Viability assay with fish gill cell line to assess acute toxicity

Commonly used acronym: RTgill-W1 cell line assay

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Organisation
Name of the organisation: Vlaamse Instelling voor Technologisch Onderzoek (VITO)
Department: Health
Country: Belgium
Geographical Area: Flemish Region

Partners and collaborations
Swiss Federal Institute of Aquatic Science and Technology (EAWAG)

SCOPE OF THE METHOD

<table>
<thead>
<tr>
<th>The Method relates to</th>
<th>Environment</th>
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</thead>
<tbody>
<tr>
<td>The Method is situated in</td>
<td>Translational - Applied Research</td>
</tr>
<tr>
<td>Type of method</td>
<td>In vitro - Ex vivo</td>
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<tr>
<td>Species from which cells/tissues/organs are derived</td>
<td>Rainbow trout, Oncorhynchus mykiss</td>
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<tr>
<td>Type of cells/tissues/organs</td>
<td>Gill tissue</td>
</tr>
</tbody>
</table>

DESCRIPTION

Method keywords

cell viability test
fish gill cell line
cell metabolic activity
lysosomal membrane integrity
cell membrane integrity

Scientific area keywords
fish acute toxicity
chemical exposure

Method description
The rainbow trout gill cell line assay quantifies cell viability using fluorescent measurements for metabolic activity (Alamar Blue, AB), cell membrane integrity (5-CarboxyFluorescein DiAcetate AcetoxyMethyl ester, CFDA-AM) and lysosomal membrane integrity (Neutral Red, NR). Chemicals are added to confluent RTgill-W1 cell monolayers in 24-well plates with L-15/ex medium (a simplified version of L-15 cell culture medium without serum). Cells are incubated for 24 hours in the incubator (19°C, normal atmosphere, in the dark). At the end of the exposure, cell viability measurements are performed with 3 fluorescent indicator dyes on the same set of exposed cells.

Lab equipment
Laminar flow ;
Incubator (room temperature, no CO2) ;
Microplate reader for fluorescence detection.

Method status
History of use
Internally validated
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages
Cell line model with limited requirements ;
Robust assay: repeatability and reproducibility is shown through inter- and
intralaboratory studies;
Alternative model to predict fish acute toxicity.

Challenges
Exposure of chemicals (bioavailability).

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References
Fischer, M; Belanger, SE; Berckmans, P; Bernhard, MJ; Blaha, L; Schmid, DEC; Dyer, SD; Haupt, T; Hermens, JLM; Hultman, MT; Laue, H; Lillo-clip, A; Minarikova, M; Natsch, A; Novak, J; Sinnige, TL; Tollefson, KE; von Niederhausern, V; Witters, H; Zupanic, A; & K. Schirmer (2019). Repeatability and reproducibility of the RTgill-W1 cell line assay for predicting fish acute toxicity. Toxicological Sciences, 169 (2), 353-3640.
ISO 21115:2019. Water quality — Determination of acute toxicity of water samples and chemicals to a fish gill cell line (RTgill-W1).