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Original Article

Acute toxicity of nonylphenol ethoxylate-6 to whiteleg shrimp, *Penaeus vannamei* (Boone, 1931) (Decapoda, Penaeidae)

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Abstract: Nonylphenol ethoxylate-6 (NP6EO) is widely used in industrial and domestic products and easily detected in the environment. The toxicity and estrogenic potency of alkylphenols have been investigated in several studies. However, to the best of our knowledge available, acute toxicity data about the effects of NP6EO on decapod and aquatic ecosystem in literature is yet scarce. Therefore, in this study the adult male and female whiteleg shrimp, *Penaeus vannamei*, were exposed to various concentrations of NP6EO (0.04, 1, 5, 25, 125, 625 μL L^{-1}) for four days. Acute toxicity potential of NP6EO on adult *P. vannamei* was assessed by calculating LC_{50} for different times. Median lethal concentration (LC_{50}) of NP6EO at 96 hours was 7.017 μL L^{-1}. The LC_{50} of this compound revealed a positive correlation between shrimp mortality and exposure periods. The data exhibited that NP6EO was considered as "toxic" to *P. vannamei* and further toxicity assessment to other species is strongly recommended.

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Introduction

Man-made chemicals are an important part of the modern life. Human beings as well as wildlife populations cannot avoid coming into contact with many of chemicals employed in variety of industries like food production (plants and meat), pathogen control (insecticides), production of modern materials (plastics), or in the built environment (insulations and fire retardants) (Bergman et al., 2012). Considering the importance of these compounds and their widespread presence in the environment, it is important that comprehensive strategies are developed to preclude widespread environmental contamination with endocrine disruptors (EDs) and protect environment (David et al., 2009; Bergman et al., 2012).

Nonylphenol ethoxylate-6 (NP6EO) is used in countless number of applications and because of its extensive use, discharged to the sewer system and make their way into wastewater and aquatic systems (Ying et al., 2002). NP6EO is a nonionic surfactant that is used in a wide range of industrial applications and consumer products, such as laundry detergents, dust-control agents and deicers, industrial liquid soaps and cleaners, cosmetics, paints, and as the dispersing agents in pesticides and herbicides (Jobling and Sumpter, 1993). Concern has recently increased about the use of alkylphenol ethoxylates (APEs) because of the relative stability of their metabolites such as nonylphenol (NP), octylphenol (OP) and nonylphenol ethoxylate-1-3 (NP1-3EO) in the environment (Giger et al., 1984) and their estrogenic effects on organisms (Ying et al., 2002) which is considered as EDs.

Testing strategies employed acute toxicity studies to evaluate and measure the effect(s) of one or more pollutants on one or more species. This implies that tests at high doses will inform us about low-dose exposures (Reish and Oshida, 1986; Bergman et al., 2012). The lethality of the EDs was used as the

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endpoint in an aquatic acute toxicity testing system (Faheem and Lone, 2013). In general, determination of lethal concentrations, such as the median lethal concentration (LC_{50}), is recognized as the first step for risk assessment of synthetic and natural chemicals (Johnson and Finley, 1980; Ura et al., 2002). These data assist in the development and application of water quality criteria for the protection of the aquatic environment.

In spite of evidence that proved the toxicity of NPnEO on many aquatic animals (Dorn et al., 1993; Lussier et al., 2000; Hirano et al., 2004; Oliveira-Filho et al., 2005; Ricciardi et al., 2008; Liu et al., 2011), there is no attempts have been made to determine the impacts of NP6EO on *Penaeus vannamei*. Hence, this study aims to determine and compare the acute toxicity of NP6EO to *P. vannamei* upon modification of the exposure conditions.

Materials and Methods

Chemicals: NP6EO (CAS No.9016-45-9) was obtained from Kimiagaran Emroz Company (Tehran, Iran). Stock solution of the NP6EO was prepared by dissolving appropriate concentration in 96% ethanol as solvent. Required concentrations were obtained by serial dilution and stored in dark at 4°C until usage. Solvent concentration was kept at 0.01% (v/v) for all treatments.

Animal maintenance and exposure to NP6EO: Adult *P. vannamei* (both sexes; body weight: 25.89±0.79 g, total length: 14.26±0.16 cm) were obtained from shrimp farms located at southern coast lines of Iran and were transferred to Kolahi Aquatic Restock Center (KARC). Adult shrimps were acclimated in 300L fiberglass tanks containing ultraviolet-filtered recirculating water (pH=7.77±0.017) for 2 weeks prior to the experiment. Feeding was done on daily basis in four times at the rate of 2.5% of the body weight by commercial feed until 24 hrs prior to the initiation of the test. After the acclimation period, 240 adult shrimp were randomly distributed among 24 tanks, comprising control, vehicle control (ethanol with a final concentration of 1:1,000 v/v water), 0.04, 1, 5, 25, 125, 625 $\mu L\ L^{-1}$ of NP6EO. The experiment was run in triplicate, without feeding and water exchange during the experiment. The mortality of shrimps in each treatments were counted and recorded over the exposure period at 24, 48, 72 and 96 hrs. Dead shrimps were removed from treatments immediately. The study was approved by the Iranian Society for the Animal Welfare.

Data analysis and statistics: Data are expressed as mean with the corresponding standard error (SE). LC values and 95% confidence intervals (95% CI) were calculated using probit analysis. Shapiro-Wilk and Levene's tests were used to check the normality of data distribution and the homogeneity of variances, respectively (Zar, 2010). If data support the prerequisites for parametric analysis, one-way analysis of variance (ANOVA) followed by Tukey's multiple range test was applied. Otherwise, Kruskal-Wallis and Mann-Whitney U test were applied to determine the statistical significance (Zar, 2010). All the analysis was performed by SPSS 16.0. The significant level in all analysis was set at $P \leq 0.05$.

Results

The percent mortality of *P. vannamei* after exposure to various concentrations of NP6EO for 24, 48, 72 and 96 hrs has been depicted in Figure 1. Mortality increased with increasing concentrations and exposure time (Fig. 1). The LC_{50} values were 437.052 ± 326.250 $\mu L\ L^{-1}$ for 24 hrs, 33.627±15.443 $\mu L\ L^{-1}$ for 48 hrs, 10.816±3.936 $\mu L\ L^{-1}$ for 72 hrs and 7.017±2.391 $\mu L\ L^{-1}$ for 96 hrs.

The LC values, their upper and lower confidence limits and slope functions for NP6EO have been given in Table 1.

Discussion

Acute toxicity tests provide a measure of the toxicity of the given compounds to experimental species under specific environmental conditions (Reish and Oshida, 1986). They also reflect the severe and rapid damage caused by sudden exposure to lethal concentrations of contaminants (Alam and Maughan, 1993).

In the present study, calculated LC_{50}-96 hrs value

Table 1. Effective dose, confidence limits, and slope function for nonylphenol ethoxylate-6 (NP6EO) at different intervals for the whiteleg shrimp, *Penaeus vannamei*.

Exposure periods	Effective dose (μL/L)	SE	limits LCL	limits UCL	Slope function	't' ratio	Heterogeneity
24 hrs	LC_1=0.006	0.009	0.000	0.052	0.479±0.081	5.920	1.135
	LC_5=0.161	0.150	0.012	0.683			
	LC_{10}=0.922	0.647	0.144	2.895			
	LC_{20}=7.642	3.976	2.346	20.537			
	LC_{50}=437.052	**326.250**	**131.656**	**3241.25**			
	LC_{80}=*	*	3340.90	*			
	LC_{90}=*	*	*	*			
	LC_{95}=*	*	*	*			
	LC_{99}=*	*	*	*			
48 hrs	LC_1=0.001	0.002	0.000	0.010	0.530±0.070	7.548	1.540
	LC_5=0.026	0.023	0.003	0.107			
	LC_{10}=0.128	0.089	0.022	0.400			
	LC_{20}=0.866	0.447	0.255	2.124			
	LC_{50}=33.627	**15.443**	**14.568**	**95.011**			
	LC_{80}=1305.031	1032.11	362.622	9758.932			
	LC_{90}=8834.585	8962.67	1755.602	*			
	LC_{95}=*	*	6344.154	*			
	LC_{99}=*	*	*	*			
72 hrs	LC_1=0.002	0.002	0.000	0.042	0.635±0.075	8.436	2.903
	LC_5=0.028	0.020	0.000	0.247			
	LC_{10}=0.104	0.063	0.001	0.664			
	LC_{20}=0.512	0.237	0.022	2.438			
	LC_{50}=10.816	**3.936**	**2.243**	**74.971**			
	LC_{80}=228.489	127.492	39.623	*			
	LC_{90}=1125.541	802.962	133.766	*			
	LC_{95}=4199.877	3578.53	348.322	*			
	LC_{99}=*	*	1984.653	*			
96 hrs	LC_1=0.003	0.002	0.000	0.035	0.680±0.078	8.755	2.538
	LC_5=0.027	0.018	0.000	0.193			
	LC_{10}=0.091	0.052	0.002	0.495			
	LC_{20}= 0.406	0.182	0.028	1.673			
	LC_{50}=7.017	**2.391**	**1.708**	**33.301**			
	LC_{80}=121.319	59.905	26.836	2593.424			
	LC_{90}=538.201	338.229	87.163	*			
	LC_{95}=1841.874	1382.53	219.975	*			
	LC_{99}=*	*	1182.462	*			

* Values more than 10000 are not shown

of NP6EO for *P. vannamei* was assessed as 7.017 μL L^{-1}, which was in agreement with the results reported for other species have been tested with NP and NPnEO (Dorn et al., 1993; Mann and Bidwell, 2000; Oliveira-Filho et al., 2005; Ricciardi et al., 2008). The acute and chronic toxicity of alkylphenol ethoxylates and their metabolites have been investigated for several freshwater and marine species (Servos, 1999; Staples et al., 2004). Previous studies showed that species sensitivity varies from 17 μg L^{-1} of para-nonylphenol (PNP) for winter flounder (*Pleuronectes americanus*) (Lussier et al., 2000) to 9.2 mg L^{-1} of NP8EO for *Litoria adelaidensis* (Mann and Bidwell, 2000). Median lethal concentrations of 4 to 6.6 mg L^{-1} of NP9EO was reported for fathead minnow, *Pimephales promelas* (Dorn et al., 1993; Staples et al., 1998). Also in the other similar studies, LC_{50}-96 hrs for NP, NP1EO and NP2EO for fathead minnow were 136, 218, and 323 μg L^{-1}, respectively (TenEyck and Markee, 2007). The varying data of available toxicity tests resulted as a function of ethoxy chain

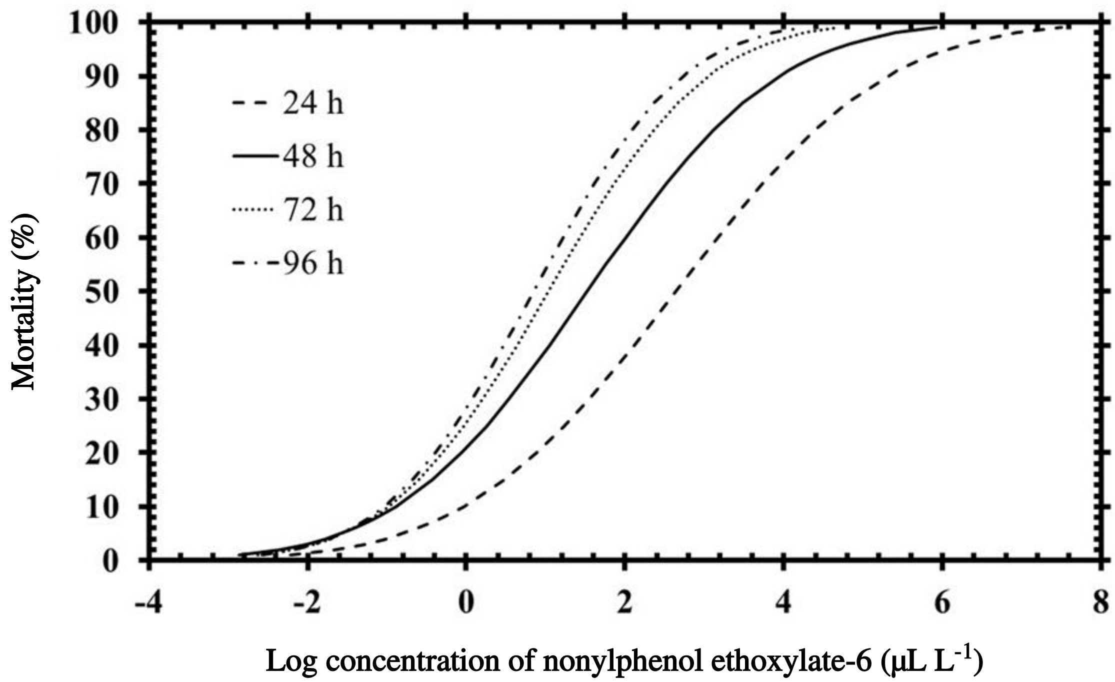

Figure 1. Percent mortality of *Penaeus vannamei* after 24, 48, 72 and 96 hrs exposure to different concentrations of nonylphenol ethoxylate-6 (NP6EO) (μL L^{-1}).

length, the type of test used, and the species tested.

Considering the relative toxicity values, or NP toxic equivalency factors (TEFs) which calculated for nonylphenol compounds, toxic concentrations for different nonylphenolic compounds such as NPnEO with various EO chain length could be matched with similar endpoints for NP for the same species. If TEF for NP be considered as 1, for example, toxic equivalency factors for NPnEO ($1<n<8$) will be considered as 0.5 (Environment Canada, 2002). Based on the present results, it can be proposed that in *P. vannamei* sensitivity to NP toxicity is approximately the same as other studied species.

According to the USEPA toxicity categories (U.S. EPA., 2015), chemicals with LC_{50} values ranging from 1 to 10 mg L^{-1} are considered as "toxic to aquatic species with long lasting effects". Therefore, our result strongly indicate that NP6EO is toxic for *P. vannamei*.

This study assessed the acute toxicity of NP6EO on *P. vannamei*, which is the first report of LC_{50} values of this chemical on this species. The work has revealed that NP6EO has a potentially harmful impact on *P. vannamei* and probably other aquatic crustaceans and invertebrates. The response of various species to same chemicals are different at the same time of exposure. Moreover, the potential of NP6EO for endocrine disruption at even lower concentrations may cause further concerns for aquatic fauna. Additional long term studies are needed to study other impacts of NP6EO on aquatics. The findings of this research suggest that the policy makers must take necessary measures to prevent the crucial damage of this compound on aquatic organisms and human beings.

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چکیده فارسی

تاثیر تعیین سمیت حاد نونیل فنل ۶-اتوکسیلات بر روی میگوی پاسفید غربی *Penaeus vannamei* (Boone, 1931) (Decapoda, Penaeidae)

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چکیده:
نونیل فنل ۶-اتوکسیلات کاربرد وسیعی در تولید محصولات خانگی و صنعتی داشته و به همین دلیل به راحتی در محیط زیست قابل سنجش می باشد. سمیت و خاصیت شبه استروژنی ترکیبات آلکیل فنل در بسیاری از مطالعات مورد ارزیابی قرار گرفته است. با این وجود کمبود اطلاعات در خصوص تاثیرات سمیت حاد نونیل فنل ۶-اتوکسیلات بر روی ده‌پایان و همچنین اکوسیستم‌های آبی به خوبی احساس می‌گردد. به همین منظور، در مطالعه حاضر هر دو جنس نر و ماده بالغ میگوی پاسفید غربی (*Penaeus vannamei*) در یک دوره چهار روزه در معرض غلظت‌های مختلف نونیل فنل ۶-اتوکسیلات (۰/۰۴، ۱، ۵، ۲۵، ۱۲۵ و ۶۲۵ میکرولیتر بر لیتر) قرار گرفت. سمیت حاد نونیل فنل ۶-اتوکسیلات با توجه به مقدار غلظت LC_{50} در زمان‌های مختلف ارزیابی شد. با توجه به نتایج حاصل، مقدار غلظت LC_{50} نونیل فنل ۶-اتوکسیلات برای ۹۶ ساعت برابر با ۷/۰۱۷ میکرولیتر بر لیتر محاسبه شد. غلظت LC_{50} محاسبه شده در این تحقیق بیانگر رابطه مستقیم بین بروز مرگ و میر در میگوها و مدت زمان تاثیر گذاری بود. نتایج نشان داد که نونیل فنل ۶-اتوکسیلات برای میگوی پاسفید غربی ترکیبی سمی به شمار می‌رود و به همین دلیل ارزیابی سمیت این ترکیب برای دیگر گونه‌های آبزی ضروری بوده و توصیه می‌شود.

کلمات کلیدی: نونیل فنل ۶-اتوکسیلات، میگو، پاسفید غربی، سمیت حاد.