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*

Created on: 30-03-2022 - Last modified on: 30-03-2022

Contact person

João Barbosa

Organisation

Name of the organisation Ghent University (UGent)
Department Department of Animal Sciencies and Aquatic Ecology
Country Belgium

SCOPE OF THE METHOD

The Method relates to	Animal health, Environment, Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Rainbow trout (Oncorhynchus mykiss)
Type of cells/tissues/organs	Gill cell line (RTgill-W1)
Specify the type of cells/tissues/organs	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 diactate 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

Coordinated by

Financed by