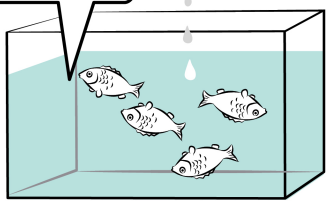
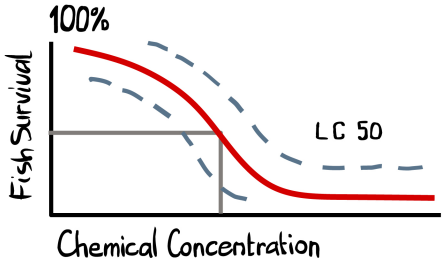
THE STORY OF THE IMMORTAL FISH

Standard fish acute toxicity test

96 h of testing! What a torture! At the end they kill me anyway

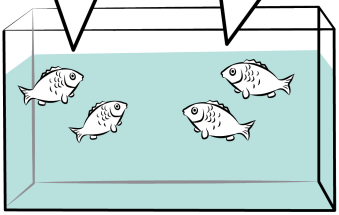
25 l chemical



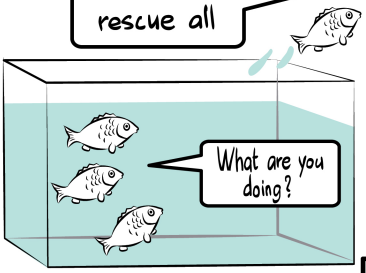


Oh No !!! We are next.

I have an idea!



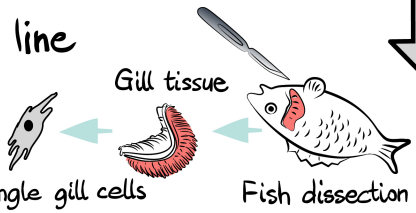
I know how to rescue all



Establishment of a fish cell line

I can double up forever

We are immortal

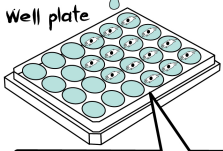


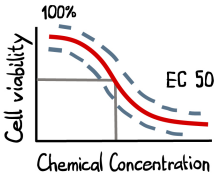
Fish cell line test

Now they can do endless testing without killing fish!

2 ml chemical

24 Well plate





More Parameters! Less Chemical! Only 24 h testing! Same results!

Thanks to our savior!

We are safe!

aquatox solutions

RTgill-W1 cell line assay

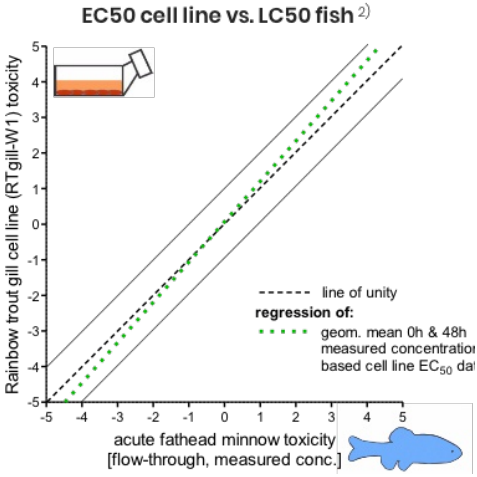
Prediction of acute fish toxicity of water samples and chemicals

Background

In acute fish toxicity testing the gill epithelia can be considered as a primary uptake and target site of water contaminants. Damage to the gill epithelia can cause impaired functioning of the organism and eventually death. This impaired functioning is reflected by a reduced cell viability, which is determined in the RTgill-W1 cell line assay.

The permanent gill cell line from rainbow trout – RTgill-W1 [1] – can be exposed directly to dilutions of test chemicals or water samples. Cell viability is determined using a combination of fluorescent indicator dyes that allow the detection of three different toxicity parameters on the same set of cells.

In screening studies, an excellent correlation of RTgill-W1 cell line toxicity data with results from fish acute (OECD 203) and fish embryo acute toxicity (OECD 236 and ISO 15088) testing has been demonstrated.[2-5]



What is possible?

- Chemical testing according to OECD 236
  - Determination of EC_x, NOEC, LOEC, NtC[6] 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

Advantages and Applications

Due to the use of the permanent RTgill-W1 cell line, no fish needs to be sacrificed for the test. Testing is performed in small test vessels (one 24-well plate per test) and therefore a very small amount of sample suffices. The test exposure duration is 24 h compared to 96 h for fish acute fish toxicity testing. Given these facts, the assay is optimally suited for pre-screening during product development or testing of any kind of water sample for environmental safety regarding fish toxicity.

Standardization

The RTgill-W1 cell line assay for testing of water samples and chemicals is currently under review by ISO. An application to OECD is in preparation.

Limitations

Given the nature of the RTgill-W1 cell line being established from epithelial gill cells of rainbow trout, chemicals that specifically affect certain pathways in fish, such as neurotransmission cannot be detected.

Further Options

Information may be obtained using the same permanent fish cell line for studies on sub-lethal effects via gene expression analysis of indicator genes for e.g. metal- or oxidative stress, immune regulation and pathogen defense, biotransformation and many others, to learn about early responses in fish to possible contaminants in water samples or to test chemicals.

The impact of chemicals on fish growth can be predicted based on in vitro data with the RTgill-W1 cell line combined with computational modelling[7]. Compared to the Fish Early-life Stage Toxicity Test (OECD 210), the in vitro prediction method is simple, inexpensive, and fast.

References

1) Bols et al., Development of a cell line from primary cultures of rainbow-trout, Oncorhynchus mykiss (Walbaum), gills. Journal of fish diseases. 1994; 17(6), pp. 601-611. **2)** Tanneberger et al., Predicting fish acute toxicity using a fish gill cell line-based toxicity assay. Environmental Science and Technology. 2013; 47, pp. 1110-1119. **3)** Dayeh et al., Applying whole-water samples directly to fish cell cultures in order to evaluate the toxicity of industrial effluent. Water Research. 2002; 36, pp. 3727-3738. **4)** Dayeh et al., Ammonia-containing Industrial Effluents, Lethal to Rainbow Trout, Induce Vacuolisation and Neutral Red Uptake in the Rainbow Trout Gill Cell Line, RTgill-W1. Alternatives to Laboratory Animals. 2009; 37, pp. 77–87. **5)** Natsch et al., Accurate Prediction of Acute Fish Toxicity of Fragrance Chemicals With the RTgill-W1 Cell Assay. Environmental Toxicology and Chemistry. 2017; 9999, pp. 1– 11. **6)** Stadnicka-Michalak et al. (2018). A validated algorithm for selecting non-toxic chemical concentrations. ALTEX-Alternatives to animal experimentation 35 (1), 37-50. **7)** Stadnicka-Michalak, et al., Toxicology across scales: cell population growth in vitro predicts reduced fish growth. Science Advances. 2015; 1(7), pp. 1-8.

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