# Sediment-Water Chironomid Toxicity Test – **Experiences Using a Flow-Through Device.**



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### Materials & Methods

For Environmental Risk Assessment in the frame of several legislations, toxicity tests with sediment dwellers have to be conducted. Internationally accepted OECD guidelines exist for Lumbriculus (OECD 225<sup>1</sup>) and Chironomus (OECD 218<sup>2</sup>/219<sup>3</sup>). All test designs represent sediment-water compartments. Even when the standard test design is static without water renewal, semi-static or flow-through systems are accepted in exceptional cases (e.g. if water quality become inappropriate for the chironomids). Such exceptional cases could also be unstable substances, or precipitating metal salts in spiked water test designs.

Despite of most other aquatic or sediment dwelling standard test organisms applied in ecotoxicity tests, chironomids show a holometabolous life cycle starting from a larvae living in and on the sediment surface and ending as a midge, emerging from a pupae at the water surface. Test vessels in tests looking for emergence rate, therefore have to be sealed with emergence traps. This, and the circumstance that the chironomids are inserted as first instar larvae into the overlying water makes the application of a flow-through test system challenging. Additionally, food availability – in most cases a fish food suspension applied via overlying water – could be limited depending on the water exchange rate, affecting the survival rate.



28-day Sediment-Water Chironomid control tests according to OECD 218<sup>2</sup>/219<sup>3</sup> in an adapted flow-through test system developed for Daphnia Reproduction Tests were applied.

300 mL glass beakers with an outlet tube, equipped with a metal gauze to provide from loss of larvae or pupae were applied as test vessels. 90 g dry mass artificial OECD sediment (height 1.5 cm) were submerged by 270 mL water (height ratio 1:4). 20 first stage larvae were applied per vessel, six replicates per treatment. Water exchange rate was 4.5 volumes per day, conducted continuously by a peristaltic pump. The test was run at 21 °C with a L:D ratio of 16:8 h.

Tubes for water and air supply were inserted to the overlying water via the emergence traps placed on the test vessels. Different kind of food sources and feeding intervals were tested under the flowthrough conditions. The first three are in accordance to the OECD 218<sup>2</sup>/219<sup>3</sup> guidelines, the fourth variety not strictly.

- A suspension of ground fish food flakes added daily
- A suspension of ground fish food flakes added three times a week
- Green plant meal (lucerne) incorporated into the sediment before test start
- Green plant meal incorporated into the sediment + ground fish food flakes (for one week) placed in a furrow on the sediment surface before test start

Figure 1: Flow-through test device for chironomids

300 mL glass beakers with outlet tube, metal gauze to provide from loss of larvae or pupae and continuous water renewal by peristaltic pump. Photo © Fh-IME



## **Results & Discussion**

The flow-through test design applied ensure remaining of all larvae and pupae in the vessels. Comparable emergence rates (with 89%) and 93% clearly surpassing the validity criterion of 70%) and development times (18.7 d and 19.8 d) were found for daily and frequently applied maximum allowed amount of fish food suspension at a water exchange rate of 4.5 volumes per day.

Incorporated lucerne green plant meal resulted in a clearly lower emergence rate of only 34%. Additional ground fish food flakes for the first week results in an increased emergence rate of 63%. Development times were comparable (18.3 d and 17.3 d).

Tests under static test conditions with the same feeding design resulted in comparable emergence rates and development times (Suspension of fish food: 86%, 89% and 18.8 d, 20.0 d; green plant meal: 41% and 18.3 d; green plant meal + fish food:  $79\frac{10}{10}$  and 16.8 d).

The results show that suspension of ground fish food flakes in a flowthrough test device lead to comparable emergence rates under control conditions. Exclusively plant meal is no sufficient food source at all. Sufficient additional fish food for the young larvae stages could balance the nutrient lack in this case.

#### Figure 2: Floating larvae

#### Water flow results in floating larvae. This can result in a loss of larvae in flow-through devices – especially for early stages. Photo © Fh-IME

### Conclusions

- Applying a suitably flow-through test device can result in a valid OECD 218/219 28-day Sediment-Water Chironomid Test when food is applied via water phase as (a suspension of) ground fish food flakes.
- Test conditions requiring incorporated green plant meal as the sole food source will presumably fail. The only possibility for a valid test would be a pimped mixture with e.g. fish food.



1 OECD 225 (16.10.07): OECD guideline for testing of chemicals – Sediment-Water Lumbriculus Toxicity Test using Spiked Sediment. 2 OECD 218 (13.04.04): OECD guideline for testing of chemicals – Sediment-Water Chironomid Toxicity Test using Spiked Sediment. **3** OECD 219 (13.04.04): OECD guideline for testing of chemicals – Sediment-Water Chironomid Toxicity Test using Spiked Water.