Assessment of median lethal concentration (LC$_{50}$ - 96h) and behavioural modification of nonylphenol in the Cichlid fish, *Etroplus maculatus* (Bloch, 1795)

K.P. Asifa, P.V. Vidya and K.C. Chitra*
Endocrinology and Toxicology Laboratory, Department of Zoology, University of Calicut, Malappuram District, Kerala, 673 635, India
Email: kcchitra@yahoo.com

**Abstract**

Nonylphenol is a toxic xenobiotic compound widely used as an element of detergents, paints, pesticides, pulp and paper processing, plastics and personal care products. In the present study, median lethal concentration (LC$_{50}$) of nonylphenol in the cichlid fish *Etroplus maculatus* was determined by probit analysis. Eight different concentrations of nonylphenol (0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.2, 1.5 mg/ L) were exposed to fish with ten animals per group for 96 h maintaining a control group. Mortality of the fish in each group was monitored throughout the experiment and the results of probit analysis indicated that the percentage of mortality is positively correlated (r = +0.94) against the concentrations of nonylphenol. The result showed that 0.89 mg i.e., 890 μg/ L of nonylphenol kills 50% of the exposed fish. In the experiment, the body weight of all treated groups remained unchanged as compared with the control group. However, mucous deposition was significantly increased in nonylphenol-treated groups in dose-dependent manner. The movement and the behaviour of the fish were closely examined throughout the study. Fish exposed to different concentrations of nonylphenol showed slow and restricted movements, loss of equilibrium, decreased opercular movements and hemorrhage in gills and throughout the body surface. In conclusion, nonylphenol at 890 μg/ L concentration is considered as 96 h LC$_{50}$ value and the modification in normal behavioural pattern due to nonylphenol can be used as an indicator of ecologically relevant monitoring of environmental contaminants.

**Keywords** : Nonylphenol, median lethal concentration, *Etroplus maculatus*, probit analysis and behaviour

**Introduction**

Nonylphenol (NP) is the commercially most important member in the group of alkyl phenols and has a large number of isomeric compounds, which are varying in the point of attachment of the nonyl group to the phenol molecule and in the degree of branching in the nonyl moiety. NP is used as a starting material in the synthesis of nonylphenol ethoxylates (NPEs), and as a monomer in polymer production. NPEs are non-ionic surfactants and widely used in detergents, personal-care products, textile processing, agricultural chemicals, pulp and paper processing, metal and mineral processing, latex paints, wetting agents, emulsifiers, foaming agents, inks, adhesives, and pharmaceuticals (Lee, 1999).

The occurrence of nonylphenol in the environment is clearly correlated with anthropogenic activities such as wastewater treatment, landfilling and sewage sludge recycling. Thus nonylphenol is found often in different environmental compartments such as surface water, sediment, groundwater, soil, air, sewage sludge and effluents from sewage treatment works. Due to its
low solubility and high hydrophobicity, nonylphenol accumulates in these matrices and where it persists (Soares et al., 2008). Several acute and chronic studies have been reported for nonylphenol toxicity in aquatic species. Fish are considered as the most susceptible species to pesticide after being exposed through gills, skin or food. In the present study *Etroplus maculatus*, an indigenous cichlid fish was used as an experimental model to evaluate the median lethal concentration of nonylphenol for 96 h by using the probit analysis method. In aquatic ecotoxicology the nexus of behavioural sciences with the study of toxicants have become predominant in recent years. Behavioural modifications are considered as a responsive sign of stress conditions in animals exposed to toxicants. Therefore the study also incorporates the behavioural endpoints because there was a lack of understanding of behavioural modification with respect to ecologically relevant issues such as growth, prey capture, stress resistance, reproduction and longevity. Behavioural endpoints in aquatic toxicology consequently provides a valuable tool for toxicity assessment of nonylphenol in *Etroplus maculatus*.

Materials and Methods

Animal

The cichlid fish, *Etroplus maculatus* weighing 8.5 ± 1.5 g and length 7.5 ± 1.5 cm were collected from fish farms, KKF Nursery, Manjeri, Vaniyambalam, Malappuram District, Kerala, India. Fishes were acclimatized to the laboratory conditions prior to experiments and were exposed to a constant supply of water and the good lighting system. They were maintained in well-aerated aquarium tanks (40 L capacity) and were regularly dechlorinated.

Preliminary tests

The physico-chemical features of the tap water were estimated as per APHA (1998). Water temperature in the test ranged from 28 ± 2°C during the experiment, oxygen saturation of water ranged between 70 and 100 %, pH is 7.6 which were monitored using standardized procedures.

Chemical

Technical grade Nonylphenol, 4-(2, 4-dimethyl-heptan-3-yl) phenol of 97% purity was purchased from SISCO Research Laboratories Pvt. Ltd., Mumbai, India.

Evaluation of median lethal concentration (LC$_{50}$ - 96 h)

The LC$_{50}$ value in 96 h time interval was determined by probit analysis, with a confident limit of 5 % level (Finney, 1971). The fishes were not fed a day prior to and during the test to reduce faecal and excess food contaminating the test solution. Five specimens were placed in each tank of replicates so that ten fishes were maintained in each test and aerated using tubed motorized pumps. Monofilament netting was used to cover the tanks so as to prevent the fishes from jumping out of test solutions. For determining LC$_{50}$ concentration of nonylphenol, separate aquarium tanks of 40 L of water capacity with 10 animals per tank were taken and eight different concentrations (0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.2, 1.5 mg/ L) of nonylphenol were added. Control tub with 40 L water capacity having 10 fishes were maintained along with treatment groups without the addition of toxicant. The lethal concentration for 50 % killing (LC$_{50}$) values was computed on the basis of probit analysis for 96 h exposure. The mortality as well as behaviour of fishes in each group was monitored throughout the experiment at varying concentrations.

Statistical Analyses

Median lethal concentration or 96 h-LC$_{50}$ value were analyzed with SPSS statistical analysis software (Version 19.0) using Probit Analysis Statistical Method.
The LC$_{50}$ values (with 95% confidence limits) were calculated using MS Excel 2007, the correlation between mortality on the Y-axis and concentrations on X-axis and the best-fit line was also obtained.

**Results and Discussion**

Nonylphenol is a toxic xenobiotic compound classified as an endocrine disruptor that is capable of causing adverse effects on aquatic animals. In the present study mortality of the fishes in each group were continuously monitored throughout the experiment and it was observed that 0.5 and 0.6 mg/L concentrations of nonylphenol did not cause mortality up to 96 h of exposure period. Nonylphenol at concentrations, 0.7, 0.8, 0.9, and 1mg/L showed death of 2, 4, 5 and 6 animals respectively at the end of 96 h exposure. All fishes were killed at 1.2 and 1.5 mg/L concentrations after 24 h and 5 h of nonylphenol treatment. The results of probit analysis indicated that the percentage of mortality is positively correlated (r = +0.94) against the concentrations of nonylphenol which showed a high degree of positive correlation. The median lethal concentration (LC$_{50}$) value of nonylphenol in male and female Medaka were reported as 0.85 and 0.87 mg/L, respectively, for 72 h (Kashiwada et al., 2002). However the LC$_{50}$-96 h of nonylphenol in Oreochromis mossambicus was determined as 1.5 mg/L by probit analysis (Balakrishnan et al., 2014).

In the present study each group of fishes were exposed to different concentrations of nonylphenol for 96h. In the experiment, the body weight of all treated groups remained unchanged as compared with the control group, but when the weights observed along with mucous secretion showed significant increase (P<0.05) in dose-dependent manner than that of control group (Fig.1 and 2). Different concentrations of nonylphenol showed a different percentage of mortality at different time intervals as shown in Table - 1.

The median lethal concentration (LC$_{50}$) of nonylphenol was computed on the basis of probit analysis for 96 h exposure, which is 890 μg/L (Table - 2 and Fig.-3).

Aquatic behavioural toxicology is the recently growing and recognized field in toxicology that provides perspective link to the physiology and ecology of an organism and its environment. Behavioural endpoints may often contribute an additional utility or biologic significance to morphologic and physiologic adaptation of an animal in the ecosystem. Behaviour also allows an organism to adjust to external and internal stimuli in order to meet the challenge of surviving in a changing environment. Fish models are widely used in behavioural studies as it offers important indices for ecosystem assessment. In the present study the behaviour of fish was continuously monitored in all treatment groups. Control fish maintained in toxicant-free water were found to be active throughout the experiment. Nonylphenol exposure irrespective of varying concentrations showed abnormal behaviour in fishes when compared to control group. Immediately after the toxicant exposure fishes showed aggressive swimming for a few hours as the toxicant triggers the stimulus response by avoiding the area containing the contaminant. Since the animal was maintained in tanks, the fishes are unable to avoid the toxicant area which would resulted in slow and restricted swimming activity with decreased rate of opercular movement thereby altering the respiratory pattern.

After 24 h of nonylphenol exposure body surfaces become reddened and hemorrhagic, unable to maintain normal posture or equilibrium and large amount of mucous secretion were also observed. As a result, fishes become lethargic and at the time of death, they exhibited transient hyperactivity before collapsing. Rainbow trout when exposed to nonylphenol has been shown to decrease the shoaling behaviour in fish and
were reported to be more likely attacked by other fishes, and also less successful in competing for food resources (Ward et al., 2006). Long term exposure to nonylphenol has been found to change in locomotor activity and made aggressive behaviour in both male and female Zebra fishes (Xia et al., 2010). However, nonylphenol at sub lethal concentrations increased the swimming behaviour in Eurytemora affinis (Cailleaud et al., 2011). Erratic activity followed by restricted movements, haemorrhage in body surface, reddening of the fin and finally loss of equilibrium in Etroplus maculatus has been observed after exposure to one of the environmental contaminants, bisphenol A (Asifa et al., 2016).

Fig. 1. Effect of nonylphenol on the body weight of fish, *Etroplus maculatus*

Fig. 2. Effect of nonylphenol on mucous secretion in *Etroplus maculatus*

Fig. 3. Median lethal concentration (LC₅₀ – 96 h) of nonylphenol in *Etroplus maculatus*

Table -1. Probit analysis of 95% confidence limits for effective concentrations of nonylphenol in *Etroplus maculatus*

<table>
<thead>
<tr>
<th>Concentration (mg)</th>
<th>95% Confidence limits</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>.01</td>
<td>.49378</td>
<td>.00802</td>
<td>.67244</td>
</tr>
<tr>
<td>.02</td>
<td>.54045</td>
<td>.09835</td>
<td>.70595</td>
</tr>
<tr>
<td>.03</td>
<td>.57005</td>
<td>.15541</td>
<td>.72745</td>
</tr>
<tr>
<td>.04</td>
<td>.59233</td>
<td>.19818</td>
<td>.74379</td>
</tr>
<tr>
<td>.05</td>
<td>.61044</td>
<td>.23286</td>
<td>.75718</td>
</tr>
<tr>
<td>.06</td>
<td>.62586</td>
<td>.26228</td>
<td>.76868</td>
</tr>
<tr>
<td>.07</td>
<td>.63938</td>
<td>.28801</td>
<td>.77884</td>
</tr>
<tr>
<td>.08</td>
<td>.65149</td>
<td>.31097</td>
<td>.78800</td>
</tr>
<tr>
<td>.09</td>
<td>.66250</td>
<td>.33180</td>
<td>.79639</td>
</tr>
<tr>
<td>.10</td>
<td>.67263</td>
<td>.35091</td>
<td>.80416</td>
</tr>
</tbody>
</table>

Table -2. Percentage of fish mortality exposed to different concentrations of nonylphenol in cichlid fish, *Etroplus maculatus* for 96 h

<table>
<thead>
<tr>
<th>Concentrations (mg/ L)</th>
<th>Mortality (%)</th>
<th>Hour of mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>96 h</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>96 h</td>
</tr>
<tr>
<td>0.6</td>
<td>0</td>
<td>96 h</td>
</tr>
<tr>
<td>0.7</td>
<td>20</td>
<td>96 h</td>
</tr>
<tr>
<td>0.8</td>
<td>40</td>
<td>96 h</td>
</tr>
<tr>
<td>0.9</td>
<td>50</td>
<td>96 h</td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>96 h</td>
</tr>
<tr>
<td>1.2</td>
<td>100</td>
<td>24 h</td>
</tr>
<tr>
<td>1.5</td>
<td>100</td>
<td>5 h</td>
</tr>
</tbody>
</table>

were reported to be more likely attacked by other fishes, and also less successful in competing for food resources (Ward et al., 2006). Long term exposure to nonylphenol has been found to change in locomotor activity and made aggressive behaviour in both male and female Zebra fishes (Xia et al., 2010). However, nonylphenol at sub lethal concentrations increased the swimming behaviour in Eurytemora affinis (Cailleaud et al., 2011). Erratic activity followed by restricted movements, haemorrhage in body surface, reddening of the fin and finally loss of equilibrium in *Etroplus maculatus* has been observed after exposure to one of the environmental contaminants, bisphenol A (Asifa et al., 2016).
and Chitra, 2015). Therefore, behavioural toxicology testing provides biologically relevant endpoints to evaluate sub lethal effects of nonylphenol and may compliment traditional toxicity testing.

**Conclusion**

The median lethal concentration of nonylphenol (LC50 - 96 h) by probit analysis for *Etroplus maculatus* was determined as 0.89 mg/L (890 μg/L). The study gives further evidence that assessment of fish behavior can constitute a highly sensitive method to indicate the toxic effect of nonylphenol.

**Acknowledgement**

The authors acknowledge the financial support from KSCSTE, Thiruvananthapuram, Kerala, India for the present study.

**References**


Corresponding Author : K.C. Chitra, Endocrinology and Toxicology Laboratory, Department of Zoology, University of Calicut, Malappuram District, Kerala - 673 635, India, Email : kcchitra@yahoo.com © 2016, IJALS. All Rights Reserved.