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Acute Toxicity of Dichlorvos (DDVP) to Fingerling Mirror Carp, *Cyprinus carpio* L.

M.-Ş. Ural,[1] M. Çalta[2]

[1] Professional School of Keban, Firat University, 23700 Elazig, Turkey
[2] Faculty of Fisheries, Firat University, 23119 Elazig, Turkey

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The increasing use of synthetic chemical pesticides is causing worldwide pollution. Although pesticides by their nature are toxic and are designed to kill unwanted organisms, they, when applied to the land, may be washed into surface waters and kill or at least adversely influence the life of aquatic organisms (Hayes and Laws 1991; Rand 1995). Because of high acute toxicity and the consequent dangers to workers, there are concerns whether safe use of pesticides is possible under such conditions. However, dichlorvos (DDVP) still is widely used in developing countries such as Turkey to control of pests.

The environmental fate and effects of DDVP have been well-summarized (EPA 2000). DDVP is generally found to be toxic to phytoplankton (EPA 2000; Raine et al. 1990), zooplankton (EPA 2000; Sanchez-Fortun et al. 1995), molluscs (Jonnalagadda and Rao 1996; Tripathi and Agarwal 1998), crustaceans (Geraldine et al. 1999; McHenery et al. 1996) and fish (Chuiko and Slynko 1995; Dutt and Guha 1988; Eisler 1970; EPA 2000; Mayer and Ellersieck 1986; Verma et al. 1981). The effects of DDVP on reproduction (Benarji and Rajebdranath 1991; Saksena and Saxena 1999), enzyme activity (Golovanova et al. 1994; Pavlov et al. 1992), respiration (Mohapatra and Noble 1993), genetics (Rishi and Grewal 1995), feeding behavior (Pavlov et al. 1992), general biochemistry (Saxena and Saksena 1996) and hematological systems (Rajeswari et al. 1989) of fishes were also reported.

In Turkey, larvae of some commercial warm-water fish species, such as carp are artificially reared and then introduced to freshwater reservoirs when they have reached the fingerling stage (approx. 5-10 cm in length). At the same time, dichlorvos (0,0 dimethyl-2,2-dichlorovinyl phosphate; DDVP) is a commonly used pesticide for pest control in the agricultural fields around these freshwater reservoirs. Therefore, the present study has aimed to determine the acute toxicity of DDVP on fingerling mirror carp at the size attained just before introduced into reservoirs. Our findings would be a useful tool for the effective management and control of regional reservoirs with respect to the imput of DDVP from agricultural areas.

Correspondence to: M. Çalta

MATERIALS AND METHODS

In this study, a static acute toxicity bioassay was performed according to standard methods (APHA 1999) to determine the 24, 48, 72 and 96 h lethal concentrations of dichlorvos (0,0-dimethyl-2,2-dichlorovinyl phosphate; DDVP) for fingerling mirror carp. Experimental animals from 3.5-4.01 (3.72±0.20) g and 6.4-7.1 (6.8±0.3) cm in length, were obtained from Keban Fish Breeding Unit of DSI Ninth Region Directorate, Elazig, Turkey. They were brought to the laboratory at Keban Professional School and acclimatized for 4 days under standard laboratory conditions. Fishes were fed with flour pellets during adaptation. However, they were not fed during the last 24 h of adaptation and throughout the duration of the test. Before the starting of the test, all experimental aquaria (60 L capacity) were cleaned and filled with 50 L of dechlorinated tap water (temperature: 20.6 °C±0.3, pH: 8.45±0.03, dissolved oxygen: 7.17±0.25 mg L^{-1}, electrical conductivity: 232.25±10.54 µS cm^{-1}, alkalinity: 142-168 mg L^{-1} and hardness: 186-240 mg L^{-1} as $CaCO_3$). Five different concentrations (4, 8, 16, 32, 64 µg L^{-1}) of DDVP, with three replicates, were used in this test. Control units with three replicates were also prepared. Aeration was applied to the aquaria for 2 h in order to obtain a homogeneous concentration of the toxic compound, and then 10 fish were transferred in each test aquarium. Mortality was assessed at 24, 48, 72 and 96 h after the start of the tests. Dead animals were removed immediately. Behavioral changes of test animals were closely followed and recorded. Toxicity was assigned based on LC_{50}, according to the guidelines given by Kamrin (1997). DDVP is classified as slightly toxic to moderately toxic for fish, with an LC_{50} >1 mg L^{-1}.

The results were expressed as mean±standard deviation (SD) or range. All replicates were used for calculation of mean values. Statistical analysis was performed with the SPSS statistical software package (Version 10.1, SPSS Inc., Chicago, Illinois, USA). The LC_{50} and 95% confidence limits were calculated by the probit method. Duncan's multiple range tests was employed for comparing the significance level for time using a significance level of 0.05.

RESULTS AND DISCUSSION

The behavioral responses of the test animals were observed at 1-10 h during the first day of exposure and then every 12 h during the last three days of exposure. Normal behavior was observed for control animals and those at 4 mg L^{-1} concentration. Initial changes in behavioral response were observed 1 h after exposure in the highest DDVP concentrations (64 mg L^{-1}). Animals exposed to 8 and 16 mg L^{-1} concentrations showed normal behavior until the end of the first day of exposure. However, afterward they showed some abnormal behavior such as reduced general activity and loss of equilibrium when compared with the control animals. The two highest concentrations (32 and 64 mg L^{-1}) showed all behavioral responses at high levels and the onset was within the first 1 h after starting the test. Abnormal behavioral responses observed at all concentrations above 16 mg L^{-1} were rapid gills movement, erratic swimming, swimming at the

water surface, air gulping at the water surface and staying motionless on the aquarium bottom later in exposure.

The calculated 24, 48, 72 and 96h LC_{50} values (95% confidence limits) for DDVP, using a static bioassay system to fingerling mirror carp were 84.99 (69.89-138.95), 67.86 (48.67-127.75), 26.18 (20.44-35.06) and 9.41 (7.54-11.49) mg L^{-1} respectively (Table 1). The results show that DDVP was slightly toxic at 24, 48 and 72 h and moderately toxic at 96 h to fingerling mirror carp. The toxicity of this compound on experiment animals increased with increasing concentration and exposure time. There were significant differences between LC_{50} values calculated for 24, 48, 72 and 96 hours ($p<0.05$). All animals exposed to 64 mg L^{-1} of DDVP were dead after 96 h of exposure (Table 1). The percentage of living animals in 32 mg L^{-1} concentration was only 10% at the end of the test. It was calculated that when the concentration of DDVP increased approximately 5.6 times, the percentage of dead animals increased by 50 %. The regression equation $y = -0.309 + 0.381\text{Log}(x)$ with a correlation coefficient of 0.976 was obtained by probit method. Where; x and y denote DDVP concentration and the corresponding probit value, respectively. This equation can provide to calculate predicted toxicity at any other concentrations of DDVP. The calculated safe concentration of DDVP for fingerling mirror carp was 94.1 μg L^{-1} (i.e., 1/100 of LC_{50} value).

Table 1. Cumulative mortality and LC_{50} values with 95% confidence limits (in parentheses) of DDVP for fingerling mirror carp.

DDVP concentrations (mg L^{-1})	No. of fish Exposed	Exposure time (hours)			
		24	48	72	96
64	30	6	15	24	30
32	30	1	6	17	27
16	30	0	2	9	24
8	30	0	1	6	15
4	30	0	0	0	2
0	30	0	0	0	0
LC_{50} values, mg L^{-1}		84.99^{a}	67.86^{b}	26.18^{c}	9.41^{d}
(95 % confidence limits)		(69.89-138.95)	(48.67-127.75)	(20.44-35.06)	(7.54-11.49)

LC_{50} values in row with different letters significantly differ ($p<0.05$)

Some pesticides used in agriculture are known to be toxic to non-target aquatic biota; DDVP belongs to this group. The toxic effects of DDVP to various fish species are summarized (Table 2). In general, the toxic effect of DDVP changes with respect to species and size of animals and the duration of exposure. The results of the present study showed that the toxicity of DDVP to fingerling mirror carp under the conditions used, was also time dependent.

Table 2. Toxicity studies for DDVP on various fish species.

Scientific Name	Fish Size	Exposure Time	LC_{50}, mg L^{-1}	Chemical Description	Experiment Type	Acute Toxicity Rating*	References
Abramis brama	NR	24 h	33.05	97.5 % AI, DDVP	Renewal	ST	Chuiko and Slynko 1995
		48 h	26.18				
		72 h	21.11				
		96 h	16.66				
Anguilla rostrata	5.9cm 0.14g	24 h	2.3	NR	Static	MT	Eisler 1970
		48 h	2.3				
		96 h	1.8				
Ophiocephalus punctata	9-11cm 40-55g	24 h	4.3	EC 76 %	Static	MT	Verma et al. 1981
		48 h	3.4				
		72 h	2.1				
		96 h	2.3				
Cyprinus carpio	4.5-5.5cm 1.1-1.4g	24 h	9.44	DDVP	Static	MT	Dutt and Guha 1988
		48 h	8.99				
		72 h	8.21				
Fundulus heteroclitus	5.5cm 1.7g	24 h	3.41	NR	Static	MT	Eisler 1970
		48 h	2.68				
		96 h	2.68				
Fundulus heteroclitus	4.0g	96 h	3.2	NR	Static	MT	EPA 2000
Fundulus majalis	0.9g	96 h	2.3			MT	
Gambusia affinis	0.2g	24 h	17.8	Technical Material	Static	ST	Mayer and Ellersieck 1986
		96 h	5.27			MT	
Lepomis macrochirus	1.5g	24 h	3.08	Technical Material	Static	MT	
		96 h	0.87			HT	
Lepomis macrochirus	0.09g	96 h	4.30	NR	Flow	MT	EPA 2000
	0.4g	96 h	0.18	NR	Static	HT	
	0.8g	96 h	0.80	NR	Static	HT	
Menidia menidia	2.1g	24 h	9.60	NR	Static	MT	EPA 2000
Mugil cephalus	8.4cm 6.4g	24 h	0.74	NR	Static	HT	Eisler 1970
		48 h	0.66			HT	
		96 h	0.20			HT	
	4.6cm 1.0g	24 h	1.75			MT	
		48 h	1.25			MT	
		96 h	0.25			HT	
Mystus vittatus	8-10cm 6-10g	24 h	0.73	EC 76%	Static	HT	Verma et al. 1981
		48 h	0.65			HT	
		72 h	0.51			HT	
		96 h	0.45			HT	
Oncorhynchus clarki	0.17g	96 h	0.213	NR	Static	HT	EPA 2000
Oncorhynchus mykiss	2.3g	96 h	0.100	NR	Static	HT	EPA 2000

*Narrative descriptions of toxicity were assigned based on LC_{50} according to the guidelines in Kamrin (1997). ST, Slightly Toxic (LC_{50} = 10-100 mg L^{-1}); MT, Moderately Toxic (LC_{50} =1-10 mg L^{-1}); HT, Highly Toxic (LC_{50} = 0.1-1 mg L^{-1}); VHT, Very Highly Toxic (LC_{50} < 0.1 mg L^{-1}); NR, Not Reported; EC, Effective Concentration; AI, Active Ingredient

In laboratory tests, the USDA National Agricultural Pesticide Impact Assessment Program's EXTOXNET document reports dichlorvos acute toxicity to fish at 96 h of 11.6 mg L^{-1} in fathead minnow, 0.9 mg L^{-1} in bluegill, 5.3 mg L^{-1} in mosquito fish and 1.8 mg L^{-1} in American eels. Pesticide Action Network (PAN) pesticides database also reports DDVP average acute toxicity for fish at 96 h of 1-10 mg L^{-1}. Our results are in good agreement with these two reports. Similar ranges of DDVP LC_{50} values for various fish species were also reported in some studies (Table 2).

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