

Optimization of a long-term toxicity flow-through setup with *Daphnia magna* to test accumulation potential of different classes of pollutants

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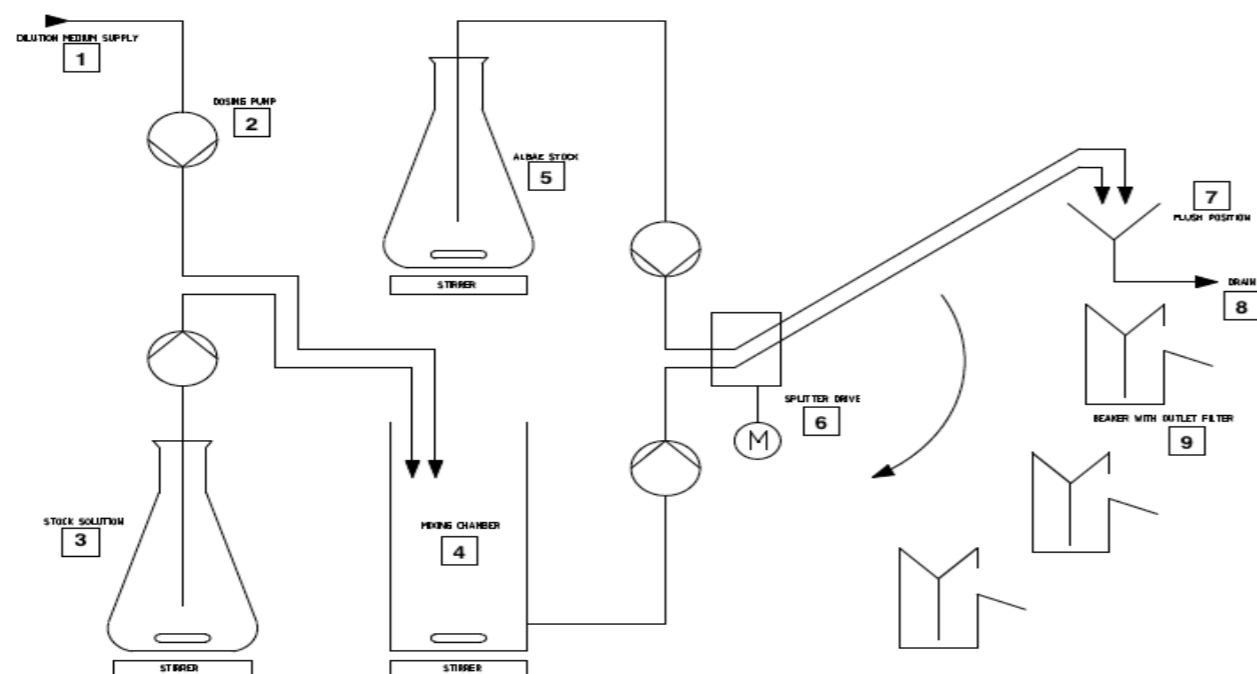
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Aim

The main goal of this project was to refine the technical design of a new flow-through system for a *daphnia magna* reproduction test (OECD211), to optimize environmental conditions for the daphnids and improve the exposure of the daphnids to unstable test items with extremely short half lives.

Apparatus



Picture 1: One module of the flow-through system by Péquitech MSR Systeme

- Separate modules for each test concentration and control
- Each module holds 10 exposure flasks (9) for individual replicates
- Food suspension (5) is pumped separately from test medium into each individual exposure flask
- Computer controlled slewing arm (6) guarantees exact distribution of the test medium to all replicates
- Exposure flasks with 50 mL volume, opening on the side; stainless steel sieve embedded in opening; small glass funnel conducts test medium to the bottom of the exposure flask, provides optimal exchange of test medium and reduces turbulence

Initial Testing:

- Design of exposure flasks, evaluation of parameters for automated test medium preparation
- Test medium exchange rate: 30 theoretical exchanges / day - upper limit for valid tests (result: mean offspring 89 per survivor)
- Food amount: high enough to ensure sufficient reproduction (mean offspring 169 per survivor at 0.45 mg TOC/*Daphnia*/day), low enough to minimize adsorption, additional feeding with e.g. TetraMin[®] not needed

Testing of Environmental Conditions under Flow Through Conditions with High Test Medium Exchange Rate

- Concept: 21-day reproduction reference test with hydrolytically stable test item Atrazine to ensure comparable test item exposure, flow-through and semi-static test design in parallel
- Goal: Comparison of environmental conditions for the daphnids in semi-static and flow-through test design
- Challenge: Extremely high theoretical medium exchange rate (30 times, conditions substantially exceeding normal requirements) in the flow-through system to discover possible side effects to daphnids due to turbulence, versus once every 2-3 days in semi-static test design
- Concentrations: 0.016, 0.08, 0.5, 2.0 and 10 mg/L Atrazine

Results:

- Mortality in both exposure designs $\leq 20\%$
- Mean offspring per survivor in both systems above OECD 211 validity criterion of 60 (flow-through: 119, semi-static: 172)
- Threshold concentrations for reproduction (NOEC / LOEC) for both exposure designs were identical
- First brood was delayed in the flow through system (5 days), this was attributed to different feeding conditions
- Dose concentrations in both systems were between 98 and 105% of nominal

Evaluation of Constant Test Item Exposure Conditions with the Highly Instable Test Item Cymoxanil

- Concept: 21-day reproduction test with fast degrading test item Cymoxanil
- Goal: Show that the new flow-through system can maintain the parent compound concentration $> 80\%$ throughout the test
- Challenge: DT_{50} of Cymoxanil in M7 Medium: 5 hours, 30 times theoretical medium exchange rate to compensate DT_{50}
- Concentrations: 0.04, 0.08, 0.16, 0.33, 0.65, 1.3 mg/L Cymoxanil

Results:

- Calculated mean concentrations: between 83 and 104% of nominal demonstrate the suitability of the flow through system
- Threshold concentrations for reproduction (NOEC / LOEC) were comparable to literature data
- The test was valid according to OECD 211 (mortality $\leq 10\%$, mean control reproduction of 93 offspring per survivor)

Conclusion:

- General advantages: reduction of secondary food effects and handling of test animals
- Dosing of test item is precise and reliable, parent compound concentration can be maintained above 80% for fast degrading test items
- Environmental conditions given by the flow-through system do not affect threshold concentrations
- Despite extreme flow conditions, system delivered valid test results and similar threshold concentrations compared to the semi-static test