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Nonylphenol: The Feminizing Free Radical Generator

by

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Abbreviations Used

APEOs	alkylphenol polyethoxylates
ER	estrogen receptor
GC/MS	gas chromatography/mass spectrometry
^{1}H-NMR	proton nuclear magnetic resonance spectroscopy
IR	infrared spectroscopy
NP	*para*-nonylphenol
PVC	poly(vinylchloride)
ROS	reactive oxygen species

Outline

Abstract

Nonylphenol is used in the production of surfactants and plastics. It is one of many xenobiotics that mimics estrogen in the body. Its phenolic group is similar to natural estrogens and its alkyl tail provides hydrophobic interactions that are necessary for binding to estrogen receptors. These properties have helped link nonylphenol to reproductive organ damage and wildlife feminization. Cells exposed to nonylphenol have also been associated with increased ROS production. Nonylphenol is degraded by light, sound and microorganisms, and its presence can be detected using common analytical methods.

Introduction

Nonylphenol is a chemical has been detected in drinking water and has been blamed for the feminization of aquatic wildlife [1]. The presence of nonylphenol in the ecosystem comes from several sources. It is used in the manufacture of many industrial products including detergents, epoxy curing, and plastic and rubber additive [1]. Effluent from sewage treatment plants contains detectable levels of nonylphenol which are thought to come from detergents used in the treatment process [2,3]. Nonylphenol can also come from polystyrene tubes that are used in laboratories everywhere [3]. It is used as a polystyrene antioxidant and has also leached from poly(vinylchloride) (PVC) tubes and from PVC used in food packaging [4]. The nonylphenol found in the environment is often a mixture of *para*- and *ortho*- nonylphenols, however, the molecule that will be discussed will be *para*-nonylphenol also known as 4-nonylphenol or NP. To understand the danger this xenobiotic presents to humans and the ecosystem, the chemistry and metabolism of this molecule must be better understood. This paper will explain the chemistry, biochemistry, degradation and detection of nonylphenol.

Chemistry and Biochemistry

4-nonylphenol (**Figure 1**) is a chemical belonging to the larger family of alkylphenolic compounds. It consists of a phenolic group with an alkyl tail attached.

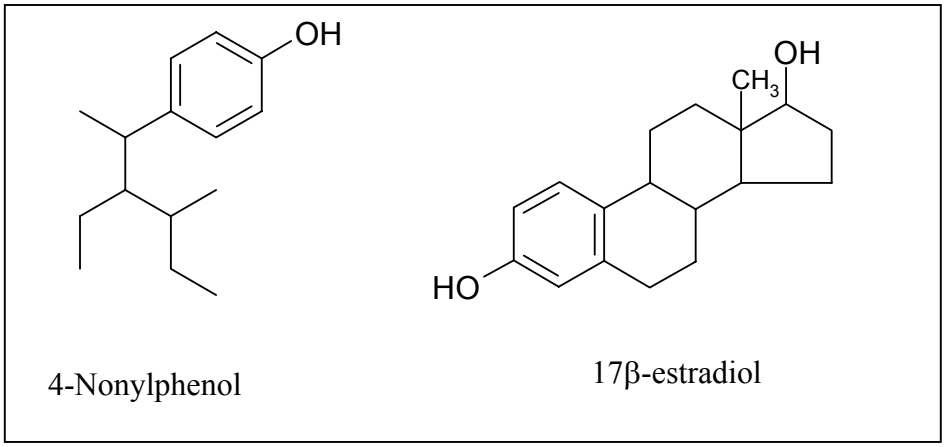

Figure 1. Nonylphenol structure is quite simple compared to the structure of 17β-estradiol. The phenolic group is present in both molecules [3,5].

Nonylphenol's structure is not particularly similar to that of the natural estrogen 17β-estradiol (**Figure 1),** although they function similarly *in vivo*. Other alkylphenolic compounds such as 4-octylphenol have very similar chemistry and biochemistry. Another related family of compounds is alkylphenol polyethoxylates (APEOs). APEOs are used as surfactants in detergents, paints, herbicides and pesticides [6]. A common application of APEOs is as a nonionic surfactant in sewage treatment. Giger and colleagues found that NP was present in sewage sludge in concentrations from 0.45 to 2.53g/kg and postulated that it was formed from the microbial biotransformation of APEOs [7]. **Figure 2** shows the formation of NP as gleaned from their research.

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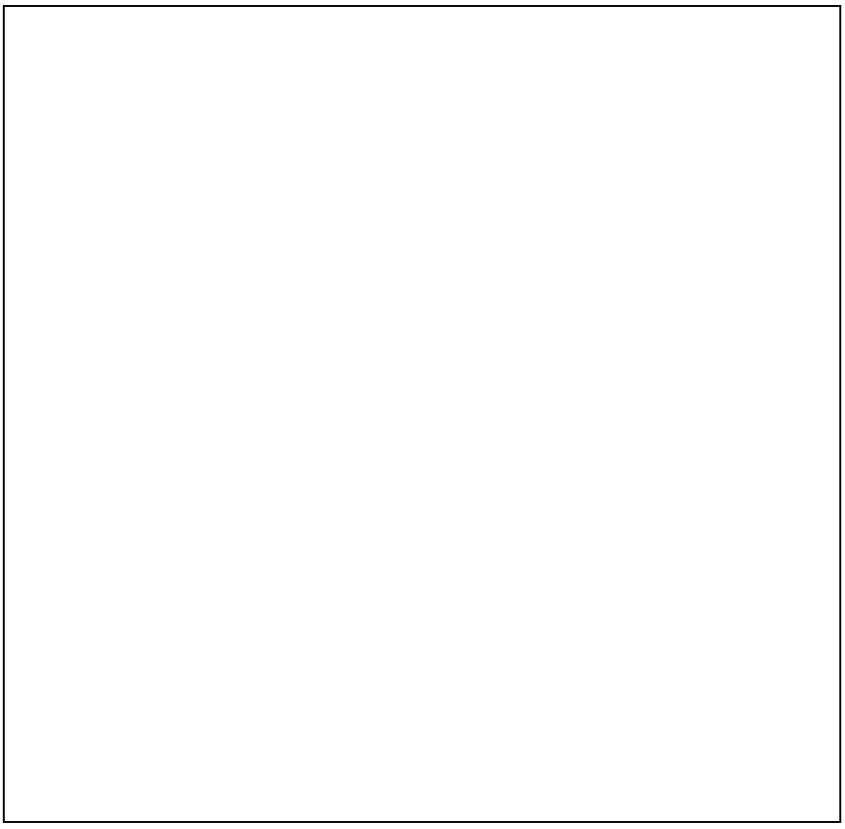

Figure 2. Nonylphenol formed through sewage sludge microbial biotransformation. A nonylphenol polyethoxylate is transformed aerobically to nonylphenol polyethoxylate with a shorter ethoxylate chain (**B**) which is anaerobically transformed to nonylphenol (**C**). Adapted from [7]

Tabira and colleagues performed experiments to determine what elements are necessary in an alkylphenol in order for it to be a good estrogen mimetic. Their *in vitro* studies found that both a phenolic group where the hydroxyl group is not hindered by large molecule and a *para*- positioned alkyl group are necessary for a high binding affinity to the estrogen receptor (ER) [8]. Although this study found that phenol binds to ER, the amount of phenol necessary to illicit a binding affinity near that of 17β-estradiol was five orders of magnitude greater than that of 17β-estradiol. This demonstrates the importance of the alkyl group. The phenolic group of alkyl phenols mimics the activity of the phenolic group of 17β-estradiol and the alkyl tail provides some unknown hydrophobic interaction with ER. The study also found that the greater the hydrophobicity of the alkyl tail, the greater the ER binding affinity [8].

The mechanism by which nonylphenol mimics estrogens in the body has been studied in great detail. It works by competitive inhibition in rainbow trout and mice: directly binding to estrogen receptors, displacing 17β-estradiol from its receptor protein. In experiments, nonylphenol bound to the β-estradiol receptor had a K_d of $5x10^{-5}$ M [1] while 17β-estradiol has a K_d of 4×10^{-10} M [9]. The pKa of nonylphenol is 10.7, which is very similar to the pKa of 10.4 for 17β-estradiol [10].

Biological Effects

There are many biological effects associated with NP exposure. White and colleagues found that nonylphenol stimulates the growth of breast cancer cells with about the same effect as that 17β-estradiol [1]. Acute exposure to nonylphenol can be toxic and even lethal. The LD_{50} of NP in rats is 1.3 kg/kg body weight and higher doses resulted in decreased weight gain and liver hemorrhages [11]. Since NP is an estrogen-mimic, it is linked to the feminization of and other reproductive dysfunction in wildlife. When male rat fetuses were exposed to NP, their reproductive systems did

not develop normally [12] while female rats exposed to nonylphenol demonstrated premature aging of mammary glands an ovariectomized female rats exposed to alkylphenols experienced vaginal cornification [13]. Nonylphenol is also associated with DNA damage and an inability to repair DNA damage [13].

Free Radical Considerations

Oxidative stress, which is characterized by a marked increase in reactive oxygen species (ROS), has been linked to several disease states such as Parkinson's disease [14]. Nonylphenol has been proven to increase the amount of hydroxyl radicals ($HO^{\bullet}$) in rat brains, thereby causing oxidative stress [15]. A mechanism for the generation of hydroxyl radicals and other ROS by natural estrogens, metabolic redox cycling, is shown in **Figure 3** [16]. Research has shown that histidine can protect these rat brain tissues that have increased free radical concentrations due to *para*-nonylphenol [17].

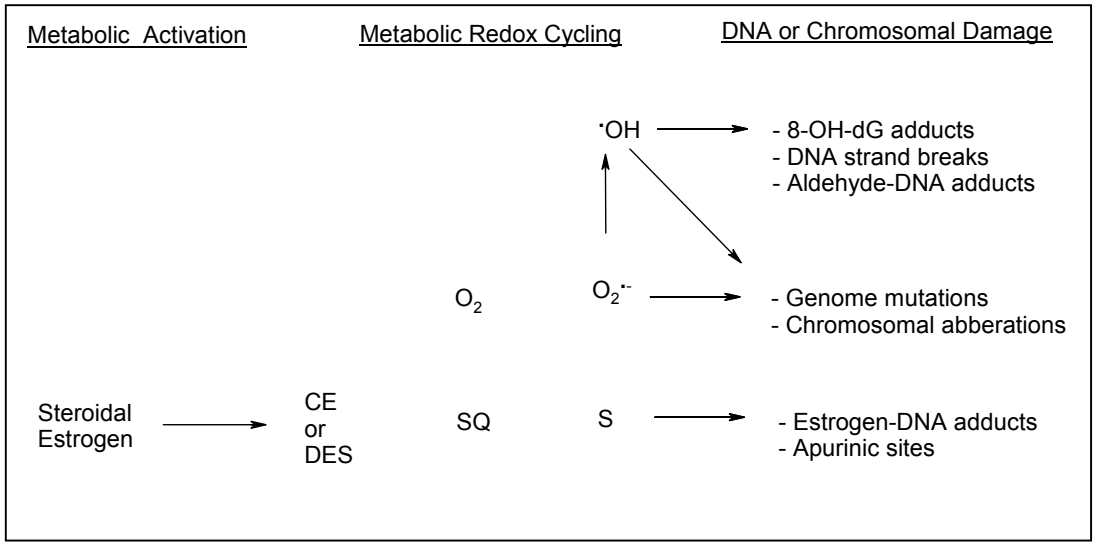

Figure 3. Hydroxyl radicals and superoxide result from estrogen metabolism and cause DNA damage [16].

Concentrations of NP from 5-50 μM have been shown to increase the generation of ROS in human blood neutrophils. Although NP increased the generation of ROS in neutrophils, it did not

seem to affect the activity of their antioxidants. When superoxide dismutase (SOD) was present, the radicals produced were effectively scavenged. Catalase, α-tocopherol and β-carotene also proved to be very effective in removing the NP-generated free radicals [18].

Similarly, NP in doses greater than 5μM caused an increase in ROS generation, inhibition of cellular growth, and ultimately cell death of *Saccharomyces cerevisae*. These effects were prevented when *S. cerevisae* cells treated and 25μM α-tocopherol or 25μM β-carotene during NP exposure, however the same dosage of the hydrophilic ascorbic acid did not provide very much protection from oxidation. *S. cerevisae* mutants with non-functional mitochondria were also spared the detrimental effects of increased ROS and cell death when exposed to NP. These results would suggest that NP causes increased generation of ROS, most likely localized in lipophilic areas of the cell such as mitochondria [19]. Another study by Okai and colleagues found that wildtype *Escherichia coli* demonstrated resistance to NP (1-200 μM), however, *E. coli* mutants with unusually low expression of catalase and superoxide dismutase suffered from increased ROS generation and marked cell death [20].

Natural estrogens including 17β-estradiol have demonstrated chain-breaking antioxidant capabilities in inhibiting lipid peroxidation [21]. Leadly and colleagues hypothesized that nonylphenol might possess the same antioxidant capability. Their study confirmed the hypothesis, finding nonylphenol inhibits liposomal membrane lipid peroxidation at high concentrations (5-50 mM) [22]. Other estrogen-mimics were shown to inhibit ox brain liposomal lipid peroxidation by decreasing the membrane fluidity, making the membrane less susceptible to oxidative damage [23]. Researchers believe that the antioxidant action of nonylphenol is due to a similar mechanism [22].

Degradation

Nonylyphenol can be degraded through microbial transformation, sound induced reactions and light induced reactions. All three methods involve the hydroxylation of the molecule. Several microorganisms have been isolated that are able to degrade NP using oxidative enzymes called laccases. Laccases (EC 1.10.3.2) are extracellular oxidoreductase enzymes that contain copper [24]. Their natural function is to break down lignin, however, they work very well to decompose NP. A number of fungi and bacteria have been identified that efficiently use laccase to degrade NP including *Clavoariopsis aquatica*, *Candida aquaetextoris,* and some *Pseudomonas* and *Sphingomonas* species [25].

Acoustic cavitation-assisted decomposition of NP occurs in water. In this method, high energy sound waves produce bubbles in water creating high temperatures, high pressures, and hydroxyl radical ($^{\bullet}OH$). The hydroxyl radical is very oxidative and attacks the NP phenolic ring, opening it and resulting in short chain organic acids. The organic acids are expected to be further be oxidized to CO_2 and water [26]. This reaction can be further catalyzed by free iron in the water. Iron will act as an electron donor for hydroxyl radical formation and an acceptor for peroxyl radical formation as shown in reactions 1-3.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\bullet} + H^+ \quad (2)$$

$${}^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \quad (3)$$

Light catalyzes similar reactions in water creating hydroxyl radicals that attack APEOs [27]. The light-induced, Fe(III)-catalyzed reaction is shown below (reaction 4).

$$Fe(OH)^{2+} \xrightarrow{h\nu} Fe^{2+} + HO^{\bullet} \quad (4)$$

Detection

Nonylphenol can be detected in several ways. It can be purified in reverse phase chromatography in a C8 column and then identified using infrared spectroscopy (IR), proton

nuclear magnetic resonance spectroscopy (^{1}H-NMR) and gas chromatography mass spectrometry (GC/MS).

Summary

Nonylphenol is a chemical that is used globally. It will continue to be present in our ecosystem and it becomes necessary to elucidate the mechanisms by which it reacts and degrades. Although it has some detrimental effects *in vivo*, it holds promise as an antioxidant in membrane systems. Continued research of environmentally friendly nonylphenol decomposition processes is necessary to gain the benefits of this molecule while preserving the quality of life for all living organisms.

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