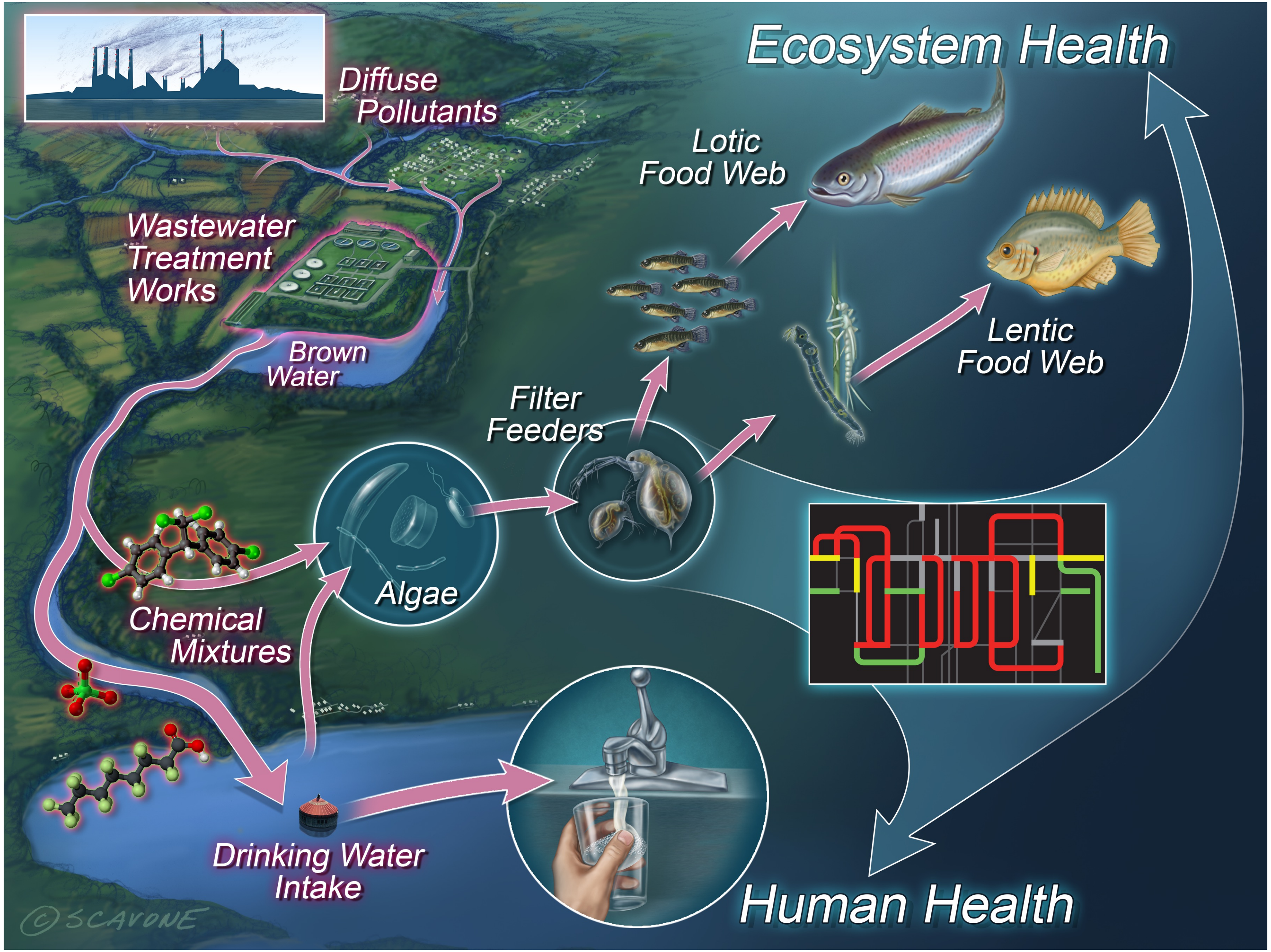
- The **molecular biomarkers** that are associated with carbamazepine and its transformed products (CBZs), are significantly enriched in the known carbamazepine metabolic pathways (e.g. drug metabolism by cytochrome P450), suggesting the modelling strategy can pull out informative molecular biomarker that are co-responsive to the chemical stressor.

- Pathway conservation analysis shows the orthologs (KO terms) in the responsive pathways in *Daphnia* are **highly shared across species**, suggesting similar exposure responses may be expected in other species.

- PrecisionTox may provide **experimental evidences** to support whether the responsive biomarkers found from different model species are functionally conserved.

Toxicity by Descent

An approach for improving the protection of human health and the environment by assessing substances' modes of action on conserved biomolecular response pathways to toxicity.



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Thank you for your attention !

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