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Acute Toxicity of Nifurpirinol, a Fish Chemotherapeutant, to Milkfish (*Chanos chanos*) Fingerlings

C. T. Tamse,* R. Q. Gacutan

Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines

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Nifurpirinol (trade name Furanace and originally known as P-7138), is a nitrofuran derivative synthesized by the Dainippon Pharmaceutical Co. Ltd., Japan, and was developed exclusively as a broad-spectrum antibiotic for fish and other aquatic organisms (Shimizu and Takase 1967). It has been shown to have bactericidal and fungicidal action *in vitro* and *in vivo* (Shimizu and Takase 1967; Amend and Ross 1970; Pearse et al. 1974; Mitchell and Plumb 1980), and was used because of its excellent potential in controlling prawn diseases (Delves-Broughton 1974; Gacutan and Llobrera 1977).

Milkfish (*Chanos chanos* Forsskal) is a widely-reared species and a very important aquaculture food crop in most parts of Southeast Asia. Thus, it was the logical choice as test animal for investigating the LC50 toxicity levels of nifurpirinol (6-hydroxymethyl-2-[2-(5-nitro-2-furyl) vinyl] pyridine) after 96 hr exposure. Changes in the normal gill architecture of milkfish after exposure to the drug were also studied (Tamse et al., in preparation).

MATERIALS AND METHODS

Milkfish fingerlings weighing 3.0-3.5 g were bought from a local fish farm, transported to the laboratory, and acclimated for 5 da. Fish were fed with adult *Artemia* but were starved 24 hr prior to and during the experiment. Water quality (dissolved oxygen levels, 6.8-8.5 ppm; salinity, 30-32 ppt; temperature, 26-29°C; pH, 7.3-8.5) were monitored daily and posed no problems throughout the toxicity tests. NH_3-N (0-0.05 ppm) and NO_2-N (0-0.003 ppm) determinations (Strickland and Parsons 1972) were also done at the start and the end of each experiment, and levels were found to be negligible.

Two runs of static 96 hr bioassay with aeration were done, with each test concentration in six replicates. The total biomass maintained in each of the 30 L glass aquaria was 1 g fish for at least 1 L seawater (Sprague 1969). Test protocols

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

of the American Public Health Association (1985) were followed. The commercial grade nifurpirinol (10% A.I.) was given to R.Q. Gacutan by Dr. K. Hatai.

The following nifurpirinol exposure groups were obtained after exploratory runs were done, and were calculated for mg/L of the active ingredient: 0.25, 0.50, 1.00, 1.50, 2.30, and 2.50 mg/L, plus 0 (control). Distribution of the test fish and concentrations were completely randomized. Fifteen minutes before the introduction of the drug, 100 mL of untreated seawater was removed from each aquarium. The pre-weighed nifurpirinol granules were then dissolved in separate lots of 100 mL distilled water and poured gradually into the corresponding aquaria. Fish were observed at regular intervals until termination at 96 hr. Mortalities were recorded and moribund fish were counted as dead with the cessation of gill movements and failure to respond to gentle prodding (APHA 1985; Rand 1985). The 24, 48, 72, and 96-hr LC50 values and 95% confidence limits were estimated using the Reed-Muench method (Reed and Muench 1938; Woolf 1968). It states that for a given set of data, the number of organisms exposed to a given level of stimulus artificially increases. It is assumed that any individual responding to a given dose or concentration would respond to all higher concentrations; likewise, any individual not responding to a given concentration would not respond to lower concentrations.

RESULTS AND DISCUSSION

The 96-hr LC50 of nifurpirinol-exposed milkfish at 27±1°C was 1.70 mg/L with 95% confidence limits at 1.52-1.89 mg/L. Figure 1 shows that the mortality response was directly proportional to the test concentration and length of exposure. Table 1 serves as a guide to the different concentrations and exposure times at which milkfish would respond to the drug, particularly at concentrations causing the least and the greatest number of deaths. It also shows that at the lower test levels (0.25, 0.50, and 1.00 mg/L), no deaths were observed for times less than 24 hr. All the fish at these concentrations were still alive even after 96 hr. This suggests that the toxicity response of milkfish fingerlings to nifurpirinol is lower compared with previous findings (Shimizu and Takase 1967; Abrahams and Brown 1977; Chandler and Marking 1979), and is closer to the LC50 values reported by Marking et al. (1977).

An interesting behavioral and swimming activity change was observed in the milkfish's response to the addition of the drug. During the first 15 min of exposure, there was a rapid increase in the rhythm of opercular movements. Fish would also swim to the surface, gasping for air. Thomas and Rice (1975), studying changes in opercular rhythms of pink salmon fry after exposure to toxicants, suggested it as a sensitive measure of physiological stress or change in metabolic needs. Aside from increased opercular rates, exposed fish also exhibited dark colorations on its dorsal side, along the entire dorsal length. Then the fish started to lose equilibrium, swimming in a vertical head down position, and settling on the bottom, ventral side up. It would lie quietly, opercular movement becoming

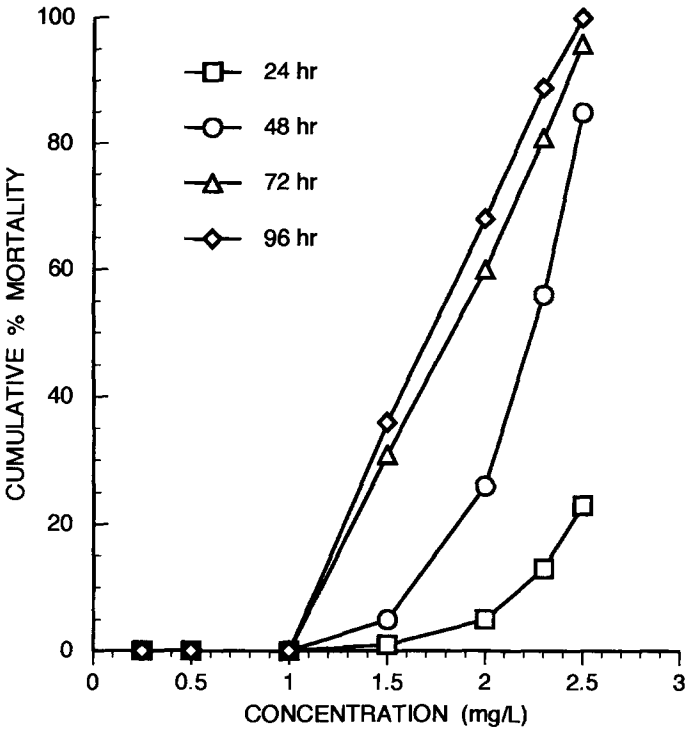

Figure 1. Mortality response of milkfish fingerlings to nifurpirinol at varying exposure times and concentrations.

irregular with occasional bursts of frenzied swimming. Fish were also less sensitive to noise or movement. Death occurred within 3-9 hr after the body darkening reaction started. This pigment modification of the dorsal surface of milkfish typically resembled fish under stress (Lingaraja et al. 1979; Holcombe et al. 1982).

Table 1. Lethal concentration values and 95% confidence limits (mg/L) for milkfish fingerlings exposed to nifurpirinol in static seawater.

	Concentration, $\bar{x}$, and 95% C.L., mg/L		
Lethal	Exposure period (hr)		
Level	48	72	96
LC5	1.48	1.07	1.06
	(1.16-1.88)	(0.84-1.35)	(0.85-1.31)
LC50	2.24	1.80	1.70
	(1.89-2.64)	(1.24-2.63)	(1.52-1.89)
LC95	2.49	2.40	
	(1.95-2.90)	(2.02-2.85)	

Our toxicity tests show that nifurpirinol is relatively non-toxic to milkfish at lower concentrations, particularly at 0.25, 0.50, and 1.00 mg/L levels. These non-lethal concentrations fall within the reported therapeutic levels used against fish and prawn diseases, which ranged from 0.5-2.5 mg/L (Amend and Ross 1970; Delves-Broughton 1974; Egidius and Andersen 1979; Mitchell and Plumb 1980) to 4.6-7.9 mg/L (Shimizu and Takase 1967). Higher concentrations where mortalities occurred in this study (1.50 through 2.50 mg/L) could still be useful for short-term bathing treatments or dipping regimes. Studies (Amend and Ross 1970; Pearse et al. 1974) have shown that fish exposed at or even in excess of 10 mg/L for 1 hr produced no adverse effects. Finally, since nifurpirinol is readily absorbed during and rapidly eliminated after exposure (Takase et al. 1968; Delves-Broughton 1974), it would appear to be a suitable chemotherapeutic for use in food fish such as milkfish.

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