Impairment of sensory organ development in petroleum-exposed zebrafish embryos - response of the visual system

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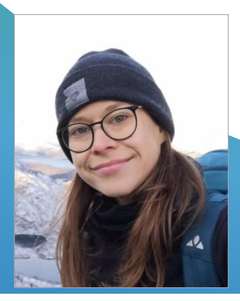
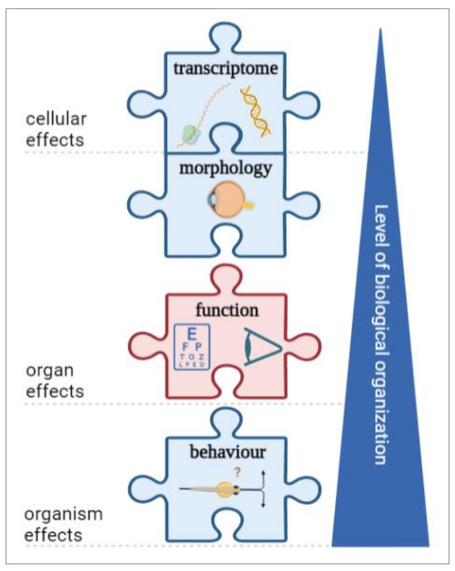


Study Background

The oculotoxic mode of action of environmentally relevant concentrations of crude oil was demonstrated on multiple levels of biological organization.[1],[2]

- Downregulation of genes associated to phototransduction
- Disruption of retinal lamina organization
- Reduced response in light/dark-transition



Aims & Objectives

- Does the oculotoxicity of crude oil also manifest in visual function?
- Are also other behavioral patterns affected by crude oil exposure?

Take-home messages

- The visual system is a main target of crude oil toxicity.
- Environmentally relevant conc. affect locomotion behavior

Experimental Setup

Preparation of water-accommodated fractions (WAF)

Napthenic North Sea crude oil

Finasol® 51 OSR dispersant

LEWAF 1:50*

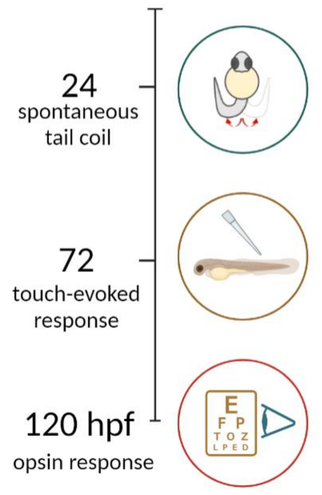
CEWAF 1:200*

HEWAF 1:2000*

* In artificial water (w/v)

LEWAF, CEWAF & HEWAF = low-energy, chemically-enhanced, & high-energy WAF

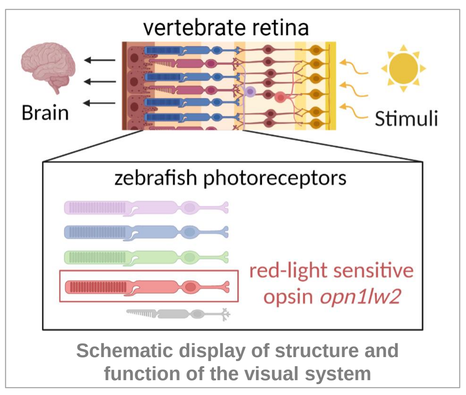
Setup



Opsin Response

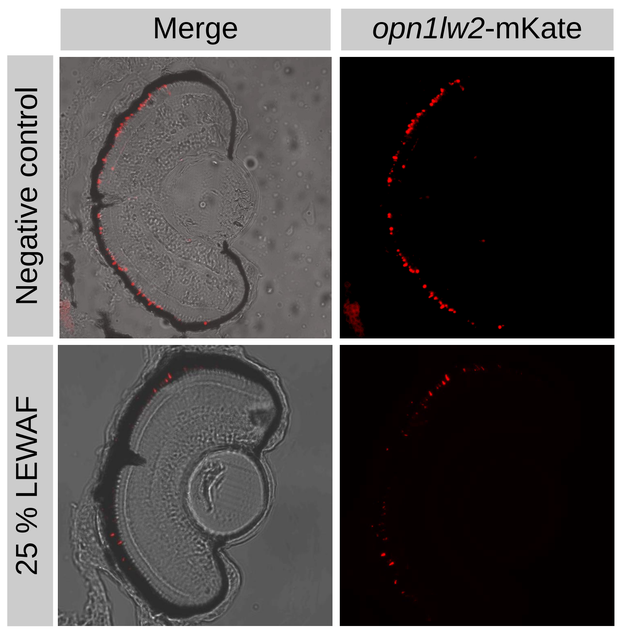
Method

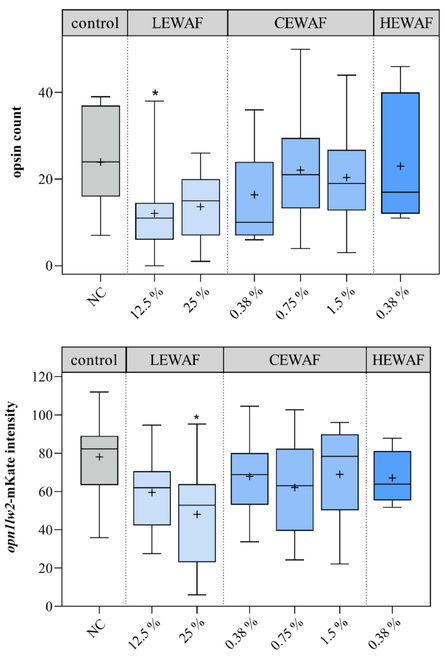
- Retinal cryosections (12µm, coronal) of tg(LWS) transgenic zebrafish [5]
- Confocal LSM of *opn1lw2*-mKate
- ImageJ quantification
  - *Opn1lw2* photoreceptor (PRC) count
  - *Opn1lw2* signal intensity



Schematic display of structure and function of the visual system

Opn1lw2-PRC count





opsin count + signal intensity per eye of zebrafish (120 hpf) exposed to CEWAF/ LEWAF. Shapiro-Wilk Normality test + non-parametric Kruskal-Wallis One-way ANOVA on ranks with Dunn's post hoc test for multiple comparison. (n=3, HEWAF n=1) *p < 0.05)

Discussion

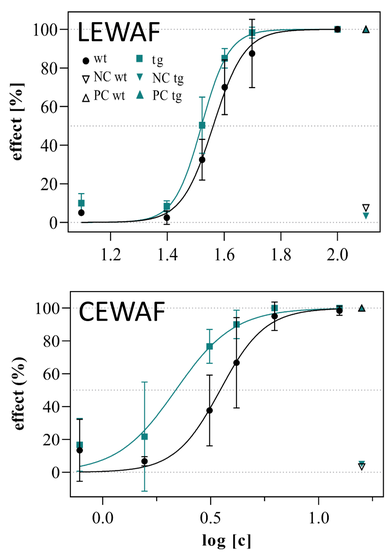
	PRC count
LEWAF	↓
CEWAF	↓
HEWAF	–

Underlying modes of action

- Oxidative stress → apoptosis in retinal pigment epithelium and photoreceptor cells [6]
- Interference with Ca^{2+} membrane permeability [7][8]
- AhR-dependent pathway [9]

Acute Toxicity

Acute Toxicity



Acute toxicity of LEWAF/CEWAF in FET with Cxcr4b:eGFP transgenic (tg) vs wildtype (wt) zebrafish at 120 hpf (LEWAF:n wt/tg=2/3, CEWAF: n wt/tg=3/3

Discussion

- EC_5 values applied for environmentally relevant exposure concentrations

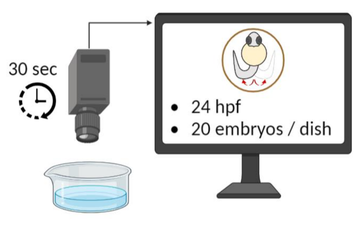
  → **LEWAF: 25%**

  → **CEWAF: 1.5%**

- Similar sensitivity of wildtype and transgenic zebrafish embryos
- Increased acute toxicity by addition of chemical dispersant

Behavioral Response

Spontaneous tail coil

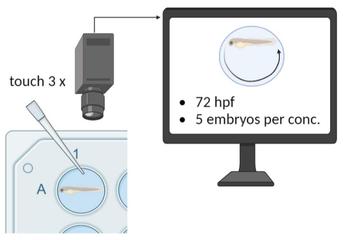


Tail coil frequency of 24 hpf embryos

Results

LEWAF	↓	↓
CEWAF	–	↓
HEWAF	↑	–

Touch-evoked response



Average swimming distance of 72 hpf embryos

- Interference of crude oil components with motoneuron connectivity [3]
- Interaction of crude oil components with neurotransmitter system [4]

Conclusion

→ Reduction of *opn1lw2-PRC count* indicates reduced visual capacities

→ Strong behavioral alterations at very low concentrations that may also affect population level [10]

The results shown here strengthen the line of evidence for an oculotoxic mode of action of crude oil.

Literature

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Illustrations created in BioRender.com

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