Optimizing OECD 236 Fish Embryo Toxicity (FET) Test for Challenging Substances: Evaluation of a Closed-System Design for Reliable Aquatic Toxicity Testing

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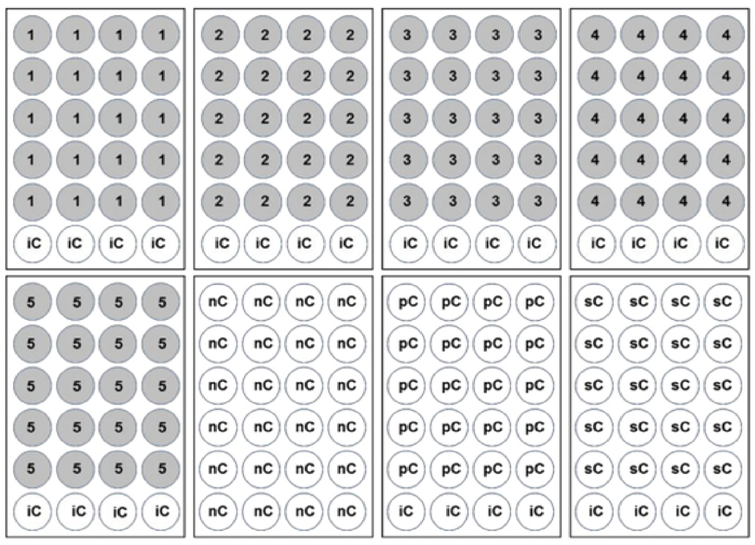
Introduction

Standardized toxicological tests are essential for assessing the intrinsic hazard of chemicals to aquatic organisms. While established test designs are effective for stable, water-soluble substances, they often fall short when applied to chemicals with **challenging physico-chemical properties**. In alignment with the **3R animal welfare principles**, we are facing an increasing demand for **Fish Embryo Toxicity** (FET) tests (OECD TG 236) [1].
The chemicals tested are often difficult test substances. One group of difficult substances are volatile compounds which could not be reliably tested using standardized 24-well polystyrene plates due to significant losses during exposure. This limitation prompted us to develop an adapted test design. Guided by existing recommendations for testing difficult substances (OECD 23 [2] and ISO 14442 [3]), our approach focuses on improving exposure consistency while maintaining **practicality**, **cost-effectiveness**, and **multipurpose suitability** for a range of challenging substances. We have used PTFE-capped screw-cap glass vials, providing an inert and closed alternative to the commonly used polystyrene 24-well plates or impractical single small petri dishes. We are currently evaluating the **versatility of the system** for a range of test substances, focusing on substances that challenge conventional set-ups such as **volatile**, **adsorbing substances** or cases where the plate material itself may interfere with total organic carbon (**TOC**) analysis.

Picture 1: Glass vial design for one treatment

Materials & Methods

Test Guideline: Fish Embryo Acute Toxicity Test (OECD TG 236)
Species: Fertilized zebrafish eggs
Exposure Duration: 96 hours at 26 ± 1°C; 16:8 h light/dark cycle
Exposure System: *PTFE screw cap glass vials (1 embryo per vial);* ***Picture 1 + 3***
Replicates: 20 embryos per concentration
Test Concentrations: 5 concentrations + controls
Endpoints: Lethal and sublethal effects assessed daily



Picture 2: Conventional multi-well design according to OECD 236 [1]
1-5= five test concentrations; nC= negative control (dilution water); iC= internal plate control (dilution water); pC= positive control (3,4-DCA 4mg/L); sC= solvent control

Picture 3: Glass vial design with test solution and embryo

Results & Discussion

Goal: Adapt FET test system for physico-chemically challenging substances to achieve **stable exposure concentrations**.

Example:

Table 1: Comparison of recoveries after incubation of a volatile substance in a conventional multi well (plate + self-adhesive foil + lid, picture 2) vs. the PTFE screw cap glass vial system (picture 3) [nominal 100 mg/L].

Incubation Hours	% Recovery	
	Multi-well	Glass vials
48 h	60	90
96 h	5	60

Advantages

- Much higher recoveries → better concentration-effect calculation possible for risk assessment (Table 1)
- Compliant with OECD Guideline (all **validity** criteria are **met)** including optimal temperature and light distribution
- Individual embryo incubation → clear developmental stage attribution
- **Replaces polystyrene** which is hydrophobic, not inert, and not gas tight
- Rack layout **with exchangeable vials and internal control**
- **Reduction of renewal periods** → stress reduction for the test organisms → practicability, less effort & costs
- **Higher** test **volumes** (10-11 mL test solution / embryo vs 2 mL in the 24-well plates) → Reduces the need for additional abiotic stability samples for analytics

Conclusion

There is currently no established guidance for testing difficult substances in the Fish Embryo Toxicity (FET) Test. To address this, we developed a modified system using PTFE screw cap glass vials as a gas-tight, inert alternative to polystyrene plates. It aims to ensure stable exposure for challenging substances while meeting OECD TG 236 validity criteria.

The system reduces organism stress, lowers costs, and is easily transferable. Future evaluations will assess its broader applicability and potential for flow-through adaptations as already discussed by Lammer *et al.* [4], using inert materials, higher volumes and in a more flexible set-up.

Contact us if you have any question

References & Acknowledgements

[1] OECD No. 236, Guideline for the Testing of Chemicals: *Fish Embryo Acute Toxicity (FET) Test*, 2013
[2] OECD No. 23, Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals, 2019
[3] ISO No. 14442, Guidelines for Algal Growth Inhibition Tests with Poorly Soluble Materials, Volatile compounds, Metals and Waste Water, 2006
[4] Lammer *et al.* 2009, Development of a flow through system for the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*)

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