

Chronic Tests with *Chironomus riparius* as sediment-dwelling organism

Registration of Plant Protection Products, Chemicals, Biocides, Veterinary Medicinal Products and Medicinal Products for Human Use according to 91/414/EC, REACH Regulation 1907/2006/EC, 98/8/EC, 2004/28/EC and 2001/83/EC

Indication

Chironomus species occur in aquatic habitats in high abundance and diversity. Larvae of these midges are typical sediment dwellers and an important prey for fish, the adults also for birds.

Long-term studies with these aquatic insects are required for the risk assessment of e.g. insect growth regulators and for active substances which absorb to and persist in sediments and additionally have a potential toxicity to invertebrates based on the results of previous acute studies.

We perform *Chironomus* studies following two different exposure scenarios:

- spiking of the water column to simulate a pesticide spray drift
- spiking of the sediment to simulate a persistence of chemicals in sediment.

Available Guidelines

IBACON has run *Chironomus* studies under full GLP conditions for several years and has acquired vast experience with both exposure scenarios. The studies are designed to comply with the following methods:

- OECD Guidelines for Testing of Chemicals, No. 218: "Sediment-Water Chironomid Toxicity Test Using Spiked Sediment", adopted April 13, 2004.
- OECD Guidelines for Testing of Chemicals, No. 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water", adopted April 13, 2004.

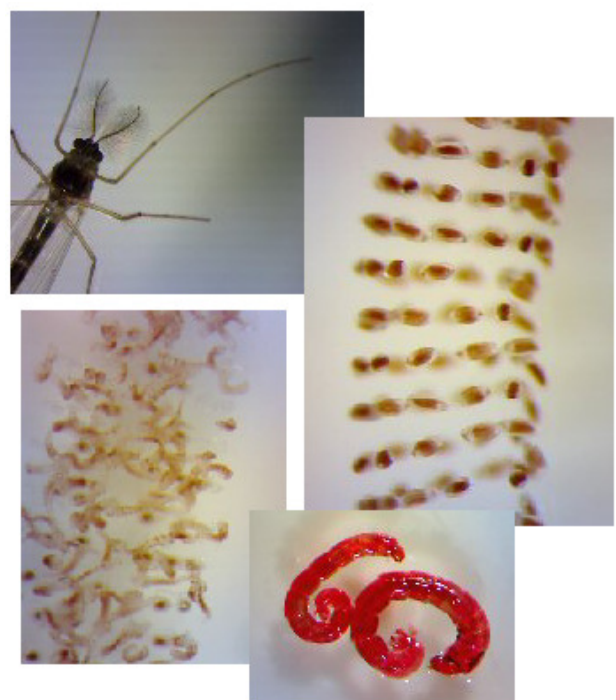
Furthermore we are already prepared to perform a full life cycle study with Chironomids which is an advantage especially with regards to possible endocrine disruptors. IBACON participated in a ring test, covering the first generation and the early part of the second generation. The aim of this ring test is the development of a new OECD Guideline ("Sediment-water Chironomid Life-Cycle Test Using Spiked Water", Draft July 16, 2009).



Besides the chronic studies we offer an acute study lasting for 48 hours to examine whether a chemical shows acute effects. This study is tailor-made and based on different guidelines, also taking into account the guidelines for chronic testing.

Test Organisms

Chironomus riparius is bred in our own laboratory culture. Freshly laid egg masses are taken from the breeding culture 4 to 5 days before test start. The larvae begin to hatch 2 to 3 days later and remain in the first instar phase for 2 to 3 days. For the chronic study designs 20 first instar chironomid larvae are exposed per test replicate.



Study Details

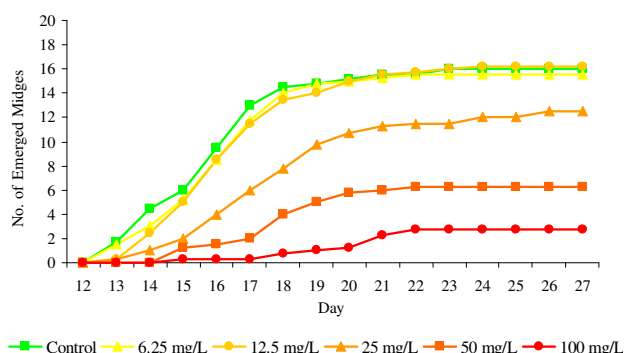
First instar larvae of *Chironomus riparius* are exposed to the test item for 28 days to assess the impact on full maturation of the larvae to adult midges. The larvae are exposed in a sediment-water system.

For the spiked water test design the first instar larvae are placed into the test beakers 24 hours before application of the test substance. In case of the spiked sediment design, the sediment is spiked first and after a stabilisation period of 48 hours the first instar larvae are introduced. The concentrations of the test substance are analysed at test start and test end to verify dosage of the test item. Additional analytical measurements during the test are possible if required. To confirm the sensitivity of the test system a toxic reference is tested once per year.



Endpoints

The measured endpoints are the total number of adults emerged (emergence rate) and the time to emergence (development rate). In the controls 70% of the larvae should emerge between day 12 and 23 after insertion to the test vessels. During the emergence period daily counts are performed. The maximum exposure duration is 28 days. The test may be terminated 5 days after emergence of the last adult in the control. The data are analysed by a regression model to calculate Effect Concentrations. Furthermore the No Observed Effect Concentration and the Lowest Observed Effect Concentration are determined.



Typical Time Frame (GLP Study)

Range-Finding Test and Protocol	4 weeks
Experimental Phase	12 weeks
Reporting	8 weeks
Total	24 weeks

Related Studies

Other important studies for the assessment of aquatic organisms are:

- **Fish**, acute toxicity test
- **Fish**, prolonged toxicity test
- **Fish**, early life stage test
- **Fish**, sexual development test
- *Daphnia magna*, acute toxicity test
- *Daphnia magna*, reproduction test
- **Algae and Cyanobacteria** growth inhibition test
- *Lemna*, growth inhibition test
- Effects on the growth of **higher aquatic plants**

About IBACON

Since 1994, IBACON has been an active partner for the global chemical industry. We gained an international reputation as a premium contract research organisation. Our highly qualified and motivated employees respond to your wishes quickly, flexibly and reliably. A comprehensive technical infrastructure, 1600 m² of lab space and field study capacities allows carrying out your assignment on time and up to the latest standards.

It is our declared aim to always offer you the highest level of security, efficiency and economy. We rely on this premise in everything we do – from the personal consultation to the development of tailored studies and their implementation.

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