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Inhibition of Plasma Cholinesterase and Acute Toxicity of Monocrotophos in a Neotropical Fish *Prochilodus lineatus* (Pisces, Curimatidae)

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Ecotoxicological evaluations are required by chemical control regulations in order to classify new substances in reference to their potential hazard to the environment. In recent years there has been growing interest in the effects of toxicants on fish health. This is of great importance for the development of fisheries, which are often located in rivers, ponds or estuaries exposed to industrialization or agricultural activities. A short period of exposure to high levels of toxicants may go unrecorded due to the impossibility of monitoring water for all possible pollutants,

Insecticides, like monocrotophos are widely used in Argentina, and the residues of these are widespread in freshwater ecosystems, contaminating water bodies. Some problems with other communities are reported by Goldstein et al. (1999). In the Argentinean territory, a big hydrographic system includes in its basins human settlement, industrial activities and most of agricultural land. The water resource in these vast areas is of major importance for man and animal use, and it is also receiver and vehicle of waste due to the result of agricultural activities.

Organophosphates compounds inhibit both erythrocyte (true cholinesterase or acetylcholinesterase) and plasma (pseudocholinesterase or serum butyrylcholinesterase) cholinesterase. Plasma cholinesterase activity returns to normal within several days to a few weeks, because it is rapidly replaced by new enzyme synthesized in the liver, the acetylcholinesterase activity of the erythrocytes, however, remains depressed for the duration of the red cell's life.

Enzyme inhibition has been suggested to evaluate the impact of the insecticide organophosphates and carbamates in aquatic ecosystems as biological indicators in the prevention of their deleterious effects (Rand and Petrocelli 1985; Heath 1987; Boudou and Ribeyre 1989; Dutta et al. 1995; Lusková 1997). Information regarding toxicity and cholinesterase inhibition effects in neotropical fish species is very scarce (Di Marzio and Tortorelli 1994; Bianchini et al. 1997).

Prochilodus lineatus ("sábalo") is subject to investigations because it is a widely

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distributed neotropical fish that represents approximately 60% of the total ichthyomass of the Paraná River and its fishery is strongly dependent on the industrial, commercial and recreational demand. (Quirós and Cuch 1989).

The object of this work was to evaluate the degree of enzymatic inhibition by the action of the organophosphate insecticide monocrotophos in juveniles exposed to lethal and sublethal concentrations.

MATERIALS AND METHODS

Samplings of specimens were made in pristine environments, such as the El Espinillo, an overflow lagoon linked to the Colastiné river and next to Santa Fe city (Argentine) to 31° 39 ' 36" LS and 60° 35 ' 26" LW. Fishes were captured during spring-summer period and transported alive in oxygenated containers. They were acclimated only for 3 days before the tests, because they are detritivorous fishes and they can not be maintained with dried or living food. Dechlorine water was used for the experiment with: pH between 8.2 and 8.5; total hardness 212 mg/L CO_3Ca; alkalinity 228 mg/L CO_3Ca and ammonium below 0,1 mg/L NH_3. For the analytic determinations a Test Kit Model FF-2 of Hatch was used.

Toxicity tests were carried out in an acclimatized laboratory to a constant temperature of 25°C, photoperiod of 12:12 hr and oxygen concentration between 5.5 and 6.5 ppm. Containers of 80 liters of capacity were used. The organophosphate selected was Icophos® 60 whose commercial formula contains 60 g of monocrotophos as active ingredient.

Juveniles of *P. lineatus* were exposed to a renewal test (Rand and Petrocelli 1985) during 72 hr to: 2.7; 3.9; 5.6; 8.0 and 11.4 mg/L concentrations and the respective controls. Test solutions and control water were renewed at 24-hr intervals. The exposure period was limited because extended starvation affected the fish.

Two fish in each of seven replicates were tested and a total of 14 fish per test concentration and control were used. The mean total length and weight registered were 210 mm (range between 165 and 245 mm) and 158.4 g (range between 72.6 and 250.0 g), respectively. The selection of large specimens was made due to the necessity of obtain an adequate blood volume for the analysis. One determination was taken for each exposed fish.

Mortality, immobility and behavior alterations were registered every 24 hr. Blood extraction was carried out individually in dying and survivors fish in different concentrations and controls by caudal peduncle dissection method (Roberts 1978; Reichenbach-Klinke 1982), using heparinised micropipette. Plasma was separated by centrifuge at 3,500 r.p.m. during 5 minutes and diluted with physiological solution, then it was used for the quantification of the enzymatic activity, expressing results in SI units, microkatals per litre (μkat/L) (Hlavová 1989).

Ellman et al. (1961) method based on the use of s-butyrylthiocholine as substrate of seric or plasmatic cholinesterase, with maximum activity at pH 7.7 was used. Liberated thiocholine reacts with the chromogen 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), producing a yellow color product. The speed of coloration appearance is directly proportional to enzymatic activity and it was measured at 405 nm using a spectrophotometer. *Colinesterasa®* of *Wiener S.A. Lab. Group, Argentine*, optimized test with butyrylthiocholine as substrate for analysis in human serum or plasma adapted for fish was used.

Degree of enzymatic inhibition by insecticide action was expressed in absolute values, and inhibition percentages found in different concentrations were calculated, whereas mean normal value of enzymatic activity was reported by Cazenave et al (2000). LC50-72 hr with confidence limits ($p \leq 0.05$) were estimated by Probit analysis according to EPA. A *t*-test to compare cholinesterase activity means between different concentrations and control ($P < 0.01$) was performed.

A second experiment was performed to test three groups of 12 specimens each to a sublethal concentration of 1.0 mg/L, during 2, 12 and 24 hr. After the exposure time, fishes were removed for cholinesterase analysis.

RESULTS AND DISCUSSION

LC50-72 hr, estimated by Probit analysis was 5.97 mg/L of monocrotophos, being lower and upper confidence limits 5.70 and 6.94 mg/L, respectively. Values of LC50-48 and 24 hr are given in Table 1.

Table 1. Acute toxicity response (LC50) of *Prochilodus lineatus* exposed to monocrotophos.

Time	LC50	Confidence Limits	
(hr)	(mkat/L)	lower	Upper
24	9.06	8.27	9.77
48	8.36	7.53	9.19
72	5.97	5.70	6.94

Mean normal values of cholinesterase activity for juveniles of *Prochilodus lineatus* reported by Cazenave et al. (2000) was 159.67 μkat/L.

In highest concentration (11.4 mg/L) mortality of 100% was registered in the first 24 hr(Fig. 1). Due to the speed in which death took place, only one dying fish could be analyzed, with a cholinesterase activity value of 16.15 μkat/L, corresponding to 89.8% inhibition. In 8 mg/L concentration was observed 78.6% of mortality with the sequence of 1 specimen at 24 hr; 4 at 48 hr and 6 at 72 hr. Mean value of enzymatic activity of dying fish analyzed was 28.83 μkat/L, corresponding to 81.9% inhibition. With regard to the survivors (3 specimens), mean value was 38.35 μkat/L and 76% inhibition. Mortality in 5.6 mg/L was

21.4% at 72 hr exposure. Eleven survived fish showed a mean activity value of 33.40 μkat/L and 79% of inhibition. Exposure to 3.9 mg/L registered low mortality and the enzymatic activity was 28.00 μkat/L, representing a inhibition percentage of 82.4%. In lowest concentration (2.7 mg/L) the survival was 100%. Mean value of cholinesterase was 27.75 μkat/L, meaning 82.6% inhibition. Controls did not register cases of death and mean value of enzymatic activity was 163.56 μkat/L.

Statistical analysis (*t*-test) showed a significant difference between control and all exposure groups.

In subletal concentration of 1 mg/L, cholinesterase activity was measured at different exposure times: 2, 12 and 24 hr. Mean values of cholinesterase activity and inhibition percentages were: 53.67, 16.65 and 33.65 μkat/L and 65.9; 89.5 and 78.9%, respectively (Table 2). Highest inhibition was registered at 12 hr of pesticide exposition.

Intoxication symptoms were hyperexcitation, erratic swimming, and jumps followed by lethargy and low breathing frequency. In dying and dead fish it was observed a great muscular rigidity and an abundant mucus secretion which were directly related to the concentrations.

Values of LC50-72 hr confirmed the high degree of monocrotophos toxicity. It is difficult to make comparisons with other species due the great range in acute toxicity levels for organophosphorous insecticides in any species. (Rand and Petrocelli 1985; Hughes et al. 1997)

Enzyme cholinesterase activity related to pesticides effects has been measured in brain (Nemcsok et al. 1984; Heath 1987; Carr et al. 1995; Dutta et al. 1995; Straus and Chambers 1995; Bianchini et al. 1997; Gruber and Munn 1998; Cunha Bastos et al. 1998), gills (Straus and Chambers 1995), liver (Straus and Chambers 1995), heart (Nemcsok et al. 1984), muscle (Nemcsok et al.1984; Straus and Chambers 1995); plasma (Nemcsok et al. 1984; Nemcsok et al. 1987; Straus and Chambers 1995; Hughes et al 1997;Cunha Bastos et al.1998) and erythrocyte of different freshwater fish species. Results obtained by different authors, independently of organs, methodologies, and species used, are quite similar in the cholinesterase inhibitory effects.

It is usually accepted that organophosphorous LC50-96 hr values are associated to cholinesterase activity reduction of 80% approximately (Hughes et al. 1997). Loss of 70-80% of enzymatic activity can be appeared before fish death occurs. A smaller enzyme inhibition would be probably associated to with wide range of effects in behavior (Heath 1987). Weiss (1961) reported that cholinesterase inhibition as low as 8% is lethal to some fish species, whereas other studies have reported that some fish are able to survive inhibition of 70-90% (Gruber and Munn 1998). On the other hand, Gruber and Munn (1998) reported that more than 70% of enzymatic inhibition in fish commonly becomes in death. Adverse effects

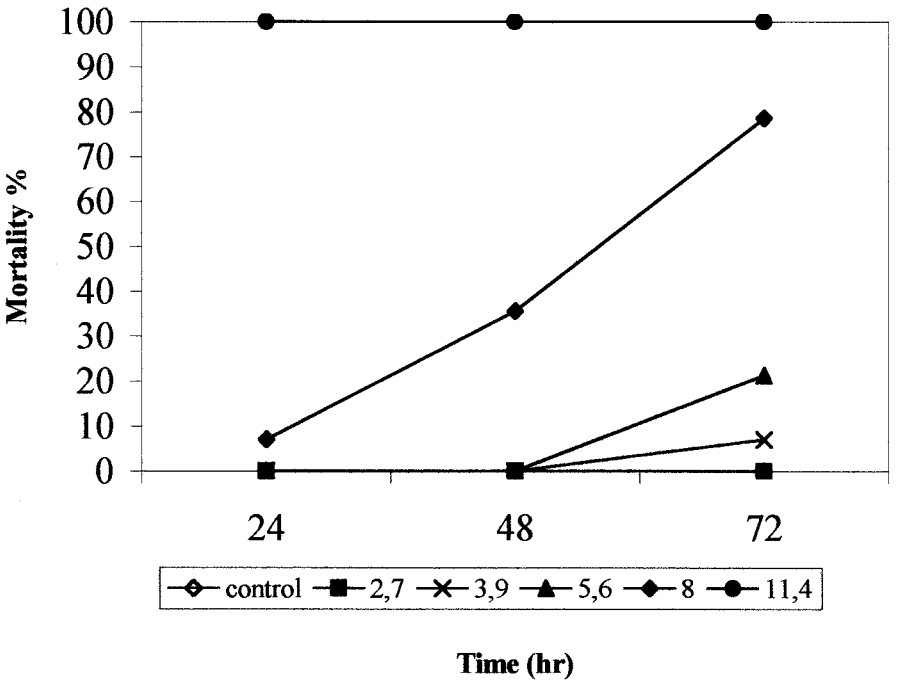

Figure 1. Percentage mortality in juveniles of *Prochilodus lineatus* exposed to different concentrations of monocrotophos

including reproduction problems and behavior alterations appears at 20 to 70 % of inhibition.

Respect to neotropical species, we can refer to Di Marzio and Tortorelli (1994) paper, who worked with paraquat concentrations between 5.6 and 24 mg/L, and found for larvae of *Cnesterodon decemmaculatus* 50 % of inhibition.

Bianchini et al. (1997), using juveniles of "pejerrey" (*Odontesthes bonaerensis*) found high levels of brain inhibition (74% and 86%) for different concentrations (75 µg/L and 750 µg/L, respectively) of methyl parathion organophosphorous.

Present work shows high inhibition percentages equally in lethal and sublethal concentrations of this pesticide. For all concentrations tested the inhibition reached was higher than 70%, and in sublethal concentrations the maximum took place within 12 hr of exposure. Straus and Chambers (1995) carried out similar observations with juvenile of *Ictalurus punctatus* exposed to different organophosphorus compounds, finding a maximum plasmatic cholinesterase inhibition within 8 hr.

Intoxication symptoms observed in juvenile of "sábalo" have already been mentioned for other teleost exposed to pesticide action (Al-Kahem et al. 1994;

Table 2. Enzyme activity in *Prochilodus lineatus* exposed at 2, 12 and 24 hours of sublethal concentration of monocrotophos.

Variable	Exposure	Mean	Deviation	Maximum	Minimum
Total length (mm)	2	180	19.03	215	151
	12	198	12.34	223	172
	24	203	13.52	219	180
Weight (g)	2	88.49	33.55	142.34	46.70
	12	110.41	21.92	161.17	77.79
	24	123.47	27.03	160.10	77.21
K Factor	2	1.44	0.14	1.71	1.20
	12	1.41	0.11	1.58	1.15
	24	1.46	0.12	1.74	1.30
Cholinesterase (µkat/L)	2	53.67	18.55	90.84	32.30
	12	16.65	9.69	38.36	4.04
	24	33.65	20.09	72.67	6.06
Percentage of inhibition	2	65.9	11.62	79.7	42.9
	12	89.5	6.09	97.5	75.9
	24	78.9	12.63	96.2	54.3

Carr et al. 1995; Bianchini et al. 1997; Hughes et al.1997). Fish hyperexcitation and abnormal swimming behavior may be due to irritating effect of toxicant as an attempt to be relieved from a stressful environment

Related to mucus secretion, there is a wide variety of chemical substances that can stimulate gills, buccal membranes and skin secretions (Heath 1987). Mucus secretion in fish probably reduces body contact with toxic media to minimize its irritating effect, or to eliminate it through epidermal mucus (Al-Kahem et al. 1994).

Results are important for evaluating pesticide's potential ecotoxical effects in aquatic environment closed to agricultural fields and possible effects on fish populations. Also, provides an easy diagnosis method before an organophosphorous contamination.

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