

ACUTE TOXICITY OF PARA-NONYLPHENOL TO SALTWATER ANIMALS

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Abstract—*para*-Nonylphenol (PNP), a mixture of alkylphenols used in producing nonionic surfactants, is distributed widely in surface waters and aquatic sediments, where it can affect saltwater species. This article describes a database for acute toxicity of PNP derived from calculating a national saltwater quality criterion. Using a flow-through exposure system with measured concentrations, we tested early life stages of four species of saltwater invertebrates and two species of fish. Static 96-h tests were also conducted on zoeal *Homarus americanus*, embryo-larval *Mulinia lateralis*, and larval *Pleuronectes americanus*. The number of organisms surviving the flow-through test was measured at 2, 4, 8, and 12 h and daily through day 7. Mortality for most species plateaued by 96 h. The ranked sensitivities (96-h 50% lethal concentrations, measured in micrograms per liter) for the species tested were 17 for *Pleuronectes americanus*, 37.9 (48-h 50% effective concentration) for *Mulinia lateralis*, 59.4 for *Paleomonetes vulgaris*, 60.6 for *Americamysis bahia* (formerly *Mysidopsis bahia*), 61.6 for *Leptocheirus plumulosus*, 70 for *Menidia beryllina*, 71 for *Homarus americanus*, 142 for *Cyprinodon variegatus*, and >195 for *Dyspanopus sayii*. Values for the seven most sensitive of these species ranged over a factor of only 4.2. The narrow range of responses for PNP implies that exceeding a threshold concentration would endanger a large proportion of the aquatic community.

Keywords—*para*-Nonylphenol 4-Nonylphenol Acute toxicity Saltwater

INTRODUCTION

para-Nonylphenol (4-nonylphenol, or PNP) is a mixture of alkylphenols that is used in producing nonionic surfactants for detergents, emulsifiers, lubricants, and oil additives. Most nonylphenols (NPs) are used as intermediates in producing nonylphenol ethoxylates (NPEs) for oil-soluble detergents and for emulsifiers that can be further refined to produce anionic detergents, lubricants, textile scouring or antistatic agents, emulsifiers for agrichemicals, antioxidants in rubber and plastics, and oil additives [1]. Because NPs are also breakdown products of alkylphenol polyethoxylates used in detergents, pesticides, herbicides, and toiletries, they are found widely in surface waters and aquatic sediments [2]. Considerable research has indicated that PNP is bioaccumulated [3], estrogenic [4], and highly toxic [5,6]. Consequently, the use of NP polyethoxylates has been banned in several European countries.

From 1980 to 1988, production of nonylphenol in the United States increased by 37% [6]. A 1989 and 1990 study of river reaches in the continental United States detected nonylphenol in 30% of water samples and 71% of sediment samples. Its concentrations in water ranged from 0.20 to 0.64 µg/L and in sediments from 10 to 3,000 µg/kg [2]. Concentrations up to 1,600 µg/L of PNP have been found in water from industrial sources [7]. Concentrations in river water downstream from a discharge ranged from 2 to 3,000 µg/L [8]. Nonylphenol and its ethoxylates have also been found in treatment-plant wastewaters and sewage sludges, where they have been produced by microbial breakdown of nonionic surfactants

during sewage treatment [9]. Their persistence in sewage effluent and industrial wastewater has been recognized for decades [10]. Degradation of nonylphenol concentration varies with conditions; biodegradation of free NP is only about 0.06% per day, especially in the absence of sediments [11].

Because NPs are ubiquitous, resist degradation, and bioaccumulate, their ability to produce physiological effects has been the subject of considerable recent attention [4,12]. For example, previous studies have shown that nonylphenol is directly toxic to fish and other aquatic animals [13,14]. The goal of this study was to measure acute toxicity of PNP to organisms representative of a saltwater community. The species, representing early life stages of nine different taxonomic families, were chosen according to the guidelines for deriving numerical national water quality criteria [15]. Documents for water quality criteria (also called aquatic-life or chemical criteria) for individual substances have been developed to protect freshwater and saltwater aquatic life and their uses. The history of the development, derivation, and use of water quality criteria has been summarized by Hansen [16].

MATERIALS AND METHODS

Biological methods

We determined the acute toxicity of PNP to early life-stages of four species of saltwater invertebrates (*Paleomonetes vulgaris*, *Americamysis bahia*, *Leptocheirus plumulosus*, and *Dyspanopeus sayii*) and two species of fish (*Menidia beryllina* and *Cyprinodon variegatus*) by using a 7-d flow-through exposure with measured concentrations. The second of these species, *Americamysis bahia*, was formerly known as *Mysidopsis bahia* and has been redescribed [17]. All species were cultured on site except *L. plumulosus*, which was cultured at the SAIC Environmental Testing Center, Narragansett, Rhode Island, USA [18–20]. Nauplii of *Artemia* sp. were fed to cultured and test organisms. In accordance with ASTM recommendations

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Contribution NHEERL-NAR-1992. Although this manuscript was reviewed in accordance with official U.S. Environmental Protection Agency procedures, its content does not necessarily reflect U.S. EPA policy. Mentioning of commercial products or facilities does not necessarily mean that U.S. EPA or the U.S. federal government endorses them.

[21], only reference *Artemia* sp. were fed to organisms during testing.

Organisms were exposed simultaneously in duplicate flow-through exposure chambers. Each species was placed into separate containers with glass petri dish bottoms and walls of Nitex® screen (mesh size 320 µm, Aquatic Eco-Systems, Apopka, FL, USA). Stock solutions were delivered to exposure chambers at each of seven concentrations by a flow-through diluter exposure system [22]. The surviving organisms were counted at hours 2, 4, 8, 12, and 24 and daily through day 7.

To obtain data for lower-temperature species, static tests were conducted with zoeal *Homarus americanus* (96 h, unmeasured concentrations), larval *Pleuronectes americanus* (96 h, measured concentrations), and embryo-larval *Mulinia lateralis* (48 h, unmeasured concentrations) [23]. The inhibition of fertilization, rather than survival, was the acute measure of toxicity in the *M. lateralis* test. Static tests were monitored daily. All flow-through and static tests used filtered (15 µm) seawater at pH levels of 7.8 to 8.2 and salinity of 30 to 31 g/kg from Narragansett Bay, Rhode Island, following ASTM procedures [24]. The salinity of the *M. lateralis* test was 32 g/kg. Exposure conditions for flow-through and static tests are summarized in Table 1. Data on survival were analyzed statistically using the Trimmed Spearman–Kärber method of estimation with Abbott's correction formula and pooled controls [25,26].

Analytical methods

We used 90% *para*-C9 phenols (CAS 84852-15-3) for all tests. The carrier solvent was a solution of 20% acetone and 80% triethylene glycol. Controls for seawater and for seawater with solvent were used in all tests. Final mean concentrations in the flow-through test were 20.2, 21, 29.5, 67.3, 70.5, 117, and 195 µg/L. For the static-measured test with *P. americanus*, final mean concentrations were 3.7, 6.9, 16.3, 19.1, 32.3, 73.2, 125, and 163 µg/L. In the static-unmeasured test with *H. americanus*, concentrations were 16, 26, 43, 72, 120, 200, and 330 µg/L; and in the *M. lateralis* test, they were 2.4, 8, 24, 80, 240, and 800 µg/L.

Before adding organisms, we measured the concentration of test material in the flow-through diluter and in one exposure chamber per treatment to verify that they were the same. On day 4 of the test, we measured the duplicate of each treatment. In the static test with *P. americanus*, we also sampled at the beginning and end of the exposure.

The sampling procedure consisted of siphoning 1-L samples from the flow-through tests into separatory funnels prerinsed with solvent. After internal standards were added, the samples were extracted three times with 50 ml of methylene chloride and dried with anhydrous sodium sulfate. Each sample was then reduced in volume, exchanged to heptane, and brought to a final volume of 1 ml. The samples were stored in 1.8-ml vials until they were analyzed.

The PNP in samples was measured by using a DB-5 capillary fused-silica column in a Hewlett-Packard 5890 gas chromatograph/flame ionization detector (Hewlett-Packard, Avondale, PA, USA). The helium carrier gas flowed at a linear velocity of 20 cm/s. The gas chromatograph oven was programmed to start at 60°C and rise at 10°C/min to 250°C. Temperatures of the injection port and detector were held at 270°C and 325°C, respectively.

We used 4-*t*-octylphenol as a surrogate internal standard and quantified the results with a five-point linear calibration.

Table 1. Test conditions for *para*-nonylphenol multispecies test exposure

Species	<i>Dyspanopeus sayi</i>	<i>Cyprinodon variegatus</i>	<i>Menidia beryllina</i>	<i>Leptocheirus plumulosus</i>	<i>Americamysis bahia</i>	<i>Palaemonetes vulgaris</i>	<i>Pleuronectes americanus</i>	<i>Homarus americanus</i>	<i>Mulinia lateralis</i>
Life stage age (d)	Zoea 4th and 5th stage	Juvenile	Juvenile	Adult	Postlarvae 1-day	Larvae 2-day	Larvae 2-day	Zoea first stage	Embryo
Test method ^a	FTM	FTM	FTM	FTM	FTM	FTM	SMN	SUR	SUN
No., size of test containers	4, 8-oz. glass jars	4, 9-cm-diameter netted cups	4, 9-cm-diameter netted cups	4, 8-oz. glass jars	4, 8-oz. glass jars	4, 8-oz. glass jars	2, 19-cm-diameter glass dishes	10, 8-oz. glass jars	3, 8-oz. glass jars
No. replicates/treatment	2	2	2	2	2	2	2	10	3
No. exposed/treatment	20	20	20	20	20	20	30	10	26 embryos/ml
Food	Reference <i>Artemia</i> sp.	Reference <i>Artemia</i> sp.	Reference <i>Artemia</i> sp.	Reference <i>Artemia</i> sp.	Reference <i>Artemia</i> sp.	Reference <i>Artemia</i> sp.	None	Reference <i>Artemia</i> sp.	None
Temperature (°C)	24–25	24–25	24–25	24–25	24–25	24–25	5	16–17	18.3

^a FTM = flow-through measured; SMN = static measured, no renewal; SUR = static unmeasured, renewed daily; SUN = static unmeasured, no renewal.

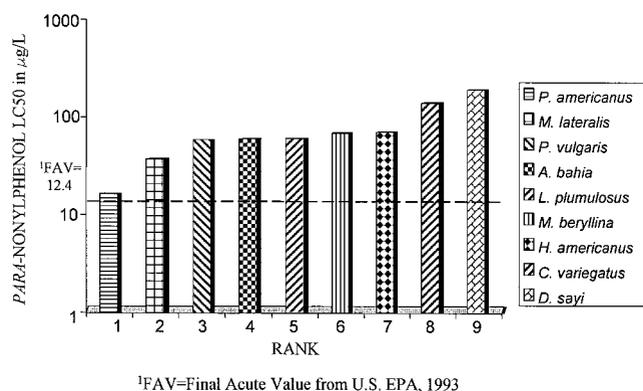


Fig. 1. Ranking of 96-h 50% lethal concentrations for saltwater species from most to least sensitive compared with the final acute value from draft water quality criterion for *para*-nonylphenol.

We verified the continuing accuracy of the results by calibrating standards at the beginning and end of the analysis and by analyzing seawater blanks, spiked seawater, and procedural blanks with each set of samples. Recoveries for six of the eight spiked seawater samples ranged from 65% to 72%; the other two were 60% and 55%. PNP was undetectable in seawater blanks and procedural blanks. The method detection limit was calculated to be 0.8 µg/L [27].

RESULTS AND DISCUSSION

Values of the 96-h LC50 for PNP (that is, the concentrations that were lethal to 50% of the organisms) for the nine species ranged from 17 to >195 µg/L (Fig. 1). Individual values in micrograms per liter were 17 for *Pleuronectes americanus*, 37.9 for *Mulinia lateralis* (48-h 50% effective concentration), 59.4 for *Paleomonetes vulgaris*, 60.6 for *Americamysis bahia*, 61.6 for *Leptocheirus plumulosus*, 70 for *Menidia beryllina*, 71 for *Homarus americanus*, 142 for *Cyprinodon variegatus*, and >195 for *Dyspanopius sayii*. Seven of the nine species responded within a range of 17 to 71 µg/L. Although these thresholds were well above concentrations in river reaches, they were within the range of concentrations in industrial effluents and sewage sludges [8,9]. This implies that PNP in receiving waters may well become concentrated enough to harm aquatic life in the mixing zone of effluents.

The nine species tested came from three different phyla—fishes, bivalves, and crustaceans. Among the most sensitive species were representatives from each of these phyla, all of which showed similarly high sensitivities to PNP. This result implies that PNP can be assimilated similarly by a broad range of species, can act similarly on all of them, and can thereby threaten a great proportion of the aquatic community.

Short-term responses were obtained for each species exposed in the flow-through system. Mortality of three species (*M. beryllina*, *A. bahia*, and *P. vulgaris*) plateaued by 96 h of exposure, with very similar 96-h LC50s of 70, 60.6, and 59.4 µg/L, respectively (Fig. 2). For *A. bahia*, Ward and Boeri had found a similar 96-h LC50 of 43 µg/L but no mortality until 72 h [28]. In contrast, effects on the most sensitive species tested, *P. americanus*, plateaued by 72 h. Continuing the test through 7 d provided little additional information except that *C. variegatus* plateaued by 6 d and *L. plumulosus* continued to respond gradually throughout the 7-d exposure, resulting in 96-h LC50s of 142 and 61.6 µg/L, respectively. A broadly

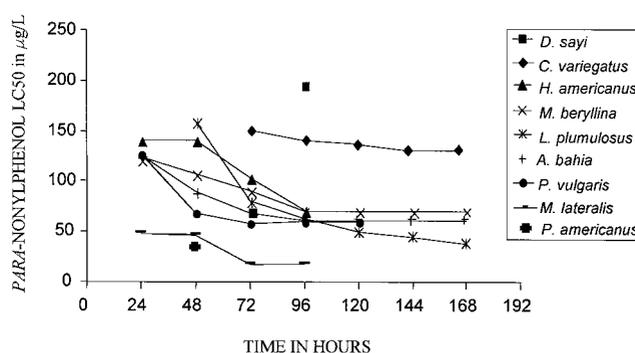


Fig. 2. 50% lethal concentrations from 24 to 168 h for saltwater species exposed to *para*-nonylphenol.

similar 96-h LC50 for *C. variegatus* (310 µg/L) was reported by Ward and Boeri [29].

LC50s for the seven most sensitive species, a well-defined group, fell within a factor of only about four. This narrow range of responses contrasts with that of a freshwater study with the detergent C₁₂ MAQ, whose LC50s for the seven most sensitive species ranged over a factor of 1,600, from 0.11 to 180 mg/L [30]. Because of PNP's narrow range of responses, it loses the advantage of having a few sensitive species that can act as sentinels for the remainder of the marine community. The narrow range provides a greater probability that small changes in the PNP concentration could harm or protect a large proportion of the community.

Results from this study provided the minimum acute saltwater database required for deriving the draft National Aquatic Life Criterion for *para*-nonylphenol, calculated to be 12.4 µg/L [15,31]. This value was based on a regression of the LC50s of the four most sensitive species tested, as required by Water Quality Criteria Guidelines [15]. It was extrapolated to a limit that protects 95% of saltwater species. Our acute exposure data presented here were recently published in a review article by Staples et al. [32] that referenced our poster presentation at the annual meeting of the Society of Environmental and Toxicology and Chemistry in November 1996. Because our methods were abbreviated in the poster, the Staples' article categorized our data as "use with care." The complete methods presented in this paper should show that our methods were sound and our results are valid.

Two other recent life-cycle tests have provided chronic data for *A. bahia*. A 1991 study found that growth, measured as length, was the most sensitive parameter [33]. The lowest-observed-effect concentration in that test was 6.7 µg/L, and the no-observed-effect concentration was 3.9 µg/L. A comparable life-cycle study with *A. bahia*, using nonylphenol from the same lot and CAS number, resulted in a no-observed-effect concentration of 9.5 µg/L and a lowest-observed-effect concentration of 15.3 µg/L for both growth (as dry weight) and reproduction (A. Kuhn-Hines, personal communication).

para-Nonylphenol can thus be characterized as having similar toxicity across a broad phylogenetic range. It also appears to exhibit sublethal toxicity at concentrations approaching the acute threshold. The threat to marine species posed by *para*-nonylphenol's narrow range of thresholds is not only among species but also between acute and sublethal responses. For example, the 96-h LC50 for *A. bahia* was 60.6 µg/L, compared with an acute lowest-observed-effect concentration of 21 µg/L from this study, and a chronic mean lowest-observed-effect con-

centration of 11 $\mu\text{g/L}$ from studies by Ward and Boeri [33] (and by Kuhn-Hines et al., personal communication). In our 48-h test with *M. lateralis*, a concentration of 37.9 $\mu\text{g/L}$ inhibited fertilization by 50%. In another study, 27 $\mu\text{g/L}$ of PNP inhibited the growth of the marine alga *Skeletonema costatum* by 50% after a 96-h exposure [34]. Estrogenic effects remain under investigation, but 10 $\mu\text{g/L}$ of PNP has been observed to elevate vitellogenin synthesis in male rainbow trout and significantly decrease the rate of growth of the testes [35]. More information is needed to establish the extent of estrogenic and other sublethal effects.

In summary, *para*-nonylphenol is a toxic chemical that is ubiquitous in surface waters. Aquatic communities can be directly exposed to it through industrial wastewaters or sewage effluents, even after they have been treated anaerobically [36]. Another common direct source is combined sewer overflows after storm events, which often result in high concentrations of PNP in receiving waters [9]. Aquatic communities can also be exposed indirectly (via nonpoint sources) through runoff from land treated with sewage sludge. Even low concentrations of PNP can lead to estrogenic effects that might have long-term consequences, such as disrupting population dynamics or interactions within aquatic communities. Further investigation in this area is clearly needed. We do know, however, that very small increases above current aquatic concentrations are sufficient to reach threshold levels that would result in sublethal effects—and even lethal effects—for many species and their life stages. Therefore, we must monitor PNP's sources and ambient concentrations very carefully. In particular, the widespread use and persistence of PNP demands close attention to our manufacturing and sewage-treatment processes as well as to our disposal practices.

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