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Toxic Effect of Parathion on *Moina macrocopa* Metabolism

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With the modernization of agricultural operations and the rapid growth of industrial activity, there has been much increase in the manufacture and utilization of insecticides which ultimately find their way into the rivers, lakes and ponds (Ridgway *et al.* 1978). Parathion, an organophosphorus insecticide, is still widely used in México (CNE 1988) and it represents a serious water pollution due to its toxic properties (Metelev *et al.* 1983).

Parathion can alter metabolism by stimulation or inhibition of enzymatic systems (Martínez-Tabche and Posadas 1989; Mourelle *et al.* 1986). Studies on the toxicity of this insecticide to fresh water organisms have concentrated on fish and have yielded only limited information concerning invertebrates metabolism. The purpose of the present study was to determine the effect of parathion exposure on the metabolism of *Moina macrocopa*. This microcrustacean is a natural component of fresh water ecosystems in México and it is a main source of food for several species of fish.

MATERIALS AND METHODS

Moina macrocopa were cultured in the laboratory from parthenogenetic females and were less than 48-hr at the start of experiment. All brood stock moinids are mantained on a diet of the green algae *Ankistrodesmus falcatus*. They were fed daily at rate of 0.625 mg dry wt/L of synthetic water. The composition of the algal medium (Table 1) is according to Kessler (1957). Erlenmeyer flasks (500-mL) are filled with algal medium. The medium is boiled for 15-min and after cooling inoculated with *A. falcatus* from stock cultures. The algal cultures are aerated with air purified by leading

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it through wash bottles with cotton wool and water. They are illuminated with fluorescent lamps. The algae are settled by centrifugation at 1,500 g and resuspended in *M. macrocopa* medium.

Table 1. Chemical composition of algal medium.

Substance	Conc. (mg/L)	Substance	Conc. (mg/L)
a. $NaNO_3$	250	h. H_3BO_3	11.92
b. $CaCl_2 \cdot 2H_2O$	25	i. EDTA	50
c. $MgSO_4 \cdot 7H_2O$	75	KOH	31
d. K_2HPO_4	75	j. $ZnSO_4 \cdot 7H_2O$	8.82
e. KH_2PO_4	175	$MnCl_2 \cdot 4H_2O$	1.44
f. NaCl	25	MoO_3	0.71
g. $FeSO_4 \cdot 7H_2O$	4.98	$CuSO_4 \cdot 5H_2O$	1.57
H_2SO_4 conc.	1.84	$Co(NO_3)_2 \cdot 6H_2O$	0.49

Moina was reared in reconstituted distilled water according Peltier and Weber (1985) at 20 ± 1°C at a natural photoperiod. Physicochemical characteristics of the reconstituted water are as follows: hardness (as $CaCO_3$), 35-45 mg/L; alkalinity (as $CaCO_3$), 20-30 mg/L; pH 7.2-7.6; dissolved oxygen (DO), 6.5-7.5 mg/L. Parathion (96.86%) was obtained from Bayer of México. Stock solutions were prepared in ethanol. Test solutions were prepared by pipetting 100 µL of stock solutions into 1 L of reconstituted water.

Acute static toxicity test consisted of exposing groups of 28 moinids to six serial concentrations of parathion (0.26-2.08 µg/L), a dilution water control and a solvent carrier control (ethanol 0.1 mL/L). All test concentrations and the controls were set in triplicate. The tests beakers were kept in an environmental chamber set at 20 ± 1°C and with a natural photoperiod. The duration of the acute test was 48-hr. The moinids were not fed nor were the solutions aerated during the testing procedures. Lack of detectable movement was used as the criterion for mortality. The method of Litchfield and Wilcoxon (1949) was used to calculate LC_{50} and its 95% confidence intervals (CI).

Five 600-mL beakers were utilized as test chambers to evaluate metabolism parameters. At procedure initiation 400 mL of the test solution and 25 moinids (5-7 d old) were added to the chamber. *Moina* was exposed to three parathion concentrations: 0.26, 0.52 and 0.78 µg/L. A reconstituted water control and a solvent carrier

control (0.1 mL/L) were used in each of four replicates (seven for hemoglobin). Selected water quality parameters were monitoring during the test. Test duration was 48-hr. Neonates and dead crustaceans were removed from beakers at 24-hr intervals. The moinids were fed daily and the test solutions were not aerated during the procedure.

Samples were collected by pipetting *Moina* from each of four replicates per concentration. Invertebrates were homogenized in 50 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 15,000 g for 3-min. Aliquots of supernatant were analyzed for soluble proteins, glucose and hemoglobin (Hb). Proteins were determinated by the method of Bradford (1976), which involves the binding of Coomassie Brilliant Blue G-250 to protein. The reaction mixture contained 2.5 mL of colored reagent and 100 µL of supernatant. The binding causes a shift in the absorption maximum of the dye at 595 nm. Glucose was evaluated by the method of Hyvärinen and Nikkilä (1962), modified. The procedure involves the reaction between o-toluidine and glucose in hot acetic acid solution to produce the corresponding Schiff base. The supernatant was mixed 1:10 with 3% trichloracetic acid. After 10-min, the suspension was centrifugated and 1 mL of the protein-free filtrate was added to 2.5 mL of Hycel o-toluidine reagent. The mixture was placed in a boiling water bath for 10-min and cooled for 3-min. The green colored end product has an absorption maximum at 630 nm. Hb was determinated by the cyanomethemoglobin technique (Crosby *et al.* 1954). The sample is diluted with a reagent containing ferricyanide and cyanide, which converts both reduced Hb and oxyhemoglobin to cyanomethemoglobin form. 100 µL of supernatant was added to 5 mL of cyanomethemoglobin reagent. After 20 min, absorbance was determinated at 540 nm. The absorbances were quantitized in a spectrophotometer Varian DMS 90.

All statistical comparisons were performed using Student's *t* test ($p<0.05$).

RESULTS AND DISCUSSION

Water quality parameters analyzed at the end of the study revealed that pH, hardness, alkalinity and DO were within the range of values reported above.

The acute toxicity of parathion to *Moina macrocopa* was estimated by determining the 48-hr LC_{50} value. The calculated 48-hr LC_{50} value of parathion was 0.94 µg/L (95% CI: 0.74-1.14). It must be emphasized that LC_{50} is based on the initial amount of the insecticide added to the reconstituted water. Controls mortality was

≤ 10% in all tests. The no observable effect level was 0.13 μg/L (mortality ≤ 10 %) and the CL_{100} was > 2.08 μg/L. This result shown that the microcrustacean *Moina macrocopa* is a very sensitive species to parathion.

The effect of different concentrations of parathion on *M. macrocopa* metabolism is shown in Table 2. Analyses were performed only on microcrustaceans surviving 48-hr of exposure. The increase of glucose content suggests that the organophosphorus insecticide interferes with some of the vital physiological functions. Reddy and Rao (1988) and Reddy *et al.* (1986) reported that the organophosphorus insecticides phosphamidon and malathion induce glycogenolysis in tissues of crustaceans and the free glucose molecules cause hyperglycemia. They found increases in the glycolytic enzymes and lactate level, and decreases in Krebs cycle enzymes that indicate depression in cellular oxidation, development of anaerobic conditions and decreased oxygen consumption at the whole animal level. Hypoxic or anoxic conditions normally increase glycogenolysis and Hb synthesis in different species. Particularly in the order cladocera, the rate of Hb synthesis augments remarkably when the microcrustaceans are exposed to low oxygen concentrations (Kobayashi and Nezu 1986).

Table 2. Effect of parathion on *Moina macrocopa* metabolism.

Parathion (μg/L)	Protein (μg/mg wet wt of flea)	Glucose	Hemoglobin
Water control	49.375 ± 0.778	2.204 ± 0.151	1.376 ± 0.308
Ethanol control	50.414 ± 1.749	2.612 ± 0.599	1.375 ± 0.280
0.26	57.230 ± 1.342	4.571 ± 0.491	4.428 ± 0.520
0.52	62.250 ± 1.433	5.742 ± 0.173	6.423 ± 0.520
0.78	78.585 ± 3.011	6.375 ± 0.404	10.699 ± 0.748

Each value is mean ± SE. All values are significantly different from water control at $p<0.025$.

At the present study, Hb levels are increased even if DO determinated throughout the test was within the range of air-saturated water. We suggest that the probable inhibition of Krebs cycle enzymes might be responsible for decreased oxygen consumption. The oxygen concentration in hemolymph may be a determinant factor to induce the Hb synthesis. Simultaneously an

increase in soluble protein level was also observed in this study. This effect may be because of induction of globin synthesis, but the precise mechanism and respective relationship involving highering in glucose and hemoglobin level together with rise in protein content by parathion in *Moina macrocopa* needs indepth investigation.

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