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Acute Toxicity of Carbofuran to a Freshwater Teleost, *Clarias batrachus*

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Paddy fields and lowland areas in northeastern India are natural habitats of the air-breathing catfish, *Clarias batrachus* (Linn.). It is an edible fish found in ponds, lakes and rivers in India. It possesses four pairs of barbles, which are sensitive to pH, salinity and temperature of the medium. Use of organocarbamate pesticides in agriculture can effect the survival, growth, reproduction and metabolism of the fish. The toxic actions of other groups of pesticides, such as organochlorine and organophosphate on the target pests and on some terrestrial populations have been evaluated extensively. However, no attempt has so far been made to study the deleterious effects of organocarbamate pesticides on non-target animals such as fish.

Carbofuran (2,3- dihydro - 2, 2 - dimethyl - 7 - benzo-furanyl methylcarbamate) is a widely used insecticide. It is known to produce hypercholinergic activity of central as well as peripheral organs (Gupta and Kadal 1989; Yadav et al 1998), by inhibiting the acetylcholinesterase enzyme at synapses in the brain and neuromuscular junction at subacute concentrations (Yadav et al 1998; Singh and Sharma 1999). Some workers have reported that carbofuran is extremely toxic to fish and its 96 hr LC_{50} is less than 1 mg/L (Trotter et al 1991). Carbofuran is also toxic to several beneficial arthropods after entering in the aquatic environment through runoff during agricultural use, greenhouse application and direct contamination of water bodies (Caro et al 1973; Parkin 1994; Saglio et al 1996; Kumari et al 1997). While reports are available on the acute toxicity of carbofuran to aquatic organisms including different fish species (Mukhopadhyay et al 1982; Jash and Bhattacharya 1983; Bhattacharya 1985; Bhaktavatsalam 1986; Trotter et al 1991; Saglio et al 1996; Kumari et al 1997), the literature regarding carbofuran toxicity of *Clarias batrachus* is scant. The present investigation describes of the effects of carbofuran on *C. batrachus* at different concentrations and durations of exposure.

MATERIALS AND METHODS

The healthy freshwater teleost, *C. batrachus* (length 10-12 cm, weight 25-30 g, mixed same age group) were obtained from a local fish breeding farm and treated

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with potassium permanganate solution (5%, w/v) for five min to remove any dermal adherent. The fish were acclimated in dechlorinated tap water for seven days under natural photoperiod and standard laboratory conditions in glass aquaria (50 L capacity) (APHA 1991). The fish were fed flour pellets and ground dried shrimp; aquaria were cleaned and water was changed every day. Only healthy fish, of both sexes, were used in the experiment. The physico-chemical characteristics of the water used were temperature ($24 \pm 2.2^{o}C$), pH (6.7 ± 0.3), DO (Dissolved oxygen, 6.2 ± 0.4 ppm), alkalinity (96 ± 6.5 ppm), hardness as calcium carbonate (120 ± 7.1 ppm) and electrical conductivity (860 ± 42 μm hos).

Carbofuran (99.8% purity and acetone soluble) was obtained (Rallis India Limited, Banglore) and dissolved in acetone (100 mg/ml). An appropriate volume of carbofuran solution was added in glass aquarium (containing 20 L water) to give the desired concentrations of pesticide. To a control aquarium, equal volume of acetone was maintained as carbofuran added to experimental aquarium was dissolved in acetone. Twenty-one fish were transferred to each aquarium (maintained in three sets) having different concentrations of carbofuran. The number of fish that died was recorded and dead fish were removed at 24, 48, 72 and 96 hr. The LC_{50} value was determined (APHA 1991). Analysis of variance was used for studying the dose- and time- dependent effect of carbofuran on percent mortality. Data were analyzed by the probit log method and expressed as mean $\pm$ SEM of percent mortality at each concentration and exposure period. LC_{50} values are expressed in ppm. Acute toxicity range, confidence limits (95%) for LC_{50} values and the slope were calculated according to Litchfield and Wilcoxon (1949). The no observed adverse effect concentration (NOAEC) for carbofuran was also calculated according to the formula suggested by Doudoroff et al (1951).

RESULTS AND DISCUSTION

In the present investigation, the percent mortality increased with increasing concentration and time of exposure (Table 1). The LC_{50} values of carbofuran at 24,48,72 and 96 hr were found to be 0.30, 0.245, 0.23 and 0.20 ppm, respectively. The estimated NOAEC (safe) concentration was 0.025 ppm (Table 2). Analysis of variance showed that an increase in carbofuran concentration and time of exposure significantly enhanced the percent mortality of fish. A minimum percent mortality of 3% was recorded with a concentration of 0.05 ppm at 24 hr exposure and maximum of 100% mortality was observed at 1.0 ppm on 96 hr exposure. At each concentration there was an increase in percent mortality with increasing exposure time. The LC_{50} was found to be maximum at 24 hr with slope function of 2.835 and minimum at 96 hr with slope function of 2.055. The slope of carbofuran LC_{50} values were steep ranging from 2.055 to 2.835. LC_{50} values at all exposure times had a fairly narrow 95% confidence limit. Chi-square tests did not indicate any heterogeneity in the data ($p<0.05$). These results clearly demonstrated that *C. batrachus* is susceptible to carbofuran.

Table 1. Toxicity of carbofuran against *Clarias batrachus*

Carbofuran Concentration (ppm)	Percent mortality (mean ± SEM) after exposure periods (hr)			
	24	48	72	96
0.05	3.4 ± 2.0	4.3 ± 2.0	5.0 ± 2.7	11.7 ± 2.0
0.1	15.0 ± 3.5	11.3 ± 2.4	15.0 ± 3.5	23.3 ± 4.1
0.2	33.3 ± 4.0	38.7 ± 3.3	41.7 ± 4.9	55.0 ± 3.5
0.4	57.7 ± 2.4	62.4 ± 4.1	65.3 ± 4.1	68.3 ± 2.0
0.8	69.7 ± 4.0	75.2 ± 2.2	85.0 ± 4.1	88.3 ± 2.0
1.0	94.3 ± 3.3	96.5 ± 2.7	96.6 ± 3.7	100.6 ± 0.0

The values represent mortality (mean ± SEM of three different observations) of *C. batrachus* at 24, 48, 72 and 96 hr.

Table 2. Toxicity of carbofuran against *Clarias batrachus*

Time of Exposure	LC_{50} (ppm)	95% of confidence limit Lower	Upper	Slope function (s)
24	0.30	0.225	0.399	2.84
48	0.245	0.189	0.315	2.28
72	0.23	0.177	0.297	2.30
96	0.20	0.16	0.249	2.06

Calculated NOAEC = 0.025 ppm
Chi-square values were not significant ($p < 0.05$)

These findings could not be compared because of lack of published information about the toxicity of carbofuran in *C. batrachus*. However, the results of the present investigation are comparable with that of various workers who have reported more or less similar values for other fish species. Saglio et al (1996) obtained a 96 hr LC50 value of carbofuran on gold fish of 0.5 to 1.0 mg/L. In cyprinids the 96 hr LC_{50} of carbofuran has been determined to be below 1 mg/L (Trotter et al 1991). Verma et al (1979) showed LC50 values of 0.637, 0.598, 0.570 and 0.568 mg/L for 24, 48, 72 and 96 hr, respectively for *Heteropneustes fossilis* exposed to carbofuran. In a comparative study on the toxicity of commercial grade carbofuran to the major fish species, *Catla catla, Labio rohita* and *Cirrhinus mrigala*, the LC_{50} values for 96 hr were 5.1, 4.8 and 4.7 mg/L, respectively (Kulshrestha et al 1986). Bakthavatsalam et al (1984) found no mortality for 12 hr at a concentration of 0.56 mg/L of carbofuran (commercial grade) where as after 24 hr all the fishes were killed at concentration of 1.456 mg/L of the exposure.

These data show that the range of acute toxicity of carbofuran to different species of fish is small. However, the acute toxicity of the carbamate compound is species specific and it is influenced by predisposing factors like temperature, pH, hardness, BOD, dissolved solids, fish size, solubility, partition coefficient and other physicochemical characteristics of water. The decrease in the LC_{50} value with increasing in exposure time may be due to the effects of environmental factors and degradation of carbofuran.

In the present investigation the estimated NOAEC (0.025 ppm) for carbofuran, is about one 8th of 96 hr LC_{50} value. This is useful information for the environmental biologist and agriculturist in regulating carbofuran use to control its presence in effluents and protect fish and other species in nature. Ram and Singh (1988) observed the safe concentration of the commercial formulation of furadan (G)(carbofuran) as 4.5 mg/L for *Channa punctatus* but no reports have been published for *Clarias batrachus*. The results of this study show that an adequate risk assessment for aquatic life must be under taken prior to large scale application of carbofuran.

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