

Comparing the sensitivity of field collected lentic and lotic mayfly species after chronic exposure to the insecticide Fipronil in the laboratory

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Introduction

Aquatic insects are often exposed to environmental pollutants, e. g. plant protection products. Especially aquatic mayfly larvae are frequently exposed and show high sensitivities against such contaminants. For the risk assessment, often standardized test systems with *Daphnia* sp. are used but can cause an underestimation of the risk since they are less sensitive. Thus, in Europe we are still lacking standardized test systems with more sensitive species like mayflies, particular for generating chronic toxicity data.

A test system developed by gaiac^[1] was adapted to test the toxicity of substances against the lentic mayfly species *Cloeon dipterum* (Baetidae) (Fig. 1). Results of a 38-day study with the phenylpyrazole Fipronil are presented. An additional study using an established test system^[2] with the lotic mayfly species *Epeorus assimilis* (Heptageniidae) was performed to compare the sensitivities of both mayfly species.



Figure 1: *Cloeon dipterum* larvae

Test design

Test system:	4 quadratic 1.5 L-glass test vessels per treatment filled with 500 ml test solution (Fig. 2)
Test organisms:	Field-collected <i>Cloeon dipterum</i> larvae (from a local pond), 5 individuals per test vessel
Test substance:	Fipronil
Concentrations:	Control + 0.038 – 0.075 – 0.15 – 0.30 – 0.60 µg/L
Medium:	Elendt M4-medium ^[3]
Medium renewal + Control of endpoints:	Twice per week (semi-static)
Food:	<i>Navicula pelliculosa</i> grown on small tiles (Fig. 3) + carrots (0.45 – 0.55 g per test vessel)
Test duration:	38 days
Test conditions:	Temperature: 20 °C (± 1 °C), Light intensity: < 70 lux (light dark cycle 16:8 h)
Endpoints:	Development (based on wing pad development ^[4]), emergence, wing length and mortality

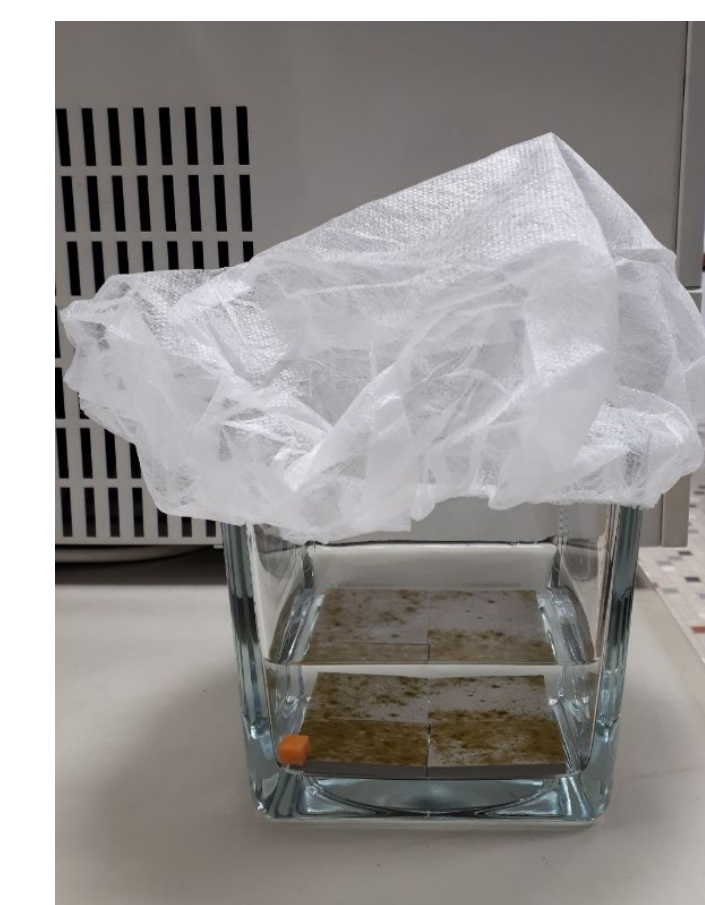


Figure 2: Test vessel for chronic toxicity testing with *C. dipterum*

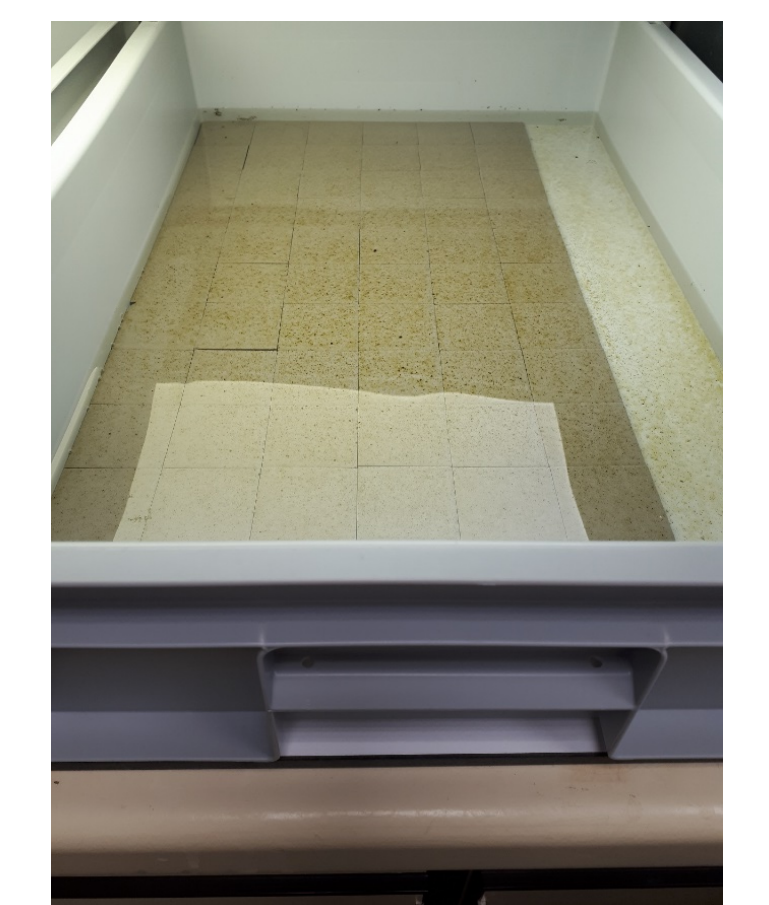


Figure 3: *Navicula pelliculosa* grown on tiles as food source

Results

- Control survival 90 % (Fig. 4)
- Concentration dependent substance effect on development, emergence and mortality (Fig. 4; Table 1); no effect on wing length
- High effect on larval development after short exposure (Table 1)

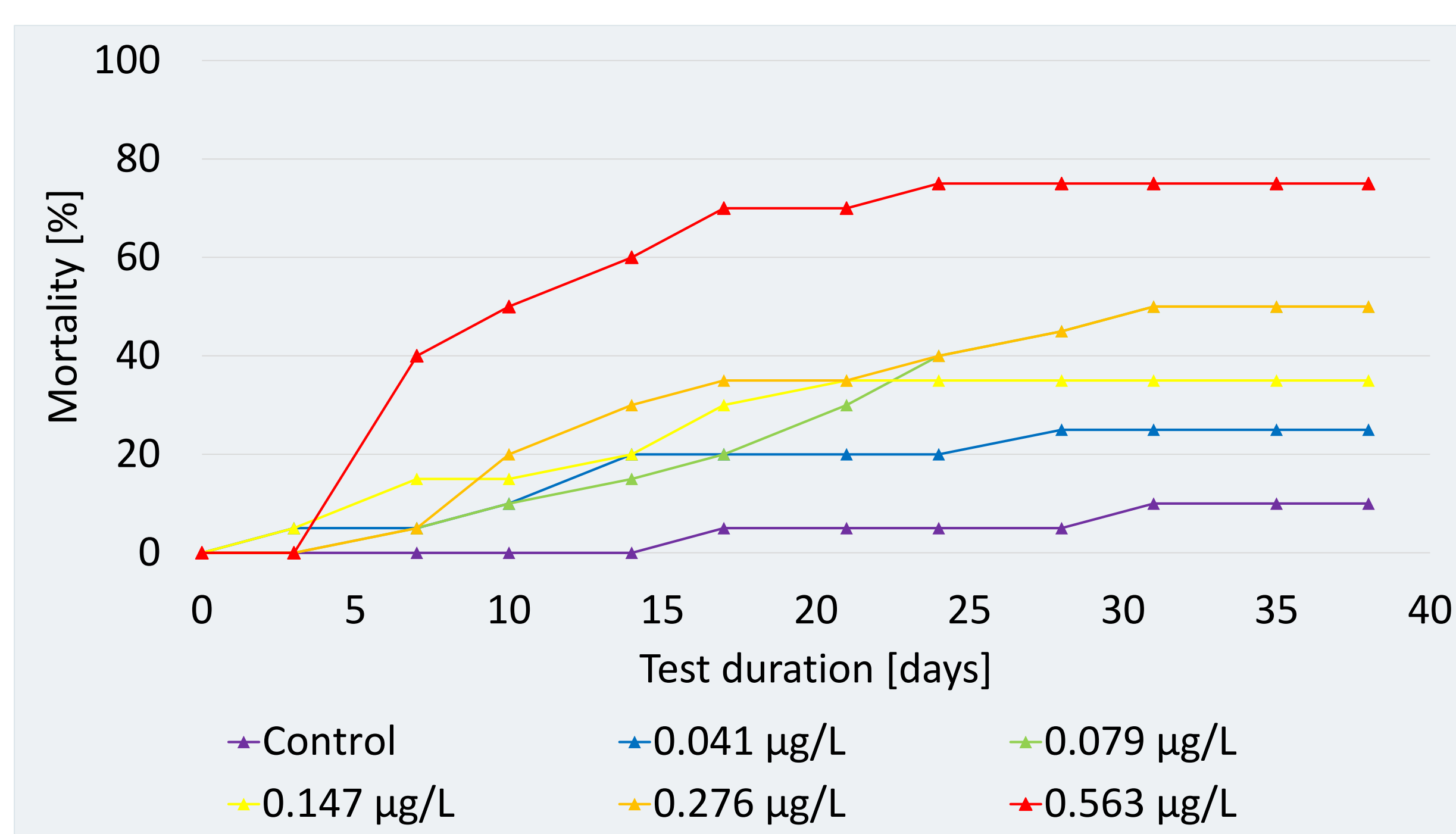


Figure 4: Mortality per treatment

Table 1: Effect of Fipronil on the development of *C. dipterum*

Day of the test	Day 10	Day 21	Day 28
EC10 [µg/L] (95 %-cl)	0.064 (0.002 – 0.133)	0.125 (0.024 – 0.207)	0.238 (0.068 – n. d.)
NOEC [µg/L]	0.041	0.147	≥ 0.563

n. d. = not determined
cl = confidence limit

Comparison of sensitivities

- To compare sensitivities, a test system established at Fraunhofer IME^[2] was used to test the toxicity of Fipronil to the lotic mayfly species *E. assimilis* in a 21-day study (semi-static test design with medium renewal twice per week). As endpoints, the growth, immobilization and mortality of the individuals was assessed.
- E. assimilis* was more than 25 times more sensitive than *C. dipterum*, although the test duration was shorter (Table 2)

Table 2: Comparison of the sensitivities of two mayfly species *C. dipterum* and *E. assimilis* to Fipronil

	<i>C. dipterum</i>	<i>E. assimilis</i>
Test duration	38 days	21 days
LC50 [µg/L] (95 %-cl)	0.181 (0.097 – 0.339)	0.007 (0.004 – 0.014)
NOEC [µg/L]	0.041	< 0.005

Conclusion & Outlook

A concentration dependent effect of Fipronil on the mayfly larvae *C. dipterum* was found regarding the endpoints development, emergence and mortality. Given the high survival rate of the individuals (90%) throughout the study, the development of the test system for *C. dipterum* can be considered successful. The test system provides an option for standardized chronic toxicity testing with mayflies that can also be used within the context of SSD (species sensitivity distribution). In further studies the test should be repeated to investigate the reproducibility of the study results. Still, lotic mayfly species show higher sensitivities and should therefore also be taken into account during risk assessment.

¹ Gaiac – Research institute for Ecosystem Analysis and Assessment (05.05.2021). Chronic toxicity test with *Cloeon dipterum* – test protocol for the ringtest

² Brüggemann, M., Hund-Rinke, K., Böhmer, W., & Schaefers, C. (2021). Development of an Alternative Test System for Chronic Testing of Lotic Macroinvertebrate Species: A Case Study with the Insecticide Imidacloprid. *Environmental Toxicology and Chemistry*, 40(8), 2229–2239. <https://doi.org/10.1002/etc.5070>

³ OECD. (2004). Test guideline No. 202: *Daphnia* sp., Acute Immobilisation Test. *OECD Guidelines for the Testing of Chemicals*.

⁴ Cianciara, S. (1976). Some study on the biology and bioenergetics of *Cloeon dipterum* (L.), Ephemeroptera (pp. 175–195). Institute of Ecology PAS.