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Effects of Two Insecticides on Survival, Growth and Emergence of *Chironomus riparius* Meigen

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Abstract Effects of chlorpyrifos and cypermethrin on the survival, growth and emergence of *Chironomus riparius* were determined on a life-cycle study. Although neither cypermethrin nor chlorpyrifos affected larval survival or growth, a decrease in the number of emerged midges and in the number of females over males, as well as in female biomass, were found for both insecticides. The results from this study indicate that multiple biological endpoints and extended periods of exposure are needed in order to achieve a better screening of insecticide toxicity.

Keywords Chlorpyrifos · Cypermethrin · Life-cycle test · *Chironomus*

Insecticides are used to control insects in both agricultural and urban settings. Freshwater systems in close proximity to agricultural fields or urban areas can be contaminated by these compounds by direct overspray, runnoff or accidental spillage thus aquatic invertebrates that live in rivers and lakes can be exposed to concentrations of insecticides ranging from sublethal to lethal (Crane et al. 1999; Relya 2005). Chlorpyrifos, an acetylcholinesterase ihnibitor belonging to the organophosphate class, and cypermethrin, a synthetic pyrethroid insecticide, are two of the most intensively used insecticides because of its versatility and broad-spectrum insecticidal activity against a wide range of pests and low mammalian toxicity (Curtis and Horne 1995). Although exposure may occur through the dissolved phase in pore waters, sediments will serve as the primary ecological repository of these compounds (Amweg et al. 2005; Crane et al. 1999).

Several standard methods using sediment-dwelling organisms such as amphipods, chironomids, polychaetes, oligochaetes and mayflies have been developed to assess the toxicity of contaminants associated with sediments (ASTM 2000; OECD 2001). One of the most common tests with chironomids used to assess both lethal and sublethal toxicity in sediments is the 10-days larval growth and survival test. Growth is one of the most used parameters to study the chronic toxicity of contaminants and in midges is considered to be a very sensitive one (Sibley et al. 1997). Recently other types of endpoints besides survival and growth have been reported in several studies, such as mouthpart deformities (Meregalli et al. 2001), feeding inhibition (Faria et al. 2006) reproduction and emergence (Péry et al. 2002). However, survival and growth continue to be the most commonly endpoints reported even though they may not be able to identify marginally contaminated sediments. The evaluation of other sublethal parameters besides growth is very important in the evaluation of risk associated with certain pesticide exposures given the fact that these compounds can degrade very quickly and even so exert hazardous effects on aquatic biota. Adult emergence of *Chironomus* species, for instance, have been successfully used to measure the effect of pesticides at low concentrations (Sibley et al. 1997).

This study examined the effects of two insecticides on the survival, growth and emergence of the aquatic midge *Chironomus riparius*. The objectives of the present work were (1) to evaluate the effects of each insecticide on the survival of *C. riparius* larvae in a 10-days exposure time starting from the first instar (2) to assess the effects of each insecticide on the sublethal endpoints growth (10-days exposure) and adult emergence (28-days exposure); (3) to

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compare information obtained in the two types of exposures, the 10-days growth test and the 28-days emergence test, in terms of sensitivity of the endpoints used and extrapolation to effects at population level.

Materials and Methods

The method for assessing the chronic effects of insecticides on *C. riparius* was based on the protocol described by the OECD for water-sediment tests with spiked water (OECD 2001). The contamination of water-column was selected since the aim was to simulate an insecticide contamination event either by aerial dispersion or runoff from agricultural fields. Additionally, recommendations from Ingersoll et al. (1995) associated with standard procedures of larval growth were followed. Chironomids were exposed to 0, 0.01, 0.05 and 0.1 μgL^{-1} of chlorpyrifos (ChemService) and cypermethrin (Riedel-de-Haën®) in ASTM hardwater medium (ASTM 2000) and with sterilized sand as substrate. Stock solutions were made by dissolving a known weight of each insecticide in methanol (technical grade (GC) = 99.8%) and in Milli Q water. Actual concentrations of stock solutions were confirmed by GC-MS according to Fernández-Gutiérrez et al. (1998). All the solutions were prepared immediately before each experiment and were stored at 4°C in the dark for no longer than a week. Exposure treatments were made by adding known volumes of stock solution to ASTM medium using a microsyringe, followed by mixing. The volume of methanol never exceeded 0.5% in the highest concentration of test solutions. Possible solvent effects were examined using a solvent control and a full control (dilution medium) was also set-up.

To initiate testing, in day-1, 1 L glass chambers (9 cm ∅) were filled with 500 mL of ASTM medium and 150 g of sand substrate and the contamination event was performed. Chambers were leave to settle overnight and on Day 0 the water was replaced with fresh test solutions in order to minimize losses of insecticides due to absorption to test chamber surfaces, and 20 first-instar organisms were added to each chamber. Chambers were covered and a gentle aeration (1 air bubble per second) was provided. Tetramin (from TetraWerck) was the sole source of food added; the ration was set at 0.2 mg per larvae per day in order to provide enough nutrition for the midges (Sibley et al. 1997). Physical–chemical parameters (pH, conductivity, temperature, dissolved oxygen) were measured at the beginning (day 0) and at the 10th day of the exposures in three of the six replicates for each treatment and controls. The pH was measured with a pHmeter WTW pH 330i/SET (WTW, Weilheim, Germany), the conductivity with a conductivity meter WTW COND 330i/SET (WTW, Weilheim, Germany) and the dissolved oxygen with a dissolved oxygen meter WTW OXI 330/SET (WTW, Weilheim, Germany). Each treatment was replicated six times, three replicates to determine growth at the end of 10 days of exposure and the other three replicates to determine emergence at 28 days of exposure. The light regime was 16 h light and 8 h dark and the temperature was 20 ± 1°C.

At day 10 the remaining larvae in three of the six chambers were collected for body length and head capsule width measures in a stereomicroscope MS5 (Leica Microsystems, Houston, USA) fitted with a calibrated eyepiece micrometer. Growth (body length increase) of larvae was calculated by subtracting the average initial length from each individual final length. After measurements, larvae were transferred individually to pre-weighted foil cups, dried at 60°C for 48 h and weighted on a microbalance (Mettler UMT2). Biomass was quantified as dry weight per larvae. At each of the three remaining beakers a cylinder was attached to the top of each beaker to capture emerged midges. The number of emerged males and females was recorded on a daily basis and midges were captured individually in eppendorfs, ice dried and its weight determined on a microbalance. The test was finalized 28 days after the exposure to the insecticides. Results of the experiments were analysed using one-way ANOVA and Tukey HSD post hoc test. The significance level was set at 0.05. The percentages of emerged midges were compared after arcsine square root transformations to stabilize the variance.

Results and Discussion

The physical parameters pH, temperature, dissolved oxygen and conductivity averaged 7.54 ± 0.40, 20.1 ± 0.4, 5.88 ± 0.4, 495 ± 30.1, respectively, during the test. The concentration range used for both insecticides was representative of the concentrations typically found in aquatic environments. Predicted environmental concentrations of cypermethrin in surface waters, assumed to be caused by spray-drift over 0.25 m deep water, have been estimated to 0.02–3 $\mu g\ L^{-1}$ cypermethrin following a single application (Linders et al. 2002). Chlorpyrifos is currently detected in a range of 0.01–1.6 $\mu g\ L^{-1}$ in surface waters (CDFG 1994). The concentrations used (up to 0.1 $\mu g\ L^{-1}$) did not cause significant effects on *C. riparius* survival during the 10-days exposure (Table 1). In fact, in CDFG (1994) it was found a 48-h LC_{50} value for chlorpyrifos of 0.3 $\mu g\ L^{-1}$ for *C. tentans*, whereas for cypermethrin, Stephenson (1982) found a LC_{50} of 0.2 $\mu g\ L^{-1}$ for *C. thummi*, which justify the selection of our chronic concentrations range.

Growth (body dry weight and body length) of control midges and the ones exposed to insecticide treatments was

Table 1 Percent survival of *C. riparius* exposed to chlorpyrifos and cypermethrin

Concentration (μg L^{-1})	Percent survival	
	Cypermethrin	Chlorpyrifos
0	81.7 ± 1.47	79.5 ± 2.17
0.005	66.7 ± 4.93	66.7 ± 1.53
0.01	73.3 ± 2.08	67.0 ± 1.73
0.05	76.7 ± 0.58	65.7 ± 3.06
0.1	66.7 ± 3.06	66.6 ± 5.13

Results are means ± standard errors (SE) of 3 replicates (n = 3) each with 20 larvae

Table 2 Effects of both insecticides on the development and growth of *C. riparius*

Treatment	Parameters	*df*	*F*	*p*
Chlorpyrifos	Head capsule width	4, 164	5.732	<0.001
	Body length	4, 164	1.398	0.237 (NS)
	Biomass	4, 164	0.617	0.651 (NS)
Cypermethrin	Head capsule width	4, 128	3.931	<0.05
	Body length	4, 128	0.744	0.564 (NS)
	Biomass	4, 128	2.107	0.084 (NS)

Head capsule width is a measure of development and body length increase and biomass are measures of growth (*df* degrees of freedom, *p* probability, *NS* no significant)

not significantly different (Table 2) showing that those insecticides did not affect the midge growth at the tested concentrations. One of the reasons for the absence of effects on chironomid dry weight could be associated with potential weight bias linked with the ingestion of different sized sand grains by the individual midges (Sibley et al. 1997). Chlorpyrifos at 0.05 and 0.1 μg L^{-1} and cypermethrin at 0.1 μg L^{-1} caused a significant reduction on the larval head capsule width (Table 2). However, larvae from all treatments were in the 4th instar after 10 days of exposure, showing that a delay in the development did not occurred, which was confirmed by the emergence times.

Midge emergence started at day fifteen for both insecticides showing that both insecticides, at the tested concentrations, did not retard emergence time. The pattern of the percent cumulative emergence was similar between controls, with a total mean emergence of approximately 60% in both cypermethrin and chlorpyrifos exposures (Table 3). This percentage of emergence in controls met the OECD criterion of 50% of adult emergence for acceptability of the test (OECD 2001). In this study no effect on total emergence was observed for the tested concentrations of cypermethrin. In contrast, the emergence of adults at 0.05 and 0.1 μg L^{-1} of chlorpyrifos were significantly decreased. In fact the total adult emergence was reduced in 50% in these chlorpyrifos exposure levels, showing that chlorpyrifos influenced midge emergence. Regarding the sex ratio of *C. riparius* emerged adults, it was found that exposure to 0.1 μg L^{-1} of cypermethrin produced a change in the sex ratios (male/female) from 1 (control conditions) to 4 although this shift was not statistically significant. For chlorpyrifos, exposure to 0.1 μg L^{-1} caused a significant shift on the sex ratio from 1.39 (at 0 μg L^{-1}) to 2.33.

The reduction in the number of emerged adults along with the increased number of males over females can have direct consequences in terms of population structure and dynamics, leading to a population reduction due to the decreased number of females for egg deposition. In the case of chlorpyrifos this reduction in the reproductive success is enhanced by a significant decrease in female fresh weight compared to control females. According to Sibley et al. (2001) which assessed the influence of adult

Table 3 Effects of both insecticides in the *C. riparius* adult emergence

Insecticide	Concentration (μg L^{-1})	Adult emergence (%)	% Emerged adults		Sex ratio
			Males	Females	
Chlorpyrifos	0	61.7 ± 1.7	54 ± 4.9	46 ± 4.9	1.39
	0.005	33.3 ± 4.4	63 ± 13.2	37 ± 13.2	1.78
	0.01	45.0 ± 5.0	46 ± 12.7	54 ± 12.7	0.94
	0.05	28.3 ± 8.8*	65 ± 4.4	35 ± 4.4	1.77
	0.1	30.0 ± 8.7*	71 ± 17.7	29 ± 17.7	2.33*
Cypermethrin	0	58.3 ± 16.8	50 ± 9.62	50 ± 9.62	1.17
	0.005	30.0 ± 15.3	20 ± 16.67	80 ± 16.67	0.30
	0.01	40.0 ± 15.3	30 ± 23.73	70 ± 23.73	0.67
	0.05	23.3 ± 8.8	30 ± 16.67	70 ± 16.67	0.69
	0.1	26.7 ± 3.3	80 ± 7.78	20 ± 7.78	4.33

Results are mean ± standard errors (SE) of 3 replicates (n = 3). Means for the same insecticide followed by an *asterisk* (*) are significantly different from the control ($p < 0.05$) according to Tukey HSD multiple comparison test

size and other factors on the reproductive output of *C. tentans* in sediment toxicity tests, the adult female size is the most important factor affecting reproductive output in this species, and this can be also be the case for *C. riparius* exposure to chlorpyrifos. Sibley et al. (1997) shown that a reduction in growth during larval stages in *C. tentans* was associated with a proportional decline in reproductive output of adult females.

In this study exposure to chlorpyrifos and cypermethin affected reproductive aspects of *C. riparius* as adult emergence, sex ratio and female midge weight without directly affect larval growth. Growth was considered as a poor indicator of effects. These results illustrate the usefulness of combining the standard 10-days test with a longer-term evaluation (28-days life-cycle test) in order to achieve a better screening of sublethal toxicity.

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