

An Eawag Spin-Off

aQUATOX solutions

Zebrafish embryo toxicity tests

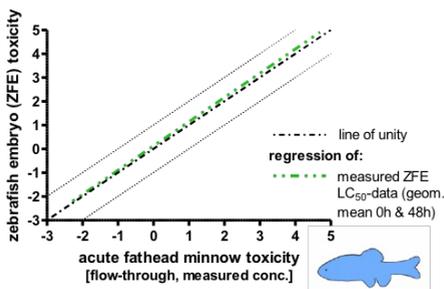
Prediction of acute fish toxicity of water samples and chemicals according to OECD 236 and ISO 15088

Background

The use of zebrafish (*Danio rerio*) embryos for the prediction of fish acute toxicity of chemicals and effluents has become a valuable and increasingly accepted alternative to the animal test with juvenile or adult fish. Up to 5 days after fertilization, until the onset of independent feeding, the embryo does not fall under animal welfare regulation and is suspected to not suffer pain as adult animals because its nervous system is still in development. Yet, it holds the complexity of a whole organism.



Over the past years, many studies showed the excellent correlation of effective concentrations causing 50% lethality (LC50s) obtained in fish and zebrafish embryos e.g. 1 and 2). The zebrafish embryo instead of fish is used for effluent testing in Germany since 2005 (according to DIN EN ISO 15088³⁾); and testing of chemicals with the zebrafish embryo was adopted in July 2013 by the OECD (guideline 236⁴⁾).



What is possible?

- Chemical testing according to OECD 236
 - Determination of LC_x , EC_x , NOEC, LOEC, Ntc^5) and other statistical values
 - Range-finding test with or without chemical analytics
 - Follow-up detailed testing with or without chemical analytics
 - Consultation regarding test chemical stability
- Water/Effluent sample testing according to ISO 15088
 - Determination of fish toxicity of any kind of water sample (effluents, surface water, extracts, etc.)
 - Determination of LID (lowest ineffective dilution)
- Flexible test designs according to client needs and sample characteristics.

Test principle

Fish Embryo Acute Toxicity (FET) Test (OECD 236) for assessing the acute toxicity of chemicals:

Fresh fertilised zebrafish eggs are exposed to the test chemical for a period of 96 hrs. Every 24 hrs, up to four apical observations are recorded as indicators of lethality: coagulation of fertilised eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac, and lack of heartbeat. At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the four apical observations recorded, and the LC50 is calculated.



Fish egg test (DIN EN ISO 15088) for assessing the acute toxicity of water constituents and effluents:

Fresh fertilised zebrafish eggs are exposed to the water/effluent samples for a period of 48 hrs. After 24 hrs and 48 hrs, four apical observations are recorded as indicators of lethality: coagulation of fertilised eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac, and lack of heartbeat. At the end of the exposure period, acute toxicity is determined based



on a positive outcome in any of the four apical observations recorded and the lowest ineffective dilution (LID - dilution at which 90% of the fertilised eggs survive) is given as test result.

Sub-lethal effects:

Scoring of apical observations resembling sub-lethal effects, such as occurrence of edema, malformations, deformations or uncontrolled movements are routinely included for both tests.

Further Options

- **Molecular responses:** Gene expression analysis of indicator genes for e.g. biotransformation, metal- or oxidative stress, immune regulation, pathogen defense and many others
- **Impact on growth:** Embryo growth over 5 days
- **Cardio effects:** Heart beat rate
- **Developmental effects:** Head and eye size
- **Neurotoxic effects:** Behavior

References

1) Knöbel et al., Predicting adult fish acute lethality with the zebrafish embryo: relevance of test duration, endpoints, compound properties, and exposure concentration analysis. *Environmental Science and Technology*. 2012; 46, pp. 9690-9700. **2)** Belanger et al., Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. *Environmental Toxicology and Chemistry*. 2013; 32(8), pp. 1768-83. **3)** International Standard ISO 15088:2007(E), Water quality – Determination of the acute toxicity of waste water to zebrafish eggs (*Danio rerio*), International Organization for Standardization, 2007. **4)** OECD Guideline for Testing of Chemicals. Test No. 236: Fish Embryo Acute Toxicity (FET) Test; Organization for Economic Cooperation and Development: Paris, 2013. **5)** Stadnicka-Michalak et al. (2018). A validated algorithm for selecting non-toxic chemical concentrations. *ALTEX-Alternatives to animal experimentation* 35 (1), 37-50.

