

## METABOLIC EFFECTS OF THE PESTICIDE METHYL PARATHION

(FOLIDOL 600<sup>®</sup>) ON *Brycon cephalus* (matrinxã), A TELEOST FISH.

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### Introduction

The poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental long-term effect may result in the incidence of poisoning of fish and other aquatic life forms. (Jyothi and Narayan, 1999).

Folidol 600<sup>®</sup> is a widely used commercial product, which contains methyl parathion as the active substance. It is heavily employed in agricultural fields as well as in fish culture tanks to kill aquatic larva of insects. It usually causes different kinds of environmental contamination and accidents concerning humans and native fauna. (Silva et al., 1993).

The knowledge of sublethal effects of xenobiotic compounds on hematological parameters, enzyme activities and metabolite concentrations is very important to delineate the health fish status and provide a future understanding of ecological impacts. These pesticides act by causing inhibition of cholinesterase enzymes (ChE) by formation of enzyme inhibitor complex (O'Brien, 1976) and damaging the nervous system. These effects may result in metabolic disorders.

Associated to cholinesterase activities, a study of other enzymes such as phosphatases and aminotransferases closed to intermediary metabolite determination provides a wider view of metabolism. Therefore, the aim of this work was the determination of the effects of different Folidol 600<sup>®</sup> concentrations on *B. cephalus* metabolism.

## **Materials and Methods**

### *Animals and treatments*

*B. cephalus*, a native tropical fish of the Amazon region, weighing about 270g, were collected from CEPTA-IBAMA, Pirassununga (SP) – Brazil and acclimated to laboratory conditions for two weeks. During the acclimation period, fish were maintained in tanks of 2,000 liters with continuous water flow, artificial aeration under controlled temperature (25 - 27°C), natural light cycle and fed *ad libitum* with a commercial feed. Fish were not fed 12 h prior to the experiment to prevent organic matter in the water.

### *Experimental design*

After acclimation, fish were divided in groups and transferred to the laboratory into six glass aquaria (six fish per aquarium) and maintained during 24h to recovery of handling stress with continuous water flow and controlled temperature. One aquarium was used as control, where six fish were maintained for 4h in a static environment, with constant aeration. The other five fish groups were exposed to 0.5, 1.0, 2.0, 5.0 and 7.0 ppm of Folidol 600<sup>®</sup> for 4h in a static environment with the same aeration conditions. After 4h of exposure to pesticide, blood samples were collected from caudal vein, the fish were killed and the tissues were excised.

### *Blood samples*

A blood sample was used for hematological parameters determination. Hematocrit (HTC) was determined by microhematocrit centrifugation technique. The red blood cell counting (RBC) was done in a Newbauer chamber and the hemoglobin concentration was measured spectrophotometrically by Drabkin's method. Another sample of blood was centrifuged at 12,000g for five minutes and the plasma was used for enzymatic assays and metabolites were determined after suitable dilution.

### *Tissues samples*

Liver and muscle samples were removed and quickly chilled in liquid nitrogen and stored at - 20°C for subsequent analysis of enzymes and metabolites. Prior to the assays the tissues were properly homogenized and centrifuged at 5° C.

### *Enzyme assays*

Alkaline and acid phosphatase activities were quantified in liver and plasma, by modification of Breaudiere and Spilman, (1983) and Moss, (1983) and Bergnoyer and Beach, (1983) colorimetric method, using p-nitrophenil phosphate as substrate. Liver was used to give final assay concentration of 1,5 mg/ml of buffer. Plasma was used without dilution.

Plasma aminotransferase activities of alanine (ALAT) and aspartate (ASAT) were quantified by a modification of Reitman and Frankel's method (1957).

Acetyl (AChE), butyryl (BChE) and propionylcholinesterase (PChE) activities were quantified by modification of Ellman et al. (1961), using acethylthiocholine, butyrylthiolcholine and propionylthiocholine as substrate respectively and 5,5'-dithiobis – 2 nitrobenzoic acid (DTNB) as the chromogen. These activities were measured in plasma centrifuged at 14,000 rpm for 20 minutes.

### *Metabolic intermediaries*

Lactate (Harrower and Brown, 1972), piruvate (Lu, 1939) and glucose (Buboie et al., 1956) were quantified in plasma and muscle of *B. cephalus*. For this analysis, tissues were homogenized in perchloric acid 0.6N and centrifuged at 3,000 rpm for 1 minute, to give a final concentration of 5µl/ml and 5mg/ml respectively. The liver and muscle glycogen was measured as reported in Bidinotto et al. (1998).

### *Statistics*

Enzymatic activities, metabolic intermediates and hematological data were analyzed by Mann-Whitney Test and the accepted level of confidence was 5%. (Zar, 1984).

## Results

Concerning the hematological parameters, it was observed that fish exposed to lower concentrations of Folidol exhibits increase of HTC values and higher haemoglobin percentage. The group exposed to higher concentrations of Folidol shown different responses (table1).

Table 1. Hematologicals parameters from *B. cephalus* exposed to Folidol 600®.

Treatments	Hematologicals parameters		
	RBC x 10 <sup>6</sup> mm <sup>-3</sup>	HTC %	Hemoglobin%
Control	37.50 ± 2.65	33.2 ± 1.47	4.13 ± 0.22
0.5ppm	36.08 ± 1.12	38.3 ± 1.68 *	5.47 ± 0.39 *
1.0ppm	35.90 ± 2.11	41.4 ± 1.29 *	4.50 ± 0.60
2.0ppm	30.90 ± 1.25 *	34.5 ± 1.21	4.96 ± 0.33
5.0ppm	28.90 ± 1.21 *	30.7 ± 1.22	5.18 ± 0.10 *
7.0ppm	31.90 ± 1.29 *	39.6 ± 3.34	5.42 ± 0.21 *

High levels of plasma inhibition for cholinesterases were observed in *B. cephalus* exposed to Folidol (table 2). At lower concentration (0.5ppm) it was observed 50 – 54% of inhibition of these enzymes, ranging 90% as fish were exposed to 5.0 and 7.0ppm of pesticide.

Table 2. Values for plasma activity of cholinesterases of *B. cephalus* exposed to Folidol 600®.

Treatments	Cholinesterase activities (mU/ml)		
	AChE	PChE	BChE
Control	67.5 ± 6.41	26.5 ± 3.99	6.1 ± 0.85
0.5ppm	36.9 ± 8.84*	14.4 ± 3.30*	3.1 ± 0.55 *
1.0ppm	13.7 ± 5.08*	5.64 ± 1.48*	2.08 ± 0.31*

2.0ppm	16.5 ± 3.33*	6.73 ± 0.93*	2.6 ± 0.16*
5.0ppm	7.2 ± 1.36*	3.49 ± 0.6*	1.47 ± 0.3*
7.0ppm	6.7 ± 1.74*	2.8 ± 1.05*	0.66 ± 0.1*

The values are means ± S.E.

\* Values statistically significant compared to control values at P<0.05.

The plasma aminotransferase activities were decreased for fish exposed to higher Folidol concentrations (fig.1).

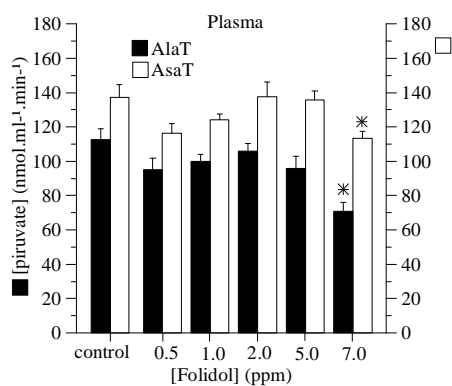


Figure1. AlaT and AsaT plasma activities of *B. cephalus* for different Folidol concentrations.

It was observed that there was an increase of alkaline and acid phosphatases on plasma and liver of matrinxã exposed to higher Folidol concentrations (fig.2 and fig.3). The white muscle and plasma glucose contents did not change in fish groups exposed to Folidol concentrations (fig.4).

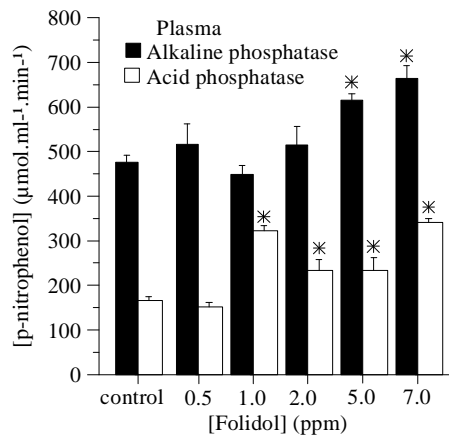


Figure 2. Alkaline and acid phosphatases activities in plasma of *B. cephalus* in different Folidol concentrations.

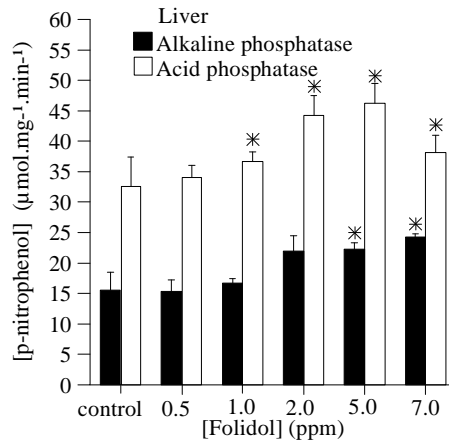


Figure 3. Liver alkaline and acid phosphatase activities of *B. cephalus* in different Folidol concentrations.

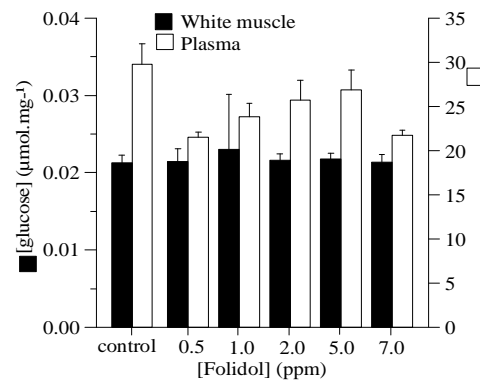


Figure 4. Plasma and white muscle glucose contents of *B. cephalus* in different Folidol concentrations.

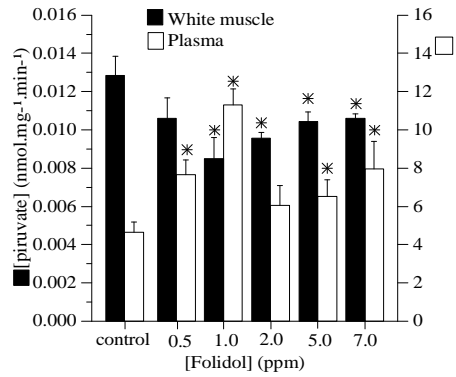


Figure 5. Plasma and white muscle pyruvate contents in *B. cephalus* for different Folidol concentrations.

The plasma pyruvate contents were higher in the groups exposed to Folidol as compared with control group but the white muscle showed opposite metabolic behavior (fig.5).

The white muscle lactate values were significantly higher for all exposed groups as compared with the control, but there were not differences in plasma lactate contents between the control and the exposed groups (fig.6).

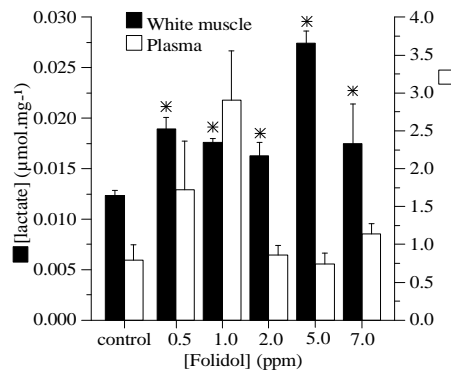


Figure 6. Plasma and white muscle lactate contents of *B. cephalus* in different Folidol concentrations.

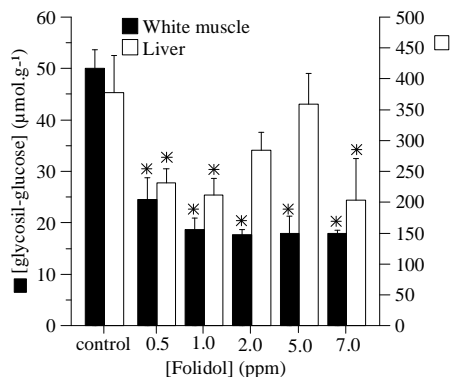


Figure 7. Liver and white muscle glycogen contents in *B. cephalus* for different Folidol concentrations.

The liver and white muscle bulk of *B. cephalus* glycogen exposed to Folidol were significantly lower than control values (fig.7).

### Discussion

Our results show that the fish group exposed to the lower concentration of Folidol increases the HTC and haemoglobin contents without increase of RBC. This increase of haematological parameters is classically showed by fish submitted to organophosphate pollution (Natarajan, 1984 and Lal *et al.*, 1986 apud Heath, 1995). On the basis of such results we can suggest that 4 hours exposure to low pesticide concentration (0.5 – 1.0ppm) leads the fish to usual haematological response from pesticide stress. According to Areechon and Plumb, (1990) and Heath, (1995) this response probably occurs due to damage to gill tissues, producing internal hypoxia and stimulation of erythropoiesis. However, the fish groups exposed to higher concentrations of pesticide developed responses probably resulting from the lack for keeping haematological parameters, probably due to exhaustion or damage of haematopoietic tissue.

#### *Effect on Cholinesterases*

A significant inhibition level of ChE was detected for *B. cephalus* exposed to Folidol. The AChE, PChE and BChE activities of *B. cephalus* were inhibited by 50 – 54% in 0,5ppm group exposed, reaching 90% of inhibition in 5ppm group exposed, which reveal the high methyl parathion toxicity for the specie. Higher activity of AChE than BChE and PChE was observed due to the substrate specificity (Frobert *et al.*, 1997).

According to Silva Filho *et al.*, (1999), plasma AChE of *P. mesopotamicus* (115g) exhibited high inhibition (73, 88 and 90% for AChE, PChE and BChE respectively) when exposed to 0.2ppm of methyl parathion for 4 hours. These high values of inhibition for *P. mesopotamicus* compared to *B. cephalus* may be due to differences of weight, rate of uptake, detoxification and activation of the pesticide in the body.

The treatments containing the higher insecticide concentration showed very high inhibition of enzymes, however in the same order of the lower concentration treatments. Strauss and Chambers, (1995) suggest, in this case, that AChE saturation may be reached with the lower concentrations of pesticide and the fish should have some mechanisms for disposing of or adapting to higher exposure levels.

#### *Effects on enzyme activities*

The decrease of aminotransferases may be attributed to liver damage. This response has been reported for *Carassius auratus* exposed to lead poisoning (Fantin et al., 1988). The increase of plasma and liver acid phosphatase may be associated either with the decrease in stability of liver lysosome membranes or with tissue damage. This enzyme is associated with lysosomal activity. Gill *et al.*, (1992) speculate that acid phosphatase elevation reflects proliferation of lysosomes in attempt to sequester the toxic xenobiotic.

The increase of alkaline phosphatase was observed in *B. cephalus*. Such result is observed as a consequence of osteoblastic activity increase or due to intra and extra hepatic obstructions of biliary passage (Jyothi and Narayan, 1999).

#### *Effects on intermediary metabolites*

It was observed a decrease of liver and muscle glycogen in some treated groups and unchanged concentrations of white muscle and plasma glucose. That means a glycogen mobilization, probably to maintain the glucose level and the glycolysis, as usually observed for other fish exposed to a variety of sub-lethal concentrations of organophosphorous compounds (Rany et al., 1990; Gill *et al.*, 1990, 1991). The pyruvate decreases and the lactate contents increases in white muscle. These responses suggest a fermentative strategy of matrix when submitted to methyl parathion.

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### **Acknowledgements**

*The authors are thankful to Dr. Jaime Bastos and Moacelio V. Silva Filho from Dep. of Biochemistry-Universidade do Estado do Rio de Janeiro, Brazil, to CAPES (Coordenação de Amparo à Pesquisa - Brazil) and CNPq (Conselho Nacional de Pesquisas - Brazil).*