Bull. Environ. Contam. Toxicol. (1994) 52:360–366

Environmental Contamination and Toxicology

Parathion Effect on Acetylcholinesterase from Fish through an Artificial Trophic Chain: *Ankistrodesmus falcatus–Moina macrocopa–Oreochromis hornorum*

L. Martínez-Tabche,[1] C. I. Galar,[2] M. B. Ramírez,[1] R. A. Morales,[1] F. C. Germán[1]

[1]Department of Pharmacy, National School of Biological Sciences—IPN, Apdo. Postal 105–314, Del M. Hidalgo, CP 11340, México, D.F., Mexico
[2]Department of Biophysics, National School of Biological Sciences—IPN, Apdo. Postal 105–314, Del M. Hidalgo, CP 11340, México, D.F., Mexico

Received: 1 September 1992/Accepted: 30 July 1993

Compounds used as pesticides have been found as underground and aquatic pollutants and their bioaccumulation may cause damage to the organisms of the trophic chains (Eichelberg 1971). The parathion, an organophosphorous insecticide, is widely used in México and this pesticide residues are widespread in freshwater ecosystems, contaminating the water bodies.

The parathion elimination, in fresh water, follows a first order kinetic relationships and 50 d are necessary to decrease on 90% its concentration (Badaway 1984). In this work, the toxic effect produced by parathion on an artificial trophic chain was evaluated. This chain was: *Ankistrodesmus falcatus* (alga), *Moina macrocopa* (aquatic flea) *and Oreochromis hornorum* (fish). The evaluated parameters were: cells/mL and protein concentration in the alga; lipid, protein and glucose concetrations, in the aquatic flea, and acetylcholineserase activity in fish. The only one organism exposed to the pesticide was alga.

MATERIALS AND METHODS.

In order to evaluate parathion toxicity, a water sample from Chapultepec Lake (México, Lat. N 19° 26' 05''; Long. W 99° 07' 58") was withdrawn and the alga *Ankistrodesmus falcatus* was isolated to obtain a monospecific growth, which was taken as basic culture. Sterile basic culture (100 mL) was added to 800 mL of nutrient medium. The composition of the algae medium (Table 1) was according to Kessler (1957). The culture was divided into two equal samples (450 mL each)), one was exposed to parathion (purity 98 %, Bayer de México, México City). Parathion was previously dissolved in acetone and water to give a final concentration of 7.5 µg/L. The other part of cells was grown in a pesticide free-medium.

Correspondence to: L. Martínez-Tabche

Table 1. Chemical composition of algal medium

Sustance	mg/L	Sustance	mg/L
$NaNO_3$	250.00	H_3BO_3	11.92
$CaCl_2 \cdot 2H_2O$	25.00	EDTA	50.00
$MgSO_4 \cdot 7H_2O$	75.00	KOH	31.00
K_2HPO	75.00	$ZnSO_4 \cdot 7H_2O$	8.82
KH_2PO_4	175.00	$MnCl_2 \cdot 4H_2O$	1.44
NaCl	25.00	MoO_3	0.71
$FeSO_4 \cdot 7H_2O$	4.98	$CuSO_4 \cdot 5H_2O$	1.57
H_2SO_4 (concen)	1.84	$Co(NO_3)_2 \cdot H_2O$	0.49

All cultures were kept in the same conditions of temperature (20 °C), ilumination (3900 lux, with fluorescent lamps) and oxygenation during 8 d. In order to eliminate the parathion from the medium, after the exposure period, the alga was filtered and then washed three times with deionized water. The cell pellet was suspended in 250 mL of culture media. Then two experiments were performed. For the first one , two aliquotes of 5 mL each, were taken and cell number/mL were measured, as well as the weight and protein concentration by the Bradfort method (1976), which involves the binding of Coomasie Brilliant blue G-250 to protein. In this determination, the cell suspension was sonicated and centrifuged. Then 100 μL of supernatant were added to 2.5 mL of colored reagent. The binding causes a shift in maximum absorption of the dye at 595 nm.

In the second experiment, the cell density was adjusted to 1.5×10^6 cell/mL with reconstituted water in order to feed, during 3 d, 4 lots of 250 mg (8 organisms/mg) of *Moina macrocopa*, which were brought from Texcoco Lake, Méx. and adapted to laboratory conditions. After feeding *M. macrocopa* with the intoxicated algae, 50 mg (approximately 400 individual fleas) were taken and homogenized, with 1.0 mL of deionized water, and then they were centrifuged. From the supernatant, glucose, lipid and protein concentrations were measured. Glucose was determined by Hivärinen method (1962). The procedure involves the o-toluidine and glucose reaction in hot acetic acid solution, to produce the corresponding Shiff base.

Five hundred μL of supernatant were mixed 1:10 with 3 % trichloroacetic acid. After 10 min the suspension was centrifuged and 1.0 mL of the protein-free filtered was added to 2.5 mL of Hycel o-toluidine reagent. The mixture was placed in a boiling water bath for 10 min and then cooled. The green colored finished product has its maximum absorption at 630 nm.

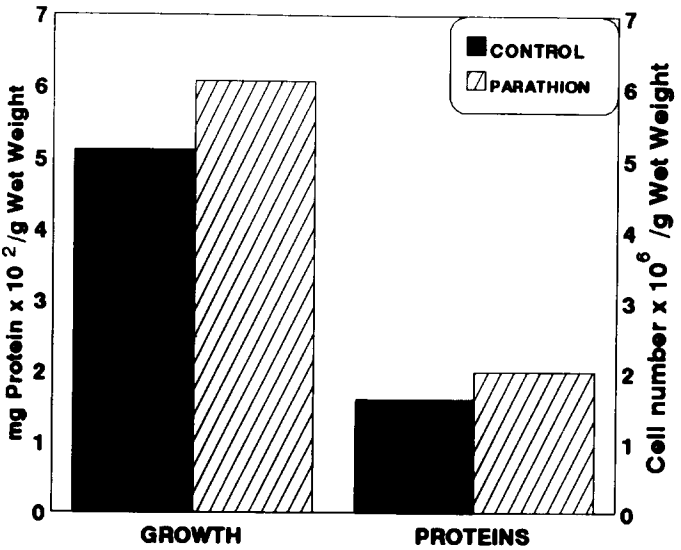

Figure 1. Parathion (7.5 µg/L) effect on protein levels and growth of the alga *Ankistrodesmus falcatus*.

Lipids were determined by the phosphovainillyn technique (Pande *et al* 1963). This method is based on the total lipids reaction with vainillyn in phosphoric acid medium. The samples were previously treated with hot sulphuric acid to obtain a pink chromophore. Two hundred µL from the supernatant were mixed with 1.0 mL of sulphuric acid. This mixture was placed in a boiling water bath for 10 min and then it was cooled. Two and a half mL of phosphovainillyn were added and the sample was placed again in the boiling water bath at 37 °C for 10 min. The pink colored finished product has its maximum absortion at 540 nm. In addition 100 µL of supernatant were used to determine protein concentration by the Bradford method (1976), as described above.

The remainder *M. macrocopa* (0.2 g/lot), was used to feed one fish during 4 d. This experiment was done nine times The average of fish weight and SEM were 3.5 and 1.0 g, respectively. Fishes were obtained from a fish farm in Zacatepec, Mor. (México) and kept in an experimental aquarium containing 2 L of water. The water quality was: temperature 20 ± 1.0 °C; alkalinity (as $CaCO_3$) 60 ± 10 mg/L, disolved oxygen 5 ± 2 mg/L; hardness (as $CaCO_3$) 129 ± 2 mg/L; pH, 7.0 ± 0.5. The fish allowed to acclimate to the aquarium for 7 d prior to the introduction of the intoxicated fleas. After feeding during 4 d, with *M. macrocopa*, the fish was decapitated, its brain was taken out and homogenized with deionized water, and acetylcholinesterase was determined. The activity of this enzyme was assayed according to the Rappaport *et al* method (1959). This method is based on the use of an indicator,

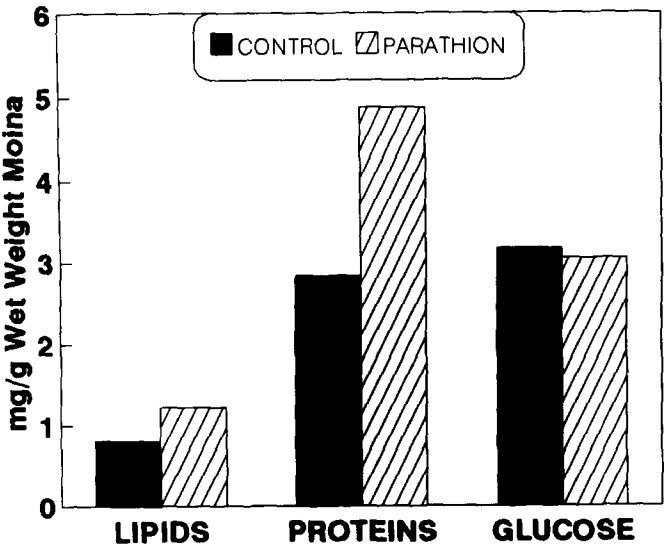

Figure 2. Parathion effect on lipid, glucose and protein concentration in *Moina macrocopa*, intoxicated through an artificial trophic chain.

m-nitrophenol, in order to measure the acetic acid produced by the enzymatic hydrolysis of acetylcholine. Thus, the supernatant of homogenized tissue was prepared in isotonic saline solution, 100 µL of sodium chloride solution (0.15 mg/L) were added to two homogenized aliquots of 200 µL (blank and test), and only the blank was placed in a 60 °C water bath for 10 min to inactivate the enzyme, 3 mL of deionized water were also added as well as 2.0 mL of nitrophenol solution (0.75 g/L in phosphate buffer, pH 7.8) and 0.2 mL of acetylcholine chloride (AChe) solution (75 mg of AChe in 500 mL of water). Solutions were kept at 25 °C 30 min after AChe solution addition, absorbance at 420±20 nm was measured. Acetic acid solution 0.02 N was used as standard. One Rappaport unit was taken as the amount of AChase able to deliver 1.0 µmol of acetic acid from AChe in 30 min at 25 °C and pH 7.8 under these test conditions.

The obtained values are presented as mean ± SEM and the mean contrast was done using the two-tailed Student's "t" test (Fisher 1950).

RESULTS AND DISCUSSION

Protein concentration of exposed *A. falcatus* was 26 % higher than control. Cells /g of wet tissue increased 19 % (Fig 1). The control and test values of protein were 160.0 ± 11.1 (n=8) and 201.4 ± 34.6 (n=7) µg prot/g of wet tissue respectively; the difference was 41.4 ± 38.9 µg/g , which is not significant (P> 0.20). Regarding cell/g, the control was (6.1 ± 0.5)x 10^6 (n=8) and with parathion it was (6.1 ± 0.63)x10^6 (n=7);

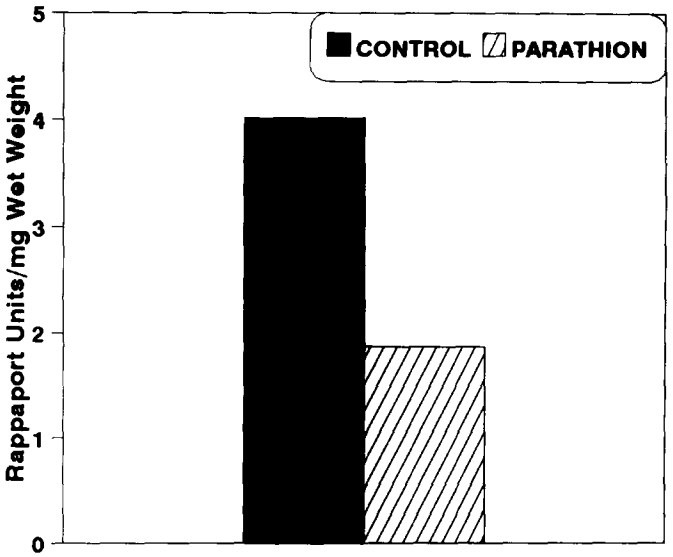

Figure 3. Parathion effect on ACHase activity in brain of *Oreochromis hornorum*, intoxicated through an artificial trophic chain.

the difference (1.0 ± 0.8)x10^6 cell/g), also was not significant (P >0.20).

Lipid and protein concentrations of *M. Macrocopa* increased 53 and 71 % respectively, when this cladocera was feed with alga *A. falcatus* previously exposed to parathion (Fig 2). The lipid average values (mg/g of flea) of 10 experiments, were 0.81 ± 0.057 and 1.22 ± 0.107 for control and tests respectively. The difference (0.41 ± 0.12) was significant (P < 0.01). Protein measurements gave 2.84 ± 0.13 in seven control experiments and 4.87 ± 0.29 in 10 experiments with parathion. Difference between means (2.03 ± 0.10) is highly significant (P < 0.001). On the other hand, difference between means of glucose values (0.14 ± 0.51 mg/g), of control versus parathion, was not statistically different of zero.

When the fish *Oreochromis hornorum* was fed with fleas *Moina macrocopa* previously fed with the alga *Ankistrodesmus falcatus* exposed to parathion, ACHase activity in the fish brain decresed 53.4 %. In this case, the control was the ACHase activity in brain from fish fed with fleas, which were fed with alga not exposed to the pesticide. The ACHase activity with parathion was 1.87 ± 0.27 nmol/mg Protein/hr, while the control value was 4.02 ± 0.38 nmol/mg Pr/hr (Fig 3). The effect was statistically significant P < 0.001). This result is in agreement with those found by Ahammad et al (1980) using other organophosphorus insecticide

(malathion) on *Tilapia mosambica*, exposing the fish directly to a medium containing malathion.

Results show that, in the alga *Ankistrodesmus falcatus*, the measured parameters, growth and protein concentration, were not significantly affected. However, when this alga was used as food for *M. macrocopa*, lipid and protein in this cladocera were significantly increased, even if the alga was washed three times with deionized water to eliminate the residual pesticide. This assay suggests a bioaccumuation of parathion by *A. falcatus*, and then it was transferred to *M. macrocopa*, because similar effect has been found by direct application of parathion to this species (Martínez-Tabche *et al* 1991). Furthermore, since the fish fed with fleas not directy exposed to parathion, showed a significant diminution in the acetylcholinesterase activity, it is suggested that the *M. macrocopa* also bioaccumulated parathion.

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