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METIER (Modular Ecotoxicity Tests Incorporating Ecological Relevance) for Difficult Substances. 5. Chlorpyrifos Toxicity to Daphnia magna in Static, Semi-Static, and Flow-Through Conditions

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The standardization of ecotoxicity tests has been an important topic for ecotoxicologists (Soares and Calow 1993). In the last few decades, there was an effort to develop protocols for standardizing the experimental conditions, mainly by running 'ring tests' ('round robin tests') (Bradley et al. 1993) with the cladoceran *Daphnia magna.* It is a suitable test species as it is easily cultured in the laboratory and is very sensitive to chemical stress (Adema 1978). Ecotoxicity tests using cladocerans are usually static and semi-static, which are simple, inexpensive, and require minimum space. The main disadvantages of these two systems are: 1) possible toxicant loss, oxygen depletion, and waste accumulation; 2) they can only be used with organisms adapted to lenthic environments. The objectives of a flow-through system are to submit test organisms to constant and continuous concentration of toxicant and to improve the assessment and standardization of ecological risks of toxic compounds. The flow-through system previously developed within the METIER project is flexible enough to meet the requirements necessary for testing difficult substances and yet sufficiently simple and cost-effective to operate using a wide range of aquatic organisms (Ribeiro et al. 1995; Diamantino et al. 1997).

Organophosphorus pesticides are generally the most toxic of all pesticides to vertebrate and are chemically unstable or non-persistent (Ware 1978). Chlropyrifos is an organophosphorus insecticide present in several formulations such as DURSBAN©, LORSBAN© and RIDLICE©. Chlropyrifos is a broad-spectrum insecticide and is widely used for control of flies, household pests, mosquitoes (larvae and adults), and of various crop pests in soil and or foliage and also used for control of ectoparasites on cattle and sheep (Allender and Keegan 1991; Worthing and Walker 1987). This compound may enter surface water by runoff, spraydrift or accidental spills (Cowgill et al. 1991). Chlorpyrifos is an insecticide toxic to aquatic life, exhibit moderate persistence in natural systems and low solubility in water (Van Winjngaarden et al. 1993; Worthing and Walker 1987) (Table 1).

We compared results of chlorpyrifos lethal and sublethal concentrations to *D. magna* in static, semi-static, and flow-through conditions.

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MATERIALS AND METHODS

All experiments were undertaken with *Daphnia magna* Straus from a single clone (clone A *sensu* Baird et al. 1989), and initiated with third to fifth brood neonates, less than 24 hr old. Parent animals were cultured individually in 100 mL of Elendt M7 medium (Elendt and Bias 1990) and were fed daily with 3 x 10^5 cells mL^{-1} of *Selenastrum capricornutum*. The photoperiod was 16:8 hr 1ight:day and the temperature was 20±1 °C. Test medium was also Elendt M7. In all tests, oxygen saturation was always above 85% and pH variation was never greater than one unit (7.0-7.9).

The flow-through system (FTS) consisted of 42 glass reservoirs for the toxicant solutions, a continuous flow pumping device, and 42 glass chambers with 43 mL capacity and containing 30 mL of solution (Diamantino et al. 1997). The flow was started 48 hr before the FTS test to allow sticking of toxicant to the FTS. During the FTS test, the flow rate was 19 mL hr^{-1} and solutions were added to reservoirs daily. In the semi-static system (SSS), test chambers were the same as those used in the FTS. Here, animals were transferred to newly prepared test solution every other day.

Both FTS and SSS tests had one control, one control with organic solvent (1 mL L^{-1} of 2-propanol) and five toxicant concentrations, with six replicates per treatment and one organism per test chamber. Chlorpyrifos concentrations were 0.043, 0.056, 0.073, 0.096, and 0.124 µg L^{-1} (with 1 mL L^{-1} of iso-2-propanol in all concentrations).

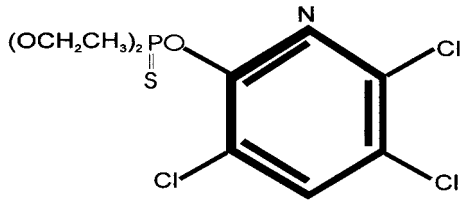
Chlorpyrifos (from Ride1 De Haen, Germany) was dissolved (1.08 mg L^{-1}) in a 10% solution of iso-2-propanol with nanopure water (conductivity < 5 µS cm^{-1}). This stock solution was maintained during 24 hr in an orbital incubator at 180 r.p.m., lights off, at 20 °C to assure complete dissolution. The stability of this stock solution, maintained during 14 d in the orbital incubator, was checked by HPLC analysis with the following equipment: MERCK-HITACHI system with a L-6200A intelligent pump, a L-426 UV - VIS detector and a D 2500 chromato-integrator, using a 200 µL loop and a silica MERCK LICHROSPHER column (5 µm, 250 x 4 mm). Wavelength was 290 nm. The eluent was dichloromethane (93%), acetonitrile (7%) and acetic acid (0.02%). Analyses were made at the beginning of the test, after 8 d and after 14 d (3 replicates for each period were made). Calibration was made with an external standard of 1.27 mg L^{-1} of chlorpyrifos dissolved in the HPLC eluent.

Test chambers were only opened for counting offspring and for taking exuviae needed to evaluate growth. Oxygen and pH were recorded twice weekly. In both test systems, organisms were fed daily with $3x10^5$ cells mL^{-1} of *Selenastrum capricornutum.* Both FTS and SSS tests were carried out for 14 d.

In the static system, 20 animals were used per treatment in groups of five organisms per 100 mL of test solution. Test chambers were made from glass with

Table 1. General information on chlorpyrifos

Chemical structure



Molecular weight	350.6
Octanol/water partition coefficient (20 °C)	5.11
Water solubility (25 °C)	2 mg L^{-1}[1a]
Half-life in water (pH 8, 25 °C)	1.5 d[1]
Half-life in water with phosphate buffer (pH 7, 15 °C)	100 d[1]
Half-life in acetone (21 °C)	1.8-4.8 d[2]
Persistence in soil	60-120 d[1]
Acute toxicity:	
LD50 rats	135-163 mg Kg^{-1}[3]
LD50 rabbits	1000-2000 mg Kg^{-1}[3]
LD50 (96 hr) rainbow rout	0.003 mg L^{-1}[1]
LC50 (48 hr) *Daphnia pulex*	0.2 µg L^{-1}[4]
LC50 (48 hr) *Daphnia longispina*	0.3 µg L^{-1}[4]
LC50 (48 hr) *Daphnia magna*	1 µg L^{-1}[4]
LC50 (96 hr) *Ceriodaphnia dubia*	0.06 µg L^{-1}[5]

[a] Values as low as 0.4 mg L^{-1} and 0.2 mg L^{-1} have also been found (Kersting and Van Winjngaarden 1992); [1] Kersting and Van Winjngaarden (1992); [2] Frank et al. (1991); [3] Cowgill et al. (1991); [4] Van Winjngaarden et al. (1993); [5] Bailey et al. (1996).

175 mL capacity. Organisms were not fed during the tests. Chlorpyrifos concentrations were 0.1, 0.16, 0.25, 0.4 and 0.5 µg L^{-1} (with 1 mL L^{-1} of iso-2-propanol in all concentrations). Bioassays were for 48 hr. and conducted twice. The oxygen concentration and pH of the solution in each test chamber were recorded at the start of the test and after 24 and 48 hr.

In the static tests, immobilization was the only checked endpoint. In SSS and FTS, tested organisms were checked for immobilisation, growth until first reproduction (body length at first reproduction minus body length at birth), total growth (body length at the end of the test minus body length at birth), and fertility (number of offsprings per surviving female). Females that died before the end of the test were not considered in the computation of growth and fertility.

Values of EC50 values were determined by probit analysis. Effects of mortality in FTS and SSS were analysed using the Fisher's Exact test (Zar 1984). Results of growth and fertility were analysed with one-way (SSS and FTS data, separately) and two-way ANOVAs (comparison between SSS and FTS) followed

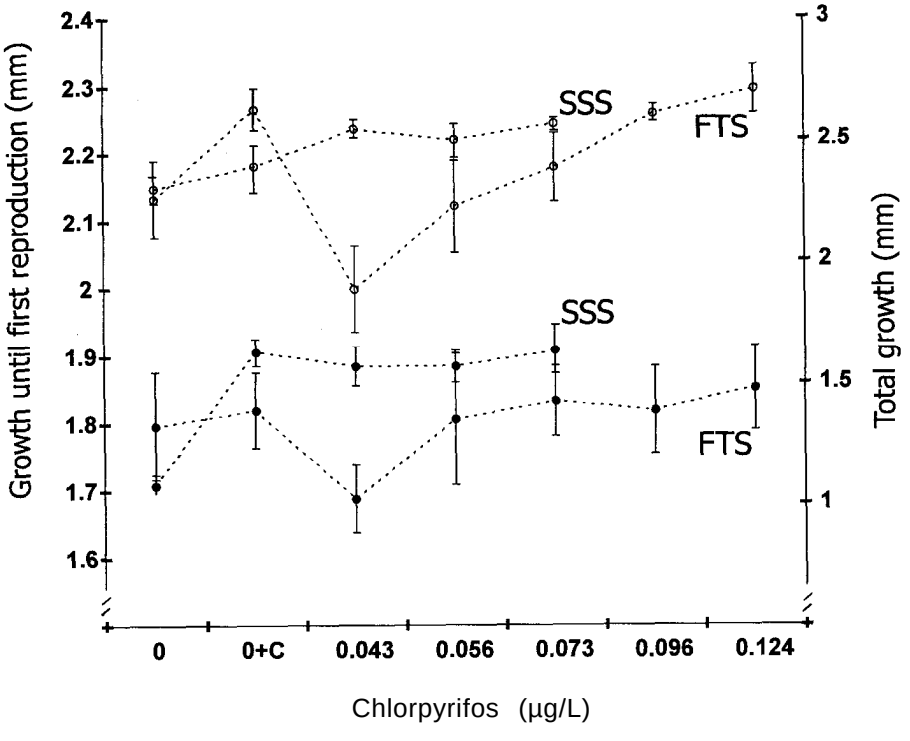


Figure 1. Growth until the first reproduction and total growth (average and standard error) under the semi-static (SSS) and the flow-through systems (FTS). 0 - control, O+C - control with 1 mL L^{-1} of 2-propanol.

by the Tukey HSD multiple comparison test (Zar 1984).

RESULTS AND DISCUSSION

At days 8 and 14, the stock solution of 1.08 (±0.09 of standard deviation) mg L^{-1} of chlorpyrifos showed a decay of 2.7% (1.05±0.09 mg L^{-1}) and 8.33% (0.99±0.12 mg L^{-1}), respectively.

Chlorpyrifos stability was found to be low by Welling and De Vries (1992) who reported that a solution of 10 µg L^{-1} chlorpyrifos without carrier and in aquaria with and without fish during 14 d (static exposure) had a half-life of 2.6 and 5.6 d, respectively.

The growth of *D. magna* until the first reproduction, both in FTS and SSS, was not significantly affected by chlorpyrifos (FTS: F=0.562; d.f.=5, 34; P=0.757, and SSS: F=2.666; d.f.=4, 29; P=0.055). However, total growth showed significant differences between toxicant concentrations (FTS: F=3.925; d.f.=5, 34; P=0.006,

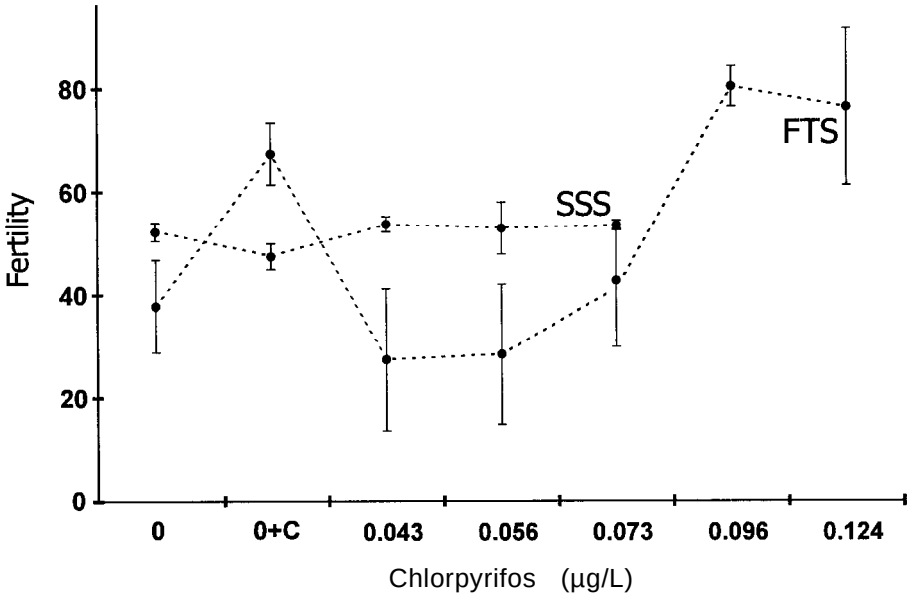


Figure 2. Fertility per surviving female (average and standard error) under the semi-static (SSS) and the flow-through systems (FTS). 0 - control, O+C - control with 1 mL L^{-1} of iso-2-propanol.

and SSS: F=2.814; d.f.=4, 29; P=0.046), though the Tukey HSD test was unable to identify homogeneous groups and, thus, no NOEC could be determined (Fig. 1).

Between the two systems, growth until the first reproduction was similar (F=3.049; d.f.=1, 64; P=0.086), although significant differences were found in total growth (F=5.032; d.f.=1, 64; P=0.028), with greater values being recorded in SSS (Fig. 1).

In SSS, total reproduction was not affected by chlorpyrifos (F=0.845; d.f.=4, 29; P=0.51), but under FTS significant differences were found (F=3.925; d.f.=5, 34; P=0.005). It was impossible to determine a NOEC value because the respective Tukey HSD test was unable to identify homogeneous groups (Fig. 2). Between the two systems, differences were found in total reproduction with greater values under SSS (Fig. 2).

Mortality was recorded in the two highest concentrations in SSS, NOEC being 0.073 µg L^{-1} and LOEC 0.096 µg L^{-1}. In FTS, no significant mortality was found. Thus, for both total growth, total reproduction and survival, FTS showed lower sensitivity to chlorpyrifos than that under SSS. This could have resulted from sorption of toxicant to the FTS materials (Silicone, Teflon, and glass) and to the

higher food availability in the FTS. Kersting and Van Wijngaarden (1992) found that the presence of algae considerably reduced the residence time of chlorpyrifos in the test solution.

In the two tests in the static system, no mortality was recorded at the 0.16 µg L^{-1} chlorpyrifos concentration. The EC50 values at 48 hr were 0.344 (95% confidence limits: 0.343-0.345) and 0.325 (0.324-0.326) µg L^{-1}. This value is just slightly higher than the NOEC for mortality found under the SSS (0.16 µg L^{-1}). These results agreed with those reported by Kersting and Van Wijngaarden (1992) and Van Wijngaarden et al. (1993) who observed an EC50 at 48 hr of 1 µg L^{-1} and a NOEC for mortality in chronic testing of 0.1 µg L^{-1}. Interestingly enough, the lethal toxicity of chlorpyrifos for this species is close to the one found to other *Daphnia* species, namely an EC50 at 48 hr for *D. longispina* of 0.3 µg L^{-1} and for *D. pulex* of 0.2 µg L^{-1} (Van Wijngaarden et al. 1993).

The fact that a sub-lethal NOEC could not be determined under SSS or FTS, which agreed with other studies where no any sub-lethal effect could be detected (Kersting and Van Wijngaarden 1992) indicated that the threshold concentration for sub-lethal toxicity of chlorpyrifos is probably close to the threshold value for mortality.

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