Histopathologic lesions in the liver of *Prochilodus lineatus* (Pisces, Prochilodontidae) exposed to a sublethal concentration of the organophosphate insecticide Dipterex 500 ®(Trichlorfon)

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ABSTRACT. Histopathologic lesions in the liver of juvenile *Prochilodus lineatus* were assessed following 24 and 48h exposure to the organophosphate pesticide Dipterex 500 ® (Trichlorfon) at a concentration of 0.2 μl/L. Livers of experimental animals were collected, processed, and stained with hematoxylin and eosin according to routine histology methods. Alterations in hepatic tissue were observed after pesticide exposure, including lateral migration of nuclei, variation in the diameter and density of nuclei, pyknosis and necrosis.

Key words: *Prochilodus lineatus*, curimbatá, liver, organophosphate, Dipterex, Trichlorfon.

RESUMO. Lesões histopatológicas em fígado de *Prochilodus lineatus* (Pisces, Prochilodontidae) exposto à concetração subletal do inseticida organofosforado Dipterex 500® (Trichlorfon). Neste trabalho foram avaliadas as alterações histopatológicas de fígados de juvenis de curimbatá, *Prochilodus lineatus* (Valenciennes, 1836) expostos a uma concentração de 0,2 μl/L do organofosforado Dipterex 500 (Trichlorfon). Após 24 e 48 horas de exposição coletaram-se os fígados dos peixes contaminados e controle, que foram processados pelas técnicas de rotina para histologia e corados pela hematoxilina e eosina (HE). As alterações hepáticas observadas foram: migração lateral do núcleo dos hepatócitos, núcleos com vários diâmetros e diferentes graus de densidade, picnose e necrose.

Palavras-chave: *Prochilodus lineatus*, curimbatá, fígado, organofosforado, Dipterex, Trichlorfon.

Organophosphates are widely used in the control of agricultural pests and in the general treatment of ectoparasites in animals. In Aquaculture their use is also extended to the control of ectoparasites. However, their appropriate concentrations with fish, for example, are still unknown. Lack of knowledge on safe concentrations for specific pesticides may lead to health risks for these organisms, and even to humans who consume them. There is no available data in the literature about how many of these products are used in Brazilian Aquaculture, and what are their effects on fish.

According to some authors (Ansari and Kumar, 1987a, Silva *et al.,* 1993, Rodrigues *et al.,* 1997, Rodrigues and Fanta, 1998), different fish species exposed to an organophosphate show some common symptoms, albeit with great differences in sensitivity, reaction speed and recovery capacity.

The aim of the current research was to examine the histopathologic lesions in the liver of *Prochilodus lineatus*, popularly called curimbatá, following exposure to a sublethal concentration of Dipterex 500 (Trichlorfon).

Material and methods

Sixty young curimbatá, *Prochilodus lineatus* Valenciennes, 1836 (Pisces, Teleostei, Prochilodontidae), were obtained from a commercial fish farm in the south region of the state of São Paulo, Brazil. The selected fish had a mean total length of 8.4 cm and mean weight of 12.3 g, with no previous exposure to pesticides. Fish were acclimated for 2 weeks in 500 L boxes of asbestos cement covered by nontoxic synthetic rubber. They continuously contained aerated filtered water at a temperature up to 25°C, pH 7 ± 0.2. A natural photoperiod was used.

After acclimation, the individuals were divided into two groups of thirty animals each, conditioned

in 81 L aquariums. One tank served as control and the other was purposely contaminated with Dipterex 500 (Trichlorfon) at a concentration of 0.2 µl/L.

The active principle of this product is Dipterex, O,O-dimethyl-(1-hydroxy-2,2,2-trichloroethyl)-phosphonate (Trichlorfon). It is an insecticide and acaricide class commercial product with a concentration of 500 g/L.

After 24 and 48h, five individuals of each treatment were anesthetized with benzocaine and killed by medullar section. The liver was fixed in Bouin's solution for 24h, embedded in paraffin and stained by hematoxylin/eosin (HE).

Results

The appearance and behavior of contaminated fish were monitored for indications of anxiety, uncoordinated movements, trismus of the pectoral fin, whitish coloration, shallow breathing, loss of appetite and dwelling at the bottom of the aquarium.

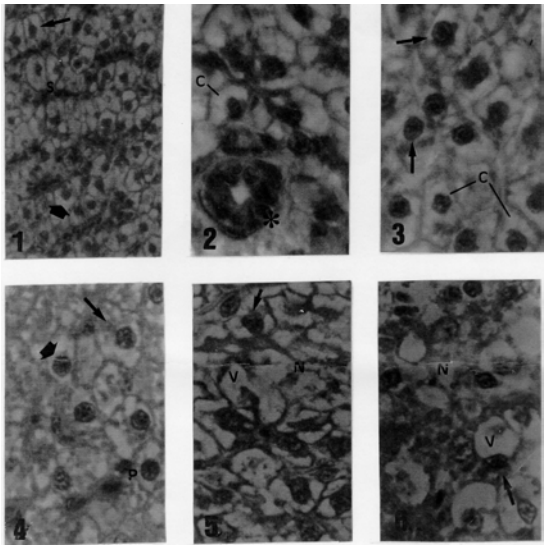
Anatomically, the livers of control individuals had the normal appearance common in fish. The contaminated ones had a friable aspect and were dark red in color.

Livers of the control group showed a typical histological pattern for fish (Figures 1-3). After 24h of exposure, the hepatocytes were tumefied with vacuolation, cytoplasmic granulation, nuclear lateralization. There were also nuclei with various diameters and densities and condensed chromatin in the central region with pyknosis (Figure 4). After 48h, vacuolated regions, hepatic cell disorganization, cells with nuclear lateralization, with or without granulations, pyknoti nuclei and areas of necrosis were registered (Figures 5 and 6).

Discussion

As a rule, organophosphates are relatively well excreted or even metabolized. However, these compounds at high concentrations act as an acetylcholinesterase inhibitor. In this case, the symptoms are similar to poisoning according to the intensity and gravity produced by a single dose or by repeated small doses; in either case a critical level may be reached which would lead to chronic and severe sequelae (Ghosh, 1987; Silva *et al*., 1993). According to Brandal and Egidius (1979), such a condition in salmon could be reversed if medication were applied at maximum tolerated doses, for 20min at a temperature 10^0C.

Pickering *et al*. (1962) postulated that the toxicity of organophosphates in different fish species varies with the animals' age, weight and sex, as well as with the composition of the products and the active principle present. As the individuals used were young and the results showed morpho-functional liver damage, alterations might be related to a decline in homeostasis. The blood and the spleen of these fish were collected and the results reported by Ranzani-Paiva *et al*. (1997) and by Rodrigues *et al*. (1997). They show a decrease in hematologic parameters and in the morpho-functionality of the spleen, while reflecting a loss of immune defense mechanisms too.



Figures 1-6. Liver cells of *Prochilodus scrofa*, H.E.. (1) control liver: central nuclei (small arrow); cytoplasmic vacuoles (short arrow); sinusoids (S) 100 X. (2,3) control liver: polygonal hepatocytes (C); nuclei and nucleoli (arrow); bile duct (*) 400 X. (4) 24 hours after treatment with the OP: lateral nuclei (short arrow); swollen hepatic cells with vacuoles and granulations (arrow); nuclei showing different sizes and shapes; small and pyknotic nuclei (P) 400 X. (5,6) 48 hours after treatment with the OP: vacuolar degeneration and granulation (V); cells with nuclear lateralization (arrow); pyknotic nuclei and areas of necrosis (N) 400 X

According to Ansari and Kumar (1987a) and Gill *et al*. (1988), the liver is an organ that frequently undergoes changes when exposed to pesticides at sublethal doses. Moreover, the pesticide may also cause deformation of the skeleton and reduction of the gonadal index (Kumar and Ansari, 1984), effects on embryonic stages and hatching of eggs (Ansari and Kumar, 1987b), and pathologic alterations in the ovary (Ansari *et al*., 1986). The study of the hepatic tissue of *Brachydanio rerio* showed morphological alterations in individuals exposed to organophosphates, even when they were submitted to sublethal doses normally considered safe (Rodrigues and Fanta, 1998).

The study of the symptomatology and histopathologic alterations in fish exposed to pesticides is vital to the public health. Results demonstrate that it is necessary to investigate the effects of organophosphates on exposed organisms, even when sublethal doses are involved.

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