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Acute Lethal Toxicity of Some Pesticides to *Brachionus calyciflorus* and *Brachionus plicatilis*

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Due to their widespread distribution and toxic nature pesticides may have a serious impact on the aquatic environment and exert adverse effects on the associated organisms. Acute bioassay tests have been used to determine the actual impact of various pesticides on aquatic life. Static acute toxicity tests provide rapid and reproductible concentration-response curves for estimating toxic effects of chemicals on aquatic organisms. These tests provide a data base for determining relative toxicity to a variety of species.

Data on sensitivity to pesticides for zooplankton species, in laboratory controlled conditions, is mainly reported to crustaceans, existing a lot of information on daphnids. However, a few information about controlled bioassays conducted with rotifers is available (Dad and Pandya 1982; Serrano et al. 1986; Snell an Persoone 1989).

Brachionus calyciflorus and *Brachionus plicatilis* are particularly useful for environmental toxicology because of their rapid reproduction, short generation time, cosmopolitan distribution and the commercial availability of the dormant eggs (cysts) (Snell and Persoone 1989).

The present study was conducted to determine the acute toxicological effects of some pesticides (trichlorfon, fenitrothion, chlorpyrifos, lindane and 3-4-dichloroaniline (3,4-DCA)) on *B. calyciflorus* and *B. plicatilis*

These two zooplanktonic species were tested to elucidate sensitivity differences between freshwater and brackish environment rotifers.

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MATERIALS AND METHODS

Brachionus calyciflorus cysts were stored in EPA medium at 6°C in the dark. Deionized water is the base for making the EPA medium (USEPA 1985). This medium is a synthetic fresh water that is prepared from reagent grade chemicals and composed of 96 mg $NaHCO_3$, 60 mg $CaSO_4.2H_2O$, 60 mg $MgSO_4$, and 4 mg KC1 per liter of water.

Cysts hatching is initiated by transferring to warmer temperatures and light (Snell and Persoone 1989).

Standard environmental conditions for acute toxicity bioassays were: temperature, 25°C; pH, 7.4-7.8; hardness, 80-100 mg $CaCO_3$/L; alkalinity, 60-70 mg/L, and darkness.

Brachionus plicatilis cysts are produced under rigorously controlled laboratory conditions (Snell and Persoone 1985) and stored in 55 ppt salinity synthetic seawater. The best storage conditions are 6°C in the dark.

Cyst hatching is initiated by transferring the cysts to lower salinity, warmer temperature and light (Snell and Persoone, 1989).

Several hundred cysts were placed in 2 mL of seawater medium (15 ppt salinity) in a 10 mL dish, the temperature was 25°C, pH 7.7 and the light intensity was 1000 lux. Under these conditions, the time required for the cysts to hatch is aproximately 24 hr.

Synthetic seawater was prepared by mixing Instant Ocean salts with deionized water.

Standard environmental conditions for acute toxicity bioassays were: temperature, 25°C; salinity, 15 ppt; pH, 7.7, and darkness.

Bioassays were conducted in sterile, 24-well polystyrene tissue culture plates which were used once and discarded.

Because of the short duration of the test (24 hr), rotifers were not fed and the medium was not renewed during the bioassay. Control survival is almost always 100% after 24h. A 24-well plate has six concentrations for one acute toxicity test. Ten neonates were placed in 1 mL in each of 3 replicate wells in a column, for a total of 30 animals per concentration (column), so the control and five concentrations for each toxicant. We carried out a total of nine replicates for each toxicant. Bioassay plates were placed in an incubator under standard conditions, the percent dead in each pesticide treatment was recorded after 24 hr, and median lethal values (24 hr-LC50s) and 95%

confidence limits were calculated from survival data using "moving-average" analysis, with an IBM computer.

RESULTS AND DISCUSSION

The 24 hr LC50 values for both species of rotifers for all tested pesticides are listed in Table 1. Comparisons of the LC50 values indicate that the organophosphorus insecticide fenitrothion was the most toxic of pesticides tested to both *B. Calyciflorus* and *B. plicatilis.* Fenitrothion was followed in order of decreasing toxicity by chlorpyrifos, lindane, trichlorfon and 3,4-DCA in *B. calyciflorus.* In *B. plicatilis* the most toxic compound after fenitrothion was also chlorpyrifos, followed by lindane, 3,4-DCA and trichlorfon.

Table 1. Mean *B. calyciflorus* and *B. plicatilis* LC50 (mg/L) values ($\bar{X}$) for five pesticides.

Compound		*B. calyciflorus*	*B. plicatilis*	Sensitivity factor LC50 B.p/LC50 B.c
Trichlorfon	$\bar{X}$	51.94	274.93	5.29
	CV	17.47	6.43	
	95%CL	42.87-61.01	257.24-292.61	
	N	9	9	
Fenitrohion	$\bar{X}$	6.68	8.87	1.32
	CV	10.93	4.75	
	95%CL	5.95-7.41	8.45-9.29	
	N	9	9	
Chlorpyrifos	$\bar{X}$	11.85	10.67	0.90
	CV	8.37	12.81	
	95%CL	10.86-12.84	9.31-12.03	
	N	9	9	
Lindane	$\bar{X}$	22.50	35.89	1.59
	CV	13.30	3.15	
	95%CL	19.51-25.49	34.76-37.02	
	N	9	9	
3,4-DCA	$\bar{X}$	61.47	57.45	0.93
	CV	9.62	7.39	
	95%CL	55.56-67.38	3.21-61.69	
	N	9	9	

B. calyciflorus was more sensitive to trichlorfon, fenitrothion and lindane than was *B. plicatilis.* But the toxicity of chlorpyrifos and 3,4-DCA in both species was the same. Sensitivity factors, derived by dividing the 24h LC50 value for *B. plicatilis* by the 24 hr LC50 for *B. calyciflorus* ranged from 5.29 for trichlorfon to 0.90 for chlorpyrifos (Table 1).

Trichlorfon 24 hr LC50 values were 51.94 and 274.93 mg/L for *B. calyciflorus* and *B. plicatilis,* respectively, in the present study. The median lethal concentration (LC50) for both species are much higher than those registered in the literature for the same organophosphorus insecticide in other aquatic invertebrates and fishes. Johnson and Finley (1980) determined a 24 hr LC50 of 0.002 mg/L for *Daphnia pulex* exposed to trichlorfon in static tests, and 0.040 mg/L in *Gammarus lacustris.* With respect to fishes, the resistance of both species was also higher. Johnson and Finley (1980) found a 24 hr LC50 of 0.003 mg/L for *Lepomis machrochirus* and Ferrando et al. (1987) determined a 96 hr LC50 of 3.38 mg/L in *Anguilla anguilla.*

The 24 hr LC50 for fenitrothion in the present study were 6.68 mg/L for *B. calyciflorus* and 8.87 mg/L for *B. plicatilis.* Our results of *B. plicatilis* are very close to the LC50 value of 9.8 mg/L reported for the same species exposed to fenitrothion by Serrano et al. (1986). If we compare our results with others from the literature we find both rotifer species less sensitive than other aquatic species. The 24 hr LC50 for fenitrothion was 0.011 mg/L for *Daphnia magna* and 0.003 mg/L for *Gammarus fasciatus* (Johnson and Finley 1980). The resistance of both species was also higher than that of most of the bioassayed fish species. Ferrando et al. (1987) conducted tests on *Anguilla anguilla* and found 96 hr LC50 of 0.20 mg/L for this organophosphorus insecticide. Johnson and Finley (1980) determined 96 hr fenitrothion LC50 values of 4.3 mg/L in *Lepomis machrochirus.*

Chlorpyrifos 24 hr LC50 values were similar in both rotifer species, 11.85 and 10.67 mg/L for *B. calyciflorus* and *B. plicatilis,* respectively. Our results indicate that both species are more resistant than other species like *Gammarus lacustris* with a LC50 of 0.011 mg/L (Johnson and Finley 1980). Also they are less sensitive than fishes like *Lepomis machrochirus* which have a LC50 of 0.024 mg/L for this organophosphorus insecticide (Johnson and Finley 1980).

Lindane 24 hr LC50 values from the present tests were 22.5 mg/L and 35.89 mg/L for *B. calyciflorus* and *B. plicatilis* respectively. Johnson and Finley (1980) also tested this organochlorine insecticide with *Daphnia pulex* and they found a LC50 (48 hr) of 0.460 mg/L for this compound. Lindane 24 hr LC50 data generated by Johnson and Finley (1980) on *Gammarus fasciatus* and *Lepomis machrochirus* were 0.01 and 0.068 mg/L, respectively. Ferrando et al. (1987) determined, also, the LC50 for this pesticide on *Anguilla anguilla* and they found a value of 0.67 mg/L.

3,4-dichloroaniline (3,4-DCA) is a chemical which may enter surface waters as a contaminant in applications of agricultural herbicides, as a metabolite of several herbicides, or in industrial effluents from dye manufacturing plants. It is also a highly useful fungicide on a wide range of fruits and vegetables. It is the less effective pesticide on *B. calyciflorus*: the LC50, in this species, is 61.47 mg/L. On the other hand, in *B. plicatilis* the less toxic compound is

trichlorfon, but after it, is 3,4-DCA, with a LC50 of 57.45 mg/L. The acute toxicity of 3,4-DCA in *Pimephales promelas* is 12 mg/L (Call et al. 1987). The molluscs, *Lymanaea stagnalis* and *Dreissena polymorpha* were somewhat more tolerant of 3,4-DCA when tested at the life-stages of the first cleavage egg (48 hr LC50 > 32 mg/L) (Adema and Vink 1981). Also, in *Daphnia magna* the LC50 for 3,4-DCA was less than in our rotifers. Adema and Vink (1981) reported a 48 hr LC50 of 12 mg/L for adults of this species.

Several short-term acute toxicity tests with a variety of sustances have been conducted using rotifers: insecticides (Dad and Pandya 1982; Serrano et al. 1986), heavy metals (Snell and Persoone 1989), free ammonia (Snell and Persoone 1989). However, quantitative data on the effects of xenobiotic compounds on this important category of zooplankton is actually quite limited.

B. calyciflorus was more sensitive to the most pesticides tested than *B. plicatilis*. The species sensitivity difference between both species for trichlorfon was 5.29 times the present study.

B. plicatilis is an euryhaline species, so it must have a higher capacity of osmoregulation which contributes to a higher resistance to toxics. But also, the toxicity of a pesticide depends on the medium composition. There are some important factors of the medium that influence the toxicity of a compound, for example, pH, salinity and hardness.

In our case, the toxicants are less effective in the medium with highest salinity, that is why the LC50 is higher. Inman and Lockwood (1977) determined how the salinity of the medium influenced the toxicity of lindane on *Gammarus duebeni* and they found that the pesticide was more toxic in the media with less salinity.

Fenitrothion and chlorpyrifos, organophosphorus insecticides, were the most toxic of the pesticides tested. And the organochlorine compounds were the least. But trichlorfon, another organophosphorus insecticide, had a similar toxicity than 3,4-DCA in *B. calyciflorus* and less toxicity to 3,4-DCA in *B. plicatilis*. Generally, organophosphorus pesticides are more toxic than organochlorine compounds in aquatic species (Ware 1983).

Our data demostrated that *B. plicatilis* is a more resistent species to pesticides than *B. calyciflorus* And, for both species, fenitrothion (organophosphorus insecticide) was the most toxic compound.

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