

Acute Toxicity of Dichlorvos on Fingerling European Catfish, *Silurus glanis*

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The widespread use of synthetic pesticides can lead to the exposure of manufacturing workers, field applicators, the ecosystem, and the public to the possible toxic effects of these pesticides. Pesticides applied to the land may be washed into surface waters and may kill or at least adversely influence the life of aquatic organisms (Bradbury and Coats 1989; Hayes and Laws 1991; Köprücü and Aydın 2004).

Dichlorvos (DDVP), an organophosphorus pesticide, is toxic to fish (Verma et al. 1982; Devillers et al. 1985; Dutt and Guha 1988; Yokoyama et al. 1988; Benarji and Rajendranath 1990; McHenery et al. 1991; Chuiko and Slynko 1995; Office of Pesticide Programs 2000) and aquatic arthropods are more sensitive than fish (URL 1). It is highly toxic to birds and to honey bees. Environmental quality standards have been proposed in the UK for the protection of freshwater and marine aquatic life. In setting the standards for dichlorvos, the Department of the Environment noted that insufficient environmental data are available to verify the proposed standards for the protection of aquatic life. However, by applying an arbitrary safety factor of 100 to the toxic dose for the most sensitive species, the Water Research Centre has recommended an annual average level of $0.001 \mu\text{g L}^{-1}$ for freshwater species, and $0.04 \mu\text{g L}^{-1}$ for marine life in saline waters (URL 1).

The European catfish, *Silurus glanis*, is widespread and presently cultured in ponds all over Asia, and central and Eastern Europe, in association with carp, for more than 100 years. The total production of European catfish in aquaculture from ten European countries was about 2000 tons in 2002. Production is increasing from intensive pond culture or in heated/geothermal waters (URL 2; Linhart et al. 2002).

In Turkey, larvae of European catfish are artificially reared and then introduced to their natural freshwater reservoirs when they have reached the fingerling stage. At the same time, dichlorvos 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 dichlorvos on fingerling European catfish at the size attained just before introduction into reservoirs. Finally, our findings would be a useful tool for the control of regional reservoirs and their

effective management with respect to the input of dichlorvos from agricultural areas.

MATERIALS AND METHODS

In this study, a static acute toxicity bioassay was performed according to the standard method (APHA 1999) to determine the 1, 12, 24, 48, 72 and 96 h LC₅₀ values of dichlorvos (purity 55%, dissolved in 45% acetone), 0,0 dimethyl-2,2-dichloro-vinylphosphate, (Didifos 55 EC) for fingerling European catfish.

Fish mean weighing 13 ± 2 g and having mean length of 15 ± 1 cm were obtained from the Keban Fish Breeding Unit of IX Region Directorate of the State Hydraulic Works in Turkey. They were brought to the laboratory and acclimatized to laboratory conditions for 7 days. Water temperature in the aquaria was maintained at 17 ± 1 °C using a heater and the fish were subjected to a 12 h photoperiod using fluorescent lights. Fishes were fed with pellet feeds (Ecobio Inc. trout grower feed) during acclimating, but they were not fed during the last 24 h before starting of the test and throughout the test. Before starting the test period, all experimental aquaria with 280 L capacity were cleaned and filled with 270 L of dechlorinated tap water. The experimental water was kept in the tank for 24 h before dichlorvos was added.

Water quality characteristics in the control units were determined according to APHA (1999). Dissolved oxygen, pH and conductivity were determined by a digital oxygen meter and a pH meter. The mean quality parameters of water used for preparation of test solutions were: temperature 17 ± 1 °C, pH 8.3 ± 0.1 , dissolved oxygen 6.9 ± 0.2 mg L⁻¹, electrical conductivity 225 ± 7.6 µS cm⁻¹, alkalinity 145 ± 19 mg L⁻¹ and total hardness 190.2 ± 11 mg L⁻¹ as CaCO₃.

Dichlorvos was prepared from a stock solution weighed in a glass boat and transferred to a volumetric flask containing experimental water. Dilutions of the defined stock solution were used for tests described below. Two control groups received acetone at a concentration used in the dilution of the maximum dichlorvos concentration.

Seven different concentrations of dichlorvos (8, 16, 24, 32, 40, 48 and 56 mg L⁻¹) and a control, with five replicates were used in the test series. Exceeding aeration was applied to the aquarium for 2 h in order to obtain a homogeneous concentration of the toxic compound, and then 20 fish were transferred into each aquarium. Mortality was assessed at 1, 24, 48, 72 and 96 h after the start and dead fishes were removed immediately. Behavioral changes of test animals were closely followed and recorded. Dichlorvos is classified as slightly toxic to moderately toxic for fish, with an LC₅₀ > 1 mg L⁻¹.

Statistical analyses were performed with the SPSS 10.1 computer program (SPSS Inc. Chicago, Illinois, USA). Data obtained from the dichlorvos acute toxicity tests were evaluated using probity analysis. The LC₅₀ and 95% confidence limits were calculated. All replicates were used for calculation of mean values. The chi-

square test was employed for comparing mean mortality values using a significance level of 0.05.

RESULTS AND DISCUSSION

The results show that the toxicity of dichlorvos on fingerling European catfish, *Silurus glanis* increased with increasing concentration and exposure time. For example, when fish were exposed to 16 mg L⁻¹ dichlorvos, only 49% died at 96 h whereas 95% died at 1 h when exposed to the 56 mg L⁻¹ concentration. In addition, the 1, 24, 48, 72 and 96 h LC₅₀ values of dichlorvos, using a static bioassay system to fingerling European catfish were determined as 33.27 (25.11-37.20), 29.45 (24.96-32.07), 25.24 (22.72-27.06), 18.85 (16.61-20.63) and 16.67 (no data because of $p > 0.05$) mg L⁻¹, respectively. There were significant differences in LC₅₀ values obtained at different times of exposure ($p < 0.05$, Table 1). The regression equation $y = -0.878 + 1.126\text{Log}(x)$ with a correlation coefficient of 0.954 was obtained by the probity method, with x and y denoting dichlorvos concentration and the corresponding probity value, respectively. This equation can be used to calculate predicted toxicity at any other concentration of dichlorvos.

There are differences in the acute toxicity of dichlorvos for various fish species. McHenery et al. (1991) reported a 96h LC₅₀ value of dichlorvos for Atlantic herring, *Clupea harengus* larvae as 0.12 mg L⁻¹ (highly toxic). Verma et al. (1981) determined 24, 48, 72 and 96 h LC₅₀ values for fry striped catfish, *Mystus vittatus* as 0.73, 0.65, 0.51 and 0.45 mg L⁻¹ (highly toxic), respectively. Chuiko and Slynko (1995) estimated 24, 48, 72 and 96 h LC₅₀ values of dichlorvos for bream, *Abramis brama*, as 33.05, 26.18, 21.11 and 16.66 mg L⁻¹ (slightly toxic), respectively. Yokoyama et al. (1988) found 24 and 48 h LC₅₀ values for Japanese eel, *Anguilla japonica*, as 11.0 (slightly toxic) and 1.5 mg L⁻¹ (moderately toxic), respectively. Benarji and Rajendranath (1990) and Verma et al. (1983) estimated 48 and 96 h LC₅₀ values of dichlorvos for walking catfish, *Clarias batrachus*, as 8.88 and 4.40 mg L⁻¹ (moderately toxic), respectively. The Office of Pesticide Programs (2000) reported 24 h LC₅₀ values for guppy, *Poecilia reticulata*, as 5.81 mg L⁻¹ (moderately toxic) and for fry Atlantic silverside, *Menidia menidia*, as 9.60 mg L⁻¹ (moderately toxic), 96 h LC₅₀ values for fry rainbow trout, *Oncorhynchus mykiss*, as 0.10 mg L⁻¹ (highly toxic), for sheepsheep minnow, *Cyprinodon variegatus*, as 3.2 mg L⁻¹ (slightly toxic) and for mummichog, *Fundulus heteroclitus*, as 14.40 mg L⁻¹ (slightly toxic). Dutt and Guha (1988) determined 24, 48 and 72 h LC₅₀ values for fingerling common carp, *Cyprinus carpio*, as 10.23 (slightly toxic), 8.99 and 8.21 mg L⁻¹ (moderately toxic), and for fingerling Mozambique tilapia, *Tilapia mossambica*, as 16.82, 16.03 and 15.57 mg L⁻¹ (slightly toxic), respectively. Verma et al. (1982) estimated 24, 48, 72 and 96 h LC₅₀ values for Indian catfish, *Heteropneustes fossilis*, as 8.13, 7.66, 7.24 and 6.61 mg L⁻¹ (moderately toxic), respectively. Devillers et al. (1985) found 24 h LC₅₀ value for zebrafish, *Danio rerio*, as 35 mg L⁻¹ (slightly toxic). Perschbacher and Sarkar (1989) reported 24 h LC₅₀ value for snake-head catfish, *Channa punctata*, as 6.00 mg L⁻¹ (moderately toxic). Our LC₅₀ values were similar with the reports of Chuiko and Slynko (1995) for bream, Dutt and Guha (1988) for

Table 1. Cumulative mortality of fingerling European catfish (n = 100 in five replicates).

Concentrations (mg L ⁻¹)	Number of dead fish				
	1 h	24 h	48 h	72 h	96 h
Control	-	-	-	-	-
8	-	-	-	-	-
16	-	-	-	41	49
24	-	-	45	61	71
32	-	61	68	85	92
40	72	76	90	ND	
48	80	93	ND		
56	95	ND			
Chi-Square value	1.56	1.64	1.68	2.02	2.12
p	<0.05	<0.05	<0.05	<0.05	<0.05
LC ₅₀ values	33.27 ^a	29.45 ^b	25.24 ^c	18.85 ^d	16.67 ^e
(95 % confidence limits)	(25.11-37.20)	(24.96-32.07)	(22.72-27.06)	(16.61-20.63)	(*)

LC₅₀ values with different letters significantly differ (p<0.05).

ND: No data because of 100% mortality.

(*): No data because of p>0.05

(-): Not dead.

Mozambique tilapia fingerling, Office of Pesticide Programs (2000) for mummichog, and Devillers et al. (1985) for zebrafish as stated above.

Some pesticides used in agriculture are known to be toxic to non-target aquatic biota (Ward et al. 1995), and dichlorvos belongs to this group. In general, this toxic effect changes with respect to species and size of fish and the duration of exposure. The results of the present study show that toxicity of dichlorvos for fingerling European catfish under such conditions is also time-dependent.

In the present study, behavioral responses of the test fish were observed at 1-12 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 fish and fish at 8 mg L⁻¹. Fingerling fish exposed to 16 mgL⁻¹ of dichlorvos also showed normal behavior during exposure, but afterwards some abnormalities were observed, such as less general activity and loss of equilibrium, when compared with the control fish. Initial changes in behavior were observed 30 min after exposure to the five highest dichlorvos concentrations (24, 32, 40, 48 and 56 mg L⁻¹).

The abnormal behavioral responses observed at all concentrations higher than 8 mg L⁻¹ were loss of equilibrium, hanging vertically in the water, erratic swimming, swimming at the water surface, rapid gill movement, air gulping from the water surface, or staying motionless on the aquarium bottom. In addition to

these, it was observed that there was lightening in skin color of the fingerling European catfish.

Similar behavioral responses determined in this study have been observed on guppy (Viran et al. 2003), freshwater catfish, *Heteropneustes fossilis* (Saha and Kaviraj 2003) and young mirror carp, *Cyprinus carpio* (Calta and Ural 2004) exposed to various concentrations of the synthetic pyrethroids cypermethrin and deltamethrin.

For this study, it was concluded that dichlorvos is a slightly toxic pesticide to fish. The adverse effect was dependent on concentration and duration of exposure. Dichlorvos contamination is dangerous to the aquatic ecosystem and this should be taken into consideration when this insecticide is used in agriculture or in the control of mosquito populations.

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