

ACUTE TOXICITY OF BUTACHLOR TO *RUTILUS RUTILUS CASPICUS* AND *SANDER LUCIOPERCA* IN VIVO CONDITION

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ABSTRACT

Herbicides are used to control weeds and are usually targeted to sites that are specific to plants. Butachlor is an herbicide of the acetanilide class, which is widely used in agricultural fields. At the present study, lethal concentration (LC50) of butachlor was calculated for *Rutilus rutilus caspicus* and *Sander lucioperca* with a mean weight of 4 ± 1 g (mean \pm SD). Our results indicated that LC50 96 h butachlor for *R. rutilus caspicus* and *S. lucioperca* were 0.342 and 0.760 ppm respectively. These findings suggest that butachlor is moderately toxic and moderately irritating for these two species. Clinical symptoms included irregular protrusion of the eyes and irregular swimming.

RESUMEN: Toxicidad aguda in vivo en la pez (*Rutilus rutilus caspicus*) y la lucioperca (*Sander lucioperca*) al butacloro.

Los herbicidas se utilizan para controlar el crecimiento de hierba mala y suelen ser dirigidos a los procesos y sitios de destino que son específicos de las plantas. El butacloro es un herbicida de la clase acetanilida que se utiliza comúnmente en campos agrícolas. En el presente estudio se calculó la concentración letal (LC50) de butacloro para especímenes de *Rutilus rutilus caspicus* y *Sander lucioperca* cuyo un peso medio fue de 4 ± 1 g (media \pm DE). Nuestros resultados fueron que se obtuvo 0.342 y 0.760 ppm de LC50 96 h butacloro en *R. rutilus caspicus* y *S. lucioperca*, respectivamente. Estos hallazgos sugieren que el butacloro es moderadamente tóxico y moderadamente irritante para estas dos especies. Se observaron síntomas clínicos incluyendo protrusión irregular de los ojos y natación irregular.

REZUMAT: Toxicitatea acută in vivo a butaclorului la *Rutilus rutilus caspicus* și *Sander lucioperca*.

Erbicidele sunt folosite pentru a controla buruienile și sunt de obicei orientate spre procesele și site-urile țintă specifice plantelor. Butaclor este un erbicid din clasa acetanilida, care este utilizat pe scară largă în terenuri agricole. În prezentul studiu, concentrația letală (CL50) de butaclor a fost calculată pentru *Rutilus rutilus caspicus* și *Sander lucioperca* cu o greutate medie de 4 ± 1 g (valoare medie \pm SD). Rezultatele noastre au indicat că LC50 96 h butaclor pentru *Rutilus rutilus caspicus* și *Sander lucioperca* sunt 0,342 și 0,760 ppm. Aceste rezultate sugerează ca butaclor este moderat toxic și moderat iritant pentru aceste două specii. Au fost observate simptome clinice, inclusiv proeminența neregulată a ochilor și înot neregulat.

INTRODUCTION

Aquatic ecosystems are environments rich in biodiversity (Paltenea et al., 2008; Pekarik et al., 2009; Lengyel et al., 2012; Ong'oa et al., 2013; Sanguila et al., 2015; Balasaheb et al., 2017) but constantly faced human activities threats. However, these environments are not the target for herbicide; nevertheless some studies sighted the presence of herbicides and their metabolites in surface water (Mansingh and Wilson, 1995; Tsuda et al., 1996). Herbicides are the most prevalent environmental pollutants worldwide (Khoshnood, 2017). Herbicides are used to control weeds and are targeted to sites specific to plants, exceptions are uncouples of oxidative phosphorylation and herbicides that interfere with cell division (Solomon et al., 2013).

Butachlor is an herbicide of the acetanilide class and is used as a selective pre-emergent herbicide with $C_{17}H_{26}ClNO_2$ molecular formula. Butachlor is one of the most widely used chloro-acetanilide herbicide for the control of annual grasses in rice fields and many broadleaf weeds (Nwani et al., 2013). The herbicide has contaminated river water via the effluents from rice paddy fields. Fish accumulate these chemicals by directly exposure to the chemicals present in water or indirectly through the ecosystem food chain (Ateeq et al., 2002). Butachlor is persistent in agricultural soil and water systems, posing a potential threat to the agro-ecosystem and human health (Debnath et al., 2002; Vajargah et al., 2013).

Rutilus rutilus caspicus (Caspian roach) is one of the most valuable inhabitants of the Caspian Sea, however, the Caspian roach population has recently declined in the Caspian Sea because of overfishing, a degraded habitat, and pollution (Hoseini and Nodeh, 2012). *Sander lucioperca* (Pikeperch) is found in freshwater and brackish water, which is a semi-anadromous, cool-water species in the Caspian Watershed (Ural, Volga, Kura, and Sefid Roud rivers) and in the basins of the Black, Azov, Aral, and Baltic Seas (Abdolmalaki and Psuty, 2007). As a predator and commercially valuable species, pikeperch constitute an important component of the Caspian ichthyofauna, both ecologically and commercially (Abdolmalaki and Psuty, 2007).

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 fish (Tilak et al., 2007; Geng et al., 2010; Chang et al., 2013; Nwani et al., 2013; Vajargah et al., 2014) but data on acute toxicity of this herbicide on Pikeperch and Caspian roach is scarce. Despite the large use of butachlor in agriculture and the potential ecotoxicological impact, there is a scarcity of data on its effects on many Caspian Basin fish species like Caspian roach or Pikeperch. Sensitivity of various fish species is different on toxic substances, so toxicology tests are needed for different fish. For this purpose, LC50 96 h is required of any ecotoxicology studies.

MATERIAL AND METHODS

231 live specimens of Caspian roach and Pikeperch were obtained from hatchery and ponds in Gorgan Province. Samples weighted 4 ± 1 g acclimatized in $60 \times 55 \times 30$ cm aquarium for 10 days. In order to measure biological capability and determine survival, fish were kept in natural and toxin-free environment to determine natural mortality. Physical and chemical parameters of water are represented in table 1. The fish 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 faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Commercial butachlor (Machete, EC 60%) was purchased from Exir Keshavarzi Co, Iran. All Experiments were performed with 16-hours of light and eight hours of darkness. Static acute toxicity test was performed following guideline the OECD standard method (OECD, 1989). 10 treated aquariums with concentration ranges 0.11, 0.22, 0.33, 1, 1.66, 2.33, 3.33, 6.66, 13.33, 16.66 and control groups (no toxic concentration) were

performed. Mortality rates were recorded after 24, 48, 72 and 92 hours and dead fish 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.

This work was approved by the ethical committee of GAU University. To minimize suffering of the fish, all animals were exposed to clove essence, and a low dose of anesthesia; hypothermia prior to euthanasia and eventually spinal cord dislocation for euthanasia.

Table 1: Physico-chemical properties of the test water.

Physico-chemical indices	24 h	48 h	72 h	96 h
pH	8.22	8.28	8.25	8.30
BOD	69%	68%	67%	68%
Temperature (C°)	17.9	17.8	17.9	17.7
Total hardness (MgCaCO ₃)	210	210	210	210

RESULTS

No mortality was observed during acclimation (Tabs. 2 and 3). Result showed that within a 96 h test, the LC50 value declined with increasing toxin concentration and duration of exposure. This shows that an LC50 value in the first 24 hours of the experiment was always higher than LC50 96 h. According to the results LC50 96 h Butachlor for *Rutilus rutilus caspicus* and *Sander lucioperca* were obtained 0.342 and 0.760 ppm respectively. Fish had a 100% mortality rate only hour 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 (Tab. 4). It was significantly different between experiment fish, that in all steps, mortality was higher for Caspian roach during exposure to butachlor, Caspian roach is more sensitive in comparison with Pikeperch.

Table 2: Mortality rate in acute toxicity (LC₅₀ 96h) rate for Caspian roach (n = 21 each treatment).

Concentration (mg/l)	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.11	0	0	3	5
0.22	0	3	4	7
0.33	2	4	11	17
1	2	3	15	19
1.66	5	9	19	21
2.33	4	9	21	21
3.33	4	21	21	21
6.66	15	21	21	21
13.33	21	21	21	21
16.66	21	21	21	21

Table 3: Mortality rate in acute toxicity (LC₅₀ 96 h) rate for Pikeperch (n = 21 each treatment).

Concentration (mg/l)	24 h	48 h	72 h	96 h
Control	0	0	0	0
0.11	0	0	2	4
0.22	0	0	3	7
0.33	1	3	9	13
1	2	4	10	13
1.66	3	6	11	17
2.33	3	11	15	19
3.33	6	15	21	21
6.66	11	19	21	21
13.33	21	21	21	21
16.66	21	21	21	21

Table 4: Lethal concentration of butachlor (mg/l) (95% confidence intervals) depending on exposure time for Caspian roach and Pikeperch.

LC	96 h		95% confidence intervals			
	Exposure time		Caspian roach			
	Caspian roach	Pikeperch		Caspian roach	Pikeperch	
LC ₁₀	–	–	LC ₁₀	–	–	LC ₁₀
LC ₂₀	0.052	–	LC ₂₀	0.052	–	LC ₂₀
LC ₃₀	0.162	0.247	LC ₃₀	0.162	0.247	LC ₃₀
LC ₄₀	0.255	0.512	LC ₄₀	0.255	0.512	LC ₄₀
LC ₅₀	0.342	0.760	LC ₅₀	0.342	0.760	LC ₅₀
LC ₆₀	0.430	1.008	LC ₆₀	0.430	1.008	LC ₆₀
LC ₇₀	0.523	1.273	LC ₇₀	0.523	1.273	LC ₇₀
LC ₈₀	0.632	1.584	LC ₈₀	0.632	1.584	LC ₈₀
LC ₉₀	0.784	2.015	LC ₉₀	0.784	2.015	LC ₉₀
LC ₉₅	0.909	2.370	LC ₉₅	0.909	2.370	LC ₉₅

DISCUSSION

Acute and chronic toxicity tests are widely used to evaluate the toxicity of chemicals on non-target organisms (Yalsuyi and Vajargah, 2017). Exposure time is one of the effective factors in toxic ratios (Vajargah and Hedayati, 2014). When fish are exposed to a constant concentration of the toxin, fish tolerance is diminished over time and the toxin has a greater effect (Hedayati et al., 2014). However, when the toxin accumulates in fish tissue it increases the adverse effects on the body and causes a decrease in LC₅₀ 96 h. Overall LC₅₀ for butachlor in Caspian roach and Pikeperch showed a decreasing trend over 96 hours and in listed physicochemical conditions. Result of LC₅₀ 96 h for toxin showed that the rate of LC₅₀ decreases with increasing toxin concentration and duration of exposure. The results of the acute toxicity of diazinon and Deltamethrin on *Cyprinus carpio* (common carp) concedes shows decreasing trend in LC₅₀ 96 h (Svoboda et al., 2001, 2003).

Herbicides can influence aquatic organisms 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 (Solomon et al., 2013). Results are limited on acute toxicity of butachlor in fish. The influence of butachlor on fish toxicity was evident. Chang et al. (2013) studied the effects of butachlor on reproduction and hormone levels in adult zebra fish (*Danio rerio*). They declared that butachlor adversely affected the normal reproductive success of zebra fish and disrupted the thyroid and sex steroid endocrine systems. Ateeq et al. (2002, 2006) stated that butachlor causes anisochromasia and anisocytosis of erythrocytes in *Clarias batrachus*. They reported that this herbicide is genotoxic and cytotoxic and is able to induce apoptosis both at molecular as well as cytological level. Lasheidani et al. (2008) reported that butachlor has adverse effect on density and volume of Caspian Kutum *Rutilus frisii kutum*. They stated that the number of abnormal sperm increased by $28.6 \pm 1.92\%$ in fish exposed to high butachlor toxicity (75% of its LC₅₀ value).

Previous studies demonstrated LC₅₀ values of butachlor for *Heteropneustus fossilis* (Ateeq et al., 2006) and *Channa punctatus* (Tilak et al., 2007) 2.34 ppm and 247.46 ppb, respectively. Also Geng et al. (2005) determined the LC₅₀ 96 h values of butachlor in *Rana japonica* 1.40 mg/l while Gobic and Gunasekaran (2010) obtained 96 h LC₅₀ of 0.515 mg/l for *Eisenia fetida*.

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 in aquatic organisms (Nwani et al., 2010; Vajargah et al., 2013). The safe level obtained for butachlor in the present study varied from 0 to 0.157 mg/l concentration for *Rutilus rutilus caspicus* and *Sander lucioperca* in 68% BOD, pH 8.28, $17 \pm 1^\circ\text{C}$ temperature and 210 ppm hardness. However these values are determined in vivo condition.

Herbicides are rapidly absorbed in fish gills which cause respiratory limitations (Masud and Singh, 2013). Fish that were exposed to butachlor had respiratory disorders which quickly opened and closed their gill cover. Fish were anxious, had a harmonic breathing and unusual semi-circular swimming. These findings coincide with studied acute toxicity of butachlor in other fish species, such as *Tilapia zillii* (Nwani et al., 2013) and *Oreochromis niloticus* (Bekeh et al., 2011).

According to the results and Toxicity category rating inhalation LC₅₀, butachlor for *Rutilus rutilus caspicus* and *Sander lucioperca* are lying in toxicity category II: is Moderately toxic and Moderately irritating (Toxicity Category, 2009). Due 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 severe risk associated with the use of the pesticide.

CONCLUSIONS

Few surveys have focused on the effects of butachlor on mortalities indices of various fish species, the current investigation examined LC₅₀ of this herbicide on Caspian roach and Pikeperch. Since the species is edible, infections in turn affect human health. Furthermore, the results show that butachlor is too poisonous for these edible fishes and also Caspian roach is more sensitive than pikeperch.

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