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Effects of Paraquat on Survival and Total Cholinesterase Activity in Fry of *Cnesterodon decemmaculatus* (Pisces, Poeciliidae)

W. D. Di Marzio, M. C. Tortorelli

Laboratory of Ecotoxicology, Department of Basic Sciences, National University of Luján, P.O. Box 221, (6700) Luján, Argentina

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Paraquat (PQ) (1,1'-dimethyl-4,4'-bipyridilium dichloride) is a broad-spectrum herbicide amply used in Argentina for crop desiccation and weed control. The application rate in water for aquatic weed control is 0.1 to 2 mg/L (Newman 1970; Calderbank 1972). *Cnesterodon decemmaculatus* is a native Argentine fish, which is widespread in freshwater environments of the Buenos Aires province (Ringuelet et al 1967). This area has the highest urban concentration, along with the greatest number of industrial, agricultural and cattle centers of Argentina. *Cnesterodon sp* is in the same family as *Poecilia reticulata*, a fish species frequently used in toxicity testing (Zagatto et al 1985).

This herbicide produces gill, kidney and liver alterations in fishes. Inhibition about 40% of the serum cholinesterase activity was reported when carps were treated with 10 mg/L PQ concentration (Nemcsók 1985). Shinohara and Seto (1986) show that PQ cause reversible inhibition of acetylcholinesterase activity.

The purpose of this study was to evaluate the effects of PQ on survival and cholinesterase activity (TCHA) of the fish species *Cnesterodon decemmaculatus*.

MATERIALS AND METHODS

The 96-hr acute toxicity test was conducted according to U.S.E.P.A. (1975) standards. *Cnesterodon sp* adults (250 individuals) were captured from an artificial pond culture and acclimated in tap water (pH: 7.70, conductivity: 380 µmhos/cm, dissolved oxygen concentration: 8.40 mg/L, hardness: 80 mg/L as CO_3Ca) at 22°C for 30 d. The fry were obtained by natural spawning and were immediately placed in artificial pond water (APW) for 10 d (pH: 7.17, conductivity: 180 µmhos/cm, dissolved oxygen concentration:8.90 mg/L, hardness: 90 mg/L as CO_3Ca). A batch of 500 fry was used in the experiments. The assayed product was Osaquat® (OSA Buenos Aires, Argentina) a commercial formulation containing 27.6 % of PQ. In the survival acute toxicity test and enzymatic assay the concentrations used were: 5.6, 13.5, 24, 32, 42 and 5.6, 7.5, 13.5 and 24 mg of PQ/L . Tests were conducted in glass bowls (8 cm diameter and 4 cm height) each containing 200 mL of test solution and 10 fry kept at 21°C and 14-10 light-dark. Fry incubated in APW served as controls. Both control and test solutions were in triplicate. Solutions were renewed daily.

Correspondence to: W. D. Di Marzio

Mortality was recorded every 24 hr. LC50's with confidence limits ($p \leq 0.05$) were estimated by using a probit analysis program based on Finney (1971). The total cholinesterase activity (acetylcholinesterase + butyrylcholinesterase) was measured at 48 and 96 hr using the spectrophotometric method of Ellman et al (1961). The measurements were taken from composites of 4-5 individuals, using 4 composites for each concentration. The weight range of the composites was 15 to 40 mg. The protein content was measured by the method of Lowry et al (1951). The enzymatic activity was expressed as m*M* of substrate hydrolyzed (acetylthiocholine) per min per mg of protein. Data of control and experimental groups were analyzed by one-way analysis of variance in conjunction with LSD test (Sokal and Rohlf 1979).

RESULTS AND DISCUSSION

Toxic effects were noticed on *Cnesterodon sp* fry treated with several PQ concentrations (Figure 1). The 96-hr LC50 is three fold lower with respect to the 24-hr LC50 (Table 1). It means that there was an increase of PQ toxicity as the exposition time was longer. Under the experimental conditions the 96-hr LC50 for *Cnesterodon decemmaculatus* fry was 9.41 mg of PQ/L, which was higher than the recommended individual application rate (0.1 to 2 mg/L) of PQ for aquatic weed control. However, the PQ requires, in water, more than 12 d for its natural deactivation (Calderbank 1972) and one continuous application of PQ could result in a ecotoxicological risk to natural populations of *Cnesterodon decemmaculatus.*

The enzymatic assay shows approximately a 50 % inhibition of total cholinesterase activity (TCHA) for all concentration assayed, at 96 hr, with respect to the control

Table 1. Acute toxicity response (LC50) of *Cnesterodon decemmaculatus* fry exposed to paraquat, n=30.

Time (hr)	LC50 mg PQ/L	Confidence Limits	
		lower	upper
24	27.80	22.16	38.88
48	17.07	13.80	21.05
72	12.45	9.57	15.76
96	9.41	6.84	11.76

(Figure 2). However, its behavior during the test was different with respect to the finding by other authors (Nemcsók et al. 1984; Shinohara et al. 1986) who showed that PQ inhibits the enzymatic activity from the beginning of the test. An interesting feature in the TCHA was a significant increase at 48 hr of exposure to PQ (Figure 2).

We think that this increase in the TCHA could be a response mechanism to PQ due to an increase the cholinesterase enzyme synthesis. On the other hand, 50 %

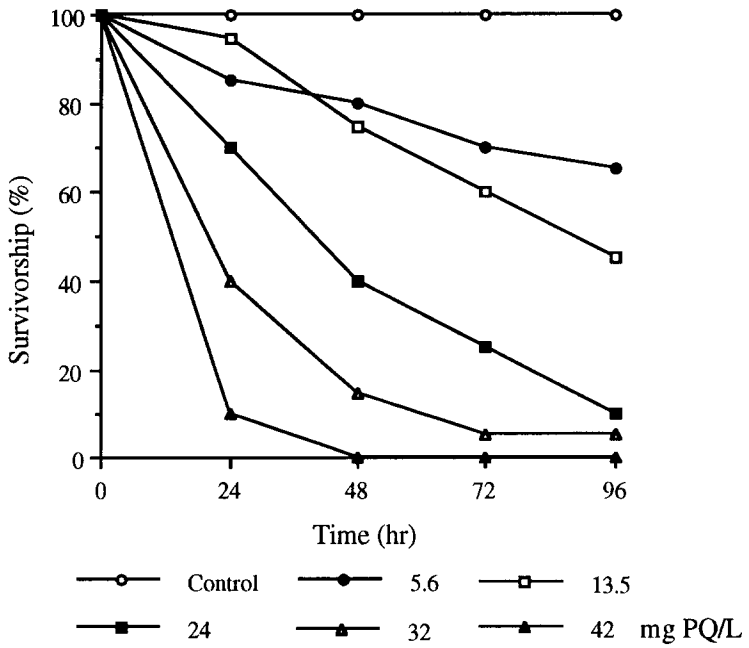

Figure 1. Survival curves for fry *Cnesterodon decemmaculatus* exposed to several concentrations of paraquat.

inhibition of TCHA could affect the natural survival of the fry by cardiorespiratory alterations (Weiss 1961). Tortorelli et al (1990) have observed cardiorespiratory alterations in fry of *Plecostomus commersoni* exposed to PQ, and Howe and Wright (1965) show that primarily respiratory and nervous systems seem to be impaired by paraquat.

We propose, when comparing the 96-hr LC50 value with the concentrations that produce a 50 % inhibition of TCHA, that the fry mortality results from inhibition of some cholinesterase dependent activity such as the cardiorespiratory mechanism. Nevertheless, the mortality wasn't correlated with the inhibition of TCHA at 96 hr. Thus, the mortality increased as the concentration of PQ was higher, but the inhibition of TCHA at 96 hr was the same (about 50 % with respect to the control) for all assayed concentrations (Figure 2). Tortorelli et al (1990) have shown that the PQ produces epithelial gill alterations and that this effect depends on the concentration and the exposure time. So, we can say that the death the fishes was caused by a combined effect between the TCHA inhibition and gill alterations, this last effect was greater at higher concentrations, producing a major mortality.

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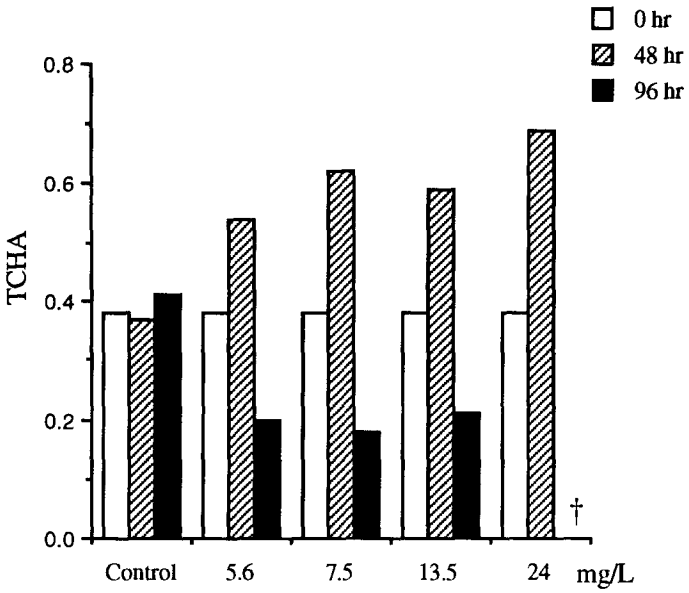

Figure 2. Effect of several paraquat concentrations on total cholinesterase activity in fry *Cnesterodon decemmaculatus*. The values are expressed as m*M* of acetylthiocholine hydrolyzed per min per mg of protein and are averages of measurements from 4 composites, each composite formed for 4-5 individuals.

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