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Changes Induced in the Gills of Milkfish (*Chanos chanos* Forsskål) Fingerlings after Acute Exposure to Nifurpirinol (Furanace; P-7138)

C. T. Tamse,[1,*] R. Q. Gacutan, A. F. Tamse[2,*]

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines
[2]College of Fisheries, University of the Philippines in the Visayas, Miag-ao, Iloilo 5023, Philippines

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The need for a chemotherapeutant used specifically for fish disease became increasingly apparent with intensive fish culture practices, and with the possibility of bacterial resistance against drugs used for human and animal medicine (Austin 1985). With this in mind, Nifurpirinol (6-hydroxymethyl-2(2-(5-nitro-2-furyl)vinyl)pyridine (P-7138) (trade name Furanace; P-7138) was developed by the Dainippon Pharmaceutical Co., Ltd., Japan (Shimizu and Takase 1967), and is currently manufactured in the United States as Prefuran (Forsythe et al. 1990). Studies have proven that the drug is effective against bacterial and fungal pathogens in a wide variety of aquatic animals (Amend and Ross 1970; Abrahams and Brown 1977; Egidius and Andersen 1979; Mitchell and Plumb 1980; Lio-Po et al. 1982; Hanlon et al. 1984).

Most of the Nifurpirinol studies done in the past have dealt on its antimicrobial activity, tissue uptake, and effective treatment levels ranging from 0.5-2.5 mg/L (Delves-Broughton 1974; Egidius and Andersen 1979; Mitchell and Plumb 1980; Forsythe et al. 1990). The 96-hr median lethal concentration (LC50) to channel catfish (*Ictalurus punctatus* Rafinesque) has also been determined at 0.945-1.90 mg/L (Marking et al. 1977; Mitchell et al. 1978), and at 1.70 mg/L for milkfish, *Chanos chanos* Forsskål (Tamse and Gacutan 1994). However, there have only been two studies that have examined the histological effects on treated fish. Histopathologically, Mitchell et al. (1978) found hypertrophy and hyperplasia of the lamellar epithelium in channel catfish gills exposed to 0.5 mg/L for 4 d or longer at 24±2°C, while Amend and Ross (1970) working at 21±1°C observed no apparent changes in the gills of coho salmon (*Oncorhynchus kisutch)* exposed intermittently to 1 mg/L of Nifurpirinol.

This paper describes the histological changes observed in the gills of milkfish fingerlings used in static, 96-hr Nifurpirinol toxicity tests. Milkfish was used because of its economic importance as a widely cultured food fish in Asia. The gills were chosen as target organs because aside from its respiratory and

Present address: Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA
Correspondence to: C. T. Tamse

osmoregulatory functions (Randall 1970), they were also found to be the main site of Nifurpirinol absorption (Takase et al. 1968).

MATERIALS AND METHODS

Static 96-hr toxicity tests on milkfish fingerlings (3.0-3.5 g) obtained from a local fish farm, were done in 30-L glass aquaria with aeration, after a five-day acclimation period to natural seawater under laboratory conditions (temperature: 27±1°C; salinity: 31±1 ppt; pH: 7.9±0.6). Fish were fed live adult *Artemia* but were starved 24 hr before and during the tests. The toxicity test protocols of the American Public Health Association (APHA 1985) were followed including the 1-g fish/L aquarium biomass, which was 7-10 fish per tank based on the weight of the fish.

Seven Nifurpirinol (A.I.= 10%) concentrations were tested: 0.25, 0.5, 1.0, 1.5, 2.0, 2.3, and 2.5 mg/L plus control, each in six replicates, and two experimental runs were done. The distribution of test fish and concentrations were completely randomized. Test fish remained exposed to Nifurpirinol for 96 hr and were observed at frequent intervals.

A total of 12-14 fish were sampled from each test concentration after 24, 48, and 96 hr. Control fish were sampled only after 96 hr. The sampled and freshly dead fish were dissected for histological examination of the gills. All remaining fish at 96 hr were transferred to untreated seawater, observed, and fed with live adult *Artemia* for another 240 hr (10 days). At termination of recovery period, all fish were processed for histological studies.

Gills were fixed in 10% buffered Formalin, decalcified with 3-5 drops of concentrated nitric acid, dehydrated in a series of alcohol, cleared in xylene, embedded in paraffin, sectioned at 8 µm thickness, and stained with Harris' hematoxylin (Luna 1977) and triosin. Terminology for gill histopathology as suggested by Eller (1975), Mallatt (1985), and Meyers and Hendricks (1985), was used as reference guides in describing and analyzing any tissue changes observed.

RESULTS AND DISCUSSION

The results of our experiments show that histopathological changes in the gills of milkfish occurred after acute exposure to Nifurpirinol. It also showed that fish exposed to all the test concentrations exhibited an initial reaction of slight epithelial hyperplasia, together with clavate-globate (clubbing) lamellae. Moreover, it clearly indicated that the severity of gill damage progressed with higher drug concentrations of and longer exposure times to Nifurpirinol. No fish mortalities were observed in the lower concentrations (0.25-1.0 mg/L), while mortality rates ranging from 58-100% occurred at the higher concentrations (1.5-2.5 mg/L, respectively).

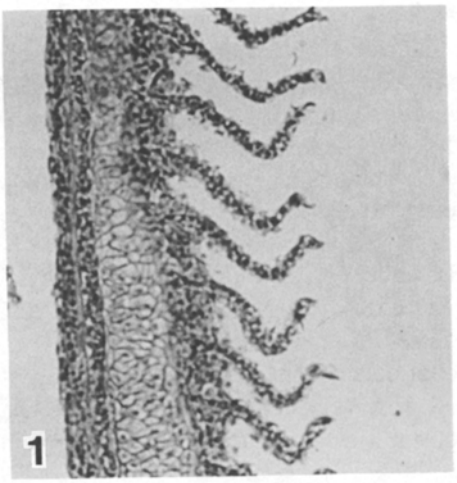
At the lower test concentrations (0.25 and 0.5 mg/L), lamellar clubbing and fusion were observed after 24 hr with some lamellar detachment after 96 hr exposure. Epithelial regeneration occurred in fish exposed to the lowest concentration (0.25 mg/L) when allowed to recover in untreated seawater for 10 days, and exhibited normal gill structure like that of the control fish. At 0.5 mg/L, slight hyperplasia was still present until after 10 days post-exposure. Fish from the control group exhibited no changes in the gill tissues throughout the experiment (Fig. 1).

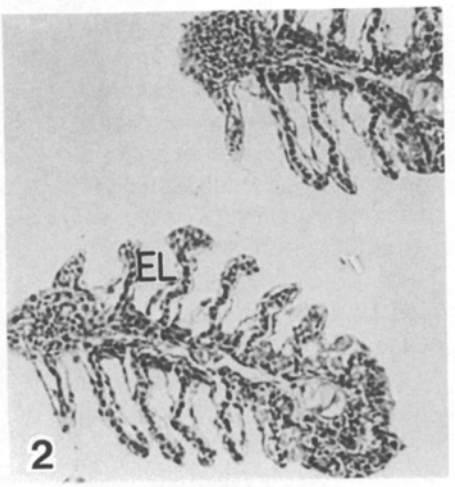
The degenerative branchial changes seen at higher concentrations (1.5 through 2.5 mg/L) were exaggerated forms of those caused by lower concentrations. Lifting of lamellar epithelium (Fig. 2), hyperplasia resulting in interlamellar fusion (Fig. 3), clavate-globate lamellae (Fig. 4), lamellar detachment, complete disruption of gill filaments (Fig. 5), and general necrosis were very much evident in these test levels. The gill lesions occurred at all exposure times and persisted even through the 10-d recovery period.

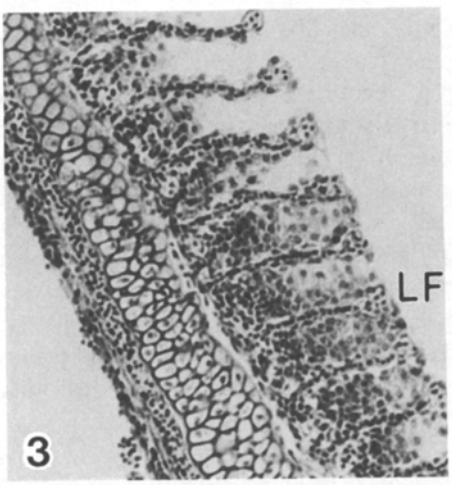
The Nifurpirinol-induced changes observed in the gills of milkfish were similar to those reported by Eller (1975) in response to a wide range of chemical agents. Mallatt (1985), in an exhaustive review of toxicant/irritant-induced changes in the gills, showed that such changes tend to be largely non-specific, and seem to reflect physiological adaptation to stress, and in this study, stress induced through exposure to Nifurpirinol. Epithelial hyperplasia resulting in lamellar fusion and clavate-globate lamellae, could be seen as a defensive response (Smart 1976) against prolonged exposure to irritants. Epithelial lifting and lamellar fusion were also suggested as a protective measure by decreasing the vulnerable surface area of the gills, to maintain its osmoregulatory function while sustaining a progressive loss of its basic functions (Abel 1976). However, such reactions that help slow down toxicant uptake could result in dysfunctional or even non-functional gills, and eventually asphyxiate the fish.

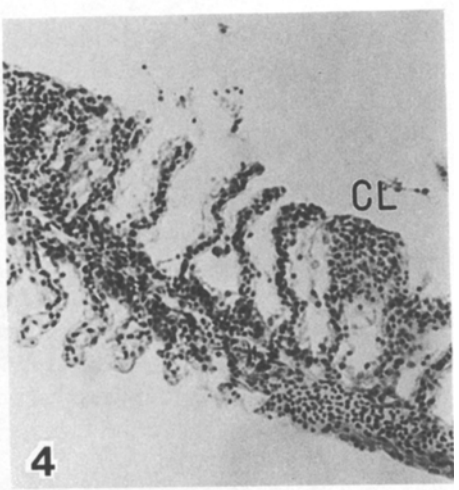
The restoration of normal gill architecture of fish exposed to 0.25 mg/L, the lowest concentration, after 10 d in untreated seawater suggests that gill damage was reversible and that the animal can recover from the exposure. It has been demonstrated that normal gill morphology was regained after toxicant-exposed goldfish were allowed to recover in uncontaminated water for 10 days (Fukuda 1983). On the other hand, the slight hyperplasia observed at 0.5 mg/L after the recovery period indicated that regeneration of epithelial cells would take more than 10 days, and that it would take longer for fish to acclimate to stressful conditions. This agrees with the work of Mitchell et al. (1978) who found that fish gills exposed 4 or 14 days to 0.5 mg/L Nifurpirinol had hypertrophied and hyperplastic lamellar epithelium.

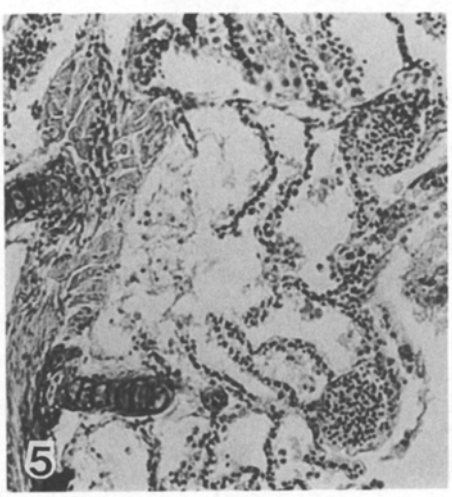
Severe gill lesions at higher concentrations (1.0 through 2.5 mg/L), such as lifting, hyperplasia, and general necrosis of gill epithelium were very much evident in these test levels. These responses can impair (Mitchell et al. 1978) gill functions and could eventually lead to the death of fish (Eller 1975). Furthermore, unlike











Figures 1-5. Nifurpirinol-induced changes in the gills of milkfish(*Chanos chanos* Forsskål) fingerlings. Fig. 1. Gill structure of control milkfish fingerlings. x250. Fig. 2. Distinct lifting (EL) of the lamellar epithelium after 96 hr in 1.5 mg/L Nifurpirinol. x250. Fig. 3. Lamellar fusion (LF) in gills exposed to 1.5 mg/L Nifurpirinol after 24 hr. x290. Fig. 4. Hyperplasia resulting in clavate-globate (CL) lamellae in milkfish gills exposed to 2.0 mg/L Nifurpirinol after 24 hr. x250. Fig. 5. Disruption of milkfish gill filaments with lamellar cells in a disorganized mass after 24 hr in 2.5 mg/L Nifurpirinol. x250.

the lower concentrations, restoration of normal gill architecture was not seen in fish that survived at these higher test levels, since lesions persisted even through the 10-day recovery period. This indicates that the regenerative capacity of the gill epithelium could not keep up with the rapid and widespread cellular degeneration that occurred after exposure to the higher Nifurpirinol concentrations. Fish mortalities observed at these test levels may then be related to asphyxiation, partial or complete loss of gill physiological functions, or to a loss of cellular ions or proteins from exposed gill lesions (Eller 1975).

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