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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SETAC Europe 28th Annual Meeting
Rome, Italy, May 12th-17th, 2018

Introduction

Daphnids are playing a role as representative or indicator species for aquatic invertebrates in the environmental risk assessment for plant protection products, chemicals and pharmaceuticals. To investigate chronic toxicity, semi-static *Daphnia magna* reproduction tests following Test Guideline OECD 211 have to be performed. So far in many cases this test design is also used for highly degrading substances, despite the fact that over time (2-3 days) the exposure concentration of the parent test compound is decreasing and the metabolic products are accumulating. To avoid these disadvantages and aim for an ensured, steady exposure level, studies can be performed in flow-through systems. We are presenting a new flow-through system for reproduction testing with *Daphnia magna*.

Apparatus

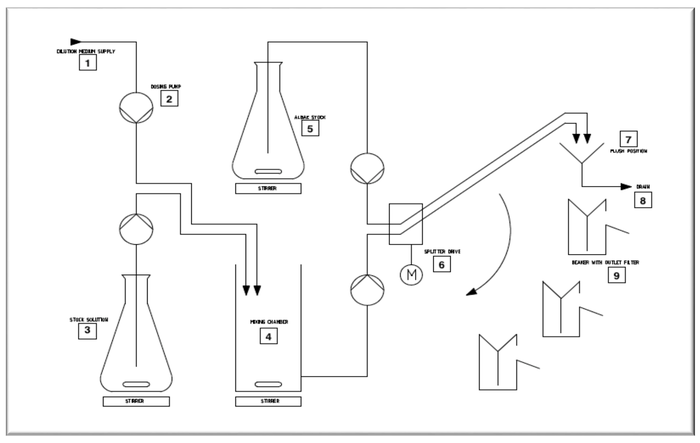

Figure 1: Mechanical drawing of one module of the flow-through system by Péquitec; Dilution medium supply, 2: Dosing pump, 3: Stock solution, 4: Mixing chamber, 5: Algae stock, 6: Splitter drive, 7: Flush position, 8: Drain, 9: Exposure flask

- 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

Test organisms:

- Less than 24 hours old daphnids of the species *Daphnia magna* Straus (bred in the IES Laboratories)

Figure 2: Exposure flask

Medium exchange efficiency

The actual replacement rate of the culture medium in the exposure flasks is lower than the theoretical replacement rate. This phenomenon occurs since old culture media in the flasks is mixed with the freshly incoming culture medium and is therefore not flushed out completely. To determine the true replacement-rate each exposure flask of one module (ten flasks total) was filled with 50 ml of EPA-Medium. The conductivity of the EPA-Medium was measured. Then ultrapure water (Conductivity: 78 µS/cm) was pumped through the system with 40 mL/min (60 renewals per day) and the conductivity of the mixture was measured at defined time points. The conductivity was measured only once per beaker to avoid turbulences. Results: After 50 minutes of dosing ultrapure water, only one percent of the initial conductivity was measured. At 75 minutes the value was at zero percent of initial conductivity. As seen in Figure 3 the first time the conductivity was zero between 50 and 75 minutes. 60 minutes were calculated as the time necessary to completely replace the EPA-Medium by the ultrapure water in the exposure flask. This resulted in an actual medium exchange rate of 24 times a day.

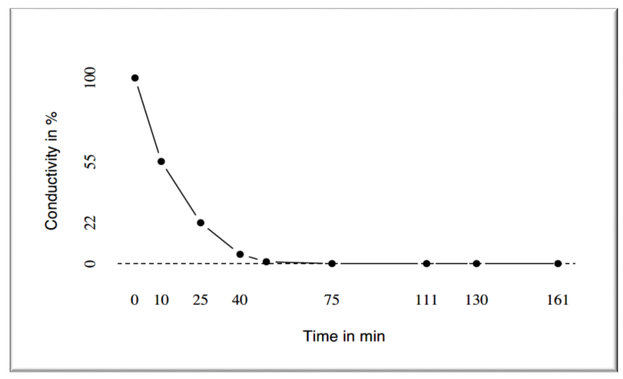

Figure 3: Medium exchange efficiency of the new flow-through system; decline of conductivity within the exposure flask; 60 times theoretical exchange rate per day

Reproduction rates

The mean number of offspring per parent animal in the controls at the end of the test were between 119 and 169 when fed with an algae concentration of 0.45 mg TOC per daphnia per day and at a test medium renewal rate of 30. With an increased food amount of 0.60 mg TOC per daphnia per day at 30 renewals per day the mean number of offspring per daphnia could be increased to 186.

Dosing precision

The stock solutions are automatically dosed into the mixing chambers via PTFE tubes. The diameter of the PTFE tubes is chosen rather small to minimize the residence time of the test item in the tube. The test medium, consisting of culture medium and test item, is stirred for a defined time before it is distributed into the exposure flasks. The stirring ensured that the test item is completely dissolved in the culture medium.

Hydrolytically stable test item – DT_{50}: 86 days

Atrazine was chosen as a model compound because of its presence in the environment in eco-toxicological relevant concentrations and it's stability in water. Concentrations of 0.016, 0.08, 0.5, 2 and 10 mg/L as well as a control (M7 media) and a solvent control (M7 media + 0.008 % DMF) were used for the test. Per dosing cycle 500 mL of culture medium were pumped into the mixing chamber and 40 µL of the stock solution containing the corresponding concentration of test item were dosed into the culture medium. The test medium was stirred for four minutes before it was distributed into the ten replicate exposure flasks. A theoretical renewal rate in the exposure flasks of 30 renewals per day was chosen. Samples were taken from the exposure flasks at 12 different time points to cover a test period of 21 days.

Results: The calculated time weighted means of the dosed concentrations of Atrazine were 0.0163 mg/L (102.1% of nominal), 0.0812 mg/L (101.4% of nominal), 0.4954 mg/L (99.1% of nominal), 1.9052 mg/L (95.3% of nominal) and 9.7241 mg/L (97.2% of nominal) (see Figure 4).

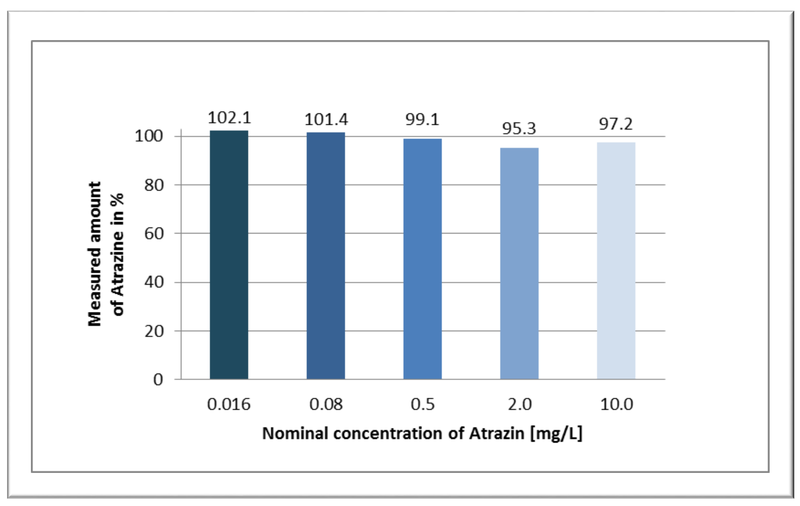

Figure 4: Dosing precision of the new flow-through system; Dosing of Atrazine dissolved in DMF

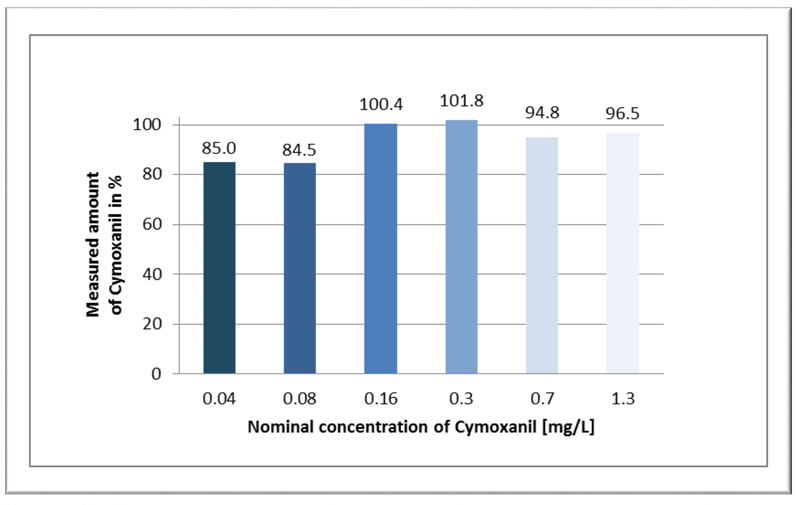

Figure 5: Dosing precision of the new flow-through system; Dosing of Cymoxanil dissolved in DMF

Hydrolytically instable test item – DT_{50}: 5 hours

Cymoxanil was chosen as a model compound for a hydrolytically instable test item. Hydrolysis of Cymoxanil is pH dependent. At pH 5, the chemical is relatively stable. However, in neutral and alkaline waters, it hydrolyzes quickly (pH 7 half-life: 34 hours, pH 9 half-life 31 minutes) (US EPA 1998). The DT_{50} in M7 Media was determined to be 5 hours.
Mixing chambers of 150 mL volume were used to reduce the residence time of the test medium in the mixing chambers. Per dosing cycle 75 ml of M7 media were pumped into the mixing chamber and 6 µL of the corresponding stock solution were dosed into the media. The nominal concentration of solvent was 0.008% DMF. The test medium was stirred shortly and was then distributed into the exposure flasks. The mixing chambers were emptied at the end of every cycle and refilled for the next. A theoretical renewal rate in the exposure flasks of 30 renewals per day was chosen. Samples were taken from the exposure flasks at 12 different time points to cover a test period of 21 days.

Results: The calculated time weighted means of the dosed concentrations of Cymoxanil were 0.034 mg/L (82.8% of nominal), 0.0676 mg/L (84.5% of nominal), 0.1606 mg/L (100.4% of nominal), 0.336 mg/L (103.5% of nominal), 0.616 mg/L (94.82% of nominal) and 1.255 mg/L (96.51% of nominal) (see Figure 5).

Improvements:

- 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% even for fast degrading test items
- Environmental conditions given by the flow-through system do not affect threshold concentrations
- Despite extreme flow conditions, system delivers valid test results and similar threshold concentrations compared to semi-static tests