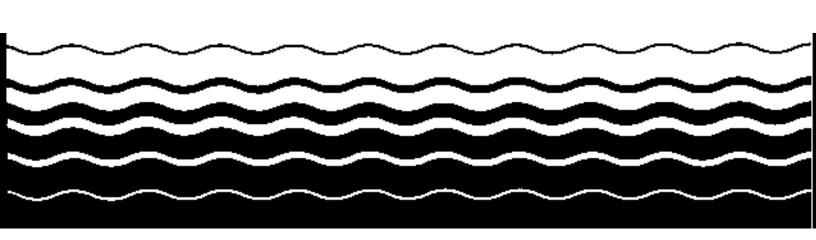


# Ambient Aquatic Life Water Quality Criteria for Nonylphenol - Draft



# AMBIENT AQUATIC LIFE WATER QUALITY CRITERIA FOR ${\tt NONYLPHENOL-DRAFT}$

(CAS Registry Number 84852-15-3) (CAS Registry Number 25154-52-3)

U.S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF WATER
OFFICE OF SCIENCE AND TECHNOLOGY
HEALTH AND ECOLOGICAL CRITERIA DIVISION
WASHINGTON D.C.

#### **NOTICES**

This document has been reviewed by the Health and Ecological Criteria Division, Office of Science and Technology, U.S. Environmental Protection Agency, and is approved for publication.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This document is available to the public through the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161. It is also available on EPA's web site: http://www.epa.gov/waterscience/criteria/nonylphenol/

#### **FOREWORD**

Section 304(a)(l) of the Clean Water Act of 1977 (P.L. 95-217) requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water. This document is a revision of previous drafts based upon consideration of comments received from US EPA staff and independent peer reviewers. Criteria contained in this document replace any previously published EPA aquatic life criteria for the same pollutant.

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304(a)(l) and section 303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. Criteria presented in this document are such scientific assessments. If water quality criteria associated with specific stream uses are adopted by a state as water quality standards under section 303, they become enforceable maximum acceptable pollutant concentrations in ambient waters within that state. Water quality criteria adopted in state water quality standards could have the same numerical values as criteria developed under section 304. However, in many situations states might want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. It is not until their adoption as part of state water quality standards that criteria become regulatory. Guidelines to assist the states and Indian tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 1994). This handbook and additional guidance on the development of water quality standards and other water-related programs of this agency have been developed by the Office of Water.

This draft document is guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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#### Introduction<sup>1</sup>

Nonylphenol ( $C_{15}H_{24}O$ ) is produced from cyclic intermediates in the refinement of petroleum and coal-tar crudes. It is manufactured by alkylating phenol with mixed isomeric nonenes in the presence of an acid catalyst. The product is a mixture of alkylphenols, predominantly para-substituted (4-nonylphenol; CAS No. 104-40-5) and occasionally ortho-substituted (2-nonylphenol; CAS No. 136-83-4), with various isomeric, branched-chain nonyl (nine carbon) groups. (Commercial mixtures containing specified amounts of nonylphenol isomers and 2,4-dinonylphenol are given specific CAS Numbers, either 25154-52-3 or 84852-15-3. These products were used for deriving the water criteria for nonylphenol.) There is little direct use for nonylphenol except as a mixture with diisobutyl phthalate to color fuel oil for taxation purposes and with acylation to produce oxime as an agent to extract copper. Most nonylphenol is used as an intermediate chemical which, after etherification by condensation with ethylene oxide in the presence of a basic catalyst, produces the nonionic surfactants of the nonylphenol ethoxylate type. The nonionic surfactants are used as oil soluble detergents and emulsifiers that can be sulfonated or phosphorylated to produce anionic detergents, lubricants, antistatic agents, high performance textile scouring agents, emulsifiers for agrichemicals, antioxidants for rubber manufacture, and lubricant oil additives (Reed 1978).

Nonylphenol is produced at a high annual tonnage rate. Its production in the U.S. was 147.2 million pounds (66.8 million kg) in 1980 (USITC 1981), 201.2 million pounds (91.3 million kg) in 1988 (USITC 1989), 230 million pounds (104 million kg) in 1998 (Harvilicz 1999), and demand is increasing about 2 percent annually. Nonylphenol has an approximate molecular weight of 215.0 to 220.4 g/mole, is a pale yellow highly viscous liquid with a slight phenolic odor and a specific gravity of 0.953 g/mL at  $20^{\circ}$ C (Budavari 1989). It has a dissociation constant (pK<sub>a</sub>) of  $10.7 \pm 1.0$ ; octanol/water partition coefficient (Log K<sub>ow</sub>) of 3.80 to 4.77; pH-dependent water solubility of 4,600 : g/L at pH 5.0, 6,237 : g/L at pH 7, 11,897 : g/L at pH 9 and 3,630 : g/L in seawater; soluble in many organic solvents; and has a vapor pressure of  $4.55 \times 10^{-3}$  ( $\pm 3.54 \times 10^{-3}$ ) Pa (Roy F. Weston Inc. 1990). Ahel and Giger (1993) measured the solubility of nonylphenol at different temperatures in distilled water and demonstrated a nearly linear relationship in solubility of 4,600  $\mu$ g/L at  $2^{\circ}$ C to 6,350  $\mu$ g/L at  $25^{\circ}$ C.

Nonylphenol has been studied for its acute and chronic toxicity to aquatic organisms and results of many studies are well summarized in a review article (Staples et al. 1998). Additionally, this review

<sup>&</sup>lt;sup>1</sup>A comprehension of the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses" (Stephen et al. 1985), hereafter referred to as the Guidelines, is necessary to understand the following text, tables and calculations.

article addresses the ability of nonylphenol to bioaccumulate in aquatic organisms.

Nonylphenol and nonylphenol ethoxylates have been found in the environment and a review of studies describing their distribution has been published (Bennie 1999). Shackelford et al. (1983) reported 4-nonylphenol at average concentrations ranging from 2 to 1,617: g/L in eleven water samples associated with various industrial sources. Bennie et al. (1997) measured water concentrations from 0.01 to 0.92 ug/L in 25 percent of the sites sampled in the Great Lakes. They found nonylphenol in all sediment samples and the concentrations ranged from 0.17 to 72 μg/g (dry weight). Studies have shown the presence of nonylphenol and its ethoxylates in treatment plant wastewaters (Ellis et al. 1982, Giger et al. 1981) and in sewage sludges (Giger et al. 1984). A study was conducted of thirty river reaches in the continental U.S. in 1989 and 1990 to determine the frequency and concentrations of nonylphenol and its ethoxylates in water and sediments. Nonylphenol was found in approximately 30 percent of the water samples and concentrations in water ranged from about 0.20 to 0.64: g/L. Approximately 71 percent of the sites had measurable concentrations of nonylphenol in the sediments and the concentrations ranged from about 10 to 2,960: g/kg. Various ethoxylates of nonylphenol were found in 59 to 76 percent of the water samples, varying by extent of ethoxylation (Naylor 1992, Naylor et al. 1992, Radian Corp. 1990). Keith et al. (2001) measured nonylphenol in fish tissues of seven species from the Kalamazoo River and the river's mouth at Lake Michigan. They found 41 percent of the samples had measurable concentrations of nonylphenol with a range of 3.3 to 29.1 µg/kg, and a mean value of 12.0 µg/kg.

Most nonylphenol enters the environment as 4-alkylphenol polyethoxylate surfactants which are degraded to 4-alkylphenol mono- and diethoxylates in active sewage sludges (Giger et al. 1984). It was theorized by Giger et al. (1984) that further transformation of 4-alkylphenol mono- and diethoxylates to 4-nonylphenol is favored by anaerobic environments. They conducted experiments with stabilized (anaerobic) and raw (aerobic) sewage sludge and found that concentrations of 4-nonylphenol increased four to eight times in the stabilized versus two times in the raw sludge, a finding which supported their theory.

Persistence of nonylphenol in sewage effluents and the environment has been studied and a review has been written (Maguire 1999) of published studies. Gaffney (1976) determined that 1 mg/L nonylphenol did not degrade during a 135-hr incubation with domestic wastewater. He also measured no change in nonylphenol concentration at 24 hr in industrial wastewater, but after 135 hr there was a 45 percent degradation of the compound.

Sundaram and Szeto (1981) studied nonylphenol fate in stream and pond waters when incubated in open and closed containers. They found no degradation of nonylphenol when incubated in open containers of the pond or stream waters and a half-life of 2.5 days, probably due to volatization. After

three days of incubation in pond or stream waters in closed containers, a breakdown product was measured and half-lives were estimated of 16.5 and 16.3 days, respectively. The same study demonstrated that nonylphenol in pond water with sediment present resulted in about 50 percent of the nonylphenol appearing in the sediment after 10 days. About 80 percent of the nonylphenol in the sediment was degraded in 70 days. No degradation of nonylphenol occurred when autoclayed water and sediment samples were used. Staples et al. (1999) measured a half-life of 20 days at 22°C for nonylphenol at a concentration of 31 mg/L. They suggested that the temperature of water and the initial concentration of the nonylphenol both affect the degradation rate of the chemical. Ahel et al. (1994a,b) studied the fate and transport of alkylphenol polyethoxylates (APnEO) and their metabolites in the Glatt River system in Northern Switzerland from the Greifensee to the Rhine River. Water samples were collected at eight sites along the river hourly and seasonally. They found nonylphenol concentrations to be lower than other metabolites and nonylphenol concentrations were most common in the 1 to 3 µg/L range. Metabolite concentrations of APnEO's varied with time of day reflecting wastewater treatment plant discharge fluctuations. Metabolite concentrations of APnEO's also varied seasonally, and were higher in the winter due to lower water temperature. Nonylphenol had less season variability than other metabolites of APnEO's. Sediments were investigated and nonylphenol was the predominant nonylphenolic compound with concentrations of 364 to 5,100 times that found in the river water. Treatment conditions within the treatment plants along the Glatt River system were studied and the abundance of particular metabolites of APnEO's were dependent on the treatment conditions (Ahel et al. 1994a; Ahel et al. 2000). Another study by Ahel et al. (1996) demonstrated that nonylphenol can be reduced in ground water probably by biological processes provided that the ground water temperature does not become too cold for biological degradation. It has been demonstrated (Ahel et al. 1994c) that nonylphenol can be degraded by photochemical processes in 10 to 15 hrs (half-life) in bright summer sun when nonylphenol is near the water surface.

Heinis et al. (1999) studied the distribution and persistence of nonylphenol in temperate climate zone natural pond systems. They reported that nonylphenol partitioned to the pond enclosure wall material, macrophytes, and sediments within two days. After 440 days, the primary sink for nonylphenol was the sediment. Dissipation time from the sediment for 50 and 95 percent were estimated at 66 and 401 days, respectively. Measurable concentrations of nonylphenol can persist for many years in sediments. Hale et al. (2000) measured nonylphenol concentrations in sediments below wastewater outfalls and found one site that had a sediment concentration of 54,400 µg/kg more than twenty years after the treatment plant ceased operation. Bennett and Metcalfe (1998; 2000) found that nonylphenol was widely distributed in the lower Great Lakes sediments and reached 37,000 µg/kg in sediments near sewage

treatment plants.

It appears that degradation of nonylphenol in sea water may be slower than in fresh water. This was observed in both water and sediments. Ekelund et al. (1993) found that initial nonylphenol degradation was slow in sea water, but after microorganism adaptation occurred, the degradation rate increased. Approximately 50 percent of the nonylphenol was degraded after 58 days. In marine sediments, the rate of degradation was initially faster than in water, but about the same percentage was degraded in 58 days as in sea water. Ethoxylated nonylphenol, in marine sediments, has a half-life of 60 days similar to nonylphenol (Shang et al. 1999).

Nonylphenol is metabolized by hepatic cytochrome P450 enzymes in the rainbow trout (*Oncorhynchus mykiss*), and bile from the fish contained the glucuronic acid conjugates of nonylphenol (Meldahl et al. 1996; Thibaut et al. 1999). Arukwe et al. (2000) found that bile was the major route of nonylphenol excretion with a half-life of 24 to 48 hrs in both waterborne and dietary exposures. The Log P of nonylphenol ranges from 3.80 to 4.77, indicating the possibility of bioaccumulation in aquatic organisms. Bioconcentration was measured in two saltwater organisms, blue mussel (*Mytilus edulis*) and Atlantic salmon (*Salmo salar*). The estimated bioconcentration factor (BCF) for the blue mussel ranged from 1.4 to 7.9 (McLeese et al. 1980a), and the Atlantic salmon estimated BCF was 75 (McLeese et al. 1981). Ahel et al. (1993) measured the bioconcentration of nonylphenol in rivers in Switzerland. They found that nonylphenol was bioconcentrated approximately 10,000 times in algae, but this concentration was not further concentrated up the food chain. Instead, they measured lower bioconcentration factors in fish and ducks than in plants.

Nonylphenol has been tested for its ability to bind to estrogen receptors. There are several review articles that describe disruption of endocrine function by nonylphenol (Servos 1999; Sonnenschein and Soto 1998; Sumpter 1998). It has been found to bind to estrogen receptors in cell cultures (Flouriot et al. 1995; Hewitt et al. 1998; Jobling and Sumpter 1993; Lutz and Kloas 1999; Routledge and Sumpter 1996; Soto et al. 1991, 1992; White et al. 1994) and whole animals (Jobling et al. 1996). Optimal estrogenic activity requires a single tertiary branched alkyl group composed of six to eight carbons located at the *para* position on an otherwise unsubstituted phenol ring (Routledge and Sumpter 1997). Tabira et al. (1999) found that when using human estrogen receptors, the receptor binding of alkylphenols was maximized when the number of alkyl carbons was nine as it is with nonylphenol. Nonylphenol is able to stimulate the liver of male and immature female fish to produce the egg-yolk precursor protein vitellogenin, which is normally found in high concentrations only in mature female fish. Islinger et al. (1999) estimated the estrogenic potential of nonylphenol to stimulate vitellogenin production in male rainbow trout at 2,000 to 3,000 times less potent than 17\$-estradiol. It is also able to cause changes in the

spermatogenesis cycle of male fish. Ren et al. (1996a) demonstrated significant increases in the estrogenic effects in rainbow trout exposed to nonylphenol at 100 µg/L for 72 hr using vitellogenin production as a biomarker. In another study, Ren et al. (1996b) demonstrated that nonylphenol could stimulate the production of vitellogenin mRNA in four hr at a concentration as low as 10 : g/L. Nonylphenol at concentrations of 50 and 100 µg/L caused 50 and 86 percent, respectively, of the male Japanese medaka (*Oryzias latipes*) fish to develop an intersex condition (both testicular and ovarian tissues in the gonad) with a three month exposure (Gray and Metcalfe 1997). The sex ratio shifted in favor of females at the highest treatment. Purdom et al. (1994) found that rainbow trout held in cages in the outfalls of sewage treatment plants had increased vitellogenin concentrations in the blood. They suggested that the two most likely estrogenic substances present in the effluents were ethynylestradiol and nonylphenol. Several studies (Allen et al. 1999, Harries et al. 1997, Lye et al. 1999, Tanghe et al. 1999) conducted in Europe have attempted to demonstrate that waters in various rivers and estuaries below sewage treatment plants have the ability to induce estrogenic effects in a yeast assay and in fish. Effects were seen in most areas sampled and the possibility of mixture effects with nonylphenol, other xenoestrogens, and human estrogens exists.

A comprehension of the "Guidelines" for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephan et al. 1985), hereinafter referred to as the "Guidelines," and the response to public comments concerning that document (U.S. EPA 1985) is necessary to understand the following text, tables, and calculations. Results of such intermediate calculations as recalculated LC50s and Species Mean Acute Values are given to four significant figures to prevent roundoff error in subsequent calculations, not to reflect the precision of the value. The U.S. Environmental Protection Agency has modified its original intention of requiring testing for nonylphenol-mixed isomers (CAS No. 25154-52-3) and now recommends testing to be conducted with the chemical substance comprised of mostly *para*-branched C<sub>9</sub>-alkylphenols with CAS No. 84852-15-3 (Federal Register 1990). The criteria presented herein are the agency's best estimate of maximum concentrations of the chemical of concern to protect most aquatic organisms, or their uses, from any unacceptable short-or long-term effects. Freshwater criteria were derived using nonylphenol of CAS numbers 25154-52-3 and 84852-15-3; saltwater criteria were derived using only nonlyphenol of CAS number 84852-15-3. The latest comprehensive literature search for fresh- and saltwater information for this document was conducted in November, 1999. Some newer information has been included.

#### **Acute Toxicity To Aquatic Animals**

Data that are suitable, according to the "Guidelines," for the derivation of a freshwater Final

Acute Value (FAV) are included in Table 1. Eighteen species and two subspecies representing fifteen genera were tested with nonylphenol to determine its acute toxicity to these species. Acute toxicity test results ranged from 55.72 μg/L for an amphipod (*Hyalella azteca*) to 774 μg/L for a snail (*Physella virgata*).

The most sensitive freshwater species tested was an amphipod, *Hyalella azteca* (Tables 1 and 3). Brooke (1993a) and England and Bussard (1995) tested this species under similar conditions, except for water hardness levels which were 51.5 and 148-154 mg/L as CaCO<sub>3</sub>, respectively. An LC50 of 20.7 μg/L was calculated in the lower hardness water and 150 μg/L in the higher hardness water. However, insufficient data exist to demonstrate an effect of water hardness on the toxicity of nonylphenol.

Tadpoles of the boreal toad, *Bufo boreas*, were ranked second in sensitivity to nonylphenol (Dwyer et al. 1999a) and had a 96-hr LC50 of 120 μg/L. Data for one cladoceran species (*Daphnia magna*) are available. Brooke (1993a) reported an EC50 of 84.8 μg/L from a test that had the solutions renewed daily and Comber et al. (1993) reported an EC50 of 190 μg/L in a static test. The *Daphnia magna* Species Mean Acute Value is 126.9 μg/L.

Freshwater fish species were in the mid-range of toxicity to nonylphenol. Toxicity test results are available for eleven species representing eight genera. Their sensitivity to nonylphenol ranged from 133.9 µg/L for the fathead minnow (*Pimephales promelas*) to 289.3 µg/L for the bonytail chub (*Gila elegans*). Three trout species of the genus *Oncorhynchus* and two subspecies were tested and had similar LC50s ranging from 140 to 270 µg/L. Dwyer et al. (1995, 1999a) exposed nine species of fish that were surrogates of threatened or endangered fish species or were threatened and endangered. Acute toxicity test results were based upon static tests with unmeasured nonylphenol concentrations and the LC50s ranged from 110 µg/L for the fountain darter, *Etheostoma rubrum*, to a geometric mean of 289.3 µg/L for the bonytail chub.

The least sensitive freshwater species to nonylphenol toxicity were invertebrates. The annelid worm (*Lumbriculus variegatus*) had an LC50 of 342  $\mu$ g/L, nymphs of the dragonfly *Ophiogomphus* sp. had an LC50 of 596  $\mu$ g/L and the least sensitive species tested was a snail, *Physella virgata*, which had an LC50 of 774  $\mu$ g/L. The lower sensitivity to nonyphenol occurs even though this species of snail does not have an operculum and would not be able to completely enclose its body and thus protect itself against nonylphenol exposure.

Freshwater Species Mean Acute Values (SMAV) and Genus Mean Acute Values (GMAV) were derived from available acute values (Tables 1 and 3). GMAVs were available for 15 genera; the most sensitive was the amphipod, *H. azteca*, which was 13.9 times more sensitive than the least sensitive species, a snail *P. virgata* (Figure 1). The four most sensitive species were within a factor of 2.4 of one

another. The freshwater Final Acute Value (FAV) for nonylphenol is  $55.71 \,\mu\text{g/L}$  and was calculated using the procedure described in the "Guidelines" and the GMAVs in Table 3. The FAV is equal to the lowest freshwater SMAV of  $55.72 \,\mu\text{g/L}$  for the amphipod *H. azteca*.

The acute toxicity of nonylphenol to saltwater animals has been tested with seven invertebrate and three fish species (Table 1). The range of SMAVs extends from 17 µg/L for the winter flounder, *Pleuronectes americanus*, to 209.8 µg/L for the sheepshead minnow, *Cyprinodon variegatus* (Lussier et al. 2000; Ward and Boeri 1990b), a difference of 12.3 times. Fish (winter flounder), bivalves (coot clam, *Mulinia lateralis*) and crustaceans (the mysid, *Americamysis bahia*) were all among the most sensitive species.

Data for nine of the twelve saltwater test values reported in Table 1 were from a single multi-species test (Lussier et al. 2000). Nonylphenol concentrations were measured in seven of the nine tests (Table 1), with measurements made at test initiation and at the end of the test (48 or 96 hr). Test organisms were fed brine shrimp, *Artemia* sp., during test chemical exposure. Normally this is not acceptable for data used to derive Final Acute Values. However, the tests reported by Lussier et al. (2000) were designed to extend beyond the usual 48 or 96-hr acute test interval to 168 hr. The extended exposure time required feeding to ensure survival of animals not affected by nonylphenol. The brine shrimp fed during the tests were "reference grade" and not likely to change the exposure to nonylphenol. Two animal species were tested in two laboratories allowing comparison of results from a study with food added and another without food. In the case of the mysid, 96-hr LC50s were estimated at 43 and 60.6 µg/L for the non-fed and fed studies, respectively. The sheepshead minnow had 96-hr LC50s of 310 and 142 µg/L for the respective non-fed and fed studies. Because feeding during the tests did not consistently raise or lower the LC50 estimates, feeding is assumed not to have altered the results in these tests. Therefore, the data from the Lussier et al. (2000) tests were acceptable for deriving a saltwater Final Acute Value.

GMAVs for the four most sensitive saltwater species differ by a factor of only 3.5 (Table 3 and Figure 2). Using the method of calculation specified in the "Guidelines," the saltwater FAV is 13.35  $\mu$ g/L. The FAV is lower than the lowest SMAV of 17  $\mu$ g/L for the winter flounder.

#### Chronic Toxicity To Aquatic Animals

The available data that are usable according to the "Guidelines" concerning the chronic toxicity of nonylphenol are presented in Table 2. England (1995) exposed neonates of a cladoceran, *Ceriodaphnia dubia*, to nonylphenol for seven days in a renewal test. The results showed a significant reproductive impairment at 202 µg/L, but not at 88.7 µg/L, and survival was reduced at 377 µg/L, but not

at 202 µg/L. Based upon reproductive impairment, the Chronic Value for *C. dubia* was 133.9 µg/L. At the end of 48 hr in the same test, effects were observed and an EC50 of 69 µg/L was calculated. However, the animals had received food and according to the "Guidelines," acute tests with this species must not receive food during an acute toxicity test if the test is to be valid and used to compute an Acute-Chronic Ratio (ACR).

Fliedner (1993) exposed 4 to 24 hr-old *Daphnia magna* neonates to nonylphenol for 22 days in a 20°C life-cycle test. Test solutions were renewed three times each week during which a 52.2 to 65.5 percent decrease in nonylphenol concentration was measured. Mean measured nonylphenol test concentrations were: 0, 0, 1.55, 1.34, 3.45, 10.70, and 47.81  $\mu$ g/L. No effects were observed during the study on the mortality, the number of offspring per female, or the mean day of the first brood. A significant effect was measured for the total number of young per concentration on day nine of the study. Consequently, the No Observed Effect Concentration (NOEC) was 10.7  $\mu$ g/L and the Lowest Observed Effect Concentration (LOEC) was 47.8  $\mu$ g/L with a chronic value (geometric mean of the NOEC and LOEC) of 22.62  $\mu$ g/L. An acute test was not conducted to calculate an ACR.

Brooke (1993a) also reported a chronic exposure for the cladoceran *Daphnia magna*, but for 21-days. Test solutions were renewed three times per week and concentrations of nonylphenol declined an average of  $57.4 \pm 5.8$  percent between solution renewals. The author concluded that *D. magna* were significantly impaired in growth and reproduction at 215 µg/L, but not at 116 µg/L. Survival was reduced to 60 percent at 215 µg/L; however, this survival rate was not a significant reduction from the control survival rate because only 80 percent survived in the control group. The Chronic Value estimated from the geometric mean of the lower (116 µg/L) and upper (215 µg/L) chronic limits based upon reproductive impairment was 157.9 µg/L. Division of the chronic value for this test (157.9 µg/L) into the 48-hr EC50 from a companion test (84.8 µg/L) resulted in an ACR of 0.5370. The calculated ratio of 0.5370 was changed to 2.000 as suggested in the "Guidelines" because acclimation to nonylphenol probably occurred during the chronic test.

A third *D. magna* life-cycle 21-day exposure to nonylphenol was conducted by Comber et al. (1993). They found no significant effects in survival, reproduction or growth at concentrations #24  $\mu$ g/L. Reproduction was significantly reduced at concentrations \$39  $\mu$ g/L when the number of live young produced was compared to control reproduction. Growth was reduced at concentrations \$71  $\mu$ g/L and survival of adults was reduced at concentrations \$130  $\mu$ g/L. Based upon reduced reproduction at 39  $\mu$ g/L but not at 24  $\mu$ g/L, the Chronic Value was 30.59  $\mu$ g/L. Division of the Chronic Value (30.59  $\mu$ g/L) for this test into the 48-hr EC50 of 190  $\mu$ g/L from a companion study resulted in an ACR of 6.211.

Because two ACRs were available for D. magna, the geometric mean of the two values was used

as the species-mean ACR. The species-mean ACR for *D. magna* is 3.524.

The midge, *Chironomus tentans*, was exposed to five concentrations of nonylphenol and a control from #24-hr old larva through emergence (53 days) as adults (Kahl et al. 1997). Nominal exposure concentrations ranged from 12.5 to 200  $\mu$ g/L, but mean measured concentrations were lower. Neither growth or reproductive (sex ratio, emergence pattern, and egg production and viability) measurements were negatively affected at any of the exposure concentrations. There was a significant effect upon survival of larvae during the first 20 days of exposure, but none after 20 days. The LOEC was 91  $\mu$ g/L, based upon survival at 20 days, and the NOEC was 42  $\mu$ g/L. The Chronic Value is 61.82  $\mu$ g/L. An acute exposure was not conducted; therefore, an ACR can not be calculated for this species.

A 91-day early life-stage test was conducted with embryos and fry of the rainbow trout, *Oncorhynchus mykiss* (Brooke 1993a). Five nonylphenol exposure concentrations were tested and they ranged from 6.0 to 114 μg/L in the flow-through test. Time to hatch and percent survival at hatch were not affected by the nonylphenol concentrations; however, nearly all of the larvae were abnormal at the two highest exposure concentrations (\$53.0 μg/L). At the end of the test, survival was significantly reduced at concentrations \$23.1 μg/L but not at 10.3 μg/L. Growth (both weight and length) was a more sensitive chronic endpoint than survival. At the end of the test, the fish were significantly shorter (14 percent) and weighed less (30 percent, dry weight) at concentrations \$10.3 μg/L than the controls, but not at 6.0 μg/L. Based upon growth, the Chronic Value for rainbow trout was 7.861 μg/L. A companion acute test was available for this species. Division of the Chronic Value (7.861 μg/L) into the Acute Value (221 μg/L) yielded an ACR of 28.11 for rainbow trout.

An early-life-stage toxicity test was conducted with nonylphenol and the fathead minnow, *Pimephales promelas* (Ward and Boeri 1991c). Embryos and larvae were exposed for a total of 33 days to five concentrations of nonylphenol that ranged from 2.8 to 23 μg/L. Embryos in the control and those in the three lowest nonylphenol exposure concentrations (2.8, 4.5, and 7.4 μg/L) began to hatch on the third day of exposure, while the two higher concentrations (14 and 23 μg/L) began hatching on the fourth day. Growth (length or weight) was not significantly different from the control organisms at any of the treatment exposures. Survival of the fish at the end of the test was significantly reduced at nonylphenol concentrations \$14 μg/L. Fish survival averaged 56.7 percent at the 23 : g/L exposure, 66.7 percent at the 14 μg/L exposure, and 76.7 percent at the 7.4 μg/L exposure, only concentrations #7.4 : g/L did not differ from the control that averaged 86.7 percent survival. Based upon survival, the LOEC for the fathead minnow was 14 μg/L and the NOEC was 7.4 μg/L. The Chronic Value was 10.18 μg/L (Table 2). No companion acute toxicity test was conducted with the fathead minnow with which an ACR can be calculated.

The chronic toxicity of nonylphenol to saltwater animals was determined in a 28-day life-cycle test with mysids, *Americamysis bahia* (Ward and Boeri 1991b). There was no effect on survival or reproduction at  $6.7 \,\mu g/L$ , but there was a 18 percent reduction in survival and a 53 percent reduction in reproduction at  $9.1 \,\mu g/L$ . Effects on survival at the highest concentration tested ( $21 \,\mu g/L$ ) were observed before the end of the third week of the test. Test organisms of each sex were measured separately for length and weight. The data show no obvious difference between the length of male and female mysids for all of the concentrations tested. The growth analysis was based on combined length data for both sexes. Growth (length) was the most sensitive endpoint for mysids. There was a 7 percent, but statistically significant, reduction in the length of mysids exposed to  $6.7 \,\mu g/L$  of nonylphenol relative to control mysids. There was not a significant difference in growth for mysids exposed to  $3.9 \,:\, g/L$  nonylphenol when compared to control animals (Table 2). The Chronic Value, based on growth, for mysids was the geometric mean of the lower ( $3.9 \,\mu g/L$ ) and the upper ( $6.7 \,\mu g/L$ ) Chronic Values and was  $5.112 \,\mu g/L$ . The ACR of 8.412 was calculated using the acute value of  $43 \,\mu g/L$  from a companion study and dividing by the Chronic Value of  $5.112 \,\mu g/L$ .

Three valid ACRs are available for nonylphenol using the third and eighth (Table 3) most sensitive tested species of freshwater animals and the third most sensitive saltwater animal. Two ACRs were available for the cladoceran *Daphnia magna*, which differed by a factor of approximately 3.1 times. The geometric mean of these two values is 3.524. The cladoceran, *Ceriodaphnia dubia*, had an ACR ratio of 0.515 when using the tests of England (1995). However, this ratio was derived using the results of the companion acute test during which the organisms were fed. According to the "Guidelines," acute tests with this species must be done without food present in the test solutions. Therefore, the *C. dubia* ACR was not used. The three valid ACRs (3.524, 8.412 and 28.11) differed by a maximum of 7.98 times (Table 3). The largest ACR was for a fish (rainbow trout) that represented the eighth most sensitive genera of the fifteen tested from fresh water. The geometric mean of the three valid ACRs was 9.410, which is the Final Acute-Chronic Ratio (FACR).

#### **Toxicity To Aquatic Plants**

Only a single species of freshwater plant has been tested that meets the requirements for inclusion in Table 4 according to the "Guidelines." Ward and Boeri (1990a) exposed green algae (*Selenastrum capricornutum*) to nonylphenol for four days. They calculated an EC50 of 410 µg/L based upon cell counts. At the end of the toxicity test, algae from the highest exposure concentration (720 µg/L) were transferred to fresh media solution. During the next seven days, cell counts increased exponentially indicating that nonylphenol treatment at this concentration for four days did not have a persistent

algistatic effect.

Acceptable data on the toxicity of nonylphenol to saltwater plants were available for one species of marine algae (Table 4). The EC50 value for vegetative growth of the planktonic diatom, *Skeletonema costatum*, was 27 µg/L (Ward and Boeri 1990d). Although this value was lower than nearly all of the acute values for animals, it is for vegetative growth, which can recover rapidly. *Skeletonema* transferred from the highest nominal concentration of nonylphenol with survivors (120 µg/L) into control medium grew to a 76-fold increase in cells/mL within 48 hr (Ward and Boeri 1990d).

Based on the vegetative growth test using the saltwater planktonic diatom, *Skeletonema costatum*, the Final Plant Value for nonylphenol is 27: g/L. This plant species is more sensitive to nonylphenol than any tested species of freshwater animal and more sensitive than all but one tested saltwater animal species.

#### **Bioaccumulation**

Three studies were conducted to measure the bioconcentration of nonylphenol in freshwater animals that, according to the "Guidelines," meet the requirements for inclusion in this section of the document (Table 5). Ward and Boeri (1991a) measured the whole body burden in juvenile fathead minnows, with bioconcentration determined at two exposure concentrations (4.9 and 22.7  $\mu$ g/L) after 27 days of exposure. The bioconcentration factors were not lipid normalized and were similar at 271 and 344 times for the respective lower and higher exposure concentrations.

Brooke (1993b) exposed juvenile fathead minnow (*Pimephales promelas*) and juvenile bluegill (*Lepomis macrochirus*) to nonylphenol each at five concentrations for four and twenty-eight days. Lipid concentrations were measured (Brooke 1994) for the test fish and the bioconcentration results were lipid normalized which reduced the bioconcentration factors from 4.7 to 4.9 times. Nonylphenol concentrations that proved lethal to the organisms were not used to compute bioconcentration factors. The short-term (4 day) tests showed that plateau tissue concentrations were reached within two days in both the fathead minnow and the bluegill. Therefore, there was generally good agreement between the 4-and 28-day tests. Normalized bioconcentration factors for the fathead minnow ranged from 128.3 to 209.4 (Table 5). Normalized bioconcentration factors for the bluegill ranged from 38.98 to 56.94.

Giesy et al. (2000) measured the concentration of nonylphenol in the whole bodies of the fathead minnow following a 42-day exposure. Three sublethal concentrations allowed nonylphenol to bioaccumulate 203 to 268 times in exposure concentrations ranging from 0.4 to 3.4 µg/L.

Bioconcentration factors are available (Ekelund et al. 1990) for three species of saltwater animals, *Mytilus edulis, Crangon crangon* and *Gasterosteus aculeatus* (Table 5). Dosing was with <sup>14</sup>C-labeled

nonylphenol, but the CAS number was not listed. (*Crangon crangon* is a non-resident species, but the data are included since very little bioaccumulation data are available.) Exposures lasted 16 days followed by an elimination period of 32 days. Lipid normalized bioconcentration factors based on wet weight ranged from 78.75 for *C. crangon* to 2,168 for *M. edulis*. The steady state tissue concentration for *M. edulis* was estimated since it did not reach steady state after only 16 days of exposure.

No U.S. FDA action level or other maximum acceptable concentration in tissue, as defined in the "Guidelines," is available for nonylphenol. Therefore, a Final Residue Value cannot be calculated.

#### Other Data

Additional data on the lethal and sublethal effects of nonylphenol on freshwater species that do not comply with data requirements described in the "Guidelines" for inclusion in other tables are presented in Table 6. Three plant species (*Chlamydomonas reinhardtii*, *Salvinia molesta*, *Lemna minor*) were exposed in studies using media solutions that were not described. The results generally showed the plant species to be less sensitive to nonylphenol than animals. One test with the duckweed, *Lemna minor*, was an exception and showed a four-day reduction in vegetative growth at 125 µg/L (Prasad 1986). McLeese et al. (1980b) reported LC50s of 5,000 µg/L for a clam, *Anodonta cataractae*, in a 144-hr exposure and 900 µg/L for the Atlantic salmon, *Salmo salar*, in a 96-hr exposure. The values were higher than those reported in Table 1 for similar species. The test organisms were fed in both tests.

Three long-term (21 day) tests with *Daphnia magna* (Baer and Owens 1999, Baldwin et al. 1997, LeBlanc et al. 2000) and a single long-term (30 day) test with *D. galeata mendotae* (Shurin and Dodson 1997) are included in this section because the tests were conducted without measurement of nonylphenol concentrations in the test water. The results in the unmeasured tests agree reasonably well with those measured and reported in Table 2 for *D. magna*. Negative effects on survival or reproduction were observed in all three tests between 25 and 200 µg/L. The cladoceran, *Daphnia pulex*, was exposed for 48 hr in tests in which nonylphenol concentrations decreased more than 50 percent during the exposures (Ernst et al. 1980), but resulting LC50s ranged from 140 to 190 µg/L, which agreed with LC50s for other cladoceran species. The cladoceran, *Ceriodaphnia dubia*, gave similar LC50 results of 276 and 225 µg/L for the respective exposure durations of 48 hr and 7 days (England 1995). The LC50 values reported in this table for the species are slightly higher than the Chronic Value for the species of 134 µg/L (Table 2). England and Bussard (1993) reported an EC50 and an LC50 for larva of the midge, *Chironomus tentans*, of 95 and 119 µg/L, respectively. These values were slightly more sensitive than values reported in a similar study in which food was not available (Table 1). In a pair of tests in which the test organisms were fed, Brooke (1993b) measured a 96-hr LC50 for the fathead minnow, *Pimephales promelas*, of 138

 $\mu$ g/L and a 96-hr LC50 for the bluegill, *Lepomis macrochirus*, of 135  $\mu$ g/L. The LC50 values for these species from tests in which the fish were fed, agree well with data from tests in which the fish were not fed (Table 1).

Five fish species (rainbow trout, lahontan cutthroat trout, apache trout, Colorado squawfish and fathead minnow) were exposed to nonylphenol for 96 hr to determine if nonylphenol inhibited brain acetylcholoinesterase enzymes. Response to AchE inhibition was measured by a decrease in the number of muscarinic cholinergic receptors which is a compensatory response to an acetycholine buildup (Jones et al. 1998). Responses at exposure concentrations #220 µg/L were observed in the rainbow trout, lahontan cutthroat trout, and apache trout.

Brooke (1993b) measured the bioconcentration of nonylphenol in the fathead minnow and bluegill at concentrations near lethality. The fathead minnow BCF was 100.4 and the bluegill BCF was 35.31. The values were slightly less than the BCFs measured in the fish from lower exposure concentrations (Table 5). Lewis and Lech (1996) found that bioconcentration of nonylphenol was highest (BCF=98.2) in the viscera of rainbow trout and 24.21 in the remainder of the carcass. They also measured the half-life of nonylphenol in various tissues and found that fat and muscle similarly depurated nonylphenol to half concentrations in about 19 hr. The liver depurated to half concentrations in about 6 hr.

Mesocosm studies were conducted with nonylphenol in which zooplankton, benthic macroinvertebrates, and fish were observed for effects. The exposure was for 20 days with four nonylphenol concentrations. Zooplankton populations (O'Halloran et al. 1999) and benthic macroinvertebrate populations (Schmude et al. 1999) showed no negative effects at the 23  $\mu$ g/L nonylphenol exposure concentration, and were negatively affected at 76  $\mu$ g/L. Various species of zooplankton and macroinvertebrates exhibited differences in sensitivity to nonylphenol. The authors of the zooplankton study stated that a MATC for the protection of all zooplankton taxa is - 10  $\mu$ g/L. The fish (bluegill) in the mesocosms (Liber et al. 1999) were unaffected at nonylphenol exposures #76  $\mu$ g/L, but survival was reduced at 243  $\mu$ g/L. In one exposure replicate with a mean nonlyphenol concentration of 93  $\mu$ g/L, survival of the fish was reduced after 20 days of exposure indicating that concentrations near 100  $\mu$ g/L may be maximal for this species. The mesocosm studies demonstrated that the freshwater Final Chronic Value of 5.920  $\mu$ g/L should be protective of aquatic life.

Nonylphenol does have estrogen-like qualities. Vitellogenin is a protein produced in the liver of female oviparous vertebrate species and deposited in the ovaries as the primary material for yolk in the ova. Male fish normally produce very little vitellogenin. Jobling et al. (1996) demonstrated significant increases in vitellogenin in male rainbow trout, *Oncorhynchus mykiss*, at three weeks of exposure to 20.3

and 54.3  $\mu$ g/L of nonylphenol. Lech et al. (1996) observed a significant increase in mRNA for the vitellogenin gene in rainbow trout at 14.14  $\mu$ g/L. A long-term study was conducted with rainbow trout, *Onchorynchus mykiss*, exposing female fish immediately after hatch to 1, 10, and 30 or 50  $\mu$ g/L of nonylphenol (Ashfield et al. 1998). They found reduced growth in fish exposed to 1  $\mu$ g/L for 22 days and grown for 86 days beyond treatment. Growth was not reduced in the 10  $\mu$ g/L treatment but was in the 50  $\mu$ g/L treatment. A second study was conducted and exposure was for 35 days and grow-out was for 431 days beyond the last treatment day. On day 55 of the study, reduced growth was observed at the 10 and 30  $\mu$ g/L treatments, but not at the 1  $\mu$ g/L. At day 466, the fish exposed to 10  $\mu$ g/L recovered the growth reductions seen earlier and only the 30  $\mu$ g/L exposed fish showed reduced (- 25 percent) growth. The ovosomatic index (increase in ovary size relative to the control fish ovaries) increased in the fish exposed to 30  $\mu$ g/L at day 466. The authors speculated that the growth reduction may have been caused by the use of energy for precocious sexual development.

A non-resident fish species, Japanese medaka (*Oryzias latipes*), was exposed to nonylphenol for 28 days following hatching (Nimrod and Benson 1998). The survivors were monitored for the following 55 days. At the highest exposure concentration of 1.93  $\mu$ g/L, survival, growth, egg production, egg viability, and gonadosomatic index (GSI) were not altered. In another study with the same species of fish, development of testis-ova, an intersex condition, occurred after a three month exposure to 50  $\mu$ g/L of nonylphenol (Gray and Metcalf 1997). An increase in the number of Sertoli cells may have occurred in the male fathead minnow exposed to nonylphenol at 1.6  $\mu$ g/L for 42 days (Miles-Richardson et al. 1999). The evidence was not complete, but indicated the possibility of increased phagocytic action and Sertoli cell tissue in testes. The condition may negatively affect sperm production or survival. In a companion study with the fathead minnow, Giesy et al. (2000) found that nonylphenol exposures of >0.4  $\mu$ g/L depressed fecundity, concentrations of #3.4  $\mu$ g/L did not change vitellogenin concentrations in the blood of males, and raised the 17\$-estradiol titers in the blood of male and female fish at concentrations >0.05  $\mu$ g/L. The characteristic of nonylphenol to induce estrogenic effects has seldom been reported at concentrations below the freshwater Final Chronic Value of 5.920  $\mu$ g/L. More studies are needed to achieve a better understanding of the role of nonylphenol in estrogen mimicry.

Additional data on the lethal and sublethal effects of nonylphenol on saltwater species that do not comply with data requirements described in the "Guidelines" for inclusion in other tables are presented in Table 6. Results from a sexual reproduction test with red alga species, *Champia parvula*, indicated that reproduction was not inhibited at the highest measured concentration tested, 167 µg/L (Tagliabue 1993). Cypris larva of the barnacle, *Balanus amphitrite*, were exposed to nonylphenol for 48 hr and the settlement of the larva was reduced at 1.0 µg/L (Billinghurst et al. 1998). The soft-shell clam, *Mya* 

arenaria, showed no adverse effects on survival from a 360-hr exposure at 700 μg/L (McLeese et al. 1980b). Nonylphenol reduced byssus thread strength in the blue mussel *Mytilus edulis* (Granmo et al. 1989) at concentrations \$56 μg/L. Nonylphenols also show promise as antifouling agents when compared with other alkylphenols, copper, and tributyl tin (Takasawa et al. 1990). The antifouling test results, however, are qualitative. Nonylphenol concentrations extracted from sediments in the Venice, Italy lagoon were higher in areas with large masses of decomposing macroalgae (primarily *Ulva rigida*) than in areas not associated with the decomposition (Marcomini et al. 1990). This suggests that nonylphenol bioaccumulated by the macroalgae was transferred to the sediment as the algae died and decomposed.

McLeese et al. (1980b) reported 96-hr test results for the Atlantic salmon, *Salmo salar*, that were in general agreement with freshwater trout test results. In four tests, LC50 values ranged from 130 to 900 μg/L. Ward and Boeri (1990c) found similar toxicity results for sheepshead minnow, *Cyprinodon variegatus*, exposed in brackish water as those reported for salt water (Table 1). In brackish water, LC50's ranged from >420 μg/L for a 24-hr exposure to 320 μg/L for a 72-hr exposure. Killifish (Kelly and Di Giulio 2000) were exposed as embryos and larva to nonylphenol for 96 hrs. Even though the solvent concentration used in the exposures exceeded the 0.5 mL/L recommended limit, the data are included in Table 6 because the results reported for the solvent controls do not show decreased hatching success or increased abnormalities at 10 days post-hatch. Embryos exposed to 2,204 μg/L for 96 hr were all abnormally developed at 10 days post-fertilization. The LC50 for the same exposure period was 5,444 μg/L. Killifish larva were similar in sensitivity to nonylphenol exposures at post hatch ages of 1, 14, and 28 days with LC50's of 214, 209, and 260, respectively.

Additional data on the effect of nonylphenol on saltwater species do not indicate greater sensitivities than indicated previously. Some of the data presented in Table 6 were from the same acute tests listed in Table 1 (Lussier et al. 2000; Ward and Boeri 1990a,b), but for exposure durations other than 96 hr.

#### **Unused Data**

Some data concerning the effects of nonylphenol on aquatic organisms and their uses were not used because the tests were conducted in mixtures of chemicals (i.e., Ahel et al. 1993; Amato and Wayment 1998; Dwyer et al. 1999a; Escher et al. 1999; Larsson et al. 1999; Moore et al. 1987; Purdom et al. 1994; Sundaram et al. 1980; Turner et al. 1985) or in sediments (i.e., Fay et al. 2000; Hansen et al. 1999; Ward and Boeri 1992). Andersen et al. (1999); Celius et al. (1999); Jobling and Sumpter (1993); Knudsen and Pottinger (1999); Lamche and Burkhardt-Holm (2000); Levine and Cheney (2000); Loomis

and Thomas (1999); Milligan et al. (1998); Petit et al. (1997, 1999) exposed excised cells in tissue cultures. Data were not used when organisms were dosed by injection (i.e., Arukwe et al. 1997a,b, 1998; Christiansen et al. 1998a,b,c; Coldham et al. 1997, 1998; Haya et al. 1997; Madsen et al. 1997; Nimrod and Benson 1996, 1997; Spieser et al. 1998; Yadetie et al. 1999) or gavage (i.e., Rice et al. 1998; Thibaut et al. 1998). Data were not used when generated in an artificial medium (i.e., Weinberger et al. 1987). Tsuda et al. (2000) measured tissue concentrations from feral fish, but water concentrations greatly varied. Some studies were conducted with only the ethoxylated nonylphenols (i.e., Baldwin et al. 1998; Braaten et al. 1972; Dorn et al. 1993; Maki et al. 1998; Manzano et al. 1998, 1999; Patoczka and Pulliam 1990). Bearden and Schultz (1997, 1998); Lewis (1991); Liber et al. (1999); Varma and Patel (1988) and Veith and Mekenyan (1993) compiled data from other sources. Results were not used when the test organism or the test material were not adequately described (e.g., Folmar et al. 1998; Hansen et al. 1998; Kopf 1997; Magliulo et al. 1998; Muller 1980; Palmer et al. 1998; Weinberger and Rea 1981).

#### Summary

Acute toxicity of nonylphenol was tested in eighteen species and two subspecies representing fifteen genera of freshwater organisms. Toxicity values ranged from 55.72 μg/L for the amphipod *Hyalella azteca* to 774 μg/L for the snail *Physella virgata*. For the four most sensitive tested freshwater species, two were invertebrates and two were vertebrate species (Figure 1). No relationships have been demonstrated between water quality characteristics (such as hardness and pH) and toxicity. Eleven species of fish were tested and were in the mid-range of sensitivity (133.9 to 289.3 μg/L) of tested species. The freshwater Final Acute Value (FAV) is 55.71 μg/L which is equal to the LC50 for the most sensitive tested species, *Hyalella azteca*. Acute toxicity has been tested with ten species of saltwater organisms (Figure 2). Species Mean Acute Values ranged from 17 μg/L for the winter flounder, *Pleuronectes americanus*, to 209.8 μg/L for the sheepshead minnow, *Cyprinodon variegatus*. The saltwater FAV is 13.35 μg/L.

Chronic toxicity of nonylphenol was tested in five freshwater species and one saltwater species (Figure 3). The most sensitive species tested was the mysid *Americamysis bahia* and it had a Chronic Value (CV) of 5.112 µg/L based on reduced growth. Two freshwater fish were tested; the rainbow trout, *Oncorhynchus mykiss*, had a CV of 7.861 µg/L based on growth, and the fathead minnow, *Pimephales promelas*, had a CV of 10.18 µg/L based on survival. Two species of freshwater cladocerans were tested and CVs ranged from 22.62 to 157.9 µg/L based on reproduction. One species of freshwater midge was tested and its CV was 61.82 µg/L. Data were available to calculate a Final Acute-Chronic Ratio (FACR) for *Daphnia magna*, a freshwater cladoceran, saltwater mysid, *Americamysis bahia*, and rainbow trout.

The FACR for nonylphenol is 9.410.

Two species of aquatic plants were exposed to nonylphenol. Plants were as sensitive as animals, showing effects that ranged from 27 to 410  $\mu$ g/L. Based on the vegetative growth test using the saltwater planktonic diatom *Skeletonema costatum*, the Final Plant Value for nonylphenol is 27: g/L.

Nonylphenol bioaccumulates in aquatic organisms to low levels. In freshwater fish, lipid-normalized bioconcentration factors ranged from 39 to 209 times. Bioaccumulation was apparently greater in saltwater organisms where bioconcentration factors ranging from 78.75 to 2,168 were measured.

Nonylphenol is considered an endocrine disruptor chemical and induces production of vitellogenin in male rainbow trout. This is a process normally occurring in female fish in response to estrogenic hormones during the reproductive cycle. It also induces precocious development of ovaries and an intersex condition in some fish species.

#### National Criteria

The procedures described in the "Guidelines" for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, freshwater organisms and their uses should not be affected unacceptably if the four-day average concentration of nonylphenol does not exceed 5.9  $\mu$ g/L more than once every three years on the average and if the one-hour average concentration does not exceed 27.9  $\mu$ g/L more than once every three years on the average. Saltwater organisms and their uses should not be affected unacceptably if the four-day average concentration of nonylphenol does not exceed 1.4  $\mu$ g/L more than once every three years on the average and if the one-hour average concentration does not exceed 6.7  $\mu$ g/L more than once every three years on the average.

#### Implementation

As discussed in the Water Quality Standards Regulation (U.S. EPA 1983) and the Foreword to this document, a water quality criterion for aquatic life has regulatory impact only after it has been adopted in a State water quality standard. Such a standard specifies a criterion for a pollutant that is consistent with a particular designated use. With the concurrence of the U.S. EPA, States designate one or more uses for each body of water or segment thereof and adopt criteria that are consistent with the use(s) (U.S. EPA 1987, 1994). Water quality criteria adopted in State water quality standards could have the same numerical values as criteria developed under Section 304 of the Clean Water Act. However, in many situations States might want to adjust water quality criteria developed under Section 304 to reflect

local environmental conditions and human exposure patterns. Alternatively, States may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. State water quality standards include both numeric and narrative criteria. A State may adopt a numeric criterion within its water quality standards and apply it either state-wide to all waters designated for the use the criterion is designed to protect or to a specific site. A State may use an indicator parameter or the national criterion, supplemented with other relevant information, to interpret its narrative criteria within its water quality standards when developing NPDES effluent limitations under 40 CFR 122.44(d)(1)(vi).2

Site-specific criteria may include not only site-specific criterion concentrations (U.S. EPA 1994), but also site-specific, and possibly pollutant-specific, durations of averaging periods and frequencies of allowed excursions (U.S. EPA 1991). The averaging periods of "one hour" and "four days" were selected by the U.S. EPA on the basis of data concerning how rapidly some aquatic species react to increases in the concentrations of some pollutants, and "three years" is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions (Stephan et al. 1985; U.S. EPA 1991). However, various species and ecosystems react and recover at greatly differing rates. Therefore, if adequate justification is provided, site-specific and/or pollutant-specific concentrations, durations and frequencies may be higher or lower than those given in national water quality criteria for aquatic life.

Use of criteria which have been adopted in State water quality standards for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Although dynamic models are preferred for the application of these criteria (U.S. EPA 1991), limited data or other considerations might require the use of a steady-state model (U.S. EPA 1986).

Guidance on mixing zones and the design of monitoring programs is available (U.S. EPA 1987, 1991).

Figure 1. Ranked Summary of Nonylphenol GMAVs - Freshwater.

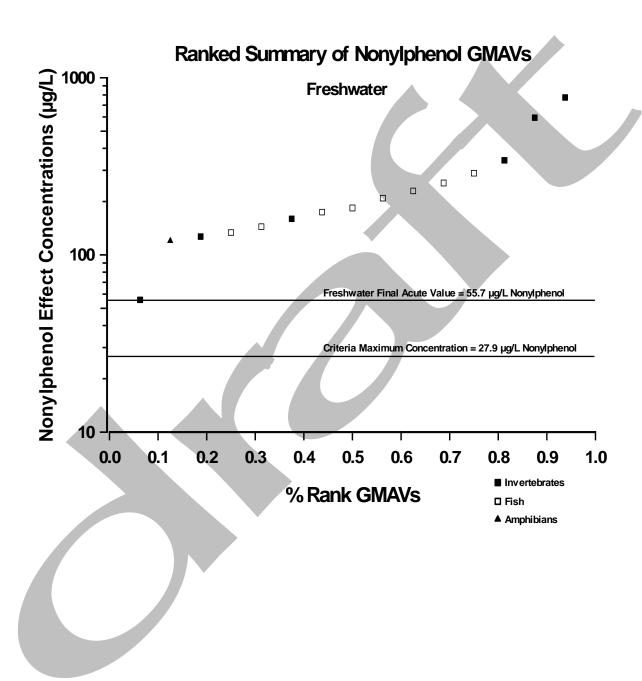


Figure 2. Ranked Summary of Nonylphenol GMAVs - Saltwater.

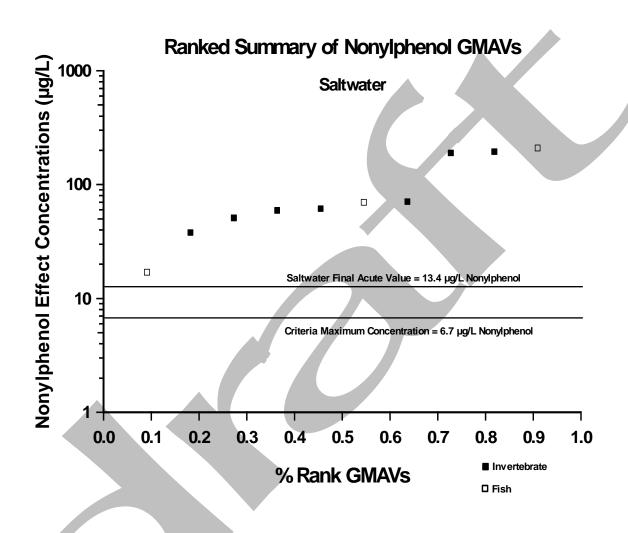


Figure 3. Chronic Toxicity of Nonylphenol to Aquatic Animals.

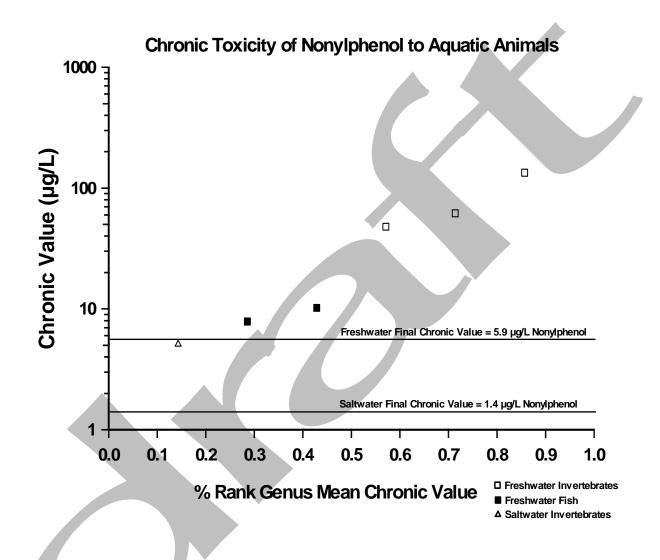


Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals

<u>Species</u>	Method <sup>a</sup>	<u>Chemical</u>	<u>pH</u>	LC <sub>50</sub> or EC <sub>50</sub> <sup>b</sup> (: g/L)	Species Mean Acute Value (: g/L)	<u>Reference</u>
		FRESHWA	TER SPE	<u>CIES</u>		
Annelid (adult), Lumbriculus variegatus	F,M	>90%	6.75	342	342	Brooke 1993a
Snail (adult), Physella virgata	F,M	>90%	7.89	774	774	Brooke 1993a
Cladoceran (<24-hr old), Daphnia magna	R,M	>90%	7.87	84.8	-	Brooke 1993a
Cladoceran (<24-hr old), Daphnia magna	S,M	91.8%	8.25	<u>190</u>	126.9	Comber et al. 1993
Midge (2nd instar), Chironomus tentans	F,M	>95%	8.0-8.4	<u>160</u>	160	England and Bussard 1995
Dragonfly (nymph), <i>Ophiogomphus</i> sp.	F,M	>90%	8.06	<u>596</u>	596	Brooke 1993a
Amphipod, (juvenile, 2mm TL), <i>Hyalella azteca</i>	F,M	>90%	7.80	<u>20.7</u>	-	Brooke 1993a
Amphipod (juven., 2-3mm TL), Hyalella azteca	F,M	>95%	7.9-8.7	<u>150</u>	55.72	England and Bussard 1995
Rainbow trout (0.67 ±0.35 g), Oncorhynchus mykiss	S,U	85%	7.8-7.9	190	-	Dwyer et al. 1995
Rainbow trout (1.25 ±0.57 g), Oncorhynchus mykiss	S,U	85%	7.5-7.7	260	-	Dwyer et al. 1995
Rainbow trout (0.27 ±0.07 g), Oncorhynchus mykiss	S,U	85%	7.9	140	-	Dwyer et al. 1995
Rainbow trout (1.09 ±0.38 g), Oncorhynchus mykiss	S,U	85%	7.7-7.9	270	-	Dwyer et al. 1995
Rainbow trout (0.48 ±0.08 g), Oncorhynchus mykiss	S,U	85%	7.5-7.9	160	-	Dwyer et al. 1995

Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals (continued)

Species Species	Methoda	Chemical	pН		Species Mean Acute Value (: g/L)	<u>Reference</u>
Rainbow trout (0.50 ±0.21 g), Oncorhynchus mykiss	S,U	85%	6.5-7.9	180	-	Dwyer et al. 1995
Rainbow trout (45 d), Oncorhynchus mykiss	F,M	>90%	6.72	221	221	Brooke 1993a
Apache trout $(0.85 \pm 0.49 \text{ g})$ , Oncorhynchus apache	S,U	85%	7.8-7.9	180	-	Dwyer et al. 1995
Apache trout $(0.38 \pm 0.18 \text{ g})$ , Oncorhynchus apache	S,U	85%	7.3-7.7	160	169.7	Dwyer et al. 1995
Greenback cutthroat trout (0.31 ±0.17 g), Oncorhynchus clarki stomais	S,U	85%	7.5-7.6	<u>150</u>		Dwyer et al. 1995
Lahontan cutthroat trout (0.34 ±0.08 g), Oncorhynchus clarki henshawi	S,U	85%	7.9	<u>140</u>	-	Dwyer et al. 1995
Lahontan cutthroat trout (0.57 ±0.23 g), Oncorhynchus clarki henshawi	S,U	85%	7.6-7.7	<u>220</u>	166.6	Dwyer et al. 1995
Fathead minnow (0.32 ±0.16 g), Pimephales promelas	S,U	85%	7.7-8.1	210	-	Dwyer et al. 1995
Fathead minnow (0.56 ±0.19 g), Pimephales promelas	S,U	85%	7.8-8.1	360	-	Dwyer et al. 1995
Fathead minnow (0.45 ±0.35 g), Pimephales promelas	S,U	85%	7.6-7.8	310	-	Dwyer et al. 1995
Fathead minnow (0.40 ±0.21 g), Pimephales promelas	S,U	85%	7.5-7.9	330	-	Dwyer et al. 1995

Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals (continued)

<u>Species</u>	<u>Method<sup>a</sup></u>	<u>Chemical</u>	<u>pH</u>	$LC_{50}$ or $EC_{50}^{b}$ (: $g/L$ )	Species Mean Acute Value (: g/L)	<u>Reference</u>
Fathead minnow (0.34 ±0.24 g), Pimephales promelas	S,U	85%	7.5-7.6	170		Dwyer et al. 1995
Fathead minnow (0.39 ±0.14 g), Pimephales promelas	S,U	85%	7.8-8.2	290		Dwyer et al. 1995
Fathead minnow (32 d), Pimephales promelas	F,M	99%	7.29	140	-	Holcombe et al. 1984; University of Wisconsin- Superior 1985
Fathead minnow (25-35 d), Pimephales promelas	F,M	>90%	7.23	<u>128</u>	133.9	Brooke 1993a
Bonytail chub (0.29 ±0.08 g), Gila elegans	S,U	85%	7.7-7.9	270	-	Dwyer et al. 1995
Bonytail chub $(0.52 \pm 0.09 \text{ g})$ , Gila elegans	S,U	85%	7.4-7.6	<u>310</u>	289.3	Dwyer et al. 1995
Colorado squawfish (0.32 ±0.05 g), Ptychocheilus lucius	S,U	85%	8.1-8.2	<u>240</u>	-	Dwyer et al. 1995
Colorado squawfish (0.34 ±0.05 g), Ptychocheilus lucius	S,U	85%	7.8-8.0	<u>270</u>	254.6	Dwyer et al. 1995
Razorback sucker (0.31 ±0.04 g), Xyrauchen texanus	S,U	85%	7.8-8.1	<u>160</u>	-	Dwyer et al. 1995
Razorback sucker (0.32 ±0.07 g), Xyrauchen texanus	S,U	85%	7.9-8.0	<u>190</u>	174.4	Dwyer et al. 1995
Gila topminnow (0.219 g, 27.2 mm), Poeciliopsis occidentalis	S,U	85%	8.0	<u>230</u>	230	Dwyer et al. 1999a
Fountain darter (0.062 g, 20.2 mm), Etheostoma rubrum	S,U	85%	8.0-8.1	<u>110</u>	110	Dwyer et al. 1999a

Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals (continued)

Species	<b>Method</b> <sup>a</sup>	Chemical	pН	$     \begin{array}{c}             LC_{50} \\             or EC_{50}^{b} \\             (\underline{:} \ g/L)     \end{array} $	Species Mean Acute Value (: g/L)	Reference
Greenthroat darter (0.133 g, 22.6 mm), Etheostoma lepidum	S,U	85%	8.0-8.2	190	190	Dwyer et al. 1999a
Bluegill (juvenile), Lepomis macrochirus	F,M	>90%	7.61	<u>209</u>	209	Brooke 1993a
Boreal toad (0.012 g, 9.6 mm), Bufo boreas	S,U	85%	7.9-8.0	120	120	Dwyer et al. 1999a
		SALTWA	TER SPEC	<u>IES</u>		
Coot clam (embryo/larva), <i>Mulinia lateralis</i>	S,U	90%	7.8-8.2	<u>37.9</u>	37.9	Lussier et al. 2000
Copepod (10-12 d), Acartia tonsa	S,U	-		<u>190</u>	190	Kusk and Wollenberger 1999
Mysid (<24-hr old), Americamysis bahia	F,M	>95%	7.3-8.2	<u>43</u>	-	Ward and Boeri 1990a
Mysid (<24-hr old), Americamysis bahia	F,M	90%	7.8-8.2	<u>60.6</u>	51.05	Lussier et al. 2000
Amphipod (adult), Leptocheirus plumulosus	F,M	90%	7.8-8.2	<u>61.6</u>	61.6	Lussier et al. 2000
Grass shrimp (48-hr old), Palaemonetes vulgaris	F,M	90%	7.8-8.2	<u>59.4</u>	59.4	Lussier et al. 2000
American lobster (1st stage), Homarus americanus	R,U	90%	7.8-8.2	<u>71</u>	71	Lussier et al. 2000
Mud crab (4th and 5th stages), Dyspanopeus sayii	F,M	90%	7.8-8.2	<u>&gt;195</u>	>195	Lussier et al. 2000
Winter flounder (48-hr-old), Pleuronectes americanus	S,M	90%	7.8-8.2	<u>17</u>	17	Lussier et al. 2000

Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals (continued)

<u>Species</u>	Method <sup>a</sup>	<u>Chemical</u>	<u>pH</u>	LC <sub>50</sub> or EC <sub>50</sub> <sup>b</sup> (: g/L)	Species Mean Acute Value (: g/L)	<u>Reference</u>
Sheepshead minnow (juvenile), Cyprinodon variegatus	F,M	>95%	7.4-8.1	<u>310</u>		Ward and Boeri 1990b
Sheepshead minnow (juvenile), Cyprinodon variegatus	F,M	90%	7.8-8.2	142	209.8	Lussier et al. 2000
Inland silversides (juvenile), <i>Menidia beryllina</i>	F,M	90%	7.8-8.2	<u>70</u>	70	Lussier et al. 2000

 $<sup>^{</sup>a}$  S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.



<sup>&</sup>lt;sup>b</sup> Each Species Mean Acute Value was calculated from the associated underlined number(s) in the preceding column.

Table 2a. Chronic Toxicity of Nonylphenol to Aquatic Animals

Species	Test <sup>a</sup>	Chemical	_pH_	Chronic Limits (µg/L) <sup>b</sup>	Chronic Value (µg/L)	Reference
Cladoceran, Ceriodaphnia dubia	LC	>95%	8.3-8.6	88.7-202	133.9	England 1995
Cladoceran, Daphnia magna	LC	93.1	8.04	10.7-47.8	22.62	Fliedner 1993
Cladoceran, Daphnia magna	LC	>90%	8.46	116-215	157.9	Brooke 1993a
Cladoceran, Daphnia magna	LC	91.8%	8.25	24-39	30.59	Comber et al. 1993
Midge, Chironomus tentans	LC	95%	7.73	42-91	61.82	Kahl et al. 1997
Rainbow trout, Oncorhynchus mykiss	ELS	>90%	6.97	6.0-10.3	7.861	Brooke 1993a
Fathead minnow, Pimephales promelas	ELS	>95%	7.1-8.2	7.4-14	10.18	Ward and Boeri 1991c
Mysid, Americamysis bahia	LC	>95%	7.4-8.3	3.9-6.7	5.112	Ward and Boeri 1991b

 <sup>&</sup>lt;sup>a</sup> LC = life-cycle or partial life-cycle; ELS = early life-stage.
 <sup>b</sup> Based upon measured concentrations of nonylphenol.

**Table 2b. Acute-Chronic Ratios** 

# **Acute-Chronic Ratios**

<b>Species</b>	рН	Acute Value (μg/L)	Chronic Value (µg/L)_	Ratio	Reference
Cladoceran, Daphnia magna	7.87-8.46	84.8	157.9	2.000ª	Brooke 1993a
Cladoceran, Daphnia magna	8.25	190	30.59	6.211	Comber et al. 1993
Mysid, Americamysis bahia	7.3-8.3	43	5.112	8.412	Ward and Boeri 1990a, 1991b
Rainbow trout, Oncorhynchus mykiss	6.72-6.97	221	7.861	28.11	Brooke 1993a

<sup>&</sup>lt;sup>a</sup> Acute-Chronic Ratio calculated as 0.5370 but changed to 2.000 (see text).



Table 3. Ranked Genus Mean Acute Values with Species Mean Acute-Chronic Ratios

Rank <sup>a</sup>	Genus Mean Acute Value (µg/L)_	<u>Species</u>	Species Mean Acute Value (µg/L) <sup>b</sup>	Species Mean Acute-Chronic <u>Ratio</u> c
		FRESHWATER SPECI	<u>ES</u>	
15	774	Snail, <i>Physella virgata</i>	774	-
14	596	Dragonfly, Ophiogomphus sp.	596	-
13	342	Annelid, Lumbriculus variegatus	342	
12	289.3	Bonytail chub, Gila elegans	289.3	-
11	254.6	Colorado squawfish, Ptychocheilus lucius	254.6	-
10	230	Gila topminnow, Poeciliopsis occidentalis	230	-
9	209	Bluegill, Lepomis macrochirus	209	-
8	184.2	Rainbow trout, Oncorhynchus mykiss	221	28.11
		Apache trout, Oncorhynchus apache	169.7	-
		Lahontan cutthroat trout,  Oncorhynchus clarki henshawi, and	166.6	-
		Greenback cutthroat trout, Oncorhynchus clarki stomais	-	-
7	174.4	Razorback sucker, Xyrauchen texanus	174.4	-
6	160	Midge, Chironomus tentans	160	-
5	144.6	Greenthroat darter, Etheostoma lepidum	190	-
		Fountain darter, Etheostoma rubrum	110	-
4	133.9	Fathead minnow, Pimephales promelas	133.9	-

Table 3. Ranked Genus Mean Acute Values with Species Mean Acute-Chronic Ratios (continued)

<u>Rank</u> a	Genus Mean Acute Value (µg/L)	<u>Species</u>	Species Mean Acute Value (µg/L) <sup>b</sup>	Species Mean Acute-Chronic <u>Ratio</u> c
3	126.9	Cladoceran, Daphnia magna	126.9	3.524
2	120	Boreal toad, Bufo boreas	120	-
1	55.72	Amphipod, Hyalella azteca	55.72	-
		SALTWATER SP	<u>ECIES</u>	
10	209.8	Sheepshead minnow, Cyprinodon variegatus	209.8	-
9	>195	Mud crab, Dyspanopeus sayii	>195	-
8	190	Copepod, Acartia tonsa	190	
7	71	American lobster, Homarus americanus	71	-
6	70	Inland silversides, Menidia beryllina	70	-
5	61.6	Amphipod, Leptocheirus plumulosus	61.6	-
4	59.4	Grass shrimp, Palaemonetes vulgaris	59.4	-
3	51.05	Mysid, Americamysis bahia	51.05	8.412
2	37.9	Coot clam, Mulinia lateralis	37.9	-
1	17	Winter flounder, Pleuronectes americanus	17	-

 $<sup>^{\</sup>rm a}$  Ranked from the most resistant to the most sensitive based on Genus Mean Acute Value.  $^{\rm b}$  From Table 1.

<sup>&</sup>lt;sup>c</sup> From Table 2.

## Table 3. Ranked Genus Mean Acute Values with Species Mean Acute-Chronic Ratios (continued)

## Fresh Water

Final Acute Value =  $55.71 \,\mu g/L$ Criterion Maximum Concentration =  $55.71/2 = 27.86 \,\mu g/L$ Final Acute-Chronic Ratio = 9.410 (see text) Final Chronic Value =  $(55.71 \,\mu g/L)/9.410 = 5.920 \,\mu g/L$ 

## Salt Water

Final Acute Value =  $13.35 \,\mu\text{g/L}$ Criterion Maximum Concentration =  $13.35/2 = 6.675 \,\mu\text{g/L}$ Final Acute-Chronic Ratio = 9.410 (see text) Final Chronic Value =  $(13.35 \,\mu\text{g/L})/9.410 = 1.419 \,\mu\text{g/L}$ 



Table 4. Toxicity of Nonylphenol to Aquatic Plants

Chemical	рН_	Duration (days)	<b>Effect</b>	Concentration (µg/L)	Reference
	<u>FI</u>	RESHWATEI	R SPECIES		
>95%	7.8	4	EC50	410	Ward and Boeri 1990a
	<u>S</u> .	ALTWATER	SPECIES		
>95%	30ª	4	EC50, number of cells	27	Ward and Boeri 1990d
	>95%	>95% 7.8 S	Chemical pH (days)  FRESHWATEI  >95% 7.8 4  SALTWATER	ChemicalpH(days)EffectFRESHWATER SPECIES>95%7.84EC50SALTWATER SPECIES>95%30a4EC50, number of	Chemical         pH         (days)         Effect         (μg/L)           FRESHWATER SPECIES           >95%         7.8         4         EC50         410           SALTWATER SPECIES           >95%         30a         4         EC50, number of         27

<sup>&</sup>lt;sup>a</sup>Salinity (g/kg).



Table 5. Bioaccumulation of Nonylphenol by Aquatic Organisms

<u>Species</u>	<u>Chemical</u>	Conc. in Water (μg/L) <sup>a</sup>	<u>pH</u>	Duration (days)	<u>Tissue</u>	Percent <u>Lipids</u>	BCF or BAF <sup>b</sup>	Normalized BCF or BAF <sup>c</sup>	Reference
			<u>I</u>	FRESHWAT	ER SPECI	<u>ES</u>			
Fathead minnow (0.5-1 g), Pimephales promelas	>95%	4.9	7.0-7.6	27	Whole body		271	·	Ward and Boeri 1991a
Fathead minnow (0.5-1 g), Pimephales promelas	>95%	22.7	7.0-7.6	27	Whole body		344		Ward and Boeri 1991a
Fathead minnow (4-wk old), Pimephales promelas	99%	18.4	7.62	4	Whole body	4.7±1.7	751	159.8	Brooke 1993b
Fathead minnow (4-wk old), Pimephales promelas	99%	41.9	7.62	4	Whole body	4.7±1.7	677	144.0	Brooke 1993b
Fathead minnow (4-wk old), Pimephales promelas	99%	82.1	7.62	4	Whole body	4.7±1.7	945	201.1	Brooke 1993b
Fathead minnow (4-wk old), Pimephales promelas	99%	9.3	7.60	28	Whole body	4.7±1.7	769	163.6	Brooke 1993b
Fathead minnow (4-wk old), Pimephales promelas	99%	19.2	7.60	28	Whole body	4.7±1.7	984	209.4	Brooke 1993b

Table 5. Bioaccumulation of Nonylphenol by Aquatic Organisms (continued)

Species Species	<u>Chemical</u>	Conc. in Water (µg/L) <sup>a</sup>	<u>рН</u>	Duration (days)	<u>Tissue</u>	Percent <u>Lipids</u>	BCF or BAF <sup>b</sup>	Normalized BCF or <u>BAF</u> <sup>c</sup>	<u>Reference</u>
Fathead minnow (4-wk old), Pimephales promelas	99%	38.1	7.60	28	Whole body	4.7±1.7	876	186.4	Brooke 1993b
Fathead minnow (4-wk old), Pimephales promelas	99%	77.5	7.60	28	Whole body	4.7±1.7	603	128.3	Brooke 1993b
Fathead minnow (adult), Pimephales promelas	>98%	0.4 1.6 3.4	-	42	Whole body	-	203 252 268		Giesy et al. 2000
Bluegill (4-wk old), Lepomis macrochirus	99%	21.6	7.79	4	Whole body	4.9±1.5	279	56.94	Brooke 1993b
Bluegill (4-wk old), Lepomis macrochirus	99%	43.9	7.79	4	Whole body	4.9±1.5	257	52.45	Brooke 1993b
Bluegill (4-wk old), Lepomis macrochirus	99%	86.5	7.79	4	Whole body	4.9±1.5	223	45.51	Brooke 1993b
Bluegill (4-wk old), Lepomis macrochirus	99%	5.6	7.55	28	Whole body	4.9±1.5	231	47.14	Brooke 1993b
Bluegill (4-wk old), Lepomis macrochirus	99%	12.4	7.55	28	Whole body	4.9±1.5	253	51.63	Brooke 1993b
Bluegill (4-wk old), Lepomis macrochirus	99%	27.6	7.55	28	Whole body	4.9±1.5	250	51.02	Brooke 1993b

Table 5. Bioaccumulation of Nonylphenol by Aquatic Organisms (continued)

Species	Chemical	Conc. in Water (µg/L) <sup>a</sup>	<u>pH</u>	Duration (days)	<u>Tissue</u>	Percent <u>Lipids</u>	BCF or BAF <sup>b</sup>	Normalized BCF or BAF°	Reference	
Bluegill (4-wk old), Lepomis macrochirus	99%	59.5	7.55	28	Whole body	4.9±1.5	191	38.98	Brooke 1993b	
Bluegill (juvenile), Lepomis macrochirus	96.4%	1.0 3.0 30.0	7.7	20	Whole body	0.72 ±0.46	76 60 37	105.6 83.33 51.39	Liber et al. 1999	
SALTWATER SPECIES										
Blue mussel, Mytilus edulis	<sup>14</sup> C- labeled	5.9	-	16	Whole body	1.6	2,740	1,712	Ekelund et al. 1990	
Blue mussel, Mytilus edulis	<sup>14</sup> C- labeled	6.2	-	16	Whole body	1.9	4,120	2,168	Ekelund et al. 1990	
Common shrimp, Crangon crangon <sup>d</sup>	<sup>14</sup> C- labeled	6.4	-	16	Whole body	1.4	110	78.75	Ekelund et al. 1990	
Common shrimp, Crangon crangon <sup>d</sup>	<sup>14</sup> C- labeled	7.4		16	Whole body	1.7	900	529.4	Ekelund et al. 1990	
Three-spined stickleback, Gasterosteus aculeatus	<sup>14</sup> C- labeled	4.8		16	Whole body	6.7	1,200	179.1	Ekelund et al. 1990	
Three-spined stickleback, Gasterosteus aculeatus	<sup>14</sup> C- labeled	4.9	-	16	Whole body	7.8	1,300	166.7	Ekelund et al. 1990	

<sup>&</sup>lt;sup>a</sup> Measured concentration of nonylphenol.

Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are based on measured concentrations of nonylphenol in water and in tissue.

When possible, the factors were normalized to 1% lipids by dividing the BCFs and BAFs by the percent lipids.

d Non-resident species.

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms

<u>Species</u>	<u>Chemical</u>	pH_	<b>Duration</b>	<u>Effect</u>	Concentration (µg/L)	<u>Reference</u>				
FRESHWATER SPECIES										
Green alga, Chlamydomonas reinhardtii	-	-	24 days	100% algistatic	6,250	Weinberger and Greenhalgh 1984				
Floating moss, Salvinia molesta	-	-	9 days	Reduced frond production	2,500	Prasad 1986				
Duckweed, Lemna minor	-	5.6	96 hr	IC50	5,500	Weinberger and Iyengar 1983				
Duckweed, Lemna minor	-	-	4 days	Reduced frond production	125	Prasad 1986				
Ciliate protozoan, Tetrahymena pyriformis	-	-	24 hr	EC50	460	Yoshioka 1985				
Ciliate protozoan, Tetrahymena pyriformis	-	7.40	40 hr	Reduced population growth 50%	747	Schultz 1997				
Rotifer (4 to 6 hr-old female) Brachionus calyciflorus	Technical	7.5	96 hr	Sexual reproduction reduced	50	Preston et al. 2000				
Clam (15 g), Anodonta cataractae	-		144 hr	LC50	5000	McLeese et al. 1980b				
Zooplankton	96.4%	7.5 - 8.2	20 days	NOEC LOEC	23 76	O'Halloran et al. 1999				
Benthic macro- invertebrates	96.4%	7.5 - 8.2	20 days	NOEC LOEC	23 76	Schmude et al. 1999				
Cladoceran (<24-hr old), Daphnia magna		8.0	21 days	NOEC LOEC (reduced fecundity)	50 100	Baldwin et al. 1997				
Cladoceran (<24-hr old and adults), Daphnia magna	~85%	7.8 - 8.4	96 hr (fed)	MATC (young) MATC (adults)	302 136	Gerritsen et al. 1998				

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

					Concentration	
<b>Species</b>	<u>Chemical</u>	<u>pH</u>	<b>Duration</b>	<u>Effect</u>	(μg/L)	Reference
Cladoceran (<24-hr old), Daphnia magna	~85%	7.7±0.02	21 days	No sex ratio change (high food rate) Increased ratio of males (low food rate)	25	Baer and Owens 1999
Cladoceran (<24-hr old), Daphnia magna	Technical	-	21 days	50% adult mortality NOEC (deformed offspring)	200.5	LeBlanc et al. 2000
Cladoceran (<36-hr old), Daphnia galeata mendotae	-	-	30 days	NOEC LOEC (deformed offspring)	10 50	Shurin and Dodson 1997
Cladoceran (>48-hr old), Daphnia pulex	Practical grade	-	48 hr	LC50	140	Ernst et al. 1980
Cladoceran (>48-hr old), Daphnia pulex	Practical grade	-	48 hr	LC50	176	Ernst et al. 1980
Cladoceran (>48-hr old), Daphnia pulex	Practical grade	-	48 hr	LC50	190	Ernst et al. 1980
Cladoceran (<24-hr old), Ceriodaphnia dubia	>95%	8.3-8.6	48 hr	LC50 (fed)	276	England 1995
Cladoceran (<24-hr old), Ceriodaphnia dubia	>95%	8.3-8.6	7 days	LC50 (fed)	225	England 1995
Midge (2nd instar), Chironomus tentans	>95%	8.2	14 days	LC50 EC50	119 95	England and Bussard 1993
Sea lamprey (larva), Petromyzon marinus	-	7.5-8.2	14 hr	LT100	5,000	Applegate et al. 1957

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

Species	Chamical	ъU	Duration	Effort	Concentration (µg/L)	Doforonoo
Species Brook trout	<u>Chemical</u>	<u>pH</u>	<u>Duration</u> 96 hr	Effect LC50	<u>(μg/L)</u> 145	Reference Holmes and
(juvenile), Salvelinus fontinalis	-	-	90 III	LC30	143	Kingsbury 1980
Lake trout (juvenile), Salvelinus naymaycush	-	-	35 days	LC50 (fed)	>40	Holmes and Kingsbury 1980
Brown trout (fingerling), Salmo trutta	-	7.0	2 hr	LT100	5,000	Wood 1953
Atlantic salmon (4 g), Salmo salar	-	-	96 hr	LC50	900	McLeese et al. 1980b
Chinook salmon (juvenile), Oncorhynchus tshawytscha	-	7.2	3 hr	LT100	10,000	MacPhee and Ruelle 1969
Coho salmon (juvenile), Oncorhynchus kisutch	-	7.2	3 hr	LT100	10,000	MacPhee and Ruelle 1969
Rainbow trout (juvenile), Oncorhynchus mykiss		7.5-8.2	4 hr	LT100	5,000	Applegate et al. 1957
Rainbow trout (juvenile), Oncorhynchus mykiss	Practical grade		96 hr	LC50	920	Ernst et al. 1980
Rainbow trout (juvenile), Oncorhynchus mykiss	Practical grade	-	96 hr	LC50	560	Ernst et al. 1980
Rainbow trout (juvenile), Oncorhynchus mykiss	-	-	96 hr	LC50	230	Holmes and Kingsbury 1980

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

					Concentration	
<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<b>Duration</b>	<b>Effect</b>	(μg/L)	<u>Reference</u>
Rainbow trout (adult males), Oncorhynchus mykiss	-	-	3 wk	Increased vitellogenin production	20.3	Jobling et al. 1996
Rainbow trout (adult males), Oncorhynchus mykiss	-	-	3 wk	Increased vitellogenin production	54.3	Jobling et al. 1996
Rainbow trout (50 - 200 g), Oncorhynchus mykiss	-	-	72 hr	LC50	193.65	Lech et al. 1996
Rainbow trout (50 - 200 g), Oncorhynchus mykiss	-	-	72 hr	Increased vitellogenin mRNA	14.14	Lech et al. 1996
Rainbow trout, (40 - 60 g), Oncorhynchus mykiss	>99%	-	8 hr	Tissue half-life fat 19.8 hr muscle 18.6 hr liver 5.9 hr	18	Lewis and Lech 1996
Rainbow trout (40 - 60 g), Oncorhynchus mykiss	>99%		2 - 5 hr	Eviscerated carcass BAF = 24.21	18	Lewis and Lech 1996
Rainbow trout (40 - 60 g), Oncorhynchus mykiss	>99%		12 - 24 hr	Viscera BAF = 98.2	18	Lewis and Lech 1996
Rainbow trout (juvenile), Oncorhynchus mykiss		-	4 hr	Vitellogenin mRNA production	10	Ren et al. 1996a
Rainbow trout (juvenile), Oncorhynchus mykiss		-	72 hr	Vitellogenin production	100	Ren et al. 1996b
Rainbow trout (& juvenile),	-	6.5	22 days	Reduced growth at 108 days	50	Ashfield et al. 1998
Oncorhynchus mykiss			35 days	Reduced growth at 466 days	30	

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

<b>Species</b>	<u>Chemical</u>	pH_	<u>Duration</u>	<b>Effect</b>	Concentration (µg/L)	Reference
Rainbow trout (juvenile), Oncorhynchus mykiss	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	220	Jones et al. 1998
Rainbow trout (35-50 g, immature), Oncorhynchus mykiss	-	8.0 - 8.4	21 days	Increased vitellogenin in blood plasma	50	Tremblay and Van Der Kraak 1998
Rainbow trout (adult males), Oncorhynchus mykiss	-	-	3 wk	BCF = 116 BCF = 88	63 81	Blackburn et al. 1999
Rainbow trout (juvenile, 103- 168 g), Oncorhynchus mykiss	99%	-	9 days	No vitellogenin induction	109	Pedersen et al. 1999
Rainbow trout (adult males), Oncorhynchus mykiss	Technical		10 days per month for 4 months	Epidermal mucous cell granulation	1	Burkhardt-Holm et al. 2000
Rainbow trout (598 g; juvenile females), Oncorhynchus mykiss	99%		18 wk	Reduced GSI; Reduced HSI; Induced vitellogenin; Lowered plasma	85.6 85.6 8.3	Harris et al. 2001
				estradiol; Lowered plasma FSH	8.3	

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

<b>Species</b>	<b>Chemical</b>	рН	<b>Duration</b>	<b>Effect</b>	Concentration (µg/L)	<u>Reference</u>
Rainbow trout (1667 ±201.6 g; F <sub>0</sub> 3 yr-old adults), Oncorhynchus mykiss	98%	7.6	4 months (exposed 10 days/month)	Reduced embryo survival; Reduced hatch; F <sub>o</sub> Males increased vitellogenin; F <sub>1</sub> Females increased vitellogenin and testosterone; F <sub>1</sub> Males increased estradiol	1 10 1 10 10	Schwaiger et al. 2002
Lahontan cutthroat trout (juvenile), Oncorhynchus clarki henshawi	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	220	Jones et al. 1998
Apache trout (juvenile), Oncorhynchus mykiss	-		96 hr	Decreased number of muscarinic cholinergic receptors in brain	>130	Jones et al. 1998
Northern squawfish (juvenile), Ptychocheilus oregonensis	-	7.2	3 hr	LT100	10,000	MacPhee and Ruelle 1969
Colorado squawfish (juvenile), Ptychocheilus lucius		-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	>220	Jones et al. 1998
Goldfish (juvenile), Carassius auratus	-	7.0	5 hr	LT100	5,000	Wood 1953
Common carp (15.2 ±3.8 g juvenile), Cyprinus carpio	Technical (90% 4-NP)	7.6	70 days	Decreased erythrocytes; Increased reticulocytes	10 10	Schwaiger et al. 2000

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

	~				Concentration	- 0
<u>Species</u>	<b>Chemical</b>	<u>pH</u>	<b>Duration</b>	<b>Effect</b>	(μg/L)	<u>Reference</u>
Common carp (50-150 g mature males), Cyprinus carpio	95%	7.57 ±0.03	28-31 days 11 °C	BCF = 546.5 No change in 17\$-estradiol, testosterone, or vitellogenin	5.36	Villenueve et al. 2002
Fathead minnow (4-wk old), Pimephales promelas	99%	7.62	4 days	LC50 (fed)	138	Brooke 1993b
Fathead minnow (4-wk old), Pimephales promelas	99%	7.60	28 days	BCF=100.4	193	Brooke 1993b
Fathead minnow, Pimephales promelas	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	>220	Jones et al. 1998
Fathead minnow (mature), Pimephales promelas	>98%		42 days	Possible increased number of Sertoli cells in males	1.6	Miles- Richardson et al. 1999
Fathead minnow (mature), Pimephales promelas	>98%		42 days	Decreased fecundity	>0.4	Giesy et al. 2000
Fathead minnow (mature), Pimephales promelas	>98%	_	42 days	Increased % vitellogenin	>3.4	Giesy et al. 2000
Fathead minnow (mature), Pimephales promelas	>98%	-	42 days	Increased 17\$-estradiol	>0.05	Giesy et al. 2000
Bluegill (juvenile), Lepomis macrochirus	-	7.0	2 hr	LT100	5,000	Wood 1953

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

Species	<u>Chemical</u>	_pH_	<u>Duration</u>	<b>Effect</b>	Concentration (µg/L)	<u>Reference</u>
Bluegill (juvenile), Lepomis macrochirus	-	7.5-8.2	14 hr	LT100	5,000	Applegate et al. 1957
Bluegill (4-wk old), Lepomis macrochirus	99%	7.79	4 days	LC50 (fed)	135	Brooke 1993b
Bluegill (4-wk old), Lepomis macrochirus	99%	7.55	28 days	BCF=35.31	126	Brooke 1993b
Bluegill (juvenile), Lepomis macrochirus	96.4%	7.7 - 7.9	20 days	NOEC LOEC (survival)	76 243	Liber et al. 1999
Southern platyfish (adult, 0.62 to 1.15 g), Xiphophorus maculatus	Technical 85%	-	28 days	Reduced GSI	960	Kinnberg et al. 2000
Green Swordtail (adult males), Xiphophorus helleri	Technical		96 hr 72 hr	LC50 Vitellogenin induced	206 4	Kwak et al. 2001
Green Swordtail (juvenile 30-d-old males), Xiphophorus helleri	Technical		60 days	Reduced sword length	0.2	Kwak et al. 2001
African clawed frog (larva), Xenopus laevis	ACS Grade	7.8 - 8.0	21 days	NOEC LOEC (increased rate of tail resorption)	25 50	Fort and Stover 1997
African clawed frog (larva), Xenopus laevis	-	-	12 wk	Increased female phenotypes	22	Kloas et al. 1999

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

<u>Species</u>	<u>Chemical</u>	рН_	<u>Duration</u>	<u>Effect</u>	Concentration(µg/L)	<u>Reference</u>
		<u>s</u>	ALTWATER	R SPECIES		•
Red alga, Champia parvula	>95%	-	2 days	No effect on sexual reproduction	167	Tagliabue 1993
Barnacle (cypris larva), Balanus amphitrite	-	-	48 hr	Reduced cyprid settlement	1.0	Billinghurst et al. 1998
Soft-shell clam, Mya arenaria	-	-	360 hr	No mortality	700	McLeese et al. 1980b
Coot clam, Mulinia lateralis	90%	30-31 <sup>a</sup>	24 hr	LC50	- 50	Lussier et al. 2000
Coot clam, Mulinia lateralis	90%	30-31 <sup>a</sup>	48 hr	LC50	- 50	Lussier et al. 2000
Coot clam, Mulinia lateralis	90%	30-31 <sup>a</sup>	72 hr	LC50	- 40	Lussier et al. 2000
Blue mussel,  Mytilus edulis	-	32ª	96 hr	LC50	3,000	Granmo et al. 1989
Blue mussel, Mytilus edulis		32ª	360 hr	LC50	500	Granmo et al. 1989
Blue mussel, Mytilus edulis	-	32ª	13 days	Reduced byssus strength	56	Granmo et al. 1989
Blue mussel,  Mytilus edulis	-	32ª	30 days	Reduced byssus strength	56	Granmo et al. 1989
Blue mussel, Mytilus edulis		32ª	30 days	No byssus threads formed	100	Granmo et al. 1989
Blue mussel, <i>Mytilus edulis</i>		32ª	32 days	Reduction in growth	56	Granmo et al. 1989
Blue mussel, Mytilus edulis	-	32ª	24 hr	No effect on fertilization	200	Granmo et al. 1989
Blue mussel, Mytilus edulis	-	32ª	72 hr	No effect on development	200	Granmo et al. 1989

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

<b>Species</b>	Chemical	pН	Duration	Effect	Concentration (µg/L)	Reference
Blue mussel (40-50 mm length), Mytilus edulis	-	-	50 days	BCF = 350	40	Granmo et al. 1991a,b
Blue mussel, Mytilus edulis galloprovincialis	-	-	2 days	Repelled attachment	22	Etoh et al. 1997
Mysid, Americamysis bahia	90%	30-31 <sup>a</sup>	24 hr	LC50	- 114	Lussier et al. 2000
Mysid, Americamysis bahia	90%	30-31 <sup>a</sup>	48 hr	LC50	- 82	Lussier et al. 2000
Mysid, Americamysis bahia	90%	30-31 <sup>a</sup>	72 hr	LC50	- 66	Lussier et al. 2000
Mysid, Americamysis bahia	90%	30-31 <sup>a</sup>	120 hr	LC50	- 60	Lussier et al. 2000
Mysid, Americamysis bahia	90%	30-31 <sup>a</sup>	144 hr	LC50	- 60	Lussier et al. 2000
Mysid, Americamysis bahia	90%	30-31 <sup>a</sup>	168 hr	LC50	- 60	Lussier et al. 2000
Mysid, Americamysis bahia	>95%	20ª	24 hr	LC50	>47	Ward and Boeri 1990a
Mysid, Americamysis bahia	>95%	20ª	48 hr	LC50	>47	Ward and Boeri 1990a
Mysid, Americamysis bahia	>95%	20ª	72 hr	LC50	44	Ward and Boeri 1990a
Copepod (10-12 d), Acartia tonsa	-	18ª	48 hr	LC50 synthetic media	360 280	Kusk and Wollenberger 1999

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

Species	Chemical	pH	Duration	<u>Effect</u>	Concentration (µg/L)	Reference
Amphipod, Leptocheirus plumulosus	90%	30-31 <sup>a</sup>	48 hr	LC50	- 160	Lussier et al. > 2000
Amphipod, Leptocheirus plumulosus	90%	30-31 <sup>a</sup>	72 hr	LC50	- 80	Lussier et al. 2000
Amphipod, Leptocheirus plumulosus	90%	30-31 <sup>a</sup>	120 hr	LC50	- 50	Lussier et al. 2000
Amphipod, Leptocheirus plumulosus	90%	30-31 <sup>a</sup>	144 hr	LC50	- 40	Lussier et al. 2000
Amphipod, Leptocheirus plumulosus	90%	30-31 <sup>a</sup>	168 hr	LC50	-30	Lussier et al. 2000
Grass shrimp, Palaemonetes vulgaris	90%	30-31ª	24 hr	LC50	- 125	Lussier et al. 2000
Grass shrimp, Palaemonetes vulgaris	90%	30-31 <sup>a</sup>	48 hr	LC50	- 60	Lussier et al. 2000
Grass shrimp, Palaemonetes vulgaris	90%	30-31 <sup>a</sup>	72 hr	LC50	- 60	Lussier et al. 2000
Grass shrimp, Palaemonetes vulgaris	90%	30-31 <sup>a</sup>	120 hr	LC50	- 60	Lussier et al. 2000
Shrimp, Crangon septemspinosa	>95%	-	96 hr	LC50	300	McLeese et al. 1980b
Shrimp, Crangon septemspinosa	>95%	-	96 hr	LC50	300	McLeese et al. 1980b
Shrimp, Crangon septemspinosa	>95%	-	96 hr	LC50	300	McLeese et al. 1980b
American lobster, Homarus americanus	90%	30-31ª	24 hr	LC50	- 140	Lussier et al. 2000

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

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<u>Species</u>	<b>Chemical</b>	<u>pH</u>	<b>Duration</b>	<b>Effect</b>	(μg/L)	<u>Reference</u>
American lobster, Homarus americanus	90%	30-31 <sup>a</sup>	48 hr	LC50	- 140	Lussier et al. 2000
American lobster, Homarus americanus	90%	30-31 <sup>a</sup>	72 hr	LC50	- 100	Lussier et al. 2000
American lobster, Homarus americanus	>95%	-	96 hr	LC50	170	McLeese et al. 1980b
Atlantic salmon, Salmo salar	-	-	96 hr	LC50	190	McLeese et al. 1980b
Atlantic salmon, Salmo salar	-	-	96 hr	LC50	160	McLeese et al. 1980b
Atlantic salmon, Salmo salar	-	-	96 hr	LC50	130	McLeese et al. 1980b
Atlantic salmon, Salmo salar	-	-	96 hr	LC50	900	McLeese et al. 1980b
Sheepshead minnow, Cyprinodon variegatus	90%	30-31 <sup>a</sup>	72 hr	LC50	- 150	Lussier et al. 2000
Sheepshead minnow, Cyprinodon variegatus	90%	30-31 <sup>a</sup>	120 hr	LC50	- 125	Lussier et al. 2000
Sheepshead minnow, Cyprinodon variegatus	90%	30-31 <sup>a</sup>	144 hr	LC50	- 120	Lussier et al. 2000
Sheepshead minnow, Cyprinodon variegatus	90%	30-31 <sup>a</sup>	168 hr	LC50	- 120	Lussier et al. 2000
Sheepshead minnow, Cyprinodon variegatus	>95%	15-17 <sup>a</sup>	24 hr	LC50	>420	Ward and Boeri 1990c

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

<b>Species</b>	<u>Chemical</u>	pH_	<u>Duration</u>	<u>Effect</u>	Concentration (µg/L)	Reference
Sheepshead minnow, <i>Cyprinodon</i> variegatus	>95%	15-17 <sup>a</sup>	48 hr	LC50	340	Ward and Boeri 1990c
Sheepshead minnow, Cyprinodon variegatus	>95%	15-17 <sup>a</sup>	72 hr	LC50	320	Ward and Boeri 1990c
Killifish (embryo), Fundulus heteroclitus	85 - 90% (technical)	20ª	10 days	100% abnormal development	2,204	Kelly and Di Giulio 2000
Killifish (embryo), Fundulus heteroclitus	85 - 90% (technical)	20ª	96 hr	LC50	5,444	Kelly and Di Giulio 2000
Killifish (1-day old larva), Fundulus heteroclitus	85 - 90% (technical)	20ª	96 hr	LC50 (fed)	214	Kelly and Di Giulio 2000
Killifish (14-day old larva), Fundulus heteroclitus	85 - 90% (technical)	20ª	96 hr	LC50 (fed)	209	Kelly and Di Giulio 2000
Killifish (28-day old larva), Fundulus heteroclitus	85 - 90% (technical)	20ª	96 hr	LC50 (fed)	260	Kelly and Di Giulio 2000
Threespine stickleback Gasterosteus aculeatus	Commercial (para- substituted with branched nonyl chain)	32ª	96 hr	LC50	370	Granmo et al. 1991a
Inland silversides, Menidia beryllina	90%	30-31 <sup>a</sup>	24 hr	LC50	- 120	Lussier et al. 2000
Inland silversides, Menidia beryllina	90%	30-31 <sup>a</sup>	48 hr	LC50	- 100	Lussier et al. 2000
Inland silversides, Menidia beryllina	90%	30-31 <sup>a</sup>	72 hr	LC50	- 80	Lussier et al. 2000
Inland silversides, Menidia beryllina	90%	30-31 <sup>a</sup>	120 hr	LC50	- 60	Lussier et al. 2000

Table 6. Other Data on Effects of Nonylphenol on Aquatic Organisms (continued)

<b>Species</b>	Chemical	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	Concentration(µg/L) Reference
Inland silversides, Menidia beryllina	90%	30-31 <sup>a</sup>	144 hr	LC50	- 60 Lussier et al. 2000
Inland silversides, Menidia beryllina	90%	30-31 <sup>a</sup>	168 hr	LC50	- 60 Lussier et al. 2000

<sup>&</sup>lt;sup>a</sup>Salinity (g/kg).



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