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Effect of Lethal Concentration of Commercial Gasoline on Caspian Roach (*Rutilus caspicus*)

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Abstract

In this study, the mortality effects of commercial gasoline at different tested concentrations were evaluated on the common roach (*Rutilus caspicus*) and LC_{50} values for each time period (24, 48, 72, and 96 hours) have been determined. Roach with an average weight of 3.1 $\pm$ 0.45 g and lengths of 4 $\pm$ 0.25 cm were used in this study. After transferring the 200 fish to the laboratory, they were kept in tanks of 100 liters for one week to adapt them to the experimental conditions. After the adaptation period, 100 fish were selected randomly and divided into 14 treatments (0, 2, 5, 10, 20, 40, 60, 80, 100, 200, 400, 600, 800, and 100 ppm commercial gasoline). The treatments were completed in triplicate. The results of this study show that the 96-hour LC_{50} of commercial gasoline is 600.2 $\pm$ 0.44 ppm and the maximum allowable concentration (MAC) is 60.02 mg/L. The study demonstrates the deadly effects of commercial gasoline on the Caspian roach. Spillage of diesel and gasoline fuels from transport tankers can enter rivers and eventually the marine ecosystem, and reach nursery and spawning areas where it can become a serious threat to fish survival.

Keywords: Pollution, LC_{50}, Common Roach, MAC

1. Introduction

Acute toxicity is extremely varied even within an individual species and in relation to a single toxicant. The differences in acute toxicity may be due to changes in water quality and test species conditions, such as size, age, and health (1). These variables, controlled by abiotic, biotic, and genetic parameters, can be affected by the quality of the environment, the differential susceptibility of individuals to the toxicant as a result of genetic capacity or the physiological health of individual members of the host population, and the presence and concentration of the toxicant.

Acute toxicity information can help to recognize the mode of toxic action and may provide data on doses affiliated with organ lethality and toxicity that can be used in setting dose levels for further dose studies. This information may also be applied in the assessment and treatment of toxic responses in humans. The results from acute toxicity tests can provide data for the evaluation of toxicity and dose response among similar chemicals and help in the selection of new materials for better work (2).

Extracted from natural gas, the mixture of hydrocarbons that makes up natural gasoline is mostly pentanes; including isopentane (C_5H_{12}) which is a saturated branch-chain hydrocarbon obtained by fractionation of natural gasoline or isomerization of pentane. This mixture must meet the vapor pressure, end-point, and other specifications for natural gasoline set by the gas processors association. Commercial gasoline is extensively used all over the world for fuel. The pollution of water by commercial gasoline is mainly attributed to the small but continuous leakage from storage tanks into water bodies, so it is important to understand the toxicity of gasoline to aquatic organisms (3).

Gasoline is used as a fuel, finishing agent, industrial solvent, and diluent (4). Automotive commercial gasoline is an intricate combination of relatively fugacious hydrocarbons with or without additives taken by shuffling suitable refinery flows (5). Dissolved oxygen, pH, size and age, water quality, species, formulation, and concentration of test chemicals are the major factors in the affecting toxicity of chemicals to aquatic organisms (6).

The roach (*Rutilus caspicus*), also known as the common roach, is a freshwater and brackish water fish of the

Cyprinidae family, native to most of Europe and western Asia. The name "roach" is not unique-fishes called roach can be any species of the genera Rutilus and Hesperoleucus, depending on locality (7).

In this study, we demonstrate the mortality effects of commercial gasoline at different concentrations on the Caspian roach and determine the LC_{50} values for each time period (24, 48, 72, and 96 hours).

2. Materials and Methods

2.1. Fish Preparation and Experimental Conditions

The study involved 200 roach with an average weight of 3.1 ± 0.45 g and length of 4 ± 0.25 cm. After being transferred to the laboratory, the fish were kept for one week in 200 liter tanks to adapt to the laboratory conditions. The fish were fed three times a day (with feed from Biomar.co) at a 2% of average body weight feeding rate. The experiment was conducted according to the standard method for determining the lethal concentration of commercial gasoline in a short time (96 hours) under constant (static) exposure (8).

2.2. Experimental Setup and Procedure

After the adaptation period, 100 fish were selected randomly and were divided into 14 treatments (0, 2, 5, 10, 20, 40, 60, 80, 100, 200, 400, 600, 800, and 1000 ppm of commercial gasoline) with 7 fish in each treatment. Fish were exposed to different concentrations of commercial gasoline for 96 hours. The mortality rate was recorded at intervals of 24, 48, 72, and 96 hours and dead fish were removed from the experimental tanks. Chemical and physical conditions (such as water temperature, salinity, pH, and dissolved oxygen) were kept constant throughout the experiment. All experiments were performed in triplicate (9).

2.3. Analysis

Data were analyzed with SPSS statistical analysis software version 20 using probit analysis. The lethal concentration required to kill 50% of a tested population (LC_{50}) values with 95% confidence limits were calculated. Differences among the results were considered to be statistically significant when the P value was less than 0.05.

3. Results and Discussion

In this present study no mortality was observed in the control group (0 ppm of commercial gasoline). However, 100% mortality was observed for the concentration of 1000

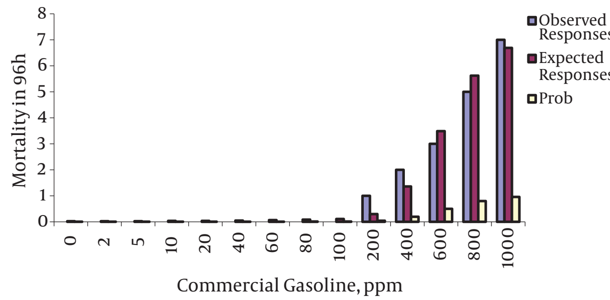

Figure 1. Commercial Gasoline Reaction Diagram of Roach Exposed to 96-Hour LC_{50} Experiment

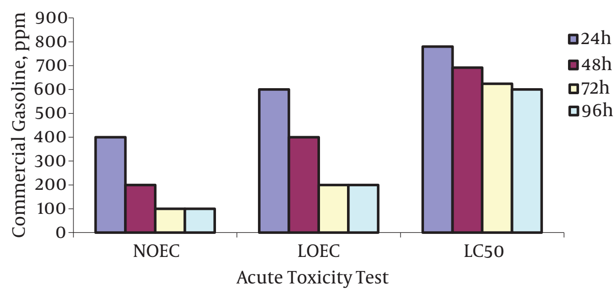

Figure 2. Acute Toxicity testing Statistical Endpoints of Commercial Gasoline

mg/L of commercial gasoline (Table 1). The impacts of different concentrations of commercial gasoline on the common roach at various times are shown in Tables 1 and 2. Through preliminary experiments, the lethal concentration of commercial gasoline for the common roach was calculated for concentrations ranging from 0 - 1000 mg/L. The mortality rates of roach during the four days (24, 48, 72, and 96 hours) are presented in Table 1.

The LC_1, LC_{10}, LC_{20}, LC_{30}, LC_{40}, LC_{50}, LC_{60}, LC_{70}, LC_{80}, LC_{90}, LC_{95}, and LC_{99} values of commercial gasoline were calculated during the times of 24, 48, 72 and 96 hours with probit analysis (Table 2). The results of this study show that the 96-hour LC_{50} of commercial gasoline for the common roach is 600.2 ± 0.44 ppm and the maximum allowable concentration (MAC) of this toxin was calculated as 60.02 mg/L (10% of LC_{50}).

The lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) were identical for all studied roach, although the median lethal concentration (LC_{50}) at different times showed a significant difference (Figure 2). Based on the 96-hour LC_{50} results, the MAC in natural environments is 0.1 LC_{50}.

For assessment of the risk of environmental pollutants to aquatic organisms, LC_{50} values were used from different concentrations (7). The NOEC was calculated for 24 and 48

Table 1. Mortality Rates of the Common Roach Under Exposure to Different Concentrations of Commercial Gasoline (7 Fish Exposed to Each Concentration)

Concentration (ppm)	No. of Deaths, h			
	24	48	72	96
0.00	0	0	0	0
2.00	0	0	0	0
5.00	0	0	0	0
10.00	0	0	0	0
20.00	0	0	0	0
40.00	0	0	0	0
60.00	0	0	0	0
80.00	0	0	0	0
100.00	0	0	0	0
200.00	0	0	1	1
400.00	0	1	2	2
600.00	1	2	2	3
800.00	3	4	5	5
1000.00	7	7	7	7

Table 2. Lethal Concentrations (LC_{1-99}) of Commercial Gasoline (Mean ± Standard Deviation) for Different Time Intervals (24, 48, 72, and 96 Hours) for the Common Roach

Point	Concentration (ppm) (95% Confidence Limit), h			
	24	48	72	96
LC_1	474.4 ± 1.9	230.9 ± 0.85	66.6 ± 0.45	0.01 ± 0.44
LC_{10}	611.6 ± 1.9	440.3 ± 0.85	317.0 ± 0.45	301.0 ± 0.44
LC_{20}	669.3 ± 1.9	528.5 ± 0.85	422.4 ± 0.45	403.7 ± 0.44
LC_{30}	711.0 ± 1.9	592.0 ± 0.85	498.4 ± 0.45	477.7 ± 0.44
LC_{40}	746.6 ± 1.9	646.4 ± 0.85	563.4 ± 0.45	541.0 ± 0.44
LC_{50}	779.9 ± 1.9	691.7 ± 0.85	624.1 ± 0.45	600.2 ± 0.44
LC_{60}	813.1 ± 1.9	747.9 ± 0.85	684.8 ± 0.45	659.3 ± 0.44
LC_{70}	848.7 ± 1.9	802.2 ± 0.85	749.8 ± 0.45	722.6 ± 0.44
LC_{80}	890.4 ± 1.9	865.8 ± 0.85	825.8 ± 0.45	796.7 ± 0.44
LC_{90}	948.2 ± 1.9	954.0 ± 0.85	931.2 ± 0.45	899.4 ± 0.44
LC_{99}	1085.4 ± 1.9	1163.4 ± 0.85	1181.6 ± 0.45	1143.3 ± 0.44

hours as 200 mg/L and for 72 and 96 hours as 100 mg/L. The LOEC was calculated as 400 mg/L for 24 and 48 hours and 200 mg/L for 72 and 96 hours. The lethal concentration required to kill 50% of a tested population (LC50) is the most widely accepted basis for acute toxicity experiments, and is defined as the mortality of 50% of the test water organisms after a specific period of exposure, usually 96 hours (10). The individual variability in acute toxicity even within a species and under the influence of the same toxicant depends on the age, size, and condition of the examined organism as well as on experimental factors (11).

The 96-hour LC_{50} results for commercial gasoline showed that it decreases with increasing toxin concentration and duration of exposure (Figure 2). This means that the more exposure time, the lower the toxin concentration required to kill 50% of a common roach population. Therefore the LC_{50} at the first 24 hours of the experiment occurred at a higher concentration than that required for the 96-hour LC_{50}. It is well known that disposal time is one of the effecting factors in lethal toxicity (12).

When fish are exposed to a fixed concentration of toxins, their tolerance decreases over time and the toxin has more time to affect them. Lai and Kessler used a static condition to test the acute toxicity of the same raw oil to black tiger shrimp postlarvae and sea bass fry under tropical conditions. Their experiment showed that the lethal toxicity (96-hour LC_{50}) of water soluble fractions (WSF) of raw oil to black tiger shrimp and seabass were 20.3 and 23.1 ppm, respectively (13).

A change in breathing rate is one of the common physiological answers to toxicants (14). Immerse air at the surface and swimming at the water surface were observed in the acute toxicity of malathion to Labeo rohita (15). The results of this study showed that the 96-hour LC_{50} of commercial gasoline for the common roach is 600.2 $\pm$ 0.44 ppm and the MAC (10% of LC_{50}) was calculated as 60.02 mg/L. A wide range for the 96-hour LC_{50} values of petroleum compounds has been reported in different fish species. Fishes are sensitive to aquatic pollution and serious concerns remain due to the pollutants' potential to cause harmful effects on human and wildlife populations.

The NOEC was calculated as 200 mg/L at 24 and 48 hours and 100 mg/L at 72 and 96 hours. LOEC was calculated as 400 mg/L at 24 and 48 hours and 200 mg/L at 72 and 96 hours. Tarkhani et al. (16) showed very low LC_{50} amounts for diazinon (17.5 $\pm$ 1.32 ppm) compared to deltamethrin (0.05 $\pm$ 0.027 ppm) and confirmed that deltamethrin has a higher toxicity compared to the other toxin in zebrafish (Danio rerio). Both diazinon and deltamethrin had a toxic effect on Danio rerio and the related mortality rates increased with the increasing concentration and exposure time. Also, Danio rerio was more sensitive to lower values of deltamethrin than diazinon. Our results showed that commercial gasoline (1000 ppm) had a higher toxicity compared to other treatments at similar experimental conditions for the common roach, and the rate of mortality increased with the increasing concentration and exposure time.

Horsefall and Spiff (17) showed that a decreased amount of oxygen in the water is often the main cause of toxicity in organic pollutants. Bob-Manuel showed that the average deadly dose of diesel fuel dissolved in water is slightly toxic to fishes (18). The exposure of Periophthalmus koelreuteri to WSF of diesel fuel caused mortality even at low concentrations. This agrees with earlier reports on the effect of WSF hydrocarbon compounds on aquatic life (19-21). In our study, the toxicity of gasoline to the Caspian roach increased with increasing concentrations and exposure time. We used a variety of methods to detect the acute and chronic toxicity of gasoline by preparing various water concentrations. This makes comparisons between fish species difficult.

4. Conclusion

The study demonstrated that commercial gasoline is deadly for the Caspian roach. Spillage of diesel and gasoline fuels from transport tankers can enter rivers and eventually the marine ecosystem, and reach nursery and spawning areas where it can become a serious threat to fish survival. Our results demonstrated that commercial gasoline has harmful effects on the behavior of fish. High concentrations of the tested toxicant cause death within a short time of exposure.

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