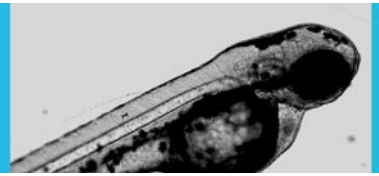
SETAC EUROPE2021
SETAC EUROPE 31ST ANNUAL MEETING
3–6 MAY 2021 | VIRTUAL CONFERENCE

Similar Target, Similar Toxicity?

Toxicogenomic Profiles of Neuronal Targeting Insecticides in the Zebrafish Embryo Model



Speaker: Hannes Reinwald(1;2)

Eco'n'OMICs ATTRACT
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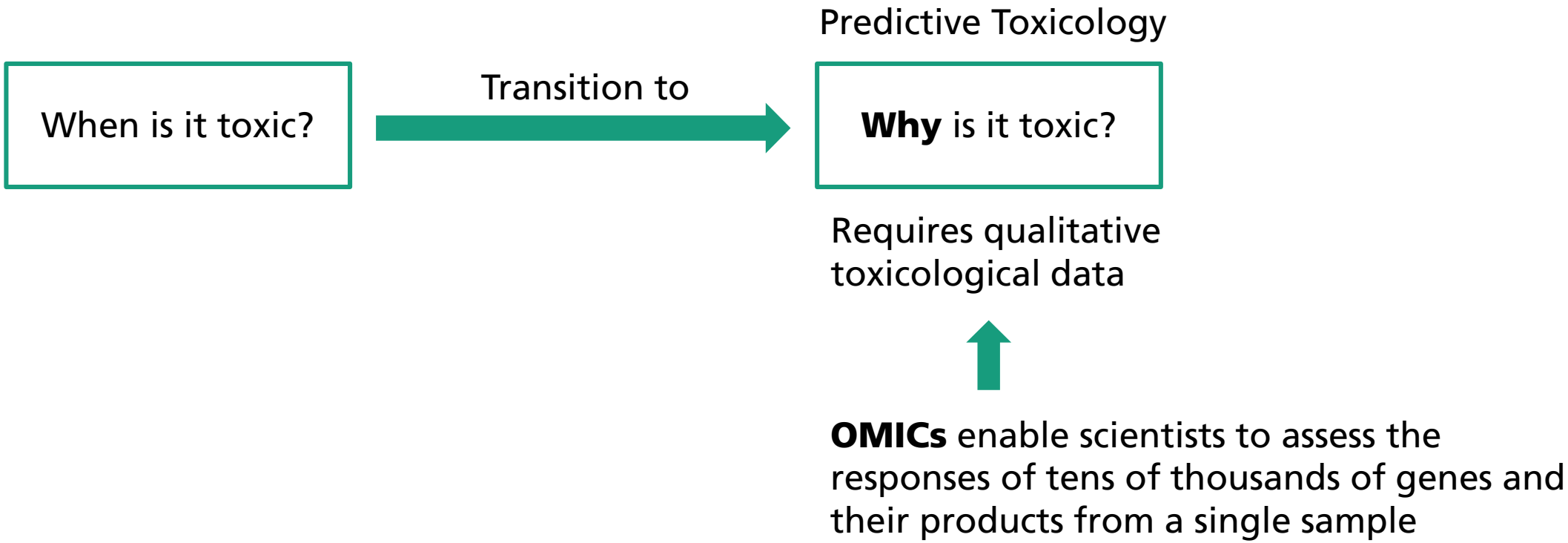
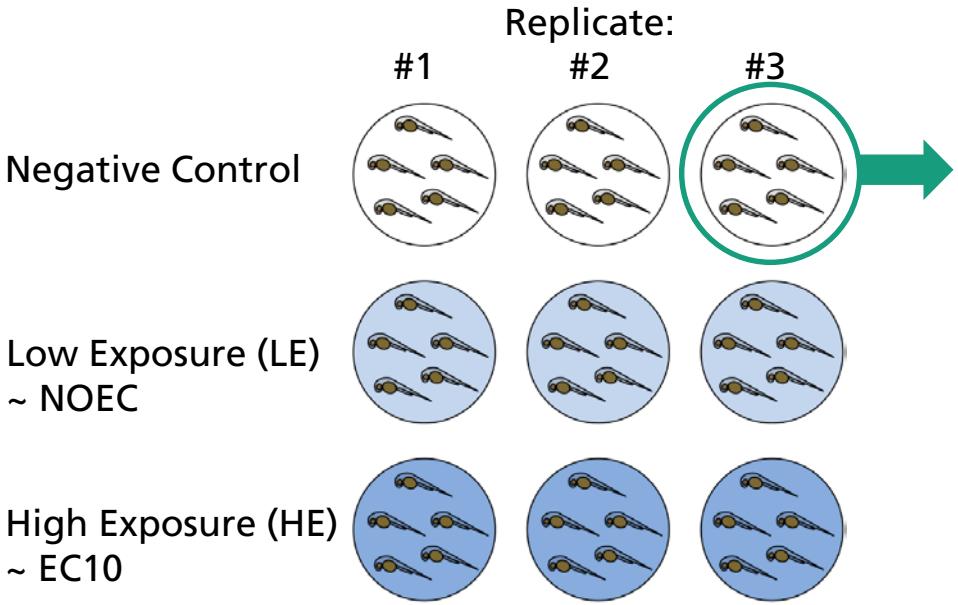
OMICs in Ecotoxicology – a potential game changer

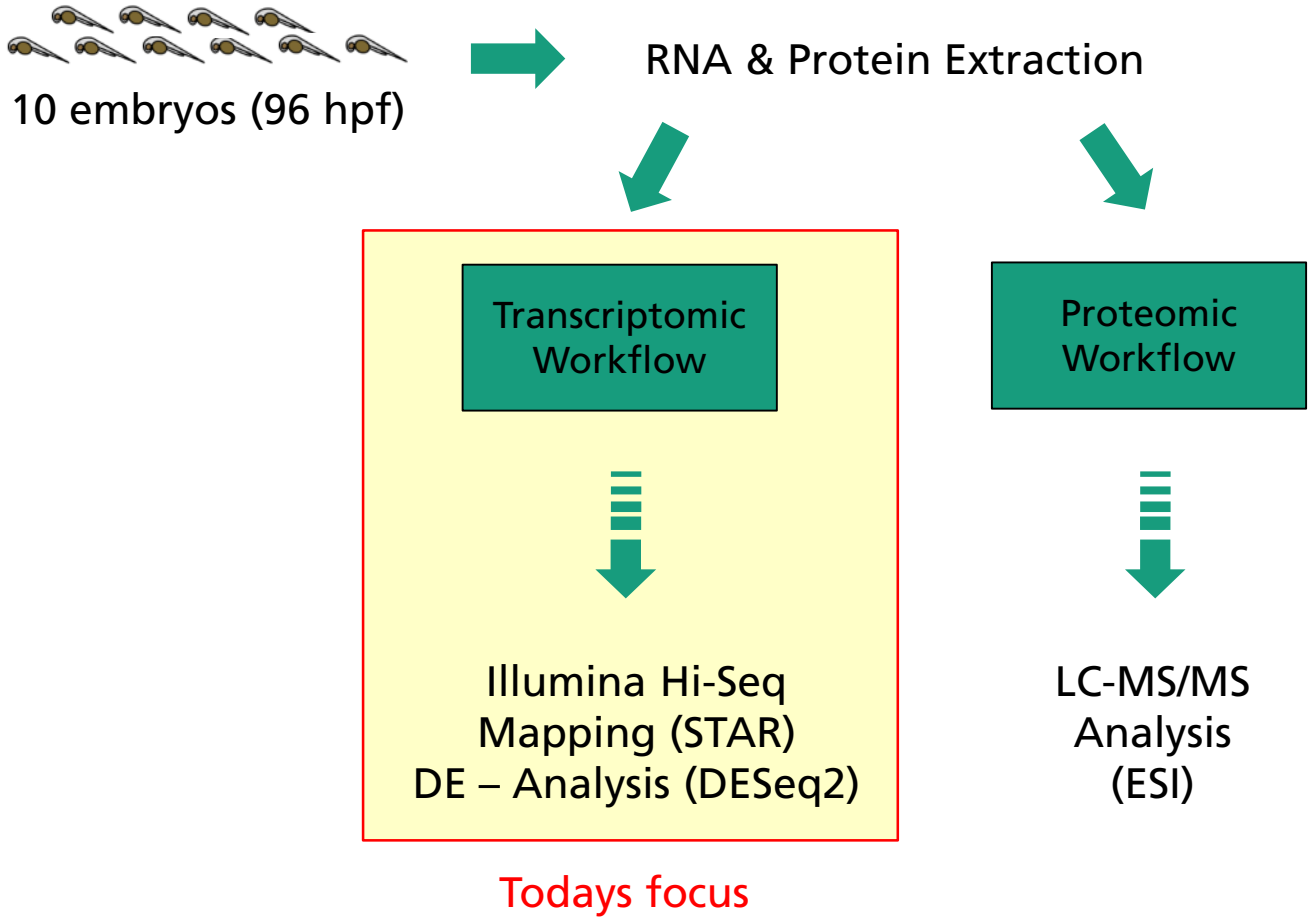


(Brockmeier et al 2017, Bencic 2015)

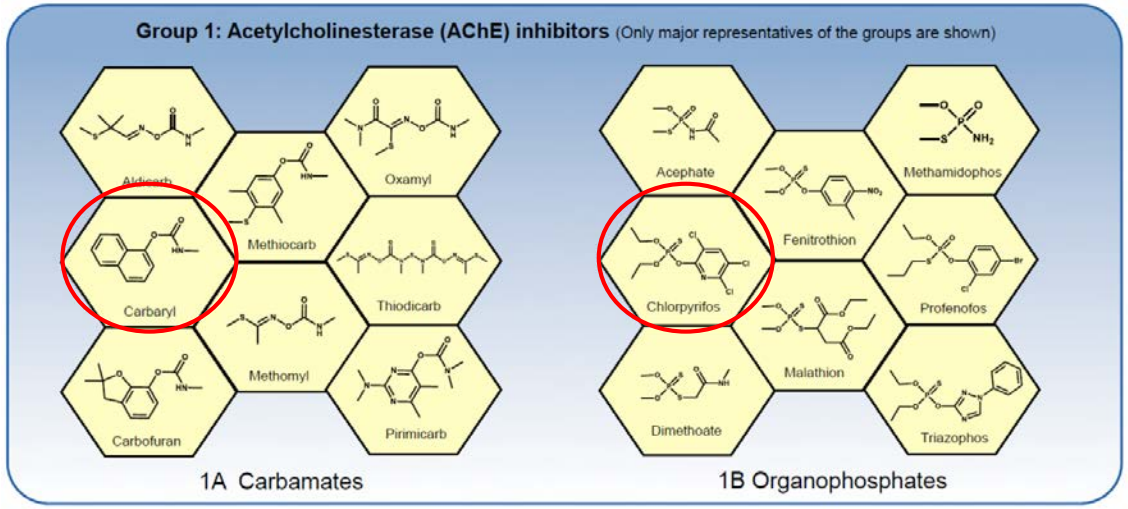
Screening for ecotoxicogenomic fin(ger)-prints

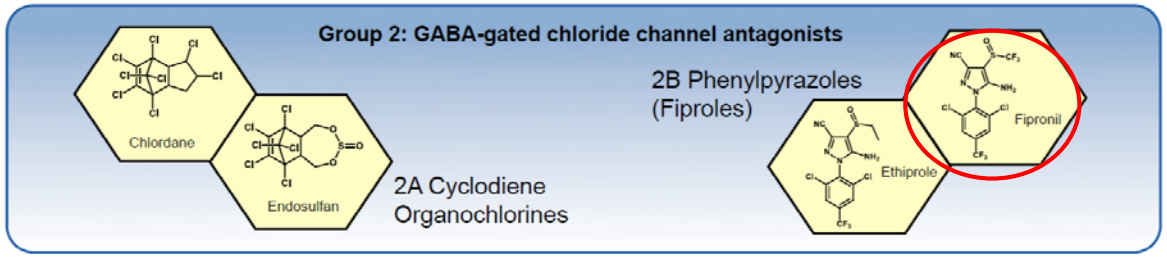


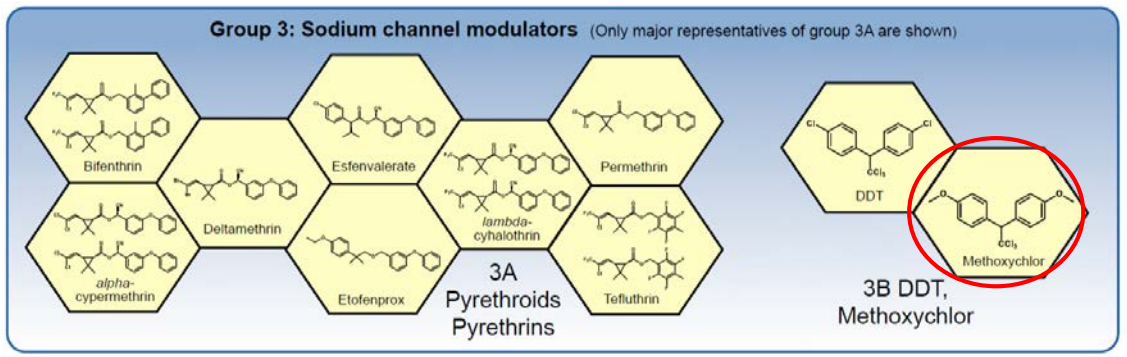
- Incubation in **glass petri dishes** (preincubated 24 h pre exposure)
- 15 embryos (2 hpf) per well
- 3 biological replicates from different spawning groups
- semi-static incubation for 96 h (Medium exchange 48 hpf)

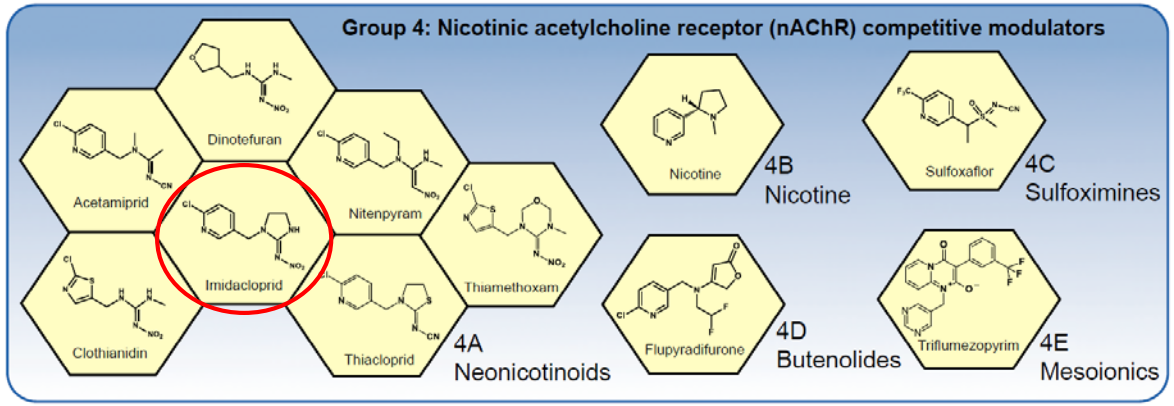


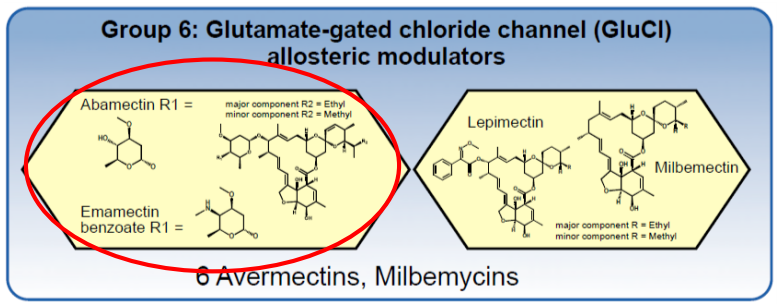
Neuronal targeting insecticides with classified Mode of Action (MoA)











Mode of Action Classification
IRAC
Insecticide Resistance Action Committee

(Sparks & Nauen, 2015)

Test concentrations and experimental scope

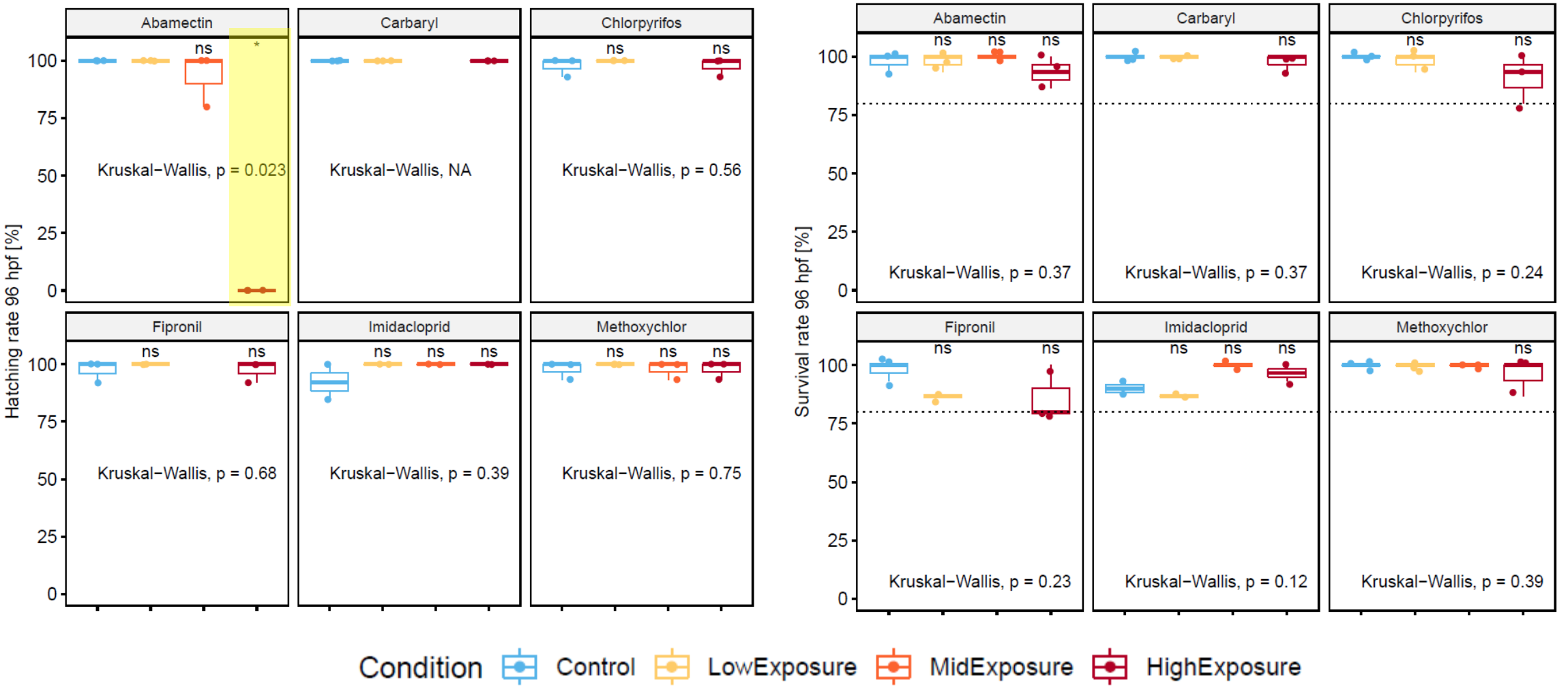
	Low exposure	*Mid exposure*	*High exposure*
		µg/L	
Abamectin*	110	220	440
Carbaryl	275	/	1100
Chlorpyrifos*	750	/	3000
Fipronil*	75	/	300
Imidacloprid	15000	30000	60000
Methoxychlor*	20	60	180

*: Stocks prepared in acetone

- Can we **identify** potential **early response biomarkers** as indicator for neuronal toxicity in the zebrafish embryo model?
- Can we use the overall signatures to **differentiate between** the IRAC classified **MoAs**?

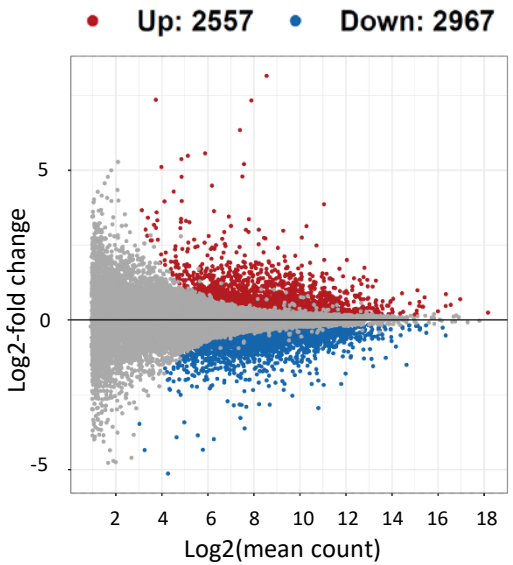
CURRENT RESULTS

Observed physiological effects post exposure - 96 hpf

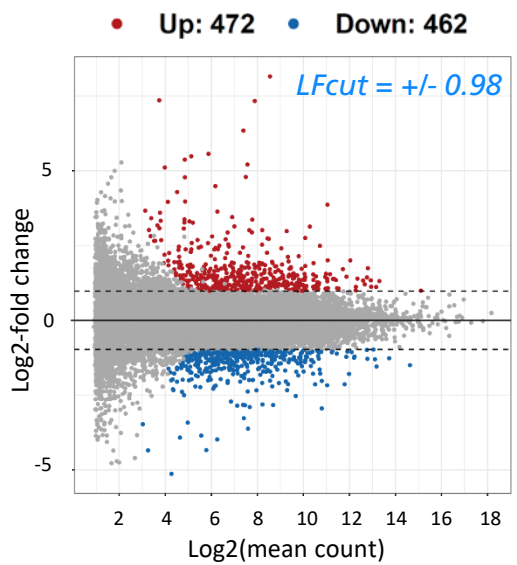


Definition of Differentially Expressed Genes (DEGs)

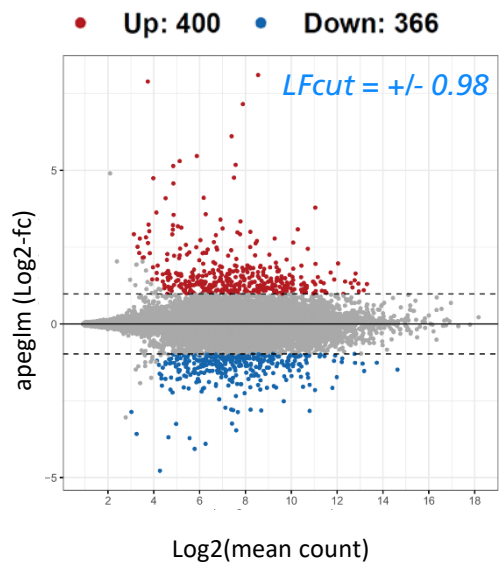
Comparing a treatment against control:



Effect size cut off



apeglm shrinking



$$LFcut = quantile(abs(x), 0.9)$$

padj < 0.05

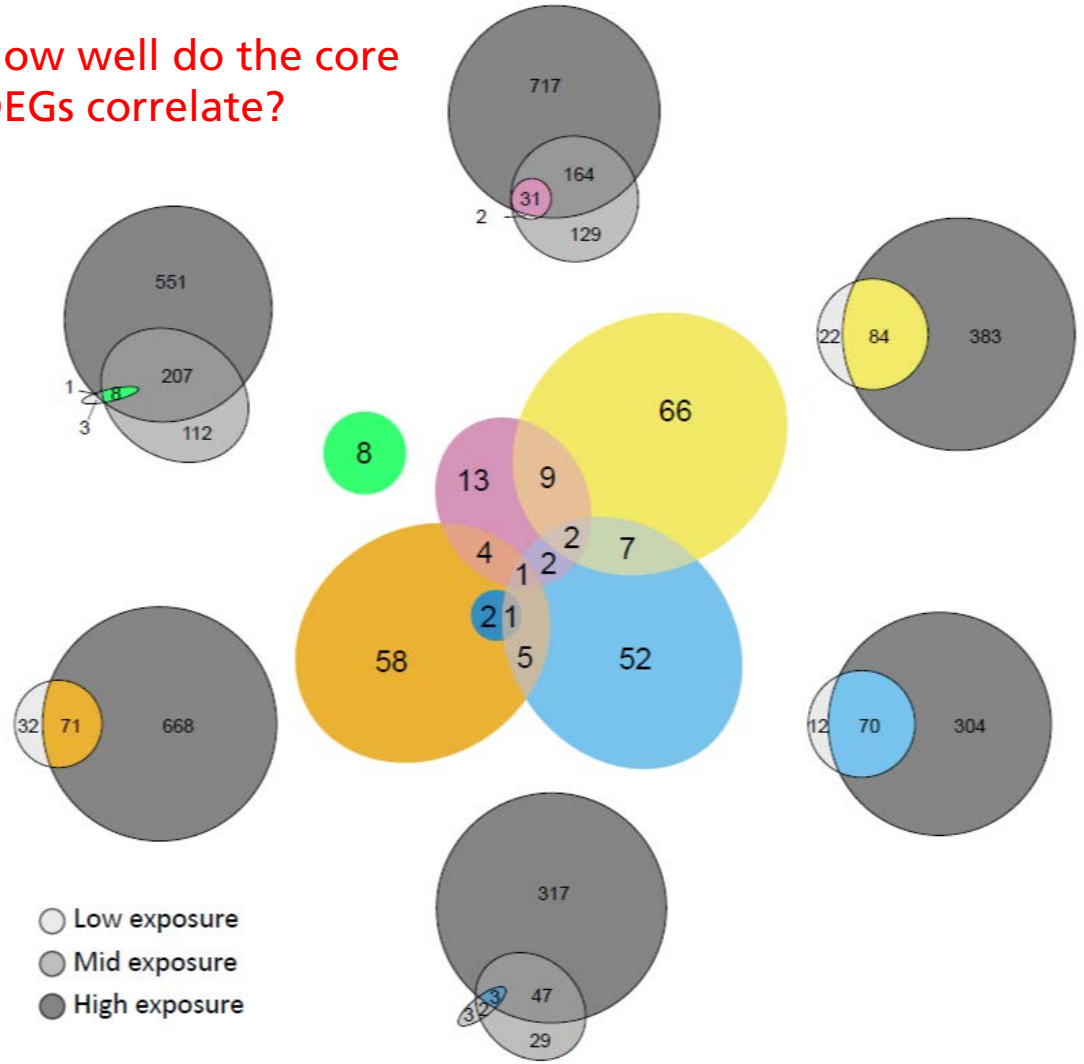
Wald's t-test using IHW (**I**ndependent **H**ypothesis **W**eighting) (Ignatiadis et al 2016) for a weighted Benjamini-Hochberg correction.

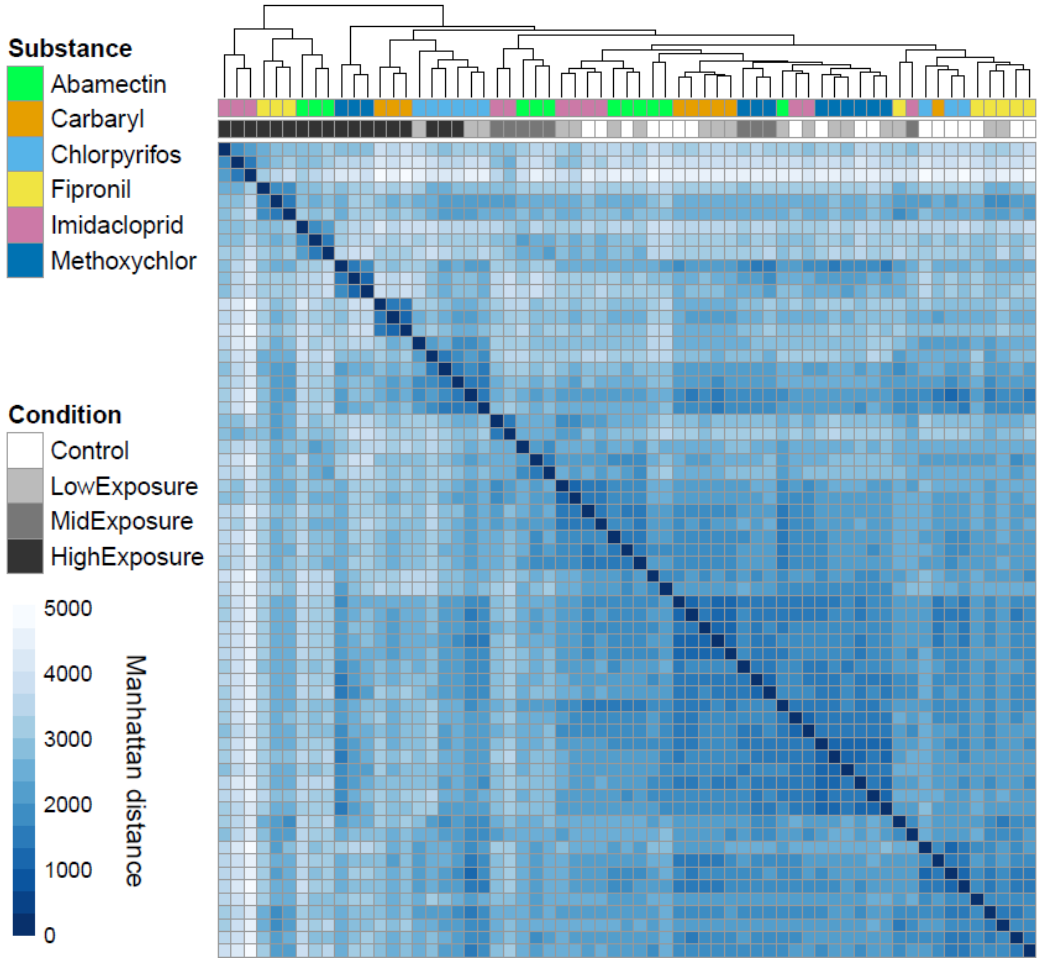
apeglm : **A**pproximate **P**osterior **E**stimation for **g**eneralized **l**inear **m**odel (Zhu et al 2019)

766 DEGs

Identified DEGs and global sample distance

How well do the core DEGs correlate?

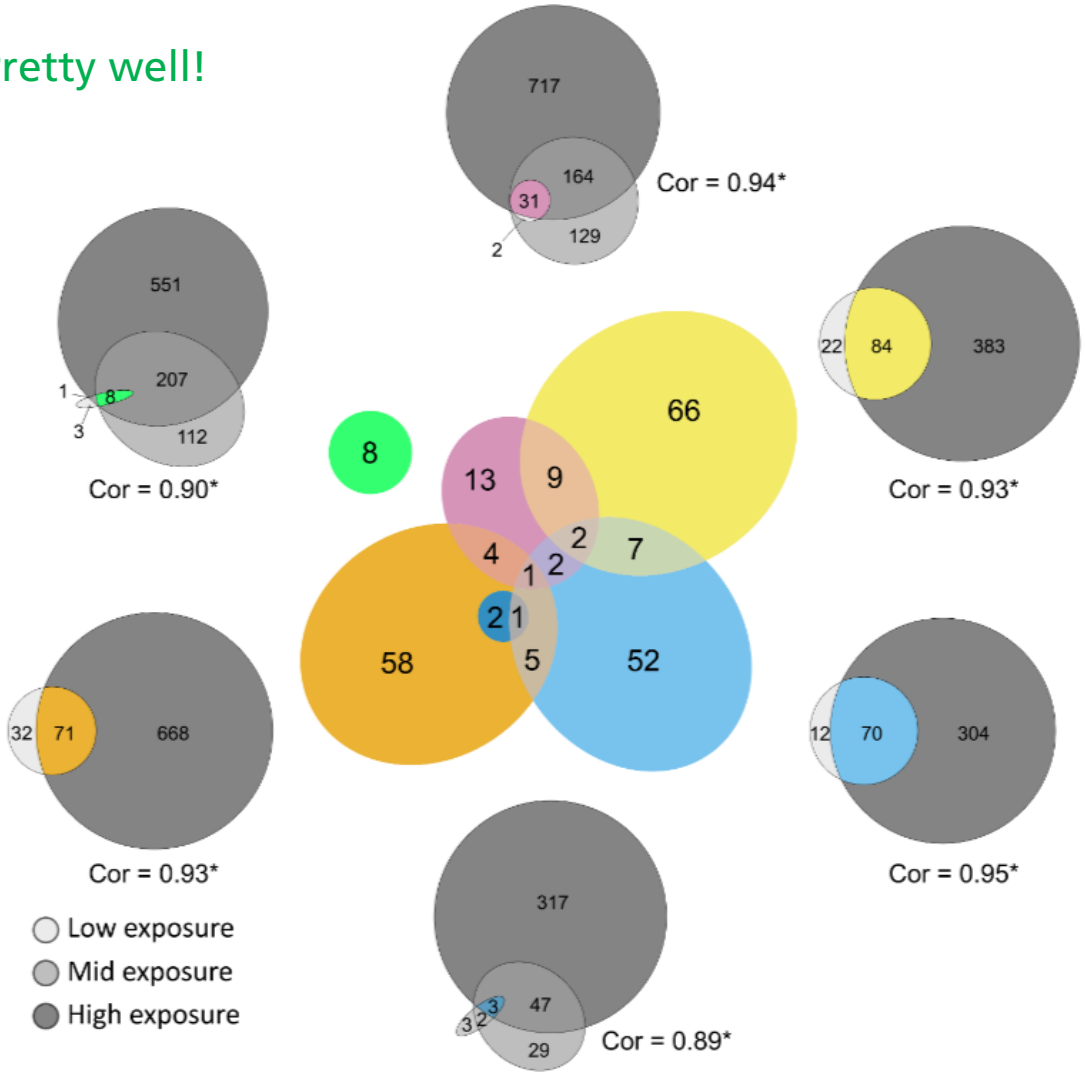


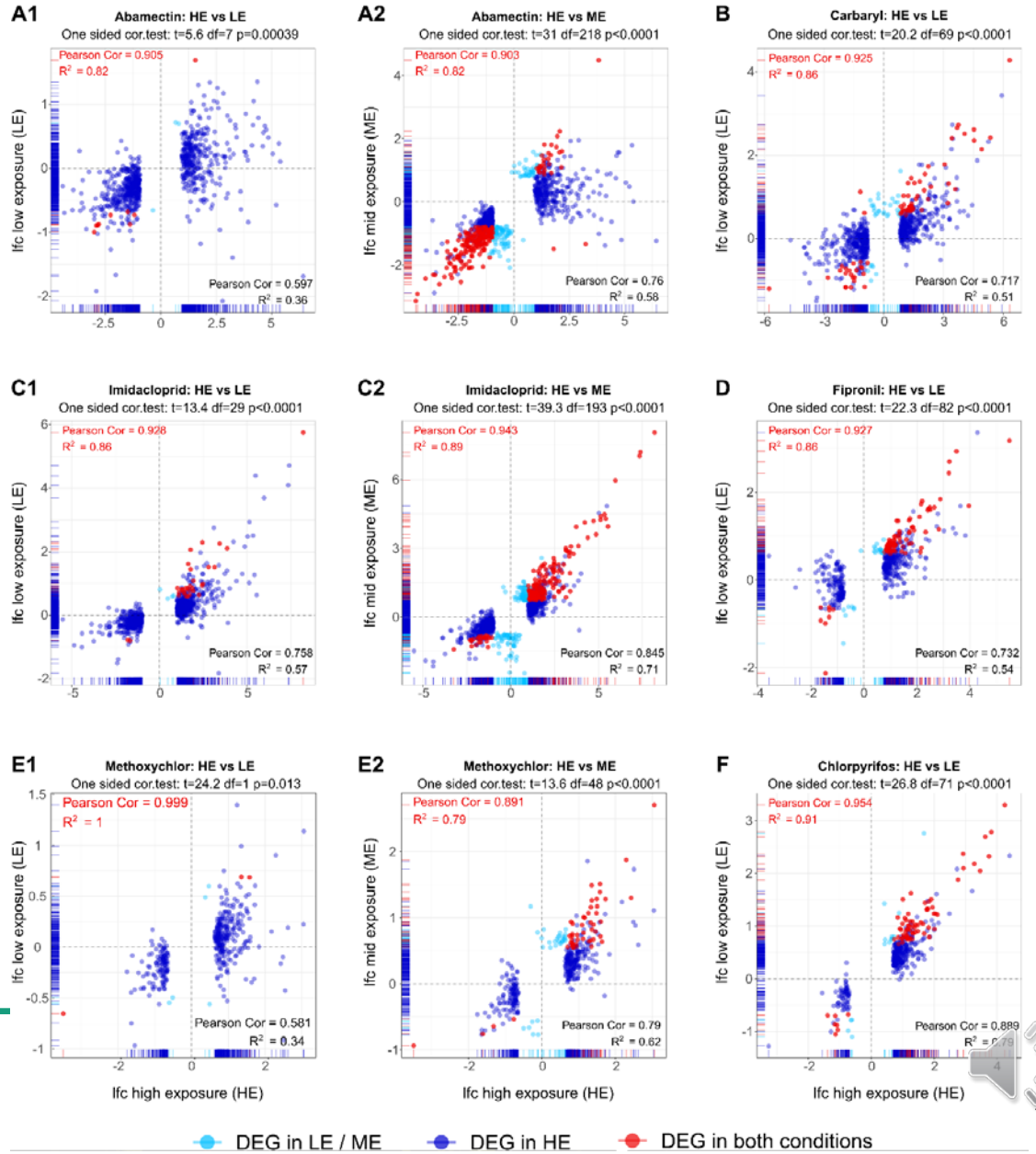


Manhattan distance of normalized variance stabilized transformed gene counts (ward.D2 clustered)

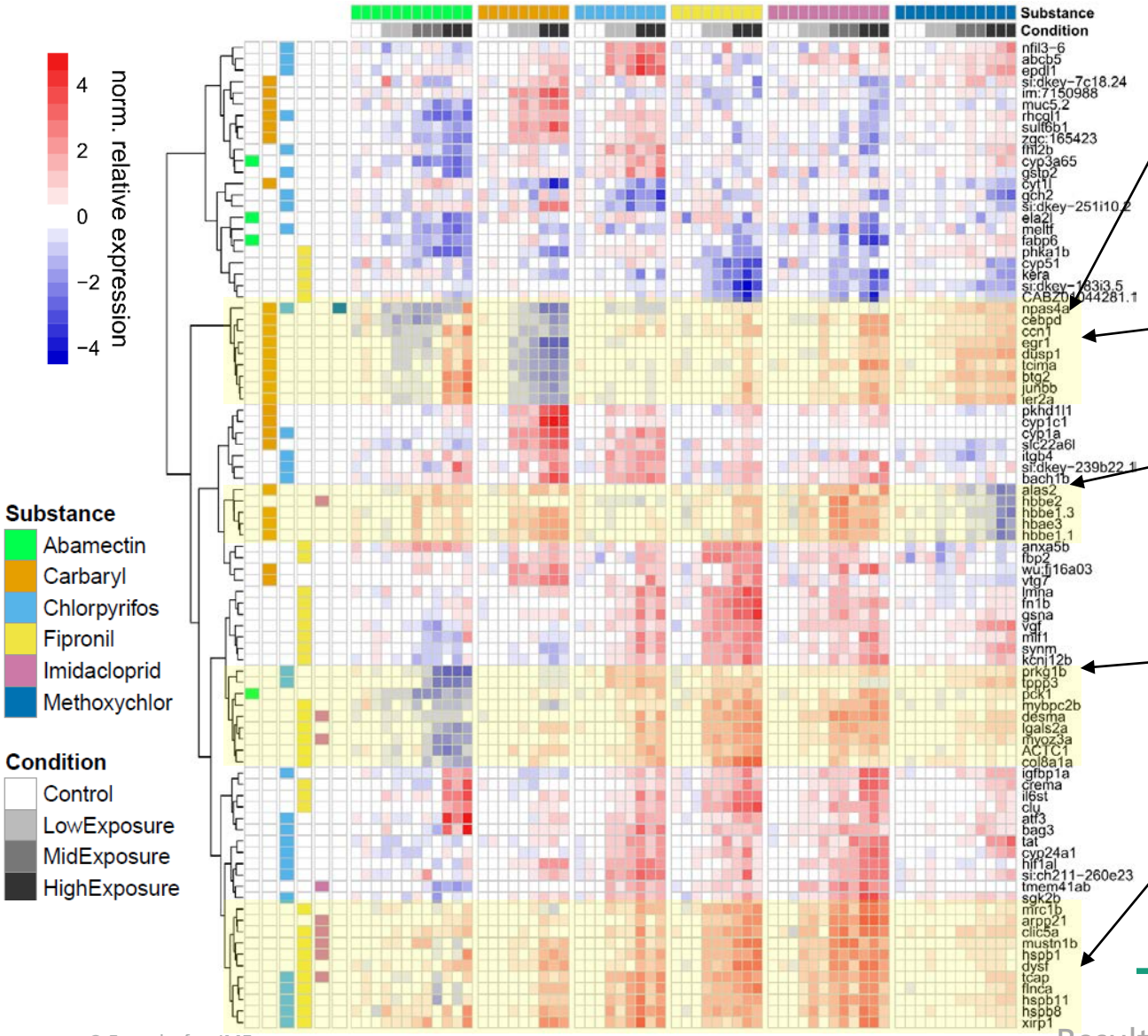
Correlation of core DEGs for each tested insecticide

Pretty well!





Gene clustered heat map of all overlapping DEGs with baseMean > 500 (top 67% Quantile)



npas4a: *neuronal PAS domain protein 4a*
Involved in T-tubule organization and heart contraction. Human ortholog(s) of this gene implicated in autosomal recessive limb-girdle muscular dystrophy type 2G and hypertrophic cardiomyopathy

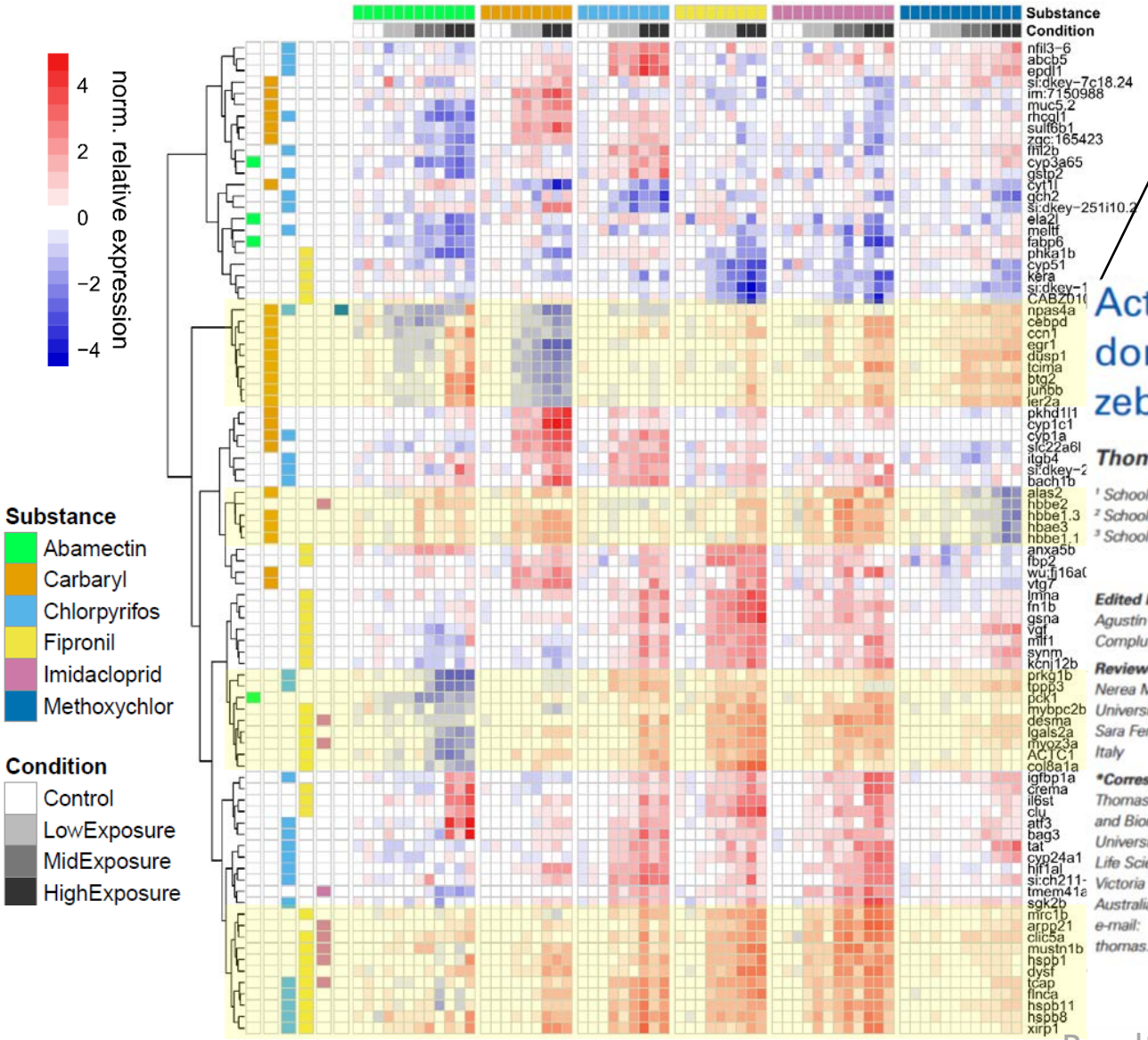
egr1: Involved in embryonic retina morphogenesis in camera-type eye; expressed in head; heart; hindbrain neural keel; mesoderm; and nervous system.

alas2: *Aminolevulinate, delta-, synthase 2*
Involved in heme / hemoglobin biosynthetic process
hbbe: hemoglobin beta embryonic
-> ortholog(s) in human implicated in sickle cell anemia

prkg1b: *Protein kinase cGMP-dependent 1b*
Expressed in heart; integument; nervous system. Human ortholog(s) of this gene implicated in thoracic aortic aneurysm.

tcap: *Titin-cap* ; Involved in T-tubule organization and heart contraction. Human ortholog(s) of this gene implicated in autosomal recessive limb-girdle muscular dystrophy type 2G and hypertrophic cardiomyopathy

Gene clustered heat map of all overlapping DEGs with baseMean > 500 (67% Quantile)



npas4a: *neuronal PAS domain protein 4a*
Involved in T-tubule organization and heart contraction. Human ortholog(s) of this gene implicated in autosomal recessive limb-girdle muscular dystrophy type 2G and hypertrophic cardiomyopathy

Activity-dependent expression of neuronal PAS domain-containing protein 4 (npas4a) in the developing zebrafish brain

Thomas Klarić[1]*, Michael Lardelli[1], Brian Key[2], Simon Koblar[3] and Martin Lewis[1] (2014)

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In rodents, the Npas4 gene has recently been identified as being an important regulator of synaptic plasticity and memory. Homologs of Npas4 have been found in invertebrate species though their functions appear to be too divergent for them to be studied as a proxy for the mammalian proteins. The aim of this study, therefore, was to ascertain the suitability of the zebrafish as a model organism for investigating the function of Npas4 genes. We show here that the expression and regulation of the zebrafish Npas4 homolog, npas4a, is remarkably similar to that of the rodent Npas4 genes. As in mammals, expression of the zebrafish npas4a gene is restricted to the brain where it is up-regulated in response to neuronal activity. Furthermore, we also show that knockdown of npas4a during embryonic development results in a number of forebrain-specific defects including increased apoptosis and misexpression of the forebrain marker genes dlx1a and shha. Our work demonstrates that the zebrafish is a suitable model organism for investigating the role of the npas4a gene and one that is likely to provide valuable insights into the function of the mammalian homologs. Furthermore, our findings highlight a potential role for npas4a in forebrain development.

Keywords: npas4a, zebrafish, neurodevelopment, dlx1, shh, PTZ

TAKE HOME MESSAGE

Take home message

- We observed consistent transcriptomic changes in zebrafish embryos (96hpf) exposed to neuronal targeting insecticides well below acute toxicity levels.

  Sensitive and informative approach

- Can we **identify** potential **early response biomarkers** as indicator for neuronal toxicity in the zebrafish embryo model?

  Neuronal associated genes such as ***npas4a, tcap, egr1 & prkg1b*** responded to very low levels of exposure for multiple neuronal targeting insecticides and tested concentrations.

- Can we use the overall signatures to **differentiate between** the IRAC classified **MoAs**?

  We observed gene clusters demonstrating opposing regulation for different substances. (i.e. “hbbe”cluster of Carbaryl against Methoxychlor & Imidacloprid)

  However, substances from the same MoA (Chlorpyrifos & Carbaryl) do not necessarily show more similar expression profiles compared to other neuronal targeting insecticides.

Thank you SETAC Europe for having us online today!

The ATTRACT Eco'n'OMICs Group

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SCAN ME

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