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Chronic Toxicity of Methylparathion to *Daphnia magna*: Effects on Survival, Reproduction, and Growth

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Cladocerans, especially *Daphnia sp.*, are among the most favourable test animals in aquatic toxicology. The many advantages of daphnids, i.e., sensitivity to toxicants, parthenogentic reproduction and the short reproductive cycle and life span, can hardly be found in combination in any other species.

The evaluation of the effects of hazardous chemicals on aquatic organisms usually includes chronic toxicity tests (Sprague 1976; Gersich *et al.* 1985). Daphnids, especially *Daphnia magna*, have been used for many years in standard tests of acute toxicity (Buikema *et al.* 1980; Lawrence 1981; Nebeker *et al.* 1983; Ferrando *et al.* 1992). It is well recognized that this type of information is not enough to identify an acceptable, non-toxic, concentration that protects against chronic effects on organism growth and reproduction (Sprague 1976).

Effects of pollutants on the reproduction of *Daphnia magna* have been noticed in several studies (Van Leeuwen *et al.* 1985a; Day and Kaushik 1987; Bodar *et al.* 1988; Gersich *et al* 1989; Kühn *et al* 1989). In most cases the reproduction was affected negatively by the toxic agent, but stimulatory effects have been reported as well. Very low concentrations of heavy metals, i.e., cadmium have proven particularly beneficial to reproduction, i.e., the number of neonates per female increased (Bodar *et al.* 1988).

One of the reasons daphnia species have been used extensively in toxicity tests is the sensitivity to a broad spectrum of toxicants. Their short life-cycle and the good correlation of 21-d chronic tests with chronic fish toxicity data made these tests an attractive alternative to long-term fish toxicity studies (Maki 1979).

The objective of this study was to assess the effects of low levels of the organophosphorus pesticide, methylparathion, on survival, reproduction and growth of *Daphnia magna* using a standard 21-d semi-static procedure.

MATERIALS AND METHODS

Daphnia magna organisms were obtained from continuous laboratory cultures at 22±1°C, in dechlorined tap water (total hardness, 250 mg/L as $CaCO_3$; pH 7.9±0.2; alkalinity, 4.1 mmol/L), 12 hr:12 hr light:dark photoperiod and a density of below 50 animals/L . The medium was renewed three times each week and the daphnids were fed daily *ad libitum* with the alga *Nannochloris oculata*. This alga is also continuously cultivated in our laboratory using a nutrient medium (Bischoff and Bold 1983).

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Offspring separated at regular intervals, taken from cultures 3-5 wk old (brood 5-10), were used as test animals 6-24 hr juveniles.

Technical grade (80% purity) methylparathion (Bayer Hispania) was used as test chemical. Stock solutions were prepared by dissolving the toxicant in acetone (1mL/L) immediately prior to each experiment. Because acetone was required as a carrier, a control with acetone was included with a concentration equivalent to the amount in the highest test concentration (1µL/L acetone).

Prior to the chronic experiments, the acute toxicity of methylparathion was studied in order to evaluate the sensitivity of the species and to establish the range of concentrations to be used. The experiment was carried out three times, according to the EEC standard procedure (EEC 1984) for determining the 24-hr LC50. Six test concentrations plus a control with acetone were used. A single brood was used for each test. For each replicate, ten neonates (< 24 hr old) were placed in a 30-mL glass beaker containing 25 mL of test solution. Because of the short duration of the test (24 hr) the organisms were not fed and the medium was not renewed during the experiments. Observations were made at 24 hr and the mortality results recorded. Control survival was always 100%.

Based on the results of the acute toxicity tests, five test concentrations of methylparation (approximately 1/4, 1/2, 2/3, 3/4 and 5/6 24-hr LC50, which was 0.31 ng/L) were chosen for the chronic study. Daphnids were exposed to 0 (control), 0.07, 0.15, 0.20, 0.25 and 0.27 ng/L methylparathion and a control with acetone (1µL/L).

Fifteen juveniles (< 24 hr old) were obtained randomly from the stock cultures and raised individually in 60-mL glass beakers containing 50 mL of dechlorined tap water and the appropiate pesticide concentration. The studies were designed to use a semi-static procedure where all daphnids were transfered every other day to a clean beaker containing fresh medium and food. The experimental conditions were the same as used in the culture. During the test, the daphnids were fed daily with *Nannochloris oculata* at a density of $5x10^5$ cell/mL.

Each test animal was observed daily and progeny were counted and discarded for a period of 21 d. Longevity, time to the first reproduction, total number of neonates per female, number of broods, and brood size were the criteria used.

The intrinsic rate of natural increase, r, was calculated using successive approximations of the formula of Lotka (1913): $\sum \mathbf{lx \cdot mx \cdot e^{-r.x} = 1}$ where: x=time interval observed; e=base of natural logarithm; lx=the probability of surviving to age x; mx=the number of female offpring per female of age x born during the interval x to x+1 and r=the intrinsic rate of natural increase. "r" calculated after 21 days is indistinguishable from "r" estimated for the entire lifespan, due to the great importance of early reproduction (van Leeuwen *et al.* 1985a).

Growth of surviving adults was determined after 21 d of exposure. The length of each surviving animal was measured at 21 d, from the apex of the helmet to the base of the tail spine.

The results of the chronic test are expressed as the maximum acceptable toxicant concentration (MATC), this value is defined as the estimated toxic threshold concentration falling between the highest concentration showing no effect (NOEC)

and the next lowest concentration showing a toxic effect (LOEC) when compared with the control (McKim 1977). Additionally, pH, dissolved oxygen and temperature were measured and recorded on each renewal day.

The 24-hr LC50 values with the 95% confidence limits were calculated according to Litchfield and Wilcoxon (1949) with an IBM computer program.

In the chronic experiments, data from control, acetone control and test concentrations were compared by analysis of variance (ANOVA), where differences were significant ($p \leq 0.05$), then mean values were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Methylparathion 24-hr LC50 mean value for *Daphnia magna* was 0.31 ng/L with a standard deviation of 0.07 ng/L, its corresponding 95% confidence interval was (0.13-0.63) and a coefficient of variation of 22.5%.

Methylparathion 24-hr LC50 value for *Brachionus calyciflorus* was 29.19 mg/L (Fernández-Casalderrey *et al* 1992). Johnson and Finley (1980) also tested this organophosphorus insecticide with *Daphnia magna* and they found a 48-hr LC50 of 0.14 µg/L; for *Gammarus fasciatus* a 96-hr LC50 of 3.8 µg/L and for *Simocephalus sp.* the 48-hr LC50 value was 0.37 µg/L. We found that *D. magna* was much more sensitive to methylparathion short-term exposure than other species of aquatic invertebrates. Kühn *et al.*, (1989) conducted tests with *Daphnia magna* exposed to ethylparathion finding 48-hr LC50 of 2 µg/L. These data show that the toxicity of methylparathion is greater than the toxicity of the insecticide ethylparathion on *D. magna.*

In the analyses of chronic toxicity tests, it is important that all major demographic parameters be examined. In the present study, the effects of several concentrations of methylparathion on survival, time to first reproduction, reproductive frequency, number of young per brood and intrinsic rate of natural increase were studied.

All the parameters tested were influenced by the sublethal concentrations of methylparation to which the organisms were exposed (Table 1). The longevity of daphnids declined with increasing methylparathion concentrations, but only the differences between control and 0.25 and 0.27 ng/L were statistically significant ($p \leq 0.05$). The survivorship curves (Fig. 1) show that the longevity was only affected by 0.25 and 0.27 ng/L methylparathion.

Several researchers (Daniels and Allan 1981; Ingersoll and Winner 1982) have suggested that survival in chronic toxicity tests is the best index of toxicity because it is more sensitive and less variable than reproductive parameters. Day and Kaushik (1987), however, suggested that survival is only a good indicator when exposure to toxicants continues throughout the entire life-cycle of the organism. He found that survival of *D. galeata mendotae* exposed to 0.01 µg/L fenvalerate did not differ significantly from that of the controls for approximately 30-32 d.

Similar effects to those observed on the survival of *D. magna*, were also found on the rotifer *Brachionus calyciflorus* when exposed to the pesticide methylparathion. The survivorship was decreased from 11 d in controls, to 8 days after the treatment with 5 mg/L (Fernández-Casalderey *et al.* 1993).

The average total production of young per female was also affected, being significantly smaller in the two highest methylparathion concentrations (0.25 and 0.27 ng/L). Mean brood size and mean number of broods were also reduced in daphnids exposed to 0.25 and 0.27 ng/L methylparathion (Table 1). Reproduction was significantly reduced with 0.25 and 0.27 ng/L methylparathion, as a result of a decreasing numbers of broods and neonates per brood. Number of neonates born declined significantly ($p \leq 0.05$) from 51.92 young (control) to 33.81 and 25.36 young with 0.25 and 0.27 ng/L, respectively.

The onset of reproduction of *D. magna* was not delayed with 0.07, 0.15 and 0.20 ng/L methylparathion (range 8.4-8.5 d). The time required to reach reproductive maturity with 0.25 and 0.27 ng/L increased compared with control, but the differences were only statistically significant ($p \leq 0.05$) at the highest level (0.27 ng/L).

Populations of *D. magna* under control conditions and exposed to the acetone control had "r" values of 0.26 and 0.28, respectively. Concentrations of 0.07, 0.15, 0.20 and 0.25 ng/L methylparathion did not delay reproductive maturity, thus "r" at these concentrations (0.27, 0.28, 0.27 and 0.25, respectively), was not significantly different from that for controls . Methylparathion had little effect on "r" (0.19) up to 0.27 ng/L, a concentration which also reduced survivorship and limited reproduction. The magnitude of "r" is affected by time of first reproduction, numbers of broods and brood size, and a change in one of these parameters caused by sublethal stress could change the "r" values for that population (Allan 1976; Stearns 1976).

Day and Kaushik (1987) found reduction in total young per female, mean brood size and number of broods when *Daphnia galeata mendotae* was exposed to 0.01 and 0.05 µg/L fenvalerate, but they did not observed any effect in the numbers of days to first reproduction with the fenvalerate levels tested. Similar effects on reproduction were observed by Crossland and Hillaby (1985) in *D. magna* exposed to sublethal levels (20, 50 and 100 µg/L) of DCA. They also observed an increase in the first day of production of young from 8 d (control) to 10.75 and 11 d with 20 and 50 µg/L, respectively.

The intrinsic rate of natural increase "r" was a relatively insensitive parameter of toxicity due to the delayed effect of methylparathion on reproduction. The importance of early reproduction in contributing to the estimation of "r" has been demonstrated clearly by Birch (1948). For populations such as daphnids with several overlapping generations, "r" will be determined primarly by the number of young per brood and the frequency of the first few broods will contribute relatively little to "r". Only toxicants which cause a decrease in the number and/or size of the first few broods of daphnids will cause "r" to decrease significantly. Examination of mean brood size per successive brood and cumulative brood size of *D. magna* exposed to toxicant levels below 0.27 ng/L indicates that the first few broods do not have significantly lower numbers than those of controls but numbers in later broods are reduced. This results in "r" values are not significantly different from the control. A concentration of 0.27 ng/L metylparathion significantly reduced the mean number of young in the first clutches and reduced survivorship, resulting in a reduction in "r".

Daniels and Allan (1981) found that cohorts of *D. pulex* exposed to increased concentrations of dieldrin showed little reduction in "r" until a concentration of 5 µg/L was reached which reduced survivorship, fecundity and number of broods. A reduction in the intrinsic rate of natural increase "r" resulted as a consequence of

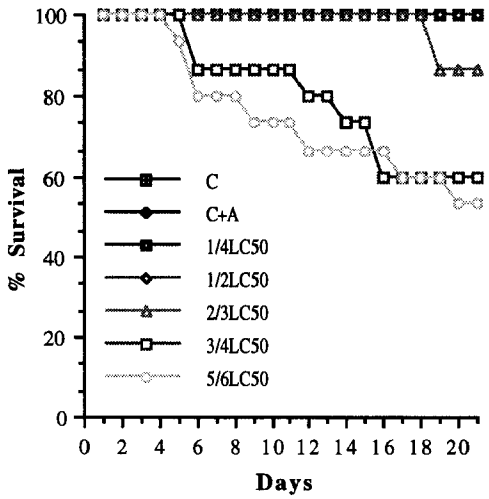

Figure 1. Survivorship curves of *D. magna* at seven concentrations of methylparathion.

chronic toxicant stress of fenvalerate on *D. magna* (Day and Kaushik 1987). The "r" values for populations of *D. magna* under control and acetone control conditions (0.26 and 0.27, respectively) compare favourably to those of Day and Kaushik (1987) who found "r" to range from 0.23 and 0.24 for *D. galeata mendotae* in the laboratory under different levels of food at 20°C. Similar results were found by Francis *et al.* (1986) on *D. magna* after a 21 d chronic test. The "r" values observed by these authors were 0.36 and 0.38 in a static-renewal test and in a flow-through test, respectively.

A significant reduction in mean caparace length of 21-d-old daphnids was detected at the highest methylparation concentrations: a decrease was found from 0.76 cm (controls) to 0.68 and 0.69 cm (0.25 and 0.27 ng/L, respectively). The lowest concentrations of methylparathion tested did not produce any significant ($p>0.05$) effect compared with the control. van Leewen *et al.* (1985b) found that these effects occurred at levels comparables with their LC50 values when *D. magna* was exposed to the carbamates, ziram and thiram. The length of *D. magna* was also reduced significantly ($p\leq0.05$) after exposure to 20 mg/L 4-nitrophenol (Francis *et al.* 1986).

The data used to estimate the chronic value (MATC) for methylparation are presented in Table 1. Interpretation of the data indicates that MATC lies between 2/3 and 3/4 LC50 (0.20 and 0.25 ng/L) methylparathion. Another estimate of the MATC value expressed as the geometric mean of 0.20 and 0.25 ng/L is 0.22±0.02 ng/L. The determination of the MATC was based on the endpoints: survival, mean total young per female, mean brood size, mean number of broods and length of adults, all of which were significantly ($p<0.05$) different from the control at the 0.25 ng/L (3/4 LC50) level.

The chronic data from this study can be used for formulating an acute/chronic ratio. Dividing the chronic value generated during this study (0.22 ng/L) into the daphnid acute LC50 value (0.31 ng/L) results in a daphnid acute/chronic ratio for

methylparathion of approximately 1.4. Acute/chronic ratios play an important role in the development of water quality standards. Based on the acute/chronic ratio calculated in our study for methylparathion it would be unlikely to observe chronic invertebrate effects much below levels that are acutely toxic.

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