

# Implementation of Test Guideline No. 249 - Fish Cell Line Acute Toxicity: The RTgill-W1 cell line assay

*Commonly used acronym:* Implementation of OECD TG 249

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## SCOPE OF THE METHOD

<b>The Method relates to</b>	Animal health, Environment, Human health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Animal derived cells / tissues / organs
<b>Species from which cells/tissues/organs are derived</b>	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
<b>Type of cells/tissues/organs</b>	Gill cell line (RTgill-W1)
<b>Specify the type of cells/tissues/organs</b>	Colon adenocarcinoma cell line (Caco2) and hepatocellular carcinoma cell line (HepG2)

## DESCRIPTION

### Method keywords

in vitro

in vivo

extrapolation

Cell viability

acute toxicity

human health

### Scientific area keywords

Toxicology

Ecotoxicology

ecology

biology

cell biology

### Method description

OECD TG 249 was developed by Prof. Dr. Kristin Schirmer, from Eawag. The OECD 249 aims at assessing the acute toxicity of single or mixtures of chemicals using the permanent rainbow trout gill cell line, RTgill-W1. RTgill-W1 cells in a confluent monolayer in 24-well plates are exposed to the test chemical(s) for 24 hours. The assays are performed in a defined, protein- and animal-component-free buffer, L-15/ex. Acute toxicity is assessed via the combination of three cell viability assays targeting distinct cell compartments. Specifically, the assays use resazurin-based dye for measuring metabolic activity, 5-carboxyfluorescein diacetate acetoxy methyl ester to evaluate the integrity of the cell membrane and Neutral Red to assess the integrity

of the lysosomal membrane. The resulting concentration-response curves allow the determination of the effective concentration causing 50% loss in cell viability, EC50, as well as no-toxic concentrations, i.e. the lowest-observed effect concentration (LOEC) and no-observed effect concentration (NOEC). The assay allows the extrapolation of *in vitro* to *in vivo* toxicity with the derived EC50 corresponding to the predicted 96 hours lethal concentration to 50% of the tested organisms (LC50) in fish. Moreover, the described methodology was adapted to allow the testing of two human cell lines, Caco2 and HepG2, hence promoting the assessment of effects on human health. The adaptations include the seeded cell density, the test media and the exposure period.

### **Lab equipment**

The method requires the following special equipment: biosafety cabinet, incubator, inverted microscope, fluorescent plate reader.

### **Method status**

Published in peer reviewed journal

Validated by an external party (e.g. OECD, EURL ECVAM,...)

### **PROS, CONS & FUTURE POTENTIAL**

#### **Advantages**

Allows the determination of *in vivo* acute toxicity using an *in vitro* assay.

The use of three viability assays targeting different cell compartments provides an indication on the chemical mode of action.

#### **Challenges**

Does not allow the assessment of chronic exposure effects.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

OECD 249 (2021) - OECD Guidelines for the Testing of Chemicals Dayeh et al (2004) - Ecotoxicology and Environmental Safety

### Associated documents

[OECD 249 \(2021\) - OECD Guidelines for the Testing of Chemicals.pdf](#)

[Dayeh et al \(2004\) - Ecotoxicology and Environmental Safety.pdf](#)

### Links

[OECD 249 \(2021\) - OECD Guidelines for the Testing of Chemicals](#)

[Dayeh et al \(2004\) - Ecotoxicology and Environmental Safety](#)

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** Ghent University (UGent)

**Department** Department of Animal Sciences and Aquatic Ecology

**Country** Belgium

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