An Investigation on Some Toxic Effects of Carbofuran on *Daphnia magna* (Crustacea, Cladocera)

Karbofuran'ın *Daphnia magna* (Crustacea, Cladocera) Üzerindeki Bazı Toksik Etkilerinin Araştırılması

Research Article

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ABSTRACT

The aim of this study was to determine the median effective concentration (EC_{50}) of carbofuran, a carbamate pesticide, for *Daphnia magna* and to evaluate the effects on the heart rate. In the experiment, five neonates (≤ 24 hours) were used for each negative control, solvent control (acetone) and the treatment groups. The treatment groups were exposed to five different concentrations (2.5 μg/L, 4.5 μg/L, 8.1 μg/L, 14.58 μg/L and 26.244 μg/L) of carbofuran (98%) for 48 hours. Immobilised daphnids were noted at the 24th and the 48th hour in both control and treatment groups. EC_{50} values were calculated by probit analysis as 33.814 μg/L and 3.451 μg/L for 24 and 48 h, respectively. At the end of the 48th h, all samples were observed by light microscopy and the heart beats per minute (bpm) were counted by using a video camera. Heart rate was similar in negative and solvent control groups (444.8±24.12 and 458.4±34.14, respectively). In the treatment groups heart beats were noted as 498.4±17.43, 183.8±103.83, 441.6±35.73, 293.8±101.09 and 442.4±32.94 for 2.5, 4.5, 8.1, 14.58 and 26.244 μg/L, respectively. These results show that carbofuran caused immobilisation and decreased heart rate in *D. magna*. However, heart occured independently of carbofuran concentration gradient.

Key Words
Pesticide, carbofuran, *Daphnia magna*, EC_{50}, heart rate.

ÖZET

Bu çalışmada karbamatlı bir pestisit olan karbofuran'ın *Daphnia magna* için EC_{50} değerinin bulunması ve kalp atım sayısı üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Denemede negatif kontrol, çözücü kontrol (aseton) ve uygulama grupları için beşer daphnid (24 saatlikten küçük) kullanılmıştır. Uygulama grupları karbofuran'ın (% 98 saflıkta) beş farklı derişimine (2.5 μg/L, 4.5 μg/L, 8.1 μg/L, 14.58 μg/L ve 26.24 μg/L) 48 saat boyunca maruz bırakılmıştır. Tüm gruplarda 24. ve 48. saatlerde hareketsiz bireyler kaydedilmiş ve probit analiz yöntemi ile EC_{50} değerleri sırasıyla 33.814 μg/L ve 3.451 μg/L olarak hesaplanmıştır. 48 saat sonunda, tüm örnekler ışık mikroskobu ile incelenmiş ve bir dakikadaki kalp atım sayıları video kamera ile kaydedilerek belirlenmiştir. Kalp atım sayıları negatif kontrol grubunda 444.8±24.12; çözücü uygulanan kontrol grubunda ise 458.4±34.14 olarak birbirine yakın değerlerdedir. Deneme gruplarında bu sayılar artan derişimine göre sırasıyla 2.5 μg/L için 498.4±17,43; 4.5 μg/L için 183.8±103.83; 8,1 μg/L için 441.6±35.73; 14.58 μg/L için 293.8±101.09 ve 26.244 μg/L için 442.4±32.94 olarak kaydedilmiştir. Buna göre karbofuran, *D. magna* neonatlarında hareketsizliğe ve kalp atım sayılarında azalmaya sebep olmuştur; ancak bu azalma derişim artışı ile paralellik göstermemektedir.

Anahtar Kelimeler
Pestisit, karbofuran, *Daphnia magna*, EC_{50}, kalp atım sayısı.

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INTRODUCTION

Pesticides are toxic chemicals that are frequently used for invasive species. They are transported from agricultural fields to aquatic habitats by direct use or rain and affect non-target organisms. According to the report of EPA (1990) pollution of the pond and rivers are substantially due to the agricultural processes. In general, pesticide levels on the surface waters are lower than the lethal concentration value; however the sublethal effects appear on aquatic organisms by exposing these enviromental levels [1-6].

Carbamate pesticides are one of the most preferred pesticide group in western countries; because they are highly toxic and they can be rapidly degraded. However, they don't have target specificity and they cause serious effects especially on aquatic invertebrates [7-9]. Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) (CAS Number: 1563-66-2) is one of the most toxic carbamate pesticides is widely used as nematicide and insecticide on ornamentals, corn, potatoes and sunflowers [10]. Carbofuran shows its toxicity as acethylcholinesterase (AChE) inhibition like other carbamate pesticides [11-13].

Water fleas are important members of the food chain in freshwater. They feed on primary producers like algae and are the prey of most of the fish [14]. *Daphnia magna* is a frequently used invertebrate model in ecotoxicological researches due to its small size, short life cycle, rapid maturation and reproduction, sensitivity to toxic chemicals and transparent body which makes possible observations of internal organs and it is used to determine the effects of bioactive compounds, pharmaceutics and pesicide pollution in water [15-17].

Globular heart of *D. magna* is located in the dorsal part of the body in front of the brood chamber. Unlike neurogenic hearts of other many crustaceans; *Daphnia* has a myogenic heart [18]. In neurogenic hearts, cardiac ganglion is the pacemaker that starts contraction; but in myogenic hearts the beat is started by cardiac muscle [19,20]. This feature makes its heart similar to vertebrate heart [21,22].

The aim of the present study was to determine the EC_{50} value of carbofuran for *D. magna* and to observe the effects on heart rate.

MATERIALS AND METHODS

Culture Conditions

Daphnids were obtained from a commercial supplier and *D. magna* were identified according to an image based key [23]. They were transferred into a well aerated 10 L aquarium with spring water (pH 6,52; NO_3 (mg/L)= 0; NO_2 (mg/L)= 0; Cl_2 (mg/L)= 0; GH<3°d, KH= 0°d), maintained at 22±1 °C, 14 h light/10 h dark cycle, fed with yeast sorbet (25 g granular baker's yeast+100 ml spring water) daily and cultured for three months.

Acute Toxicity Test

The experiment was conducted according to the protocol of OECD 202 (2004) [24] with some modifications. Stock solution was prepared by dissolving carbofuran (purity 98%) in acetone. Five different concentrations of test solutions (2.5 μg/L, 4.5 μg/L, 8.1 μg/L, 14.58 μg/L and 26.244 μg/L) were diluted from the stock solution. Five neonates (≤ 24 hours) were randomly choosen for each group. Negative control group was chemical free and solvent control contained 1 ml/L acetone. Neonates were transferred into the test tubes containing 10 ml test solutions. The experiment was carried out as a static method and the temperature and photoperiod conditions were the same with the culture. Samples were considered immobile when they were unable to swim within 15 seconds after gentle shaking of the test tube. The number of immobilised samples were noted at the 24th and the 48th hour. EC_{50} values for these two timepoints were calculated by using probit analysis (SPSS 20.0). Each daphnid was transferred on a slide with a drop of the media and observed by light microscopy. Heart beats of each alive sample (per minute) were recorded in slow motion by a video camera for 30 s and results were compared for statistical significance by one-way ANOVA.

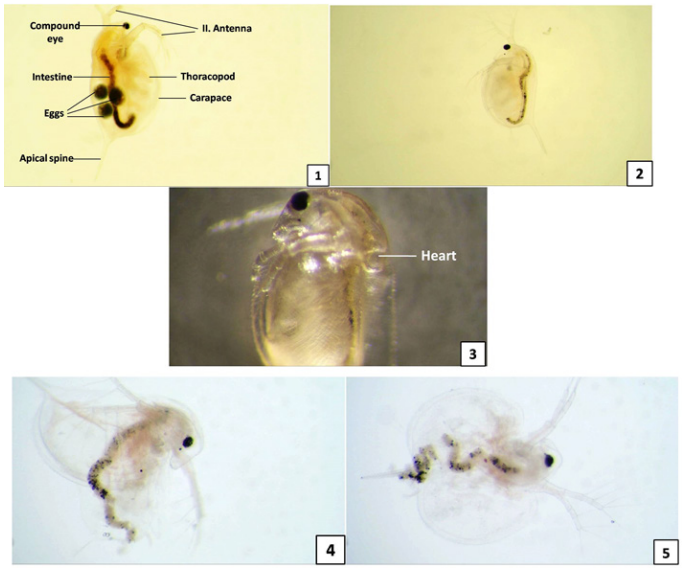

Figure 1. Adult *D. magna*.
Figure 2. A neonate from the negative control group.
Figure 3. Position of the heart.
Figure 4 and 5. Affected samples by carbofuran.

RESULTS

The samples of both negative and solvent control groups maintained their ordinary swimming behaviour during the experiment. At the 48th h, samples of all the treatment groups except for the group of 2.5 μg/L were noted as immobilised. Some of them could move their antennae; however they were unable to swim. EC_{50} values of carbofuran for 24 and 48 h were calculated as 33.814 μg/L and 3.451 μg/L, respectively. At the end of the 48th h, heart beats per minute (mean±standart deviation) were noted similar as 444.8±24.12 in the negative control group and 458.4±34.14 in the solvent control group. In the treatment groups heart beats were evaluated as 498.4±17.43 for 2.5 μg/L; 183.8±103.83 for 4.5 μg/L; 441.6±35.73 for 8.1 μg/L; 293.8±101.09 for 14.58 μg/L and 442.4±32.94 for 26.244 μg/L. Additionally, carapace deformations were observed for some daphnids in the groups of 8.1 μg/L and 26.244 μg/L.

DISCUSSION

Effects of different pesticides on water fleas were evaluated by various studies. 48 h EC_{50} values of Fenobucarb and NAC (carbamate pesticides) for *D. magna* were reported as 13 μg/L [25] and 12 μg/L [26], respectively. 48 h EC_{50} value of carbofuran was calculated as 42.5 nmol/L [27]. Also, 24 h and 48 h EC_{50} values of carbofuran were noted as 0.092 mg/L and 0.03 mg/L [28]. In the present study 24 h and 48 h EC_{50} value of carbofuran was calculated as 33.814 μg/L and 3.451 μg/L, respectively. Variations in the experimental conditions or the genotype of the populations may give rise to different EC_{50} value determinations. Additionally, maternal feeding and the culture media compound can be also effect the sensitivity of young individuals and causes inequality between the results of toxicity tests [29-31].

In general, 96 h LC_{50} values of carbofuran are lower than 1000 μg/L for different fishes [32]. These values were determined as 500 μg/L for *Cyprinus carpio* [33], 560 μg/L for *Salmo trutta* [34], 147 μg/L for *Perca fluviatilis* [34], 530 μg/L for *Oncorhynchus mykiss* [34] and 639 μg/L for *Gambusia yucatana* [35]. Based on these data points, *D. magna* is more sensitive than fishes. Sensitivity of *D. magna* and *Gammarus pulex* was compared and *D. magna* was found more sensitive to organophosphorus pesticides than G. *pulex*. However, it was noted that there was no significant difference between the sensitivity of two organisms to carbamate pesticides [27].

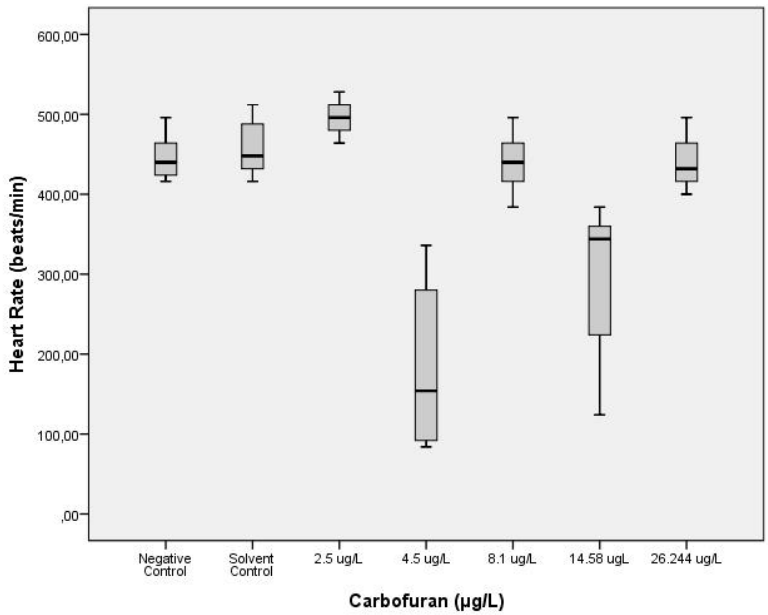

Figure 6. Box plot of heart rates of the control and the experimental groups. Median line of the box shows mean, the upper and the lower hinges of the box represents 75th and 25th percentiles of the data. The lower and higher lines in the figure represent minimum and maximum point of the data.

Table 1. Comparison of negative control and the other groups heart rates (bpm) by one-way ANOVA (Tukey HSD Test).

		Mean Difference	Std. Error	Sig.
Negative Control	Solvent Control	-13.60	19.05	0.99
	2.5 µg/L	-53.00	19.05	0.08
	4.5 µg/L	261.00*	19.05	< 0.05
	8.1 µg/L	3.20	19.05	1.00
	14.58 µg/L	151.00*	19.05	< 0.05
	26.244 µg/L	2.40	19.05	1.00

*Significance level p < 0.05

Several authors reported that heart rates of daphnids were affected by the chemicals added to the water in which they swim [16,17,36]. Arrhythmia was evaluated in D. *pulex* that was exposed to 100-200 mM lactose concentrations for 30-60 minutes [17]. 2.5 mg/L and 4 mg/L ectoin exposure caused increased heart rate (450±16 and 450±12 bpm, respectively), while 20 mg/L and 25 mg/L treatments caused decreased heart rate (350±11 and 340±11 bpm, respectively) in *D. magna* after 48 h [37]. The effects of melatonin and ethanol on the heart rate of *D. magna* was investigated and revealed that two hours exposure to melatonin (10 mg/L) and six minutes exposure to ethanol (5%) gave rise to depressed heart rate; moreover melatonin treatment prior to the addition of ethanol caused greater decrease in heart rate [22]. Carbamate pesticides caused toxicity by binding the active site and phosphorylating the B esterases such as cholinesterases and carboxylesterases [9]. Carbaryl that is a carbamate pesticide led to decrement of heart rate in zebrafish embryos [38]. In the present study carbofuran caused decrease in heart rate in the treatment groups of 4.5 µg/L (183.8±103.83 bpm) and 14.58 µg/L (293.8±101.09 bpm). Increasing acetylcholine release effects the activity of cholinergic neurons that regulates the heart and this probably caused decrease in heart rate [22]. According to our data, decrease in heart rate was not in a concentration dependent manner. This may be due to the individual differences or activation of defence mechanisms against certain concentration levels of the chemical.

More detailed studies containing different timepoints and concentrations are needed to reveal clearly the effects of carbofuran on heart rate.

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