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Toxicity Bioassay of Technical and Commercial Formulations of Carbaryl to the Freshwater Catfish, *Clarias batrachus*

G. TRIPATHI AND S. P. SHUKLA

Department of Zoology, Banaras Hindu University, Varanasi 221005, India

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The acute toxicity of the carbamate insecticide, carbaryl, in technical and commercial formulations was investigated in the freshwater catfish, *Clarias batrachus*, after exposures of 24, 48, 72, and 96 hr. The "trimmed Spearman–Karber method" with 10% trimming was employed for the determination of median lethal concentration ($LC(I)_{50}$). The $LC(I)_{50}$ values of the commercial carbaryl (162.60, 134.08, 123.36, 107.66 mg/liter) decreased gradually with increase of exposure duration from 24, to 48, 72, and 96 hr, respectively. Similarly, the $LC(I)_{50}$ values of technical grade carbaryl (61.14, 53.65, 48.58, 46.85 mg/liter) decreased with increasing length of exposure. This reflects a time-dependent adaptability of the fish to the toxicant. Further, the technical grade compound was 2.5 times more toxic than the commercial preparation. This demonstrates the involvement of carbaryl as the active principle in acute toxicity testing, rather than the additive substances. Dermal desquamation was observed as a characteristic change in response to carbaryl exposure.

INTRODUCTION

India is one of the leading manufacturers of basic pesticidal compounds among Asian countries. The main use of pesticides in India is in agricultural enterprises to combat various pests. Attention is being paid to the use of less hazardous compounds which are mainly toxic to the target species. Carbamates belong to such a category. In 1956, carbaryl (Sevin) was introduced as the first successful commercial carbamate insecticide. Carbaryl (*n*-naphthyl, 1-methyl carbamate) is an aryl *N*-methyl carbamate that is commonly used to control a number of insect pests affecting cotton, tobacco, vegetable, and other crops. It is used extensively in India because it is considered to be environmentally safe compared to organochlorine and organophosphate pesticides. It has a shorter persistence and lower mammalian toxicity (Brown, 1978).

The acute toxicity of a wide variety of pesticides has been reviewed in fishes (Konar, 1981). The aquatic toxicity (96 hr) of technical grade carbaryl to some freshwater fishes has been reported (Tilak *et al.*, 1980, 1981) using an unweighted regression method of probit analysis (Finney, 1971). Several parametric methods have been introduced to estimate the median lethal concentration (LC_{50}) and binomial confidence interval of the toxicants (Gelber *et al.*, 1985). However, the trimmed Spearman–Kaber method is advantageous even when mortality is not monotonous, whereas the other methods are not resistant to anomalies in the data. Further, there is no information on the differences between the toxicities of technical grade and commercial preparations of carbaryl to the freshwater fishes with increasing length of exposure. The present toxicity bioassay has been performed to estimate LC_{50} values and 95% confidence intervals (employing trimmed Spearman–Karber method) of

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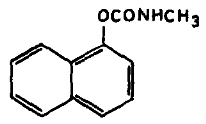

(1-Naphthalenol methylcarbamate)

FIG. 1. Structural formula of carbaryl.

technical and commercial formulations of carbaryl (Fig. 1) exposed to the freshwater catfish, *Clarias batrachus*, for 24, 48, 72, and 96 hr.

MATERIALS AND METHODS

The freshwater catfish, *C. batrachus*, weighing 65–70 g and having a body length of 17–18 cm, were collected from local ponds around Varanasi, India. They were acclimatized to laboratory conditions for 2 weeks in glass aquaria containing chlorine-free tap water (pH 7.5, temperature 27 ± 2°C). Specimens were fed with minced goat liver *ad libitum* on each alternate day. The feeding was stopped 24 hr prior to experimentation. Carbaryl was obtained from Bharat Pulverishing Mills Pvt. Ltd., India. The technical and commercial preparations of carbaryl were separately dissolved in acetone, and stock solutions were prepared in volumetric flasks. The required volume of acetone (vehicle control) or stock solutions was pipetted and poured into glass aquaria of 60-liter capacity. The water in the tanks was changed at every 24 hr and fresh acetone (in control tanks) and carbaryl solution (in pesticide-treated tanks) were mixed into the water of the glass reservoirs. Dead fish were removed from the aquaria whenever they were observed.

The pilot experiment was conducted to select the concentrations of carbaryl for the experimental purposes. Renewal bioassay tests of carbaryl were performed at 24, 48, 72, and 96 hr for each experimental group of 20 fishes. There was no mortality in the control groups. Behavioral abnormalities were also observed in response to carbaryl treatment. Since the reduction in the concentration of carbaryl in water was not determined, the term $LC(I)_{50}$ (initial concentration of toxicant which is lethal to 50% of population) was used instead of LC_{50}. The trimmed Spearman–Karber method (Hamilton *et al.*, 1977) with 10% trimming was employed to calculate $LC(I)_{50}$ values and 95% confidence limits.

RESULTS

Table 1 illustrates the acute lethality of technical and commercial formulations of carbaryl exposed to *C. batrachus* for 24, 48, 72, and 96 hr. The $LC(I)_{50}$ values of commercial carbaryl (162.60, 134.08, 123.36, 107.66 mg/liter) showed a decreasing pattern with increasing length of exposure. $LC(I)_{50}$ values of the technical grade carbaryl (61.14, 53.65, 48.58, 46.85 mg/liter) also showed a similar trend. The data further indicate that the technical compound was approximately 2.5 times more toxic than the commercial preparation at all exposures (Table 1). The acute toxicity curve of the technical as well as the commercial carbaryl (Fig. 2) tended toward an asymptotic pattern within 96 hr of exposure. The time-dependent reductions in carbaryl concen-

TABLE 1

$LC(I)_{50}$ VALUES AND 95% CONFIDENCE LIMITS OF CARBARYL TO *Clarias batrachus*

Formulation	$LC(I)_{50}$ and 95% confidence limits (mg/L)	Duration of exposure (hr)			
		24	48	72	96
Technical	$LC(I)_{50}$	61.14	53.65	48.58	46.85
	Lower limit	58.28	49.04	44.95	40.96
	Upper limit	64.14	58.28	52.22	52.77
Commercial	$LC(I)_{50}$	162.60	134.08	123.36	107.66
	Lower limit	155.82	128.62	119.81	100.83
	Upper limit	169.68	139.54	126.96	114.49

trations required for acute lethality were gradual, which rendered the toxicity curve an asymptote (Fig. 2).

Fish exposed to technical or commercial formulations of carbaryl exhibited a number of behavioral anomalies including signs of restlessness, uneasy respiration, convulsion, erratic swimming, dashing against the walls of the water reservoirs, and sinking to the bottom before death. These abnormal signs were more prominent in response to technical carbaryl. In addition, dermal desquamation was observed as a characteristic and much pronounced change in the technical grade carbaryl-treated fish.

DISCUSSION

There are great differences in the $LC(I)_{50}$ values of technical and commercial formulations of carbaryl to *C. batrachus* at all exposures, i.e., 24, 48, 72, and 96 hr

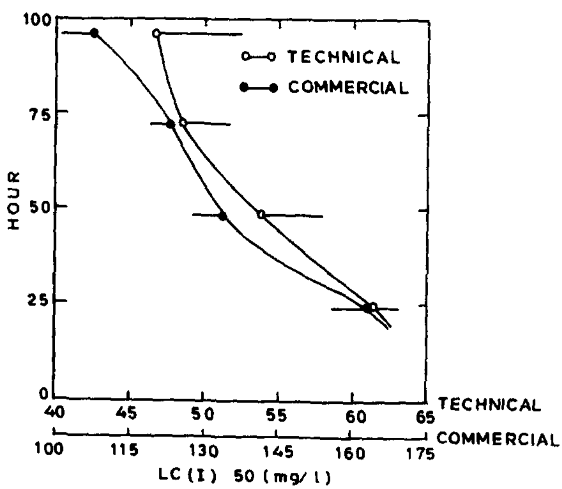

FIG. 2. Toxicity curve of carbaryl exposed to the freshwater catfish, *Clarias batrachus*, for 24, 48, 72, and 96 hr. Each point indicates an $LC(I)_{50}$ value (mg/liter) and its 95% confidence limits. (O) Technical grade carbaryl; (●) commercial carbaryl.

(Table 1 and Fig. 2). The estimated $LC(I)_{50}$ values are based on data from different groups of individuals and the given concentrations of carbaryl. Hence, these values do not reflect the true $LC(I)_{50}$ values which are the concentration that would be lethal to exactly 50% of the entire species tested. Therefore, the confidence limits were computed to each $LC(I)_{50}$ value so that the true $LC(I)_{50}$ would be somewhere within the specified range of confidence interval. The range of confidence limits for a time-dependent $LC(I)_{50}$ depends upon the distribution of deaths over the exposure concentrations and gradation of exposure concentrations, and is pertinent to results of an individual test only (Stephan, 1977). Both technical as well as commercial preparations of carbaryl showed a gradual reduction in $LC(I)_{50}$ values with increasing length of time from 24, to 48, 72, and 96 hr. This indicates a time-dependent decrease in acute lethality of carbaryl which may be largely due to the adaptability of the fish toward the toxicant. Further, the technical grade carbaryl was 2.5 times more toxic than the commercial formulation at all time intervals. This demonstrates the involvement of carbaryl, rather than the additive substances, as the active principle in acute toxicity. However, for some other pesticides, it has been reported that the commercial formulation is more toxic than its technical material (Haider and Imbaraj, 1986).

Recent reports on acute toxicity (96-hr LC_{50}) of carbaryl (Tilak *et al.*, 1980, 1981) to the freshwater fishes, *Labio rohita, Catla catla, Mystus vittatus, Mystus cavasius,* and *Anabas testutus,* were 4.6, 6.4, 2.4, 4.6, and 5.5 ppm, respectively. Comparatively, the $LC(I)_{50}$ values (96 hr) of technical grade (46.85 mg/liter), and commercial formulation (107.66 mg/liter), were much higher, indicating the lower sensitivity of carbaryl to *C. batrachus.* These differences might be due to species-specific potency of tolerance as well as to many other factors, including toxicant purity, bioassay method, individual and sample size, sex, and ecophysiological conditions. Further, the behavioral anomalies (restlessness, intense opercular movement, difficulty in respiration, erratic motion) were the cause of carbaryl toxicity leading toward uneasy survival in polluted water. More or less similar changes in behavior were reported from fishes exposed to different varieties of insecticides (Lunn *et al.*, 1976; Devi *et al.*, 1981, Singh *et al.*, 1984; Haider and Inbaraj, 1986).

CONCLUSIONS

The carbamates, in general, are absorbed by all portals including skin, and most of the aromatic carbamate insecticides have low dermal toxicity (Gupta, 1986). In contrast, carbaryl causes an intense dermal desquamation which might be due to loss of mucus secretion causing the body surface to be devoid of protective materials. Dermal desquamation was more prominent in response to technical grade carbaryl. It may be the result of the high purity of the technical grade compound compared to that of the commercial preparation. Though carbaryl is not believed to be very toxic to non-target species, the above-described toxic responses of both technical and commercial materials indicate the possibility of carbaryl being an hazardous aquatic compound.

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