NONYLPHENOL, AN INTEGRATED VISION OF A POLLUTANT.
SCIENTIFIC REVIEW

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(Received 1st Jan 2004, accepted 28th June 2005)

Abstract. Nonylphenol is a metabolic intermediate from the microbial transformation of detergents used worldwide. While nonylphenol shows some acute toxicity, it is also able to mimic important hormones resulting in the disruption of several processes by interfering with the signals that control the overall physiology of the organism. This work perform a critical reviews on the origin, environmental fate, microbial transformation, ecosystems impact and endocrine disruption capacity of nonylphenol. Due to mass production of parent products and potential toxicity, nonylphenol is an example of a microbial decay product that may pose an environmental risk. The analysis supports the need for better tests to evaluate, model and monitor the potential long-term environmental impact of single compounds produced as a result of an environmentally-mediated degradation.
Keywords. *Nonylphenol, toxicity, Daphnia, transformation, ecosystem, environment, pollution*

Introduction

Microbial transformation or degradation of pollutants is a common way to reduce their environmental impact. However, in some cases the chemical originally released into the environment is less toxic than the products of microbial transformation. In these cases, the toxicity tests on the original compound are useless, and in order to accurately evaluate their potential environmental impact it is necessary to know the metabolic pathways and intermediates during transformation in the environment.

Nonylphenol ethoxylates (NPE) are surfactants used worldwide, and are transformed in the environment by microorganisms to form more toxic compounds, such as nonylphenol (NP) and short-chain nonylphenol ethoxylates. These intermediates from microbial transformations, in addition to their intrinsic toxicity, seem to be able to mimic natural estrogens and disrupt the endocrine systems of higher organisms. The aim of this critical review is to evaluate the potential public health risk of nonylphenol and its derivatives and if they may alter the sexual development and the sex distribution of natural populations.

Chemistry, production and uses

Nonylphenol mixtures have an approximate molecular weight of 215 to 220 g mol-1, and are viscous pale yellow liquids with a specific gravity of 0.953 g mL-1 at 20ºC. They have a

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dissociation constant (pKa) of 10.7 ±1.0 and an octanol/water partition coefficient (log P) between 3.8 and 4.8. They exhibit both pH- and temperature-dependent solubility, showing values of 6,350 μg L-1 at pH 5 and 25ºC [1], and are soluble in many organic solvents with a vapor pressure of 4.55 x 10-3 Pa.

Chemical structure is the basis for both toxicity and endocrine system disruption. Hydrophobicity, measured as the octanol/water partition coefficient (P), has been recognized as an important parameter to determine toxicity. Factors such as absorption, excretion and tissue penetration may be related to the log P value of a compound and in certain cases predictions can be made. Hydrophobicity of substituted phenols was correlated to the 96-hr LC50 values [2]. In this study, log P was the more important parameter and exhibited a good correlation with log (1/LC50) at different pH levels. When the LC50 values were corrected for ionization using an empirically formulated relationship between toxicity and pH, the resulting regression equation could be used to predict the toxicity at pH values between 6 to 8. Also, when corrected for ionization, a correlation coefficient close to one was found between the logarithmic value of the bioconcentration factor (log BCF) of 8 phenols, and log P. The regression of log BCF on log P seems to explain the correlation between toxicity and phenol hydrophobicity.

The electrophilicity of the molecule also seems to be important for its acute toxicity. Quantitative structure-activity relationships (QSARs) were developed relating toxic potency with hydrophobicity (log P) and electrophilic reactivity quantified by the molecular orbital parameters, either the energy of the lowest unoccupied molecular orbital (ELUMO) or maximum acceptor superdelocalizability (Amax) [3]. For the full data set, ELUMO and Amax were colinear. A comparison of the QSARs with ELUMO and Amax revealed Amax to be the better electrophilic parameter for modeling toxicological QSARs for aromatic compounds [3].

Nonylphenol and other alkylphenols are produced from intermediates in the refinement of petroleum and coal-tar. Technical preparation of p-nonylphenol is a complex mixture of several isomers [4]. The process for the manufacture of alkylphenols comprises the following steps (Fig. 1): propylene oligomerization and separation of propylene trimers and tetramers. The post-reaction mixture, containing propylene dimers, trimers and tetramers and unconverted propylene, is separated by distillation. Then the propylene trimer is made to react with phenol in an alkylation reactor in the presence of an acidic ion-exchange resin catalyst. The alkylated phenol from the reactors is distilled and the product is a mixture of alkylphenols, predominantly para-substituted (4-nonylphenol) and occasionally ortho-substituted (2-nonylphenol), with various isomeric, branched-chain nonyl groups.

The endocrine interference potential of alkylphenols, reviewed here, is the major public health concern. In spite of the controversy on the endocrine effects of NP, the US demand is increasing at rate of 2% per year (Fig. 2). The US market is now 120,000 tons per year [5].

Most of NP is used as an intermediate chemical which, after etherification by condensation with ethylene oxide in the presence of a basic catalyst, produces the nonionic surfactants of the nonylphenol ethoxylate type. The nonionic surfactants are used as oil soluble detergents and emulsifiers that can be sulfonated or phosphorylated to produce anionic detergents, lubricants, antistatic agents, high performance textile scouring agents, emulsifiers for agrochemicals, antioxidants for rubber manufacture, and lubricant oil additives (Table 1) [5].

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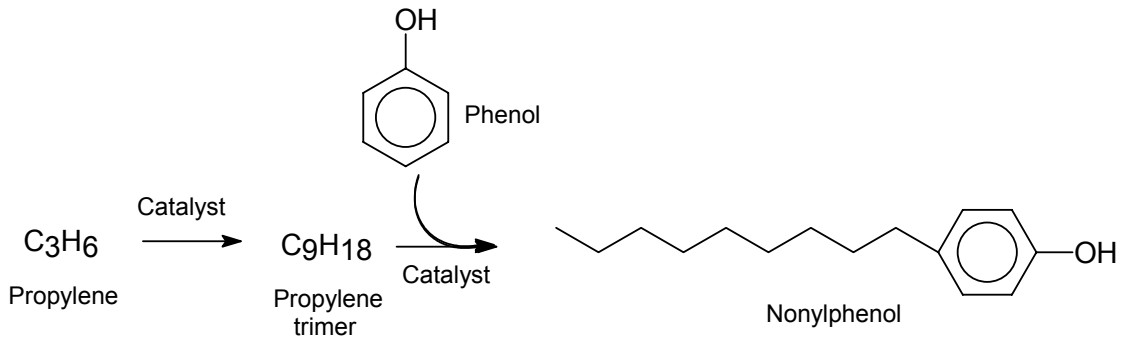

Figure 1. *Manufacture process of alkylphenols.*

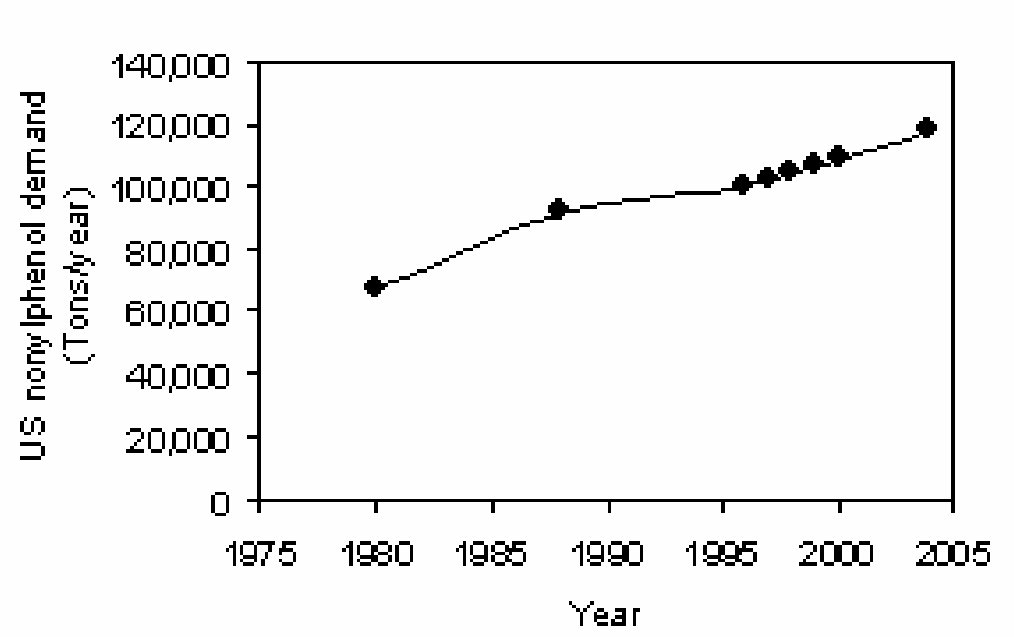

Figure 2. *Growth of the Unites States market for nonylphenol.*

Nonylphenol ethoxylates (NPE) are highly cost-effective surfactants, performing as well as or better than other nonionic detergents. Environmental and health issues continue to cast uncertainty over the future of alkylphenols and alkylphenol ethoxylates. Questions regarding their degradability have troubled producers and customers. Despite studies demonstrating their biodegradability in the environment, the regulatory future of these products still remains unclear. Major detergent suppliers do not use NPE in their household detergent products, favoring instead the more readily degradable alcohol ethoxylates.

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Table 1. *Uses of alkylphenols*

Surfactants	The largest industrial use for alkylphenols is in the manufacture of nonionic surfactants. These ethoxylated alkylphenol surfactants have good chemical stability and excellent wetting, emulsifying and detergent properties.
TNPP	Nonylphenol is reacted with phosphorus trichloride to produce trisnonylphenyl phosphite (TNPP), a common antioxidant for a wide range of polymer systems.
Phenolic Resins	Nonylphenol reacts with aldehydes to yield phenolic resins. When used with other phenols, even in small quantities, it makes the phenolic resins more water resistant, more soluble in oil, and improves electrical properties.
Rubber Chemistry	Nonylphenol sulfide has been used as a reclaiming agent for synthetic rubber.
PVC	A variety of alkylphenol derivatives have uses as polyvinyl chloride plasticizer intermediates. These intermediates include nonylphenol benzoate, nonylphenol alkanesulfonates and nonylcyclohexanol.
Epoxy Resins	Alkylphenols can be used in epoxy resin hardeners.
Miscellaneous	Other applications for alkylphenols include pharmaceuticals, corrosion inhibitors, dyestuffs, ore flotation agents, insecticides, bactericides, chemical stabilizers, and in the leather industry. Overbased calcium salt nonylphenol can also be used as a dispersant in hydraulic fluid and motor oil.

Synthesis of industrial and household liquid detergents is the major application (80% of the demand) for NP and is responsible for the anticipated steady growth for NP consumption. Other applications include lubricant oil additives (10%) and phosphite antioxidants for rubber and plastics (10%). [6]. The antioxidant tris(nonylphenol)phosphite (TNPP) has been used for decades as a stabilizer in certain plastics, such as polyethylene. TNPP contains some residual NP that can migrate out of the polymer matrix. The Food and Drug Administration (FDA) has cleared the use of these compounds in plastic food packaging. Additionally, the European Union investigated TNPP in its risk assessment of NP and concluded that TNPP does not pose a health risk. However, in January 2004, the US Environmental Protection Agency (EPA) released and requested scientific views on a draft document for NP criteria for ambient water quality. They developed draft recommendations for acute and chronic criteria for NP designed to protect aquatic life in both freshwater and saltwater. Finally, there is little direct use for NP 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.

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Toxicity

Chronic and acute toxicities of NP on aquatic organism have been recently reviewed by Servos [7], Lussier et al. [8] and Staples et al. [9]. Although the abundant data analyzed by Servos, and independently of the organism and test methods, NP showed to be toxic (LC50) to fish at concentrations from 17 to 3000 μgL-1. Invertebrates are also sensible to NP in a range of LC50 of 21 – 3000 μgL-1, and algae with LC50 between 27 to 2500 μgL-1. Evidence supporting the hazardous nature of NP is quite strong, with at least 53 chronic values representing fish, invertebrates and algae, for both freshwater and marine species (Fig. 3). In addition, embryotoxicity in crustaceans (Daphnia magna) was recorded at levels of 44 μg L-1 [10]. The range of responses for p-nonylphenol shows that exceeding a threshold concentration of 5 μgL-1 would put a large proportion of the aquatic community in risk.

Effects in mammals include the decrease of weight gain and hemorrhaging in the liver. The LD50 is 1.3 g kg-1 body weight. Short-term toxicity studies on rats exposed to 200, 650, or 2000 μgL-1 NP in their diet for 3 months were carried out [11]. A diet dose of 2000 μgL-1 caused a small decrease in body weight and food consumption, but no treatment-related clinical or histopathological changes, including effects on endocrine organs, estrous cycling, or sperm measurements were noted up to 2000 μgL-1 exposure. The no-observed-adverse-effect-level (NOAEL) was considered to be 650 μgL-1 NP in the diet (approximately 50 mg kg-1 body weight) [11]. However, an increase in uterine weight in the standard uterotrophic assays was found. Thus, the oral NOAEL value seems to range from approximately 50 to 100 mg kg-1 body weight [12, 13].

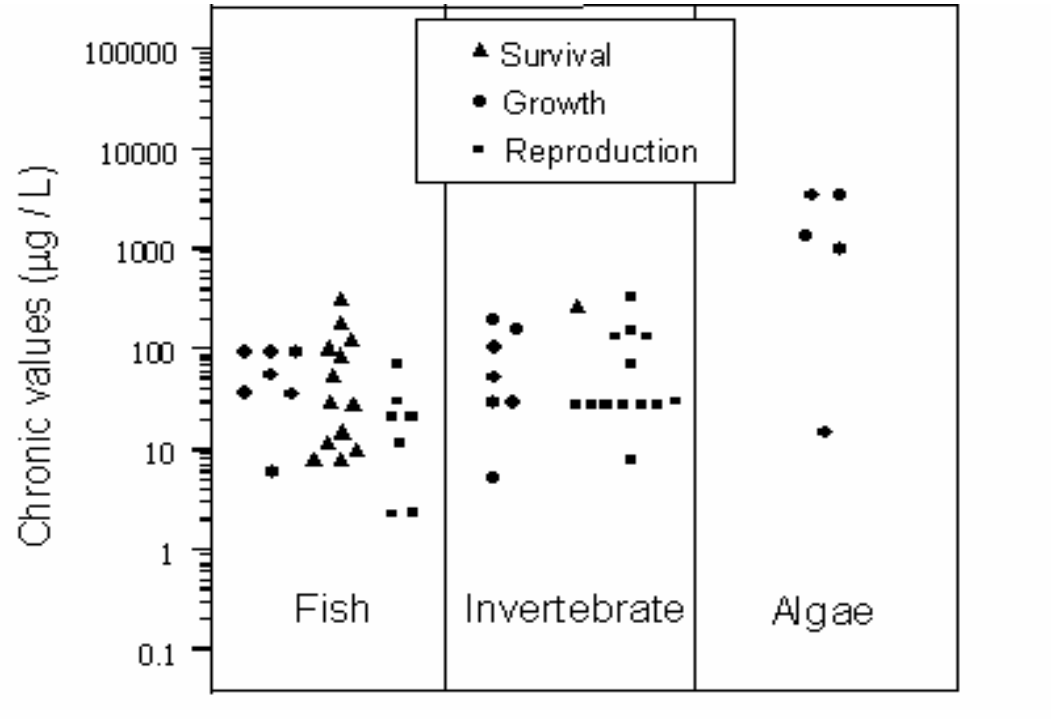

Figure 3. *Chronic toxicity values of nonylphemol on fish, invertebrates and algae, for both freshwater and marine species (adapted from Stample et al. [9]).*

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At nephrotoxic levels, NP showed limited effects on the reproductive system. Embryo- and gonadotoxicity was estimated in Sprague-Dawley rats that were treated with a NP diet [14]. Only about 1% of the oral dose entered into circulation. A reduction in body weight gain by 8 to 10% was found in the groups administered with 650 and 2000 μgL-1 NP. Vaginal opening was accelerated by approximately 2 days (650 μgL-1) and approximately 6 days (2000 μgL-1) in F1, F2, and F3 generations. Pup number, weight or viability, litter indices, or other functional reproductive parameters showed inconsistent results. Sperm indices were unchanged in F0 and F1 males. Testis and epididymis weights were unchanged [14].

Environmental fate of Nonylphenol and derivates

As mentioned above, the uses for nonylphenol ethoxylate (NPE) surfactants are diverse. In most applications, the surfactants are used briefly then disposed of into wastewater streams. Because all NPE is anthropogenic, measurement of NP and NPE provides a rapid and sensitive means for evaluating the general quality of water after human impact [15]. The environmental fate of alkylphenols and their ethoxylates has been reviewed [16], and significant concentrations of NPE and NP are found in air, waters, soils and sediments.

In the atmosphere, the presence of NP has been detected. Nonylphenol concentrations ranking from 2.2 to 70 ng m-3 in the coastal atmosphere of New York and New Jersey were detected [17]. In another study at a suburban site at New Brunswick, NP gas phase concentrations varied from 0.13 to 81 ng m-3 [18]. As expected, these values exhibited seasonal trends with higher concentrations during the summer than fall and early winter. The presence of NP concentrations in the atmosphere should be taken seriously and the potential public health risk should be evaluated for the concentrations found in urban ecosystems. So far no studies are available.

Due to their wide usage, the occurrence of NPE and NP has been reported around the world in rivers, lakes and coastal waters [16]. Water NP concentrations ranked from below detection level to significantly high concentration of 644 μgL-1 found in Spanish waters [19]. Values up to 53 μgL-1 were found in the U.K. [20] and 95 μgL-1 in U.S.A. [17]. From several studies, surface waters containing < 1 μgL-1 of NP can be considered to be of low pollution content, waters containing from 1 to 10 μgL-1 are polluted, and finally surface waters with more than 10 μgL-1 are highly contaminated. These amounts could be considered significant when with women, that are able to produce between 10 and 100 μg of estrogens daily, depending on the phase of the menstrual cycle [21]. If the presence of endocrine disruptors can easily mimic such levels, and even higher in women in their cycle, dangerous consequences may result.

NPE and NP are hydrophobic compounds (Kow 3.9-4.5) and thus it is expected that they will be rapidly adsorbed into the sediments. The concentration values for NP in sediments are several orders of magnitude higher that those found in surface waters, and can reach concentrations up to 13,700 μgkg-1. A comprehensive monitoring study designed in conjunction with the EPA, measured the levels of NP and NPE in 30 U.S. rivers. The sites, all receiving municipal or industrial wastewater, were selected randomly by a statistical procedure from the EPA's ‘river-reach’ database. Water column and bottom sediment

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samples were collected and all samples were assayed for NP and NPE1, and also higher ethoxylates (NPE2 to NPE17). Even if NP and NPE concentrations in river water were below detection limits in 60-75% of the samples (0.1 μgkg-1 for NP, NPE1 and NPE2, and 1.6 μgkg-1 for NPE3-17), a majority of sediment samples contained detectable amounts of NP and NPE1, reaching up to 3000 μgkg-1 for NP and 170 for NPE1. Nonylphenol concentrations in the sediment interstitial water were estimated to be similar to concentrations in the water column [22]. Nonylphenol ethoxylate monitoring was enhanced by including their carboxylate biodegradation intermediate metabolites. The carboxylates were measured in all significant wastewater effluent streams entering the river and in the river itself. Nonylphenol carboxylates were present in the river in low concentrations, but at levels higher than NPE, and nonylphenol carboxylate/NPE ratios in the effluents were highly variable [23]. Nonylphenoxyethoxy acetic acid was shown to be the most important carboxylate species. John et al. [24] found that NPE homologues, commonly present in commercial mixtures, were rapidly adsorbed to and desorbed from native river sediment. The adsorption partition coefficients (Kd) for the native sediment decreased progressively according the chain length of alkyl moiety from 1460 L kg-1 for NPE3 to 450 L kg-1 for NPE10. The Kd increased slightly for higher homologues. In contrast, Kd values for organic-free sediment (range 230-590 L kg-1) or kaolinite (190-490 L kg-1) increased steadily from NPE3 to NPE13. Adsorptions to silica and alumina were very weak, but all components adsorbed strongly to sewage sludge (Kd values 12,000-33,000, with maximum at NPE7). The dependence of Kd values on ethoxylate chain length impacted directly on their potency as endocrine disruptors, as discussed below.

Ferguson et al. [25] examined the concentrations and distributions of NPE surfactants and their primary neutral metabolites in sediment cores collected in 1988 and 1996. These cores were from a depositional area proximal to a wastewater treatment plant within Jamaica Bay, NY. Total NPE concentrations ranged from >50,000 μgkg-1 near the surface (deposited ca. 1990) to below detection limits (<0.1 μgkg-1) at 50 cm depth (deposited ca. 1940). NPE concentrations decreased with increasing sediment depths, reflecting increased commercial use of these compounds over the last 50 years. NPE profiles in deeper sediments were characterized by relatively higher proportions of non-metabolized, and highly ethoxylated NPE.

Fate in biological wastewater treatment facilities.

Wastewater treatment is essential for minimizing the environmental impact of residual NPE [26]. Although they are easily degraded during wastewater treatment, NPE and NP have been detected in effluents and sludge from many municipal sewage treatment plants. Doubtless, the NP concentration in the final effluents from wastewater treatment plants is indicative of the plant efficiency. Nonylphenol is the most abundant NPE derivative found in the final effluents, and concentrations up to 343 μgL-1 have been found [16].

The major NPE residue in sludge is also NP. Significant NP concentrations have been detected in sludge from wastewater treatment plants. Sewage sludge usually contains from 100 to 500 mg kg-1[19, 27]. An increasingly popular way to utilize these waste biosolids is composting to produce a useful product. Analysis of samples after 125 days of composting showed over 95% reduction of NP in the sludge and effluents [28].

The attenuation of NPE metabolites was studied at a soil aquifer treatment site [29]. One

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predominantly aerobic and one predominantly anaerobic parcel of water were monitored during infiltration. Alkylphenol ethoxycarboxylates and carboxyalkylphenol ethoxycarboxylates were detected, and no short-chained NPE were observed, even under anoxic conditions. Nonylphenol ethoxylate metabolites were rapidly removed in less than 7 days under both aerobic and anaerobic conditions. The length of the ethoxycarboxylate chain decreased with depth, and at depths greater than 3 m, only alkylphenoxy acetic acids, carboxyalkylphenoxy acetic acids, and alkylphenols remained. Octylphenol and nonylphenol concentrations decreased by approximately 80% (w/w) within 3 m of the ground surface under aerobic conditions. Under anaerobic conditions however, alkylphenol concentrations increased by approximately 38% within 3 m.

Microbial-mediated production of nonylphenol from detergents.

As mentioned above, there is limited direct use of NP and most comes from the NPE natural degradation, and any of the intermediates are considered recalcitrant to microbial attack. Ferguson and Brownawell [30] have examined the rates and pathways of NPE degradation in batch sediment slurry experiments using radiolabeled NPE mixtures. Results suggest that NPE are more persistent in sediments under anaerobic conditions than in the presence of oxygen, as reported by Brunner et al. [31]. In addition, it was illustrated that NPE degradation proceeds via separate pathways in aerobic and anoxic sediment. The aerobic biodegradation of a 14C ring-labeled NPE9 ([14C]-NPE9) was examined in a semi-continuous laboratory activated sludge and river water environments [32]. A significant portion of the 14C consisted of soluble metabolites that had degraded beyond the phenol ring. Carbon dioxide evolution and decline of radioactivity in the sludge solids both followed first order rate kinetics, with half-lives of 2.8 days and 5.8 days, respectively. A portion of the residual sludge activity was incorporated into the biomass. In the river experiment, the extent of the 14CO2 evolution from river water dosed with [14C]-NPE was monitored for 128 days. After an induction period of 21 days, 14CO2 evolution followed first order kinetics and the half-life was 22 days. This was an unequivocal demonstration that the phenolic ring of NPE is mineralized under activated sludge conditions [32]. In contrast, no evidence was observed for net production of nonylphenol from NPE during aerobic or anaerobic degradation [30]. This could be due to rapid NP degradation.

The proposed biodegradation pathway (Fig. 4) starts with the shortening of the polyethoxyl moiety leading to a short-chain NPE containing one or two ethoxy groups (NPE1 and NPE2). Further transformation proceeds by oxidation of the ethoxy chain to form nonylphenol carboxylates, such as nonylphenoxy ethoxy acetic acid and nonylphenoxy acetic acid [33]. A complete deethoxylation with formation of NP has been observed only under anaerobic conditions [34]. Nevertheless, usually NP is a mixture of different isomers. A single tertiary 14C-isomer of NP, which is believed to be one of the major branched isomers present in the NP mixtures, was synthesized for use in investigations on its metabolism [35]. The isomer was found to be resistant to biodegradation in both the lake water and sediment, showing only a slight 9% loss after 56 days and 4.2% loss after 28 days, respectively by microbial activity.

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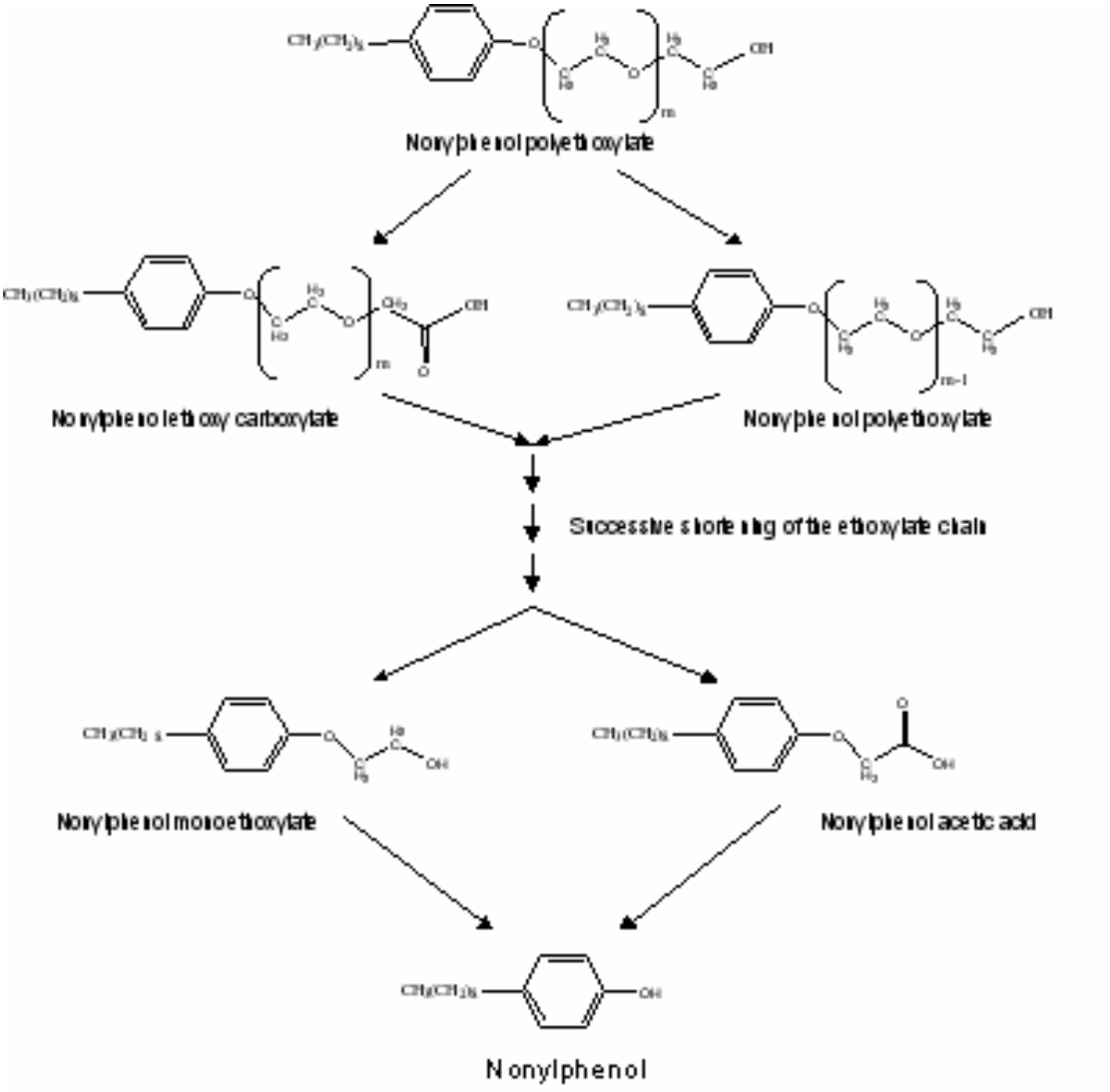

Figure 4. *Proposed microbial biodegradation pathway of nonylphenol.*

Metabolism in aquatic organisms.

Alkylphenols are lipophilic [36] and thus are accumulated in a wide range of marine and aquatic life including algae, crustacean, mollusks, and fish [37-42]. This is especially important when these organisms are part of the lower trophic level. Algae had a larger capacity for bioaccumulation of NP showing bioconcentration factors of NP reaching up to 10 000. The estimated bioconcentration factors in fish tissues ranged from 13 to 410 for NP, 3 to 300 for NPE1 and 3 to 330 for NPE2. Similar concentrations to those in the fish were determined in different tissues of a wild duck. The low NP concentrations found in some higher animals could be due to the tissue metabolism and elimination. However, information is sparse concerning the metabolic fate of alkylphenols in aquatic animals.

Biotransformation, tissue distribution, and elimination were investigated in juvenile rainbow trout following a single dose of [3H]-4-nonylphenol [43]. Total 3H-labelled residue

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concentrations in trout after 144 h were in the order: bile>> faeces >> liver >> pyloric caeca > kidney > brain, gill, gonad, heart, plasma, skeletal muscle, and skin. The depletion kinetics of [3H]-residues from tissue and plasma were shown to be biphasic with prolonged β-phase half-life in muscle and liver of 99 h. In muscle, only parent compound was found, while radio-HPLC analysis in bile, liver, pyloric caecae, and faeces samples showed similar metabolite profiles. The predominant metabolite in bile was a glucoronide conjugate of 4-nonylphenol (Fig. 5).

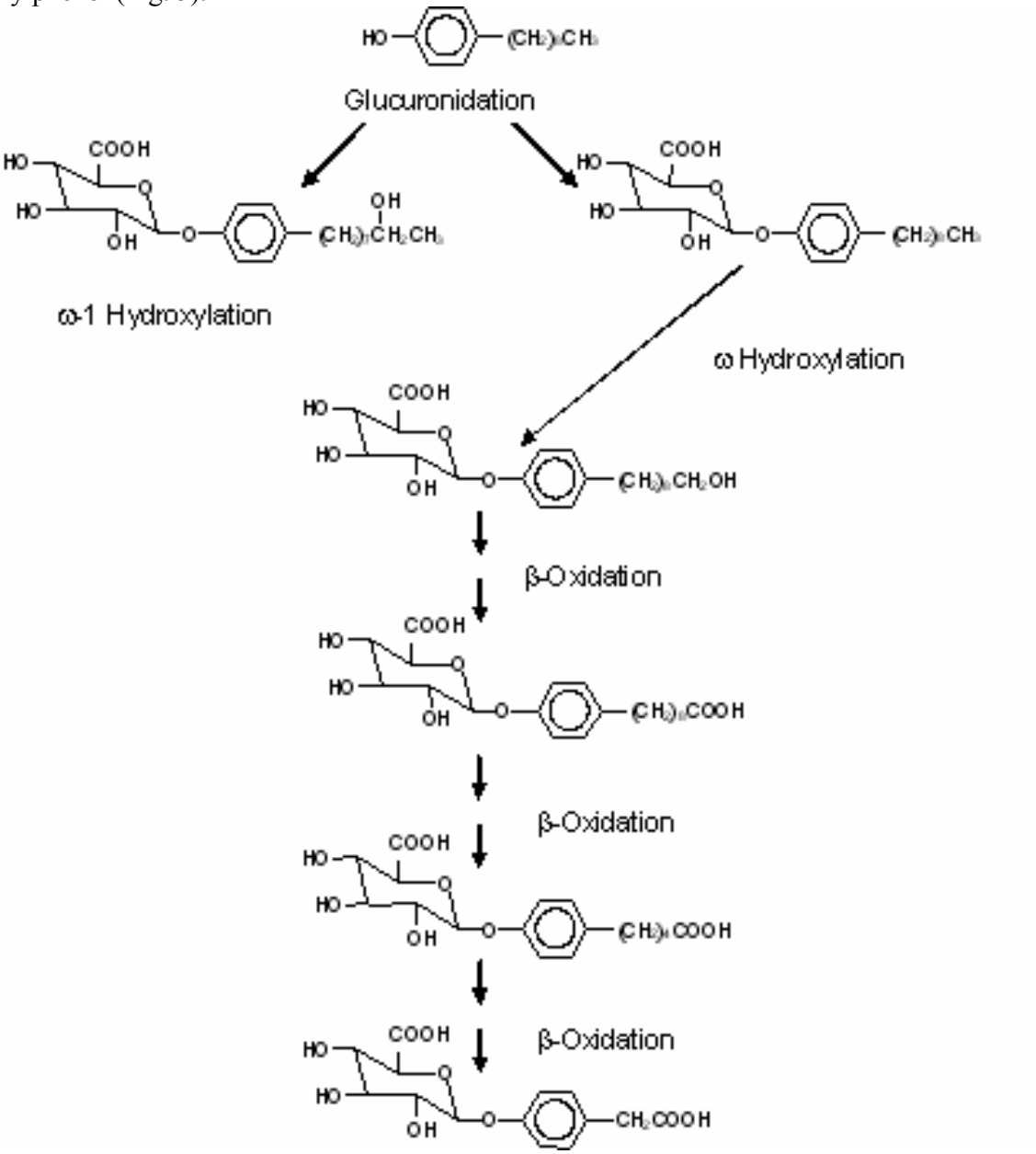

Figure 5. *Proposed pathway for the biotransformation of 4-nonylphenol by trout and by isolated hepatocytes.*

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Other metabolites included glucuronide conjugates of ring or side chain hydroxylated 4-nonylphenol. Liver contained a low level (1.7%) of covalently bound residues. Side chain (or -1) hydroxylation is a common pathway of metabolism for compounds with aliphatic side chains such as phthalates [44]. Three β-glucuronidase-sensitive metabolites of 4-nonylphenol were found in trout bile as 2-,3-, and 4-(4-hydroxyphenyl)-8-hydroxy-nonanes [45]. Metabolism studies using isolated trout hepatocytes produced a similar range of metabolites and a sulfate conjugate of hydroxylated 4-nonylphenol [46]. This may be a result of the lower KM of sulfo- compared with UDP-glucuronosyl- transferases [46], which may favor sulfonation at low substrate concentrations. Another possibility is that production of the lower molecular weight sulfate conjugate could favor excretion by the kidney and gills. It is worthwhile point out that phenol sulfotransferases, which are involved in the sulfonation of estrogenic alkylphenols, are also able to sulfonate 17-β-estradiol in human platelets, and NP inhibits this reaction [47].

Despite rapid metabolism and excretion, a substantial proportion of parent compound remained in muscle which could have implications for the maintenance of 4-nonylphenol residues and associated biological activity. After absorption, the lipophilic nature of many persistent environmental contaminants predisposes to accumulation, whereas intrinsic hormonal activity may be modulated by biotransformation. The potency of the natural estrogens, estrone and estradiol, is significantly reduced by conjugation to glucuronic and sulfuric acids [48], whereas biotransformation of certain progestogens such as PCBs may form estrogenic phenolic metabolites by the action of cytochrome P450 enzymes [49]. A reasonable scenario for the action of NP under physiological conditions would be, for instance, accumulation in fat and biotransformation in organs such as the liver, resulting in appreciable uptake into hormonally sensitive tissues. Microsomes prepared from trout liver have been used to identify three β -glucoronidase sensitive metabolites as conjugates of 4-nonylphenol hydroxylated in the C8 position of the alkyl side chain [45]. These conjugates are also present in trout bile.

The relative abundance of 4-nonylphenol metabolites in bile and isolated hepatocytes could not be accurately assigned since a proportion of the tritium was eliminated during biotransformation. However, radioactivity and ultraviolet HPLC analysis suggest that glucuronidation of the parent compound predominates over other routes of metabolism in both trout liver and hepatocytes. Relatively smaller quantities of side chain and aryl ring hydroxylates were produced, as indicated in the proposed pathway for the biotransformation of 4-nonylphenol in trout and isolated hepatocytes (Fig. 5).

Endocrin disruption

The endocrine system is a complex communication system between chemical signals and their targets responsible for regulating internal functions of the body. Any substance that alters the function of this system is termed an endocrine disruptor. Endocrine disrupting chemicals (EDCs) [46], can alter endocrine function by a variety of different mechanisms [50]: by mimicking the sex steroid hormones estrogen and androgen and binding to their natural receptors either as agonists or antagonists, by altering the synthesis and breakdown of natural hormones, and by modifying the production and functioning of hormone receptors.
Environmental estrogens include, but are not limited to, chemicals that mimic the female sex

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hormone 17-β-estradiol . Comparing structure-activity relationships for estrogenicity of 120 aromatic chemicals and 17-β-estradiol , the relative gene activation varied over eight orders of magnitude [51]. Analysis of the data compared to 17-β-estradiol structure identified three structural criteria that were related to xenoestrogen activity and potency: (a) a hydrogen-bonding ability of the phenolic ring mimicking the A-ring, (b) a hydrophobic centre similar in size and shape to the B- and C-rings, and (c) a hydrogen-bond donor mimicking the 17- β -hydroxyl moiety of the D-ring, especially with an oxygen-to-oxygen distance similar to that between the 3- and 17- β -hydroxyl groups of 17-β-estradiol . Moderately active compounds, such as NP, have a 4-hydroxyl substituted benzene ring with a hydrophobic moiety equivalent in size and shape to the B- and C-ring of 17-β-estradiol (Fig. 6). Strongly active compounds, similar to 4,4'-diethylethylene bisphenol, possess the same hydrophobic ring structure as described for moderately active compounds and an additional hydroxyl group with an oxygen-to-oxygen distance close to that exhibited by the 3- and 17-hydroxyl groups of 17-β-estradiol .

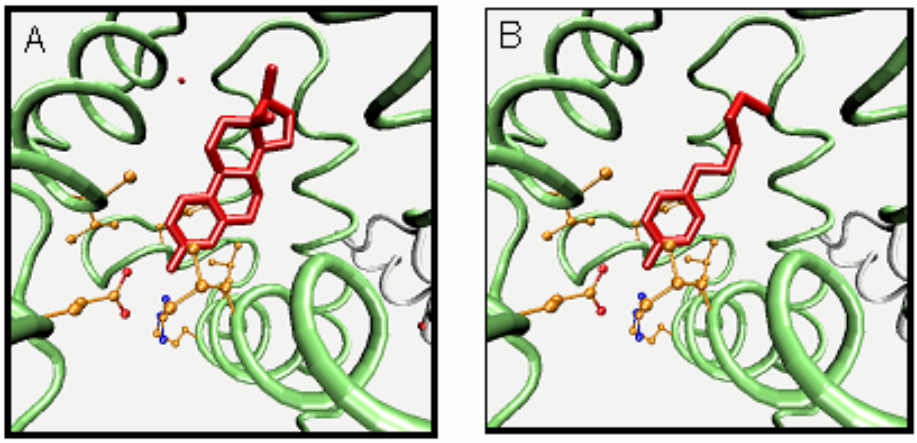

Figure 6. *17-β-Estradiol (A) and nonylphenol (B) as ligands for the estrogen receptor.*

Nonylphenol is one of the most studied estrogen mimics that appear to interact with development in several organisms. Table 2 shows several effects on different organisms including mollusks, crustaceans, fishes, amphibians, and other vertebrates. The data in Table 2 have been generated from experiments using a range of NP concentrations that are found in contaminated rivers. Incidence of hermaphroditism, delay in the settlement and metamorphosis, delay in the development to D-shape, developmental abnormalities, reduction in larval survival, and changes in the sex ratio towards females [52, 53] were found in the Pacific oyster Crassostrea gigas, which is of vital importance to the aquaculture industry.

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Table 2. *Effects on different organisms after treatment with environmental concentrations of nonylphenol.*

Nonylphenol effects	Organism	Concentration (μgL^{-1})	Ref.
- Delayed development to D-shape. - Developmental abnormalities. - Reduction in larval survival. - Delayed settlement and metamorphosis. - Changes in the sex ratio towards females. - Increase in the incidence of hermaphroditism.	*Crassostrea gigas*	0.1-10 1-100	52 53
- Perturbations of endogenous steroid metabolism. - Reduction of the testosterone elimination - Increase of the testosterone conversion rate. - Reduction of fecundity.	*Daphnia magna*	10-40	55 60 61
- Increase of fertility. - Longer second antennae.	*Corophium volutator*	10	56
- DNA adduct formation, and mutations or genomic rearrangements.	*Elminius modestus*	0.1-10	57
- Increase of the larval storage protein, cyprid major protein (CMP).	*Balanus amphitrite*		58
- Increase in egg production and a reduction in egg viability.	*Dinophilus gyrociliatus*		64
- Mentum deformities.	*Chironomous riparius*	10-100	67
- Mortality increase. - Morphologic deformations. - Apoptosis increase - Alteration of the deposition and differentiation of neural crest-derived melanocytes.	*Xenopus laevis*	0-50 0.1-10	68 69
- VTG clearance.	*Cyprinodon variegatus*	1-100	70
- Vitellogenin induction. - Choriogenin H and L mRNA induction.	*Fundulus heteroclitus* *Barbus graellsi* *Cyprinus Carpio* *Oryzia latipes*	65	71

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- Perturbation of the regulation of GTH II β gene expression. - Feminization of the phenotype. - Intersex "testis-ova" condition.	*Salmo salar*	10-125 (mg/Kg BW)	72 73 74
- Increase of mortality rates. - Stress behavior. - Decrease of body weight. - Reduction of gonadal development and reproductive function.	*Xiphophorus maculates* *Xiphophorus helleri*	14	78
- Reduction in microsomal integrity. - Negative effect on spermatogenesis and sperm quality.	mice	50-500 (drinking water)	81

Crustaceans such as Daphnia magna, benthic amphipod Corophium volutator, barnacles Elminius modestus and Balanus amphitrite, and the polychaete worm Dinophilus gyrociliatus have been studied [54-65]. Several endocrine alterations have been found after exposure to NP at concentrations ranging from 0.1 to 100 μgL-1 (Table 2). Nonylphenol has been proposed to act via the androgenic gland in Malacostracan crustacean. This gland is present only in males and regulates sexual differentiation and development of secondary sexual characteristics through a protein known as androgenic hormone [55]. An increase of fertility in C. volutator females was observed in NP exposed populations while the sex ratio was not affected. Additionally, the second antennae of exposed male animals were observed to be significantly longer that those of control animals [56]. Nonylphenol has also been suggested to induce DNA adduct formation, and/or mutations or genomic rearrangements. This DNA damage could help explain how xenoestrogens mimic the effects produced by natural estrogens, since changes at the DNA level may be the precursors of some of the numerous effects reported at higher levels of organization such as the feminization of males, developmental abnormalities, and infertility [57]. On the other hand, a significant increase of the larval storage protein (cyprid major protein, CMP) has been found in the larvae of the barnacle Balanus amphitrite after NP exposure [65]. This protein is produced during development from nauplii to cyprids, where it is necessary for the successful cyprid settlement and metamorphosis [58]. CMP and perhaps other vitellin-like proteins are potential biomarkers of low-level estrogen exposure.

A process of metabolic androgenization, in which the rate of elimination of testosterone as the glycosylated derivative is reduced, while the rate conversion of testosterone to various derivatives is increased, has been detected in Daphnia magna exposed to NP [59- 61]. Concentrations of NP that cause metabolic androgenization have been found to reduce fecundity of exposed daphnids. This reduced fecundity has been associated with developmental abnormalities and high mortality of offspring [61]. Other studies have shown that exposure to 100 and 200 μgL-1 of NP are not directly embryogenic but rather that toxicity is mediated by maternal influences during gestation [10]. However, the influence of NP on sex ratios in this organism has not been clarified [62, 63]. On the other hand, effects on survival, number of eggs produced and ratios of sexually dimorphic eggs to adults were noted in the polychaete worm Dinophilus gyrociliatus by the exposure to environmental

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concentrations of NP. No effects on growth or sex ratio were detected but NP exposure was associated with an increase in egg production and a reduction in egg viability [64].

Stimulation or antagonism effects of gonadal steroid hormones, activity changes of cytochrome P450 enzymes, effects on the thyroid hormone function or on intrinsic neuroendocrine control mechanisms, and an activation of stress response are specific mechanisms by which xenobiotic chemicals such as NP could disrupt endocrine function in vertebrate wildlife [66]. Zou and Fingerman [55] have reported that some compounds capable of disrupting hormonal processes in vertebrates, including NP, can also interfere with endocrine–mediated molting process in arthropods. This is the case of the Chironomus riparius larvae whose mouthpart deformation is often used to monitor the quality of sediments in freshwater environments. Exposure to NP increased significantly the frequency of mentum deformities [67]. This could be explained probably by physiological disturbance in the development of the buccal structures during the molting process.

Larval sexual development of amphibians is also hormone-dependent. One of the major target organs for estrogens is the liver where, in all egg-laying vertebrates, the induction of vitellogenin (VTG) is driven specifically by the female sexual steroid 17-β-estradiol . Estrogen receptors have shown a lower affinity to NP than to 17-β-estradiol using the receptor assay for [3H]-17-β-estradiol binding in the liver cytosol of Xenopus laevis [68]. Nevertheless, NP is able to bind the 17-b-estradiol receptor. Additionally, exposure to NP increased mortality, induced morphologic deformations, increased apoptosis, and altered the deposition and differentiation of neural crest-derived melanocytes in the tailbud stage of X. laevis embryos [69].

Abundant information is available on the estrogenic effects of NP on several fish, mainly freshwater species, both in vivo and in vitro. Kinetics of hepatic VTG mRNA regulation, plasma VTG accumulation, and VTG clearance have been determined during and after exposure to p-nonylphenol in sheepshead minnows (Cyprinodon variegatus), mummichog (Fundulus heteroclitus), barbs (Barbus graellsi), medaka (Oryzia latipes), and in the common carp (Cyprinus carpio) [70, 71] (see table 2). Choriogenin H and L mRNA induction has been also observed in male medaka treated with NP, showing a dose-dependant pattern. These are the precursors of the two major subunit groups of the zona radiate (ZR) proteins of medaka [71]. The influence of NP on mRNA levels of pituitary gonadotropic hormone beta subunits (leutinizing hormone beta) (GTH II β), follicle stimulation hormone beta, prolactin, growth hormone and pituitary specific transcription factor have been reported recently by Yadetie and Male [72] in individual male and female juvenile Atlantic salmon (Salmo salar). Nonylphenol seemed to have the potential to perturb the regulation of GTH II β. gene expression by mimicking exposure of male fish larvae to estrogenic hormones, during the sensitive part of gonadal development, can completely feminize the phenotype [73]. In this way, an intersex “testis-ova” condition was obtained when male medaka fish were exposed to NP [74]. The proteins of the zona radiata, estrogen receptor and VTG gene have been expressed in both NP- and 17-β-estradiol -treated juvenile rainbow trout (Oncorhynchus mykiss) showing that VTG and zona radiata protein are not independent of the increase in the transcriptional activity of the estrogen receptor early after NP and 17-β-estradiol exposure [75]. Vitellogenin mRNA has been used for the evaluation of endocrine disruption by NP in primary cultures of rainbow trout [76, 77]. Nonylphenol increased mortality rates, elicited stress behavior, decreased body weight and significantly hampered gonadal development and

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reproductive function [78]. For an extensive review of the cellular and molecular responses to general endocrine modulators on fish reproduction see Arukwe et al. [75]. Finally, the activity of the ovarian P450 aromatase and glucuronidation of testosterone and estradiol was inhibited in the carp by NP exposure of feral carp of the Ebro River. It is important to point out that in this study high and non realistic environmental concentration of NP (50 □M) was used [79].

Nonylphenol has shown microtubule-disruptive activity in Chinese hamster V79 cells. However, in whole rats no disrupting effects were observed on the cytoplasmic microtubules of Sertoli cells [80]. Nonylphenol affects the weight of reproductive organs and kidneys in parental and F1-males of CD-I mice. In females these effects were only seen in the parental organisms. A significant reduction in microsomal integrity has been reported in both generations of NP-treated mice as well as a negative effect on spermatogenesis and sperm quality [81]. The sarcoplasmic reticulum Ca2+-ATPase is thought to be a specific site of NP action in animal cells. Nonylphenol decreased the effective equilibrium constant for phosphorylation of the ATPase of rabbit skeletal muscle, probably through an increase in the effective rate of dephosphorylation of the ATPase [82].

Impact on Ecosystems

Growth retardation and low survival was observed in several organisms even at levels as low as 10 μg L-1 [56], concentrations usually found in rivers waters [16]. In an integrated evaluation of the persistence and effects of NP in a littoral enclosure, Liber et al. [83, 84] demonstrated that NP in water had a relatively short half life (≤1.2 d), whereas in sediment and macrophytes it was substantially longer (28 to 104 d and 8 to 13 d, respectively). These authors were not able to measure the half life in zooplankton and fish, but they report high survival rates. They concluded that the maximum acceptable toxicant concentration in water columns, based on the protection of the most sensitive taxa in the system, should be ≤10μg L-1. It has been demonstrated that mammals and birds are also affected by NP because it mimics the female hormone 17-β-estradiol. However there are different reports in this specific area with contradictory results. For example, Takagi et al. [85] concluded that in rats, NP at high doses (350 mg kg-1 per day), caused a decrease in maternal body weight and retardation of offspring growth, but no effect was observed on the endocrine/ reproductive endpoints of the offspring. These contradictory results may be due to a lack of a reproducible experimental design and depended on the way the NP was administered. This last study offered NP in the feed whereas in the other reports the NP was administered by injection. However, fish species have shown effects on vitellogenin synthesis in both juvenile and male Atlantic salmon [86].

Nonetheless, since rivers are primarily affected by wastewater from home and industry, most studies have focused on fresh-water organisms without considering the long-term effect on marine ecosystems and from edible fish and shellfish to man. For example, a study reported by Ekelund et al. [39] demonstrated that mussels were the most susceptible group biomagnifying NP up to 2,168 times. Growth retardation was observed in Crassostrea gigas larvae stage (D-shape) after 56 h exposure of NP even at concentrations as low as 1 μg L-1 compared to the growth observed at 0.1 μg L-1 or the controls [52], even if at 1 μg L-1 differences in survival were observed [87]. Schmude et al. [88] reported that NP

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concentrations of 41 μgL-1 resulted in low survival in the most abundant macroinvertebrates groups, Chironomidae, Oligochaeta and Mollusca. Mollusca were the most sensitive group, even if recovery was observed after 6 weeks. Oysters are extremely sensitive to NP and, combined with the frequent presence of NP in sewage discharge waters, raises concerns about the effect of this pollutant and related compounds on natural and farmed oyster populations [53].

According to a draft proposal from the EPA acute toxicity of NP was determined for several aquatic species. Fresh-water species were less sensitive with a range from 55.72 μg L-1 for an amphipod (Hyalella azteca) to 774 μg L-1 for a snail (Physella virgata), whereas salt water species ranged from 17.0 μg L-1 for the white flounder (Pleuronectes americanus) to 209.8 μg L-1 for the sheepshead minnow (Cyprinodon variegates).

Even if there is evidence that NP can be effectively removed by the organism, continuous exposure may make it difficult to be eliminated at the same efficiency that it is absorbed. For example, after 20 days of exposure to NP at concentrations from 76 to 243 μg L-1, recovery generally appeared after 1-4 weeks; however, beyond 20 days of exposure, recovery may not take place [89]. In addition, there is evidence of transgenerational effects of NP on oysters, where offspring from oysters previously grown in the presence of NP for 8 days at the larval stage, showed lower survival rates and poorer embryonic and larval development compared to those oysters that were never exposed [53].

No study on the effect of NP throughout a trophic chain is available in literature.

Public health risk

Since higher forms of life are based of the sexual recombination of genes, the effects of estrogen disruptors could be of dire importance to all members of both sexes. While life could evolve in response to other forms of pollution, environmental estrogens affect sexual differentiation, a necessary component of evolution. This facet alone may make the issue of environmental estrogen pollution important to everyone.

With solid evidence on the effects of high levels of NP on endocrine disruption, the only unanswered question is the risk of ambient NP exposure. The evaluation of risk to compounds causing endocrine may prove to be one of the greatest challenges that the risk assessment and regulatory organizations have ever faced [90]. As mentioned above, there has been only limited direct use of NP; most NP dispersed in the environment originates from the environmentally-mediated transformation of detergents. The analytical focus has been mostly on testing for acute toxicity of commercially available products and not on testing of their degradation products after dispersal in nature. Increasingly, researchers will strive to include effects on entire ecosystems, and long-term, multigenerational effects on fertility, reproductive quality, and hormonal functions. Although considerable advances have been made, there is still a great need to improve the ability to predict the environmental consequences of a new chemical on a variety of scales before the great expense of getting it into the marketplace is undertaken. There are reasonably good testing methods for acute toxicity. These tests use surrogate animals, and the correlation to humans is the weakest element. On the other hand, there is a need for better tests to assess ecological damage potentially caused by single compounds, the environmentally-transformed products, and the degradation products that find their way into the environment. Better test methods should be

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developed to evaluate, model, and monitor the potential long-term environmental impact of single compounds emitted as a result of an environmentally- mediated degradation. In vivo mammalian and fish assays could provide a comprehensive screening battery for diverse hormonal functions, such as the androgen, estrogen, and thyroid hormones. Endocrine disruption testing should include fish development and fish reproduction tests whereas a full life-cycle test could be subsequently used to refine aquatic risk assessments when necessary [91].

Some authors argue that there is no public health risk in NP, or other endocrine active compounds, due to the low concentration found in the environment [92, 93]. However, even though the biological activity of NP is weaker than of 17-β-estradiol , this compound interacts with the binding pocket of the estrogen receptor through structural similarities in the phenolic A-ring (Fig. 6). The nature and magnitude of the response is a function of the complexity of the interactions between the estrogen receptor, plasma binding, other signaling pathways, androgen antagonism, and alternate modes of estrogen action [94]. Whereas there is general agreement that high doses of NP can evoke endocrine disruption, there is no consensus that low doses of environmentally relevant levels of NP put humans at risk of endocrine disruption. Nevertheless, society is using drinking water with significant concentrations of NP up to 55.3 μgL-1 [95], and adverse consequences from exposure to NP can take an almost infinite variety of forms, including the most troubling disorders of early development.

In addition to the endocrine disruption capacity, NP shows other toxic effects. As an estrogen, NP has immunoregulatory properties that can be crucial for normal fetal development. It was hypothesized that developmental and adult exposures to NP had the potential to adversely affect the immune system. Furthermore, developmental exposure to NP might also produce differential immunomodulation in male and female rats [96] to detect the effect of NP in the immune system. Changes in splenic antibody-forming cell response, natural killer cell activity, and leukocyte numbers were used to evaluate NP immunotoxicity. The results from one study [97], indicate that dietary NP can increase splenic natural killer cell activity and splenocyte subpopulation numbers in the F1 rats, without similar changes to the F0 generation. The immunological changes that were observed in the F1 generation also appeared to be gender-specific. As we know, NP can modify hormone levels in serum, which can alter the activity of blood cells.

Nonylphenol has a direct effect on the caspase cascade and alters the cell cycle in neural stem cells [97]. Treatment with NP resulted in nuclear condensation and DNA fragmentation, morphological changes due to apoptosis, in neural stem cells after 12 h of exposure, and elevated caspase-3 after 6 h and 9 h of exposure. Furthermore, an exposure to NP led to the accumulation of cells at the G2/M phase interface and down-regulated the protein levels of cyclin A and B1, the major regulatory proteins at the G2 to M transition of the cell cycle. Together these results indicate that, in contrast to other endocrine disruptors, NP may exhibit a potent cytotoxicity through apoptosis via the caspase cascade and cell cycle arrest at the G2/M phase: thus, NP may affect neurogenesis in the central neural system.

Cancer induced by NP is not only related to spermatogenesis disorders. Vivacqua et al. [98] and Recchia et al. [99] demonstrated that NP has a direct effect in the activation of estrogen receptor alpha, which induces proliferation in breast cancer cells. Seike et al. [100] have administered NP to rats for 28 weeks. The total incidence of adenomas and carcinomas

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in the lungs of animals treated with nonylphenol and genistein were significantly higher than in the control group. They also demonstrated that NP induced formation of 8-hydroxy-2'-deoxyguanosine, a marker of oxygen radical-mediated DNA damage, which was significantly increased. These results indicate that nonylphenol has the potential to promote rat lung carcinogenesis, possibly via a mechanism involving stimulation of cell proliferation and DNA damage caused by oxygen radicals. Sakai [101] examined the effects of NP on the two-stage transformation of BALB/3T3 cells, a model of two-stage carcinogenesis. The treatment by NP in the promotion phase markedly enhanced the transformation of the cells pre-treated with a subthreshold dose of a carcinogen, 3-methylcholanthrene (MCA), but not that of non-pretreated cells. The promoting activity of NP was approximately one hundredth of that of 12-O-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter, in the cell transformation. A correlation between germ cell cancer and disorders of spermatogenesis has been proposed [102], suggesting increases in spermatogenesis disorders, and a direct effect in testicular cancer.

Conclusion

Thousands of chemicals are currently produced by our society and many are released into the environment. There is information on acute toxicity for most of them but few have been evaluated for long-term toxicity and none has a complete picture on the toxicity of their environmental intermediates. Experimental data on nonylphenol, which is the product from environmental decay of detergents used worldwide, shows that it could have a potential environmental impact and may represent a public health risk. However, still the main challenge for environmental sciences, is to develop new test methods to evaluate, model, and monitor the potential long-term environmental impact of compounds generated as result of an environmentally-mediated degradation, even if the original compound is non toxic.

Acknowledgments. We thank Professor Michael A. Pickard from the University of Alberta for the critical review of this work and discussions on environmental issues. This work was funded by the National Council for Science and Technology of Mexico (Grant FOSEMARNAT-2004-01-42).

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