

# **Aquatic Life Ambient Water Quality Criteria – Nonylphenol**

## **FINAL**

# **Ambient Aquatic Life Water Quality Criteria**

## **Nonylphenol**

(CAS Registry Number 84852-15-3)

(CAS Registry Number 25154-52-3)

**FINAL**

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U.S. Environmental Protection Agency  
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## FOREWORD

Section 304(a)(1) 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 proposed criteria based upon consideration of comments received from independent peer reviewers and the public. Criteria contained in this document replace any previously published EPA aquatic life criteria for the same pollutant(s).

The term “water quality criteria” is used in two sections of the Clean Water Act, section 304(a)(1) 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 health or ecological effects. Criteria presented in this document are such scientific assessments. If water quality criteria associated with specific waterbody uses are adopted by a state or tribe as water quality standards under section 303, they become enforceable maximum acceptable pollutant concentrations in ambient waters within that state or tribe. Water quality criteria adopted in state or tribal water quality standards could have the same numerical values as criteria developed under section 304. However, in many situations states or tribes might want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states or tribes 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 or tribal water quality standards that criteria become regulatory. Guidelines to assist the states and tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA 1994). The 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 final 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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# 1. INTRODUCTION

## 1.1. Physical-Chemical Properties

Nonylphenol (C<sub>15</sub>H<sub>24</sub>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 resulting product is a mixture of various isomers of nonylphenol, predominantly *para*-substituted nonylphenol, (phenol, 4-nonyl-branched, CAS No. 84852-15-3; 4-nonylphenol, CAS No. 104-40-5; and phenol, nonyl-, CAS No. 25154-52-3) with small amounts of *ortho*-substituted phenol (2-nonylphenol, CAS No. 136-83-4), and trace amounts of 2,4-dinonylphenol (phenol, dinonyl, branched, CAS No. 84962-08-3). Additional isomers, which represent the numerous branched structures that occur within the nonyl (nine carbon) group, add to the complexity of the compound. Commercial nonylphenol is most accurately described by CAS number 84852-15-3 (phenol, 4-nonyl-branched), but CAS numbers 104-40-5 (phenol, 4-nonyl-) and 25154-52-3 (phenol, nonyl) have also been used to describe these compounds commercially. The criteria derived in this document address the CAS numbers 84852-15-3 and 25154-52-3.

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 in the production of other chemicals. Notably, nonionic surfactants of the nonylphenol ethoxylate type are produced through etherification of nonylphenol by condensation with ethylene oxide in the presence of a basic catalyst. 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 in large quantities in the United States. Production 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 is a pale yellow highly viscous liquid with a slight phenolic odor, an approximate molecular weight of 215.0 to 220.4 g/mole, a

specific gravity of 0.953 g/mL at 20°C (Budavari 1989), and a vapor pressure of  $4.55 \times 10^{-3}$  ( $\pm 3.54 \times 10^{-3}$ ) Pa (Roy F. Weston Inc. 1990). It has a dissociation constant ( $pK_a$ ) of  $10.7 \pm 1.0$  and a log octanol/water partition coefficient ( $\log K_{ow}$ ) of 3.80 to 4.77 (Roy F. Weston Inc. 1990). The water solubility of nonylphenol is pH-dependent; 4,600 µg/L at pH 5.0, 6,237 µg/L at pH 7.0, 11,897 µg/L at pH 9.0. Nonylphenol is soluble in seawater at 3,630 µg/L and is soluble in many organic solvents (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 increase in solubility between 2°C (4,600 µg/L) and 25°C (6,350 µg/L).

## **1.2. Nonylphenol in the Environment**

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 µg/L in eleven water samples associated with various industrial sources. Bennie et al. (1997) measured nonylphenol in water in 25 percent of sites sampled in the Great Lakes at concentrations from 0.01 to 0.92 µg/L. They found nonylphenol in all sediment samples with concentrations ranging from 0.17 to 72 µg/g (dry weight). Nonylphenol and its ethoxylates have been found in treatment plant wastewaters (Ellis et al. 1982, Giger et al. 1981) and in sewage sludges (Giger et al. 1984). In a study of airport runoff, nonylphenol was measured at 0.98 and 7.67 µg/L in the runoff as a result of aircraft deicer and antiicer fluid use (Corsi et al. 2003). 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 with concentrations ranging from about 0.20 to 0.64 µg/L. Approximately 71 percent of the sampling sites had measurable concentrations of nonylphenol in the sediments at concentrations ranging from about 10 to 2,960 µg/kg. Ethoxylates of nonylphenol were found in 59 to 76 percent of the water samples, with amounts varying by extent of ethoxylation (Naylor 1992, Naylor et al. 1992, Radian Corp. 1990).

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.

A reconnaissance of 95 organic wastewater contaminants in 139 U.S. streams conducted in 1999-2000 revealed that nonylphenol was one of the most commonly occurring contaminants and was measured at higher concentrations than most of the other contaminants (Kolpin et al. 2002). Selection of streams sampled was biased toward streams susceptible to contamination. A number of studies on the persistence of nonylphenol in sewage treatment plant effluents and the environment have been conducted and are reviewed by Maguire (1999). Gaffney (1976) determined that 1 mg/L nonylphenol did not degrade during 135-hr incubation with domestic wastewater. In industrial wastewater, nonylphenol concentration was unchanged after 24 hr incubation, but decreased by 45 percent after 135 hr. Staples et al. (2001) determined that nonylphenol at 13 mg/L and 22°C was mineralized to CO<sub>2</sub> within 35 days in aerobic systems inoculated with sludge from a waste treatment plant. No intermediate compounds were formed and the calculated half-life for nonylphenol was 8.2 days.

Sundaram and Szeto (1981) studied nonylphenol fate incubated in open and closed containers of stream and pond waters. They found no degradation of nonylphenol incubated in open containers of the pond or stream waters. The observed half-life of 2.5 days, was attributed to volatilization. Incubation of nonylphenol in pond or stream waters in closed containers resulted in formation of a breakdown product. The observed half-life of nonylphenol in pond and stream water were estimated at 16.5 and 16.3 days, respectively. The same authors incubated nonylphenol in pond water with sediment present and found about 50 percent of the nonylphenol 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 autoclaved (sterilized) water and sediment samples were used. Staples et al. (1999) measured a half-life of 20 days for nonylphenol in water (31 mg/L) at 22°C. 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. (1994 a,b) studied the fate and transport of alkylphenol polyethoxylates (AP<sub>n</sub>EO)

and their degradates 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 over several seasons. They found nonylphenol concentrations to be lower than other degradates and nonylphenol concentrations were most commonly detected in the 1 to 3  $\mu\text{g/L}$  range. The concentration of APnEO degradates varied with time of day reflecting fluctuations in wastewater treatment plant discharge. Concentrations of APnEO degradates also varied seasonally, being found at higher concentrations in the winter due to lower water temperature. Nonylphenol had less seasonal variability than other APnEO degradates. Nonylphenol was the predominant nonylphenolic compound found in sediments in this study. Sediment concentrations were 364 to 5,100 times those found in the river water.

Ahel and co-workers also reported that the abundance of particular APnEO degradates is dependent on the conditions in the treatment plants studied along the Glatt River system (Ahel et al. 1994a; Ahel et al. 2000). Under aerobic conditions, the APnEOs degrade through either the loss of ethylene oxide units to form low-molecular weight ethoxylates or through the formation of carboxylated ethoxylates ultimately terminating in  $\text{CO}_2$  and water. Nonylphenol is formed during anaerobic breakdown of the APnEOs and is therefore a minor component of wastewater treatment effluents because of aerobic conditions present during treatment. Another study by Ahel et al. (1996) demonstrated that nonylphenol can be reduced in ground water. The authors propose that biological processes are responsible provided that the ground water temperature does not become too cold for biological degradation. It has also been demonstrated (Ahel et al. 1994c) that nonylphenol can be degraded by photochemical processes. In bright summer sun, nonylphenol near the water surface has a half-life of 10-15 hr.

Heinis et al. (1999) studied the distribution and persistence of nonylphenol in natural pond systems in the temperate climate zone. 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 from the sediment was estimated to be 50 and 95 percent at 66 and 401 days, respectively. Hale et al. (2000) measured nonylphenol concentrations in sediments below wastewater outfalls and found one site that had a sediment concentration of 54,400  $\mu\text{g/kg}$  more than twenty years after the treatment plant ceased operation. Bennett and Metcalfe (1998; 2000) found that nonylphenol was widely distributed in 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 and saltwater sediments may be slower than in fresh water and freshwater sediments. Ekelund et al. (1993) found that nonylphenol degradation rate was initially slow in sea water, but increased after microorganism adaptation occurred. Approximately 50 percent of the nonylphenol was degraded after 58 days. In marine sediments, the initial rate of degradation faster than in sea water, but after 58 days about the same percentage of nonylphenol was degraded. Ethoxylated nonylphenol has a half-life of 60 days in marine sediments, similar to that of nonylphenol (Shang et al. 1999). Ferguson and Brownawell (2003) conducted degradation studies with APnEOs in marine sediments and found that degradation occurred in oxic and anoxic conditions. They reported no clear evidence for net formation of nonylphenol from APnEOs under anaerobic conditions during the 120 day study, but they speculated that the time scale of their study may not have been long enough to make the observation.

### **1.3. Metabolism and Bioconcentration**

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 following either waterborne or dietary exposures.

The log  $K_{ow}$  of nonylphenol ranges from 3.80 to 4.77, indicating that moderate bioaccumulation in aquatic organisms may be expected. However, reported laboratory bioconcentration factors (BCFs) and field-derived bioaccumulation factors (BAFs) do not support the expected accumulations in tissues, indicating that some nonylphenol is metabolized. Bioconcentration was measured in two saltwater organisms, the blue mussel (*Mytilus edulis*) and Atlantic salmon (*Salmo salar*) by McLeese et al. (1980a). The estimated BCF for the blue mussel ranged from 1.4 to 7.9 and the estimated BCF for Atlantic salmon was 75 (McLeese et al. 1981). Hecht et al. (2004) reported nonylphenol BCFs for the three marine amphipod species, *Eohaustorius estuarium*, *Grandidierella japonica* and *Corophidium salmonis*, of 154, 185, and 46 to 133, respectively. Ahel et al. (1993) measured the bioconcentration of nonylphenol for

several species in rivers in Switzerland. They determined a BCF for algae of 487 (converted to a wet weight basis assuming 95 percent water in algae). Nonylphenol did not biomagnify in the food chain in the system studied; rather BCFs in fish and ducks were lower than in the algae. Keith et al. (2001) measured nonylphenol in fish tissues of seven species from the Kalamazoo River and in water at the river's confluence with Lake Michigan. They found 41 percent of the tissue samples had measurable concentrations of nonylphenol with a range of 3.3 to 29.1  $\mu\text{g}/\text{kg}$  and a mean value of 12.0  $\mu\text{g}/\text{kg}$ . A followup study was conducted in the same river (Kannan et al. 2003) to further examine the occurrence of nonylphenol and nonylphenol ethoxylates in fish, water and sediments and their association with two wastewater treatment plants. Ten fish from near each treatment plant were analyzed for nonylphenol and ethoxylated nonylphenol. Only one fish contained a measurable concentration of nonylphenol (3.4  $\mu\text{g}/\text{kg}$ ). Neither nonylphenol or its ethoxylates were detected in the sediments collected upstream of the treatment plants. However, five of twenty-four (21 %) sediment samples collected from below the treatment plants contained nonylphenol (no ethoxylates were found) at concentrations that ranged from 2 - 15.3  $\mu\text{g}/\text{kg}$  dry weight. Downstream of one treatment plant, neither nonylphenol nor nonylphenol ethoxylates were measured above the method detection limit. 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 may suggest that nonylphenol bioaccumulated by the macroalgae was transferred to the sediment as the algae died and decomposed.

#### **1.4. Estrogenicity of Nonylphenol**

There are several review articles that describe the estrogenicity of nonylphenol (Servos 1999; Sonnenschein and Soto 1998; Sumpter 1998). The majority of studies using aquatic species models report results for molecular or biochemical endpoints such as induction of the egg protein, vitellogenin, or are in vitro studies such as receptor binding assays. These types of studies and endpoints do not meet the data acceptability requirements outlined in EPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al. 1985) and hence were not used deriving

ambient water quality criteria. However, studies identified in the literature search describing effects of nonylphenol on molecular and biochemical endpoints and activity in in vitro bioassays are discussed in Section 6 of this document.

Whole organism endpoints such as reproductive and growth effects are used to derive aquatic life ambient water quality criteria for nonylphenol. To the extent that such endpoints reflect the integration of molecular, biochemical and tissue-level effects at the whole organism level, the nonylphenol criteria address the estrogenicity of nonylphenol. For example, while vitellogenin is a commonly used biomarker indicative of exposure to estrogenic compounds, measurement of this molecular/biochemical endpoint alone does not necessarily indicate adverse effect on population relevant endpoints such as survival, growth and reproduction. However, several studies have demonstrated that vitellogenin induction can be accompanied by decreased fecundity (egg production) of breeding pairs of fathead minnows exposed chronically to estrogenic compounds (Ankley et al.). The chronic toxicity studies used in deriving the nonylphenol criteria (Table 6) included assessment of effects on growth and reproduction endpoints in aquatic organisms. Hence, to the extent that these endpoints are the result of effects on the endocrine system (although this was not definitively demonstrated in any of the tests by use of a concomittant measure of a estrogen-receptor specific endpoint), the estrogenic effects of nonylphenol have been considered in deriving the aquatic life ambient water quality criteria for nonylphenol.

EPA has activities underway to develop scientific methods for considering endocrine effects, such as the estrogenicity of nonylphenol, in Agency risk assessments. Under the Federal Food, Drug and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act (FQPA), EPA is required to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) “may have an effect in humans that is similar to an effect produced by a naturally-occurring estrogen, or other such endocrine effects as the Administrator may designate”. Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC’s recommendation that the Program include evaluations of potential effects in aquatic life and wildlife. When the

appropriate screening and or testing protocols being considered under the Agency's Endocrine Disruptor Screening Program have been developed, nonylphenol may be subjected to additional screening and or testing to better characterize effects related to endocrine systems.

### **1.5. Derivation of Aquatic Life Ambient Water Quality Criteria**

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), hereafter referred to as the Guidelines, is necessary to fully understand the text, tables, and calculations presented in this criteria document. Results of intermediate calculations are presented to four significant figures to prevent round-off error in subsequent calculations, not to reflect the precision of the value. Final criteria values are presented to two significant figures.

Nonylphenol has been studied for its acute and chronic toxicity to aquatic organisms and results of many studies are summarized in a review article by Staples et al. (1998). This review article also addresses the ability of nonylphenol to bioaccumulate in aquatic organisms. Much of the data reported in the review article has been used in this document, as well as some newer data, to derive the aquatic life ambient water quality criteria. The latest comprehensive literature search for information used in developing this document was conducted in November 1999. Subsequently, forty-three newer studies have subsequently been identified and included. Data and analysis included in the U.S. EPA's Office of Pollution Prevention and Toxics nonylphenol risk assessment have also been evaluated in deriving the aquatic life criteria for nonylphenol. Freshwater criteria were derived using nonylphenol of CAS numbers 25154-52-3 and 84852-15-3; saltwater criteria were derived using only nonylphenol of CAS number 84852-15-3.

Whenever adequately justified, a national criterion may be replaced by a site-specific criterion (U.S. EPA 1983), which may include not only site-specific criterion concentrations (U.S. EPA 1994), but also site-specific averaging periods and frequencies of allowed excursions (U.S. EPA 1991).



## 2. ACUTE TOXICITY TO AQUATIC ANIMALS

### 2.1. Freshwater

The acute toxicity of nonylphenol to freshwater animals has been determined for 18 species and 2 subspecies representing 15 genera (Table 1). Species Mean Acute Values (SMAV) 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 the 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 LC<sub>50</sub> of 20.7 µg/L was calculated in the lower hardness water and 150 µg/L in the higher hardness water. Insufficient data exist to demonstrate an effect of water hardness on the toxicity of nonylphenol; therefore, the results are given equal weight for determining the SMAV. Data for one cladoceran species (*Daphnia magna*) are available. Brooke (1993a) reported an EC<sub>50</sub> of 104 µg/L from a test that had the solutions renewed daily and Comber et al. (1993) reported an EC<sub>50</sub> of 190 µg/L in a static test. The *Daphnia magna* SMAV is 140.6 µg/L.

The least sensitive freshwater species to nonylphenol toxicity were also invertebrates (Tables 1 and 3). The annelid worm (*Lumbriculus variegatus*) had an LC<sub>50</sub> of 342 µg/L, nymphs of the dragonfly *Ophiogomphus* sp. had an LC<sub>50</sub> of 596 µg/L and the least sensitive species tested was a snail, *Physella virgata*, which had an LC<sub>50</sub> of 774 µg/L (Brooke 1993a). The lower sensitivity to nonylphenol 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. The midge, *Chironomus tentans*, had an LC<sub>50</sub> of 160 µg/L (England and Bussard 1995), indicating intermediate sensitivity among invertebrate species tested (Figure 1).

The only amphibian toxicity test available was for the boreal toad, *Bufo boreas*. The toad tadpoles had a 96-hr LC<sub>50</sub> of 120 µg/L and were ranked second in sensitivity to nonylphenol (Dwyer et al. 1999a).

Freshwater fish species were in the mid-range of sensitivity to nonylphenol (Figure 1). SMAVs ranged from 110 µg/L for the fountain darter (*Etheostoma rubrum*) to 289.3 µg/L for the bonytail chub (*Gila elegans*). Three trout species of the genus *Oncorhynchus* (rainbow trout, apache trout, and cutthroat trout) and two subspecies of the species *Oncorhynchus clarki* were

tested and had similar LC50s ranging from 140 to 270 µg/L (Dwyer et al. 1995; Brooke 1993a). Dwyer et al. (1995, 1999a) exposed nine species of fish that were classified as threatened/endangered or were surrogates of threatened or endangered fish species. Acute toxicity test results were based on 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 calculated from two tests with the bonytail chub. In addition to the test conducted by Dwyer et al. (1995), two additional tests were available for the fathead minnows (*Pimephales promelas*). LC50s for this species ranged from 128 µg/L (Brooke 1993a) to 360 µg/L (Dwyer et al. 1995). The tests conducted using flow-through exposure conditions (Holcombe et al. 1984; Brooke 1993a), which is preferable to static exposure conditions (Stephan et al. 1985) were used in calculating the SMAV (158.9 µg/L). One test was available for the bluegill (*Lepomis macrochirus*) and the LC50 was 209 µg/L (Brooke 1993a).

Freshwater Species Mean Acute Values (SMAV) and Genus Mean Acute Values (GMAV) were derived from available acute values (Tables 1 and 3, respectively). 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 2.5 of one another. Based on available data for freshwater organisms summarized in Table 1 and the GMAVs presented in Table 3, the freshwater Final Acute Value (FAV) for nonylphenol is 55.69 µg/L (calculated using the procedure described in the “Guidelines”). This FAV is essentially the same as the lowest freshwater SMAV of 55.72 µg/L for the amphipod *H. azteca*.

## **2.2. Saltwater**

The acute toxicity of nonylphenol to saltwater animals has been determined for 8 invertebrate and 3 fish species (Table 1). SMAVs ranged 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-fold. Fish (winter flounder), bivalves (coot clam, *Mulinia lateralis*) and crustaceans (the mysid, *Americamysis bahia*) were the most sensitive species.

Data for nine of the thirteen 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 chemical exposure because the tests 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. Normally, data gathered from tests in which organisms are fed are not acceptable for use in deriving Final Acute Values. However, the brine shrimp fed during the tests were “reference grade” and not likely to change the exposure to nonylphenol. Further, additional tests conducted in a different laboratory are available for two of the saltwater species such that toxicity results obtained when the testing is conducted with and without food added can be compared. In a 96-hr test with the mysid, the estimated LC50 was somewhat higher when the organisms were fed (60.6 µg/L; Lussier et al. 2000) compared to when they were not fed (43 µg/L; Ward and Boeri 1990a) during the study. In contrast, in a 96-hr test with the sheepshead minnow, the LC50 determined when the organisms were fed (142 µg/L; Lussier et al. 2000) was lower than when the organisms were not fed (310 µg/L; Ward and Boeri 1990a) during the study. These data indicate that feeding during the tests did not consistently increase or decrease the LC50 estimates, and therefore feeding is assumed not to have altered the results in these tests. Hence, the data from the Lussier et al. (2000) tests were used in deriving a saltwater Final Acute Value.

Acute toxicity test data were available for a number of other saltwater species. Invertebrates tested include: coot clam, *Mulinia lateralis* (LC50 = 37.9 µg/L; Lussier et al. 2000), the copepod, *Acartia tonsa* (LC50 = 190 µg/L; Kusk and Wollenberger 1999), American lobster, *Homarus americanus* (LC50 = 71 µg/L; Lussier et al. 2000), mud crab, *Dyspanopeus sayii* (LC50 >195 µg/L; Lussier et al. 2000) and the amphipods, *Leptocheirus plumulosus* (LC50 = 61.6; Lussier et al. 2000) and *Eohaustorius estuarius* (LC50 = 138 µg/L; Hecht and Boese 2002). The test with the amphipod *E. estuarius* (Hecht and Boese 2002) was conducted as a 96-hr test with a mean LC50 for toxicity measured at 227 µg/L as the average of three tests (299, 194, 189 µg/L). The ability of the surviving organisms to bury themselves in sediment at 96 hr when placed on sediment was combined with the number of survivors to calculate an EC50. The mean EC50 for the three tests was 138 µg/L. The sensitivity of the saltwater fish inland silversides (*Menidia*

*beryllina*), was intermediate (LC50 = 70 µg/L) among the three saltwater fish species tested.

Saltwater Species Mean Acute Values (SMAV) and Genus Mean Acute Values (GMAV) were derived from available acute values (Tables 1 and 3, respectively). GMAVs were available for 11 genera; the most sensitive was the winter flounder, *Pleuronectes americanus*, which was 12.3 times more sensitive than the least sensitive species, the sheepshead minnow, *Cyprinodon variegates* (Table 1 and 3). GMAVs for the four most sensitive saltwater species differ by a factor of only 3.5 (Table 3 and Figure 2). Based on available data for freshwater organisms summarized in Table 1 and the GMAVs presented in Table 3, the freshwater Final Acute Value (FAV) for nonylphenol is 13.93 µg/L (calculated using the procedure described in the “Guidelines”). This FAV is lower than the lowest SMAV of 17 µg/L for the the winter flounder, *Pleuronectes americanus*.

### 3. CHRONIC TOXICITY TO AQUATIC ANIMALS

#### 3.1. Freshwater

The chronic toxicity of nonylphenol was determined for 5 freshwater species, two fish and 3 invertebrates (Table 2). Concentrations of nonylphenol were measured in all the tests. 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 µ/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 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 % 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 µg/L. No effects were observed during the study on mortality, the number of offspring per female, or the mean day of the first brood at any of the test concentrations. A significant effect was observed on the total number of young per concentration on day nine of the study. Based on the No Observed Effect Concentration (NOEC) of 10.7 µg/L and the Lowest Observed Effect Concentration (LOEC) of 47.8 µg/L reported, the chronic value (geometric mean of the NOEC and LOEC) for *D. magna* in this test is 22.62 µ/L. An acute test with this species conducted by the same authors was not available to calculate an ACR.

Brooke (1993a) conducted a 21-day chronic exposure for the cladoceran *Daphnia magna*. Test solutions were renewed three times per week and concentrations of nonylphenol declined, on average,  $57.4 \pm 5.8$  % between solution renewals. The author concluded that *D. magna* growth and reproduction were significantly affected 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 of organisms survived in the control group. Based on reproductive impairment, the chronic value, calculated as the geometric

mean of the lower (116 µg/L) and upper (215 µg/L) chronic limits, for this test was 157.9 µg/L. Dividing the acute value (104 µg/L), determined from a companion test for this species (Brooke 1993a; Table 1) by the chronic value (157.9 µg/L; Table 2) results in an ACR 0.6586 for *D. magna* (Table 2).

A third life-cycle test (21-day exposure) with *D. magna* was conducted by Comber et al. (1993). They found no significant effects in survival, reproduction or growth at concentrations  $\leq 24$  µg/L. The number of live young produced was significantly reduced at concentrations  $\geq 39$  µg/L when compared to controls. Growth was reduced at concentrations  $\geq 71$  µg/L and survival of adults was reduced at concentrations  $\geq 130$  µg/L. Based on reproductive impairment, the chronic value, calculated as the geometric mean of the lower (24 µg/L) and upper (39 µg/L) chronic limits, for this test was 30.59 µg/L. Dividing the acute value (190 µg/L), determined in a companion test for this species (Table 1) by the chronic value (30.59 µg/L; Table 2) results in an ACR of 6.211 for *D. magna*. Calculating the geometric mean of the two ACRs for *D. magna* (0.6586 and 6.211) results in a species mean acute-chronic ration (SMACR) of 2.023 for *D. magna*.

The midge, *Chironomus tentans*, was exposed in a continuous-flow diluter to nonylphenol from  $\leq 24$ -hr old larva through emergence (53 days) as adults (Kahl et al. 1997). Nominal exposure concentrations ranged from 12.5 to 200 µg/L, but mean measured concentrations were lower. Neither growth nor reproductive endpoints (sex ratio, emergence pattern, and egg production and viability) were negatively affected at any of the exposure concentrations. There was a significant effect on survival of larvae during the first 20 days of exposure, but no effect after 20 days. Based on survival at 20 days, the NOEC and LOEC for this study were 42 and 91 µg/L, respectively. The chronic value, calculated as the geometric mean of the NOEC and the LOEC, is 61.82 µg/L for this test. A companion acute toxicity test 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, ranging 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 tested; however, nearly all of the larvae were abnormal at the two highest exposure concentrations ( $\geq 53.0$  µg/L). At the end of

the test, survival was significantly reduced at concentrations  $\geq 23.1$   $\mu\text{g/L}$  but not at  $10.3$   $\mu\text{g/L}$ . 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 %) and weighed less (30 %, dry weight) than control fish at nonylphenol concentrations  $\geq 10.3$   $\mu\text{g/L}$ , but not at  $6.0$   $\mu\text{g/L}$ . Based on growth, the NOEC and LOEC determined in this study were  $6.0$  and  $\geq 10.3$   $\mu\text{g/L}$ , respectively. The chronic value, calculated as the geometric mean of the NOEC and the LOEC, is  $7.861$   $\mu\text{g/L}$  for rainbow trout. Dividing the acute value ( $221$   $\mu\text{g/L}$ ), determined in a companion test for this species (Table 1) by the chronic value ( $7.861$   $\mu\text{g/L}$ ; Table 2) results in an ACR of 28.11 for rainbow trout.

An early-life-stage toxicity test was available for the fathead minnow, *Pimephales promelas* (Ward and Boeri 1991c). Embryos and larvae were exposed under continuous-flow conditions for a total of 33 days to five concentrations of nonylphenol that ranged from  $2.8$  to  $23$   $\mu\text{g/L}$ . Embryos in the control group and those in the three lowest nonylphenol exposure concentrations ( $2.8$ ,  $4.5$ , and  $7.4$   $\mu\text{g/L}$ ) began to hatch on the third day of exposure, while the two higher concentrations ( $14$  and  $23$   $\mu\text{g/L}$ ) began hatching on the fourth day. Growth (length or weight) of nonylphenol exposed fish was not significantly different from the control organisms at any of the nonylphenol treatment concentrations. Survival of the fish at the end of the test was significantly reduced at nonylphenol concentrations  $\geq 14$   $\mu\text{g/L}$ . Fish survival averaged 56.7 % at  $23$   $\mu\text{g/L}$  nonylphenol, 66.7 % at  $14$   $\mu\text{g/L}$  nonylphenol, and 76.7 % at  $7.4$   $\mu\text{g/L}$  nonylphenol. At concentrations  $\leq 7.4$   $\mu\text{g/L}$  survival of nonylphenol exposed fish did not differ from the control fish survival, which averaged 86.7 %. Based on survival, the NOEC and LOEC determined in this study were  $7.4$   $\mu\text{g/L}$  and  $14$   $\mu\text{g/L}$ , respectively. The chronic value, calculated as the geometric mean of the NOEC and the LOEC, is  $10.18$   $\mu\text{g/L}$  for fathead minnow (Table 2). A companion acute toxicity test was not conducted; therefore, an ACR can not be calculated for this species.

### **3.2. Saltwater**

Two chronic toxicity tests with phenol have been conducted with the same saltwater animal species. A 28-day chronic toxicity test was conducted with mysids, *Americamysis bahia* (Ward

and Boeri 1991b). There was no effect on survival or reproduction at 6.7 µg/L, but there was a 18 % reduction in survival and a 53% reduction in reproduction at 9.1 µg/L. Effects on survival at the highest concentration tested (21 µg/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, therefore growth analysis was based on combined length data for both sexes. Growth (length) was the most sensitive endpoint for mysids. There was a 7% (statistically significant relative to control animals) reduction in the length of mysids exposed to 6.7 µg/L nonylphenol, but no difference in growth for mysids exposed to 3.9 µg/L nonylphenol. Based on growth, the NOEC and LOEC determined in this study were 3.9 µg/L and 6.7 µg/L, respectively. The chronic value, calculated as the geometric mean of the NOEC and the LOEC, is 5.112 µg/L for mysids (Table 2). Dividing the acute value (43 µg/L), determined in a companion test for this species (Table 1) by the chronic value (5.112 µg/L; Table 2) results in an ACR of 8.412 for the mysid, *Americamysis bahia*.

A second 28-day life cycle test with mysids, *Americamysis bahia* (Kuhn et al. 2001) was conducted using the ASTM standardized life-cycle test methods. Time to first brood release appeared dose dependent, but was not statistically significant. Growth of the female mysid was dose dependent and was significantly affected at concentrations  $\geq 27.56$  µg/L. The most sensitive endpoint for this test was a reproduction. The average number of young per available female reproductive days was significantly reduced at test concentration  $\geq 15.28$  µg/L, but was not affected at 9.46 µg/L. Based on reproduction, the NOEC and LOEC determined in this study were 9.46 µg/L and 15.28 µg/L, respectively. The chronic value, calculated as the geometric mean of the NOEC and the LOEC, is 12.02 µg/L. A companion acute toxicity test was not conducted; therefore, an ACR can not be calculated from this test.

### **3.3. Acute-Chronic Ratios**

Three nonylphenol ACRs, determined from the fourth (*Daphnia magna*) and eighth (rainbow trout) most sensitive freshwater species tested and the third (mysid) most sensitive saltwater species tested, are available (Table 3). Two ACRs (0.6586 and 6.211; Table 2) were available



for the cladoceran *Daphnia magna*, which differed by a factor of approximately 9.4-fold. The species mean ACR, calculated as the geometric mean of the two values, is 2.023. Acute-chronic ratios for the acutely sensitive mysid, *A. bahia*, was 8.412 and the moderately acutely sensitive rainbow trout, *Oncorhynchus mykiss*, was 28.11. An ACR of 0.515 would be calculated from the tests of England (1995) with the cladoceran, *Ceriodaphnia dubia*. However, the organisms were fed during the acute test and data demonstrating that feeding did not significantly affect the acute value were not available. 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 species mean ACRs (2.023, 8.412 and 28.11) differed by 13.9-fold (Table 3). The Guidelines stipulate that if the species mean acute-chronic ratio seems to increase or decrease as the SMAV increases, the Final Acute-Chronic Ratio (FACR) should be based on the acute-chronic ratios for species whose SMAVs are close to the Final Acute Value (FAV). Examination of the SMACRs (Table 3) relative to the SMAVs indicates that the more acutely sensitive species (*A. bahia* and *D. magna*) have 3 to 14-fold lower SMACRs than for the less acutely sensitive rainbow trout, indicating a general trend of increasing SMACR with increasing SMAV. Therefore, the FACR should be based on the SMACR for species whose SMAVs are close to the FAV. The mysid SMAV (51.05 µg/L) is closest to both the freshwater FAV (55.49 µg/L) and the saltwater FAV (13.93 µg/L). Therefore, the SMACR for the mysid is used as the FACR and is 8.412.

## 4. TOXICITY TO AQUATIC PLANTS

### 4.1. Freshwater

Acceptable data on the toxicity of nonylphenol to freshwater plants were available for one species of algae (nonvascular plant) and no acceptable toxicity data are available for vascular plants (Table 4). Ward and Boeri (1990a) exposed the green alga, *Selenastrum capricornutum*, to nonylphenol for four days. They calculated an EC<sub>50</sub> of 410 µg/L based on 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.

### 4.2. Saltwater

Acceptable data on the toxicity of nonylphenol to saltwater plants were available for one species of marine algae and no acceptable toxicity data are available for vascular plants (Table 4). The EC<sub>50</sub> 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), demonstrating that nonylphenol treatment at this concentration for four days did not have a persistent algistatic effect.

Based on the vegetative growth test using the saltwater planktonic diatom, *Skeletonema costatum*, the Final Plant Value for nonylphenol is 27 µg/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.

## 5. BIOACCUMULATION

### 5.1. Freshwater

Acceptable data on the bioconcentration of nonylphenol in two species of freshwater animals were available (Table 5). Ward and Boeri (1991a) measured the whole body concentrations of nonylphenol in juvenile fathead minnows at two exposure concentrations after 27 days of exposure. The bioconcentration factors (BCFs) were 271 and 344 (not lipid normalized) following exposure to 4.9 and 22.7 µg/L nonylphenol, respectively. Brooke (1993b) exposed juvenile fathead minnow (*Pimephales promelas*) and juvenile bluegill (*Lepomis macrochirus*) to nonylphenol 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 calculate bioconcentration factors. The short-term (4-day) tests showed that tissue concentrations reached steady-state within two days in both the fathead minnow and the bluegill. Therefore, there was good agreement between the results obtained in the 4-day and 28-day tests. Lipid-normalized BCFs for the fathead minnow ranged from 128.3 to 209.4 (Table 5) and for the bluegill ranged from 38.98 to 56.94. Giesy et al. (2000) measured the nonylphenol concentrations in whole bodies of the fathead minnow following a 42-day exposure. Exposure to sublethal concentrations of nonylphenol ranging from 0.4 to 3.4 µg/L resulted in BCFs ranging from 203 to 268

### 5.2. Saltwater

Bioconcentration factors are available for three species of saltwater animals, *Mytilus edulis*, *Crangon crangon* and *Gasterosteus aculeatus* (Ekelund et al. 1990; Table 5). *Crangon crangon* is a non-resident species, but the data are included because so little bioaccumulation data are available. Organisms were exposed to <sup>14</sup>C-labeled nonylphenol (CAS number not provided) for 16 days followed by an elimination period of 32 days. Lipid-normalized BCFs based on wet weight ranged from 78.75 for *C. crangon* to 2,168 for *M. edulis*. The BCF for *M. edulis* was an estimate, because steady-state tissue concentration was not reached during 16 days of exposure.

## 6. OTHER DATA

### 6.1. Freshwater

Additional data on the lethal and sublethal effects of nonylphenol on freshwater species that do not meet the data requirements described in the Guidelines (Stephan et al. 1985) for use in deriving aquatic life ambient water quality criteria are summarized in Table 6.

Three plant species (*Chlamydomonas reinhardtii*, *Salvinia molesta* and *Lemna minor*) were exposed in studies using media solutions that were not described. The effect levels determined for *Salvinia molesta* (2,500 µg/L) and *Chlamydomonas reinhardtii* (6,250 µg/L), indicates that these plant species are less sensitive to nonylphenol than animals (Prasad 1986; Weinberger and Greenhalgh 1984). Effect concentrations reported for the duckweed, *Lemna minor*, were highly variable, ranging from 125 to 5,500 µg/L (Weinberger and Iyengar 1983; Prasad 1986). Protozoa were affected in the concentration range from 50 to 747 µg/L (Preston et al. 2000, Schultz 1997, Yoshioka 1985).

Additional data on acute and chronic toxicity of a variety of invertebrates are summarized in Table 6. McLeese et al. (1980b) reported an LC50 of 5,000 µg/L for a clam, *Anodonta cataractae*, following a 144-hr exposure. The test organisms were fed in this test and the toxicity value is higher than those reported in Table 1 for similar species. In an acute test (96-hr) in which the cladoceran, *Daphnia magna* was fed, effect levels were reported as 136 and 302 µg/L for young and adult animals, respectively (Gerritsen et al. 1998). In a 48-hr test with the same species, EC50s ranged from 234 to 337 (Zang et al. 2003). Three 21-day tests with *Daphnia magna* (Baer and Owens 1999, Baldwin et al. 1997, LeBlanc et al. 2000), an additional *D. magna* test of 35-day duration in a high-hardness medium (Zang et al. 2003), and a 30-day test with *D. galeata mendotae* (Shurin and Dodson 1997) are included in this section because nonylphenol concentrations in the test water were not measured in these chronic tests. Negative effects on survival or reproduction were observed in all three tests with typical water hardness (i.e., between 25 and 200 µg/L). The results from these tests with *D. magna* (Table 6) agree reasonably well with those from tests with *D. magna* in which nonylphenol concentrations were measured (Table 2). Another 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). The LC50s determined in the test 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 following exposure to nonylphenol for 48 hr and 7 days, respectively (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, determined when the organisms were fed, are less than the values reported in another study by the same authors in which organisms were not fed during the test (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 µg/L and a 96-hr LC50 for the bluegill, *Lepomis macrochirus*, of 135 µg/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). McLeese et al. (1980b) reported an LC50 of 900 µg/L for the Atlantic salmon, *Salmo salar*, in a 96-hr exposure and Lech et al (1996) reported an LC50 of 193.65 for rainbow trout, *Oncorhynchus mykiss*, in a 72-hr exposure. Holmes and Kingsbury (1980) reported a 96-hr LC50 of 145 µg/L for brook trout juveniles (*Salvelinus fontinalis*), a 96-hr LC50 of 230 µg/L for rainbow trout juveniles (*Oncorhynchus mykiss*) and a 32-day LC50 of > 40 µg/L for lake trout juveniles (*Salvelinus namaycush*). Fish were fed during these studies, but the resulting toxicity values are similar to comparable studies reported for salmonids in Table 1. Ernst et al (1980) reported 96-hr LC50s ranging from 560-920 µg/L for rainbow trout exposed to practical grade nonylphenol. A number of older studies were identified that report time to lethality (LT100) values for a number of freshwater species exposed to very high concentrations of nonylphenol (Applegate et al. 1957; MacPhee and Ruelle 1969; Wood 1952)

A long-term study was conducted with rainbow trout, *Oncorhynchus mykiss*, exposing female fish immediately after hatch to 1, 10, and 30 or 50 µg/L of nonylphenol (Ashfield et al. 1998). They found reduced growth in fish exposed to 1 µg/L for 22 days and grown for 86 days beyond treatment. Growth was not reduced in the 10 µg/L treatment but was in the 50 µg/L treatment. In a second study in which exposure was for 35 days and grow-out was for 431 days beyond the last treatment day, reduced growth was observed at the 10 and 30 µg/L treatments on

day 55 of the study, but not at the 1 µg/L. At day 466, the fish exposed to 10 µg/L recovered the growth reductions seen earlier and only the 30 µg/L exposed fish showed reduced (approximately 25%) growth.

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 acetylcholinesterase enzymes. AChE inhibition was measured indirectly as a decrease in the number of muscarinic cholinergic receptors which is a compensatory response to acetylcholine buildup (Jones et al. 1998). Responses at exposure concentrations  $\leq$  220 µg/L were observed in the rainbow trout, Lahontan cutthroat trout and Apache trout. The lack of a clear connection between this sublethal biochemical endpoint and population relevant effects precludes the use of these results as core data. An effect of nonylphenol on another sub-organismal endpoint, histology of epidermal mucous cells, was observed following intermittent exposure to technical grade nonylphenol (Burkhardt-Holm et al. 2000). Other histochemical or biochemical changes have been reported following exposure to nonylphenol including hemorrhage and lymphocyte infiltration in liver tissue of rainbow trout (Ugaz et al. 2003) and blood cell composition in carp (Schwaiger et al. 2000).

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). Blackburn et al. (1999) reported BCFs for adult male rainbow trout of 116 and 88 following 3 weeks exposure to 63 and 81 µg/L nonylphenol (purity unknown), respectively. Lewis and Lech (1996) found that bioconcentration of nonylphenol after short-term exposure (2-24 hr) was higher in rainbow trout viscera (BCF = 98.2) than in the remainder of the carcass (BCF = 24.21). 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. Zooplankton populations (O'Halloran et al. 1999) and benthic macroinvertebrate populations (Schmude et al. 1999) exposed to four concentrations of nonylphenol for 20 days showed no negative effects at the 23 µg/L

nonylphenol but were negatively affected at 76 µg/L nonylphenol. 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 approximately 10 µg/L. The fish (bluegill) in the mesocosms (Liber et al. 1999) were unaffected at nonylphenol exposures  $\leq$  76 µg/L, but survival was reduced at 243 µg/L. In one exposure replicate with a mean nonylphenol concentration of 93 µg/L, survival of the fish was reduced after 20 days of exposure indicating that concentrations near 100 µg/L may be the toxicity threshold for this species. Hense et al. (2003) and Severin et al. (2003) conducted microcosm studies in Germany using 6-week exposures to nonylphenol. They found changes in phytoplankton species composition, but no change in biomass with nonylphenol concentrations up to 120 µg/L. The zooplankton in the study were not affected at concentrations ranging from 19 to 44 µg/L (mean of 30 µg/L), but species richness was affected at concentrations  $>$ 30 µg/L. Effects observed in these mesocosm studies were all above the freshwater Final Chronic Value of 5.920 µg/L.

## 6.2. Saltwater

Additional data on the lethal and sublethal effects of nonylphenol on saltwater species that do not meet the data requirements described in the Guidelines (Stephan et al. 1985) for use in deriving aquatic life ambient water quality criteria are summarized 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 (Billingham 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). Granmo et al. (1989) report LC50s of 3,000 µg/L and 500 µg/L at 96-hr and 360-hr, respectively, for the blue mussel, *Mytilus edulis*. Nonylphenol also reduced growth and byssus thread strength in the blue mussel at concentrations of  $\geq$  56 µg/L (Granmo et al. 1989) and caused effects on attachment activity at 22 µg/L (Etoh et al. 1997). Lussier et al. (2000) tested a number of saltwater invertebrates including coot clam, mysid, amphipod, grass shrimp, and American lobster and determined LC50s for various timepoints

(Table 6). Results from other studies with mysid (Ward and Boeri, 1990a) and American lobster (McLeese et al 1980b) are similar to those reported by Lussier et al (2000). The LC50 value (300 µg/L) reported by McLeese et al. (1980b) for the shrimp, *Crangon septemspinosa*, is higher than the grass shrimp values reported by Lussier et al (2000). Kusk and Wollenberger (1999) determined a 48-hr LC50 (280-360 µg/L for the copepod, *Acartia tonsa*, exposed to nonylphenol in a synthetic media. Nice et al (2000) reported developmental effects at 100 µg/L nonylphenol on the Pacific oyster (*Crassostrea gigas*) exposed for 72-hr. Nonylphenols have also been reported to have antifouling activity, but the test results are qualitative (Takasawa et al. 1990; Kitajima et al. 1995).

A fifty-five-day flow-through test with the mysid, *Americamysis bahia*, was conducted by Kuhn et al. (2001) to evaluate the efficacy of an age-classified projection matrix model for predicting population behavior. Organisms were exposed for more than three generations to nonylphenol. The measured mean concentrations of nonylphenol used for the 55-day exposure were 5.79, 7.56, 10.88, 15.75, 21.44, 33.19, and 106.00 µg/L. Thirty individuals were used in each replicate exposure chamber and the age distribution consisted of 15 (24-h newly hatched), 8 (8-d-old juveniles), 4 (17-d-old adults), 2 (23-d-old adults), and 1 (31-d-old adult) test organisms. Several generations were possible in this test (control organisms produced first brood in 14 days). It appears that the control populations grew in number of individuals for the first 28 to 36 days, then stabilized. Population growth was reduced from day 8 and beyond in all of the nonylphenol treated groups. The population exposed to 5.79 µg/L nonylphenol grew at the same rate as the control animals for the first 21 days, but then the rate fell below the control rate. The populations exposed to higher nonylphenol concentrations all decreased from day 8 and beyond. There appears to be a trend (not significant) in the shift in the sex ratios for the various treatments. At the end of the test, the sex ratios were one-third female in the control groups and half female in the 33.19 µg/L exposure group. The authors calculated a zero population growth value ( $\lambda$ ) of 19 µg/L for the 55-day multigenerational test. The chronic values from the 28-day exposure in this study was 12.02 µg/L (Table 2) and from a similar 28-day study by Ward and Boeri (1991b) was 5.112 µg/L (Table 2), which are lower than the predicted value for “population protection” from the 55-day multigenerational test.

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, LC50s ranged from > 420 µg/L for a 24-hr exposure to 320 µg/L for a 72-hr exposure. Threespine stickleback, *Gasterosteus aculeatus*, exposed to a commercial mixture of nonylphenol had a 96-hr LC50 of 370 µg/L (Granmo et al. 1991a). 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 LC50s of 214, 209, and 260, respectively.

Additional data on the effect of nonylphenol on saltwater species do not indicate greater sensitivities than the data summarized in Tables 1 and 2. Some of the data presented in Table 6 (e.g., sheepshead minnow, Inland silversides) 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.

### 6.3. Reproductive, Developmental and Estrogenic Effects of Nonylphenol

There are several review articles that describe the estrogenic activity of nonylphenol (Servos 1999; Sonnenschein and Soto 1998; Sumpter 1998). The majority of studies describing the estrogenic activity of nonylphenol using aquatic species models exposed in vivo measure molecular, biochemical, or histological endpoints such as induction of the egg protein, vitellogenin, or occurrence of egg cells within testes (a condition known as intersex or ootestis). In addition, estrogenicity is commonly characterized using in vitro studies such as estrogen receptor binding assays. Molecular, biochemical and reproductive endpoints measured following in vivo exposures to nonylphenol and that are thought to result from estrogenic activity of nonylphenol are summarized in this section. In vitro studies are listed in Section 7 of this document.

The majority of reports of estrogenic effects in aquatic organisms have been for fish, although some effects in invertebrates have also been reported. Bechmann (1999) found no effects in the marine copepod *Tisbe battagliai* exposed to nonylphenol at 55 µg/L, but estrogenic effects were reported to have occurred in the amphipod *Corophium volutator* (Brown et al. 1999) at 10 µg/L and in the larva of *Chironomus riparius* (Hahn et al. 2002) at 2,000 µg/L. The mechanism(s) by which estrogenic effects can be produced in invertebrates that do not possess estrogen receptors is unclear.

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. 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 the natural estrogen, 17β-estradiol. Ren et al. (1996a) demonstrated significant increases in vitellogenin production in rainbow trout exposed to nonylphenol at 100 µg/L for 72 hr. In another study, Ren et al. (1996b) demonstrated that nonylphenol could stimulate the production of vitellogenin mRNA (which precedes vitellogenin protein synthesis) within 4 hr at 10 µg/L. Similarly, Lech et al. (1996) observed a significant increase in vitellogenin mRNA at 72 hr in rainbow trout at 14.14 µg/L nonylphenol. Vitellogenin was induced in green swordfish, *Xiphophorus helleri*, by exposure to 4 µg/L of technical grade nonylphenol (Kwak et al. 2001).

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 µg/L of nonylphenol. Similar results were reported in another study with rainbow trout, plasma vitellogenin was increased after 21 days exposure to 50 µg/L of nonylphenol (Tremblay and Van Der Kraak 1998). Harris et al. (2001) also observed increased plasma vitellogenin levels in female rainbow trout exposed to 8.3 and 85.6 µg/L of nonylphenol. In the same study, nonylphenol also caused changes in several pituitary and plasma hormone levels. In contrast, vitellogenin induction was not observed in rainbow trout exposed for 9 days to 109 µg/L of nonylphenol (Pedersen et al. 1999) or in Atlantic salmon, *Salmo trutta*, exposed for 30 days to 20 µg/L (Moore et al. 2003). The influence of exposure route on nonylphenol-induced vitellogenin mRNA and plasma vitellogenin production in the male fathead minnow was studied by Pickford et al. (2003). Their results showed that exposure via water produced 10-fold higher vitellogenin induction than exposure via the dietary route.

In a study with the fathead minnow, Giesy et al. (2000) found that nonylphenol exposures to 0.5 to 3.4 µg/L nonylphenol were not acutely toxic to the adult fish and fecundity was variously affected over the reproductive season. When the cumulative reproduction was combined for the two experiments during different portions of the reproductive season, concentrations of > 0.3 to 0.4 µg/L did appear to reduce fecundity. However, fish exposed to 0.09 and 0.1 µg/L produced more eggs than control fish. These data appear to produce a U-shaped dose-response and indicate a possible hormetic response of fecundity to nonylphenol. Nonylphenol concentrations of 0.05 to 3.4 µg/L did not significantly change vitellogenin concentrations in the blood of males, and raised the 17β-estradiol titers in the blood of male and female fish at most treatment concentrations > 0.05 µg/L. An increase in the number of Sertoli cells may have occurred in the male fathead minnow exposed to nonylphenol at 1.6 µg/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.

A non-resident fish species, Japanese medaka (*Oryzias latipes*), was exposed to nonylphenol for 28 days following hatch and survivors monitored for the following 55 days (Nimrod and Benson 1998). At the highest exposure concentration of 1.93 µg/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 ovo-testis, an intersex condition, occurred after a

three month exposure to 50 µg/L of nonylphenol (Gray and Metcalf 1997). The sex ratio shifted in favor of females at the highest exposure concentration. Seki et al. (2003) found that in the same species of fish exposed to nonylphenol from fertilized egg to 60 days post-hatch, the lowest-observed-effect concentration for vitellogenin induction was 11.6 µg/L.

Yokota et al. (2001) conducted a two-generation flow-through study with the non-resident fish species medaka (*Oryzias latipes*). Concentrations of nonylphenol were measured during exposures that began with eggs and proceeded to 60-days post-hatch of the second (F<sub>1</sub>) generation. Five exposure concentrations of nonylphenol in quadruplicate (4.2, 8.2, 17.7, 51.5, and 183 µg/L) and water-only and solvent controls were used. In the F<sub>0</sub> generation, egg hatchability was reduced (46.7%) by 183 µg/L nonylphenol exposure, survival was significantly decreased at 60 days post-hatch by nonylphenol exposures  $\geq 17.7$  µg/L, and no differences in growth (length or weight) were observed at 60 days post-hatch. Induction of ovo-testis was observed in the 17.7 µg/L treatment, with 20% of fish displaying male characteristics externally having ovo-testis tissues. In fish from the 51.5 µg/L treatment, 40% had ovo-testis and all of these fish exhibited female characteristics externally. Spermatogenesis was observed in the fish with ovo-testis exposed to 17.7 µg/L nonylphenol, but was not observed in the fish with ovo-testis exposed to 51.5 µg/L nonylphenol. Fecundity of paired fish during the reproductive phase (days 71 to 103 post-hatch) was not affected by nonylphenol treatments. GSI of male fish was reduced at 17.7 µg/L, but not significantly, and GSI of female fish was increased significantly by exposure to nonylphenol concentrations  $> 8.2$  µg/L.

The effects of nonylphenol on F<sub>1</sub> fish from Yokota et al. (2001) were also reported. No embryological abnormalities or hatching failures of fertilized eggs were observed in any treatments. Growth was not affected at 60-days post-hatch by any of the nonylphenol exposure concentrations. The sex ratio, characterized by secondary sex characteristics, changed in treatments  $\geq 17.7$  µg/L to favor females 1:2. Induction of ovo-testis was observed at lower concentrations of nonylphenol in the F<sub>1</sub> generation than in the F<sub>0</sub> generation. Ovo-testis were observed in the 8.2 µg/L exposure group (10%) and in the 17.7 µg/L exposure group (25%). However, all fish with ovo-testis displayed external male characteristics and the degree of development of oocytes in each ovo-testis was not as severe as that in the F<sub>0</sub> generation in the 17.7 µg/L treatment. The overall results indicate a LOEC of 17.7 µg/L and a NOEC of 8.2 µg/L

with a chronic value, calculated as the geometric mean of the NOEC and LOEC, of 12.05 µg/L. The chronic value for this study is in good agreement with the Table 2 data for resident freshwater species, rainbow trout and fathead minnow.

A multi-generational exposure to nonylphenol has been conducted with rainbow trout by Schwaiger et al. (2002). Adult rainbow trout of both sexes were exposed intermittently to nonylphenol at 1 and 10 µg/L over a 4 month period. Mortality rate in the progeny was significantly reduced by parental exposure to both 1 and 10 µg/L nonylphenol and hatching in the progeny was reduced by parental exposure to 10 µg/L nonylphenol. Vitellogenin was induced (approximately 10-fold) in adult male fish exposed to both 1 and 10 µg/L nonylphenol. In the male progeny of parental fish exposed to 10 µg/L nonylphenol, no effects were observed on plasma vitellogenin or testosterone concentrations, but plasma estradiol concentrations were elevated. In the female progeny of the same parental fish, plasma vitellogenin and plasma testosterone concentrations were elevated, but plasma estradiol concentrations were not different from control levels. Testicular tissue of nonylphenol exposed adult male fish was not affected by nonylphenol. Sex ratios of the offspring of exposed fish were also unaffected by parental exposure to nonylphenol.

As summarized in this section, the ability of nonylphenol to induce estrogenic effects has seldom been reported at concentrations below the freshwater Final Chronic Value of 6.5965 µg/L.

## 7. UNUSED DATA

Data from some studies were not used in this document, as they did not meet the criteria for inclusion as specified in the Guidelines (Stephan et al. 1985). The reader is referred to the Guidelines for further information regarding these criteria.

Results were not used when the test organism is not resident to North America (Gross-Sorokin et al. 2003; Yoshimura 1986). Tsuda et al. (2000) measured tissue concentrations from feral fish, but water concentrations greatly varied.

### **Test Organism or Test Material were Not Adequately Described**

Folmar et al. (1998)	Magliulo et al. (1998)	Weinberger and Rea (1981)
Hansen et al. (1998)	Muller (1980)	
Kopf (1997)	Palmer et al. (1998)	

### **Nonylphenol was a Component of a Mixture or Sediment**

Ahel et al. (1993)	Escher et al. (1999)	Sundaram et al. (1980)
Amato and Wayment (1998)	Hansen et al. (1999)	Turner et al. (1985)
Bettinetti and Provini (2002)	Larsson et al. (1999)	Ward and Boeri (1992)
Fay et al. (2000)	Moore et al. (1987)	
Dwyer et al. (1999a,b)	Purdom et al. (1994)	

### **Studies were Conducted with Ethoxylated Nonylphenols**

Baldwin et al. (1998)	Dorn et al. (1993)	Manzano et al. (1998, 1999)
Braaten et al. (1972)	Maki et al. (1998)	Patoczka and Pulliam 1999

### **Organisms were Dosed by Injection, Gavage or in Artificial Medium**

Arukwe et al. (1997a,b;1998)	Madsen et al. (1997)	Thibaut et al. (1998)
Christiansen et al. (1998a,b,c; 1999)	Nimrod and Benson (1996; 1997)	Weinberger et al. (1987) Yadette et al. (1999)
Coldham et al. (1997, 1998)	Rice et al. (1998)	
Haya et al. (1997)	Spieser et al. (1998)	

### **Experimental Model was Plasma, Enzymes, Receptors, Tissues or Cell Cultures**

Andersen et al. (1999)	Lamche and Burkhardt-Holm (2000)	Routledge and Sumpter (1996, 1997)
Celius et al. (1999)	Levine and Cheney (2000)	Soto et al. (1991, 1992)
Flouriot et al. (1995)	Loomis and Thomas (1999)	White et al. (1994)
Hewitt et al. (1998)	Lutz and Kloas (1999)	
Jobling et al. (1996)	Milligan et al. (1998)	
Jobling and Sumpter (1993)	Petit et al. (1997, 1999)	
Knudsen and Pottinger (1999)		

### **Data were Compiled from Other Source**

Bearden and Schultz (1997, 1998)	Lewis (1991) Liber et al. (1999)	Varma and Patel (1988) Veith and Mekenyan (1993)
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## 8. SUMMARY

### 8.1. Freshwater Data

Acute toxicity of nonylphenol was tested in 18 freshwater species and 2 subspecies from 15 genera (Figure 1 and Table 3). Species Mean Acute Values (SMAV) ranged from 55.72 µg/L for the amphipod *Hyaella azteca* to 774 µg/L for the snail *Physella virgata*. Eleven species of fish were tested and were in the mid-range of sensitivity (SMAVs = 110 to 289.3 µg/L) of tested species. The four most sensitive tested freshwater species were comprised of two invertebrate species and two vertebrate species (Figure 1). No relationships have been demonstrated between nonylphenol toxicity and water quality characteristics such as hardness and pH. The freshwater Final Acute Value is 55.49 µg/L which is equal to the LC50 for the most sensitive tested species, *Hyaella azteca*.

Chronic toxicity of nonylphenol was tested in 5 freshwater species from 5 genera (Figure 3 and Table 3). Two freshwater fish were tested; the rainbow trout, *Oncorhynchus mykiss*, had a chronic value of 7.861 µg/L based on growth, and the fathead minnow, *Pimephales promelas*, had a chronic value of 10.18 µg/L based on survival. Two species of freshwater cladocerans were tested and chronic values ranged from 22.62 to 157.9 µg/L based on reproduction. One species of freshwater midge was tested and its chronic value was 61.82 µg/L based on survival.

Data were available to calculate a Final Acute-Chronic Ratio (FACR) for a freshwater cladoceran, *Daphnia magna*, saltwater mysid, *Americamysis bahia*, and rainbow trout, *Oncorhynchus mykiss*. The Final Acute-Chronic Ratio for nonylphenol was the ACR for *A. bahia* because SMARs increased with increasing SMAV and the SMAV for *A. bahia* is closest to the freshwater and saltwater FAV. The FACR for nonylphenol is 8.412.

### 8.2. Saltwater Data

Acute toxicity of nonylphenol was tested in 11 saltwater species from 11 genera (Figure 2 and Table 3). Species Mean Acute Values (SMAV) ranged from 17 µg/L for the winter flounder, *Pleuronectes americanus*, to 209.8 µg/L for the sheepshead minnow, *Cyprinodon variegatus*. These two fish species were the only fish were tested. Nine different species of



invertebrates were tested. The four most sensitive tested saltwater species were comprised of three invertebrate species and one fish species (Figure 1). No relationships have been demonstrated between nonylphenol toxicity and water quality characteristics such as hardness and pH. The saltwater Final Acute Value is 13.93 µg/L.

Chronic toxicity of nonylphenol was tested on one saltwater species (Figure 3 and Table 3). The saltwater species tested was the mysid, *Americamysis bahia*, which was also the most sensitive of all species tested, both freshwater and saltwater. Two tests were available for *A. bahia*, with chronic values of 5.112 µg/L based on reduced growth and 12.02 µg/L based on a reproductive endpoint.

Data were available to calculate a Final Acute-Chronic Ratio (FACR) for a freshwater cladoceran, *Daphnia magna*, saltwater mysid, *Americamysis bahia*, and rainbow trout, *Oncorhynchus mykiss*. The Final Acute-Chronic Ratio for nonylphenol was the ACR for *A. bahia* because SMARs increased with increasing SMAV and the SMAV for *A. bahia* is closest to both the freshwater and saltwater FAV. The FACR for nonylphenol is 8.412.

### **8.3. Plant Data**

Nonylphenol toxicity data for 2 species of aquatic plants, one freshwater alga and one saltwater diatom, were available. Algae were as sensitive as animals, showing effect concentrations that ranged from 27 µg/L for the freshwater alga to 410 µg/L for the saltwater diatom. Based on the vegetative growth endpoint in saltwater planktonic diatom *Skeletonema costatum*, the Final Plant Value for nonylphenol is 27 µg/L.

### **8.4. Bioaccumulation Data**

Nonylphenol bioaccumulation in aquatic organisms is less than would be predicted from the log  $K_{ow}$  of nonylphenol. Nonylphenol is metabolized in animals which may account for the lower than expected BCFs. In freshwater fish, lipid-normalized BCFs ranged from 39 to 209. Bioaccumulation in saltwater organisms is apparently greater, with lipid-normalized BCFs 79 to 2,168.

## **9. NATIONAL CRITERIA**

### **9.1. Freshwater**

The procedures described in the “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses” (Stephan et al. 1985) indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the one-hour average concentration of nonylphenol does not exceed 28 µg/L more than once every three years on the average and if the four-day average concentration of nonylphenol does not exceed 6.6 µg/L more than once every three years on the average.

### **9.2. Saltwater**

The procedures described in the “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses” (Stephan et al. 1985) indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the one-hour average concentration of nonylphenol does not exceed 7.0 µg/L more than once every three years on the average and if the four-day average concentration of nonylphenol does not exceed 1.7 µg/L more than once every three years on the average.

## 10. 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 or tribal 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 and tribes 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 1994, 1987). In each standard a state or tribe may adopt the national criterion, if one exists, or, if adequately justified, a site-specific criterion (if the site is an entire state, the site-specific criterion is also a state-specific criterion).

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 different 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 into state or tribal water quality standards, for developing water quality-based permit limits 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 also available (U.S. EPA 1987, 1991).

**Figure 1. Summary of Ranked Nonylphenol GMAVs**

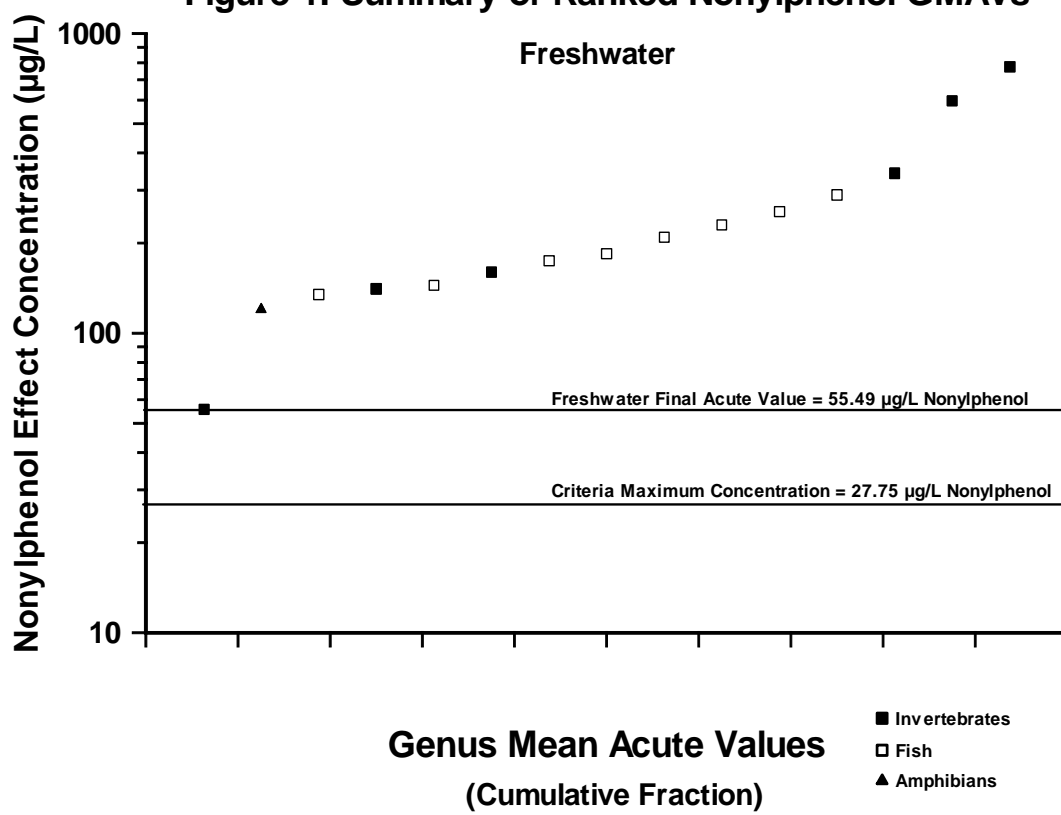


Figure 2. Summary of Ranked Nonylphenol GMAVs

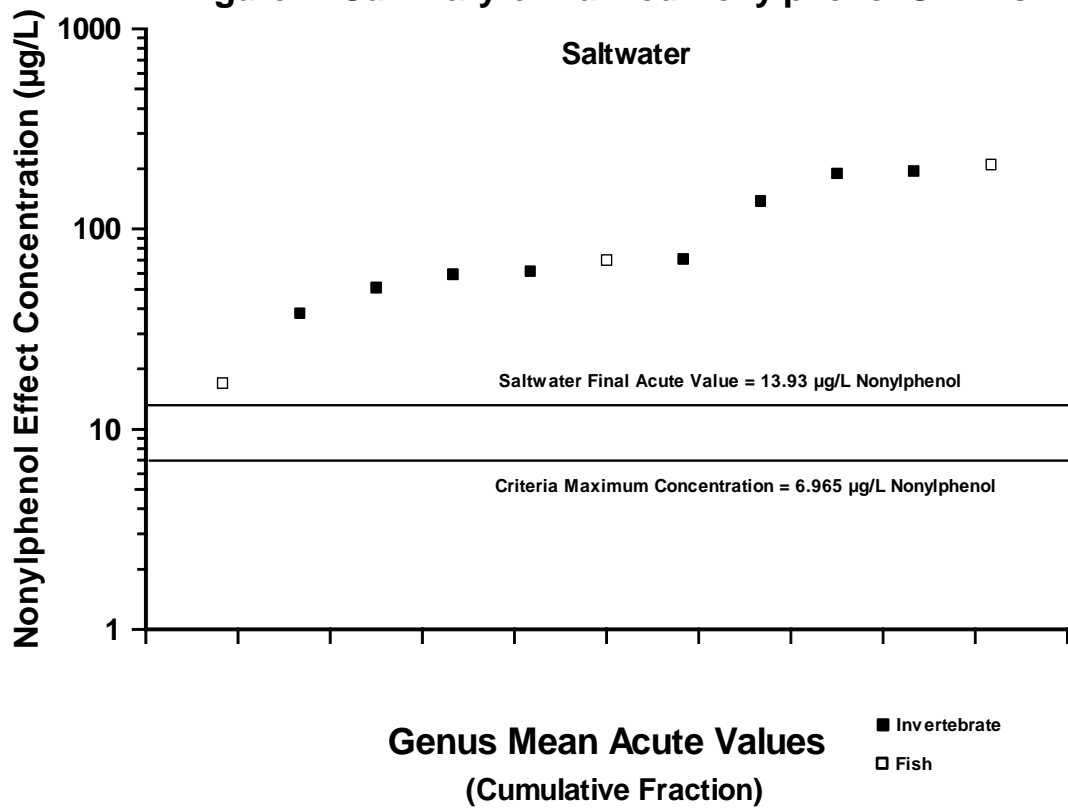
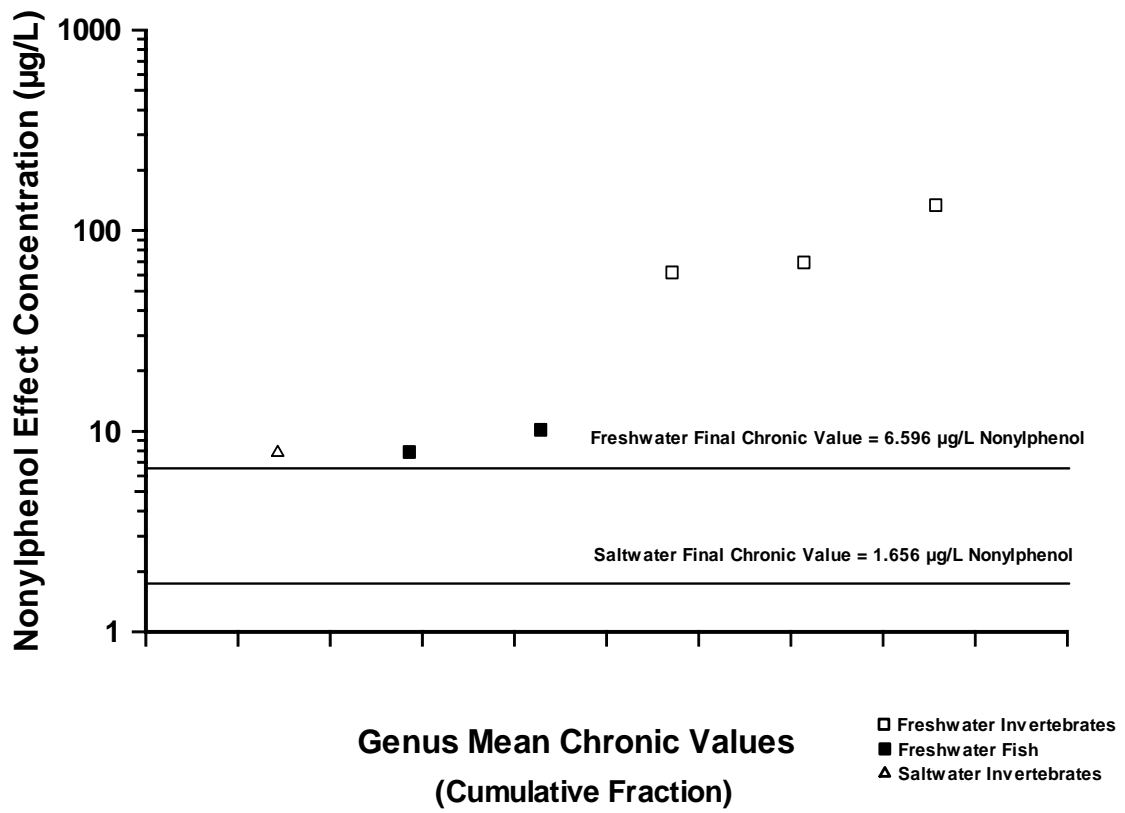


Figure 3. Chronic Toxicity of Nonylphenol to Aquatic Animals



**Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals**

<u>Species</u>	<u>Method<sup>a</sup></u>	<u>Chemical</u>	<u>pH</u>	<u>LC<sub>50</sub> or EC<sub>50</sub> (<math>\mu\text{g/L}</math>)</u>	<u>Species Mean Acute Value<sup>b</sup> (<math>\mu\text{g/L}</math>)</u>	<u>Reference</u>
<b><u>FRESHWATER SPECIES</u></b>						
Annelid (adult), <i>Lumbriculus variegatus</i>	F,M	>90%	6.75	<b><u>342</u></b>	342	Brooke 1993a
Snail (adult), <i>Physella virgata</i>	F,M	>90%	7.89	<b><u>774</u></b>	774	Brooke 1993a
Cladoceran (<24-hr old), <i>Daphnia magna</i>	R,M	>90%	7.87	<b><u>104</u></b>	-	Brooke 1993a
Cladoceran (<24-hr old), <i>Daphnia magna</i>	S,M	91.8%	8.25	<b><u>190</u></b>	140.6	Comber et al. 1993
Midge (2nd instar), <i>Chironomus tentans</i>	F,M	>95%	8.0-8.4	<b><u>160</u></b>	160	England and Bussard 1995
Dragonfly (nymph), <i>Ophiogomphus</i> sp.	F,M	>90%	8.06	<b><u>596</u></b>	596	Brooke 1993a
Amphipod, (juvenile, 2mm TL), <i>Hyalella azteca</i>	F,M	>90%	7.80	<b><u>20.7</u></b>	-	Brooke 1993a
Amphipod (juvenile, 2-3mm TL), <i>Hyalella azteca</i>	F,M	>95%	7.9-8.7	<b><u>150</u></b>	55.72	England and Bussard 1995
Rainbow trout (0.67 $\pm$ 0.35 g), <i>Oncorhynchus mykiss</i>	S,U	85%	7.8-7.9	190	-	Dwyer et al. 1995
Rainbow trout (1.25 $\pm$ 0.57 g), <i>Oncorhynchus mykiss</i>	S,U	85%	7.5-7.7	260	-	Dwyer et al. 1995
Rainbow trout (0.27 $\pm$ 0.07 g), <i>Oncorhynchus mykiss</i>	S,U	85%	7.9	140	-	Dwyer et al. 1995
Rainbow trout (1.09 $\pm$ 0.38 g), <i>Oncorhynchus mykiss</i>	S,U	85%	7.7-7.9	270	-	Dwyer et al. 1995

**Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals**

<u>Species</u>	<u>Method<sup>a</sup></u>	<u>Chemical</u>	<u>pH</u>	<u>LC<sub>50</sub> or EC<sub>50</sub> (<math>\mu\text{g/L}</math>)</u>	<u>Species Mean Acute Value<sup>b</sup> (<math>\mu\text{g/L}</math>)</u>	<u>Reference</u>
Rainbow trout (0.48 $\pm$ 0.08 g), <i>Oncorhynchus mykiss</i>	S,U	85%	7.5-7.9	160	-	Dwyer et al. 1995
Rainbow trout (0.50 $\pm$ 0.21 g), <i>Oncorhynchus mykiss</i>	S,U	85%	6.5-7.9	180	-	Dwyer et al. 1995
Rainbow trout (45 d), <i>Oncorhynchus mykiss</i>	F,M	>90%	6.72	<b><u>221</u></b>	221	Brooke 1993a
Apache trout (0.85 $\pm$ 0.49 g), <i>Oncorhynchus apache</i>	S,U	85%	7.8-7.9	<b><u>180</u></b>	-	Dwyer et al. 1995
Apache trout (0.38 $\pm$ 0.18 g), <i>Oncorhynchus apache</i>	S,U	85%	7.3-7.7	<b><u>160</u></b>	169.7	Dwyer et al. 1995
Greenback cutthroat trout (0.31 $\pm$ 0.17 g), <i>Oncorhynchus clarki</i> <i>stomais</i>	S,U	85%	7.5-7.6	<b><u>150</u></b>	-	Dwyer et al. 1995
Lahontan cutthroat trout (0.34 $\pm$ 0.08 g), <i>Oncorhynchus clarki</i> <i>henshawi</i>	S,U	85%	7.9	<b><u>140</u></b>	-	Dwyer et al. 1995
Lahontan cutthroat trout (0.57 $\pm$ 0.23 g), <i>Oncorhynchus clarki</i> <i>henshawi</i>	S,U	85%	7.6-7.7	<b><u>220</u></b>	166.6	Dwyer et al. 1995
Fathead minnow (0.32 $\pm$ 0.16 g), <i>Pimephales promelas</i>	S,U	85%	7.7-8.1	210	-	Dwyer et al. 1995
Fathead minnow (0.56 $\pm$ 0.19 g), <i>Pimephales promelas</i>	S,U	85%	7.8-8.1	360	-	Dwyer et al. 1995
Fathead minnow (0.45 $\pm$ 0.35 g), <i>Pimephales promelas</i>	S,U	85%	7.6-7.8	310	-	Dwyer et al. 1995



**Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals**

<u>Species</u>	<u>Method<sup>a</sup></u>	<u>Chemical</u>	<u>pH</u>	<u>LC<sub>50</sub> or EC<sub>50</sub> (<math>\mu\text{g/L}</math>)</u>	<u>Species Mean Acute Value<sup>b</sup> (<math>\mu\text{g/L}</math>)</u>	<u>Reference</u>
Fathead minnow (0.40 $\pm$ 0.21 g), <i>Pimephales promelas</i>	S,U	85%	7.5-7.9	330	-	Dwyer et al. 1995
Fathead minnow (0.34 $\pm$ 0.24 g), <i>Pimephales promelas</i>	S,U	85%	7.5-7.6	170	-	Dwyer et al. 1995
Fathead minnow (0.39 $\pm$ 0.14 g), <i>Pimephales promelas</i>	S,U	85%	7.8-8.2	290	-	Dwyer et al. 1995
Fathead minnow (32 d), <i>Pimephales promelas</i>	F,M	99%	7.29	<b><u>140</u></b>	-	Holcombe et al. 1984; Univ. Wisc.- Superior 1985
Fathead minnow (25-35 d), <i>Pimephales promelas</i>	F,M	>90%	7.23	<b><u>128</u></b>	133.9	Brooke 1993a
Bonytail chub (0.29 $\pm$ 0.08 g), <i>Gila elegans</i>	S,U	85%	7.7-7.9	<b><u>270</u></b>	-	Dwyer et al. 1995
Bonytail chub (0.52 $\pm$ 0.09 g), <i>Gila elegans</i>	S,U	85%	7.4-7.6	<b><u>310</u></b>	289.3	Dwyer et al. 1995
Colorado squawfish (0.32 $\pm$ 0.05 g), <i>Ptychocheilus lucius</i>	S,U	85%	8.1-8.2	<b><u>240</u></b>	-	Dwyer et al. 1995
Colorado squawfish (0.34 $\pm$ 0.05 g), <i>Ptychocheilus lucius</i>	S,U	85%	7.8-8.0	<b><u>270</u></b>	254.6	Dwyer et al. 1995
Razorback sucker (0.31 $\pm$ 0.04 g), <i>Xyrauchen texanus</i>	S,U	85%	7.8-8.1	<b><u>160</u></b>	-	Dwyer et al. 1995
Razorback sucker (0.32 $\pm$ 0.07 g), <i>Xyrauchen texanus</i>	S,U	85%	7.9-8.0	<b><u>190</u></b>	174.4	Dwyer et al. 1995
Gila topminnow (0.219 g, 27.2 mm), <i>Poeciliopsis occidentalis</i>	S,U	85%	8.0	<b><u>230</u></b>	230	Dwyer et al. 1999a

**Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals**

<u>Species</u>	<u>Method<sup>a</sup></u>	<u>Chemical</u>	<u>pH</u>	<u>LC<sub>50</sub> or EC<sub>50</sub> (<math>\mu</math>g/L)</u>	<u>Species Mean Acute Value<sup>b</sup> (<math>\mu</math>g/L)</u>	<u>Reference</u>
Fountain darter (0.062 g, 20.2 mm), <i>Etheostoma rubrum</i>	S,U	85%	8.0-8.1	<u>110</u>	110	Dwyer et al. 1999a
Greenthroat darter (0.133 g, 22.6 mm), <i>Etheostoma lepidum</i>	S,U	85%	8.0-8.2	<u>190</u>	190	Dwyer et al. 1999a
Bluegill (juvenile), <i>Lepomis macrochirus</i>	F,M	>90%	7.61	<u>209</u>	209	Brooke 1993a
Boreal toad (0.012 g, 9.6 mm), <i>Bufo boreas</i>	S,U	85%	7.9-8.0	<u>120</u>	120	Dwyer et al. 1999a
<b><u>SALTWATER SPECIES</u></b>						
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), <i>Acartia tonsa</i>	S,U	-	-	<u>190</u>	190	Kusk and Wollenberger 1999
Mysid (<24-hr old), <i>Americamysis bahia</i>	F,M	>95%	7.3-8.2	<u>43</u>	-	Ward and Boeri 1990a
Mysid (<24-hr old), <i>Americamysis bahia</i>	F,M	90%	7.8-8.2	<u>60.6</u>	51.05	Lussier et al. 2000
Amphipod (adult), <i>Leptocheirus plumulosus</i>	F,M	90%	7.8-8.2	<u>61.6</u>	61.6	Lussier et al. 2000
Amphipod (adult), <i>Eohaustorius estuarius</i>	S,U	-	-	<u>138</u>	138	Hecht and Boese 2002
Grass shrimp (48-hr old), <i>Palaemonetes vulgaris</i>	F,M	90%	7.8-8.2	<u>59.4</u>	59.4	Lussier et al. 2000
American lobster (1st stage), <i>Homarus americanus</i>	R,U	90%	7.8-8.2	<u>71</u>	71	Lussier et al. 2000
Mud crab (4th and 5th stages), <i>Dyspanopeus sayii</i>	F,M	90%	7.8-8.2	<u>&gt;195</u>	>195	Lussier et al. 2000

**Table 1. Acute Toxicity of Nonylphenol to Aquatic Animals**

<b>Species</b>	<b>Method<sup>a</sup></b>	<b>Chemical</b>	<b>pH</b>	<b>LC<sub>50</sub> or EC<sub>50</sub> (<math>\mu\text{g/L}</math>)</b>	<b>Species Mean Acute Value<sup>b</sup> (<math>\mu\text{g/L}</math>)</b>	<b>Reference</b>
Winter flounder (48-hr-old), <i>Pleuronectes americanus</i>	S,M	90%	7.8-8.2	<u>17</u>	17	Lussier et al. 2000
Sheepshead minnow (juvenile), <i>Cyprinodon variegatus</i>	F,M	>95%	7.4-8.1	<u>310</u>	-	Ward and Boeri 1990b
Sheepshead minnow (juvenile), <i>Cyprinodon variegatus</i>	F,M	90%	7.8-8.2	<u>142</u>	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>a</sup> S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.

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

**Table 2. Chronic Toxicity of Nonylphenol to Aquatic Animals**

<u>Species</u>	<u>Test</u> <sup>a</sup>	<u>Chemical</u>	<u>pH</u>	<u>Chronic Limits (µg/L)</u> <sup>b</sup>	<u>Chronic Value (µg/L)</u>	<u>Reference</u>
Cladoceran, <i>Ceriodaphnia dubia</i>	LC	>95%	8.3-8.6	88.7-202	133.9	England 1995
Cladoceran, <i>Daphnia magna</i>	LC	93.1	8.04	10.7-47.8	22.62	Fliedner 1993
Cladoceran, <i>Daphnia magna</i>	LC	>90%	8.46	116-215	157.9	Brooke 1993a
Cladoceran, <i>Daphnia magna</i>	LC	91.8%	8.25	24-39	30.59	Comber et al. 1993
Midge, <i>Chironomus tentans</i>	LC	95%	7.73	42-91	61.82	Kahl et al. 1997
Rainbow trout, <i>Oncorhynchus mykiss</i>	ELS	>90%	6.97	6.0-10.3	7.861	Brooke 1993a
Fathead minnow, <i>Pimephales promelas</i>	ELS	>95%	7.1-8.2	7.4-14	10.18	Ward and Boeri 1991c
<b><u>SALTWATER SPECIES</u></b>						
Mysid, <i>Americamysis bahia</i>	LC	>95%	7.4-8.3	3.9-6.7	5.112	Ward and Boeri 1991b
Mysid, <i>Americamysis bahia</i>	LC	-	-	9.46-15.28	12.02	Kuhn et al. 2001

<sup>a</sup> LC = life-cycle or partial life-cycle; ELS = early life-stage.

<sup>b</sup> Based upon measured concentrations of nonylphenol.

**Table 2. Acute-Chronic Ratios**

<b>Acute-Chronic Ratios</b>					
<b><u>Species</u></b>	<b><u>pH</u></b>	<b><u>Acute Value</u></b> <b><u>(µg/L)</u></b>	<b><u>Chronic Value</u></b> <b><u>(µg/L)</u></b>	<b><u>Ratio</u></b>	<b><u>Reference</u></b>
Cladoceran, <i>Daphnia magna</i>	7.87-8.46	104	157.9	0.6586	Brooke 1993a
Cladoceran, <i>Daphnia magna</i>	8.25	190	30.59	6.211	Comber et al. 1993
Mysid, <i>Americamysis bahia</i>	7.3-8.3	43	5.112	8.412	Ward and Boeri 1990a, 1991b
Rainbow trout, <i>Oncorhynchus mykiss</i>	6.72-6.97	221	7.861	28.11	Brooke 1993a

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

<u>Rank<sup>a</sup></u>	<u>Genus Mean Acute Value (µg/L)</u>	<u>Species</u>	<u>Species Mean Acute Value (µg/L)<sup>b</sup></u>	<u>Species Mean Acute-Chronic Ratio<sup>c</sup></u>
<b><u>FRESHWATER SPECIES</u></b>				
15	774	Snail, <i>Physella virgata</i>	774	-
14	596	Dragonfly, <i>Ophiogomphus sp.</i>	596	-
13	342	Annelid, <i>Lumbriculus variegatus</i>	342	-
12	289.3	Bonytail chub, <i>Gila elegans</i>	289.3	-
11	254.6	Colorado squawfish, <i>Ptychocheilus lucius</i>	254.6	-
10	230	Gila topminnow, <i>Poeciliopsis occidentalis</i>	230	-
9	209	Bluegill, <i>Lepomis macrochirus</i>	209	-
8	184.2	Rainbow trout, <i>Oncorhynchus mykiss</i>	221	28.11
		Apache trout, <i>Oncorhynchus apache</i>	169.7	-
		Lahontan cutthroat trout, <i>Oncorhynchus clarki henshawi</i> , and	166.6	-
		Greenback cutthroat trout, <i>Oncorhynchus clarki stomais</i>	-	-
7	174.4	Razorback sucker, <i>Xyrauchen texanus</i>	174.4	-
6	160	Midge, <i>Chironomus tentans</i>	160	-
5	144.6	Greenthroat darter, <i>Etheostoma lepidum</i>	190	-
		Fountain darter, <i>Etheostoma rubrum</i>	110	-
4	140.6	Cladoceran, <i>Daphnia magna</i>	140.6	2.023

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

<b>Rank<sup>a</sup></b>	<b>Genus Mean Acute Value (µg/L)</b>	<b>Species</b>	<b>Species Mean Acute Value (µg/L)<sup>b</sup></b>	<b>Species Mean Acute-Chronic Ratio<sup>c</sup></b>
3	133.9	Fathead minnow, <i>Pimephales promelas</i>	133.9	-
2	120	Boreal toad, <i>Bufo boreas</i>	120	-
1	55.72	Amphipod, <i>Hyalella azteca</i>	55.72	-
<b><u>SALTWATER SPECIES</u></b>				
11	209.8	Sheepshead minnow, <i>Cyprinodon variegatus</i>	209.8	-
10	>195	Mud crab, <i>Dyspanopeus sayii</i>	>195	-
9	190	Copepod, <i>Acartia tonsa</i>	190	-
8	138	Amphipod, <i>Eohaustorius estuarius</i>	138	-
7	71	American lobster, <i>Homarus americanus</i>	71	-
6	70	Inland silversides, <i>Menidia beryllina</i>	70	-
5	61.6	Amphipod, <i>Leptocheirus plumulosus</i>	61.6	-
4	59.4	Grass shrimp, <i>Palaemonetes vulgaris</i>	59.4	-
3	51.05	Mysid, <i>Americamysis bahia</i>	51.05	8.412
2	37.9	Coot clam, <i>Mulinia lateralis</i>	37.9	-
1	17	Winter flounder, <i>Pleuronectes americanus</i>	17	-

<sup>a</sup> Ranked from the most resistant to the most sensitive based on Genus Mean Acute Value.

<sup>b</sup> From Table 1.

<sup>c</sup> From Table 2.

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

**Freshwater**

Final Acute Value = 55.49 µg/L

Criterion Maximum Concentration =  $55.49 \div 2 = 27.75$  µg/L

Final Acute-Chronic Ratio = 8.412 (see text)

Final Chronic Value =  $55.49 \text{ µg/L} \div 8.412 = 6.5965$  µg/L

**Saltwater**

Final Acute Value = 13.93 µg/L

Criterion Maximum Concentration =  $13.93 \div 2 = 6.965$  µg/L

Final Acute-Chronic Ratio = 8.412 (see text)

Final Chronic Value =  $13.93 \text{ µg/L} \div 8.412 = 1.6560$  µg/L



**Table 4. Toxicity of Nonylphenol to Aquatic Plants**

<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration (days)</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
<b>FRESHWATER SPECIES</b>						
Green algae, <i>Selenastrum capricornutum</i>	>95%	7.8	4	EC50, number of cells	410	Ward and Boeri 1990a
<b><u>SALTWATER SPECIES</u></b>						
Diatom, <i>Skeletonema costatum</i>	>95%	30 <sup>a</sup>	4	EC50, number of cells	27	Ward and Boeri 1990d

<sup>a</sup>Salinity (g/kg).

**Table 5. Bioaccumulation of Nonylphenol by Aquatic Organisms**

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

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

**Table 5. Bioaccumulation of Nonylphenol by Aquatic Organisms**

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

**Table 5. Bioaccumulation of Nonylphenol by Aquatic Organisms**

<u>Species</u>	<u>Chemical</u>	<u>Water Conc. (<math>\mu\text{g/L}</math>)<sup>a</sup></u>	<u>pH</u>	<u>Duration (days)</u>	<u>Tissue</u>	<u>Percent Lipids</u>	<u>BCF or BAF<sup>b</sup></u>	<u>Normalized BCF or BAF<sup>c</sup></u>	<u>Reference</u>
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<sup>a</sup>Measured concentration of nonylphenol.

<sup>b</sup>Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are based on measured concentrations of nonylphenol in water and in tissue.

<sup>c</sup>When possible, the factors were normalized to 1% lipid by dividing the BCFs and BAFs by the percent lipid measured in the test organism.

<sup>d</sup>Non-resident species.

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

<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
<b><u>FRESHWATER SPECIES</u></b>						
Phytoplankton and Periphyton	>98%	8.8 - 10.6	6 wk	Dominant species changed	29 - 120	Hense et al. 2003
Green alga, <i>Chlamydomonas reinhardtii</i>	-	-	24 days	100% algistatic	6,250	Weinberger and Greenhalgh 1984
Floating moss, <i>Salvinia molesta</i>	-	-	9 days	Reduced frond production	2,500	Prasad 1986
Duckweed, <i>Lemna minor</i>	-	5.6	96 hr	IC50	5,500	Weinberger and Iyengar 1983
Duckweed, <i>Lemna minor</i>	-	-	4 days	Reduced frond production	125	Prasad 1986
Ciliate protozoan, <i>Tetrahymena pyriformis</i>	-	-	24 hr	EC50	460	Yoshioka 1985
Ciliate protozoan, <i>Tetrahymena pyriformis</i>	-	7.40	40 hr	Reduced population growth 50%	747	Schultz 1997
Rotifer (4 to 6 hr-old female) <i>Brachionus calyciflorus</i>	Technical	7.5	96 hr	Sexual reproduction reduced	50	Preston et al. 2000
Clam (15 g), <i>Anodonta cataractae</i>	-	-	144 hr (fed)	LC50	5,000	McLeese et al. 1980b
Zooplankton	96.4%	7.5 - 8.2	20 days	NOEC LOEC	23 76	O'Halloran et al. 1999
Zooplankton	>98%	8.8 - 10.4	6 wk	NOEC	19 - 44	Severin et al. 2003
Benthic macro-invertebrates	96.4%	7.5 - 8.2	20 days	NOEC LOEC	23 76	Schmude et al. 1999
Cladoceran (<24-hr old), <i>Daphnia magna</i>	-	8.0	21 days	NOEC LOEC (reduced fecundity)	50 100	Baldwin et al. 1997

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

<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Cladoceran (<24-hr old and adults), <i>Daphnia magna</i>	~85%	7.8 - 8.4	96 hr (fed)	MATC (young) MATC (adults)	302 136	Gerritsen et al. 1998
Cladoceran (<24-hr old), <i>Daphnia magna</i>	~85%	7.7±0.02	21 days	No sex ratio change (high food rate) Increased ratio of males (low food rate)	25 25	Baer and Owens 1999
Cladoceran (<24-hr old), <i>Daphnia magna</i>	Technical	-	21 days	50% adult mortality NOEC (deformed offspring)	200.5 44	LeBlanc et al. 2000
Cladoceran (<24-hr old), <i>Daphnia magna</i>	~85%	-	48 hr	EC50	234 272 337	Zang et al. 2003
Cladoceran (<24-hr old), <i>Daphnia magna</i>	~85%	-	35 day	LOEC	>50	Zang et al. 2003
Cladoceran (<36-hr old), <i>Daphnia galeata mendotae</i>	-	-	30 day	NOEC LOEC (deformed offspring)	10 50	Shurin and Dodson 1997
Cladoceran (>48-hr old), <i>Daphnia pulex</i>	Practical grade	-	48 hr	LC50	140	Ernst et al. 1980
Cladoceran (>48-hr old), <i>Daphnia pulex</i>	Practical grade	-	48 hr	LC50	176	Ernst et al. 1980
Cladoceran (>48-hr old), <i>Daphnia pulex</i>	Practical grade	-	48 hr	LC50	190	Ernst et al. 1980
Cladoceran (<24-hr old), <i>Ceriodaphnia dubia</i>	>95%	8.3-8.6	48 hr	LC50 (fed)	276	England 1995

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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Cladoceran (<24-hr old), <i>Ceriodaphnia dubia</i>	>95%	8.3-8.6	7 days	LC50 (fed)	225	England 1995
Midge (2nd instar), <i>Chironomus tentans</i>	>95%	8.2	14 days	LC50 EC50	119 95	England and Bussard 1993
Sea lamprey (larva), <i>Petromyzon marinus</i>	-	7.5-8.2	14 hr	LT100	5,000	Applegate et al. 1957
Brook trout (juvenile), <i>Salvelinus fontinalis</i>	-	-	96 hr	LC50	145	Holmes and Kingsbury 1980
Lake trout (juvenile), <i>Salvelinus naymaysush</i>	-	-	35 days	LC50 (fed)	>40	Holmes and Kingsbury 1980
Brown trout (fingerling), <i>Salmo trutta</i>	-	7.0	2 hr	LT100	5,000	Wood 1953
Atlantic salmon (4 g), <i>Salmo salar</i>	-	-	96 hr (fed)	LC50	900	McLeese et al. 1980b
Atlantic salmon (48.3 ± 2.6 mm TL), <i>Salmo salar</i>	-	-	30 days	No change in plasma vitellogenin or gill NaK ATPase activity or plasma Cl <sup>-</sup> and Na <sup>+</sup>	20	Moore et al. 2003
Chinook salmon (juvenile), <i>Oncorhynchus tshawytscha</i>	-	7.2	3 hr	LT100	10,000	MacPhee and Ruelle 1969
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	-	7.5-8.2	4 hr	LT100	5,000	Applegate et al. 1957



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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	Practical grade	-	96 hr	LC50	920	Ernst et al. 1980
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	Practical grade	-	96 hr	LC50	560	Ernst et al. 1980
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	-	-	96 hr	LC50	230	Holmes and Kingsbury 1980
Rainbow trout (adult males), <i>Oncorhynchus mykiss</i>	-	-	3 wk	Increased vitellogenin production	20.3	Jobling et al. 1996
Rainbow trout (adult males), <i>Oncorhynchus mykiss</i>	-	-	3 wk	Increased vitellogenin production	54.3	Jobling et al. 1996
Rainbow trout (50 - 200 g), <i>Oncorhynchus mykiss</i>	-	-	72 hr	LC50	193.65	Lech et al. 1996
Rainbow trout (50 - 200 g), <i>Oncorhynchus mykiss</i>	-	-	72 hr	Increased vitellogenin mRNA	14.14	Lech et al. 1996
Rainbow trout, (40 - 60 g), <i>Oncorhynchus mykiss</i>	>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), <i>Oncorhynchus mykiss</i>	>99%		2 – 5 hr	Eviscerated carcass BAF = 24.21	18	Lewis and Lech 1996
Rainbow trout, (40 - 60 g), <i>Oncorhynchus mykiss</i>	>99%		2 – 5 hr	Viscera BAF = 98.2	18	Lewis and Lech 1996

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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	-	-	4 hr	Vitellogenin mRNA production	10	Ren et al. 1996a
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	-	-	72 hr	Vitellogenin mRNA production	100	Ren et al. 1996a
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	-	6.5	22 days	Reduced growth at 108 days	50	Ashfield et al. 1998
			35 days	Reduced growth at 466 days	30	
Rainbow trout (juvenile), <i>Oncorhynchus mykiss</i>	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	220	Jones et al. 1998
Rainbow trout (35-50 g, immature), <i>Oncorhynchus mykiss</i>	-	8.0 – 8.4	21 days	Increased vitellogenin in blood plasma	50	Tremblay and Van Der Kraak 1998
Rainbow trout (adult males), <i>Oncorhynchus mykiss</i>	-	-	3 wk	BCF = 116 BCF = 88	63 81	Blackburn et al. 1999
Rainbow trout (103-168 g, juvenile) <i>Oncorhynchus mykiss</i>	99%	-	9 days	No vitellogenin induction	109	Pedersen et al. 1999
Rainbow trout (adult males), <i>Oncorhynchus mykiss</i>	Technical	-	10 days per month for 4 months	Epidermal mucous cell granulation	1	Burkhardt-Holm et al. 2000

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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Rainbow trout (598 g; juvenile females), <i>Oncorhynchus mykiss</i>	99%	-	18 wk	Reduced GSI; Reduced HSI; Induced vitellogenin; Lowered plasma estradiol; Lowered plasma FSH	85.6 85.6 8.3 85.6 8.3	Harris et al. 2001
Rainbow trout (1667±201.6 g; F <sub>0</sub> 3 yr-old adults), <i>Oncorhynchus mykiss</i>	98%	7.6	4 months (exposed 10 days/month)	Reduced embryo survival; Reduced hatch; F <sub>0</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
Rainbow trout (6-mo-old), <i>Oncorhynchus mykiss</i>	-	7.2	4 wk	Liver tissue showed hemorrhage and lymphocyte infiltration	220	Uguz et al. 2003
Lahontan cutthroat trout (juvenile), <i>Oncorhynchus clarki henshawi</i>	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	220	Jones et al. 1998
Apache trout (juvenile), <i>Oncorhynchus mykiss</i>	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	>130	Jones et al. 1998
Northern squawfish (juvenile), <i>Ptychocheilus oregonensis</i>	-	7.2	3 hr	LT100	10,000	MacPhee and Ruelle 1969

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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (<math>\mu\text{g/L}</math>)</u>	<u>Reference</u>
Colorado squawfish (juvenile), <i>Ptychocheilus lucius</i>	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	>220	Jones et al. 1998
Goldfish (juvenile), <i>Carassius auratus</i>	-	7.0	5 hr	LT100	5,000	Wood 1953
Common carp (15.2 $\pm$ 3.8 g juvenile), <i>Cyprinus carpio</i>	Technical (90% 4-NP)	7.6	70 days	Decreased erythrocytes; Increased reticulocytes	10 10	Schwaiger et al. 2000
Common carp (50-150 g mature males), <i>Cyprinus carpio</i>	95%	7.57 $\pm$ 0.03	28-31 days 11 $^{\circ}\text{C}$	BCF = 546.5 No change in 17-estradiol, testosterone, or vitellogenin	5.36	Villeneuve et al. 2002
Fathead minnow (4-wk old), <i>Pimephales promelas</i>	99%	7.62	4 days	LC50 (fed)	138	Brooke 1993b
Fathead minnow (4-wk old), <i>Pimephales promelas</i>	99%	7.60	28 days	BCF = 100.4	193	Brooke 1993b
Fathead minnow, <i>Pimephales promelas</i>	-	-	96 hr	Decreased number of muscarinic cholinergic receptors in brain	>220	Jones et al. 1998
Fathead minnow (mature), <i>Pimephales promelas</i>	>98%	-	42 days	Possible increased number of Sertoli cells in males	1.6	Miles-Richardson et al. 1999
Fathead minnow (mature), <i>Pimephales promelas</i>	>98%	-	42 days	Decreased fecundity	>3.4	Giesy et al. 2000

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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Fathead minnow (mature), <i>Pimephales promelas</i>	>98%	-	42 days	Increased ♂ vitellogenin	>3.4	Giesy et al. 2000
Fathead minnow (mature), <i>Pimephales promelas</i>	>98%	-	42 days	Increased ♂ & ♀ 17β-estradiol	>0.05 (not all test concentrations)	Giesy et al. 2000
Bluegill (juvenile), <i>Lepomis macrochirus</i>	-	7.0	2 hr	LT100	5,000	Wood 1953
Bluegill (juvenile), <i>Lepomis macrochiru</i>	-	7.5-8.2	14 hr	LT100	5,000	Applegate et al. 1957
Bluegill (4-wk old), <i>Lepomis macrochirus</i>	99%	7.79	4 days	LC50 (fed)	135	Brooke 1993b
Bluegill (4-wk old), <i>Lepomis macrochirus</i>	99%	7.55	28 days	BCF=35.31	126	Brooke 1993b
Bluegill (juvenile), <i>Lepomis macrochirus</i>	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), <i>Xiphophorus maculatus</i>	Technical 85%	-	28 days	Reduced GSI	960	Kinnberg et al. 2000
Green Swordtail (adult males), <i>Xiphophorus helleri</i>	Technical	-	96 hr 72 hr	LC50 Vitellogenin induced	206 4	Kwak et al. 2001

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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Green Swordtail (juvenile 30-d-old males), <i>Xiphophorus helleri</i>	Technical	-	60 days	Reduced sword length	0.2	Kwak et al. 2001
African clawed frog (larva), <i>Xenopus laevis</i>	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), <i>Xenopus laevis</i>	-	-	12 wk	Increased female phenotypes	22	Kloas et al. 1999
<b><u>SALTWATER SPECIES</u></b>						
Red alga, <i>Champia parvula</i>	>95%	-	2 days	No effect on sexual reproduction	167	Tagliabue 1993
Barnacle (cypris larva), <i>Balanus amphitrite</i>	-	-	48 h	Reduced cypris settlement	1.0	Billinghurst et al. 1998
Soft-shell clam, <i>Mya arenaria</i>	-	-	360 hr	No mortality	700	McLeese et al. 1980b
Coot clam, <i>Mulinia lateralis</i>	90%	30-31 <sup>a</sup>	24 hr	LC50	50	Lussier et al. 2000
Coot clam, <i>Mulinia lateralis</i>	90%	30-31 <sup>a</sup>	48 hr	LC50	50	Lussier et al. 2000
Coot clam, <i>Mulinia lateralis</i>	90%	30-31 <sup>a</sup>	72 hr	LC50	40	Lussier et al. 2000
Blue mussel, <i>Mytilus edulis</i>	90%	30-31 <sup>a</sup>	96 hr	LC50	3,000	Granmo et al. 1989
Blue mussel, <i>Mytilus edulis</i>	-	32 <sup>a</sup>	360 hr	LC50	500	Granmo et al. 1989
Blue mussel, <i>Mytilus edulis</i>	-	32 <sup>a</sup>	13 days	Reduced byssus strength	56	Granmo et al. 1989
Blue mussel, <i>Mytilus edulis</i>	-	32 <sup>a</sup>	30 days	Reduced byssus strength	56	Granmo et al. 1989

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<u>Species</u>	<u>Chemical</u>	<u>pH</u>	<u>Duration</u>	<u>Effect</u>	<u>Concentration (µg/L)</u>	<u>Reference</u>
Blue mussel, <i>Mytilus edulis</i>	-	32 <sup>a</sup>	30 days	No byssus threads formed	100	Granmo et al. 1989
Blue mussel, <i>Mytilus edulis</i>	-	32 <sup>a</sup>	32 days	Reduction in growth	56	Granmo et al. 1989
Blue mussel, <i>Mytilus edulis</i>	-	32 <sup>a</sup>	24 hr	No effect on fertilization	200	Granmo et al. 1989
Blue mussel, <i>Mytilus edulis</i>	-	-	72 hr	No effect on development	200	Granmo et al. 1989
Blue mussel (40-50 mm length), <i>Mytilus edulis</i>	-	-	50 days	BCF = 350	40	Granmo et al. 1991a,b
Blue mussel, <i>Mytilus edulis galloprovincialis</i>	-	-	2 days	Repelled attachment	22	Etoh et al. 1997
Mysid, <i>Americamysis bahia</i>	90%	30-31 <sup>a</sup>	24 hr	LC50	~114	Lussier et al. 2000
Mysid, <i>Americamysis bahia</i>	90%	30-31 <sup>a</sup>	48 hr	LC50	~82	Lussier et al. 2000
Mysid, <i>Americamysis bahia</i>	90%	30-31 <sup>a</sup>	72 hr	LC50	~66	Lussier et al. 2000
Mysid, <i>Americamysis bahia</i>	90%	30-31 <sup>a</sup>	120 hr	LC50	~60	Lussier et al. 2000
Mysid, <i>Americamysis bahia</i>	90%	30-31 <sup>a</sup>	144 hr	LC50	~60	Lussier et al. 2000
Mysid, <i>Americamysis bahia</i>	90%	30-31 <sup>a</sup>	168 hr	LC50	~60	Lussier et al. 2000
Mysid, <i>Americamysis bahia</i>	>95%	20 <sup>a</sup>	24 hr	LC50	>47	Ward and Boeri 1990a

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Mysid, <i>Americamysis bahia</i>	>95%	20 <sup>a</sup>	48 hr	LC50	>47	Ward and Boeri 1990a
Mysid, <i>Americamysis bahia</i>	>95%	20 <sup>a</sup>	72 hr	LC50	44	Ward and Boeri 1990a
Pacific Oyster (embryo-larva), <i>Crassostrea gigas</i>	-	35 <sup>a</sup>	72 hr	Delayed development to D-shape stage	100	Nice et al. 2000
Copepod (10-12 d), <i>Acartia tonsa</i>	-	18 <sup>a</sup>	48 hr	LC50 synthetic media	360 280	Kusk and Wollenberger 1999
Amphipod, <i>Leptocheirus plumulosus</i>	90%	30-31 <sup>a</sup>	48 hr	LC50	~160	Lussier et al. 2000
Amphipod, <i>Leptocheirus plumulosus</i>	90%	30-31 <sup>a</sup>	72 hr	LC50	~80	Lussier et al. 2000
Amphipod, <i>Leptocheirus plumulosus</i>	90%	30-31 <sup>a</sup>	120 hr	LC50	~50	Lussier et al. 2000
Amphipod, <i>Leptocheirus plumulosus</i>	90%	30-31 <sup>a</sup>	144 hr	LC50	~40	Lussier et al. 2000
Amphipod, <i>Leptocheirus plumulosus</i>	90%	30-31 <sup>a</sup>	168 hr	LC50	~30	Lussier et al. 2000
Grass shrimp, <i>Palaemonetes vulgaris</i>	90%	30-31 <sup>a</sup>	24 hr	LC50	~125	Lussier et al. 2000
Grass shrimp, <i>Palaemonetes vulgaris</i>	90%	30-31 <sup>a</sup>	48 hr	LC50	~60	Lussier et al. 2000
Grass shrimp, <i>Palaemonetes vulgaris</i>	90%	30-31 <sup>a</sup>	72 hr	LC50	~60	Lussier et al. 2000
Grass shrimp, <i>Palaemonetes vulgaris</i>	90%	30-31 <sup>a</sup>	120 hr	LC50	~60	Lussier et al. 2000



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Shrimp, <i>Crangon septemspinoso</i>	>95%	-	96 hr	LC50	300	McLeese et al. 1980b
Shrimp, <i>Crangon septemspinoso</i>	>95%	-	96 hr	LC50	300	McLeese et al. 1980b
Shrimp, <i>Crangon septemspinoso</i>	>95%	-	96 hr	LC50	300	McLeese et al. 1980b
American lobster, <i>Homarus americanus</i>	90%	30-31 <sup>a</sup>	24 hr	LC50	~140	Lussier et al. 2000
American lobster, <i>Homarus americanus</i>	90%	30-31 <sup>a</sup>	48 hr	LC50	~140	Lussier et al. 2000
American lobster, <i>Homarus americanus</i>	90%	30-31 <sup>a</sup>	72 hr	LC50	~140	Lussier et al. 2000
American lobster, <i>Homarus americanus</i>	>95%	-	96 hr	LC50	170	McLeese et al. 1980b
Atlantic salmon, <i>Salmo salar</i>	-	-	96 hr	LC50	190	McLeese et al. 1980b
Atlantic salmon, <i>Salmo salar</i>	-	-	96 hr	LC50	160	McLeese et al. 1980b
Atlantic salmon, <i>Salmo salar</i>	-	-	96 hr	LC50	130	McLeese et al. 1980b
Atlantic salmon, <i>Salmo salar</i>	-	-	96 hr	LC50	900	McLeese et al. 1980b
Sheepshead minnow, <i>Cyprinodon variegatu</i>	90%	30-31 <sup>a</sup>	72 hr	LC50	~150	Lussier et al. 2000
Sheepshead minnow, <i>Cyprinodon variegatu</i>	90%	30-31 <sup>a</sup>	120 hr	LC50	~125	Lussier et al. 2000

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Sheepshead minnow, <i>Cyprinodon variegatu</i>	90%	30-31 <sup>a</sup>	144 hr	LC50	~120	Lussier et al. 2000
Sheepshead minnow, <i>Cyprinodon variegatu</i>	90%	30-31 <sup>a</sup>	168 hr	LC50	~120	Lussier et al. 2000
Sheepshead minnow, <i>Cyprinodon variegatu</i>	>95%	15-17 <sup>a</sup>	24 hr	LC50	>420	Ward and Boeri 1990c
Sheepshead minnow, <i>Cyprinodon variegatu</i>	>95%	15-17 <sup>a</sup>	48 hr	LC50	340	Ward and Boeri 1990c
Sheepshead minnow, <i>Cyprinodon variegatu</i>	>95%	15-17 <sup>a</sup>	72 hr	LC50	320	Ward and Boeri 1990c
Killifish (embryo), <i>Fundulus heteroclitus</i>	85 - 90% (technical)	20 <sup>a</sup>	10 days	100% abnormal development	2,204	Kelly and Di Giulio 2000
Killifish (embryo), <i>Fundulus heteroclitus</i>	85 - 90% (technical)	20 <sup>a</sup>	96 hr	LC50	5,444	Kelly and Di Giulio 2000
Killifish (1-day old larva), <i>Fundulus heteroclitus</i>	85 - 90% (technical)	20 <sup>a</sup>	96 hr	LC50 (fed)	214	Kelly and Di Giulio 2000
Killifish (14-day old larva), <i>Fundulus heteroclitus</i>	85 - 90% (technical)	20 <sup>a</sup>	96 hr	LC50 (fed)	209	Kelly and Di Giulio 2000
Killifish (28-day old larva), <i>Fundulus heteroclitus</i>	85 - 90% (technical)	20 <sup>a</sup>	96 hr	LC50 (fed)	260	Kelly and Di Giulio 2000

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Threespine stickleback <i>Gasterosteus aculeatus</i>	Commercial (para-substituted with branched nonyl chain)	32 <sup>a</sup>	96 hr	LC50	370	Granmo et al. 1991a
Inland silversides, <i>Menidia beryllina</i>	90%	30-31 <sup>a</sup>	24 hr	LC50	~120	Lussier et al. 2000
Inland silversides, <i>Menidia beryllina</i>	90%	30-31 <sup>a</sup>	48 hr	LC50	~100	Lussier et al. 2000
Inland silversides, <i>Menidia beryllina</i>	90%	30-31 <sup>a</sup>	72 hr	LC50	~80	Lussier et al. 2000
Inland silversides, <i>Menidia beryllina</i>	90%	30-31 <sup>a</sup>	120 hr	LC50	~60	Lussier et al. 2000
Inland silversides, <i>Menidia beryllina</i>	90%	30-31 <sup>a</sup>	144 hr	LC50	~60	Lussier et al. 2000
Inland silversides, <i>Menidia beryllina</i>	90%	30-31 <sup>a</sup>	168 hr	LC50	~60	Lussier et al. 2000

<sup>a</sup>Salinity (g/kg).

## 11. REFERENCES

Ahel, M. and W. Giger. 1993. Aqueous solubility of alkylphenols and alkylphenol polyethoxylates. *Chemosphere* 26:1461-1470.

Ahel, M., J. McEvoy and W. Giger. 1993. Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. *Environ. Pollut.* 79:243-248.

Ahel, M., W. Giger and M. Koch. 1994a. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-I. Occurrence and transformation in sewage treatment. *Wat. Res.* 28:1131-1142.

Ahel, M., W. Giger and C. Schaffner. 1994b. Behavior of alkylphenol polyethoxylate surfactants in the aquatic environment-II. Occurrence and transformation in rivers. *Wat. Res.* 28:1143-1152.

Ahel, M., F.E. Scully, Jr., J. Hoigne and W. Giger. 1994c. Photochemical degradation of nonylphenol and nonylphenol polyethoxylates in natural waters. *Chemosphere* 28:1361-1368.

Ahel, M., C. Schaffner and W. Giger. 1996. Behaviour of alkylphenol polyethoxylates surfactants in the aquatic environment-III. Occurrence and elimination of their persistent metabolites during infiltration of river water to groundwater. *Wat. Res.* 30:37-46.

Ahel, M., E. Molnar, S. Ibric and W. Giger. 2000. Estrogenic metabolites of alkylphenol polyethoxylates in secondary sewage effluents and rivers. *Wat. Sci. Tech.* 42:15-22.

Allen, Y., A.P. Scott, P. Matthiessen, S. Haworth, J.E. Thain and S. Feist. 1999. Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*. *Environ. Toxicol. Chem.* 18:1791-1800.

Amato, J.R. and D.D. Wayment. 1998. Surfactant toxicity identification with a municipal wastewater. In: *Environmental Toxicology and Risk Assessment*. Little, E.S., A.J. DeLoney and B.M. Greenberg, Eds. ASTM STP 1333, Vol. 7, American Society for Testing and Materials, Philadelphia, PA. pp. 272-283.

Andersen, H.R., A. Andersson, S.F. Arnold, H. Autrup, M. Barfoed, N.A. Beresford, P. Bjerregaard, L.B. Christiansen, B. Gissel, R. Hummel, E.B. Jorgensen, B. Korsgaard, R. Le Guevel, H. Leffers, J. McLachlan, A. Moller, J.B. Nielsen, N. Olea, A. Oles-Karasko, F. Pakdel, K.L. Pedersen, P. Perez, N.E. Skakkeboek, C. Sonnenschein, A.M. Soto, J.P. Sumpter, S.M. Thorpe and P. Grandjean. 1999. Comparison of short-term

estrogenicity tests for identification of hormone-disrupting chemicals. *Environ. Health Perspect.* 107(Suppl. 1):89-108.

Applegate, V.C., J.H. Howell, A.E. Hall, Jr. and M.A. Smith. 1957. Toxicity of 4,346 chemicals to larval lampreys and fishes. Special Scientific Report-Fisheries, No. 207, U.S. Dept. Interior, Fish and Wildlife Service, Washington, DC. 157 pp.

Arukwe, A., L. Forlin and A. Goksoyr. 1997a. Xenobiotic and steroid biotransformation enzymes in Atlantic salmon (*Salmo salar*) liver treated with an estrogenic compound, 4-nonylphenol. *Environ. Toxicol. Chem.* 16:2576-2583.

Arukwe, A., F.R. Knudsen and A. Goksoyr. 1997b. Fish zona radiata (eggshell) protein: A sensitive biomarker for environmental estrogens. *Environ. Health Perspect.* 105:418-422.

Arukwe, A., T. Celius, B.T. Walther and A. Goksoyr. 1998. Plasma levels of vitellogenin and eggshell *zona radiata* proteins in 4-nonylphenol and *o,p'* nonylphenol treated juvenile Atlantic salmon (*Salmo salar*). *Mar. Environ. Res.* 46:133-136.

Arukwe, A., R. Thibaut, K. Ingebrigtsen, T. Celius, A. Goksoyr and J-P. Cravedi. 2000. *In vivo* and *in vitro* metabolism and organ distribution of nonylphenol in Atlantic salmon (*Salmo salar*). *Aquat. Toxicol.* 49:289-304.

Ashfield, L.A., T.G. Pottinger and J.P. Sumpter. 1998. Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. *Environ. Toxicol. Chem.* 17:679-686.

Baer, K.N. and K.D. Owens. 1999. Evaluation of selected endocrine disrupting compounds on sex determination in *Daphnia magna* using reduced photoperiod and different feeding rates. *Bull. Environ. Contam. Toxicol.* 62:214-221.

Baldwin, W.S., S.E. Graham, D. Shea and G.A. LeBlanc. 1997. Metabolic androgenization of female *Daphnia magna* by the xenoestrogen 4-nonylphenol. *Environ. Toxicol. Chem.* 16:1905-1911.

Baldwin, W.S., S.E. Graham, D. Shea and G.A. Leblanc. 1998. Altered metabolic elimination of testosterone and associated toxicity following exposure to *Daphnia magna* to nonylphenol polyethoxylate. *Ecotoxicol. Environ. Saf.* 39:104-111.

- Bearden, A.P. and T.W. Schultz. 1997. Structure-activity relationships for *Pimephales* and *Tetrahymena*: a mechanism of action approach. *Environ. Toxicol. Chem.* 16:1311-1317.
- Bearden, A.P. and T.W. Schultz. 1998. Comparison of *Tetrahymena* and *Pimephales* toxicity based on mechanism of action. *SAR QSAR Environ. Res.* 9:127-153.
- Bechmann, R.K. 1999. Effect of the endocrine disrupter nonylphenol on the marine copepod *Tisbe battagliai*. *Sci. Total Environ.* 233:33-46.
- Bennett, E.R. and C.D. Metcalfe. 1998. Distribution of alkylphenol compounds in Great Lakes sediments, United States and Canada. *Environ. Toxicol. Chem.* 17:1230-1235.
- Bennet, E.R. and C.D. Metcalfe. 2000. Distribution of degradation products of alkylphenol ethoxylates near sewage treatment plants in the Lower Great Lakes, North America.. *Environ. Toxicol. Chem.* 19: 784-792.
- Bennie, D.T. 1999. Review of the environmental occurrence of alkylphenols and alkylphenol ethoxylates. *Wat. Qual. Res. J. Can.* 34:79-122.
- Bennie, D.T., C.A. Sullivan, H. Lee, T.E. Peart and R.J. Maguire. 1997. Occurrence of alkylphenols and alkylphenol mono- and di-ethoxylates in natural waters of the Laurentian Great Lakes basin and the upper St. Lawrence River. *Sci. Total Environ.* 193:263-275.
- Bettinetti, R. and A. Provini. 2002. Toxicity of 4-nonylphenol to *Tubifex tubifex* and *Chironomus riparius* in 28-day whole-sediment tests. *Ecotoxicol. Environ. Safety* 53:113-121.
- Billinghurst, Z., A.S. Clare, T. Fileman, M. McEvoy, J. Readman and M.H. Depledge. 1998. Inhibition of barnacle settlement by the environmental oestrogen 4-nonylphenol and the natural oestrogen 17 $\beta$  oestradiol. *Mar. Pollut. Bull.* 36:833-839.
- Blackburn, M.A., S.J. Kirby and M.J. Waldock. 1999. Concentrations of alkylphenol polyethoxylates entering UK estuaries. *Mar. Pollut. Bull.* 38:109-118.
- Braaten, B., A. Granmo and R. Lange. 1972. Tissue-swelling in *Mytilus edulis* L. induced by exposure to a nonionic surface active agent. *Norw. J. Zool.* 20:137-140.

Brooke, L.T. 1993a. Acute and chronic toxicity of nonylphenol to ten species of aquatic organisms. Report to the U.S. EPA for Work Assignment No. 02 of Contract No. 68-C1-0034. Lake Superior Research Institute, University of Wisconsin-Superior, Superior, WI. March 24. 30 pp. Amended 18 October 2005. 34 pp.

Brooke, L.T. 1993b. Nonylphenol Toxicity. Accumulation and lethality for two freshwater fishes (fathead minnow and bluegill) to nonylphenol. Report to the U.S. EPA for Work Assignment No. 1-12 of Contract No. 68-C1-0034. Lake Superior Research Institute, University of Wisconsin-Superior, Superior, WI. September 30. 50 pp.

Brooke, L.T. 1994. Nonylphenol Analysis. Accumulation and lethality for two freshwater fishes (fathead minnow and bluegill) to nonylphenol. Report to the U.S. EPA for Work Assignment No. 2-15 of Contract No. 68-C1-0034. Lake Superior Research Institute, University of Wisconsin-Superior, Superior, WI. May 31. 49 pp.

Brown, R.J., M. Conradi and M.H. Depledge. 1999. Long-term exposure to 4-nonylphenol affects sexual differentiation and growth of the amphipod *Corophium volutator* (Pallas, 1766). *Sci. Total Environ.* 233:77-88.

Budavari, S. (Ed.). 1989. *The Merck Index: An encyclopedia of chemicals, drugs, and biologicals.* 11th ed. Merck and Co., Inc. Rahway, NJ.

Burkhardt-Holm, P., T. Wahli and W. Meier. 2000. Nonylphenol affects the granulation of pattern of epidermal mucous cells in rainbow trout, *Oncorhynchus mykiss*. *Ecotoxicol. Environ. Safe.* 46:34-40.

Celius, T., T.B. Haugen, T. Grotmol and B.T. Walther. 1999. A sensitive zogenetic assay for rapid *in vitro* assessment of estrogenic potency of xenobiotics and mycotoxins. *Environ. Health Perspect.* 107:63-68.

Christiansen, T., B. Korsgaard and A. Jespersen. 1998a. Effects of nonylphenol and 17 $\beta$ -estradiol on vitellogenin synthesis, testicular structure and cytology in male eelpout *Zoarces viviparus*. *J. Exp. Biol.* 201:179-192.

Christiansen, T., B. Korsgaard and A. Jespersen. 1998b. Induction of vitellogenin synthesis by nonylphenol and 17 $\beta$ -estradiol and effects on the testicular structure in the eelpout *Zoarces viviparus*. *Mar. Environ. Res.* 46:141-144.

Christiansen, T., K.L. Pedersen, B. Korsgaard and P. Bjerregaard. 1998c. Estrogenicity of xenobiotics in rainbow trout (*Oncorhynchus mykiss*) using *in vivo* synthesis of vitellogenin as a biomarker. *Mar. Environ. Res.* 46(1-5): 137-140.

Christensen, L.J., B. Korsgaard and P. Bjerregaard. 1999. The effect of 4-nonylphenol on the synthesis of vitellogenin in the flounder *Platichthys flesus*. *Aquat. Toxicol.* 46:211-219.

Coldham, N.G., M.J. Sauer, S. Sivapathasundaram, L. Ashfield, T. Pottinger and C. Goodall. 1997. Tissue distribution, metabolism and excretion of 4-nonylphenol in rainbow trout. *J. Vet. Pharmacol. Therap.* 20(Suppl. 1): 256-257.

Coldham, N.G., S. Sivapathasundaram, M. Dave, L.A. Ashfield, T.G. Pottinger, C. Goodall and M.J. Sauer. 1998. Biotransformation, tissue distribution, and persistence of 4-nonylphenol residues in juvenile rainbow trout (*Oncorhynchus mykiss*). *Drug Metabol. Dispos.* 26:347-354.

Comber, M.H.I., T.D. Williams and K.M. Stewart. 1993. The effects of nonylphenol on *Daphnia magna*. *Wat. Res.* 27:273-276.

Corsi, S.R., D.H. Zitomer, J.A. Field and D.A. Cancilla. 2003. Nonylphenol ethoxylates and other additives in aircraft deicers, antiicers, and waters receiving airport runoff. *Environ. Sci. Technol.* 37:4031-4037.

Dorn, P.B., J.P. Salanitro, S.H. Evans and L. Kravetz. 1993. Assessing the aquatic hazard of some branched and linear nonionic surfactants by biodegradation and toxicity. *Environ. Toxicol. Chem.* 12:1751-1762.

Dwyer, F.J., L.C. Sappington, D.R. Buckler and S.B. Jones. 1995. Use of surrogate species in assessing contaminant risk to endangered and threatened species. EPA/600/R-96/029. National Technical Information Service, Springfield, VA. 71 pp.

Dwyer, F.J., D.K. Hardesty, C.E. Henke, C.G. Ingersoll, D.W. Whites, D.R. Mount and C.M. Bridges. 1999a. Assessing contaminant sensitivity of endangered and threatened species: Toxicant classes. EPA/600/R-99/098. National Technical Information Service, Springfield, VA. 15 pp.

Dwyer, F.J., D.K. Hardesty, C.E. Henke, C.G. Ingersoll, D.W. Whites, D.R. Mount and C.M. Bridges. 1999b. Assessing contaminant sensitivity of endangered and threatened species: Effluent toxicity tests. EPA/600/R-99/099. National Technical Information Service, Springfield, VA. 9 pp.

Ekelund, R., A. Bergman, A. Granmo, and M. Berggren. 1990. Bioaccumulation of 4-nonylphenol in marine animals--A re-evaluation. *Environ. Poll.* 64: 107-120.



- Ekelund, R., A. Granmo, K. Magnusson and M. Berggren. 1993. Biodegradation of 4-nonylphenol in seawater and sediment. *Environ. Pollut.* 79:59-61.
- Ellis, D.D., C.M. Jone, R.A. Larson and D.J. Schaeffer. 1982. Organic constituents of mutagenic secondary effluents from wastewater treatment plants. *Arch. Environ. Contam. Toxicol.* 11:373-382.
- England, D.E. 1995. Chronic toxicity of nonylphenol to *Ceriodaphnia dubia*. Report No. 41756. ABC Laboratories, Inc. Columbia, MO. 409 pp.
- England, D.E. and J.B. Bussard. 1993. Toxicity of nonylphenol to the midge *Chironomus tentans*. Report No. 40597. ABC Laboratories, Inc., Columbia, MO. 2528 pp.
- England, D.E. and J.B. Bussard. 1995. Toxicity of nonylphenol to the amphipod *Hyalella azteca*. Report No. 41569. ABC Laboratories, Inc., Columbia, MO. 178 pp.
- Ernst, B., G. Julien, K. Doe and R. Parker. 1980. Environmental investigations of the 1980 spruce budworm spray program in New Brunswick. Surveillance Report EPS-5-AR-81-3, November 1980. Environment Canada, Atlantic Region, Halifax, NS.
- Escher, M., T. Wahli, S. Buttner, W. Meier and P. Burkhardt-Holm. 1999. The effect of sewage plant effluent on brown trout (*Salmo trutta fario*): a cage experiment. *Aquat. Sci.* 61:93-110.
- Etoh, H., S. Hageshita and K. Ina. 1997. An improved assay for attachment-promoting substances of the blue mussel, *Mytilus edulis galloprovincialis*. *J. Mar. Biotechnol.* 5:24-26.
- Fay, A.A., F.J. Brownawell, A.A. Elskus and A.E. McElroy. 2000. Critical body residues in the marine amphipod *Ampelisca abdita*: Sediment exposures with nonionic organic contaminants. *Environ. Toxicol. Chem.* 19:1028-1035.
- Ferguson, P.L. and B.J. Brownawell. 2003. Degradation of nonylphenol ethoxylates in estuarine sediment under aerobic and anaerobic conditions. *Environ. Toxicol. Chem.* 22:1189-1199.
- Fliedner, A. 1993. *Daphnia magna*, Reproduction test (OECD No. 202). Fraunhofer-Institute für Umweltchemie und Ökotoxikologie, Postfach 1260, W-5948 Schmallenberg – Grafschaft, Germany. Report No. UBA-002/4-22 February.

Flouriot, G., F. Pakdel, B. Ducouret and Y. Valotaire. 1995. Influence of xenobiotics on rainbow trout liver estrogen receptor and vitellogenin gene expression. *J. Molecular Endocrin.* 15:143-151.

Folmar, L., M. Hemmer, N. Denslow, K. Kroll, A. Cheek, H. Meredith, H. Richman and G. Grau. 1998. A comparison of an *in vivo* fish VTG assay with YES and E-screen to rank 'relative estrogenicity' of natural pharmaceutical and xenoestrogens. *Am. Zool.* 38:112A.

Fort, D.J. and E.L. Stover. 1997. Development of short-term, whole-embryo assays to evaluate detrimental effects on amphibian limb development and metamorphosis using *Xenopus laevis*. In: *Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment*. F.J. Dwyer, T.R. Doane and M.L. Hinman (Eds.). ASTM, STP 1317, Vol. 6, American Society for Testing and Materials, Philadelphia, PA., pp. 376-390.

Gaffney, P.E. 1976. Carpet and rug industry case study II: Biological effects. *J. Water Pollut. Control Fed.* 48:2731-2737.

Gerritsen, A., N. van der Hoeven and A. Pielaat. 1998. The acute toxicity of selected alkylphenols to young and adult *Daphnia magna*. *Ecotoxicol. Environ. Safety.* 39:227-232.

Giesy, J.P., S.L. Pierens, E.M. Snyder, S. Miles-Richardson, V.J. Kramer, S.A. Snyder, K.M. Nichols and D.A. Villeneuve. 2000. Effects of 4-nonylphenol on fecundity of biomarkers of estrogenicity in fathead minnows (*Pimephales promelas*). *Environ. Toxicol. Chem.* 19:1368-1377.

Giger, W., E. Stephanou and C. Schaffner. 1981. Persistent organic chemicals in sewage effluents: I. Identifications of nonylphenols and nonylphenol ethoxylates by glass capillary gas chromatography/ mass spectrometry. *Chemosphere* 10:1253-1263.

Giger, W., P.H. Brunner and C. Schaffner. 1984. 4-Nonylphenol in sewage sludge; Accumulation of toxic metabolites from nonionic surfactants. *Science* 225:623-625.

Granmo, A., R. Ekelund, K. Magnusson and M. Berggren. 1989. Lethal and sublethal toxicity of 4-nonylphenol to the common mussel (*Mytilus edulis* L.). *Environ. Poll.* 59: 115-127.

Granmo, A., R. Ekelund, M. Berggren and K. Magnusson. 1991a. Toxicity of 4-nonylphenol to aquatic organisms and potential for bioaccumulation. *Proceedings, Swedish Environmental Protection Agency Seminar on Nonylphenol Ethoxylates/nonylphenol, Saltsjobaden, Sweden, February 6-8*, pp. 53-75.

- Granmo, A., S. Kollberg, M. Berggren, R. Ekelund, K. Magnusson, L. Renberg and C. Wahlberg. 1991b. Bioaccumulation of nonylphenol in caged mussels in an industrial coastal area on the Swedish west coast. In: Organic Micropollutants in the Aquatic Environment. Angeletti, G. (Ed.). Proceedings of the 6<sup>th</sup> European Symposium. Pp. 71-79.
- Gray, M.A. and C.D. Metcalfe. 1997. Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to *p*-nonylphenol. Environ. Toxicol. Chem. 16:1082-1086.
- Gross-Sorokin, M.Y., E.P.M. Grist, M. Cooke and M. Crane. 2003. Uptake and depuration of 4-nonylphenol by the benthic invertebrate *Gammarus pulex*: How important is feeding rate? Environ. Sci. Technol. 37:2236-2241.
- Hahn, T., K. Schenk and R. Schulz. 2002. Environmental chemicals with known endocrine potential affect yolk protein content in the aquatic insect *Chironomus riparius*. Environ. Pollut. 120:525-528.
- Hale, R.C., C.L. Smith, P.O. de Fur, E. Harvey, E.O. Bush, M.J. La Guardia and G.G. Vadas. 2000. Nonylphenols in sediments and effluents associated with diverse wastewater outfalls. Environ. Toxicol. Chem. 19:946-952.
- Hansen, F.T., V.E. Forbes and T.L. Forbes. 1999. Effects of 4-n-nonylphenol on life-history traits and population dynamics of a polychaete. Ecol. Appl. 9:482-495.
- Hansen, P., H. Dizer, B. Hock, A. Marx, J. Sherry, M. McMaster and C. Blaise. 1998. Vitellogenin - a biomarker for endocrine disruptors. Trends Anal. Chem. 17:448-451.
- Harries, J.E., D.A. Sheahan, S. Jobling, P. Matthiessen, P. Neall, J.P. Sumpter, T. Tylor and N. Zaman. 1997. Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. Environ. Toxicol. Chem. 16:534-542.
- Harris, C.A., E.M. Santos, A. Janbakhsh, T.G. Pottinger, C.R. Tyler and J.P. Sumpter. 2001. Nonylphenol affects gonadotropin levels in the pituitary gland and plasma of female rainbow trout. Environ. Sci. Technol. 35:2909-2916.
- Harvilicz, H. 1999. NPE demand remains strong despite environmental concerns in Europe. Chemical Market Reporter. October 18, 1999. p. 15.
- Haya, K., L.E. Burrige and T.J. Benfey. 1997. The effect of cortisol and nonylphenol on growth and ornithine decarboxylase activity of juvenile Atlantic salmon, *Salmo salar*. Can. Tech. Rep. Fish. Aquat. Sci. 2192: 85-86.

Hecht, S. and B.L. Boese. 2002. Sensitivity of an infaunal amphipod, *Eohaustorius estuarius*, to acute waterborne exposures of 4-nonylphenol: Evidence of a toxic hangover. *Environ. Toxicol. Chem.* 21:816-819.

Hecht, S.A., J.S. Gunnarsson, B.L. Boese, J.O. Lamberson, C. Schaffner, W. Giger and P.C. Jepson. 2004. Influences of sedimentary organic matter quality on the bioaccumulation of 4-nonylphenol by estuarine amphipods. *Environ. Toxicol. Chem.* 23:865-873.

Heinis, L.J., M.L. Knuth, K. Liber, B.R. Sheedy, R.L. Tunell and G.T. Ankley. 1999. Persistence and distribution of 4-nonylphenol following repeated application to littoral enclosures. *Environ. Toxicol. Chem.* 18:363-375.

Hemmer, M.J., B.L. Hemmer, C.J. Bowman, K.J. Kroll, L.C. Folmer, D. Marcovich, M.D. Hoglund and N.D. Denstow. 2001. Effects of p-nonylphenol, methoxychlor and endosulfan on vitellogenin induction and expression in sheepshead minnow (*Cyprindon variegatus*). *Environ. Toxicol. Chem.* 20:336-343.

Hense, B.A., I. Juttner, G. Welzl, G.F. Severin, G. Pfister, A. Behechti and K-W. Schramm. 2003. Effects of 4-nonylphenol on phytoplankton and periphyton in aquatic microcosms. *Environ. Toxicol. Chem.* 22:2727-2732.

Hewitt, L.M., L. Tremblay, G.J. Van Der Kraak, K.R. Solomon and M.R. Servos. 1998. Identification of the lampricide 3-trifluoromethyl-4-nitrophenol as an agonist for the rainbow trout estrogen receptor. *Environ. Toxicol. Chem.* 17:425-432.

Holcombe, G.W., G.L. Phipps, M.L. Knuth and T. Felhaber. 1984. The acute toxicity of selected substituted phenols, benzenes and benzoic acid esters to fathead minnows (*Pimephales promelas*). *Environ. Pollut. (Series A)* 35:367-381.

Holmes, S. B. and P. D. Kingsbury. 1980. The environmental impact of nonylphenol and the Matacil<sup>®</sup> formulation. Part 1: Aquatic ecosystems. Report FPMX-35. Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario.

Islinger, M., S. Pawlowski, H. Hollert, A. Volkl and T. Braumbeck. 1999. Measurement of vitellogenin-mRNA expression in primary cultures of rainbow trout hepatocytes in a non-radioactive dot blot/RNase protection-assay. *Sci. Total Environ.* 233:109-122.

Jobling, S. and J.P. Sumpter. 1993. Detergent components in sewage effluent are weakly oestrogenic to fish: An in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat. Toxicol.* 27:361-372.

- Jobling, S., D. Sheahan, J.A. Osborne, P. Matthiessen and J.P. Sumpter. 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environ. Toxicol. Chem.* 15:194-202.
- Jones, S.B., L.B. King, L.C. Sappington, F.J. Dwyer, M. Ellersieck and D.R. Buckler. 1998. Effects of carbaryl, permethrin, 4-nonylphenol, and copper on muscarinic cholinergic receptors in brain of surrogate and listed fish species. *Comp. Biochem. Physiol.* 120:405-414.
- Kahl, M.D., E.A. Makynen, P.A. Kosian and G.T. Ankley. 1997. Toxicity of 4-nonylphenol in a life-cycle test with the midge *Chironomus tentans*. *Ecotoxicol. Environ. Saf.* 38:155-160.
- Kannan, K., T.L. Keith, C.G. Naylor, C.A. Staples, S.A. Snyder and J.P. Giesy. 2003. Nonylphenol and nonylphenol ethoxylates in fish, sediment, and water from the Kalamazoo River, Michigan. *Arch. Environ. Contam. Toxicol.* 44:77-82.
- Keith, T.L., S.A. Snyder, C.G. Naylor, C.A. Staples, C. Summer, K. Kannan and J.P. Giesy. 2001. Identification and quantitation of nonylphenol ethoxylates and nonylphenol in fish tissues from Michigan. *Environ. Sci. Technol.* 35:10-13.
- Kelly, S.A. and R.T. Di Giulio. 2000. Developmental toxicity of estrogenic alkylphenols in killifish (*Fundulus heteroclitus*). *Environ. Toxicol. Chem.* 19:2564-2570.
- Kinnberg, K., B. Korsgaard, P. Bjerregaard and A. Jespersen. 2000. Effects of nonylphenol and 17 $\beta$ -estradiol on vitellogenin synthesis and testis morphology in male platyfish *Xiphophorus maculatus*. *J. Exper. Biol.* 203:171-181.
- Kitajima, F., C.G. Satuito, H. Hirota, I. Katsuyama and N. Fusetani. 1995. A new screening method for antifouling substances against the young mussels *Mytilus edulis galloprovincialis*. *Fish. Sci.* 61:578-583.
- Kloas, W., I. Lutz and R. Einspanier. 1999. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. *Sci. Total Environ.* 225:59-68.
- Knudsen, F.R. and T.G. Pottinger. 1999. Interaction of endocrine disrupting chemicals, singly and in combination, with estrogen-, androgen-, and corticosteroid-binding sites in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 44:159-170.

- Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber and H.T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999 - 2000: A national reconnaissance. *Environ. Sci. Technol.* 36:1202-1211.
- Kopf, W. 1997. Wirkung endokriner Stoffe in Biotests mit Wasserorganismen. *In: Stoffe mit endokriner Wirkung in Wasser*. Bayerisches Landesamt für Wasserwirtschaft, Institut für Wasserforschung, München. 12pp.
- Kuhn, A., W.R. Munns, Jr., D. Champlin, R. McKinney, M. Tagliabue, J. Serbst and T. Gleason. 2001. Evaluation of the efficacy of extrapolation population modeling to predict the dynamics of *Americamysis bahia* populations in the laboratory. *Environ. Toxicol. Chem.* 20:213-221.
- Kusk, K.O. and L. Wollenberger. 1999. Fully defined saltwater medium for cultivation of and toxicity testing with marine copepod *Acartia tonsa*. *Environ. Toxicol. Chem.* 18:1564-1567.
- Kwak, H., M. Bae, M. Lee, Y. Lee, B. Lee, K. Kang, C. Chae, H. Sung, J. Shin, J. Kim, W. Mar, Y. Sheen and M. Cho. 2001. Effects of nonylphenol, bisphenol A, and their mixture on the viviparous swordtail fish (*Xiphophorus helleri*). *Environ. Toxicol. Chem.* 20:787-795.
- Lamche, G. and P. Burkhardt-Holm. 2000. Nonylphenol provokes a vesiculation of the Golgi apparatus in three fish epidermis cultures. *Ecotoxicol. Environ. Saf.* 47:137-148.
- Larsson, D.G.J., M. Adolfsson-Erici, J. Parkkonen, M. Pettersson, A.H. Berg, P. Olsson and L. Forlin. 1999. Ethinylloestradiol - an undesired fish contraceptive? *Aquat. Toxicol.* 45:91-97.
- Lavado, R., R. Thibaut, D. Raldua, R. Martin and C. Porte. 2004. First evidence of endocrine disruption in feral carp from the Ebro River. *Toxicol. Appl. Pharm.* 196:247-257.
- LeBlanc, G.A., S. Mu and C.V. Rider. 2000. Embryotoxicity of the alkylphenol degradation product 4-nonylphenol to the crustacean *Daphnia magna*. *Environ. Health Perspect.* 108:1133-1138.
- Lech, J. J., S. K. Lewis and L. Ren. 1996. *In vivo* estrogenic activity of nonylphenol in rainbow trout. *Fund. Appl. Toxicol.* 30:229-232.
- Levine, C. and M.A. Cheney. 2000. Metabolic responses to acute toxicity of alkylphenols and alkylphenol polyethoxylates in *Elliptio complanata* measured by calorimetry. *Environ. Toxicol. Chem.* 19:1906-1910.

- Lewis, M.A. 1991. Chronic and sublethal toxicities of surfactants to aquatic animals: A review and risk assessment. *Water Res.* 25:101-113.
- Lewis, S. K. and J. J. Lech. 1996. Uptake, disposition, and persistence of nonylphenol from water in rainbow trout (*Oncorhynchus mykiss*). *Xenobiotica* 26:813-819.
- Liber, K., J. A. Gangl, T. D. Corry, L. J. Heinis and F. S. Stay. 1999. Lethality and bioaccumulation of 4-nonylphenol in bluegill sunfish in littoral enclosures. *Environ. Toxicol. Chem.* 18:394-400.
- Loomis, A.K. and P. Thomas. 1999. Binding characteristics of estrogen receptor (ER) in Atlantic croaker (*Micropogonias undulatus*) testis: Different affinity for estrogens and xenobiotics from that of hepatic ER. *Biol. Reprod.* 61:51-60.
- Lussier, S.M., D. Champlin, J. LiVolsi, S. Poucher and R.J. Pruell. 2000. Acute toxicity of *para*-nonylphenol to saltwater animals. *Environ. Toxicol. Chem.* 19:617-621.
- Lutz, I. and W. Kloas. 1999. Amphibians as a model to study endocrine disruptors: I. Environmental pollution and estrogen receptor binding. *Sci. Total Environ.* 25:49-57.
- Lye, C.M., C.L.J. Frid, M.E. Gill, D.W. Cooper and D.M. Jones. 1999. Estrogenic alkylphenols in fish tissues, sediments, and waters from the U.K. Tyne and Tees estuaries. *Environ. Sci. Technol.* 33:1009-1014.
- MacPhee, C. and R. Ruelle. 1969. Lethal effects of 1888 chemicals upon four species of fish from western North America. Bulletin No. 3, Forest, Wildlife and Range Experiment Station, University of Idaho, Moscow, ID. 112 p.
- Madsen, S.S., A.B. Mathiesen and B. Korsgaard. 1997. Effects of 17 $\beta$ -estradiol and 4-nonylphenol on smoltification and vitellogenesis in Atlantic salmon (*Salmo salar*). *Fish Physiol. Biochem.* 17:303-312.
- Magliulo, L., M.P. Schreiber and J.L. Cepriano. 1998. Disruption of the brain-pituitary-gonad axis of platyfish due to hormone mimicking environmental pollutants. *Am. Zool.* 38:112A.
- Maguire, R.J. 1999. Review of the persistence of nonylphenol and nonylphenol ethoxylates in aquatic environments. *Wat. Qual. Res. J. Can.* 34:37-78.
- Maki, H., H. Okamura, I. Aoyama and M. Fujita. 1998. Halogenation and toxicity of the biodegradation products of a nonionic surfactant, nonylphenol ethoxylate. *Environ. Toxicol. Chem.* 17:650-654.

- Manzano, M.A., J.A. Perales, D. Sales and J.M. Quiroga. 1998. Effect of concentration on the biodegradation of a nonylphenol polyethoxylate in river water. *Bull. Environ. Contam. Toxicol.* 61:489-496.
- Manzano, M.A., J.A. Perales, D. Sales and J.M. Quiroga. 1999. The effect of temperature on the biodegradation of a nonylphenol polyethoxylate in river water. *Water Res.* 33:2593-2600.
- Marcomini, A., B. Pavoni, A. Sfriso and A.A. Orio. 1990. Persistent metabolites of alkylphenol polyethoxylates in the marine environment. *Mar. Chem.* 29:307-324.
- McLeese, D.W., D.B. Sargeant, C.D. Metcalfe, V. Zitko and L.E. Burridge. 1980a. Uptake and excretion of aminocarb, nonylphenol, and pesticides diluent 585 by mussels (*Mytilus edulis*). *Bull. Environ. Contam. Toxicol.* 24:575-581.
- McLeese, D.W., V. Zitko, C.D. Metcalf and D.B. Sargeant. 1980b. Lethality of aminocarb and the components of the aminocarb formulation to juvenile Atlantic salmon, marine invertebrates and a freshwater clam. *Chemosphere.* 9:79-82.
- McLeese, D.W., V. Zitko, D.B. Sargeant, L. Burridge and C.D. Metcalfe. 1981. Lethality and accumulation of alkylphenols in aquatic fauna. *Chemosphere* 10:723-730.
- Meldahl, A. C., K. Nithipatikom and J. J. Lech. 1996. Metabolism of several <sup>14</sup>C-nonylphenol isomers by rainbow trout (*Oncorhynchus mykiss*): *in vivo* and *in vitro* microsomal metabolites. *Xenobiotica* 26:1167-1180.
- Miles-Richardson, S.R., S.L. Pierens, K.M. Nichols, V.J. Kramer, E.M. Snyder, S.A. Snyder, J.A. Render, S.D. Fitzgerald and J.P. Giesy. 1999. Effects of waterborne exposure to 4-nonylphenol and nonylphenol ethoxylate on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelas*). *Environ. Res. (Series A)* 80:S122-S137.
- Milligan, S.R., O. Khan and M. Nash. 1998. Competitive binding of xenobiotic estrogens to rat alpha-fetoprotein and to sex steroid binding proteins in human and rainbow trout (*Oncorhynchus mykiss*) plasma. *Gen. Comp. Endocrinol.* 112:89-95.
- Moore, A., A.P. Scott, N. Lower, I. Katsiadaki and L. Greenwood. 2003. The effects of 4-nonylphenol and atrazine on Atlantic salmon (*Salmo salar L.*) smolts. *Aquaculture* 222:253-263.



- Moore, S.B., R.A. Diehl, J.M. Barnhardt and G.B. Avery. 1987. Aquatic toxicities of textile surfactants. Text. Chem. Color. 19:29-32.
- Muller, R. 1980. Fish toxicity and surface tension of non-ionic surfactants: investigations of antifoam agents. J. Fish. Biol. 16:585-589.
- Naylor, C.G. 1992. Environmental fate of alkylphenol ethoxylates. Soap Cosmetics Chemical Specialities. August.
- Naylor, C.G., J.P. Mieure, W.J. Adams, J.A. Weeks, F.J. Castaldi, L.D. Ogle and R.R. Romano. 1992. Alkylphenol ethoxylates in the environment. J. Am. Oil Chem. Soc. 69:695-708.
- Nice, H.E., M.C. Thorndyke, D. Morritt, S. Steele and M. Crane. 2000. Development of the *Crassostrea gigas* larvae is affected by 4-nonylphenol. Mar. Pollut. Bull. 40:491-496.
- Nimrod, A.C. and W.H. Benson. 1996. Estrogenic responses to xenobiotics in channel catfish (*Ictalurus punctatus*). Mar. Environ. Res. 42:155-160.
- Nimrod, A.C. and W.H. Benson. 1997. Xenobiotic interaction with and alteration of channel catfish estrogen receptor. Toxicol. Appl. Pharmacol. 147:381-390.
- Nimrod, A.C. and W.H. Benson. 1998. Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens. Aquat. Toxicol. 44: 141-156.
- O'Halloran, S. L., K. Liber, J. A. Gangl and M. L. Knuth. 1999. Effects of repeated exposure to 4-nonylphenol on the zooplankton community in littoral enclosures. Environ. Toxicol. Chem. 18:376-385.
- Palmer, B.D., S.K. Palmer, C. Burch, J. Danyo and K.W. Selcer. 1998. Effects of endocrine disrupting chemicals on amphibians. Am. Zool. 38(5): Abstract No. 177A.
- Patoczka, J. and G.W. Pulliam. 1990. Biodegradation and secondary effluent toxicity of ethoxylated surfactants. Water Res. 24:965-972.
- Pedersen, S.N., L.B. Christiansen, K.L. Pedersen, B. Korsgaard and P. Bjerregaard. 1999. In vivo estrogenic activity of branched and linear alkylphenols in rainbow trout (*Oncorhynchus mykiss*). Sci. Tot. Environ. 233:89-96.

Petit, F., P. Le Goff, J.P. Cravedi, Y. Valotaire and F. Pakdel. 1997. Two complementary bioassays for screening the estrogenic potency of xenobiotics: recombinant yeast for trout estrogen receptor and trout hepatocyte cultures. *J. Mol. Endocrinol.* 19:321-335.

Petit, F., P. Le Goff, J.P. Cravedi, O. Kah, Y. Valotaire and F. Pakdel. 1999. Trout estrogen receptor sensitivity to xenobiotics as tested by different bioassays. *Aquaculture.* 177:353-365.

Pickford, K.A., R.E. Thomas-Jones, B. Wheals, C.R. Tyler and J.P. Sumpter. 2003. Route of exposure affects the oestrogenic response of fish to 4-*tert*-nonylphenol. *Aq. Toxicol.* 65:267-279.

Prasad, R. 1986. Effects of nonylphenol adjuvant on macrophytes. In: *Adjuvants and Agrochemicals*. Chow, P.N.P. (Ed.). Vol. 1, Mode of action and physiological activity. First International Symposium. Brandon, Manitoba, Canada. August 5-7. XIV+207 pp.

Preston, B.L., T.W. Snell, T.L. Robertson and B.J. Dingmann. 2000. Use of freshwater rotifer *Brachionus calyciflorus* in screening assay for potential endocrine disruptors. *Environ. Toxicol. Chem.* 19:2923-2928.

Purdom, C.E., P.A. Hardiman, V.J. Bye, N.C. Eno, C.R. Tyler and J.P. Sumpter. 1994. Estrogenic effects of effluents from sewage treatment works. *Chem. and Ecol.* 8:275-285.

Radian Corp. 1990. Nonylphenol and nonylphenol ethoxylates in river water and bottom sediments-January 1989-August 1990. Final Report to Alkylphenol and Ethoxylates Panel, Chemical Manufacturers Association.

Reed, H.W.B. 1978. Alkylphenols. In: Kirk-Othmer: *Encyclopedia of chemical technology*. Grayson M. and Eckroth D. (Eds.). 3rd ed., Vol. 2, John Wiley and Sons, New York. pp. 72-96.

Ren, L., D. Lattier and J. J. Lech. 1996a. Estrogenic activity in rainbow trout determined with a new cDNA probe for vitellogenesis, pSG5Vg1.1. *Bull. Environ. Contam. Toxicol.* 56:287-294.

Ren, L., S.K. Lewis and J.J. Lech. 1996b. Effects of estrogen and nonylphenol on the post-transcriptional regulation of vitellogenin gene expression. *Chemico-Biol. Interact.* 100:67-76.

Rice, C.D., L.E. Roszell, M.M. Banes and R.E. Arnold. 1998. Effects of dietary PCBs and nonyl-phenol on immune function and CYP1A activity in channel catfish, *Ictalurus punctatus*. *Mar. Environ. Res.* 46:351-354.

Routledge, E.J. and J.P. Sumpter. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem.* 15:241-248.

Routledge, E.J. and J.P. Sumpter. 1997. Structural features of alkylphenolic chemicals associated with estrogenic activity. *J. Biol. Chem.* 272:3280-3288.

Roy F. Weston Inc. 1990. Determination of the vapor pressure of 4-nonylphenol. Final Report Study No. 90-047. Roy F. Weston Inc., Environmental Fate and Effects Laboratory, 254 Welsh Pool Road, Lionville, PA. 15 August 1990.

Schmude, K. L., K. Liber, T. D. Corry and F. S. Stay. 1999. Effects of 4-nonylphenol on benthic macroinvertebrates and insect emergence in littoral enclosures. *Environ. Toxicol. Chem.* 18:386-393.

Schultz, T.W. 1997. Tetratox: *Tetrahymena pyriformis* population growth impairment endpoint - A surrogate for fish lethality. *Toxicol. Methods.* 7:289-309.

Schwaiger, J., O.H. Spieser, C. Bauer, H. Ferling, U. Mallow, W. Kalbfus and R.D. Negele. 2000. Chronic toxicity of nonylphenol and ethinylestradiol: haematological and histopathological effects in juvenile common carp (*Cyprinus carpio*). *Aquat. Toxicol.* 51:69-78.

Schwaiger, J., U. Mallow, H. Ferling, S. Knoerr, T. Braunbeck, W. Kalbfus and R.D. Negele. 2002. How estrogenic is nonylphenol: A transgenerational study using rainbow trout (*Oncorhynchus mykiss*) as a test organism. *Aquat. Toxicol.* 59:177-189.

Seki, M., H. Yokota, M. Maeda, H. Tadokoro and K. Kobayashi. 2003. Effects of 4-nonylphenol and 4-tert-octylphenol on sex differentiation and vitellogenin induction in medaka (*Oryzias latipes*). *Environ. Toxicol. Chem.* 22:1507-1516.

Servos, M.R. 1999. Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. *Wat. Qual. Res. J. Can.* 34:123-177.

Severin, G.F., G. Welzl, I. Juttner, G. Pfister and K-W. Schramm. 2003. Effects of nonylphenol on zooplankton in aquatic microcosms. *Environ. Toxicol. Chem.* 22:2733-2738.

Shackelford, W.M., D.M. Cline, L. Faas and G. Kurth. 1983. Evaluation of automated spectrum matching for survey identification of wastewater components by gas chromatography-mass spectrometry. PB83-182931. National Technical Information Service, Springfield, VA.

Shang, D.Y., R.W. MacDonald and M.G. Ikonou. 1999. Persistence of nonylphenol ethoxylate surfactants and their primary degradation products in sediments from near a municipal outfall in the Strait of Georgia, British Columbia, Canada. *Environ. Sci. Technol.* 33:1366-1372.

Shurin, J.B. and S.I. Dodson. 1997. Sublethal toxic effects of cyanobacteria and nonylphenol on environmental sex determination and development in *Daphnia*. *Environ. Toxicol. Chem.* 16:1269-1276.

Sonnenschein, C. and A.M. Soto. 1998. An updated review of environmental estrogen and androgen mimics and antagonists. *J. Steroid Biochem. Molec. Biol.* 65:143-150.

Soto, A.M., H. Justicia, J.W. Wray and C. Sonnenschein. 1991. *p*-Nonylphenol: An estrogenic xenobiotic released from modified polystyrene. *Environ. Health Perspect.* 92:167-173.

Soto, A.M., T.M. Lin, H. Justicia, R.M. Silvia and C. Sonnenschein. 1992. An "in culture" bioassay to assess the estrogenicity of xenobiotics (E-SCREEN). In: *Advances in Modern Environmental Toxicology. Volume XXI. Chemically-induced alterations in sexual and functional development: The wildlife/human connection.* T. Colburn and C. Clement (Eds.). Princeton Scientific Publishing Co., Inc., NJ. 403 pp.

Spieser, O.H., J. Schwaiger, H. Ferling, W. Kalbfus and R. Negele. 1998. Effects of nonylphenol and ethinyl-estradiol on swimming behavior of juvenile carp. Abstract for Annual Meeting. International Association for Great Lakes Research. Hamilton, Ontario, Canada. pp. 37-38.

Staples, C.A., J. Weeks, J.F. Hall and C.G. Naylor. 1998. Evaluation of aquatic toxicity and bioaccumulation of C8- C9-alkylphenol ethoxylates. *Environ. Toxicol. Chem.* 17:2470-2480.

Staples, C.A., J.B. Williams, R.L. Blessing and P.T. Varineau. 1999. Measuring the biodegradability of nonylphenol, ether carboxylates, octylphenol, ether carboxylates, and nonylphenol. *Chemosphere* 38:2029-2039.

Staples, C.A., C.G. Naylor, J.B. Williams and W.E. Gledhill. 2001. Ultimate biodegradation of alkylphenol ethoxylate surfactants and their biodegradation intermediates. *Environ. Toxicol. Chem.* 20:2450-2455.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for

deriving numerical national water quality criteria for the protection of aquatic organisms and their uses.  
<http://www.epa.gov/waterscience/criteria/85guidelines.pdf>

Sumpter, J.P. 1998. Xenoendocrine disrupters -- environmental impacts. *Toxicol. Lett.* 102-103:337-342.

Sundaram, K.M.S. and S. Szeto. 1981. The dissipation of nonylphenol in stream and pond water under simulated field conditions. *J. Environ. Sci. Health (Part B)* B16(6):767-776.

Sundaram, K.M.S., S. Szeto, R. Hindle and D. MacTavish. 1980. Residues of nonylphenol in spruce foliage, forest soil, stream water and sediment after its aerial application. *J. Environ. Sci. Health (Part B)* B15(4):403-419.

Tabira, Y., M. Nakai, D. Asai, Y. Yakabe, Y. Tahara, T. Shinmyozu, M. Noguchi, M. Takatsuki and Y. Shimohigashi. 1999. Structural requirements of *para*-alkylphenols to bind to estrogen receptor. *Eur. J. Biochem.* 262:240-245.

Tagliabue, M.D. 1993. Nonylphenol *Champia* results. Memo dated February 23 to S. Poucher, Science Applications International Corporation, Narragansett, RI. 6 pp.

Takasawa, R., H. Etoh, A. Yagi, K. Sakata and K. Ina. 1990. Nonylphenols as promising antifouling agents found by a simple bioassay method using the blue mussel, *Mytilus edulis*. *Agric. Biol. Chem.* 54:1607-1610.

Tanghe, T., G. Devriese and W. Verstraete. 1999. Nonylphenol and estrogenic activity in aquatic environmental samples. *J. Environ. Qual.* 28:702-709.

Thibaut, R., L. Debrauwer, D. Rao and J. Cravedi. 1998. Characterization of biliary metabolites of 4-n-nonylphenol in rainbow trout (*Oncorhynchus mykiss*). *Xenobiotica.* 28:745-757.

Thibaut, R., L. Debrauwer, D. Rao and J.P. Cravedi. 1999. Urinary metabolites of 4-n-nonylphenol in rainbow trout (*Oncorhynchus mykiss*). *Sci. Total Environ.* 233:193-200.

Tsuda, T., A. Takino, M. Kojima, H. Harada, K. Muraki and M. Tsuji. 2000. 4-Nonylphenols and 4-tert-octylphenol in water and fish from rivers flowing into Lake Biwa. *Chemosphere* 41:757-762.

Tremblay, L. and G. Van Der Kraak. 1998. Use of a series of homologous in vitro and in vivo assays to evaluate the endocrine modulating actions of  $\beta$ -sitosterol in rainbow trout. *Aquat. Toxicol.* 43:149-162.

Turner, A.H., F.S. Abram, V.M. Brown and H.A. Painter. 1985. The biodegradability of two primary alcohol ethoxylate nonionic surfactants under practical conditions, and the toxicity of biodegradation products to rainbow trout. *Water Res.* 19:45-51.

Uguz, C., M. Iscan, A. Erguven, B. Isgor and I. Togan. 2003. The bioaccumulation of nonylphenol and its adverse effect on the liver of rainbow trout (*Onchorynchus mykiss*). *Environ. Res.* 92:262-270.

University of Wisconsin-Superior. 1985. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). Volume II. D.L. Geiger, C.E. Northcott, D.J. Call and L.T. Brooke (Eds.). Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, Superior, WI.  
326 pp.

U.S. EPA. 1983. Water quality standards regulation. *Federal Register* 48:51400-51413. November 8.

U.S. EPA 1986. Chapter I - Stream design flow for steady-state modeling. In: Book VI - Design conditions. In: Technical guidance manual for performing waste load allocation. Office of Water, Washington, DC.

U.S. EPA. 1987. Permit writers guide to water quality-based permitting for toxic pollutants. Office of Water, Washington, DC. EPA-440/4-87-005.

U.S. EPA. 1991. Technical support document for water quality-based toxics control. Office of Water, Washington, DC. EPA-505/2-90-001. Available for download at: <http://www.epa.gov/npdes/pubs/owm0264.pdf>

U.S. EPA. 1994. Water quality standards handbook. Second Edition. Office of Water, Washington, DC. EPA-823-B-94-005. Available for download at: <http://www.epa.gov/waterscience/standards/handbook/>

USITC. 1981. Synthetic organic chemicals: United States production and sales, 1980. United States International Trade Commission Publication No. 1183. U.S. Government Printing Office, Washington, DC 20402. 327 p.

USITC. 1989. Synthetic organic chemicals: United States production and sales, 1988. United States International Trade Commission Publication No. 2219. U.S. Government Printing Office, Washington, DC 20402.

Varma, M.M. and D. Patel. 1988. Nonionic surfactants in perspective. *J. Environ. Systems* 18:87-96.

Veith, G.D. and O.G. Mekenyan. 1993. A QSAR approach for estimating the aquatic toxicity of soft electrophiles (QSAR for soft electrophiles). *Quant. Struct. Act. Relat.* 12:349-356.

Villeneuve, D.L., S.A. Villalobos, T.L. Keith, E.M. Snyder, S.D. Fitzgerald and J.P. Giesy. 2002. Effects of waterborne exposure to 4-nonylphenol on plasma sex steroid and vitellogenin concentrations in sexually mature male carp (*Cyprinus carpio*). *Chemosphere* 47:15-28.

Ward, T.J. and R.L. Boeri. 1990a. Acute static toxicity of nonylphenol to the freshwater alga *Selenastrum capricornutum*. EnviroSystems Study No. 8969-CMA. Resource Analysts, Inc., Hampton, NH. 41 pp.

Ward, T.J. and R.L. Boeri. 1990b. Acute flow through toxicity of nonylphenol to the mysid, *Mysidopsis bahia*. Study Number 8974-CMA. EnviroSystems, Hampton, NH. 35 pp.

Ward, T.J. and R.L. Boeri. 1990c. Acute flow through toxicity of nonylphenol to the sheepshead minnow, *Cyprinodon variegatus*. Study Number 8972-CMA. EnviroSystems, Hampton, NH. 34 pp.

Ward, T.J. and R.L. Boeri. 1990d. Acute static toxicity of nonylphenol to the marine alga *Skeletonema costatum*. Study Number 8970-CMA. EnviroSystems, Hampton, NH. 42 pp.

Ward, T.J. and R.L. Boeri. 1991a. Bioconcentration test with nonylphenol and the fathead minnow, *Pimephales promelas*. Study Number 8975-CMA. EnviroSystems, Hampton, NH. 72 pp.

Ward, T.J. and R.L. Boeri. 1991b. Chronic toxicity of nonylphenol to the mysid, *Mysidopsis bahia*. Study Number 8977-CMA. EnviroSystems, Hampton, NH. 61 pp.

Ward, T.J. and R.L. Boeri. 1991c. Early life stage toxicity of nonylphenol to the fathead minnow, *Pimephales promelas*. Study Number 8979 CMA. EnviroSystems, Hampton, NH. 59 pp.

Ward, T.J. and R.L. Boeri. 1992. Toxicity of nonylphenol to the tadpole *Rana catesbiana*. Study Number 8981-MA. EnviroSystems, Hampton, NH. 78 pp.

Weinberger, P. and R. Greenhalgh. 1984. Some adjuvant effects on the fate of fenitrothion and aminocarb. *Environm. Toxicol. Chem.* 3:325-334.

Weinberger, P. and S. Iyengar. 1983. Effects of aminocarb, fuel oil 585 and nonylphenol on the growth and development of *Lemna minor* L. *Dev. Ecol. Environ. Qual.* Shuval, H. I. (Ed.). *Proc. Int. Meet. Isr. Ecol. Soc.* pp. 595-607.

Weinberger, P. and M. Rea. 1981. Nonylphenol: A perturbant additive. Canadian Tech. Rep. Fish Aquat. Sci. 990:370-381.

Weinberger, P., C. DeChacin and M. Czuba. 1987. Effects of nonyl phenol, a pesticide surfactant, on some metabolic processes of *Chlamydomonas segnis*. Can. J. Bot. 65:696-702.

White, R., S. Jobling, S.A. Hoare, J.P. Sumpter and M.G. Parker. 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135:175-182.

Wood, E. M. 1953. The toxicity of 3400 chemicals to fish. Report of Fish and Wildlife Service, Kearneysville, West Virginia. EPA 560/6-87-002. Office of Toxic Substances, Washington, DC, August.

Yadatie, F., A. Arukwe, A. Goksoyr and R. Male. 1999. Induction of hepatic estrogen receptor in juvenile Atlantic salmon in vivo by the environmental estrogen, 4-nonylphenol. Sci. Total Environ. 233:201-210.

Yokota, H., M. Seki, M. Maeda, Y. Oshima, H. Tadokoro, T. Honjo and K. Kobayashi. 2001. Life-cycle toxicity of 4-nonylphenol to medaka (*Oryzias latipes*). Environ. Toxicol. Chem. 20:2552-2560.

Yoshimura, K. 1986. Biodegradation and fish toxicity of nonionic surfactants. J. Amer. Oil Chem. Soc. 63:1590-1596.

Yoshioka, Y. 1985. Testing for the toxicity of chemicals with *Tetrahymena pyriformis*. Sci. Total Environ. 43:149-157.

Zang, L., R. Gibble and K.N. Baer. 2003. The effects of 4-nonylphenol and ethanol on acute toxicity, embryo development, and reproduction in *Daphnia magna*. Ecotoxicol. Environ. Safety 55:330-337.