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Establishment of a Flow-Through System for the Macrophyte Growth Inhibition Test (OECD 239) Including New Endpoints on Photosynthetic Activity

Maintaining constant exposure concentrations during ecotoxicological studies while testing rapidly degradable substances is a challenge. Stable concentrations during exposure are often achieved using flow-through systems. The water-sediment toxicity test with *Myriophyllum spicatum* (OECD TG 239) currently only includes a static and semi-static test design. The aim of our study was to establish a flow-through system for *M. spicatum* toxicity testing.

The standard test design was miniaturised from 2 L to 500 mL and a flow-through system was developed to achieve stable exposure concentration of rapidly degrading substances. The main test design and parameters such as light, temperature and pH were kept as described in the OECD TG 239. The observed endpoints were total shoot length (TSL), fresh (FW) and dry weight (DW). As an additional endpoint, measurement of photosynthetic yield (Y(II)) was established to detect early effects on photosynthesis.

The miniaturised system was compared with the standard test setup using 3,5-dichlorophenol. No significant differences could be detected after 14 days exposure. To prevent excessive growth ¼ Smart & Barko medium was tested as a flow-through medium and achieved similar growth to the standard test setup.

Two model substances were chosen to compare their toxicity in the flow-through and in the static/semi-static system. At first, using the PSII inhibitor bentazone (BT), the growth rate of FW and TSL showed 5-8 times higher EC_{50} values in the flow-through system but with overlapping confidence intervals and no difference for the EC_{50} values of TSL, FW and DW for yield. Using the PAM method with BT, the Y(II) decreased concentration-dependent from day 3. This reduction in Y(II) could be shown to be correlating with the growth rate of the plants. It suggests that the PAM method can detect effects on photosynthesis even before physiological effects can be measured. Atorvastatin, a pharmaceutical, degraded by 30% after 7 days in a semi-static test design, but remained stable in the flow-through system. Stronger visual effects (e.g. necrosis) were observed, as well as lower EC_{20} values for all endpoints, e.g. 1.8 vs. 4.4 m/L for FW, indicating that the flow-through system is suitable for testing of rapidly degradable substances.

Summed up, the miniaturised and standard tests did not differ, degradable substances can be tested more accurately using a flow-through setup, and photosynthetic yield can be an additional endpoint.