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Role of β-Naphthoflavone in the Acute Toxicity of Chlorpyrifos in Channel Catfish

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Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a chlorinated organophosphate insecticide used widely against a variety of insects in the home and on the farm. Widespread use of chlorpyrifos is likely to result in its bioconcentration in the environment including freshwater fish. Evaluation of bioconcentration of chlorpyrifos in various fish species has indicated that fish absorb, metabolize and excrete the organophosphate rapidly (Barron et al. 1993; Marshall and Roberts 1978). Chlorpyrifos is believed to be biotransformed by hepatic microsomal P450 isoforms via oxidative desulfuration to its oxygen analog, chlorpyrifos oxon, which subsequently inhibits acetyl cholinesterase and causes quick deaths of the exposed fish (Barron et al. 1993). Although fish and mammalian species possess the same metabolic enzymes, they differ in type and amount of microsomal P450 isozymes and the rate of xenobiotic metabolism resulting in higher or lower susceptibility to toxic effects of pesticides (Tate 1988). The major objective of this study was to determine the LC_{50} value of and cholinesterase inhibition by chlorpyrifos in fingerling channel catfish in the presence and absence of β-naphthoflavone. Beta-naphthoflavone is a microsomal enzyme inducer and was chosen as a representative of many environmental contaminants includiing polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), to which freshwater fish are continually exposed.

MATERIALS AND METHODS

Fingerling channel catfish (*Ictalurus punctatus*) of both sexes with an average weight of about 20-25 g were obtained from a local hatchery.

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Fish were acclimated to laboratory conditions at least for a week before using them in the experiments. They were fed commercial diet. Chlorpyrifos (purity 99%) was obtained from Dow Chemical Co., (Midland, MI) as a gift. The cholinesterase assay kit No. 421 and β-naphthoflavone (BNF) were purchased from Sigma Chemical Co., St. Louis, MO.

To determine the 96-hr LC_{50} value for chlorpyrifos, four groups of 10 fingerlings each were exposed to four different concentrations of chlorpyrifos dissolved in 2.5 ml of acetone. Each aquarium contained 0.5, 1.0, 2.0 and -4.0 ppm of the insecticide in 5 gallons water. The experiment was run in duplicate. Fish mortality was observed at 12-hr intervals for 96 hr. The LC_{50} was determined statistically using the SAS software package, version 6.03 (SAS Institute, Inc., Cary, NC). Another experiment was conducted in duplicate to determine the effect of BNF on the chlorpyrifos LC_{50} value in the fingerlings. A dose of 100 mg/kg of BNF suspended in corn oil was injected ip to the fish and 2 days later they were exposed to chlorpyrifos concentrations varying from 0.05 to 0.80 ppm. The mortality was observed over 96-hr period and the LC_{50} was determined as described above.

Effect of chlorpyrifos on the activity of serum acetyl cholinesterase was determined by treating control fish with a sublethal (1/4th of the LC_{50}) dose (0.5 ppm) of waterborne chlorpyrifos and measuring the serum cholinesterase activity at 0, 6 hr and 24 hr intervals posttreatment. A similar experiment was conducted in fish using 1/4th of the LC_{50} dose (0.05 ppm) determined in BNF-pretreated fish. In this experiment, fish were pretreated with BNF prior to the exposure to 0.05 ppm chlorpyrifos. The corresponding control group was pretreated with the vehicle (acetone) alone. Blood samples from these groups were collected at 0, 6 hr and 24 hr intervals and serum cholinesterase activity determined using the Sigma kits. The results were analyzed statistically using Student's t test.

RESULTS AND DISCUSSION

It is seen from Table 1 that the 96-hr LC_{50} value of chlorpyrifos for fingerling catfish is 2.077 ppm. This value is approximately 7- 8 times higher than the value reported for adult channel catfish (Wellborn et al. 1984). Chlorpyrifos is metabolized by microsomal cytochrome P-450

Table 1. Ninety-six hr LC_{50} value of chlorpyrifos with and without β-naphthoflavone in fingerling channel catfish

Group	LC_{50} (ppm)
Chlorpyrifos	2.077 ± 0.452
BNF + Chlorpyrifos	0.198 ± 0.047

Results are expressed as the LC_{50} ± S.E. of the mean.

system to chlorpyrifos oxon which is an extremely potent inhibitor of acetyl cholinesterase esterase enzyme (Van der Wel and Welling 1989; Sultatos and Murphy 1983). It is very likely that chlorpyrifos-activating system in the fingerlings may not have been fully developed resulting in decreased chlorpyrifos toxicity in the fingerlings. In contrast, when the fish were pretreated with BNF, the LC_{50} value of chlorpyrifos reduced by about lo-fold (Table 1). It has been reported by Andersson and Koivusaari (1985) that BNF treatment of rainbow trout resulted in a significant induction of cytochrome P450 and several other monooxygenases activities. This finding may account for the marked reduction of LC_{50} value caused by BNF treatment suggesting BNF induces cytochrome P450 activity significantly also in catfish (Tate 1988) and increases toxicity of chlorpyrifos by enhancing its conversion to chlorpyrifos oxon. Chlorpyrifos oxon is usually hydrolyzed and subsequently detoxified by A-esterases which are widely distributed in several mammalian species but not in fish (Abbas and Hayton 1997). Thus, the possible lack of A-esterases in catfish also may have contributed to the enhanced toxicity of chlorpyrifos.

To test the hypothesis that BNF induces cytochrome P450 activity which in turn biotransforms chlorpyrifos to its oxon form causing extensive inhibition of cholinesterase activity, we treated the fish with sublethal doses of chlorpyrifos in the presence and absence of BNF and the serum cholinesterase activity was determined. As can be seen from Table 2, there was nearly 41 and 43% inhibition of serum cholinesterase enzyme activity by chlorpyrifos (0.5 ppm) at 6 and 24 hr intervals, respectively in the fingerlings. However, the inhibition by

Table 2. Effect of chlorpyrifos (0.5 ppm) on serum acetyl cholinesterase activity in fingerling channel catfish

Group	Serum acetyl cholinesterase activity[a]		
	0 hr	6 hr	24 hr
Untreated	30.73 ± 0.47	33.84 ± 0.32	28.49 ± 1.20
CPS-treated	29.89 ± 3.53	17.72 ± 1.01*	16.99 ± 0.83*

Results are expressed as the mean ± SEM, *P<0.05. [a]Expressed as units per liter. CPS denotes chlorpyrifos.

Table 3. Effect of chlorpyrifos (0.05 ppm) in presence and absence of BNF on serum acetyl cholinesterase activity in fingerling channel catfish

Group	Serum acetyl cholinesterase activity[a]		
	0 hr	6 hr	24 hr
CPS	22.58 ± 1.49	17.72 ± 0.10	14.93 ± 0.68
BNF + CPS	28.96 ± 2.23	12.44 ± 0.56*	13.83 ± 0.14

Results are expressed as the mean ± SEM, *P<0.05. [a]Expressed as units per liter. CPS and BNF denote chlorpyrifos and β-naphthoflavone, respectively.

chlorpyrifos at ten times lower level (0.05 ppm) in BNF-pretreated fingerlings at 6 and 24 hr posttreatment was 57 and 52%, respectively (Table 3). This marked increase in the inhibition of cholinesterase activity resulted from BNF pretreatment of the fingerlings indicates

that BNF treatment could have significantly induced microsomal enzymes that biotransform chlorpyrifos to its oxon form, a potent inhibitor of cholinesterase enzyme.

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