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Study of toxicity and behaviour of freshwater fish, *Channa punctatus* (Bloch) after intoxication of carbaryl (1-naphthalenyl methylcarbamate)

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Abstract
The effects of carbaryl (1-naphthalenyl methylcarbamate), commonly used in agricultural operations, have been studied with reference to mortality and behavior of the freshwater fish, *Channa punctatus.* Pesticide was administered to glass aquaria containing the experimental fish by glass rod without disturbing the test animal. Thus the pesticide was imbibed via the gastrointestinal tract and the surface of gill and skin of the experimental fish. Among the five concentrations (4, 6, 8, 10, 12ppm) prepared, the concentration of 12 ppm, carbaryl caused 100% mortality within 48 hr. LC_{50} of carbaryl is 8.71 ppm to *C. punctatus.* But such sublethal concentrations increased the frequency of opercular beats and abnormal and erratic swimming movements. The latter response was dose-dependent. These behavioral changes were the immediate response to the toxicant and were indicators of possible stress. The fishes tried to avoid the toxic water with fast swimming and jumping. Their fins became hard and stretched due to stretching of body muscles. They secreted mucus from whole body continuously and soon thick layer of mucus was found deposited in the buccal cavity and gills. Finally fish lost their balance and consciousness, engaged in rolling movement and became exhausted and lethargic. Soon they settled at the bottom of the tank, and after some time their bellies turned upward and the fish died, while the opercula remained wide open exposing the gills. They were lethargic and at the time of death exhibited transient hyperactivity before collapsing.

Keywords: Carbaryl, *Channa punctatus*, lethargic

Introduction
The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman *et al*., 2002) [19]. Numerous reports on the effects of pesticides on various fish species are available (Campagna *et al*., 2006; Omitoyin *et al*., 2006 and many others) [12, 4]. Pesticides as carbaryl and malathion are useful to control economically important crops (Saeed *et al*., 2005) [21], but are found very much hazardous to the aquatic flora and fauna and hence ultimately to human being as they depend on aquatic foods like fishes (Mellanby, 1967) [9]. Carbaryl is an organophosphate pesticide widely used an alternative to carbamate pesticide (Svoboda *et al*., 2001) [23]. Ii is less soluble in water and highly soluble in organic solvents.
Increased use of these pesticides in most tropical countries has been reported to results in severe toxicities and bioaccumulation (Palmer, 1972; Parish, 1985) [15, 16]. Therefore there is a need to investigate the toxicity of carbaryl which are oftenly used for pest management in agricultural and run off in aquatic habitat. Snakehead, *Channa punctatus* (Bloch), a common air breathing freshwater fish has omnivorous feeding habits, easy and abundant availability and its adaptability to varied environmental conditions. Literature review reveals that little work has been carried out on toxicity of these pesticides on fishes (Omitoyin *et al*., 2006) [12]. Hence there is a need to study the toxicity of carbaryl pesticides on *C. punctatus.*

Material and Methods
Experimental Animal
Snakehead, *Channa punctatus* (Bloch), a common air breathing freshwater fish. By virtue of its omnivorous feeding habits, and easy and abundant availability and adaptability to varied environmental conditions, was selected as an ideal experimental animal for the present

investigation. Healthy fishes were collected from the local ponds and swamps and brought to laboratory for acclimatization. The fish were stored in glass aquaria containing dechlorinated tap water for fifteen days for acclimatization under laboratory conditions. Water was changed after every 24h. Commercial fish food was supplied to fish during acclimatization period. To avoid water fouling dead fish (if any) were removed from the aquaria as soon as possible. Adult fishes of nearly similar weight (12 ± 2cm & 8 ± 2 g) were selected for experiments. To avoid introducing disease into stock tanks, the fishes were briefly bathed in a potassium permagnate solution (5ppm) before introducing them into the experimental area. Laboratory conditions were maintained following the standard protocol; described in literature by various authors. Water characteristics were temperature 22 ± 2^0C, pH 7.4 ± 0.6 alkalinity 106 ± 6.8 ppm, and dissolved oxygen 7.3 ± 0.4^{0}C

Test Chemicals

Carbaryl was chosen for present study owing to its extensive application among the carbamates for the control of nearly 100 pests.

IPUC Name = 1-naphthyl methylcarbamate,

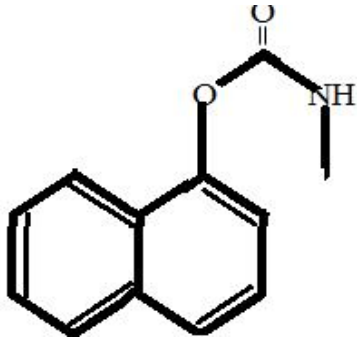
CAS number = 63-25-2,

Trade Name = Sevin

Molecular formula-CNC (OC_2=CC=CC1=CC=CC=C_{12})=O,

Molecular Weight = 201.2

Structural formula



Physical Property: Colorless white crystalline solid, m.p.142^{0}C.

Experimental Habitat

Fiber glass aquaria were set up in the laboratory. All the aquaria were of the capacity of 80 liters. Aquaria were provided with all the necessary equipment such as aerator, artificial light, and facial matter extraction tube, water removing pipes to maintain possible conditions for the test organism.

Test solution: Technical grade samples of carbaryl (99% W/W) was used. Since it is not completely soluble in water little of acetone was used as solvent. A stock solution of 1000ppm (mg/m) of carbaryl was prepared in acetone. For working concentrations required dilutions were made with tap water. Fresh stock solutions and required dilutions were prepared for each exposure. Since a small quantity of acetone was used for stock solution, it is reported to be notoxic to fish (Pickering *et al.*, 1962) [18], acetone controls were also maintained to nullify the possible effects.

Maintenance of fishes: For experimentation laboratory acclimatized fish were sorted into batches of 8 each and kept in 80L glass aquaria containing tapwater to maintain biomass theory (1g/L).The fish were starved for 24h prior to the experimentation and then the water was renewed (Monteiro *et al*., 2006) [10].

Test Concentration: For LC_{50} determination (Finney 1971) [6] five concentrations of carbaryl (4ppm to 12ppm) were selected.

Number of fishes: Fishes of uniform size and weight were selected. For each concentration 12 fishes were used. Bioassay method: Pesticide was administered to glass aquaria containing the experimental fish. It was mixed thoroughly with the test medium by glass rod without disturbing the test animal. Thus the pesticide was imbibed via the gastrointestinal tract and the surface of gill and skin of the experimental fish. All experiments were conducted at room temperature and the tanks aerated. Fishes were not fed during the experiment (Reish and Oshida 1986) [20]. Observations were recorded every 12 hours, number of dead fish were removed. Fishes were considered dead if there were no visible movement (e.g. gill movement) and if touching the caudal peduncle produces no reaction. Records were kept of visible abnormalities (e.g. loss of equilibrium, swimming behavior, pigmentation etc).

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Result and Discussion

Acute toxicity is discernible effect induced in an organism within a short time of exposure to a substance. In the present test, acute toxicity is expressed as the median lethal concentration (LC_{50}) that is the concentrations in water (test medium) which killed 50% of a test batch of fishes within continuous period of exposure. The chronic toxicity of a pesticide is determined by subjecting test animals to long-term exposure to the active ingredient. Any harmful effects that occur from small doses repeated over a period of time are termed "chronic effects." The chronic toxicity of a pesticide is more difficult than acute toxicity to determine through laboratory analysis LC_{50} of the test compound.

In the present studies the dose mortality studies were conducted for 48hrs. This fish mortality was nil upto 6ppm, 33.3% mortality in 8 ppm, 83.3% mortality in 10 ppm and 100% mortality in 12 ppm. (Table-1). Further the slope, 95% confidence limits were calculated with statistical formula given in Finney (1971) [6] and are presented in table

2. Similar results were obtained by Wasu *et al*. (2009) [26] when the toxicity of carbaryl to *Clarius batrachus* was studied in a static renewal bioassay for 48 hrs. The LC_{50} value of carbaryl for 48 hours was 13.24 ppm and 5.248 ppm respectively. The effects of carbaryl commonly used in agricultural operations, have been studied with reference to survival and behavior of the catfish *Mystus vittatus* (Arunachalam *et al*., 1980) [2]. At a concentration of 32.5 ppm, carbaryl caused 100% mortality within 24 hr; the 72 hr LC_{50} was 17.5 ppm. At concentrations of 12.5 ppm and below, it caused no mortalities within 72 hr.

The LC_{50} values were determined using different concentrations of pesticides. The LC_{50} of carbaryl is 8.71 ppm to *C. punctatus* in present studies. LC_{50} values obtained through computer analysis were also found to be 8.56 ppm. Sambasiva Rao (1999) [22] also reported 8.5 ppm LC_{50} value of carbaryl to *C punctatus* after 48 hours exposure. Similar results were obtained by Pickering and Henderson (1996) [17]. Vittozi and De Angelis (1991) [24] summarized the 96 hrs LC_{50} values of malathion 0.091 to 22.09 ppm for different species. The difference in toxicity to the different species mentioned above might be due to differences in absorption pesticide, their accumulation, biotransformation and excretion. Differences in metabolic pathways among species may result in different patterns of biotransformation leading to more or less toxic metabolites (Johnson and Taledo, 1993) [7].

Behavioural Toxicity

Behaviour is also considered a promising tool in ecotoxicology. Behaviour is an integrated result of endogenous and exogenous processes and low level of exposures have been implicated in various behavioural and physiological impairments. In the present studies the effect of carbayl is studied on the behavioural toxicity of the *C. punnctatus*. The fish showed increased opercular movement and abnormal and erratic swimming movements owing to sudden changes in the surrounding environment. Fish was stressed progressively. After sometime they tried to avoid the toxic water with fast swimming and jumping. Faster opercula activity was observed as surfacing and gulping of air. Similar results were obtained by Wasu *et al*., (2009) [26]. Their fins became hard and stretched due to stretching of body muscles. Earlier Barton and Iwama, (1991) [3] had observed that under stress, physiological and biochemical responses may be compromised, becoming detrimental to the fish's health and well being at which point the fish is termed distressed. Similar had been the findings of Morgan *et al*., (1991) [11] and others. They too have reported that Fishes in a contaminated environment show some altered behavioral patterns which may include avoidance, locomotive activity and aggression and these may be attempts by the fish to escape or adjust to the stress condition.

Many morphological changes have been reported following the exposure of fish either to high concentrations for brief periods, or to sub lethal concentrations for extended periods. In the present studies it was observed the fishes exposed to carbaryl secreted copious amounts of mucus from whole body continuously and soon thick layer of mucus was found deposited in the buccal cavity and gills. Body pigmentation was increased. Ligia *et al*., (2008) [8] also reported that exposure of a predatory fish *Tilapia jarbua* to 2 µg/l of DDT for 15 days resulted in the darkening of skin and formation of a brown spot on the forehead.

The treatment of fish with carbaryl ultimately lost the balance of fish and their consciousness in present studies. They became exhausted and lethargic. Lastly they remained in vertical position for a few minutes with anterior side or terminal mouth up near the surface of the water trying to gulp air and tail in a downward direction. Soon they settled at the bottom of the tank, and after some time their bellies turned upward and the fish died, while the opercula remained wide open exposing the gills. They were lethargic and at the time of death exhibited transient hyperactivity before collapsing. Resting at bottom, excess secretion of mucus, colour change was observed by Wasu *et al*., (2009) [26] in the chronic dose of 45 days. The loss of equilibrium of fish were also seen in the 45 days dose of malathion. These findings agreed with the studies of Alkahem *et al*., (1998) [1]; Omitoyin *et al*., (1999) [13]; Fafioye, (2001) [5]; Waiwood and Johnson, (1974) [25]; and Sambasiva Rao, (1999) [22]. Palawski *et al*., (1983) [14] found that Rainbow trout that survived 96-hr exposure to methyl parathion were immobile and had distended abdomens.

Conclusion

The result of the present study indicates that carbaryl exerts toxic effect on fish and show quick and lethal response to this toxicants. Thus the use of carbaryl should be properly and strictly control and regulated by appropriate legislation in order to prevent its bioaccumulation in the environment, aquatic animals and ultimately to the human being.

Table 1: Mortality of Channa punctatus at different concentration of Carbaryl for 48hrs exposure period

Concentration of Carbaryl (ppm)	Log Concentration	Fish exposed	Fish dead	Percent kill	Probit kill
4	0.6021	12	0	0	3.0
6	0.7782	12	0	0	3.0
8	0.9081	12	4	33.3	4.56
10	1.0000	12	10	83.3	5.95
12	1.0792	12	12	100	8.09

Table 2: 48he LC_{50}, slope and 95% confidence limit values of Carbaryl

Pesticide	LC5 value/mg/l	Slope	95% Confidence limit	
			Lower limit	Upper limit
Carbaryl	8.71 0.425	12.00	7.876	9.543

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