Transcriptomic profiles of a respiratory inhibitor and growth targeting insecticide reveal links to impaired bone mineralization and lipid homeostasis in zebrafish embryos

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OMICs in Ecotoxicology – a potential game changer

Classical Toxicology

When is it toxic?

Transition to understanding

Predictive Toxicology

Why is it toxic?

OECD 202, 203, 221, 236, …

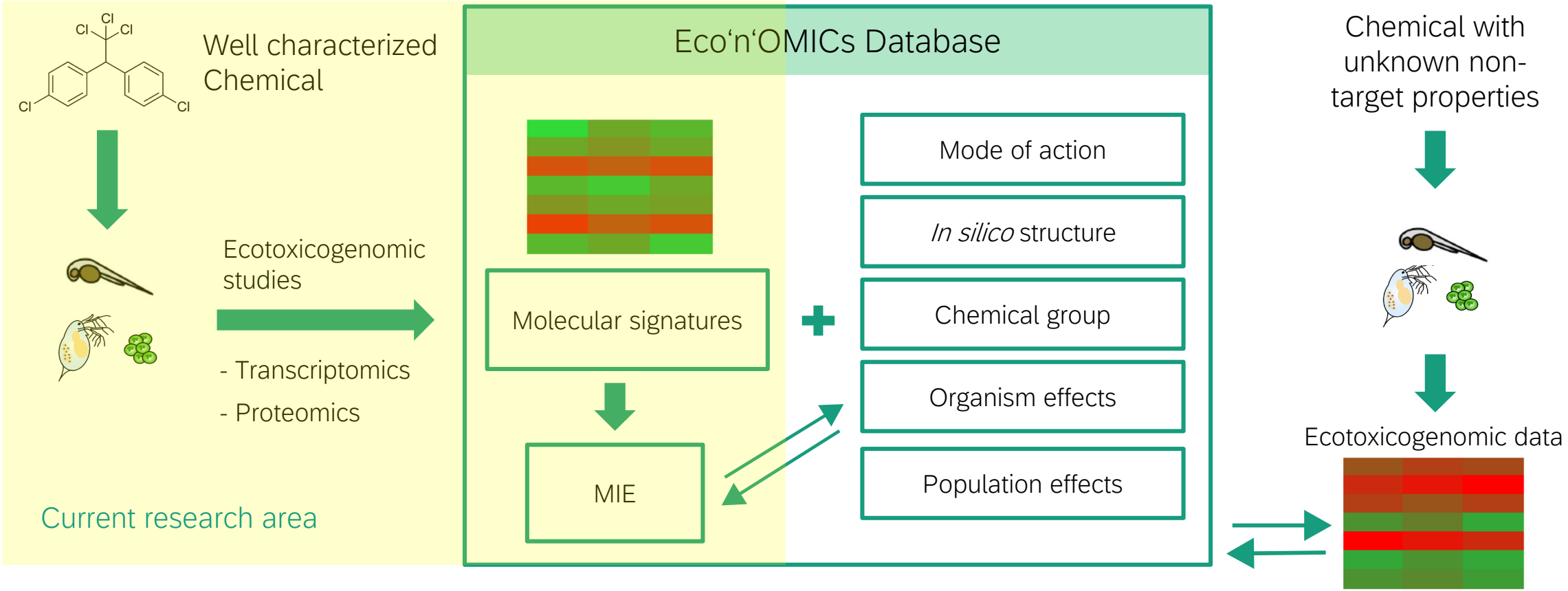
- Non-human animal tests
- Time & cost intensive
- Not able to detect hazardous and regulatory relevant toxic MoAs (e.g. EDCs)

Requires qualitative toxicological data

OMICs enable scientists to assess the responses of tens of thousands of genes and their products from a single sample.

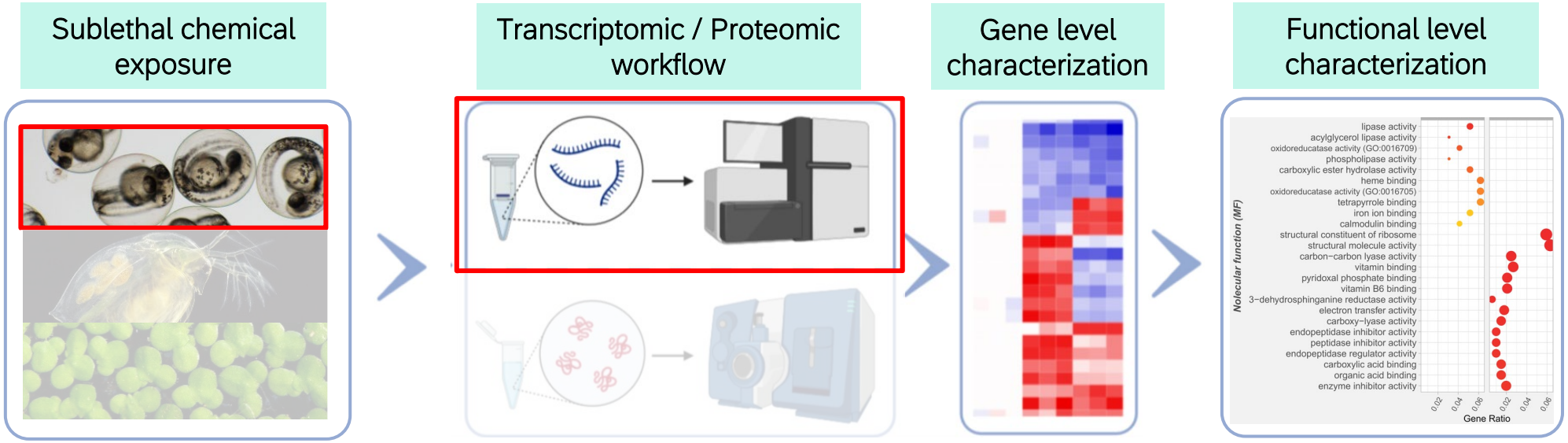
(Brockmeier et al 2017, Bencic 2015)

Our vision - the *Eco'n'OMICs* Database

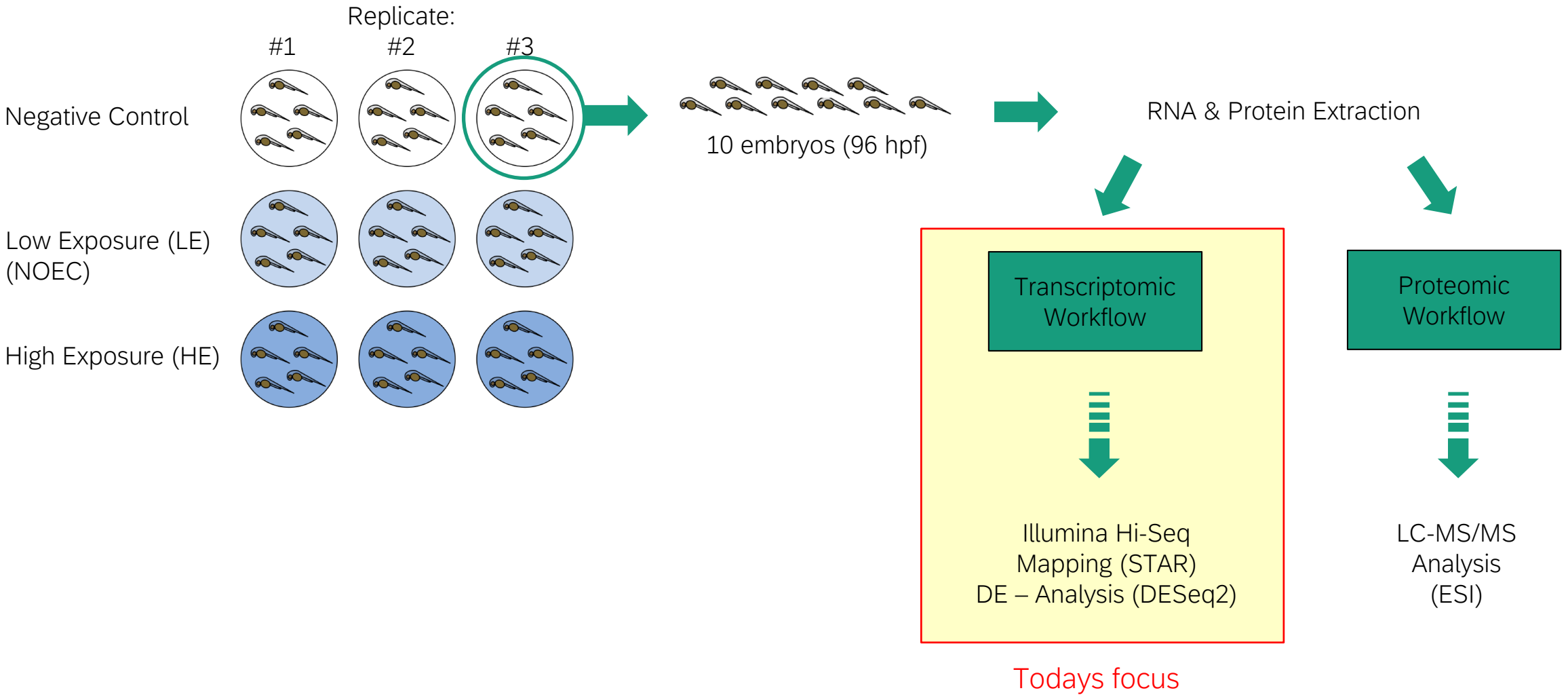


Overall research goal

- Combining non-target OMIC methods with standardized test guidelines (**OECD 236,** 221, 202) to screen for potential biomarkers, characteristic for specific MoAs.
- Identification of early affected key pathways associated with the respective MoA.
- Providing high content ecotoxicogenomic data for future data mining and meta-studies. (Also public for the scientific community on ArrayExpress)



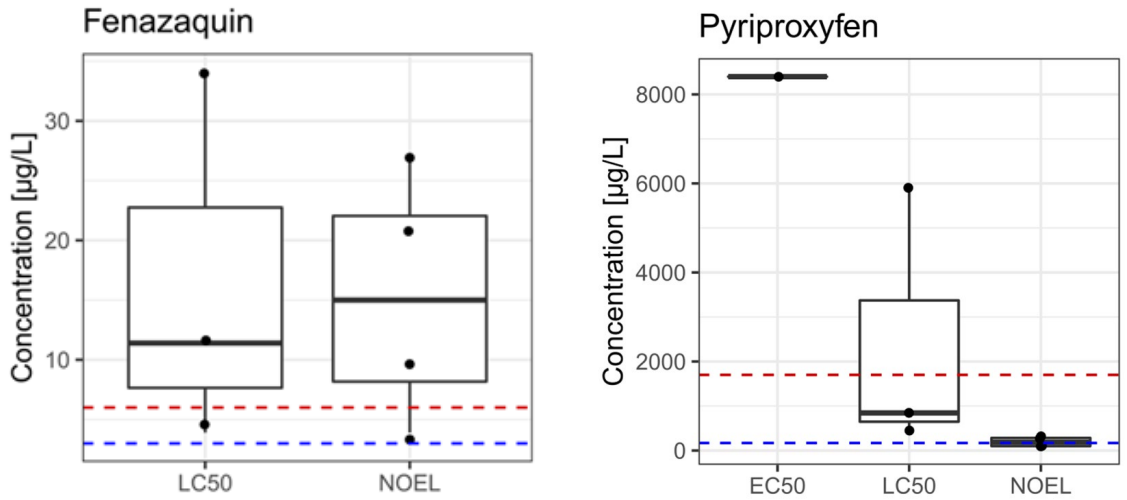
Ecotoxicogenomic fin(ger)-printing workflow setup



Ecotoxicogenomically evaluated insecticides in zebrafish embryos

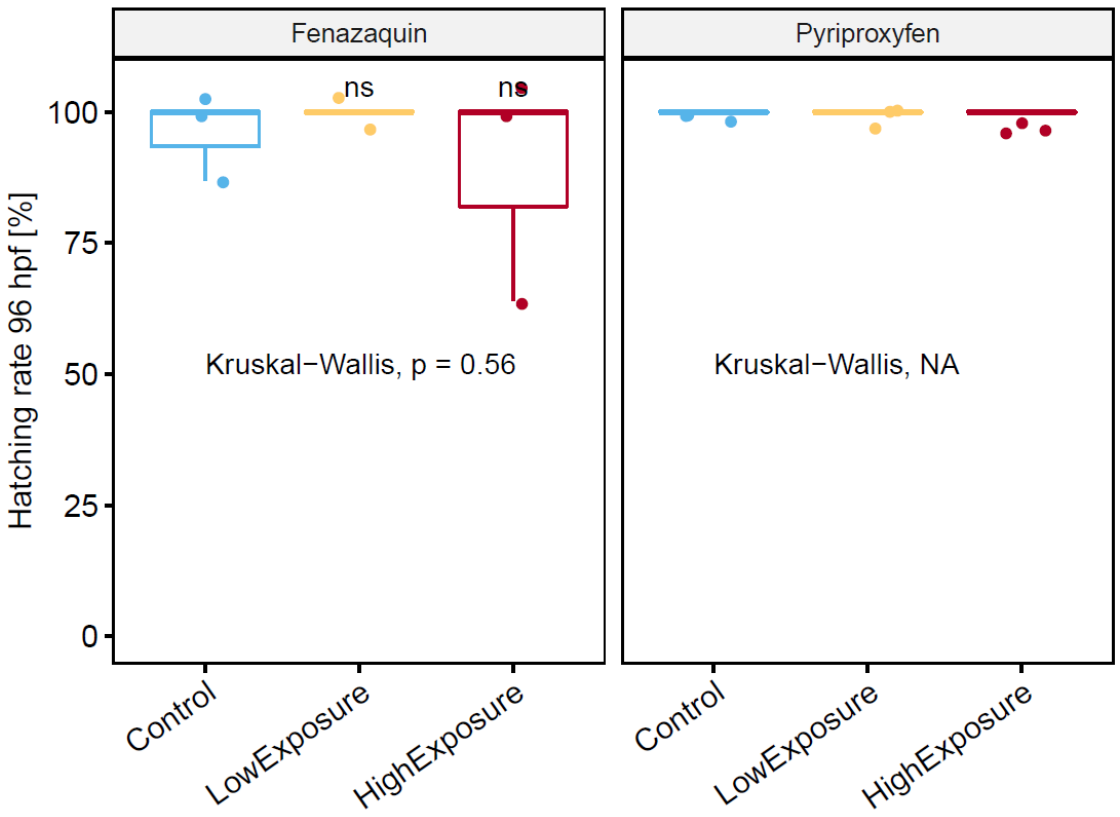
Table 1: Tested insecticides with respective mode of action in the target organism and test concentrations. (*:96h acute fish test NOEL values median from EPA's Ecotox database; **: nominal conc.)

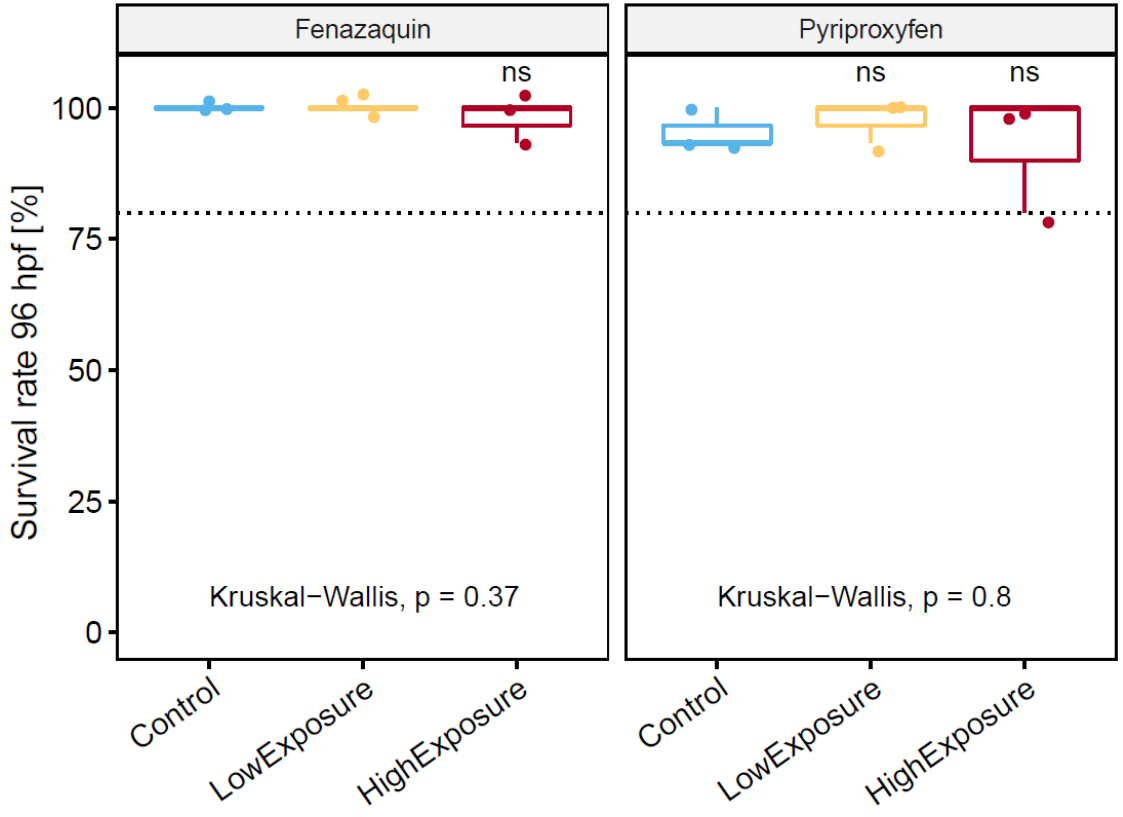
Substance	Mode of Action (MoA) (IRAC classification)	NOEL*	Test conc. [µg/L]**	
			Low (LE)	High (HE)
Fenazaquin	Mitochondrial complex I electron transport	9,6 ppm	3	6
Pyriproxyfen	Juvenile hormone mimic	270 ppm	170	1700



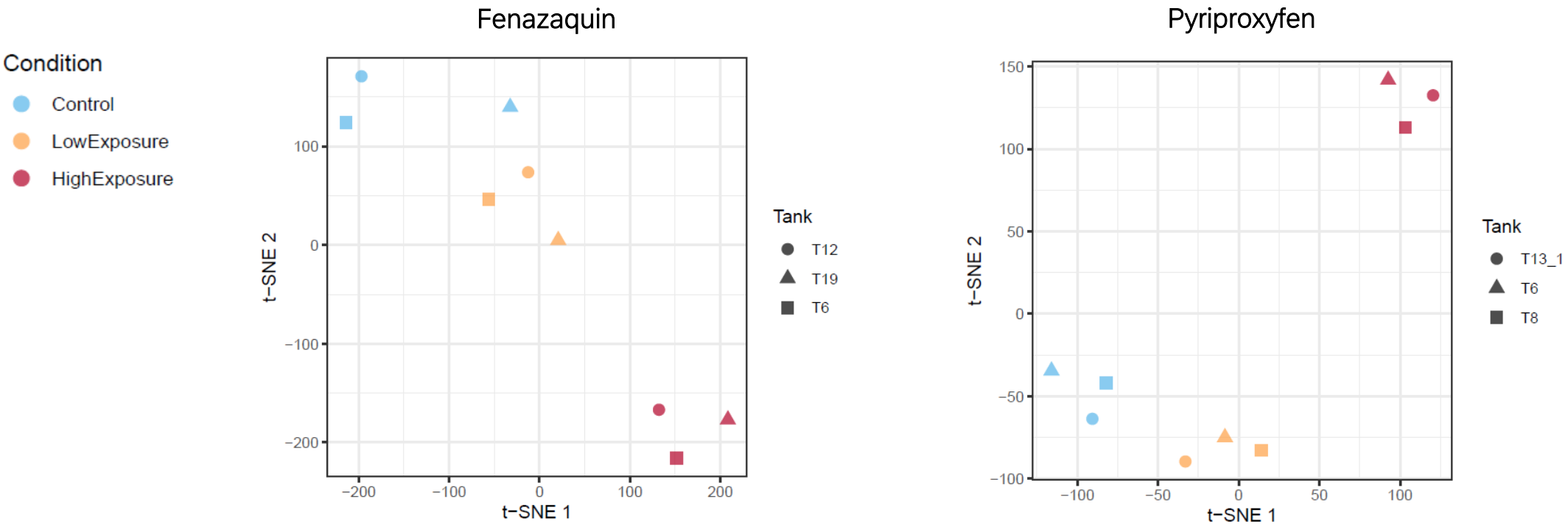
Publicly available toxicity data from EPA's knowledgebase in cyprinidae & cypriniformes for 96h & 120h exposure compiled via *StandaRtox* (Scharmüller et. al 2020).

Survival and hatching rates post 96 h exposure





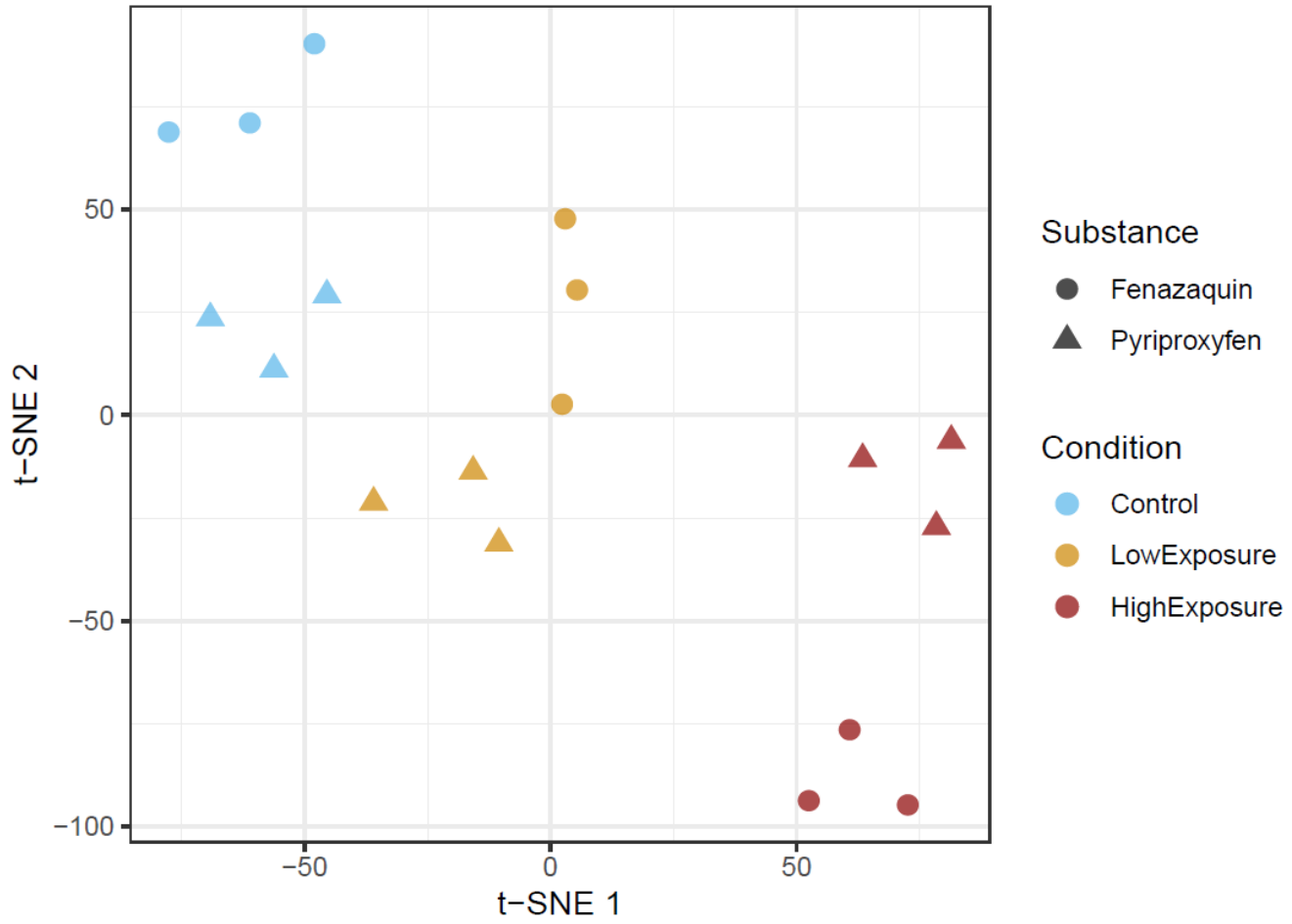
Reduced dimensional clustering of biological samples



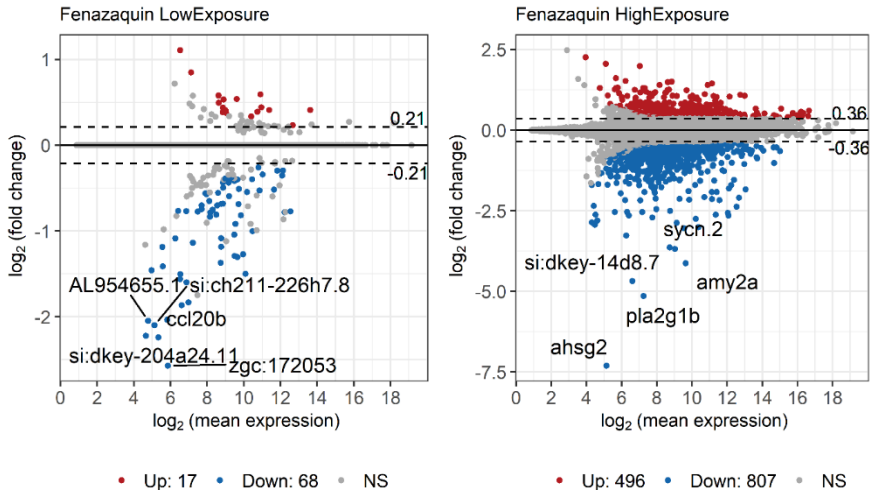
t-SNE clustering for the 1500 gene transcripts with largest variance with rlog transformed DESeq2 normalized gene counts.

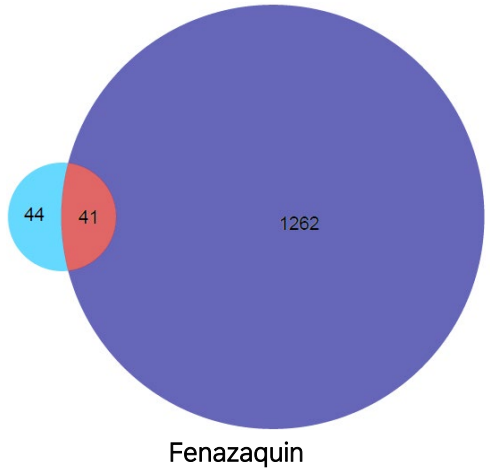
- Clearly separated clusters of sample groups (biological replicates).

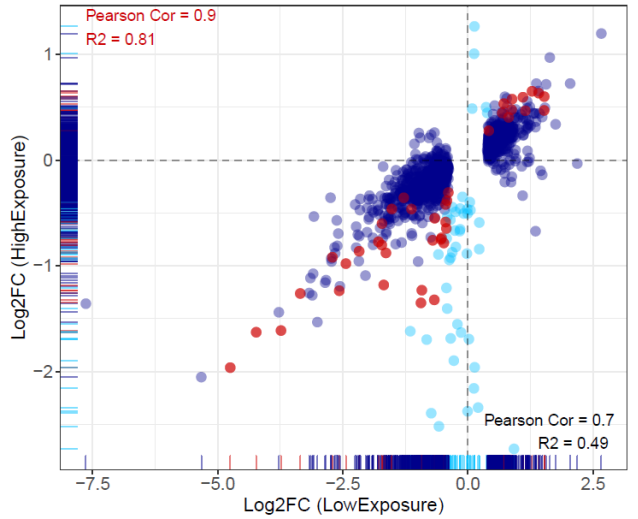
Reduced dimensional clustering of biological samples

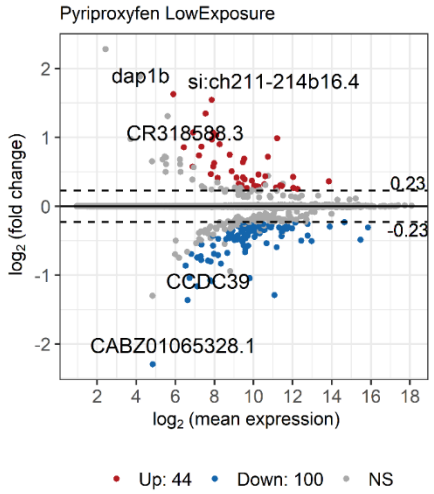


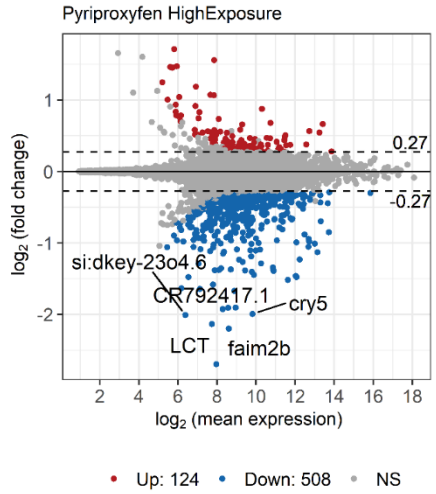
Differential expression signals for the different exposure conditions

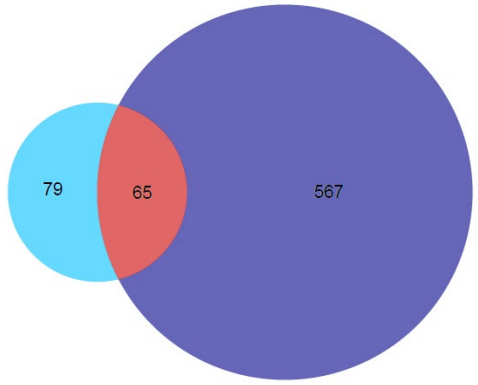


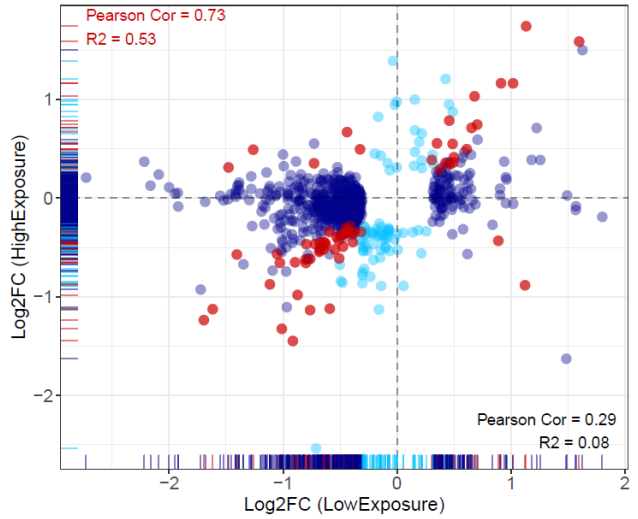






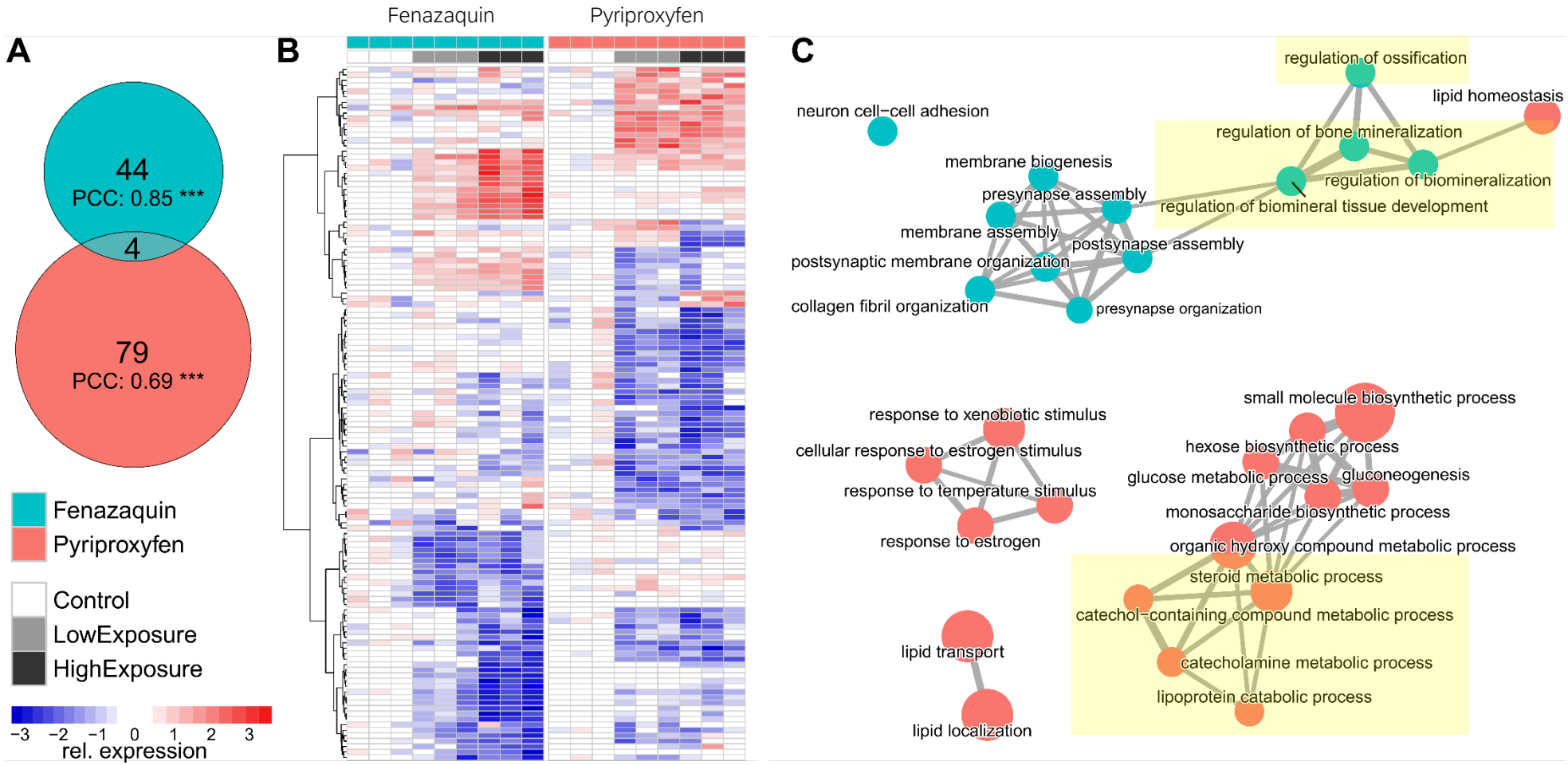






DEGs in **LE** | core DEGs | DEGs in **HE**

Ecotoxicogenomic signatures in the 96 hpf zebrafish embryo



Ecotoxicogenomic signatures in the 96 hpf zebrafish embryo

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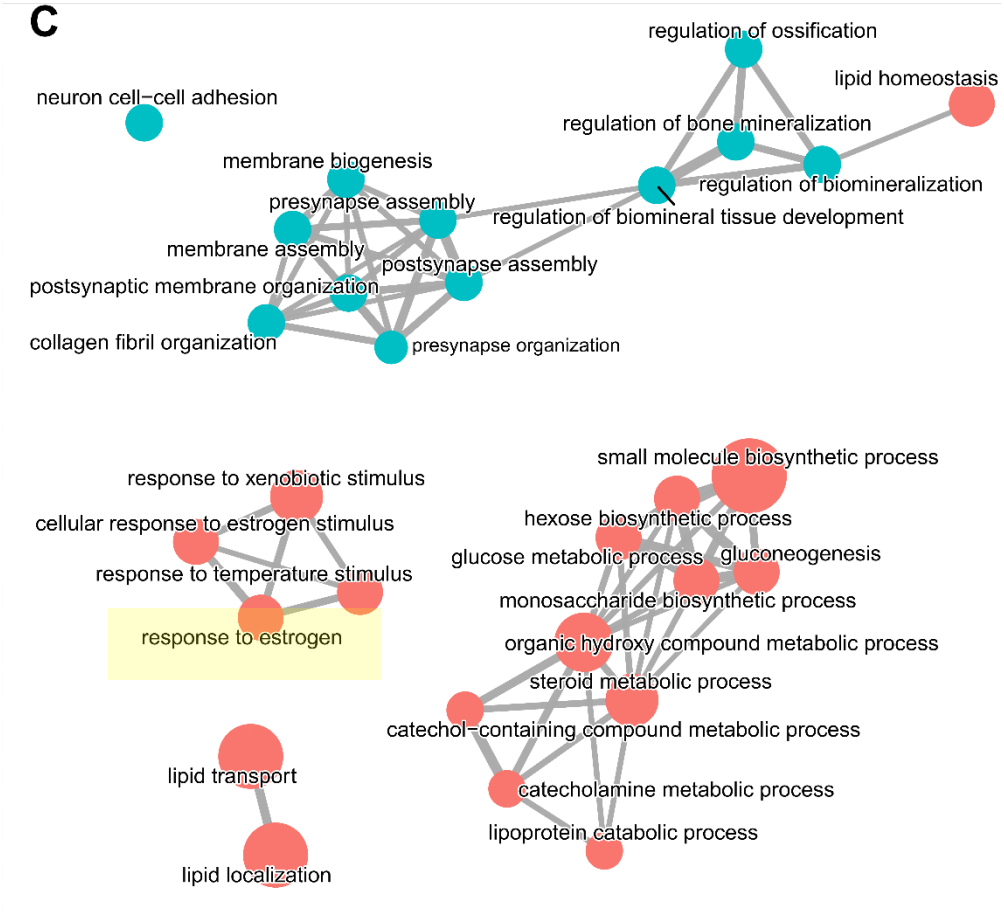
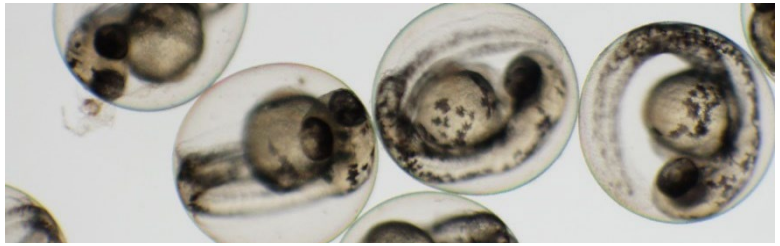
Pyriproxyfen induced impairment of reproductive endocrine homeostasis and gonadal histopathology in zebrafish (*Danio rerio*) by altered expression of hypothalamus-pituitary-gonadal (HPG) axis genes

Kannan Maharajan [a, b, e], Sellamani Muthulakshmi [a], Chinnannan Karthik [a], Bojan Nataraj [b], Kanthan Nambirajan [c], Devan Hemalatha [d], Swaminathan Jiji [a], Krishna Kadirvelu [a], Ke-chun Liu [e], Mathan Ramesh [b]



TAKE HOME MESSAGE



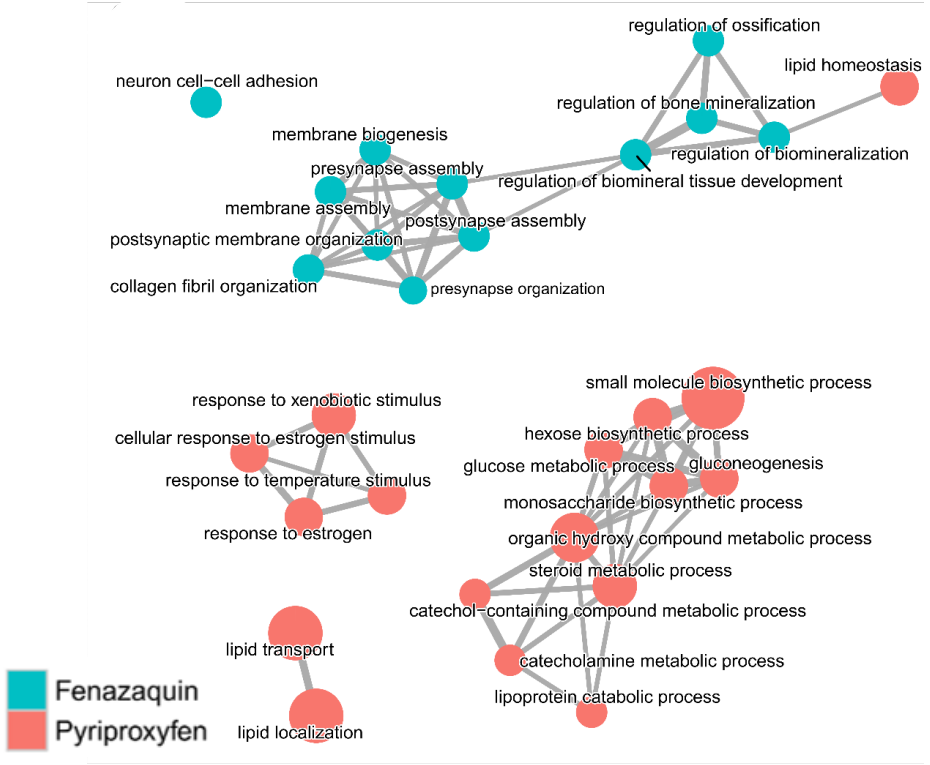
Questions still remain …

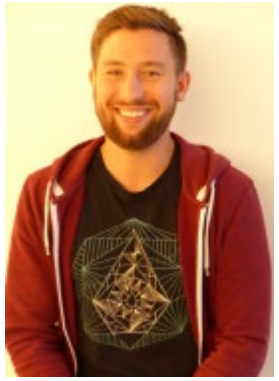
Although OMICs are a powerful tool to gain mechanistic insights about adverse MoAs, what are these insides worth?

- Are the observed ecotoxicogenomic signatures **MoA-specific** in the applied model?
- Are the observed profiles **reproducible among similar MoA-targeting compounds**?

Conclusion

- Combining *omics* with OECD standardized test methods has the potential to characterize molecular profiles in aquatic non-target organisms.
- Such profiles **provide mechanistic insights** and allow for **identification of MoA-specific** molecular **biomarkers**.
- Molecular biomarker-based screening approaches offer:
  - **Cost and resource effectiveness**
  - **High sensitivity**
  - Transition to *in vitro* cell culture based methods as **alternative to non-human animal testing**.





THANK YOU FOR YOUR INTEREST!

TIME FOR QUESTIONS NOW!