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The Comparative Study on the Acute Toxicity of Dichlorvos on Guppy (*Poecilia reticulata*, P., 1859) and Carp (*Cyprinus carpio*, L., 1758)

Dichlorvos'un Lepistes (*Poecilia reticulata*, P., 1859) ve Sazan (*Cyprinus carpio*, L.,1758) Üzerine Akut Toksik Etkisinin Karşılaştırılması

Research Article

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ABSTRACT

Dichlorvos, which is extensively used organophosphate insecticide, was investigated in the present study for acute toxicity. Guppy *(Poecilia reticulata)* and carp (*Cyprinus carpio)* were selected for the bioassay experiments. The experiments were repeated 3 times and 180 guppy and 180 carp were used. Behavioral changes of fishes at each dichlorvos concentrations were observed for the individual fish. Lethal concentration of the insecticide on two fish species were determined using the Finney Probit Analysis Statistical Method. The 96-h LC_{50} values for guppy and carp were estimated as 1.84 mg/L and 2.51 mg/L respectively.

Key Words
Dichlorvos, *Poecilia reticulata, Cyprinus carpio,* acute toxicity

ÖZET

Yaygın kullanıma sahip bir organofosfat pestisit olan dichlorvos, bu çalışma kapsamında akut toksik etkisi bakımından incelenmiştir. Deneylerde lepistes (*Poecilia reticulata*) ve sazan (*Cyprinus carpio*) balıkları kullanılmıştır. Deneyler, 3'er tekrarlı olarak yapılmış ve 180 adet lepistes, 180 adet sazan balığı kullanılmıştır. Her bir dichlorvos konsantrasyonu için, balıklarda meydana gelen davranışsal değişiklikler kaydedilmiştir. İnsektisitin her iki balık türü için letal konsantrasyon değeri Finney Probit Analizi istatistiksel metodu kullanılarak tayin edilmiştir. Dichlorvos'un 96 saatlik LC_{50} değeri lepistes ve sazan balıkları için sırasıyla; 1.84 mg/L ve 2.51 mg/L olarak bulunmuştur.

Anahtar Kelimeler
Dichlorvos, *Poecilia reticulata, Cyprinus carpio*, akut toksisite

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INTRODUCTION

Insecticides are used commonly in agriculture and public health for the warfare of harmful insects. Insecticides cause to the death of the target insects within a few hours by inflicting irrevocable damage on the peripheral and central nervous systems [1, 2].

Pollutants can react with each other and develop new forms when entering the water, and they have undesirable toxic effects on living organisms, i.e., fish and the arthropods [3].

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) or DDVP is an organophosphate compound used to control household, public health, and stored product insects. Organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Their ability to degrade made them an attractive alternative to the persistent organochloride pesticides, such as DDT and aldrin. Although organophosphates degrade faster than the organochlorides, they have greater acute toxicity, poisoning risks to people who may be exposed to large amounts [4].

The EPA has classified dichlorvos as toxicity class I - highly toxic, because it may cause cancer and there is only a small margin of safety for other effects. Products containing dichlorvos must bear the Signal Words Danger-Poison. Dichlorvos is a Restricted Use Pesticide (RUP) and may be purchased and used only by certified applicators [4].

Dichlorvos has a high acute toxicity: the oral LD_{50} values in rats are between 56 and 108 mg/kg. It is classified by the WHO as a Class 1 B, 'highly hazardous. The dermal toxicity is similar to oral toxicity, and dermal exposure is a cause for concern. Cholinesterase inhibition has been reported from exposure by inhalation after the use of dichlorvos in non-ventilated or poorly ventilated areas [4].

There is evidence that dichlorvos can induce delayed neuropathy in hens at very high doses; and neurophysiological and behavioural changes in rats [5]. No study is considered adequate to assess the teratogenic potential of dichlorvos in any species [6]. IARC (The International Agency for Research on Cancer) places dichlorvos in Group 2B (possibly carcinogenic to humans) based on what it considers to be sufficient evidence in animals, but inadequate evidence in humans. The US Environmental Protection Agency classifies it in category 2 B (possibly carcinogenic to humans) but the result of further testing is awaited and it may be reclassified. There is evidence that dichlorvos is mutagenic in bacteria, fungi, and mammalian cells in vitro, but that there is no evidence for mutagenicity in whole animals, when it is rapidly degraded [7].

Dichlorvos is toxic to fish, but aquatic arthropods are more sensitive than fish. The Water Research Centre has recommended an annual average level of 0.001 μg/L for freshwater species, and 0.04 μg/L for marine life in saline waters. Dichlorvos is licensed as a veterinary medicine by the Veterinary Medicines Directorate for use against sea lice (***Lepeophteirus salmonis*** and ***Caligus elongatus***) that afflict salmon [8]. There have been concerns about the discharge of dichlorvos as a result, and the possible effects of dichlorvos on wild salmon [5]. The 96-hour LC_{50} values for dichlorvos were reported as between 0.004-11.6 mg/L [8].

To compare the acute effects of dichlorvos on ***Poecilia reticulata*** and ***Cyprinus carpio,*** the present study was conducted.

MATERIALS AND METHODS

Guppy (1.1±0.2 cm) were obtained from a local breeder in Ankara, Turkey and brought to the laboratory one month before the study within 20 min in plastic bags with sufficient air. Carp (15±2.3 cm) were obtained from Bolu, Turkey and brought to the laboratory one month before the study within 2 hours in fish tanks with sufficient air. The fishes were kept in glass aquaria and all the aquaria were aerated by air compressors.

Test chambers were filled 25 L of dechlorinated tap water. The temperatures of the guppy aquaria was regulated at 22 ± 1 ºC and carp aquaria was regulated at 25 ± 1 ºC with the help of aquarium heaters [10, 11]. Some characteristics of guppy aquarium water were: dissolved oxygen concentration, 7.2-7.9 mg/L, pH 7.5-7.7 and conductivity 0.212-0.260 mS.

Some characteristics of carp aquarium water were: dissolved oxygen concentration, 7.5-8.0 mg/L, pH 7.6-7.7 and conductivity 0.212-0.260 mS [10].

Following the preliminary experiments, all the tests were repeated three times. Each aquarium contained ten fish, which were selected randomly. Feeding was done once a day and was stopped 48 h before the experiments. After 48 h of adaptation, different concentrations of dichlorvos were added to the guppy and carp aquaria.

Technical grade dichlorvos (98%) was supplied from the Insecticide Testing Laboratory of Hacettepe University, Ankara, Turkey. Stock solutions were prepared by weighing a certain amount analytical grade dichlorvos, stored at +4 ºC, dissolved in acetone. Test concentrations were prepared by using appropriate amount of stock solutions to give the dosing concentration 0,5; 1; 3; 5; 8 mg/L to guppy and 1; 2; 3; 4; 5 mg/L to carp. The control groups received acetone at the maximum acetone volumes used in the dissolving of the dosing concentrations. The bioassay system was as described in standardized methods [11] and the national regulation [12]. LC_{50} values and 95 % confidence limits of the dichlorvos on guppy and carp were determined using the Finney Probit Analysis Statistical Method [13]. The statistical significance of the difference between LC_{50} values of dichlorvos for the guppy and carp was evaluated by using a independent Mann-Whitney Test and SPSS 15.0 programme. The significance of the results was ascertained at $p < 0.05$.

RESULTS

The calculated 96 h acute LC_{50} values (95 % confidence limits) of technical dichlorvos, dissolved in acetone, using a static bioassay system to guppy and carp were 1.84 mg/L and 2.51 mg/L respectively. Control mortality was zero. Guppy results are in Table I and carp results are in Table II.

Observations of behavioral response of fishes were conducted at 1–8, and every 6 h during the acute toxicity tests. The control groups showed normal behavior during the test period. The behavioral changes in guppy started 30 min after dosing. The 0.5 mg/L (lowest) concentration had similar behavior with the control group. Observed behavioral effects loss of equilibrium, erratic swimming and staying motionless at a certain location generally at mid-water level for prolonged periods. Fish were exposed to 1 mg/L showed less general activity with occasional loss of equilibrium. Loss of equilibrium become more frequent in the 3 mg/L concentration. The 5 mg/L concentration group stayed motionless close to the water surface and later fell to the aquarium bottom in an uncontrolled manner. The highest concentration of 8 mg/L showed all responses at high intensities: the loss of equilibrium, hanging vertically in water, rapid gill movement, erratic swimming, sudden swimming motion in a spiral fashion, after long periods of motionlessness lying down on the aquarium bottom and suddenly starting to move.The behavioral changes in carp started 1 h after dosing. The lowest concentration had similar behavior with the control group. Fish were exposed to 2 mg/L showed less general activity. The 3 mg/L concentration group stayed motionless close to the water surface and later fell to the aquarium bottom in an uncontrolled manner. The highest concentration group showed the loss of equilibrium, hanging vertically in water, after long periods of motionlessness lying down on the aquarium bottom and suddenly starting to move.

The differences between LC values of dichlorvos in guppy and carp were compared using independent Mann-Whitney Test and it indicates that the differences between LC values of dichlorvos on guppy and carp were not statistical important ($p > 0.05$) [14].

DISCUSSION

Organophosphate pesticides are widely used in public health, agriculture and protecting stored product from insects. Organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Their ability to degrade made them an attractive alternative to the persistent organochloride pesticides. Although organophosphates degrade faster than the organochlorides, they have greater acute toxicity. Organophosphate pesticides irreversibly inactivate acetylcholinesterase, which is essential

Table I. Acute 96-h toxicity of technical dichlorvos in guppies (*Poecilia reticulata*)

Point	Concentration (mg/L)	95% Confidence Limits	Slope±SE	Intercept±SE
LC/EC 1.00	0.108	0.030-0.222	1.888±0.278	4.496±0.150
LC/EC 5.00	0.249	0.097-0.430		
LC/EC 10.00	0.387	0.178-0.614		
LC/EC 15.00	0.522	0.267-0.785		
LC/EC 50.00	1.847	1.352-2.443		
LC/EC 85.00	6.535	4.603-11.314		
LC/EC 90.00	8.813	5.915-16.908		
LC/EC 95.00	13.724	8.498-30.942		
LC/EC 99.00	31.499	16.518-97.565		

Note: Control group (theoretical spontaneous response rate) = 0.0000

Table II. Acute 96-h toxicity of technical dichlorvos in carps (*Cyprinus carpio*)

Point	Concentration (mg/L)	95% Confidence Limits	Slope ± SE	Intercept ± SE
LC/EC 1.00	0.148	0.012-0.379	1.891±0.448	4.241±0.217
LC/EC 5.00	0.340	0.055-0.674		
LC/EC 10.00	0.529	0.125-0.920		
LC/EC 15.00	0.713	0.216-1.138		
LC/EC 50.00	2.516	1.858-3.308		
LC/EC 85.00	8.887	5.733-26.775		
LC/EC 90.00	11.979	7.103-46.277		
LC/EC 95.00	18.642	9.702-104.674		
LC/EC 99.00	42.734	17.272-487.811		

Note: Control group (theoretical spontaneous response rate) = 0.0000

Table III. Acute toxic studies with *Poecilia reticulata*

Pesticide	Assay	Result	Reference
Fenitrothion	96 h LC_{50}	0.32 mg/L	[17]
Alpha-cypermethrin	96 h LC_{50}	9.43 μg/L	[18]
Alpha-cypermethrin	96 h LC_{50}	3.5 μmol/L	[19]
Beta-cypermethrin	96 h LC_{50}	21.4 μg/L	[20]
Deltamethrin	48 h LC_{50}	5.13 μg /L	[9]

Table IV. Acute toxic studies with *Cyprinus carpio*

Pesticide	Assay	Result	Reference
Diazinon	48 h LC_{50}	0.09 mg/L in embryos	[21]
Diazinon	24 h LC_{50}	0.36 mg/L in larvae	[21]
Diazinon	48 h LC_{50}	0.29 mg/L in larvae	[21]
Diazinon	72 h LC_{50}	0.23 mg/L in larvae	[21]
Diazinon	96 h LC_{50}	0.15 mg/L in larvae	[21]
Deltamethrin	24 h LC_{50}	9.41 μg/L in adult	[16]
Deltamethrin	48 h LC_{50}	4.47 μg/L in adult	[16]
Deltamethrin	72 h LC_{50}	2.37 μg/L in adult	[16]
Deltamethrin	96 h LC_{50}	1.65 μg/L in adult	[16]
Deltamethrin	48 h LC_{50}	0.021 μg/L in embryos	[22]
Deltamethrin	48 h LC_{50}	0.007 μg/L in larvae	[22]

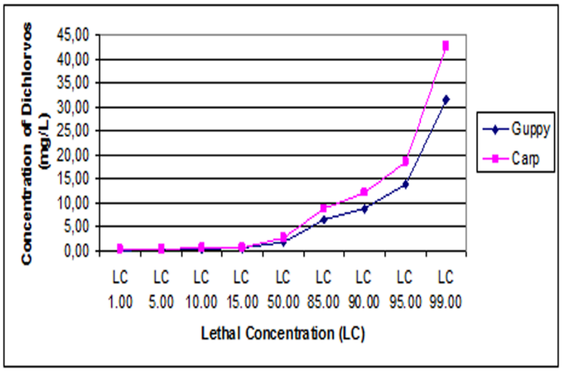

Figure I. The LC values of dichlorvos in guppy and carp

to nerve function in insects, humans, and many other animals. Dichlorvos is one of the most toxic organophosphate pesticides, but it is interesting to note that only a few studies on the acute toxicity of it to fish exist in the open literature.

Sisman [16], studied examined effects of the pesticide dichlorvos on embryonic development of zebrafish. Developmental abnormalities have been observed in embryos and larvae, such as no blood flow, cardiac edema, delayed hatching, and vertebra malformations. The acute toxic effects of dichlorvos in different species fish were studied by some researchers. They reported that 96 h LC_{50} values for dichlorvos: 11.6 mg/L in fathead minnow, 0.9 mg/L in bluegill, 5.3 mg/L in mosquito fish, 0.004 mg/L in sand shrimp, 3.7 mg/L in mummichogs, and 1.8 mg/L in American eels. The LC_{50} (24-hour) for dichlorvos in bluegill sunfish is 1.0 mg/L. The 96-h LC_{50} values of dichlorvos in fish were found between 0.004-11.6 mg/L [7]. The 96-h LC_{50} values of dichlorvos were found as 1.84 mg/L in guppies and 2.51 mg/L in carps in the present work. The 96-h LC_{50} values of guppy and carp were very similar. Comparison of the LC values of dichlorvos in guppy and carp were showed in Figure I.

The acute toxicity of dichlorvos in guppies and carps has been assessed separately with 96 h toxicity tests and were compared in present study. The 96-h LC_{50} values of pesticide showed that there was no significant difference in the sensitivity of guppies and carps to this compound. Mann-Whitney Test indicates that the differences between LC values of dichlorvos in guppy and carp were not statistical important [14].

It is necessary to discuss and compare the sensitivity of guppy and carp with the results of more assays. However, there are very limited compare study about effects of pesticides on fish. Acute toxic studies with guppy and carp were showed in Table III and Table IV. Viran et al [19], studied acute toxic effects of deltamethrin in guppies and they found 48 h LC_{50} value as 5.13 μg/L. Calta and Ural [16], reported 48 h LC_{50} value of deltamethrin in carps as 4.47 μg/L. They found similar LC_{50} value and there was no significant difference in the sensitivity of guppy and carp to deltamethrin.

Our reported measurements of 96 h LC_{50} values suggest that the dichlorvos is one of the most toxic organophosphate pesticides for fish. These results may indicate species-specific toxicity, with the conclusion that ecotoxicological tests performed only on one fish species may lead to an erroneous classification of chemical compounds.

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