

Apical and molecular endpoints in zebrafish embryos exposed to cadmium

Bruna Vieira¹, Janan Gawra², Énia Correia¹, Joana Luísa Pereira¹, Inês Domingues¹ inesd@ua.pt, Laia Navarro-Martín²

¹ CESAM - Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, Portugal.
² Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain

Multiparametric approaches can result in a more accurate environmental risk assessment of contaminants.

This can be pursued by looking at effects of chemicals from the individual and biochemical levels to the transcriptomic and epigenetic levels, with the aim of establishing a link between phenotypic and molecular endpoints.

Aim

Using zebrafish eleutheroembryos the aim of this work was to evaluate developmental, behavioural and biochemical effects to later relate to molecular effects resulting from **cadmium (Cd)** exposure.

TAKEAWAY

The integration of molecular and apical endpoints will allow the identification of biomarkers of exposure to cadmium. This data will contribute to understand the **Cd adverse outcome pathway** and validate epigenomic effects as a valuable tool to understand effects of contaminants at larger ecological scales.

Acknowledgements

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METHODS – CADMIUM EXPOSURE

96 hours post fertilization zebrafish (*Danio rerio*) eleutheroembryos exposed for 24 h to cadmium (Cd)

Preliminary range-finding tests were performed to determine LC₅₀ and EC₅₀ for swimming bladder effects. In subsequent tests concentrations used were NOEC (no observed effect concentration for swimming bladder effects), NOEC/3, NOEC/10, NOEC/30, NOEC/100 and control)

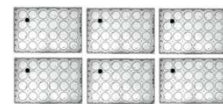


Developmental, biochemical and molecular endpoints: 10 organisms per well, 16 replicates (wells) per treatment

8 Pools of 10 organisms collected for determination of glutathione-s-transferase (GST), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), lipid peroxidation (LPO), and acetylcholinesterase (AChE)

8 Pools of 10 organisms preserved for transcriptomic and DNA methylation analysis

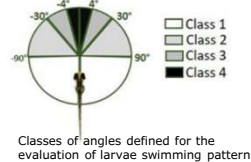
At present, we are performing transcriptomic and DNA methylation analysis to evaluate the effects of Cd exposure at the genomic level



Behavioural endpoints: 24 organisms (individually exposed) per treatment

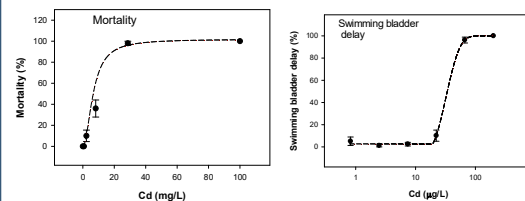
Basal locomotor activity and swimming patterns assessed using the **Zebbox** video tracking system.

Swimming patterns evaluated through the larvae path angles



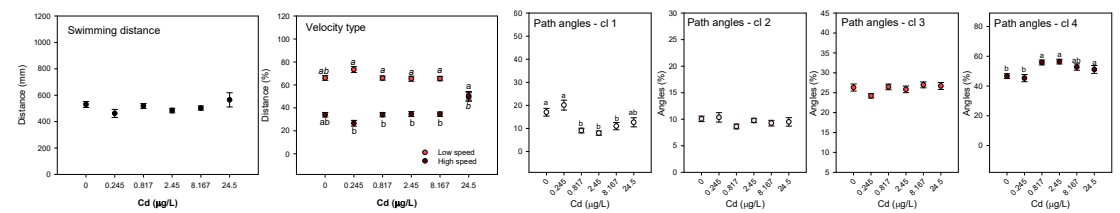
Classes of angles defined for the evaluation of larvae swimming pattern

Mortality and developmental endpoints



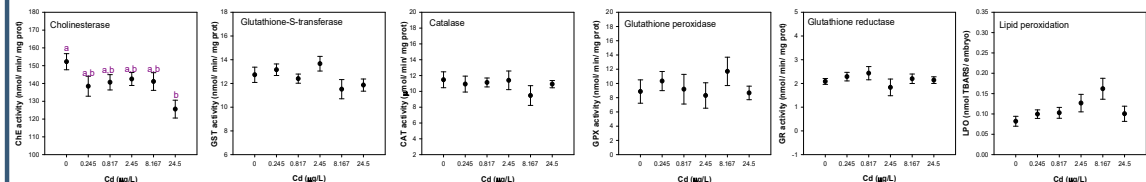
- ✓ LC₅₀ = 10.32 mg/L (standard error = 1.09)
- ✓ EC₅₀ = 34.38 µg/L (st err = 1.33x10⁸)

Behavioural endpoints



- ✓ Behavioural parameters showed at the highest concentration changes in the proportion of rapid/slow movements
- ✓ Changes in the proportions of zigzag movements (class 1 angles) and straight movements (class 4 angles) suggest effects in the fish swimming patterns

Biochemical endpoints



- ✓ AChE activity was decreased suggesting neurotoxicity elicited by the tested Cd concentrations
- ✓ The antioxidant system did not seem to have been activated (no changes in GST, CAT, GPx or GR activities were detected).
- ✓ No lipid peroxidation detected

OUTCOME