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Histopathological Effects of Paraquat and Gill Function of *Puntius gonionotus*, Bleeker

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Paraquat (1, 1-dimethyl-4, 4-dipyridylium) is an herbicide widely used in many countries including Thailand. Man appears to be a species highly susceptible to the toxicity of Paraquat which is characterized by delayed progressive pulmonary fibrosis (Bus et al. 1976; Webb 1983). Light microscope studies of rat lungs exposed to p.o. administration of Paraquat 400 mg/kg BW indicate intra-alveolar hemorrhage, perivascular lymphocyte infiltration and prominent alveolar epithelial cells with dark nuclei (Kimbrough and Gaines 1970).

Extensive use of pesticides has created concerns over their detrimental effects on living aquatic resources, including fish in Thailand. During a freshwater fish epidemic in 1983, the possibility of Paraquat producing lesions in fish which might be followed be secondary infection was a speculative controversy (Menasavet et al. 1983). Many investigators have reported the acute toxicity of Paraquat in many fresh water fish species of Thailand (Anutarakul 1977; Menasavet et al. 1983; Chiwaporn et al. 1984; Kanjanopas 1984). The present study describes the histopathological effects of Thai Silver barb Puntius gonionotus, Bleeker, exposed to Paraquat, under laboratory conditions. This species of fish was studied because they are commonly present in the natural environment, are of economics importance and are readily available, all of which make them standard fish for toxicological testing in Thailand.

MATERIALS AND METHODS

Puntius gonionotus, Bleeker, average weight 3.45±0.5 g, were purchased from a fish farm (Sang Sawang Pan Pra). All animals were acclimated for 15 days prior to exposure to Paraquat in the Laboratory condition (renewal static condition in dechrolinated Bangkok tap water with physical and chemical properties described below). They were fed daily on pelleted food until the day before the experiment.

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Table 1. Paraquat concentration in test solutions during 24 hours and 48 hours.

Paraquat Nominal concentrations (mg/L)	Paraquat concentration in test solutions (mg/L)	
	24 h	48 h
1.0	n = 2 average 0.8	n = 2 average 0.7
4.0	n = 2 average 3.8	n = 2 average 3.3

Analytical grade (99.6% w/w) Paraquat Dichrolide was provided by the plant protection division of Industrial Chemical International, Fernhurst, England. A stock solution was prepared in distilled water. Concentrations of Paraquat in test solutions were determined in duplicates throughout the experiment by dithionite reduction and detected using a Shimudzu UV visible spectrophotometer at 369 nm. (Baselt 1980). During 48 hours, Paraquat concentrations in the test solutions were within 70% of the original concentrations (Table 1).

Static bioassays were conducted using Bangkok dechlorinated tap water (total hardness 100-115 ppm as $CaCO_3$, total alkalinity 84-104 mg/L as $CaCO_3$, Paraquat <10 µg/L, pH 6.5 at 22±1 °C) in glass aquaria containing 20 L of test solution, aerated throughout the experiment to ensure adequate oxygen supply. Toxicant renewals were done on alternate days.

Fish were exposed to Paraquat at concentrations of 0,1,4 mg/L (12 fish in each aquarium, two duplicate tanks at each concentration of Paraquat). These concentrations were chosen because they are lower than lethal concentrations previously determined (Menasavet et al. 1984). After 5 and 12 days of exposure, 6 fish from each tank were sampled.

All fish were killed by decapitation. Organs taken for histological examination were skin, muscle, gills, heart, liver, kidney, spleen, stomach, intestine and brain. Tissues were fixed in 10% buffered formalin, embedded in paraplast, sectioned at 5.6 um and stained with hematoxylin and eosin for light microscopic examination.

RESULTS AND DISCUSSION

After 5 and 12 days of exposure, no abnormal behavior was noticed and none of the fish died before the termination of the experiment. Histological examination of tissues from fish of the control and exposed groups revealed that all tissues, with the exception of gills of fish at 4 mg/L for 12 days, were quite normal. The morbid changes found in the gills were characterized

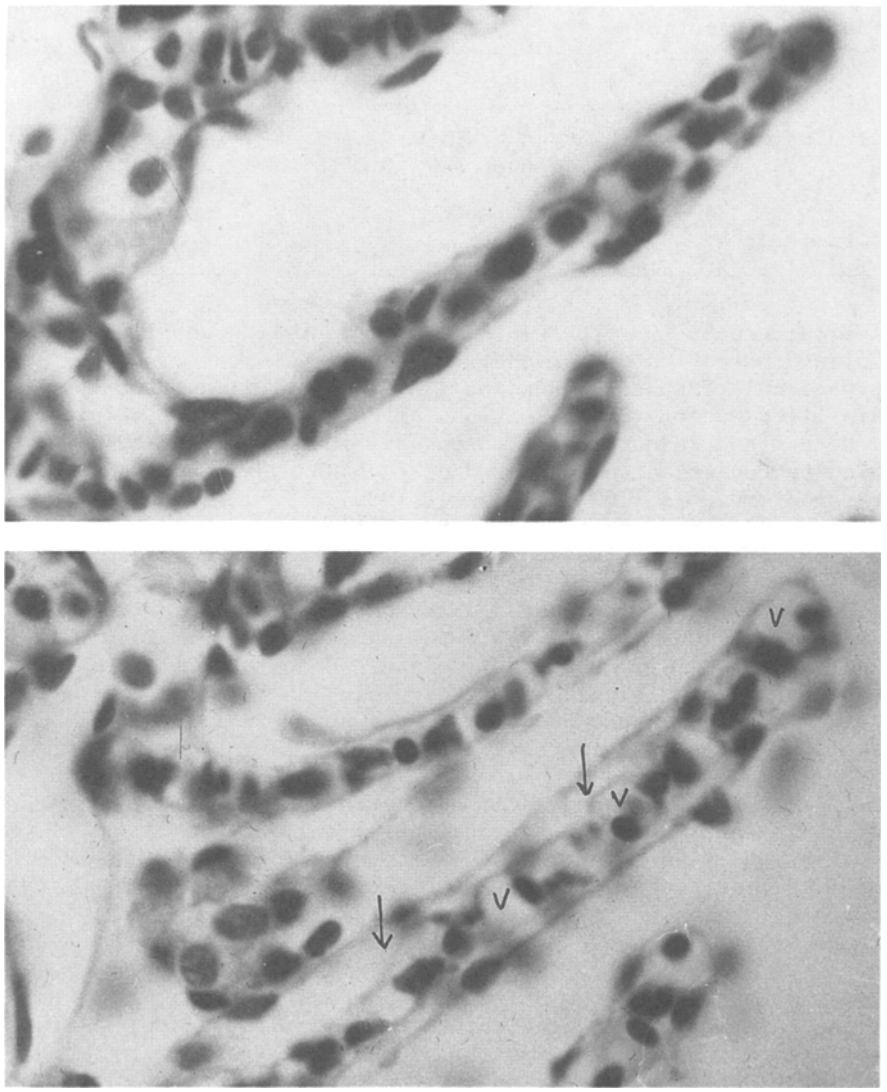

Figure 1. Photomicrograph of a HE section 400X through a gill filament from Puntius gonionotus, Bleeker.
a) normal fish (upper) b) fish exposed to 4 mg/L for 12 days (lower).
Note a marked swelling of the 2nd gill lamella under epithelial and endothelial basement membrane (arrow) and vacuolation within epithelial cells (V).

by marked swelling of the second gill lamella especially under the basement membrane of endothelial and epithelial cells in which hydropic vacuolation was present (Figure 1).

Gills are the only organ of Puntius gonionotus, that were detected to have any histopathological changes following Paraquat exposure of 4 mg/L for 12 days. In fish, gills are the primary site for both passive and active exchange of gases and ions (Lagler et al. 1977). The efficient passive transfer of molecules from water to blood of fish involves permeation of the mucous coat, epithelial cell, basal lamina and the pillar cell flange (Steen and Kruysse 1964). Paraquat itself is an ionized compound with highly hydrophilic nature. Toxicant with these properties are thought to be poorly absorbed through cell membranes by simple diffusion (Klaassen 1980). Several investigators have reported poor absorption and rapid secretion of Paraquat from fish (Conning et al. 1969; Funderburk and Bozarth 1967).

Cellular swelling of gill filaments, although it implies an early and completely reversible manifestation of injury, usually becomes apparent only sometime after the critical damaging biochemical event and function derangement have occured. The increased permeability of cell membrane with or without loss of intracellular reserves of energy may be involved. It is therefore likely that the exposure of 4 mg/L of Paraquat for a longer period of time can cause detrimental effects in fish.

During the freshwater fish epidemic which occurred in Thailand in 1983, the levels of Paraquat detected in the environment were between 0.030-0.051 mg/L (Menasavet et al. 1984). It has been previously accepted that the potential hazards of Paraquat to freshwater fish are considerably reduced by its rapid inactivation in the environment (Calderbank. 1968). Therefore the controversial possibility of Paraquat acute toxicity producing lesions in fish followed by bacterial infection causing widespread epidemic seems unlikely.

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