Global Veterinaria 8 (3): 280-284, 2012
ISSN 1992-6197

Acute Toxicity Assessment of Tribenuron-Methyl Herbicide in Silver Carp (*Hypophthalmicthys molitrix*), Common Carp (*Cyprinus carpio*) and Caspian Roach (*Rutilus rutilus caspicus*)

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Abstract: A short term definitive tests by the static non-renewal bioassay method was made inthe laboratory to determine the acutetoxicity (LC_{50}) of technical grade of tribenuron-methyl herbicide (95%) in three non-target freshwater fish. Groups of ten silver carp (*Hypophthalmicthys molitrix*), common carp (*Cyprinus carpio*) and caspian roach (*Rutilusrutiluscaspicus*) were exposed to the tribenuron-methyl (0, 50, 100, 200, 300 and 500 ppm). Data obtained from the tribenuron-methyl acute toxicity tests were evaluated using the probitanalysis statistical method. The estimated 96 hrs LC_{50} values (95% confidence limits) of tribenuron-methyl for caspian roach, common carp and silver carpwere152.74 (112.10-197.98), 289.08 (240.93 - 357.29) and 139.45 (105.32- 176.69) ppm, respectively.At various concentrations of tribenuron-methyl (100-500 ppm), fish showed abnormal behavioral responses (rapid gill movement, gulping air at the surface, erraticswimming, loss of equilibrium, reduction of body pigment and increased mucus secretion). Results of the present study indicate that technical grade of tribenuron-methyl is toxic to studied fish.

Key words: Acute Toxicity · Herbicide · Tribenuron-Methyl · Behavioral Changes

INTRODUCTION

With exploding population and increasing industrialization and urbanization, water pollution by agricultural run-off, municipal and industrial sources has become a great major concern for the welfare of humanity [1]. Pesticides are used in agriculture to control weed (herbicides), pests including insects (insecticides) and plant diseases (fungicides). In present industrialized world, people use large amounts of herbicides [2]. These herbicides may enter from agricultural run-off, industrial effluent and other sources into aquatic media and produce undesirable side effects on biological and functional properties by changing the species composition of an algal community [2]. Tribenuron-methyl is a post emergence sulfonylurea herbicide, acting through foliar uptake with little or no soil activity [3]. Sulfonylureas are widely used herbicides that have characters of high selectivity, very low dosage rates and low acute mammalian toxicity [4, 5]. A major route of dissipation in the environment is aqueous hydrolysis. Hydrolysis half-lives for tribenuron-methyl were less than one day at pH 5 and 15.8 days at pH 7 but it is reported to be stable at pH 9 [3]. High durability and leaching potential of some sulfonylureas, however, also increases the risk of effects on non-target organisms such as aquatic micro algae and other aquatic organisms [6]. Effect of herbicides in terrestrial ecosystems and the toxicity of herbicides to animals have been well documented. However, less is known about herbicides toxicity to aquatic ecosystems, including fish [7]. Toxicity tests allow the determination of pollution effects, providing direct evidence of the biological response of aquatic organisms to contaminants [8]. Thus, the aim of this study was to determine the acute

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toxicity of technical grade of tribenuron-methyl on some valuable fish and evaluated behavioral changes of: silver carp (*Hypophthalmicthys molitrix*), common carp (*Cyprinus carpio*) and caspian roach (*Rutilusrutiluscaspicus*) exposed to different concentrations.

MATERIALS AND METHODS

Experimental Animals: Live specimens of the silver carp (40.68 ± 5.87 g, 15.34±3.12 cm), common carp (15.35±2.36g, 12.14±2.31cm) and caspian roach (3.5±0.32 g, 7.3±0.65 cm) were purchased from Bony Fish Propagation and Rearing Center of Sijeval (Bandar Torkaman,Gorgan, Iran). Fish were transferred to Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources and acclimated to the laboratory conditions for 2 weeks. Fishes were parceled in 12 fiberglass tanks (500 L). Fish were fed commercial trout food (Biomare, 0.8 mm in diameter, 45% crude protein and 24% crude lipid) during acclimationperiod under continuous aeration condition and were fasted for 24 hrs before the start of the experiments.

Water: Water (dechlorinated tap water) was renewed daily and the water quality parameters were measured twice a week (using portable pH/temp meter, oxygen meter, photometer 7100, Wagtecch, UK) during acclimation period. Moreover, water quality parameters were monitored daily during experiments period with no substantial changes recorded throughout acclimation and experiments period (Table 1).

Toxicant: Technical grade tribenuron-methyl (95% pure) manufactured Shanghai MIO Chemical Co., Ltd, China and supplied by Gol Sam Co, Iran, was used for evaluation of its toxicity to fish.

Acute Toxicity Experiment: The stock solution of tribenuron-methyl was prepared by dissolving 50 gtechnical grade tribenuron-methyl in 100 L aerated and dechlorinated tap water (solubility in water, 2.04 g/L at 20°C at pH 7). The acute toxicity test was conducted following the Organization for Economic Cooperation and Development guideline under static non-renewable test conditions [9]. Uniform sized rectangular glass aquaria (120 L capacity) were used for acute toxicity test. In each glass aquarium total volume of water was maintained at 100 L and was provided with non-stop aeration. 10 fish (for each species) were exposed to 0, 50, 100, 200, 300 and 500 ppmtribenuron-methyl. Fish mortality was recorded at an interval of 24 hrs over a period of four days. Dead fish were immediately removed by dip net to avoid possible deterioration of the water quality. For statistical analyses, the statistical software EPA Probit Analysis V. 1.5 was used. Data obtained from the tribenuron-methyl acute toxicity tests were evaluated using Finney's probit analysis statistical method. All data were accepted if calculated chi square for heterogeneity was lower than the tabular value at the 0.05 level [10].

RESULTS AND DISCUSSION

The physico-chemical characteristics of the test water are given in Table 1. Water quality parameters throughout the acclimation and experiments period were similar and relatively stable.

An increase in number of mortalities with an increase in concentration of the herbicide was observed and has been presented in Tables 2, 3 and 4. There were 100% mortality at 500 ppm concentration within the 24hrs after dosing for all fishes and no mortality at 50 ppm within the exposure times for all species. No mortality was observed in the control group during the experiment. The changes in behavioral response are the most sensitive indicators of potential toxic effects [10]. Fish in the control experiment appeared active and healthy throughout the test period. The changes in behavioral response started 5hrs after dosing. The 50 ppm (lowest) concentration showed behavior similar to the control group. The higher concentrations (over 50 ppm) showed abnormal behavioral responses (rapid gill movement, gulping air at the surface, erratic swimming, loss of equilibrium, reduction of body pigment and increased mucus secretion) in all fishes. At the highest concentration (500 ppm), all these responses were observed at high

Table 1: Water quality parameters during the experimental period

	Temperature (°C)	Total hardness (mgL^{-1} as $CaCO_3$)	pH	DO (mgL^{-1})
Acclimationperiod	19±1	293±10	7.59±0.53	7.8±0.5
Experiments period	19.75±1.50	301±15.45	7.88±0.76	7.98±0.25

Table 2: Cumulative mortality of caspianroach after 24, 48, 72 and 96 hrs of exposure to different tribenuron-methyl concentrations (n=10, each concentration)

	No. of mortality			
Concentration (ppm)	24 hrs	48 hrs	72 hrs	96 hrs
Control	-	-	-	-
50	-	-	-	-
100	2	3	3	3
200	4	5	5	5
300	10	10	10	10
500	10	10	10	10

Table 3: Cumulative mortality of common carp after 24, 48, 72 and 96 hrs of exposure to different tribenuron-methyl concentrations (n=10, each concentration)

	No. of mortality			
Concentration (ppm)	24hrs	48 hrs	72 hrs	96 hrs
Control	-	-	-	-
50	-	-	-	-
100	-	-	-	-
200	-	-	-	1
300	4	5	5	5
500	10	10	10	10

Table 4: Cumulative mortality of silver carp roach after 24, 48, 72 and 96 hrsof exposure to different tribenuron-methyl concentrations (n=10, each concentration)

	No. of mortality			
Concentration (ppm)	24 hrs	48 hrs	72 hrs	96 hrs
Control	-	-	-	-
50	-	-	-	-
100	1	2	2	2
200	4	6	6	8
300	10	10	10	10
500	10	10	10	10

intensities. The abnormal behavioral responses increased with increasing concentration and exposure time. Similar behavioral responses determined in this study have been observed with the spotted snakehead *Channa Punctatus*exposed to various concentrations of atrazine herbicide [11].

Median lethal concentration (LC_{50}) is the most widely accepted basis for acute toxicity test and it is the concentration of a test chemical which kills 50% of the test organisms after a particular length of exposure, usually 96 hrs [11]. The estimated 96 hrs LC_{50} values (95% confidence limits) of tribenuron-methyl for caspian roach, common carp and silver carp were152.74 (112.10-197.98), 289.08 (240.93 - 357.29) and 139.45 (105.32-176.69) ppm, respectively (Table 5). Moreover, toxicity testing statistical endpoints is in Figure 1. As there can found 96 h LC_{50} of tribenuron-methyl in common carp was higher than other species, however silver carp had the lowest one. In other words,tribenuron-methyl was more toxic to silver carp andcaspian roach.

Tribenuron-methyl has not been classified according to IPCS hazard. Whereas European Union classifications are N R 50/53 ECB, very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment [3].

The present results show that technical grade of tribenuron-methyl is toxic to studied fish. Despite the common use of tribenuron-methyl and sulfonylurea herbicides in aquaculture, there is little information about their influence in fish. Review of toxicity of tribenuron-methyl by FAO documents 96hrs LC_{50} value of tribenuron-methyl for rainbow trout *Oncorhynchusmykiss* as 738 ppm [3]. The effects of herbicides will depend upon depth of water reservoir, rate of flowing water, fish densities and fish stock composition [12]. Hardness of water, pH and temperature may also be key factors [13]. The toxicity of sulfonylurea herbicides on micro-algae and periphyton communities are well documented [2-6]. Based on such studies it is claimed that sulfonylureas are toxic to micro-algae and periphyton communities in the micromolar range. A few reports involved the differential responses of fish to herbicides. Glusczak *et al*. [14] observed increase in hepatic glycogen, but a reduction in muscle glycogen in silver catfish (*Rhamdiaquelen*) exposed to the herbicide glyphosate (0.2 and 04.0 ppm) after 96 hrs. Fonseca *et al.* [15] found a decrease in brain AChE activity in piava freshwater fish (*Leporinusobtusidens*) following 2, 4-D herbicide intoxication. The tribenuron-methyl is a widely used herbicide in Iran due to its low cost and good effect. Tribenuron-methyl is used for the control of a wide range of broad-leaf weeds in cereal crops including wheat (winter, spring and durum), barley (winter and spring), oats (winter and spring), rye and triticale (2004). The results indicate that presence oftribenuron-methyl in the aquatic environment may have a significant effect on studied fish populations.

Table 5: Lethal concentration (LC 1-99) 96 h of tribenuron-methyl for caspian roach, common carpand silvercarp.

	Concentration (ppm) (95 % of confidence limits)		
Point	Caspian roach	Common carp	Silver carp
LC 1.00	48.86(16.27-75.91)	159.50(66.21-204.31)	60.34(22.64-85.99)
LC 5.00	68.23(29.65-97.09)	189.85(100.69-231.01)	77.12(36.66-102.89)
LC 10.00	81.52(40.61-111.30)	208.33(125.25-247.94)	87.90(47.15-113.79)
LC 15.00	91.93(50.04-122.47)	221.80(144.57-261.08)	96.02(55.70-122.20)
LC 50.00	152.74(112.10-197.98)	289.08(240.93-357.29)	139.45(105.32-176.69)
LC 85.00	253.79(196.05-409.96)	376.77(317.17-618.98)	202.54(161.89-314.22)
LC 90.00	286.18(217.36-501.36)	401.14(333.01-716.53)	221.24(174.93-368.91)
LC 95.00	341.93(250.94-681.82)	440.17(356.47-893.67)	252.16(194.63-471.71)
LC 99.00	477.44(323.19-1233.72)	523.92(402.11-1362.26)	322.28(234.27-759.10)
Chi-Square (calculated)*	3.95	0.390	0.358
Intercept ± SE	-5.26±2.46	-17.16 ±6.73	-8.71±3.61
Slope ± SE	4.70 ±1.10	9.00 ±2.74	6.39 ±1.65

*Chi-Square for Heterogeneity (tabular value at 0.05 level) = 7.81

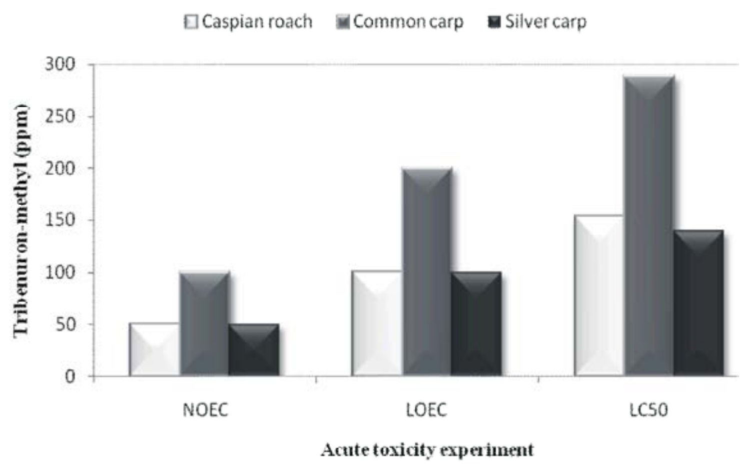

Fig. 1: Acute toxicity testing statistical endpoints of tribenuron-methyl

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