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Environmental Contamination and Toxicology

Effect of Thiophanate-Methyl on the Reproduction and Survival of the Freshwater Rotifer *Brachionus calyciflorus* Pallas

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Rotifers, especially *Brachionus calyciflorus* and *B. plicatilis*, are among the most favourable test animals in aquatic toxicology (Snell and Janssen 1995). In recent years, there is an increasing trend in the use of rotifers as bioassay organisms for ecotoxicological studies. These include determination of LC_{50} values using various toxicants, life table demographic studies, feeding studies, behavioral studies such swimming speed, population growth aspects, prey-predator interactions, biochemical studies including biomarker, and trophic level studies using mesocosm and microcosm (Snell and Janssen 1995, Charoy et al. 1995, Ferrando et al. 1996, Giddings et al. 1996, Jak et al. 1996, Del Valls et al. 1997, Sarma et al. 1998, 2001, Preston et al. 1999).

Thiophanate-methyl is an important agricultural pesticide widely used in China. Up to now, however, its toxic effects on non-target organisms, especially zooplankton, have not been reported.

The main purpose of the present study was to assess the effects of different levels of thiophanate-methyl on survival and reproduction of the freshwater rotifer *Brachionus calyciflorus* using the life table demographic technique.

MATERIALS AND METHODS

The rotifer *Brachionus calyciflorus* was used as the test species. This species was obtained by hatching resting eggs collected from sediments of Lake Jinghu and thereafter clonal culturing. Stock rotifer cultures were kept at 26 ± 1 ℃ on a 16hr: 8hr light: dark photoperiod at 130 lx provided by a fluorescent light in a illumination incubator. Rotifer cultures were daily fed on *Chlorella pyrenoidosa*. Before the experiment commenced, the rotifer cultures were fed on 4.0×10^6 cells/mL of *Scenedesmus obliquus* for at least two weeks. For mass cultures as well as for experiments, reconstituted hardwater (EPA medium)(USEPA 1985) was used as the medium. Algae were grown in a semi-continuous culture using HB-4 medium (Li et al. 1959) renewed daily at 40%. Algae in exponential growth were centrifuged and resuspended in EPA medium.

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The pesticide thiophanate-methyl (commercial grade, Nisson, Japan) was used as the toxicant. Stock solutions of 1000 mg/L were prepared using distilled water immediately prior to each experiment. In order to choose appropriate toxicant concentrations for life table experiments, seven concentrations of thiophanate-methyl (1.2, 2.4, 3.6, 4.8, 6.0, 7.2, and 8.4 mg/L) were prepared through serial dilution from the stock solution using EPA medium to obtain median lethal concentration at 24 hr of assay. LC_{50} tests were conducted in 2-mL volume of the medium in 3-mL containers each with 4.0×10^6 cells/mL of *Scenedesmus obliquus* and 10 neonates (<2 hr old). Three replicates were used for each concentration of the pesticide. After 24hr, the number of rotifers alive in each test container was counted. The LC_{50} value was derived following the probit method (Finley 1971).

Based on LC_{50} value, for the life-table experiments we selected seven toxicant concentrations (0.6, 1.2, 1.8, 2.4, 3.0, 3.6, and 4.2 mg/L) and a control, each consisting of four replicates of 10 rotifers. Life-table experiments were conducted in 24-well tissue culture plated (made of plexiglass) and started by introducing one neonate (<2 hr old) into each well which contained 1 mL of test solution and 4.0×10^6 cells/mL of *Scenedesmus obliquus.* The rotifers were checked every 2 hr during the initial 24 hr, and thereafter every 8 hr. The numbers of original test individuals alive from each cohort and neonates produced were recorded. The parental females were transferred into freshly prepared test solution every 24 hr. All life-table experiments were conducted at 26 ± 1 ℃, in darkness. Since the exposure period for chronic toxicity tests with *B. calyciflorus* can be reduced to only 3-4 days without loss of information when r_m is the best parameter (Janssen et al. 1994), all calculations were based on 4-day (96-hr) experiments.

Based on the data collected, the durations of juvenile periods, and embryonic development as well as average lifespan of the rotifers were obtained. Survivorship (l_x) and fertility (m_x) tables were constructed for each cohort (replicate) using conventional life-table techniques (Poole 1974). Net reproductive rate (R_0), generation time (T), life expectancy (e_0), and intrinsic rate of natural increase (r_m) were calculated, according to Krebs (1985) and Lotka (1913).

RESULTS AND DISCUSSION

Based on a 24-hr acute toxicology assay for thiophanate-methyl, the LC_{50} value of *Brachionus calyciflorus* was 5.02 mg/L.

Compared to the control, 0.6 mg/L thiophanate-methyl did not prolong the duration of juvenile period of the rotifers, however, both 1.2 mg/L and 1.8 mg/L thiophanate-methyl delayed significantly the onset of reproduction of the rotifers ($P<0.01$)(Fig. 1). A single factor ANOVA showed that the duration of juvenile period was significantly influenced by the concentration of thiophanate-

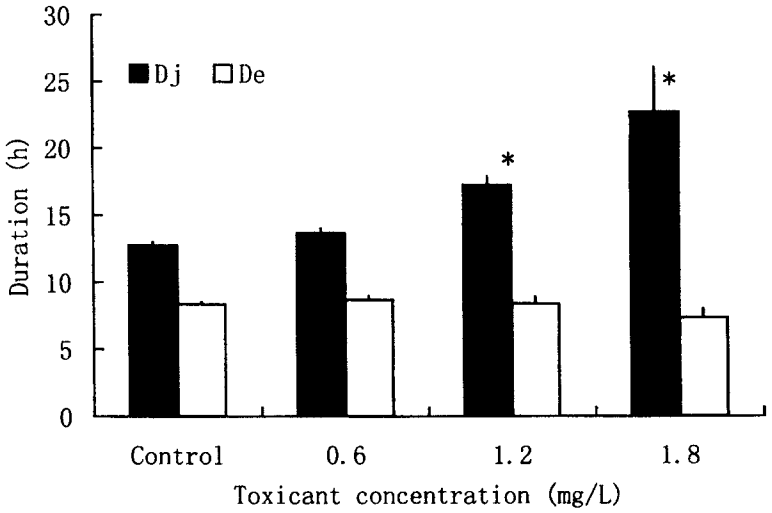

Figure 1. The effects of thiophanate-methyl on durations of juvenile period (Dj) and embryonic development (De) of *B. calyciflorus*. (* significantly different from the control, $P<0.05$)

methyl ($P<0.01$), but the reverse was the duration of embryonic development ($P>0.05$). The relationship between the duration of juvenile period (Y, hr) and the concentration (X, mg/L) of thiophanate-methyl could be described as:

$$Y=3.1956X^2-0.1355X+12.7195 \qquad R^2=0.5430, P<0.01$$

Pourriot (1986) thought that the rate at which planktonic rotifers multiply during the parthenogenetic phase, providing there is sufficient food, is due more to the short period of embryonic development and the early period of life than to the net reproduction rate. It is well known that some environmental factors such as temperature, food quality and quantity, pH, and quality of culture medium can significantly influence the duration of juvenile period of rotifers (Galkovskaja 1987, Awaiss and Kestemont 1992, Xi and Huang 2000, Xi et al. 2001a, b). In present study, we found that as little as 1.2 mg/L thiophanate-methyl prolonged significantly the duration of juvenile period of *B. calyciflorus*. The prolonged juvenile period caused by thiophanate-methyl might be one of the factors that led to lower population growth rate of *B. calyciflorus*.

The effects of thiophanate-methyl on the age-specific survival (l_x) and fertility (m_x) are presented in Fig. 2. When the toxicant concentration increased from 0.6 to 1.2, 1.8, 2.4, 3.0, 3.6, and 4.2 mg/L, toxicant-inflicted morality occurred after 32, 24, 16, 16, 16, 16, and 16 hr, respectively. However, the morality in the control appeared after 40 hr. Both the lifespan and the survivorship of the cohorts decreased significantly ($P<0.05$) with increasing concentration of thiophanate-

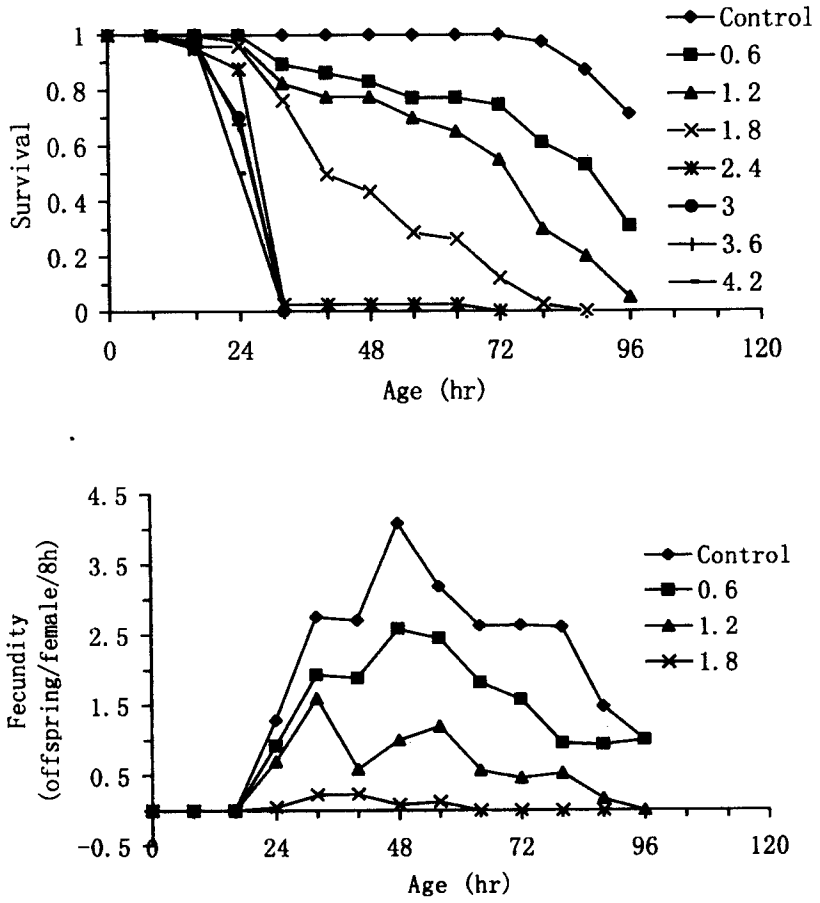

Figure 2. The effect of thiophanate-methyl on the age-specific survivorship and fecundity of *B. calyciflorus*

methyl (Table 1, 2), which was similar to the effects of pentachlorophenol (PCP), copper (Cu), 3,4-dichloroaniline (DCA), and lindane on *B. calyciflorus* (Janssenet al. 1994). The rotifers exposed to 2.4, 3.0, 3.6, and 4.2 mg/L thiophanate -methyl could not reproduce. Compared to the control, the age-specific fertility of the rotifers decreased significantly as thiophanate-methyl concentration increased to 0.6 mg/L or more.

All demographic parameters of the rotifers treated with 1.2-4.2 mg/L thiophanate-methyl were significantly lower than the controls. Exposure to 0.6 mg/L thiophanate-methyl did not significantly affect T and e_o, but all other demographic parameters were significantly lower than the controls (Table 1). Several authors have proposed the use of life-table techniques and the subsequent calculation of the population statistic r_m as effect criterion in chronic toxicity

Table 1. The demographic parameters (±SD) of *B. calyciflorus* exposed to several concentrations of thiophanate-methyl in a 96-hr life table study.

Toxicant (mg/L)	r_m (/hr)	R_o (ind.)	T (hr)	e_o (hr)	Longevity (hr)
Control	0.0689	23.91	46.16	87.79	92.32
	(0.0018)	(0.53)	(0.90)	(5.70)	(1.06)
0.6	0.0552	12.64	44.79*	71.44*	75.24
	(0.0049)	(2.32)	(1.23)	(4.72)	(4.05)
1.2	0.0389	5.06	39.86	56.32	62.40
	(0.0031)	(1.06)	(1.83)	(2.64)	(3.75)
1.8	-0.0344	0.45	35.82	38.77	42.29
	(0.0183)	(0.15)	(2.97)	(3.42)	(2.89)
2.4	-	0	-	25.28	23.60
	-	-	-	(1.29)	(1.21)
3.0	-	0	-	23.36	21.20
	-	-	-	(0.55)	(0.73)
3.6	-	0	-	23.36	21.20
	-	-	-	(0.67)	(0.67)
4.2	-	0	-	22.24	19.80
	-	-	-	(0.48)	(0.70)

* no significant difference with the controls ($P>0.05$)

studies (Gentile et al. 1982, Rao and Sarma 1986, Day and Kausiki 1987, Boyum and Brooks 1988). Since r_m integrates age-specific survival and several aspects of the reproduction such as: age of the first reproduction, reproductive frequency, brood or clutch size, and reproductive longevity, r_m is a more realistic criterion to study than the currently used separate measures of survival and reproduction. Although Janssen et al. (1994) thought that r_m is not always the most sensitive parameter in toxicity tests, we found that in the present study, r_m is one of the most sensitive parameters which can be used to indicate the effect of thiophanate-methyl on the population growth of *B. calyciflorus*. In addition, R_o and longevity are also sensitive parameters in this study. Of course, it should be investigated whether all those parameters including r_m, R_o, and longevity are sensitive to lower concentration (<0.6 mg/L) of thiophanate-methyl.

Table 2. The relationships between r_m (Y, /hr), R_o (Y, offspring/female), T (Y, hr), e_o (Y, hr), and longevity (Y, hr) of *B. calyciflorus* and thiophanate-methyl concentration (X, mg/L).

Parameter	Regression equation	Significant test
r_m	$Y=-0.0414X^2+0.0202X+0.0661$	$R^2=0.83$, $P<0.01$
R_o	$Y=4.6272X^2-21.3237X+23.8780$	$R^2=0.94$, $P<0.01$
T	$Y=-1.8481X^2-2.6632X+46.3850$	$R^2=0.60$, $P<0.01$
e_o	$Y=0.6319X^2-4.6668X+11.2658$	$R^2=0.94$, $P<0.01$
longevity	$Y=5.0062X^2-39.2962X+95.7897$	$R^2=0.76$, $P<0.01$

All demographic parameters of the rotifers were significantly influenced by the concentration of thiophanate-methyl, and the relationships between those and thiophanate-methyl concentration were curvilinear (Table 2).

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