TU058

Setting up aquatic toxicity testing using zebrafish, daphnia and algae

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OBJECTIVE

To optimize cost and time of assays for PRESCREENNG evaluation of effects on aquatic organisms based on OECD guidelines

INTRODUCTION

Industries are responsable of collecting information on the properties and the uses of substances that they manufacture or import at or above one ton per year. They have to make an environmental hazard and risk assessment presented by the substance. The OECD Guidelines are a tool for assessing the potential effects of chemicals on human health and the environment. Accepted internationally as standard methods for safety testing, the Guidelines are used by professionals in industry, academia and government involved in the testing and assessment of substances.

Companies request miniaturized version of these guidelines that allows pre-screening of hazard and risks related to the substances in order to discard the ones that display toxicity in early phases of development.

In order to prevent the deterioration of the aquatic environment caused by chemicals, **fast and cost/time-effective screening assays** are needed in the early discovery phases of industries to assess the effects of chemicals candidates in the environment. In this case, **aquatic toxicity high throughput screening assays** based on OECD guidelines are being developed as an advantageous prior step to later phases of discovery, fulfilling the **3R principles** at the same time.

Methodology

High-Throughput Alga Microassay

Scaled down version of OECD 201

Test species: *P. subcapitata*

Endpoint: Cell growth inhibition

Test design

✓ Reduced sample and alga volume

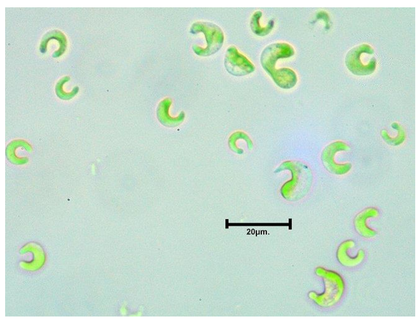
- 96 wells microplate
- 10 test concentrations per compound

✓ Minimal handling for Data acquisition

- One plate per compound
- Fluorescence on Plate reader

✓ Automated data acquisition and analysi

- Integrated data analysis workflow
- NOEC, LOEL, IC50 determination



Enhanced FET Acute Toxicity Assay

Based on OECD 236

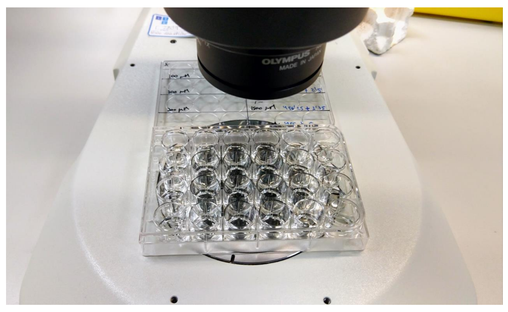
Test species: Zebrafish (*D. rerio)*

Endpoint: Mortality + Morphological alteration

Test design

✓ Optimized plate layout

- 24 well microplate
- 5 embryos per well, 3 wells per condition
- 8 test concentrations per compound
- One plate per compound



Morphological alteration endpoints

Head Malformations	Head shape alteration, Jaw Morphology, Microphtalmia
Heart Malformations	Heartbeat alterations, Irregular shape, Edema
Body shape Malformations	Notochord morphology, Somite Morphology, Curvature, Length
Yolk Malformations	Irregular shape, Yolk opacity, Edema
Others	Fins morphology, Hatching delay, Necrotic tissue, Lethality

✓ Additional sublethal endpoints considered

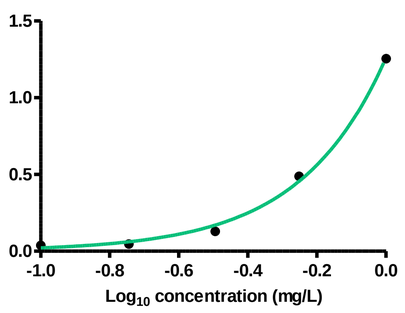
- Morphological alterations

Case study

Determination of IC_{50}

Potassium dichromate

OECD Reference Toxicant



IC_{50}= 11.8 mg/L

✓ **Increased efficiency in sample processing**

✓ **Cost-effective method of aquatic toxicity assessment**

✓ **Production of relevant information for rapid decision-making process**

Determination on EC_{50} and LC_{50}

Difenoconazole

Fungicide

Concentration µM	Dead	Affected
0.1	20%	20%
1	10%	10%
10	30%	100%
100	100%	100%
1000	100%	100%

Figure 2: Results from range-finding test

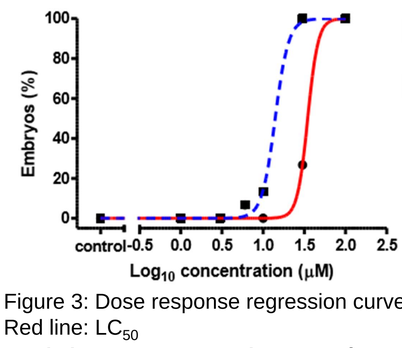


Figure 3: Dose response regression curve. Blue line: EC_{50} Red line: LC_{50}

EC_{50} = 4.61 µM

LC_{50} = 17.12 µM

Zebrafish embryos are exposed to eight concentrations selected from the range finding test results. EC_{50} value is obtained considering morphological alteration and mortality (blue line) while standard LC_{50} calculation is only based on mortality data.

✓ **Higher sensibility than FET in toxic effect assessment**

✓ **Increased substance testing capacity**

✓ **Minimized use of testing animal (3Rs)**

Work in progress

- **Alga Microassay validation with an array of reference toxicants**
- **Computer image analysis for the evaluation of mortality and morphological alterations on Enhanced FET Acute Toxicity Assay**
- **Development and validation of aquatic toxicity assay on *Daphnia magna* with continuous video tracking on a multi-cell exposure system**
- **Data analytics, reporting and integration platform for the integrated evaluation of aquatic toxicity**

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