Caspian J. Env. Sci. 2014, Vol. 12 No.2 pp. 225~232

[Research]

Assessing the sensitivity of Caspian Kutum (*Rutilus kutum*) and the endangered Caspian trout (*Salmo trutta caspius*) to acute toxicity of nonylphenol

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(Received: July. 29. 2013, Accepted: Dec. 27. 2013)

ABSTRACT

Toxicity tests are commonly used as a tool to determine the standards of water quality for chemicals and to discover appropriate organisms as bioindicators in toxicological studies, and also could be used as an essential tool for evaluation of the pollutant effects in aquatic ecosystems. The aim of the present study was to evaluate the sensitivity of two Caspian fish species, Caspian trout as an endangered species and Caspian Kutum using the static acute toxicity in response to nonylphenol, which is widely discharged into the Caspian Sea environment.The fish were exposed to various concentrations of nonylphenol for 96 hours to determine the LC_{50}. The experimental nonylphenol concentrations were consisted of five exposure groups for each species: 100, 150, 200, 250, and 300 µg/l for Caspian trout; 400, 800, 1200, 1600 and 2000 µg/l for Caspian Kutum, with a control group for each of them.Using probit analysis, the 96 h LC_{50} of nonylphenol to Caspian trout and Caspian Kutumwas determined to be 204.78 and 1262.36 µg/l, respectively. In addition, Caspian trout was approximately 6 times more sensitive than Caspian Kutum. Nonylphenol was reflected to be "highly toxic" to Caspian trout and "moderately toxic" to Caspian Kutum. The results could be considered in preparing plans for conservation and restocking management of Caspian Kutum and the endangered Caspian trout.

Keywords: *Nonylphenol,Toxicity test, Caspian kutum, Caspian trout*

INTRODUCTION

Widespread use of industrial, household and agricultural chemicals potentially poses a hazard to threatened and endangered species for the reason that the distribution of endangered species is limited and further adverse effects on their populations could lead to more extinction. Conservation of endangered species from chemical hazards is principally based on standardized toxicity tests employing standard test organisms as surrogates for other species. Nevertheless, the sensitivity of threatened and endangered species populations may not be represented by surrogate species (Sappington *et al.*, 2001). Therefore, determining the sensitivities of endangered and threatened species, could help us to protect their populations by using standard regulatory approaches (Dwyer *et al*., 2005).

Toxicity tests are widely being utilized as a tool to determine water quality standards for chemicals and to discover appropriate organisms as bioindicators in toxicological studies, and also it could be used as an essential tool for evaluation of the pollutant effects in aquatic ecosystems. The median lethal concentration (96 h LC_{50}) tests carried out to determine the vulnerability and survival potential of organisms to particular toxicant (Boudou and Ribeyre, 1997). LC_{50} values are indicative of the sensitivity and susceptibility of an organism to a particular toxicant. A species showing lower levels of LC_{50} in response to a particular toxic material could be considered as a suitable sentinel organismin the field studies concerning that toxicant.Nonylphenol is a toxic microbial degradation product of nonylphenolethoxylate, a compound which is extensively utilized in the production of non-ionic surfactants, paints, emulsifiers, lubricants, pesticides and detergents (Soares *et al.*, 2008). Nonylphenol is more stable and persistent in the aquatic environment than its parent compound (Soares *et al.*, 2008). Nonylphenol and its ethoxylates are acutely toxic to various organisms, including fish, mollusks, crustaceans and amphibians (Servos, 1999). Widespread use of afore-mentioned products, have been led to release of nonylphenol into the aquatic ecosystems through industrial, agricultural and municipal effluents (Mao *et al.*, 2012). In the Southern Caspian Sea waters, high concentrations of nonylphenol have been reported with concentrations ranging from 0.05 to 29 μg/g dry weight of surface sediment samples in the vicinity of urban areas(Mortazavi *et al.*, 2012).

Caspian trout (*Salmo trutta caspius*) and Caspian Kutum (*Rutilus kutum*) are brackish water species of the southern Caspian Sea that their population hassharply been declined over the past two decades (Shirangi *et al.*, 2011; Vera *et al.*, 2011; Ghaninejad & Abdulmaleki, 2007). A variety of factors, especially pollution, are contributing to decline and extinction of these fish population (Kiabi *et al.*, 1999). These species are artificially being propagated in freshwater and in order to recruit their stock, released to the entering rivers of the Caspian Sea.

Regarding to the existence of high concentrations of nonylphenol in the southern Caspian Sea environment (Mortazavi *et al.*, 2012), short-term exposure to this substance could threat the survival of the released fish during the critical period in which they adapt to their vicinity environmental conditions.

To author's knowledge, the sensitivity of the Caspian species in response to acute toxicity of nonylphenolhas not been investigated to date. Thus, by simulating the freshwater condition of the rivers, the present study aimed to compare the sensitivity of Caspian trout and Caspian Kutum, the representative species of Salmonidae and Cyprinidae families, with high andlower sensitivity (Naylor, 1995; Dwyer *et al.*, 2005), respectively, against nonylphenol, which is widely being discharged into the Caspian Sea environment.

MATERIALS AND METHODS

Test water characteristics

Salinity, conductivity, pH and dissolved oxygen were measured by Consort multi-parameter analyzer (Consort C863, Belgium). Total hardness was measured using PalintestHardicol test by Palintest Photometer (Palintest 8000, England) based on a colorimetric method.

Acute toxicity test

Juvenile Caspian trout and Caspian Kutum (5 ± 2 g) were purchased from a local commercial hatchery and transported to laboratory in winter 2013, and maintained in dechlorinatedtap water (Dechlorination using sodium thiosulphate together with aeration at 24 hours before using the water). The fish were acclimated for one week prior to the experiment and fed twice daily at the rate of 2% body weight by commercial feed until 24h prior to the initiation of the test. Over the experimental and acclimation period, fish were maintained in 12:12 (Light:Dark) photoperiod and average temperature of 13±2 °C for Caspian trout and 20±2 °C for Caspian Kutum. Before the experiment, a stock solution of nonylphenol (Sigma-Aldrich, PESTANAL, analytical standard, technical mixture, 85%, linear formula: $CH_3(CH_2)_8C_6H_4OH$ was prepared by dissolving in absolute ethanol. Static acute toxicity test in this study was conducted according to the procedure as described in the OECD (Organization for Economic Co-operation and Development) guideline (OECD guideline No. 203, 1992). Preliminary tests were carried out to determine the appropriate range of toxicity. According to these preliminary tests, concentrations of nonylphenol for acute toxicity test were selected for each species.In order to determine the median lethal concentration, fishes (10 fish per concentration for each species) were exposed for 96 h to various nominal concentrations of nonylphenol. The fish were maintained in 50L fiberglass tanks containing aerated water and were not fed during the toxicity test. The experiments were consisted of a control group and five nonylphenol concentration groups (100, 150, 200, 250 and 300 µg/l) forCaspian trout and five concentrations (400, 800, 1200, 1600 and 2000 µg/l) for Caspian Kutum. Animal were exposed to these concentrations for 96 h without feeding and water exchange. The survival of fish in relation to different nonylphenolconcentrations were evaluated and counted over the exposure period at 24, 48, 72 and 96 h, then the dead fish were removed immediately. Median lethal concentrations of nonylphenol for these two species were calculated by Probit analysis using SPSS software (version 17.0).

RESULTS

Test water characteristics

The physical and chemical properties of the test water are presented in Table 1.

Table 1. Physical and chemical properties of the test water

Parameter	Values
pH	7.9-8.2
Salinity	0.4 (ppt)
Dissolved Oxygen	9±0.3 (mg/l)
Conductivity	800 (µS/cm)
Total Hardness	102 (mg/l $CaCO_3$)

Acute toxicity test

There was a concentration-dependent increase and a time-dependent decrease in the mortality rate in each of the tests. For both species, it was observed that most of the mortalities occurred within the first 24h of exposure. With increasing concentrations of nonylphenol, the mortality of both species was increased (Fig. 1). No mortality was observed in the control group for Caspian trout and Caspian kutum, andalso

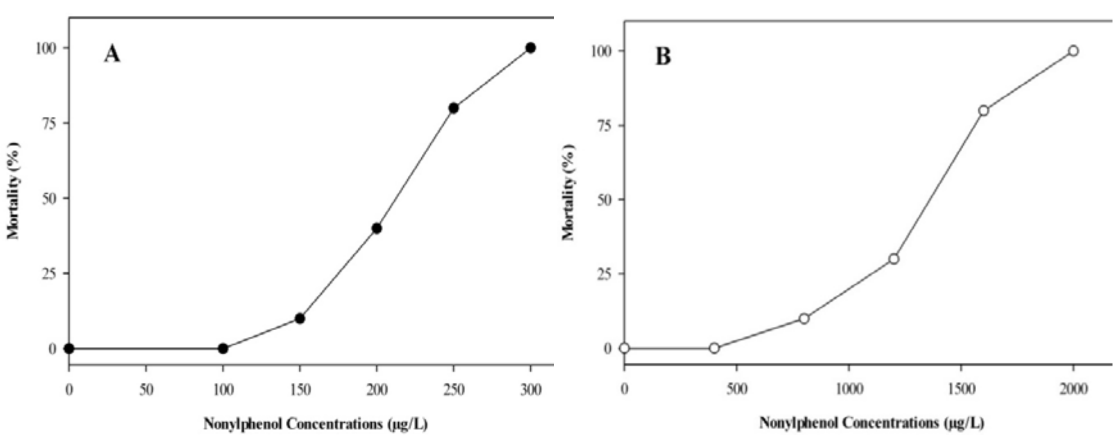

Fig 1. Mortality of Caspian trout (A) and Caspian Kutum (B) after 96 h exposure to nonylphenol

The 24, 48, 72 and 96 h LC_{50} (with 95% confidence limits) values for each species are shown in Tables 2 and 3. Calculated 96 h LC_{50} values of nonylphenol for Caspian 72h, were 218.78, 214.01 and 209.37 µg/l trout and Caspian Kutum was 204.78 and 1262.36µg/l, respectively. Also, the concentrations of nonylphenol that caused mortality in 50% of the fish within the 24, 48and l to Caspian trout, and 1414.29, 1299.94 and 1262.36 µg/l to Caspian Kutum, respectively.

Results show that, Caspian trout appeared to be approximately six times more sensitive than Caspian Kutum. Moreover, maximum acceptable toxicant concentration (MATC: one tenth of 96 h LC_{50}), no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) of nonylphenol were determined as 20.48, 100 and 126.17µg/l to Caspian trout and 126.24, 400 and 670.08µg/l to Caspian Kutum, respectively.

Table 2. Lethal concentrations (LC_{1-99}) of nonylphenol for Caspian trout

	Concentrations (µg/l) (95% of Confidence Limits)							
LC	**24 h**	**95% C.L.**	**48 h**	**95% C.L.**	**72 h**	**95% C.L.**	**96 h**	**95% C.L.**
LC_1	134.30		128.64		130.72		126.17	
LC_{10}	167.20		161.68		161.52		156.83	
LC_{20}	183.37		178.02		176.57		171.87	
LC_{30}	195.99		190.81		188.28		183.60	
LC_{40}	207.45		202.47		198.90		194.26	
LC_{50}	**218.78**	193.97-245.93	**214.01**	188.56-240.84	**209.37**	185.12-234.11	**204.78**	179.99-229.11
LC_{60}	230.72		226.21		220.39		215.88	
LC_{70}	244.22		240.03		232.83		228.41	
LC_{80}	261.02		257.28		248.28		244.00	
LC_{90}	286.26		283.28		271.41		267.41	
LC_{99}	356.41		356.04		335.36		332.39	

Table 3. Lethal concentrations (LC_{1-99}) of nonylphenol for Caspian Kutum

	Concentrations (µg/l) (95% of Confidence Limits)							
LC	**24 h**	**95% C.L.**	**48 h**	**95% C.L.**	**72 h**	**95% C.L.**	**96 h**	**95% C.L.**
LC_1	942.90		705.23		670.08		670.08	
LC_{10}	1131.20		928.14		890.55		890.55	
LC_{20}	1221.34		1041.93		1003.86		1003.86	
LC_{30}	1290.77		1132.54		1094.41		1094.41	
LC_{40}	1353.20		1216.19		1178.22		1178.22	
LC_{50}	**1414.29**	1234.64-1583.82	**1299.94**	1107.69-1504.29	**1262.36**	1066.38-1462.83	**1262.36**	1066.38-1462.83
LC_{60}	1478.13		1389.46		1352.50		1352.50	
LC_{70}	1549.63		1492.08		1456.08		1456.08	
LC_{80}	1637.71		1621.84		1587.42		1587.42	
LC_{90}	1768.21		1820.68		1789.40		1789.40	
LC_{99}	2121.35		2396.16		2378.14		2378.14	

DISCUSSION

Caspian trout population has been disappeared from a large numbers of its historic areas in the southern part of the Caspian Sea, and its wild populations are close to complete extinction(Shirangi *et al.*, 2011; Vera *et al.*, 2011). In addition, Caspian kutum populations have been sharply declined over the past decades (Ghaninejad & Abdulmaleki, 2007). Elevated levels of pollutants, overfishing and destruction of natural spawning areas are considered as the major causes of the population decline for both species (Kiabi *et al.*, 1999). Evaluation of the tolerance of endangered and threatened species against toxicants could be helpful for protecting their populations(Dwyer *et al.*, 2005). This study was conducted to compare the sensitivity of two Caspian fish species, Caspian trout (as an endangered species) and Caspian Kutum, to nonylphenol using the 96 h LC_{50} values.

In the present study, calculated 96 h LC_{50} values of nonylphenol for Caspian trout and Caspian kutum was 204.78 and 1262.36 µg/l, respectively, which were in the ranges observed in the previous studies. Previous studies using different test methods in various organisms showed that nonylphenol is acutely toxic to fish with 96 h LC_{50} values ranging from 17 to 3000 µg/l and for most species range from 100 to 500 µg/l (Baldwin *et al*., 1997; Servos, 1999; Bhattacharya *et al*., 2008). Winter flounder (*Pleuronectes americanus*) was reported to be the most sensitive fish species in response to nonylphenol acute exposure, with a obtained 96 h LC_{50} of 17 µg/l (Lussier *et al*., 2000), whereas the African catfish (*Clariasgariepinus*) was the least sensitive fish, with calculated 96 h LC_{50} values of 3480 µg/l (Kumaran *et al*., 2011). A recent study has reported 221, 128 and 209 µg/l as 96 h LC_{50} values of nonylphenolto rainbow trout alevin (240 mg), juvenile fathead minnow (*Pimephales promelas*) and juvenile bluegill, respectively(Spehar *et al*., 2010). Kashiwada *et al*. (2002) reported that the adult medaka LC_{50} for *p*-nonylphenolat 72h was 850 µg/l. A study with rainbow trout and apache trout (*Oncorhynchus apache*) as well as two subspecies of the species *Oncorhynchus clarki*, reported LC50s from 140 to 270 µg/l (Brooke, 1993; Dwyer *et al*., 1995). Moreover, McLeese *et al*. (1981) reported a 96h LC50 of 190 µg/l for nonylphenol in *Salmo salar*. Lech *et al*. (1996) obtained a median lethal concentration of 193.65 for rainbow trout after 72 h exposure to nonylphenol. According to the USEPA toxicity category, chemicals with LC_{50} values ranging from 100 to 1000 µg/lhave been considered as"highly toxic", and also chemicals with LC_{50} values within 1000-10000 µg/l as "moderately toxic" (USEPA, 2010). Therefore, our results clearly indicated that nonylphenolis highly toxic to Caspian trout and moderately toxic to Caspian Kutum.

Our results also showed that the median lethal concentration of nonylphenol for Caspian kutum was much higher than nonylphenol LC_{50} for Caspian trout. These results were consistent with previous studies which showed that salmonid and coldwaterspecies are more sensitive than cyprinid and warmwaterspecies (Naylor, 1995; Sappington *et al*, 2001; Dwyer *et al*, 2005). Sappington *et al*. (2001) studied several cold and warm water species to compare their sensitivity in response to acute 4-nonylphenol exposure and reported 96 h LC_{50} values of 190, 170, 270 and 290 µg/l for rainbow trout, apache trout, fathead minnow and bonytail chub (*Gila elegans*), respectively. Their results showed that coldwater species were more sensitive than warmwater species. Naylor (1995) presented 96 h LC_{50} values of 300, 310 and 230 µg/l for fathead minnow, sheepshead minnow and rainbow trout, respectively. It is concluded from these values that a salmonid fish is more sensitive than a cyprinid fish against nonylphenol. Dwyer *et al.* (2005) conducted 4-nonylphenol toxicity tests with 18 fish species from seven families including: Cyprinidae, Cyprinodontidae, Salmonidae, Acipenseridae, Catostomidae, Percidae and Poeciliidae. Their results showed that species of the families Acipenseridae and Salmonidae were the most sensitive species with 96 h LC_{50} values ranging 50-80 and 150-190 µg/l, respectively. Fathead minnow, bonytail chub and Colorado pikeminnow representing Cyprinidae family showed 96 h LC_{50} values of 270, 290 and 260 µg/l, respectively. Based on our results, it is obvious that Caspian trout and Caspian Kutum exhibited different tolerances in response to nonylphenol exposure. These results clearly demonstrated that much higher concentrations of nonylphenolare required to induce mortality in the 50% of Caspian Kutum than those needed in Caspian trout.

As mentioned above, various species show different sensitivity in response to a given toxicant. Also inter-laboratory variations may be observed in the toxicity of a particular chemical to a particular species(Sappington *et al*., 2001). The differences in acute toxicity even for a particular toxicant and particular species may be due to the changes in water quality, method for preparation of the toxicant, method of data analysis, fish size, age and condition of the test species as well as the experimental factors, formulation and purity of the test chemicals(Lemke, 1981; Rathore and Khangarot, 2002).

CONCLUSION

According to these acute toxicity tests (96h LC_{50} values), it is concluded thatnonylphenol is highly toxic to Caspian trout. So, it could be introduced as suitable sentinel fish for assessing the nonylphenol toxicity in the field studies. In addition, LC_{50} values determined in the present study can be used to determine thenonylphenolchronic toxicity concentrations for Caspian troutand Caspian Kutum. Moreover, these findings may be useful in preparing appropriate regulations for protection and restocking management of these species.

ACKNOWLEDGMENTS
The authors would like to thank their colleagues at the Faculty of Marine Sciences, Tarbiat Modares University, Iran, for their financial support in this project.

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ارزیابی حساسیت ماهی سفید دریای خزر (*Rutilus frisii kutum*) و ماهی در معرض خطر آزاد دریای خزر (*Salmo trutta caspius*) در برابر سمیت حاد نونیل‌فنول

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(تاریخ دریافت:92/4/7 - تاریخ پذیرش: 92/10/6)

چکیده

آزمون‌های سمیت به طور معمول برای انتخاب گونه‌های مناسب به عنوان شاخص‌های زیستی در مطالعات سم‌شناسی و همچنین جهت ارزیابی اثرات آلاینده‌ها بر اکوسیستم‌های آبی به‌کار گرفته می‌شوند. در مطالعه حاضر حساسیت دو گونه‌ی، ماهی سفید دریای خزر و ماهی آزاد دریای خزر در برابر آلاینده نونیل‌فنول و با به‌کارگیری آزمون سمیت حاد به‌صورت ساکن مورد ارزیابی قرار گرفت. به‌منظور تعیین غلظت نیمه‌کشنده،ماهی‌ها به مدت 96 ساعت در معرض غلظت‌های مختلف نونیل‌فنول قرار داده شدند. غلظت‌های به‌کار گرفته شده شامل 100، 150، 200، 250 و 300 میکروگرم در لیتر برای ماهی آزاد و 400، 800، 1200، 1600 و 2000 میکروگرم در لیتر برای ماهی سفید بود. غلظت نیمه‌کشنده (LC_{50}) نونیل‌فنول در مدت زمان 96 ساعت برای ماهی آزاد و سفید با استفاده از آنالیز Probit محاسبه شد که به ترتیب برابر 204/78 و 1262/36 میکروگرم در لیتر بود. به علاوه مشخص شد که ماهی آزاد در برابر نونیل‌فنول 6 برابر حساس‌تر از ماهی سفید می‌باشد. بر اساس طبقه‌بندی سمیت USEPA، نونیل‌فنول برای ماهی آزاد دریای خزر بسیار سمی و برای ماهی سفید، دارای سمیت متوسط می‌باشد. نتایج این تحقیق می‌تواند در تهیه برنامه‌های حفاظتی و بازسازی ذخایر این دو گونه مورد استفاده قرار گیرد.

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