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Toxicity and Residue Studies of Fenvalerate to the Freshwater Fish *Channa punctatus* (Bloch)

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The pyrethroids with alkenyl methyl cyclopentenolene alcohols are the esters of cyclopropane carboxylic acids. Their activity depends on the ester, and is influenced by the absolute configuration of the asymmetrical carbon atom at C_1 of the cyclopropane ring and at C_4 of the cyclopentenolene ring. All very active pyrethroids have the R configuration at C_1 and the S configuration at C_4. The presence of dimethyl group at C_2 of the cyclopropane ring is essential for a high insecticidal activity. Substituents at C_3 of the cyclopropane ring, whether at the cis or trans position, are not absolute requirements as even without these substituents the insecticidal action is present, but only unsaturated side chains give high activity. The replacement of the isobutenyl group by an isobutyl group decreases activity whereas a butadienyl group may enhance potency (Wouters and Bercken 1978) (Fig.1).

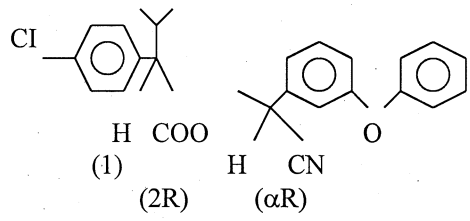

Figure 1. Chemical structure of fenvalerate.

The important pyrethroids are allethrin, bioresmethrin, permethrin, decamethrin, cypermethrin and fenvalerate. All are highly active and stable insecticidal pyrethroids, so they are used extensively in agriculture, for controlling insect pests, vectors of endemic diseases, protecting seeds during storage and fighting household insects.

Due to widespread use, these pyrethroids are transported into aquatic environments. Aquatic toxicity has become an integral part of the process of environmental hazard evaluation of toxic chemicals. Generally, the potential impact of pollutants is greater for aquatic organisms (Murty 1986).

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Among the pyrethroids, the neurotoxic organic insecticide fenvalerate (alpha cyano-3-phenoxy-phenyl) methyl-4-chloro-(1 methylethyl benzene acetate) is used to control boll worms on cotton, tobacco plants and vegetables. The impact of the toxicant to freshwater fish was examined via determining its LC_{50} value for 96h and via residue studies using thin-layer chromatography (TLC) and gas liquid chromatography (GLC). Generally pesticides accumulate in fish tissue due to their lipophilic nature and become magnified in the food chain. This biomagnification is of concern since they are consumed by humans.

MATERIALS AND METHODS

Channa punctatus, 6-9 cm length and 6.5-7.4 g weight were received from fisherman. The fish were acclimated to laboratory conditions in well-aerated unchlorinated tap water at $28\pm2^{o}C$. During the period of acclimatization and experimentation, the fish were not fed. If the number of deaths exceeded 5% in any batch of fish during acclimatization, that batch was discarded. The toxicity studies were conducted using the technical grade formulation and a 20% emulsifiable concentrate (EC), employing static and continuous flow systems as recommended in the report of the committee on methods for toxicity tests with aquatic organisms (EPA 1975). Solutions of desired concentration were prepared in 95% acetone to yield a concentration of 200 mg/100 ml of acetone i.e. 2 mg/ml for 20% EC and 100 mg/100 ml i.e. 1 mg/1 ml for technical grade fenvalerate and diluted with distilled water to get a working solution. The other precautions such as use of acetone in control as recommended by EPA (1975) were followed.

For the flowthrough system, test solutions of desired concentrations were prepared once every five hours in glass reservoirs and let into the test containers through thin-walled polyethelene tubes. The flow rate was adjusted with regulators such that 4 L of water passed through containers in one hour. The conditions of the test medium were: temperature $28^{o}\pm2^{o}C$, oxygen 6-8 ppm, hardness 80 mg/L, alkalinity-425 mg/L and pH 8.3. All the precautions laid down in the report of committee on toxicity tests were followed (EPA 1975).

Pilot experiments were conducted to determine the concentrations causing 10 to 90% mortality of test fish. For each concentration, 10 fish were tested and the experiment was repeated thrice. Probit analysis (Finney 1971) as recommended by Roberts and Boyce (1972) was followed to calculate the LC_{50} values. Technical grade fenvalerate and the 20% EC were supplied by Searle (India) Limited, Mumbai.

After exposure to pesticide, tissues from surviving fish were taken for residue analysis. Brain, muscle, liver, kidney and gill tissue (each 100 mg) were homogenised and centrifuged individually, using acetone as a solvent. The extracted solvent was cleaned-up through a column, packed with florisil as absorbent. The first three 15 ml aliquots of acetone were pooled and concentrated to 1 ml (EPA 1974).

The concentrated solvent and technical grade pesticide were spotted on a thin layer chromatographic plate (5 x 20 cm size) with silver nitrate-impregnated silica gel (Moats 1966). The spotted plates were developed in a solvent system consisting of 3:1 hexane and acetone. The developed plates were exposed to UV light for 10 minutes and the spots were observed. For residue analysis, the precautions laid down by Thompson (1974) were followed. The entire procedure was standardised in the laboratory, by adding the pesticide fenvalerate to the fish tissue and the same extraction procedure as described above is followed. The 100 x Rate of flow (Rf in cm) values were calculated for different solvent systems with different ratios and the one that has the best resolution was considered as a mobile solvent for thin layer chromatography.

Quantitative analysis by gas liquid chromatography was performed following Bradbury and Coats (1982) method. Residues of fenvalerate were determined by using the GLC – 17 "A" A.F.W. (Advanced Flow) and Auto control system (SHIMADZU, JAPAN), equipped with electron capture detactor and a coiled glass column S.E.- 30 silica gel in length of 25 m. Nitrogen was used as the carrier gas through a 0.31 internal diameter column MMID at a flow rate 2.0 ml/minutes. The temperatures of the detector, injector and oven were 290°C, 290°C and 285°C, respectively. The relative retention time of fenvalerate was 1.93 and 2.05 min. The amount of fenvalerate residue was calculated against known standard values.

$$\text{Concentration} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{Amount of standard}$$

RESULTS AND DISCUSSION

The 96 h LC_{50} values for static and continuous flow (CF) systems for the fish are given in Table 1.

The results of the whole body tissue analysis of the fish by gas liquid chromatography (GLC) are given in Table 2 and pesticide residue confirmation was done by thinlayer chromatography.

The LC_{50} values for fenvalerate to different fish were given by Anderson (1982), Chapman et al. (1985), Bradbury and Coats (1982), Bradbury et al. (1985; 1986; 1987), Mulla et al. (1978), Coats and O Donnell (1979), Zitko et al. (1977; 1979), Tripathi (1992), Anitha et al. (1999a & 1999b). These authors showed that fenvalerate is highly toxic to fish and formulations are more toxic than technical-grade fenvalerate. When static and continuous flow-through system values of technical fenvalerate are compared, the static values are higher for the 20% EC in both types of tests, for the different fish.

Table 1. LC_{50} values (ppm) of technical grade (TG) and 20% EC formulation of fenvalerate to *Channa punctatus* using static(s) and continuous flow (CF) systems

	24 h		48 h		72 h		96 h	
	S	CF	S	CF	S	CF	S	CF
TG	197.3	187.2	164.0	159.3	167.0	127.7	128.1	110.7
20% EC	243.7	256.6	214.8	178.0	196.8	164.6	126.7	86.1

ppm=parts per million S=Static CF=Continuous Flow through system

Each value is Mean of 5 Individual Observations; tested for chi-square and are significant at $P < 0.05$.

Table 2. Residues of fenvalerate

Fish	Tissue	Amount of residues µg/g of tissue weight
Channa punctatus	Gill	598.40
	Liver	301.00
	Kidney	76.80
	Brain	64.86
	Muscle	16.26

Generally, there are three main chemical reactions involved in the degradation of the toxicant (i.e. isomerization, hydrolysis and oxidation) (Demoute 1989) to form the metabolites. Fenvalerate has a common degradation pathway for mammals, birds and amphibians. The basic metabolic reactions are similar in all, i.e., cleavage of the ester bond and oxidation of the released acid and alcohol fragments. The initial transformations are followed by conjugation with sugars or amino acids which allow faster excretion.

The same type of degradation is reported in fish by Bradbury et al. (1987) who studied the toxicokinetics of fenvalerate in rainbow trout and reported uptake in the gill. Residue analysis of gill tissue confirmed that 2S isomer of fenvalerate, a degradation product is more toxic. The qualification and quantification of the residues demonstrated that fenvalerate accumulated and concentrated in fish tissues. In vertebrates, except fish, pyrethroids, as opposed to organochlorines, have the same lipophilicity and are readily metabolised and excreted. The half-life periods never go beyond a few days. Birds have a larger capacity to eliminate their products than mammals. The high toxicity to fish is partly explained by their poor ability to metabolize them. Bradbury et al. (1985) reported that the lower rates of elimination and metabolism of fenvalerate in fathead minnows (*Pimephales promelas*) are the factors responsible for higher toxicity.

Pyrethroids metabolism is largely oxidative in fish (Edwards and Millburn 1985). The fenvalerate acid moeity is very different from the other groups of pesticides and the ester cleavage releases the acid fragment which is mainly excreted as a

glucuronide conjugate. The cyanide ion is transformed into thiocyanate and also carbon dioxide.

The toxicity of fenvalerate is due to metabolism and accumulation. The results of the present study revealed that, prolonged exposure to sublethal concentrations of fenvalerate in *Channa punctatus* leads to increased accumulation of residues. Anitha et al 1999a & 1999b reported that repeated or continuous exposure to low concentration of pesticides can lead to high residue concentrations without mortalities. Table 1 data confirm that the commercial formulation is more toxic than the technical compound. The cis isomer is more toxic than trans isomer (Wouters and Bercken, 1978). Since 20% EC contains $1/5^{th}$ of the technical grade fenvalerate, metabolite formation due to storage of the pesticide after manufacture, is inevitable. Although the pyrethroids are the safest insecticides, when administered orally, they may become highly toxic when they reach the central nervous system in sufficient concentration. The observations are in agreement with earlier reports (Coats and O'Donnell 1979; Bradbury et al 1985; Haya 1989, Tripathi 1992).

The quantitative data of Table 2 show the uptake of the toxicant in the tissues of the fish. In other animals also (Tilak et al 2001), the residues are known to bioaccumulate via the food chain to humans. Hence, the risk to the health of the people who consume these fishes seems to be considerable. The concentrations of these residues will also show impact on reproductive impairment of the commercially important fishes and to the higher carnivores, especially to the birds.

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