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Acute Toxicity of 4-Nonylphenol to Aquatic Invertebrates in Taiwan

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Abstract Acute toxicity of 4-nonylphenol (NP) was examined in six freshwater species from three phyla – platyhelminthes, arthropoda and mollusca. Values of the 48-hour LC_{50} of NP for six species ranged from 20 to 508 ug/L and values of the 96-hour LC_{50} for four species ranged from 120 to 457 ug/L. The most sensitive species tested was a water flea, *Ceriodaphnia cornuta*, with a 48-hour LC_{50} of 20 μg/L. The no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) values of NP found were in the range of <10 to 400 μg/L and 10 to 450 μg/L after 48 hours of exposure, respectively.

Keywords 4-nonylphenol · Freshwater planarian · Water flea · Freshwater shrimp · Freshwater snail · NOAEL · LOAEL · LC_{50}

4-Nonylphenol (NP) is widely used in producing nonionic surfactants, such as nonylphenol ethoxylates (NPEs), for detergents, emulsifiers, pesticides, lubricants, and oil additives in our daily life (Ying et al., 2002). On the other hand, NP is also a breakdown product of NPEs that are commonly discharged in large quantities by sewage treatment plants or directly into the aquatic environment in areas where there is no sewage treatment. Consequently, NPEs and their degradation products, especially NP, have been found worldwide in wastewater discharges, sewage treatment plant effluents, natural water, and sediments (Bennie, 1999; Ying et al., 2002). Because NP and NPEs are ubiquitous, persistent, and easily bioaccumulated, potential toxic effects of these chemicals have attracted much research attention in the last few years. Previous investigations have shown that NP was estrogenic (Nimrod and Benson, 1996) and highly toxic to aquatic animals both acutely and chronically (Lussier et al., 2000; Staples et al., 2004). In addition, NP appeared to have reproductive and developmental effects to both vertebrates (Nimrod and Benson, 1998) and invertebrates (Nice et al., 2000; Tanaka and Nakanishi, 2002).

NPEs and NP are one of the major classes of nonionic surfactants that have been widely used and produced in Taiwan. According to the Taiwan Environmental Protection Administration, NPEs/NP encompassed about 80% of the annual nonionic surfactants market in Taiwan, and two major industrial producers, China Man-Made Fiber Corporation and Formosan Union Chemical Corporation, were estimated to produce about 16,000 tons of NP annually (Taiwan EPA, 2005a). Several studies have been conducted to analyze NPEs and/or NP concentrations in river waters of Taiwan in the past decade (Ding et al., 1999; Wang et al., 2001; Cheng et al., 2006). In fact, the concentrations of NP presented in the rivers of Taiwan were usually higher than the values reported in the other countries (Cheng et al., 2006). However, very little data exists on the toxicity of NP to aquatic organisms in Taiwan.

In this study, we selected six freshwater species to evaluate the acute toxicity for NP. These aquatic invertebrates were chosen as study species because of their widespread presence in the rivers and/or lakes of Taiwan as well as other parts of the world. The objective of this study was to examine the acute toxicity of NP to aquatic invertebrates that occur widely in the aquatic environment of Taiwan. The results of this study can provide some useful information about the acute toxicity of NP to some aquatic

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animals in Taiwan and help to assess the potential effects of NP in subtropical and tropical aquatic ecosystems.

Materials and Methods

4-Nonylphenol (CAS no. 84852-15-3) was purchased from Riedel-de Haën (Sigma-Aldrich, USA), with a chemical purity of 94%. High-performance liquid chromatography (HPLC)-grade acetone was purchased from Mallinckrodt. NP was dissolved in acetone and the test solution was replaced every other day to maintain treatment concentrations. In order to eliminate the risk of leached potential endocrine disruptors, plastic materials were avoided in all aquaria and plumbing. Dechlorinated tap water was stored in a stainless-steel tank before use. All animals were kept in different sizes of glass beakers or stainless-steel containers in our laboratory.

Planarians (*Dugesia japonica*) were collected from the Nanshi stream located in Wulai, in the Taipei prefecture, Taiwan in 2004. Since then, the planarians have been maintained in dechlorinated tap water in our laboratory. Animals were fed raw chicken liver once a week. Culture medium was renewed after weekly feeding. Red cherry shrimps (*Neocaridina denticulate*) were collected from the Pingguang stream located in the Guangxing borough of the Taipei prefecture, Taiwan in April of 2006. Freshwater snails (*Physa acuta*) were collected from a ditch located in Shilin in Taipei City in June 2005. Snails were fed with lettuce and half of the culture medium was changed with dechlorinated water every two weeks. Water fleas (*Ceriodaphnia cornuta*) were collected from the campus lake of National Taiwan University in May 2006. In addition, the other test animals, including atyid shrimps (*Caridina pseudodenticulata*) and water fleas (*Moina macrocopa*), were all purchased from local suppliers. Both water fleas were fed with algal powder twice a day and half of the medium was renewed with dechlorinated water once a week. As for the two species of freshwater shrimps, they were fed commercial fry food T850S from the Hai Feng feed company and half of their culture medium was renewed with dechlorinated water every two weeks.

Acute toxicity tests on shrimps and water fleas were performed according to the Taiwan EPA standard protocol (Taiwan EPA, 2005b) and the Organisation for Economic Co-operation and Development (OECD) test guideline 202 (OECD, 1984) with slight modifications, respectively. In brief, test animals were exposed to NP at five to seven concentrations or to acetone alone at a nominal concentration of 1,000 ppm as a solvent control. All acute toxicity experiments were conducted in a temperature incubator at 28 ± 2°C without illumination. For each concentration, five animals were kept in 50 ml of test solution in a glass beaker for planarians (body length 0.7 cm), freshwater snails (shell length < 0.1 cm), and water fleas (younger than 24 hours) or in 1,000 ml of test solution in a glass beaker for shrimps (body length 1.5 cm). Each treatment was replicated five times during the experiments. The animals were not fed and were inspected every 24 hours for mortality during the entire 96-hour experimental period; we refreshed the medium with the same concentration at the 48th hour. Organisms without detectable movement were considered dead and removed from the test solution.

The concentrations that were lethal to 50% of the organisms (LC_{50}) to each species for 24, 48, 72, or 96 hours were calculated using probit analysis where possible, and trimmed Spearman-Karber analysis when conditions for probit analysis were not met (Hamilton et al., 1977). Probit analyses were made using the Probit Program (version 1.5) and trimmed Spearman-Karber analyses with trimmed Spearman-Karber program (version 1.5); both programs were obtained from the environmental monitoring systems laboratory (USEPA, Cincinnati, Ohio). The LOAEL (the lowest concentration producing animal mortality significantly different from the controls) and NOAEL (the highest concentration producing no mortality significantly different from the controls) were determined by Dunnett's multiple comparison procedure (USEPA, 1989) using the Minitab statistical program (version 13.2).

Results and Discussions

In this study, the most sensitive freshwater species was a water flea, *Ceriodaphnia cornuta*, with a 48-hour LC_{50} of 20 μg/L, and the species least sensitive to NP toxicity was a planarian, *Dugesia japonica*, with a 48-hour LC_{50} of 508 μg/L and a 96-hour LC_{50} of 457 μg/L (Table 1). Compared with the published data on the acute toxicity of NP, the 48-hour LC_{50} of 20 μg/L found for *Ceriodaphnia cornuta* in our study is at the low end of the range, which is from 20 to 3,000 μg/L for invertebrates, as cited in Servos (1999). Acute toxicity tests with NP showed that crustaceans were the most sensitive organisms with reported 96-hour LC_{50} values of 20.6 and 69 μg/L in the amphipod *Hyalella azteca*, and the cladoceran *Ceriodaphnia dubia*, respectively (Jumel et al., 2002). In addition, 48-hour LC_{50} values of 190 and 67 μg/L were reported for *Daphnia magna* (Comber et al., 1993), and *Daphnia galeata* (Tanaka and Nakanishi, 2002), respectively. Therefore, the estimated 48-hour LC_{50}, of 20 μg/L for *Ceriodaphnia cornuta* is considerably lower than published LC_{50} values for other water fleas. This suggests that the cladoceran *Ceriodaphnia cornuta* is relatively sensitive to NP exposure compared with other widely researched cladocerans. Indeed, this observation is also in good agreement with findings of Do

Table 1 LC_{50} (μg/L) from 24 to 96 hours for different aquatic invertebrates exposed to NP

Species	LC_{50} (μg/L)			
	24 hours	48 hours	72 hours	96 hours
Dugesia japonica	525 (506–539)[a]	508 (419–569)[a]	463 (408–458)[a]	457 (400–451)[a]
Physa acuta	>750	319 (225–484)[a]	128 (81–182)[a]	120 (73–171)[a]
Neocaridina denticulate	431 (352–608)[a]	371 (290–528)[a]	252 (204–311)[a]	220 (89–390)[a]
Caridina pseudodenticulata	>500	366 (292–459)[b]	211 (138–266)[a]	195 (146–259)[b]
Moina macrocopa	>500	104 (78–139)[b]	——	——
Ceriodaphnia cornuta	26 (21–31)[a]	20 (16–23)[a]	——	——

[a] Determined using probit analysis and values in parentheses are 95% confidence intervals

[b] Determined using Trimmed Spearman-Karber analysis and values in parentheses are 95% confidence intervals

Hong et al. (2004). They found that *Ceriodaphnia cornuta* was more sensitive than *Daphnia magna* to acute toxicity of potassium dichromate, diazinon, methyl parathion, and mercury. Since *Ceriodaphnia cornuta* is a widely distributed tropical species and an easily cultivable organism, it may be suitable for use in ecological toxicity testing in tropical and subtropical aquatic environments.

According to the literature, the actual test water NP concentrations have been reported to be approximately 1/2-1/3 of the nominal concentration after 48 hours (Gray and Metcalfe, 1997; Kinnberg et al., 2000). This might be helpful to explain why the LC_{50} values between 48 and 72 hours is disproportionably greater than LC_{50} values in the other 24-hour periods in freshwater shrimps and snails under our 48-hour test-medium renewal scheme. In addition, the aquatic juvenile snail, *Physa acuta*, had a rather high sensitivity to NP with a 96-hour LC_{50} of 120 μg/L. The LC_{50} result for juvenile *Physa acuta* was much lower than the LC_{50} values of other mollusks reported in literature. For example, 96-hour LC_{50} values of 774 and 3,000 μg/L were reported for an adult snail (*Physella virgata*) (Brooke 1993, as cited in Servos, 1999) and for an adult mussel (*Mytilus edulis*) (Granmo et al., 1989) exposed to NP, respectively. One possible explanation for the increased sensitivity of *Physa acuta* to NP may be that a juvenile snail would not be able to enclose its body completely and thus protect itself against NP exposure. On the other hand, freshwater shrimps appeared to be less sensitive than cladocerans. Two species of freshwater shrimps tested had very similar 96-hour LC_{50} values of 220 and 195 μg/L for *Neocaridina denticulate*, and *Caridina pseudodenticulata*, respectively. These were in a similar range of reported acute toxicity to NP as other marine shrimps. For example, 96-hour LC_{50} values of 300 and 59.4 μg/L were reported for a marine shrimp (*Crangon septemspinosa*) (McLeese et al., 1980), and a grass shrimp (*Palaemonetes vulgaris*) (Lussier et al. 2000), respectively. This suggests that freshwater shrimps have intermediate sensitivity to NP among the aquatic invertebrate species tested. Overall our LC_{50} results for NP obtained from the six aquatic invertebrates tested are in a similar range to the published LC_{50} of NP for aquatic invertebrates.

Freshwater concentrations of NP have been reported to be in the range of <0.01 to 180 μg/L and the average concentrations of NP are usually less than 10 μg/L (Bennie, 1999). In recent years, a number of research projects supported by the Taiwan Environmental Protection Administration have focused on the occurrence of NP in the rivers of Taiwan. In the 40 rivers of Taiwan, NP was detected in 54.2% of water samples with average concentrations of 4.87 μg/L, in the range of 0.89 to 50.0 μg/L (Wang et al., 2001). Cheng et al. (2006) measured the concentrations of NP in 18 major rivers and also found that average NP concentrations in rivers ranged from 0.7 to 5.1 μg/L in 2000. In 2003, NP was found in 100% of the Kaoping River water samples with concentrations ranging from 0.19 to 183.4 ug/L (Taiwan EPA, 2005a). In the same year, NP was also measured in seven other rivers, including Lanyang River, Houlung River, Taichia River, Wu River, Yanshuei River, Bajhang River, and Fongshan River, at concentrations ranging from undetected to 1992 μg/L (Taiwan EPA, 2005a). The NP analytical results of the aquatic environment showed a wide variation in measured NP levels depending on the human activities and locations of the measurement in Taiwan.

In relation to ecological effects of measured NP levels and what they indicate about the magnitude of environmental damages, the risk assessment defines the predicted no-effect concentration (PNEC), which is based on standard toxicity data on LC_{50} or NOAEL with the application of a safety factor, can be used to evaluate possible ecological effects. The NOAEL values of NP found in this study were in the range of <10 to 400 μg/L (Table 2). If we used NOAELs divided by a safety factor of 10 to calculate PNECs, the PNECs of NP were determined to be in the range of <1 to 40 μg/L based on the results of this study. In fact, the measured NP concentrations of Taiwan river waters are still higher than the range of the

Table 2 NOAEL (μg/L) and LOAEL (μg/L) based on mortality determined using Dunnett's test for different aquatic invertebrates exposed to NP after 48 and 96 hours exposure

Species	48 hours		96 hours	
	NOAEL (μg/L)	LOAEL (μg/L)	NOAEL (μg/L)	LOAEL (μg/L)
Dugesia japonica	400	450	250	400
Physa acuta	100	250	50	100
Neocaridina denticulate	250	350	250	350
Caridina pseudodenticulata	100	250	100	250
Moina macrocopa	50	100	——	——
Ceriodaphnia cornuta	< 10	10	——	——

PNECs for NP. Therefore, an environmental risk due to NP is expected to exist in the aquatic environment of Taiwan. Currently, the Taiwan Environmental Protection Administration is considering including NP on the list of the Toxic Chemical Substances Control Act to restrict its use. Nevertheless, historical and existing use of NP/NPEs is likely to result in elevated and ecologically significant levels of NP in a significant proportion of Taiwan rivers and these NP concentrations can be expected to stay at significant levels for a number of years after cessation of its use.

To our knowledge, this is the first study to report LC_{50} values of NP for these six aquatic invertebrates tested in Taiwan. Based on the results of this study, NP in river water has a potentially harmful impact on the aquatic environment of Taiwan. Moreover, the potential of NP for endocrine disruption at even lower concentrations may cause further concerns for aquatic fauna. Information on the toxicological effects of NP on aquatic fauna of Taiwan and additional investigations into NP concentrations in different aquatic environments are warranted to provide relevant data to assess the ecological risk of NP adequately. Furthermore, the uses of NP/NPEs in Taiwan should be managed to minimize their ecological impact on aquatic ecosystems.

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