Systems toxicology approach for the assessment of zebrafish neurotoxicity

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Introduction and Objectives

Recent years have seen an unprecedented rise in the use of zebrafish as a model to study chemical toxicity. This is due in part to the following advantages:

- External development
- Amenability to high-throughput assays
- Rich and defined behavioral repertoire
- Compliance with the 3Rs (replacement, reduction, refinement) principles
- High-quality genome sequence (GRCz11)
- 70% gene orthology with humans
- Remarkably similar responses to chemicals in zebrafish and humans

Taking advantage of these benefits, we have selected zebrafish larvae for a comprehensive systems toxicology assessment of the insecticide Imidacloprid.

Biological Expression Language (BEL)

Our systems toxicology approach involves the construction of a computable network. The network consists of molecular relationships curated from literature. Such molecular relationships are scripted in BEL, a language for representing scientific findings in a computable form.

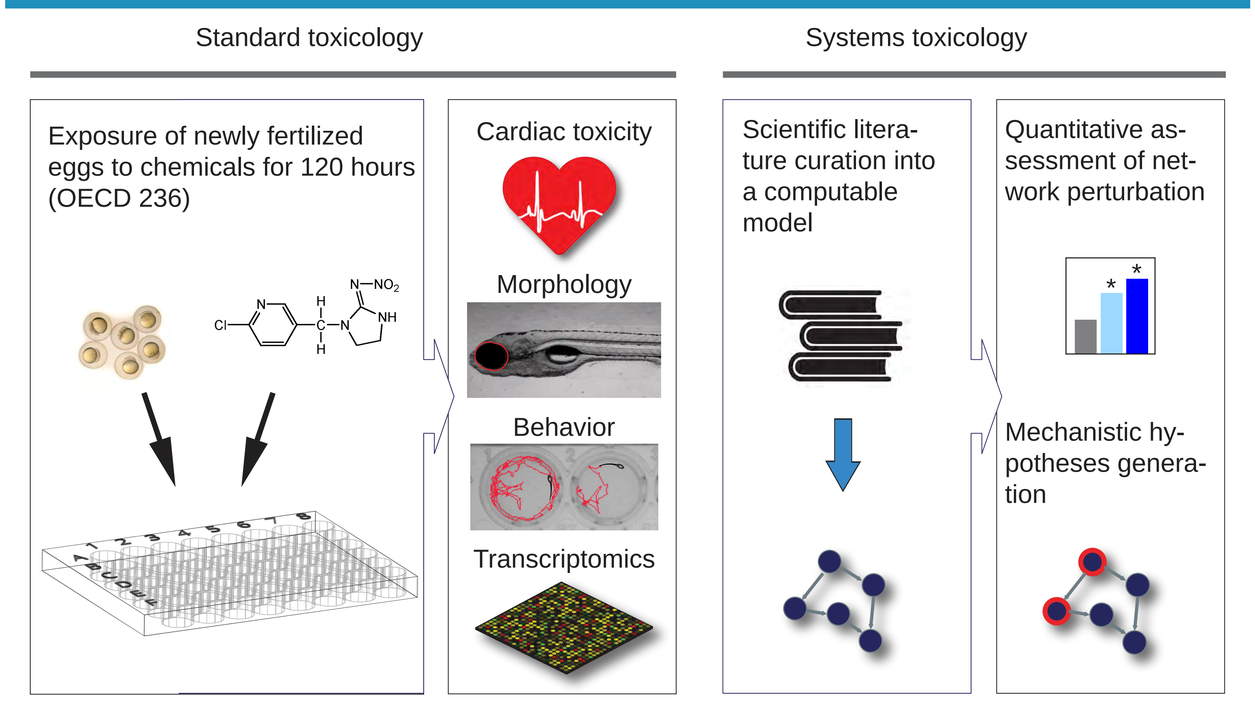
Evidence found in a publication (PMID 25568117): "Expression of tlx2 was downregulated in both sox9a and atoh1a morphants at 24 hpf (Fig. 5A-C)."

BEL statement: `act(p(ZFIN:atoh1a)) increases r(ZFIN:tlx2)`

	BEL function:	Namespace:	Relationship	Entity:
	-Abundance	-HGNC		-Gene
	-Processes	-ZFIN		-Protein
	-Activity	-CHEBI		-Biological process

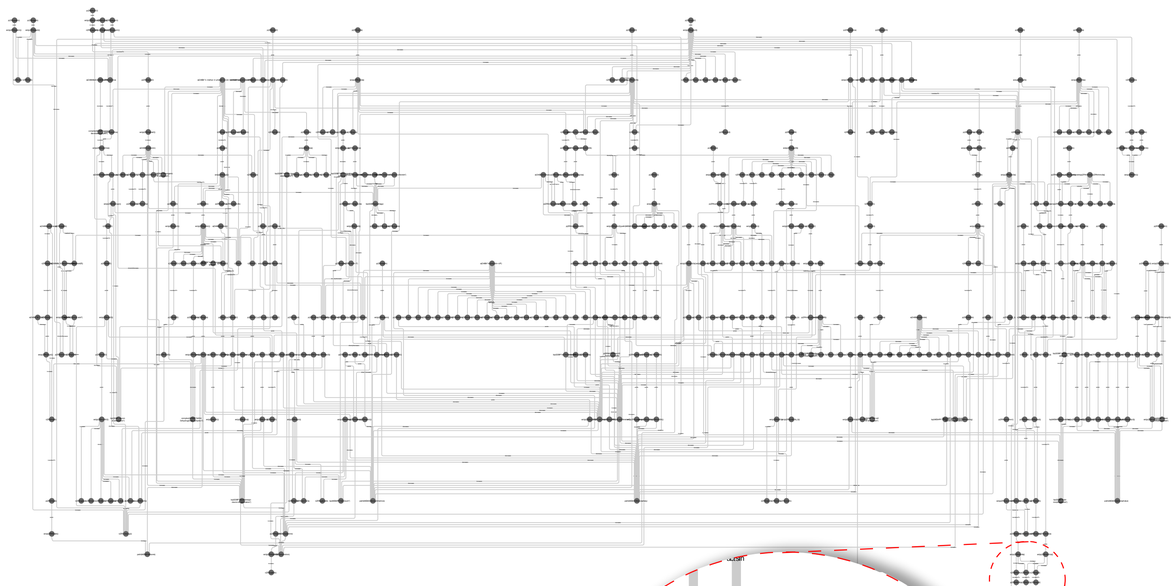
openbel.org

Methods



Zebrafish Neurotoxicity Network

BEL statements can be assembled into a computable biological network. The visualization of the zebrafish neurotoxicity model was created in Cytoscape and contains 660 BEL statements curated from 90 scientific publications.

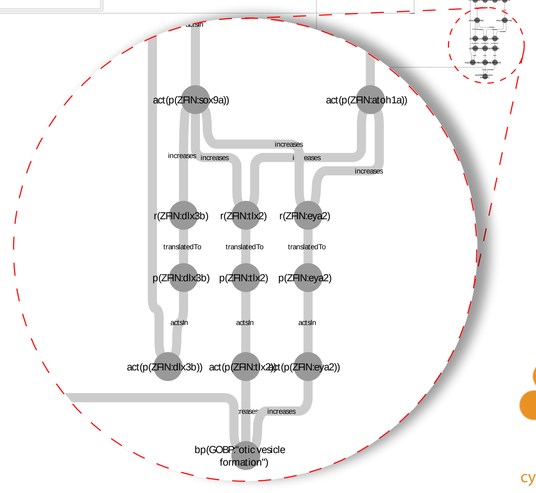


Pathological endpoints represented in the model:

- Microcephaly
- Megalencephaly
- Microphthalmos
- Seizures
- Hydrocephalus
- Neurogenic inflammation

Biological processes represented in the model:

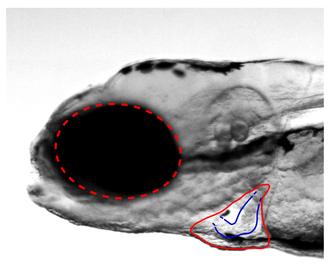
- Neuron differentiation
- Autophagy
- Locomotion
- Phototaxis
- Oxidative stress
- Notch signaling pathway



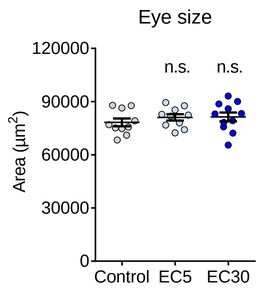
cytoscape.org

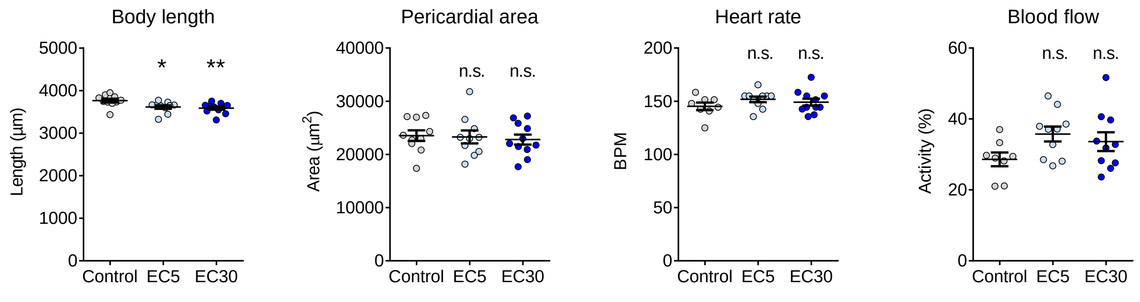
Standard toxicology

Zebrafish embryos were exposed to imidacloprid at EC5 (126.2 mg/L) and EC30 (222.7 mg/L) as determined by the OECD fish embryo toxicity test. At 120 hours of exposure the below morphological, cardiological, and behavioral endpoints were measured.



Representative image of morphological measurements





Network Scoring

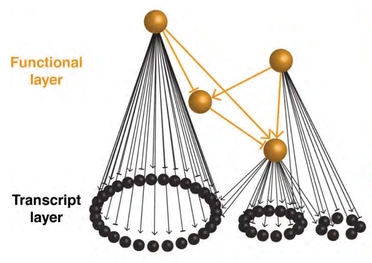
The above model constitutes the functional layer (yellow spheres in this panel), where each node represents a biological entity.

Additionally, the model contains a downstream transcript layer (black spheres), which are transcripts that have been described in the literature to be regulated by a given molecular entity.

By measuring transcript abundance in transcriptomic datasets, activity of the upstream node in the functional layer can be quantified.

The most affected nodes can provide a mechanistic understanding of the molecular events that occur upon experimental treatment.

Overall changes in the functional layer can be used to calculate the network perturbation amplitudes (NPA).



Martin, F., Sewer, A., Talikka, M., Xiang, Y., Hoeng, J., & Peitsch, M. C. (2014). Quantification of biological network perturbations for mechanistic insight and diagnostics using two-layer causal models. BMC Bioinformatics, 15, 238. doi: 10.1186/1471-2105-15-238

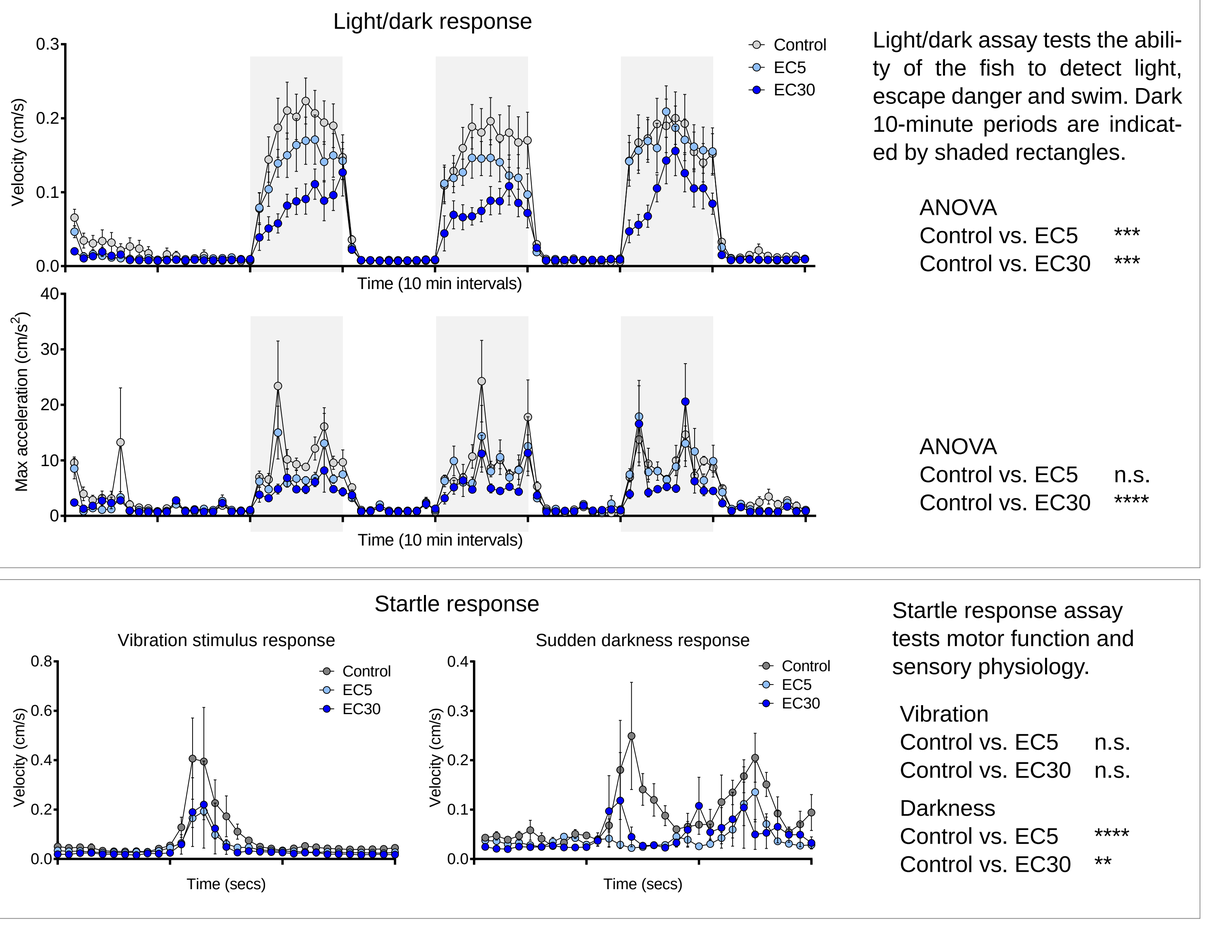
Conclusions

We have constructed a zebrafish specific biological network. After testing the acute toxicity of imidacloprid, we are currently sequencing the transcriptomes of exposed zebrafish embryos.

Transcriptomics data will be used to quantify the NPA and to generate mechanistic hypotheses into imidacloprid toxicity.

Together with the standard toxicology approaches, which describe phenotypical changes, and systems toxicology methods, which explore the molecular detail underlying such phenotypes, we aim to gain mechanistic insights into causes of adverse outcomes induced by imidacloprid as well as other chemicals.

This method may be applied to toxicological evaluation of chemicals of environmental concern and chemicals of interest in human health as well as chemical mixtures.



Light/dark assay tests the ability of the fish to detect light, escape danger and swim. Dark 10-minute periods are indicated by shaded rectangles.

ANOVA
Control vs. EC5 ***
Control vs. EC30 ***

ANOVA
Control vs. EC5 n.s.
Control vs. EC30 ****

Startle response assay tests motor function and sensory physiology.

Vibration
Control vs. EC5 n.s.
Control vs. EC30 n.s.

Darkness
Control vs. EC5 ****
Control vs. EC30 **

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