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# Acute toxicity of diazinon on the common carp (*Cyprinus carpio* L.) embryos and larvae

Rahmi Aydın [a,*], Kenan Köprücü [b]

[a] *University of Firat, College of Bingol, Fisheries Programme, Bingol, Turkey*
[b] *University of Firat, Faculty of Fisheries, 23119 Elazig, Turkey*

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**Abstract**

In the present study, the toxic effects on the embryos and larvae of the common carp were used as a model to investigate the organophosphorus pesticide, diazinon, which contaminates aquatic ecosystems. Data obtained from the diazinon acute toxicity tests were evaluated using the Probit Analysis Statistical Method. The control and six test experiments were repeated five times. The number of dead embryos significantly increased in response to diazinon concentrations 0.25, 0.5, 1, 2, 4, and 8 mg $L^{-1}$ ($p < 0.05$ for each case). The 48 h $LC_{50}$ value (with 95% confidence limits) of diazinon for common carp embryos was estimated at 0.999 (0.698–1.427) mg $L^{-1}$. Dose–response decreases in hatching success were recorded as 84.60, 75.2, 54.1, 31.0, 6.0, and 0.0%, respectively ($p < 0.05$). The number of dead larvae significantly increased with increasing diazinon concentrations exposed for 24–96 h ($p < 0.05$). The 24, 48, 72, and 96 h $LC_{50}$ values (with 95% confidence limits) of diazinon for common carp larvae were estimated at 3.688 (2.464–8.495), 2.903 (2.019–5.433), 2.358 (1.672–4.005), and 1.530 (1.009–3.948) mg $L^{-1}$, respectively. There were significant differences in the $LC_{50}$ values obtained at different exposure times ($p < 0.05$). The results of the study suggest that low levels (0.25 mg $L^{-1}$) of diazinon in the aquatic environment may have a significant effect on the reproduction and development of carp.


*Keywords:* Common carp; Embryo; Larvae; Acute toxicity; Hatching success; Organophosphorus pesticide; Diazinon

## 1. Introduction

During the last decades, significant amounts of pesticides belonging to the classes of organophosphates have been released into the environment. The organophosphorus pesticides were developed by chemical manipulation of nerve gases and further modifications have resulted in chemicals with greater species selectivity. Organophosphate compounds are useful as pesticides due to their ability to inhibit acetylcholinesterase, an enzyme

* Corresponding author.
*E-mail address:* raydin@firat.edu.tr (R. Aydın).

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responsible for inactivating the neurotransmitter acetylcholine [1,2].

Diazinon is a contact organophosphate pesticide and extensively used, both in agriculture and households to control insects in soil, plants, fruit and vegetable crops. Agricultural spray contains 85–90% diazinon [3]. After its application on crops and plants, diazinon is easily washed into surface waters and enters the ground water. Eventually, it enters the aquatic environment in large quantities [4–9]. Diazinon degrades rapidly, but under conditions of low temperature, low moisture, high alkalinity, and lack of suitable microbiological degraders, it may remain biologically active in soils for six months or longer, Because of its aquatic distribution, diazinon affects a wide range of non-target organisms, like invertebrates, mammals, birds, and fishes, especially those inhabiting aquatic environment [7,10,11].

In fishes, exposure to diazinon in sublethal doses is known to affect the nervous system by inhibition of acetylcholinesterase activity [12]. Sublethal doses may lead to reduced growth and reproduction in aquatic invertebrates [10]. Acute toxicity tests of adult fish using diazinon have shown that 96 h sublethal values vary by several orders of magnitude between species [13–15]. While experience shows that early life stages of fishes are often the most sensitive to toxic effects, little is known about the toxicity of diazinon to fish during these developmental stages. This is important since during development sensitivity may change with some compounds showing higher sensitivity in embryos [7,16] whereas others are more toxic to larvae [17–19]. Takimoto et al. [20] reported that timing of exposure of killifish (*Oryzias latipes*) embryos to sublethal concentrations of the organophosphate fenitrothion resulted in significantly different degrees of mortality and hatching success.

The present study was performed to determine acute toxicity of diazinon on the embryos and larvae of common carp (*Cyprinus carpio*). The common carp was selected for the bioassay experiments because of it is widespread and presently cultured all over Asia, in most parts of Europe, and on a small scale in some countries of Africa and Latin America.

## 2. Materials and methods

### 2.1. Adult fish and chemical supply

Four- to five-year-old male and female common carp samples weighing 1.8–5.2 kg, total length 45.4–65.2 cm, were obtained from a fish hatchery unit of the State Hydraulic Works, Elazig, Turkey. The broodfish were kept at 24 ± 1 °C [21], in 500 L fiberglass tanks and in natural light conditions.

Basudin 60 EM, with the active molecule diazinon [0,0-diethyl-0-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate], purity 63% (dissolved in 80% acetone) was purchased from Chem Services (Elazig, Turkey).

### 2.2. Collection of gametes, artificial fertilization, and incubation of eggs

Carp gametes were obtained through the hormonal induction of ovulation and spermiation by the intramuscular injection of carp pituitary powder [22] suspended in a 0.9% NaCl solution. The suspended carp pituitary was administered at a dose of 0.5 mg $L^{-1}$ of fish for males and 1.0 mg $L^{-1}$ of fish for females. The 'dry method' of fertilization was used in this study [21]. Twenty-four hours after injection the gametes of both sexes were stripped into separate bowls, by applying gentle pressure on the inflated belly. Before the stripping, broodfish were anaesthetized with benzocaine dissolved in water at the concentration of 1000 ppm for 15 min [23].

The stripped eggs and milt were then mixed in a bowl. Fertilization and degumming of eggs was performed using the modified Woynarovich method. Eggs were activated with a solution of 4 g NaCl and 3 g urea $L^{-1}$ and 5 min later they were transferred into a solution of 4 g NaCl and 20 g urea $L^{-1}$ [24]. Fertilised eggs were washed by the addition of water (24 °C) for 5 min. The fertilised eggs were rinsed and transported from the hatchery unit to the reproduction laboratory of the Fisheries Faculty of Firat University within 30 min. These eggs were immediately placed in experimental units. Each experimental unit had five incubation chambers (each containing approximately 200 eggs) containing 2.5 L of medium. The levels of medium in both aquaria