Environmental Pollution (Series A) **34** (1984) 47–54

Relative Toxicity of Technical Grade and Formulated Carbaryl and 1-Naphthol to, and Carbaryl-Induced Biochemical Changes in, the Fish *Cirrhinus mrigala*

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ABSTRACT

In a study on the relative toxicity of technical grade material and formulations of carbaryl and its primary degradation product (1-naphthol), to the fish Cirrhinus mrigala, *1-naphthol was found to be the most toxic followed by technical grade carbaryl, 85% wettable powder and 50% WP; the calculated 96-h LC_{50} values (with the 95% confidence limits in parentheses) were, respectively, 1·46 (1·3 to 1·6), 2·5 (1·9 to 3·2), 5·7 (5·5 to 5·9) and 5·9 (5·7 to 6·3) mg litre^{-1}. In fish exposed to 2 mg litre^{-1} carbaryl for 96 h, the protein metabolism was significantly altered in the brain, gills, kidney and muscle, the glycogen content of the liver was significantly increased and the lipid content of the brain was significantly lowered.*

INTRODUCTION

Carbaryl (1-naphthyl-*N*-methylcarbamate; Sevin®, Union Carbide (India) Ltd) is a carbamate pesticide that has been used successfully in the control of over 150 major pests of a wide variety of crops (Mount & Oehme, 1981). It has been reported to have low persistence in soil (about 4 months; Caro *et al.*, 1974) and water (about 60 days in freshwater and more than 63 days in sea water; Odeyemi, 1982). Carbaryl has been shown to have low toxicity to fish, but its toxicity to aquatic insect larvae and crustaceans, which form a major part of the food of many important

Environ. Pollut. Ser. A. 0143-1471/84/$03·00

species of fish, is very high (Johnson & Finley, 1980). Further, there have been a few reports on the effect of sublethal concentrations of carbaryl on fish. Weis & Weis (1974) observed disruption of schooling behaviour of the silverside *Menidia menidia* exposed to 100 μg litre^{-1} of carbaryl. Normal schooling behaviour was not restored until 72 h after the fish were transferred to clean water, which, the authors suggested, may be attributable to the accumulation of 1-naphthol, the primary degradation product of carbaryl. Statham & Lech (1975) reported that simultaneous exposure of *Salmo salar* to sublethal concentrations of carbaryl increased the acute toxicity of several other toxicants. Weis & Weis (1976) noted optic and skeletal malformations in the eggs of the silverside *Menidia menidia* exposed to a 10 μg litre^{-1} concentration of carbaryl from early development stages through hatching. Arunachalam *et al.* (1980) considered carbaryl to be a metabolic stressor in the case of the freshwater fish *Mystus cavasius*.

In the aquatic environment, 1-naphthol has been reported to be the main degradation product of carbaryl (Karinen *et al.*, 1967). In the few instances when the toxicity of 1-naphthol was evaluated along with that of carbaryl, the former was found to be more toxic than the parent compound to fish and molluscs. For instance, Butler *et al.* (1968) reported that carbaryl was 1·4 times less toxic than 1-naphthol to the cockle clam *Clinocardium nuttalli*. Stewart *et al.* (1967) found that, on the basis of the 24-h LC_{50} values, carbaryl was 3·1, 1·6 and 2·1 times less toxic than 1-naphthol, respectively, to the shiner perch *Cymatogaster aggregata*, English sole *Parophrys vetulus* and threespine stickleback *Gasterosteus aculeatus*. Tilak *et al.* (1980) reported that when the 96-h LC_{50} values were compared, carbaryl was 1·8 times less toxic than 1-naphthol to the 1 to 2·5 cm size group and 2·5 times less toxic than 1-naphthol to the 4 to 6 cm size group of *Labeo rohita*. Tilak *et al.* (1981) also reported that 1-naphthol was more toxic than its parent compound to four species of freshwater fish.

In India, carbaryl is one of the most commonly employed insecticides for controlling the pests of cotton, tobacco, paddy and a number of other crops. The relative toxicity of technical grade carbaryl, its formulations and 1-naphthol to fish has not been evaluated and carbaryl induced biochemical changes in fish are little known. We are reporting the relative toxicity of technical grade material, 1-naphthol and two formulations—i.e. carbaryl 85 % wettable powder (WP) and carbaryl 50 % WP—to the freshwater fish *Cirrhinus mrigala* (Ham.). The effect of the technical grade carbaryl on the total protein, glycogen and lipid content of some selected tissues of *C. mrigala* is also reported.

MATERIALS AND METHODS

Toxicity studies

The fish, *C. mrigala* (4 to 6 cm in standard length and 11 g in average weight), were caught in the Guntur channel, near Nagarjuna University campus (Guntur district, South India). The fish were acclimatised to the laboratory conditions at $28 \pm 2\,°C$. Preliminary analysis of the tissues of randomly chosen fish showed that there were no detectable residues in the tissues (limits of detection, 0·1 mg litre^{-1}). Toxicity tests were conducted employing the continuous flow systems as recommended by the committee on *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians* (Anon., 1975) to calculate the 96-h LC_{50} values. Chemical characteristics of the test water and other conditions of test were the same as described elsewhere (Ananda Swarup *et al.*, 1981). Unchlorinated water drawn from a deep well in the Nagarjuna University campus was used for acclimatization as well as for conducting the tests. The fish were not fed during the period of acclimatization and tests. Any batch of fish in which the mortality exceeded 5 % during the period of acclimatization was discarded.

Technical grade carbaryl, carbaryl 85 % WP and carbaryl 50 % WP were kindly supplied by Union Carbide Corporation, India. Analytical grade 1-naphthol was purchased from BDH Chemicals, India. Stock solutions of the toxicants (100 mg m^{-1} and 10 mg ml^{-1}) were prepared in acetone. Desired concentrations of the toxicants (based on active ingredient in the case of formulations) were added to water taken in large glass reservoirs (24–30 litres capacity) and the toxicant containing water was let into the test chambers at a rate of 4 litre h^{-1}. Acetone was added to the controls at the same concentration as that present in the highest concentration of the toxicant used and the maximum concentration of acetone in test and control tanks was less than 0·1 ml litre^{-1}.

Pilot experiments were conducted to choose concentrations that resulted in mortality in the range of 10 to 90 % to suit the method of calculation of the 96-h LC_{50} value (unweighted regression method of probit analysis, Finney, 1971). The chi-square test was employed to test the difference between the observed and calculated values for significance. Ten fish were exposed to each concentration and the experiments were repeated three times. Since the observed mortality in any concentration was the same in each attempt, data for each concentration were pooled to calculate the 96-h LC_{50}.

Biochemical studies

The fish that survived 96 hours' exposure to a 2 mg litre^{-1} concentration of technical grade carbaryl in the above experiments and control fish were used to estimate the total protein, glycogen and total lipid contents of some selected tissues. The fish were quickly killed by decapitation and the tissues were removed immediately and lightly dried between the folds of a filter paper. Tissues were weighed and frozen. The total protein and glycogen content of brain, gills, kidney, liver and muscle, and the lipid content of brain, liver and muscle were estimated by methods already described (Murty & Priyamvada Devi, 1982). Analyses of gills, liver and muscle (always taken from the base of the dorsal fin, on the left side), were made on tissues collected from individual fish, whereas, in the case of kidney and brain, tissues from several fish were pooled to yield enough material for analyses (25 mg for protein, 50 to 100 mg for glycogen and 1 g for lipid). Bovine serum albumin, purchased from Sigma Chemical Co., USA, was used to prepare the protein standards

RESULTS AND DISCUSSION

Toxicity studies

The calculated 96-h LC_{50} values (with the 95% confidence limits in parentheses) of technical grade carbaryl, 1-naphthol, carbaryl 85% WP and carbaryl 50% WP were, respectively, 2·5 (1·9 to 3·2), 1·46 (1·3 to 1·6), 5·7 (5·5 to 5·9) and 5·9 (5·7 to 6·3) mg litre^{-1} (Fig. 1). The calculated χ-values showed that there was no significant difference between the observed and the calculated mortality values. The regression equations for calculating the 96-h LC_{50} values were $y = 3{\cdot}58x - 3{\cdot}58$ for technical grade carbaryl, $y = 17{\cdot}7x - 33{\cdot}3$ for 1-naphthol, $y = 23{\cdot}95x - 61$ for carbaryl 85% WP and $y = 17{\cdot}6x - 43{\cdot}8$ for carbaryl 50% WP, where y is

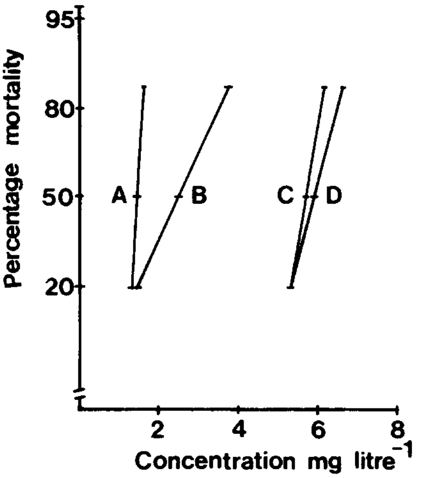

Fig. 1. Dose mortality regression lines of A, 1-naphthol, B, technical grade carbaryl, C, carbaryl 85% wettable powder (WP) and D, carbaryl 50% WP, with *Cirrhinus mrigala*.

the probit value of the observed percentage mortality and x is the log of dose times 100.

Present work showed that both 1-naphthol and carbaryl are moderately toxic to the fish *C. mrigala*. The relative toxicity of the various components tested was in the following decreasing order: 1-naphthol, technical carbaryl, 85% WP formulation and 50% WP formulation. It is evident that the inert material present in the formulations is responsible for the decreased toxicity of the formulated products. Earlier, Woodward & Mauck (1980), in their studies on the toxicity of technical grade material and field formulation (49%) of carbaryl to the cutthroat trout *Salmo clarki*, found that the formulation was less toxic than the technical grade material. Further, the present finding that 1-naphthol is more toxic than the parent compound to fish is in accordance with the few earlier reports on the toxicity of the degradation product (Stewart *et al.*, 1967; Butler *et al.*, 1968; Tilak *et al.*, 1980, 1981) and necessitates a re-examination of the long-held belief that the metabolite of carbaryl is not toxic to the non-target organisms.

Biochemical studies

The total protein content showed a significant decrease in the brain, gills and kidney and a significant increase in the muscle of the test fish

TABLE 1
Changes Induced in the Glycogen, Protein and Lipid Contents ($mg\,g^{-1}$ Wet Weight) of Some Selected Tissues of *Cirrhinus mrigala*, Exposed to $2\,mg\,litre^{-1}$ Carbaryl[a]

Tissue	*Glycogen*		*Protein*		*Lipid*	
	Control	*Test*	*Control*	*Test*	*Control*	*Test*
Brain	$7{\cdot}2 \pm 3{\cdot}1$	$7{\cdot}7 \pm 2{\cdot}4$	$98{\cdot}7 \pm 1{\cdot}9$	$93{\cdot}3 \pm 1{\cdot}9$*	$155{\cdot}6 \pm 5{\cdot}2$	$67{\cdot}2 \pm 1$*
Gills	$15{\cdot}8 \pm 6{\cdot}4$	$13{\cdot}5 \pm 5{\cdot}8$	$102{\cdot}6 \pm 5$	$76 \pm 1{\cdot}6$*	Not investigated	
Kidney	$16{\cdot}3 \pm 3{\cdot}7$	$14{\cdot}3 \pm 2{\cdot}3$	$168 \pm 3{\cdot}3$	$126{\cdot}6 \pm 5$*	Not investigated	
Liver	$69{\cdot}5 \pm 6{\cdot}8$	$92{\cdot}7 \pm 7{\cdot}9$*	$113{\cdot}3 \pm 5$	$109{\cdot}3 \pm 3{\cdot}8$	$29 \pm 1{\cdot}4$	$27{\cdot}1 \pm 2{\cdot}2$
Muscle	$8{\cdot}5 \pm 2{\cdot}7$	$9{\cdot}6 \pm 5{\cdot}8$	$138{\cdot}6 \pm 5$	$156 \pm 3{\cdot}3$*	$18{\cdot}9 \pm 1$	$20{\cdot}2 \pm 2$

[a] Each value is the mean of three replicates, with the standard deviation indicated.
* Change significant at $p = 0{\cdot}05$.

(Table 1). These observations indicate severe disturbances in the protein metabolism of the fish exposed to carbaryl. The glycogen content of liver of test fish showed a significant increase. The total lipid content of brain was significantly lowered. It is surprising to note that carbaryl, like the non-polar organochlorines, is capable of causing disturbances in the lipid metabolism.

The long-range effects of the chronic exposure of fish to carbaryl and 1-naphthol are largely unknown. The present study emphasises the need for a thorough study of the biochemical changes induced by sublethal doses of carbamate pesticides in fish.

In conclusion, it may be reiterated that the greater toxicity of 1-naphthol than carbaryl to the fish *Cirrhinus mrigala*, observed in the present study, confirms the earlier reports that the primary degradation product is more toxic to fish than the parent compound. Carbaryl is known to be highly toxic to aquatic invertebrates. The toxicity of 1-naphthol to the aquatic invertebrates and many more species of fish remains to be investigated.

ACKNOWLEDGEMENTS

The authors thank Professor K. V. Jagannadha Rao of the Chemistry Department of Nagarjuna University for his many helpful suggestions, the authorities of the Council of Scientific and Industrial Research, New

Delhi, for financial assistance and the Union Carbide Corporation of India for providing the technical material and the formulations of carbaryl.

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