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Acute toxicity of Butachlor to Caspian Kutum (*Rutilus frisii Kutum* Kamensky, 1991)

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Abstract

Herbicides are used to control weeds and are usually targeted to processes and target sites that are specific to plants. Butachlor is an herbicide of the acetanilide class, which is used widely in agricultural fields. At the present study, lethal concentration (LC_{50}) of butachlor was calculated for *Rutilus frisii Kutum*. Samples weighted 4 ± 1 [mean ± SD] gr. The experiment were carried out in static condition and based on instructions of Organisation for Economic Co-operation and Development (O.E.C.D) in 4 days under controlled water physicochemical factors with pH was 8-8.3, BOD 690 mg/l total hardness 210 mg$CaCo_3$ and temperature was 17 ± 0.1 °C. All fishes were acclimatized in 60×55×30 cm aquarium for 10 days. Treated aquariums with concentration ranges 0.11, 0.22, 0.33, 1, 1.66, 2.33, 3.33, 6.66, 13.33, 16.66 mg/l of butachlor and control group (no toxic concentration) were performed. LC_1, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99} were calculated for 24, 48, 72 and 96 hours. LC50 96h butachlor for *R. frisii Kutum* was obtained 0.258 ppm. These findings suggest that butachlor is moderately toxic and moderately irritating for this species. Clinical symptoms including irregular protrusion of the eyes and irregular swimming were observed.

Keyword: Butachlor, Caspian Kutum, Acute toxicity

1 Introduction

Man-made herbicides are among the most common sources of environmental pollutants worldwide. Herbicides are used to control weeds and are usually targeted to processes and target sites that are specific to plants. Exceptions to this general rule are uncouplers of oxidative phosphorylation and some herbicides that interfere with cell division [1]. Environmental Assessment indicates that up to one percent of used herbicides are expenditure to remove herbs and the residue causes contamination [2].

Butachlor is an herbicide of the acetanilide class and used as a selective pre-emergent herbicide with $C_{17}H_{26}ClNO_2$ molecular formula, which is the most commonly used in Iran to control a wide range of annual grasses and some broadleaf weeds. The herbicide has contaminated river water via the effluents from rice paddy fields [3]. Fish accumulates these chemicals by directly exposing itself to the chemicals present in water or through the food chain of ecosystem indirectly [4]. Butachlor is persistent in agricultural soil and water systems [5], posing a potential threat to the agro-ecosystem and human health [6; 7].

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Rutilus is a genus of fishes in the family Cyprinidae, are found in Europe and western Asia where there are about 15 species [8]. *Rutilus frisii Kutum* Kamensky, 1991, (Caspian Kutum) is native of Caspian Sea and rivers leading to. *R. frisii Kutum* main habitat is the southern part of the catchment area, particularly the coast of Iran [9]. In March and April, *R. frisii kutum* species migrate from Iranian waters (southern part of Caspian Sea) into estuaries and rivers for spawning [10]. Overfishing, Pollutants, overexploitation of bottom sediments in the rivers and dams which changed or blocked natural spawning locations of this species in Caspian Sea basins.

The contamination of aquatic ecosystems by butachlor has gained increased attention and several studies have been conducted on acute toxicity and the destructive effect of this herbicide in some fishes (11, 12, 13; 14] but data on acute toxicity of this herbicide on Caspian Kutum is scarce. In spite of the wide application of butachlor to farmlands and the possible ecotoxicological impact attached to its use, there is paucity of information on its effects on many Caspian basin fish species like Caspian Kutum. Sensitivity of various fish species is different on toxic substances, so toxicology tests are needed for different fish. LC_{50} is the lethal concentration required to kill 50% of the population and can be measured in vivo conditions. For this purpose, LC_{50} 96h is required of any ecotoxicology studies.

2 Materials and Methods

231 live specimens of Caspian Kutum were obtained from hatchery and ponds in Gorgan Province, Iran. Samples weighted 4±1 gr which acclimatized in 60×55×30 cm aquarium for 10 days. In order to measure biological capability and determine survival, fishes were kept in natural and toxin-free environment to determine natural mortality. Biological Oxygen Demand (BOD) was fixed on 690 mg/l pH: 8 to 8.28, temperature: 17±1 C° and hardness: 210 ppm (mg $CaCo_3$). Fishes were fed twice daily with Biomar feed at 2% body weight before the test, but feeding was stopped 24 h prior to and throughout the test. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. All experiments were performed 16-hr light and 8 hours of darkness. Static acute toxicity test was performed following guideline the Organisation for Economic Co-operation and Development OECD standard method [15]. 10 treated aquariums with concentration range 0.11, 0.22, 0.33, 1, 1.66, 2.33, 3.33, 6.66, 13.33, 16.66 mg/l butachlor and control group (no toxic concentration) were performed (21 fishes in each treatment). Mortality rates were recorded after 24, 48, 72 and 92 hours and dead fishes were quickly removed from the aquarium. The nominal concentration of toxin causing mortality (LC1،LC10 ، LC30 ،LC50 ،LC70 ، LC90 and LC99) within 24, 48, 72 and 92 hours for each toxin was calculated separately. . LC50 values for 24, 48, 72 and 96 h exposures were computed on the basis of probit analysis version 16/0 [16].

3 Results and Discussion

No mortality observed during acclimation. Result showed that within 96 h test, LC_{50} value declined with increasing toxin concentration and duration of exposure. It means that an LC_{50} value in the first 24 hours of the experiment always was higher than LC_{50} 96h (Table 1). According to the results LC50 96h Butachlor for *Rutilus frisii Kutum* were obtained 0.258. Hundred percent mortality of fishes were occurred only hours after exposure in 13.33 and 16.66 ppm concentration. The nominal concentration of toxin causing mortality (LC1،LC10 ، LC30 ،LC50 ،LC70 ،LC90 and LC99) within 24, 48, 72 and 92 hours for each toxin was calculated (Table 2). Clinical observation showed that fishes were exposed to butachlor had respiratory disorders which quickly opened and closed their gill cover. Fishes were anxious, had anharmonic breathing and unusual semi-circular swimming.

Table 1: Mortality rate in acute toxicity (LC_{50} 96h) rate for Caspian Kutum (n=21 each treatment)

Concentration (mg/l)	Number of Mortalities			
	24h	48h	72h	96h
Control	0	0	0	**0**
0.11	0	0	3	**5**
0.22	3	7	7	**11**
0.33	4	5	11	**19**
1.00	3	4	21	**21**
1.66	3	7	21	**21**
2.33	3	10	21	**21**
3.33	4	21	21	**21**
6.66	18	21	21	**21**
13.33	21	21	21	**21**
16.66	21	21	21	**21**

Table 2: Lethal concentration of butachlor (mg/l) (95% confidence intervals) depending on exposure time for Caspian roach and Pikeperch.

LC	96 h Exposure time	95% confidence intervals	
		Lower band	Upper band
LC_{10}	-	-	0.053
LC_{20}	0.020	-	0.142
LC_{30}	0.110	-	0.228
LC_{40}	0.186	-	0.353
LC_{50}	**0.258**	**0.134**	**0.552**
LC_{60}	0.330	0.213	0.805
LC_{70}	0.406	0.275	1.099
LC_{80}	0.496	0.337	1.453
LC_{90}	0.620	0.416	1.952
LC_{95}	0.723	0.479	2.366

Herbicides can influence aquatic organism directly and indirectly. Indirect effects are mediated by herbicide-induced changes in food webs or in the physical environment. Indirect effects can only occur if direct effects occur first and would be mediated by the killing of plants by herbicides [1]. As a popular herbicide, butachlor shows low toxicity to terrestrial animals following acute dermal, oral, and inhalation exposure [17]. The acute oral LD_{50} is 2000 mg kg^{-1} for rats, >10 000 mg kg^{-1} for ducks,>5 010 mg kg^{-1} for rabbits and >100 mg kg^{-1} for bees [18]. But the acute toxicity of butachlor is found to be highly toxic to aquatic organisms. Contrasting results seals the acute toxicity of butachlor in low concentrations. The LC_{50} 96h of butachlor is 0.52 mgL^{-1} for *Oncorhynchus mykiss*, 0.44 mgL^{-1} for *Lepoimis macrochirus*, 0.14 mgL^{-1} for *Ictalurus punctatus* and 0.32 mgL^{-1} for *Cyprinus carpio* [19]. Also Ateeq et al [4] reported that the acute toxicity of butachlor is 1.25 mgL^{-1} to *Tilapia zillii*. They stated that LC_{50} values of butachlor for *Heteropneustus fossilis* [4] and *Channa punctatus* [14] 2.34 ppm and 247.46 ppb, respectively. Also Geng et al. [20] determined the LC_{50} 96h values of butachlor in *Rana japonica* 1.40 mg/l while Gobic and Gunasekaran [21] obtained 96h LC_{50} of 0.515 mg/l for *Eisenia fietida*.

Dissolved oxygen, pH, size and age, type of species, water quality, concentration and formulation of test chemicals are the major factors in affecting toxicity of chemicals to aquatic organisms [22; 23; 11]. The safe level obtained for butachlor in the present study varied from 0 to 0.157 mg/l concentration for *Rutilus frisii Kutum* in 690 mg/l BOD, pH 8 to 8.3, 17±1 C° temperature and 210 ppm hardness. However these values are determined in vivo condition.

According to the results and Toxicity category rating inhalation LC_{50}, Butachlor for *Rutilus frisii Kutum* is lying in Toxicity category II: is Moderately toxic and Moderately irritating [24]. Dou to the vicinity of these two species location to farmland and orchards, further studies should be conducted on acceptable level of this herbicide and the usage must be restricted to avoid the sever risk associated with the use of the pesticide.

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