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Research Article

# Acute Toxicity of Carbaryl, Methiocarb, and Carbosulfan to the Rainbow Trout (*Oncorhynchus mykiss*) and Guppy (*Poecilia reticulata*)

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**Abstract:** The acute toxicity of carbaryl, methiocarb and carbosulfan to juvenile rainbow trout (*Oncorhynchus mykiss*; 1.92 ± 0.5 g) and guppies (*Poecilia reticulata*; 0.45 ± 0.04 g) was evaluated in 96-h toxicity tests under static conditions. Concentrations in the toxicity test ranged from 0.85 to 8.5 mg/l for carbaryl, from 0.7 to 28.0 mg/l for methiocarb, and from 0.031 to 12.5 mg/l for carbosulfan. Carbaryl and methiocarb were more toxic to rainbow trout than to guppies. However, compared with rainbow trout, guppies were more sensitive to carbosulfan. The incidence of mortality in fish increased significantly ($P < 0.05$) with increasing pesticide concentrations. The time required to kill 50% ($LT_{50}$) of the rainbow trout at the lowest concentrations of carbaryl, methiocarb, and carbosulfan was 51 h 12 min, 74 h 35 min, and 107 h 57 min, respectively. As insecticide concentrations were increased, $LT_{50}$ values decreased ($P < 0.05$).

**Key Words:** Acute toxicity test, carbamate, pesticides, rainbow trout, guppies

## Karbaril, Metiyokarb ve Karbosülfan'ın Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) ve Lepistes (*Poecilia reticulata*) Balıkları Üzerine Akut Toksik Etkileri

**Özet:** Karbaril, metiyokarb ve karbosülfan aktif maddelerini içeren insektisitlerin gökkuşağı alabalıkları (*Oncorhynchus mykiss*; 1,92 ± 0,5 g) ve lepistes balıkları (*Poecilia reticulata*; 0,45 ± 0,04 g) üzerine, akut toksik etkileri, statik test yöntemi (96 saatlik) kullanılarak belirlenmiştir. Test çözeltilerindeki nominal insektisit miktarları karbaril için 0,85-8,5 mg/l, metiyokarb için 0,7-28,0 mg/l ve karbosülfan için 0,031-12,5 mg/l olacak şekilde ayarlanmıştır. Karbaril ve metiyokarbın gökkuşağı alabalıkları üzerine toksik etkilerinin, lepistes balıklarına göre daha fazla olduğu tespit edilmiştir. Ancak lepistes balıklarının karbosülfan aktif maddesine karşı, alabalıklara göre daha hassas oldukları belirlenmiştir. Test edilen balıklardaki ölüm oranlarının, akvaryumlardaki çözeltiler içersinde bulunan aktif maddelerin konsantrasyonuna bağlı olarak önemli derecede ($P < 0,05$) arttığı saptanmıştır. En düşük karbaril, metiyokarb ve karbosülfan konsantrasyonlarına maruz bırakılan gökkuşağı alabalıklarının % 50'sinin ölmesi için gereken zaman sırasıyla 51 saat 12 dakika, 74 saat 35 dakika ve 107 saat 57 dakika olarak tespit edilmiştir. İnsektisit konsantrasyonu arttıkça $LT_{50}$ değerinin düştüğü görülmüştür ($P < 0,05$).

**Anahtar Sözcükler:** Akut toksisite testi, karbamat, pestisit, gökkuşağı alabalığı, lepistes

## Introduction

Carbamate pesticides are used widely for agricultural and residential applications as insecticides and fungicides. Their worldwide annual use is estimated to range from 20,000 to 35,000 t. This family of chemicals replaced the organochlorine pesticides, which have been banned throughout the world. Unlike organochlorine pesticides, carbamate insecticides do not persist long in the environment, and they tend not to bioaccumulate. However, carbamate pesticides are toxic to non-targeted wildlife and fish and birds appear to be the more sensitive than mammals to these pesticides (1).

The carbamate ester derivatives used as insecticides are generally stable and have a low vapor pressure and low water solubility. Although carbamates are not very stable under aquatic conditions and will not persist long

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in the environment, bioaccumulation to some extent occurs in fish due mainly to their slow metabolism (2). Pesticides that are soluble in water are easily transported into surface waters. Pesticides adsorbed into soil particles can be carried from application sites on sediment. The runoff water can dissolve the active ingredients, which are subsequently transported to unintended sites. Either dissolved in water or carried by sediment, pesticides that are carried off-site can contaminate surface waters (3,4).

Other than targeted pests, insecticides can affect non-targeted species such as aquatic animals. In general, methiocarb is moderately toxic to aquatic organisms and the toxic dose depends on species (5). The toxicity of carbamate substances results primarily from inhibition of acetylcholinesterase (AChE), a key enzyme of the nervous system. The inhibition causes an accumulation of acetylcholine in synapses with disruption of the nerve function, which can result in death (6).

There are limited numbers of studies comparing the acute toxicity of carbaryl, carbosulfan and methiocarb in various fishes, while information on the mechanisms of toxicity to different fish species has been reported often (2,6). Carbaryl can cause a decrease in the liver glutamine level (7), and a decrease in the number of leukocytes in *Gorra gothyla gothyla* (8). In carbaryl toxicity, characteristic behavioral changes associated with changes in physiological parameters induced by carbaryl exposure can be seen. Histologically, methiocarb-exposed fish had lamellar edema, separation of epidermis from lamellae, lamellar fusion, telangiectasis, swelling of the epithelial cells and increased cytoplasmic granularity. Gills also had scattered areas of focal lamellar hyperplasia. Methiocarb-exposed fish had necrosis between the molecular and granular layer of cerebellum where Purkinje cells are present (9).

The present study sought to determine the acute toxicity of commonly used pesticides carbaryl, methiocarb, and carbosulfan to rainbow trout (*Oncorhynchus mykiss*) and guppies (*Poecilia reticulata*) using a static test system. As studies on the lethal times of carbaryl, methiocarb, and carbosulfan for rainbow trout and guppy in the literature are limited, the other objective of this study was to determine the acute toxicity of carbaryl, methiocarb, and carbosulfan concentration to rainbow trout and guppies over 96 h.

## Materials and Methods

### Experimental Animals

Rainbow trout (*Oncorhynchus mykiss*; 1.92 ± 0.5 g) were obtained from KTU, Faculty of Marine Sciences, Research Facility in Trabzon, Turkey. The fish were acclimated to laboratory conditions in flow through fiberglass tank (100 l) under a natural photoperiod for 2 weeks. During the acclimation period, the fish were fed 5 times a day with commercial fish feed at 5% body weight. Guppies (*Poecilia reticulata*; 0.45 ± 0.04 g) were obtained from a local fish store and acclimated to laboratory conditions in glass aquaria (50 l) filled with aged tap water under a natural photoperiod for 2 weeks. The fish were fed twice a day with commercial flakes and once a week with *Daphnia* sp.

### Experimental Design

After acclimation, the rainbow trout were randomly transferred to 20-l aquaria and the guppies to 5-l aquaria. The experiments were performed in static water (10 fish per aquarium). Test solutions were prepared from commercial formulations (Bayer Crop Science AG, Frankfurt, Germany) containing 85% (active ingredient) carbaryl, 50% methiocarb, and 25% carbosulfan. The concentrations tested for carbaryl were 0 (control), 0.85, 1.53, 2.72, 4.76, and 8.5 mg/l. The methiocarb concentrations were 0 (control), 0.7, 1.4, 2.8, 5.0, 9.0, 16.0, and 28.0 mg/l. The rainbow trout were not exposed to 0.7 or 1.4 mg/l methiocarb. The concentrations tested for carbosulfan were 0 (control), 0.125, 0.625, 1.25, 6.25, 12.5 mg/l for rainbow trout, with additional 0.031 and 0.063 mg/l treatments for guppies. Triplicate aquaria were designated for each concentration. Carbaryl and methiocarb were dissolved in 10 ml of ethanol, and then added to the aquaria. The control tanks received 10 ml of distilled water. Since carbosulfan was an emulsifiable concentrate, it was directly added to the aquaria. This study was conducted under OECD Guideline No. 203 under static test conditions (10). During the 96-h acute toxicity experiment, the water in each aquarium was aerated and had the following characteristics for rainbow trout: dissolved oxygen 9.40 ± 0.3 mg/l, temperature 15.3 ± 0.9 °C, pH 7.40 ± 0.21, total hardness 99 ± 5 mg/l as $CaCO_3$, and alkalinity 87 ± 3 mg/l as $CaCO_3$. The water characteristics for the guppies were dissolved oxygen 8.58 ± 0.2 mg/l, temperature 21 ± 0.5 °C, pH 7.35 ± 0.11, total hardness 95 ± 1 mg/l as $CaCO_3$, and

alkalinity 75 ± 2 mg/l as $CaCO_3$. Fish were considered dead when gill opercula and body movement ceased, and the dead fish were removed immediately. The incidence of fish mortality was recorded 0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 36, 48, 60, 72, 84, and 96 h after exposure to insecticides.

### Water Quality

During exposure to carbamate insecticides water quality characteristics (temperature, dissolved oxygen, pH, total hardness, and alkalinity) in each treatment were measured daily. Total hardness and total alkalinity were measured by titration (11). Temperature and dissolved oxygen concentrations were determined with an YSI B51 polarographic oxygen meter, and thermistor (Yellow Spring Instrument, Yellow Spring, Ohio, USA). Water pH was determined with a glass electrode (Thermo Orion, Beverly, Massachusetts, USA).

### Statistical Analyses

Statistical test analysis was described previously by Altinok (12). Briefly, the concentration of insecticides and lethal time values estimated to kill 10%, 50%, and 90% of rainbow trout and guppies within 6 (6-h $LC_{50}$), 12, 24, 48, 72, and 96 h were calculated by probit analysis (SPSS 2002, SPSS Inc. Chicago, Illinois, USA). After exposing the fish to different concentrations of insecticides, survival of fish were analyzed by Kaplan-Meier survival and failure time analysis tests (KMSFTAT). After analyzing the survival data with KMSFTAT, when significant differences were found ($P < 0.05$), comparisons among means were carried out with a Cox-Mantel test (Statistica, Statsoft Inc., Tulsa, Oklahoma, USA).

## Results

No fish died during the acclimation period prior to insecticide exposure, and no control fish died during the toxicity tests. Carbamate-exposed fish had excessive mucus, rapid respiration rates, hyperactivity, and erratic movements. The fish first crowded at the water surface and then at the bottom of the aquarium, and finally became laterally recumbent on the bottom of the aquarium. At the time of death, the mouth and operculum stayed open. The incidence of death in fish was observed 1 h after exposure to the highest concentrations of insecticides except in carbaryl-exposed guppies, in which the first case of mortality was observed 2 h after exposure. The toxicity of carbaryl, methiocarb, and carbosulfan to rainbow trout and guppies increased with increasing pesticide concentration and duration of exposure. Carbaryl and methiocarb were found to be more toxic to rainbow trout than to guppies, while guppies were more susceptible to carbosulfan than rainbow trout (Tables 1-4). The mortality rate in fish increased significantly with increasing concentrations of pesticides. The carbaryl concentrations lethal to 50% of the rainbow trout were 1.28 mg/l (24 h), 1.14 mg/l (48 h), 1.02 mg/l (72 h), and 0.85 mg/l (96 h) (Table 1).

After exposing rainbow trout to different concentrations of insecticides for 24 h, the concentrations lethal to 10% ($LC_{10}$) and 90% ($LC_{90}$) differed by a factor of 2.06, 2.06, and 9.83 for carbaryl, methiocarb and carbosulfan, respectively (Table 1). $LC_{10}$ values of carbaryl were 5.88 times, of methiocarb were 4.95 times, and of carbosulfan were 3.32 times higher than the $LC_{90}$ values for guppies (Table 3). There were significant differences in numbers of dead fish between the duration of 1 and 96 h in each concentration ($P < 0.05$). The highest concentration of insecticides caused the highest fish mortality. Significant differences were found in $LC_{10\text{-}90}$ values obtained at different times of exposure ($P < 0.05$). The time required to kill 50% ($LT_{50}$) of the rainbow trout and guppies exposed to 0.85 mg/l carbaryl was 51 h 12 min and 107 h 59 min, respectively (Tables 2 and 4). In contrast, the $LT_{50}$ values were 2 h 36 min for rainbow trout and 30 min for guppies exposed to 8.5 mg/l of carbaryl. The time required to kill 50% of the rainbow trout at the lowest concentrations of methiocarb and carbosulfan was 74 h 35 min and 107 h 57 min, respectively. As insecticide concentrations were increased, $LT_{50}$ values decreased ($P < 0.05$).

## Discussion

We concluded that the toxicity to rainbow trout and guppies increases with increasing pesticide concentration and duration of exposure, and this is consistent with earlier reports (13-15). Although carbamate pesticides tend to undergo fairly rapid degradation in the environment, repeated input into the aquatic environment may result in harmful exposures. The degradation metabolite 1-naphthol is considered more toxic than its parent compound (16). However, Armstrong and Millemann (17) found that the $EC_{50}$ levels of carbaryl and 1-naphthol were similar.

Table 1. Lethal concentrations ($LC_{10-90}$) of carbaryl, methiocarb, and carbosulfan depending on time (1-96 h) for rainbow trout (n = 30 in 3 replicates).

| | | Concentrations (mg/l) (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|---|
| | Point | 6 h | 12 h | 24 h | 48 h | 72 h | 96 h |
| Carbaryl | $LC_{10}$ | 1.295 (0.888-1.554) | 0.754 (0.501-0.896) | 0.620 (0.250-0.759) | 0.454 (0.327-0.639) | 0.340 (0.251-0.532) | 0.198 (0.128-0.372) |
| | $LC_{50}$ | 2.126 (1.902-2.385) | 1.110 (0.981-1.248) | 0.947 (0.825-1.092) | 0.795 (0.580-0.919) | 0.679 (0.448-0.801) | 0.522 (0.341-0.662) |
| | $LC_{90}$ | 2.956 (2.647-3.486) | 1.466 (1.315-1.746) | 1.275 (1.121-1.704) | 1.137 (0.991-1.695) | 1.017 (0.886-1.332) | 0.846 (0.705-1.055) |
| Methiocarb | $LC_{10}$ | 2.604 (1.827-2.888) | 0.754 (0.501-0.896) | 0.619 (0.250-0.759) | 0.454 (0.327-0.638) | 0.339 (0.251-0.532) | 0.198 (0.128-0.372) |
| | $LC_{50}$ | 3.241 (2.940-5.033) | 1.110 (0.981-1.248) | 0.947 (0.825-1.091) | 0.795 (0.580-0.918) | 0.678 (0.448-0.801) | 0.522 (0.341-0.661) |
| | $LC_{90}$ | 3.877 (3.348-7.884) | 1.466 (1.315-1.746) | 1.275 (1.120-1.704) | 1.136 (0.991-1.695) | 1.017 (0.886-1.332) | 0.846 (0.705-1.055) |
| Carbosulfan | $LC_{10}$ | 0.906 (0.429-1.655) | 0.229 (0.087-0.419) | 0.166 (0.138-0.351) | 0.115 (0.029-0.210) | 0.067 (0.026-0.128) | 0.043 (0.063-0.102) |
| | $LC_{50}$ | 2.172 (1.516-7.015) | 0.989 (0.818-1.242) | 0.899 (0.740-1.117) | 0.432 (0.346-0.534) | 0.249 (0.181-0.401) | 0.231 (0.165-0.384) |
| | $LC_{90}$ | 3.439 (2.258-12.721) | 1.750 (1.441-2.348) | 1.631 (1.353-0.148) | 0.749 (0.628-0.952) | 0.432 (0.317-0.746) | 0.418 (0.302-0.758) |

Table 2. Lethal time (hours and minutes) ($LT_{10-90}$) of various concentrations of carbaryl, methiocarb, and carbosulfan for rainbow trout (n = 30 in 3 replicates).

| | | Lethal time (95% confidence intervals) | | | | |
|---|---|---|---|---|---|---|
| | Point | 0.85 mg/l | 1.53 mg/l | 2.72 mg/l | 4.76 mg/l | 8.5 mg/l |
| Carbaryl | $LT_{10}$ | 19 h 45 min (12 h 07 min-25 h 54 min) | 04 h 58 min (04 h 12 min-05 h 30 min) | 03 h 00 min (02 h 23 min-03 h 26 min) | 02 h 09 min (01 h 38 min-02 h 30 min) | 01 h 18 min (00 h 43 min-01 h 40 min) |
| | $LT_{50}$ | 51 h 12 min (45 h 18 min-58 h 12 min) | 08 h 01 min (07 h 38 min-08 h 31 min) | 04 h 54 min (04 h 37 min-05 h 18 min) | 03 h 28 min (03 h 12 min-03 h 42 min) | 02 h 36 min (02 h 18 min-02 h 52 min) |
| | $LT_{90}$ | 82 h 38 min (73 h 30 min-95 h 36 min) | 11 h 12 min (10 h 33 min-12 h 01 min) | 06 h 55 min (06 h 28 min-07 h 31 min) | 04 h 47 min (04 h 25 min-05 h 19 min) | 03 h 54 min (03 h 32 min-04 h 25 min) |
| | | 2.8 mg/l | 5.0 mg/l | 9.0 mg/l | 16.0 mg/l | 28.0 mg/l |
| Methiocarb | $LT_{10}$ | 49 h 06 min (41 h 38 min-54 h 30 min) | 12 h 37 min (07 h 24 min-16 h 55 min) | 03 h 01 min (00 h 10 min-04 h 31 min) | 01 h 25 min (00 h 48 min-01 h 51 min) | 0 h 05 min (-) |
| | $LT_{50}$ | 74 h 35 min (70 h 17 min-79 h 23 min) | 32 h 49 min (28 h 22 min-38 h 37 min) | 06 h 36 min (05 h 17 min-08 h 03 min) | 03 h 01 min (02 h 42 min-03 h 19 min) | 01 h 03 min (00 h 09 min-01 h 35 min) |
| | $LT_{90}$ | 100 h 01 min (93 h 17 min-109 h 54 min) | 52 h 59 min (45 h 51 min-63 h 53 min) | 10 h 09 min (08 h 34 min-13 h 47 min) | 04 h 36 min (04 h 13 min-05 h 12 min) | 03 h 18 min (02 h 48 min-04 h 09 min) |
| | | 0.125 mg/l | 0.625 mg/l | 1.25 mg/l | 6.25 mg/l | 12.5 mg/l |
| Carbosulfan | $LT_{10}$ | 39 h 37 min (30 h 19 min-48 h 09 min) | 04 h 43 min (00 h 36 min-08 h 10 min) | 00 h 58 min (00 h 01 min-03 h 48 min) | 01 h 31 min (01 h 01 min-01 h 51 min) | 0 h 08 min (-) |
| | $LT_{50}$ | 107 h 58 min (93 h 12 min-131 h 20 min) | 32 h 25 min (29 h 13 min-36 h 14 min) | 15 h 21 min (12 h 55 min-18 h 51 min) | 02 h 38 min (02 h 23 min-02 h 54 min) | 0 h 30 min (-) |
| | $LT_{90}$ | 176 h 21 min (148 h 48 min-221 h 47 min) | 60 h 12 min (54 h 23 min-67 h 45 min) | 29 h 42 min (24 h 47 min-38 h 27 min) | 03 h 45 min (03 h 24 min-04 h 15 min) | 02 h 14 min (01 h 36 min-03 h 12 min) |

Table 3. Lethal concentrations ($LC_{10-90}$) of carbaryl, methiocarb, and carbosulfan depending on time (1-96 h) for guppy (n = 30 in 3 replicates).

| | | Concentrations (mg/l) (95% confidence intervals) | | | | | |
|---|---|---|---|---|---|---|---|
| | Point | 6 h | 12 h | 24 h | 48 h | 72 h | 96 h |
| Carbaryl | $LC_{10}$ | na | 5.367 (1.715-9.612) | 3.152 (0.016-6.791) | 0.615 (-) | na | na |
| | $LC_{50}$ | 13.802 (6.537-29.205) | 14.064 (9.843-18.888) | 10.854 (7.217-15.923) | 4.207 (-) | 3.209 (-) | 1.383 (-) |
| | $LC_{90}$ | 28.430 (18.804-72.127) | 22.761 (18.101-31.466) | 18.557 (13.993-27.513) | 7.799 (-) | 6.419 (-) | 4.229 (-) |
| Methiocarb | $LC_{10}$ | 1.165 (0.949-1.893 | 1.010 (0.395-1.443) | 0.699 (0.170-1.050) | 0.649 (0.280-0.885) | 0.440 (0.029-0.689) | 0.417 (0.005-0.660) |
| | $LC_{50}$ | 10.388 (6.826-7.328) | 2.797 (2.413-3.261) | 2.080 (1.775-2.463) | 1.482 (1.270-1.759) | 1.318 (1.109-1.584) | 1.256 (1.054-1.511) |
| | $LC_{90}$ | 19.611 (14.143-37.22) | 4.583 (3.988-5.523) | 3.460 (2.972-4.283) | 2.314 (1.984-2.909) | 2.197 (1.865-2.802) | 2.094 (1.775-2.691) |
| Carbosulfan | $LC_{10}$ | 0.040 (0.000-0.197) | 0.130 (-) | 0.130 (-) | na | na | na |
| | $LC_{50}$ | 0.419 (0.179-1.101) | 0.151 (-) | 0.151 (-) | 0.141 (-) | 0.126 (-) | 0.122 (-) |
| | $LC_{90}$ | 0.879 (0.551-2.818) | 0.432 (-) | 0.432 (-) | 0.425 (-) | 0.413 (-) | 0.410 (-) |

na: not applicable

Table 4. Lethal time (hours and minutes) ($LT_{10-90}$) of various concentrations of carbaryl, methiocarb, and carbosulfan for guppy (n = 30 in 3 replicates).

| | | Lethal time (95% confidence intervals) | | | | |
|---|---|---|---|---|---|---|
| | Point | 0.85 mg/l | 1.53 mg/l | 2.72 mg/l | 4.76 mg/l | 8.5 mg/l |
| Carbaryl | $LT_{10}$ | 39 h 37 min (30 h 20 min-48 h 08 min) | 04 h 43 min (00 h 36 min-08 h 11 min) | 00 h 59 min (00 h 15 min-3 h 48 min) | 01 h 31 min (01 h 01 min-01 h 50 min) | na |
| | $LT_{50}$ | 107 h 59 min (93 h 12 min-131 h 21 min) | 32 h 27 min (29 h 13 min-36 h 15 min) | 15 h 22 min (12 h 55 min-18 h 52 min) | 02 h 38 min (02 h 23 min-02 h 54 min) | 00 h 30 min (00 h 05 min-00 h 58 min) |
| | $LT_{90}$ | 176 h 20 min (148 h 48 min-221 h 48 min) | 60 h 12 min (54 h 22 min-67 h 45 min) | 29 h 43 min (24 h 45 min-38 h 27 min) | 03 h 45 min (03 h 25 min-4 h 14 min) | 02 h 14 min (01 h 36 min-03 h 13 min) |
| | | 0.7 mg/l | 1.4 mg/l | 2.8 mg/l | 5 mg/l | 9 mg/l |
| Methiocarb | $LT_{10}$ | 39 h 57 min (28 h 00 min-50 h 21 min) | 20 h 31 min (5 h 32 min-31 h 13 min) | 2 h 43 min (00 h 19 min-4 h 32 min) | 1 h 35 min (00 h 25 min-2 h 26 min) | 1 h 43 min (00 h 43 min-2 h 28 min) |
| | $LT_{50}$ | 103 h 08 min (86 h 58 min-131 h 26 min) | 82 h 40 min (68 h 47 min-106 h 5 min) | 15 h 47 min (14 h 4 min-18 h 2 min) | 5 h 58 min (5 h 25 min-6 h 29 min) | 5 h 30 min (5 h 00 min- 6 h 00 min) |
| | $LT_{90}$ | 166 h 20 min (136 h 34 min-221 h 51 min) | 144 h 48 min (117 h 57 min-195 h 3 min) | 28 h 50 min (25 h 20 min-34 h 1 min) | 10 h 20 min (9 h 32 min-11 h 25 min) | 9 h 18 min (8 h 37 min-10 h 13 min) |
| | | 0.0313 mg/l | 0.0625 mg/l | 0.125 mg/l | 0.625 mg/l | 1.25 mg/l |
| Carbosulfan | $LT_{10}$ | 92 h 11 min (76 h 22 min-127 h 59 min) | 23 h 14 min (02 h 19 min-56 h 14 min) | 01 h 13 min (00 h 7 min-02 h 01 min) | 00 h 27 min (00 h 22 min-00 h 31 min) | 0 h 26 min (0 h 00 min-0 h 56 min) |
| | $LT_{50}$ | 155 h 23 min (122 h 19 min-259 h 10 min) | 68 h 26 min (50 h 57 min-108 h 00 min) | 05 h 10 min (04 h 38 min-05 h 40 min) | 03 h 19 min (02 h 42 min-03 h 50 min) | 02 h 21 min (01 h 46 min-02 h 48 min) |
| | $LT_{90}$ | 219 h 00 min (164 h 27 min-394 h 10 min) | 160 h 6 min (116 h 52 min-276 h 47 min) | 09 h 07 min (08 h 25 min-10 h 4 min) | 07 h 05 min (06 h 26 min-08 h 01 min) | 05 h 07 min (04 h 63 min-05 h 57 min) |

The patterns of trout and guppy behavior were similar among the controls and considered normal. Abnormal patterns of behavior were observed in rainbow trout and guppies in all midrange to high concentrations. Loss of equilibrium was the most common type of abnormal behavior in both groups of fish. Decreased swimming behavior and increased respiration rate were other effects of pesticides in the present study. Scott and Sloman (18) found that contaminants such as pesticides disturb normal fish behavior after exposure. Beauvais et al. (19) found that exposure of rainbow trout to carbaryl caused a decrease in brain ChE activity and this decrease was positively correlated with decreased swimming behavior. Brewer et al. (20) also found a positive correlation between changes in swimming speed and brain ChE activity following exposure to diazinon and malathion. Pesticides such as carbamates are known to affect fundamental physiological systems such that they affected salmonid olfactory- mediated behaviors in Jarrad et al.'s (15) study.

The concentrations at which a compound is lethal can depend upon many contributing factors including species and water quality. Little et al. (21) found the $LC_{50}$ level of carbaryl was 1.95 mg/l for rainbow trout, whereby Carlson et al. (22) determined it as 9.4 mg/l for medaka (*Oryzias latipes*). Similar results were found in the present study. The $LC_{50}$ (96 h) values of carbaryl, methiocarb and carbosulfan for rainbow trout were 0.522, 0.522, and 0.231 mg/l, and for guppies were 1.383, 1.256, and 0.122 mg/l, respectively. A comparison of the 96h-$LC_{50}$ values between the 2 species indicates that guppies are more resistant to carbaryl and methiocarb than rainbow trout, but more sensitive to carbosulfan, a result similar to studies with other species (23,24).

Viran et al. (25) estimated the 48-h $LC_{50}$ value of deltamethrin for guppies as 5.13 µg/l and they found that deltametrin was highly toxic to fish. In another study, the 48-h $LC_{50}$ value of beta-cypermethrin in guppies was found to be 21.4 µg/l (26). These values showed that the pesticides used in both studies are more toxic compared to those used in the present study.

Johnson and Finley (5) estimated the $LC_{50}$ (96 h) value of methiocarb for rainbow trout ranging from 0.630 to 0.890 mg/l. In another study, the $LC_{50}$ value (96 h) of methiocarb for the same fish was found to be 0.750-0.893 mg/l (27). In the present study, the $LC_{50}$ value for 96 h was 0.522 mg/l, which is lower than the values reported by Johnson and Finley (5) and Mayer and Ellersieck (27). The difference might be related to the weight of the tested fish as the toxicity of compounds on the organism may decrease with increasing body size (12,28). The differences may also be associated with water quality parameters and the purity of the chemical (28,29).

In conclusion, carbaryl, methiocarb, and carbosulfan are toxic for the guppy and rainbow trout and the toxicity increases with increasing pesticide concentration as well as exposure time. Compared to the rainbow trout, the guppy is more resistant to carbaryl and methiocarb, but less sensitive to carbosulfan. The results of the present study also indicate that carbaryl and methiocarb are less toxic than carbosulfan.

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