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Overview of Alkylphenols as Emerging Pollutants on Fish: A Critical Review of Classical and Novel Endpoints

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ABSTRACT

Numerous studies have examined the effects of phenolic compounds on the fish endocrine system, commonly using plasma vitellogenin (VTG) expression and zona radiata proteins as biomarkers of estrogenic exposure. However, comparatively little attention has been given to the effect of endocrine-disrupting chemicals on additional endocrine pathways in fish. This review summarizes key research on the role of alkylphenols across different fish species and offers explanations to address existing discrepancies. Beyond the conventional understanding of alkylphenols as endocrine disruptors, novel aspects are also discussed. Overall, this review aims to integrate current knowledge on the effects of alkylphenols on fish and to highlight areas where further investigation is needed to complete our understanding of these compounds.

INTRODUCTION

Estrogens are hormones present in nearly all phyla of terrestrial and marine vertebrates and some invertebrates **(Zhu *et al*., 2003; Osada *et al*., 2004)**. Many endogenous forms of estrogens exist in organisms. Among them, 17β-estradiol (E2) which is considered the most biologically active, whereas estrone (E1) and estriol (E3) exhibit comparatively weaker effects **(Pinto *et al*., 2014)**. Estrogens are most widely recognized for their role in regulating gonadal differentiation, reproduction and maturation. Nonetheless, they regulate the development and balance of several organs. Additionally, estrogens participate in cell regulation and multiplication; they stimulate uterine development and are implicated in neuronal development and differentiation **(Cheshenko *et al*. 2008)**.

Recently, researchers have shown great interest toward environmental pollutants capable of mimicking the activity of endogenous estrogens. These substances, termed environmental estrogens, are believed to alter the endocrine regulation and contribute to

adverse health outcomes in both humans and wildlife, including male reproductive disorders. Consequently, they could be considered endocrine disrupting compounds (EDCs), or simply endocrine disruptors **(El-Sayed Ali *et al.*, 2014; El-Sayed Ali & Kheirallah, 2016; Cunha *et al*. 2022)**.

In the recent two decades, various review articles have documented the occurrence of novel emerging pollutants **(Deblonde *et al.*, 2011)**. Thus, this critical review aims to highlight recent advances and emerging perspectives on the classical and innovative aspects of alkylphenol and its derivatives as examples of environmental toxicants – widely used worldwide - which have severe effects on the fish not only from the estrogenic point of view but also from the non-estrogenic mechanism of action and to evaluate the potential risk of their usage. Moreover, this review furnishes the researchers with a wealthy body of literatures on the alkylphenol-fish interaction on the classical and novel endpoints that may be directly or indirectly contribute to the research development. Although all the information is published, it is disparate and placed in various journals that may not typically be consulted.

Endocrine disruptors: action and mode of action

Endocrine disrupters or EDCs are exogenous substances that interfere with normal hormonal regulation and can alter one or more functions of the endocrine system, causing severe effects on the health of intact organisms, or its progeny **(Golden *et al.*, 1998; IPCS/OECD, 1998; Damstra *et al.*, 2002; Marty *et al.*, 2011)**.

Extensive research shows that worldwide exposure to EDCs is impacting wildlife and may endanger the survival of certain species within ecosystems **(Ramasre, 2024)**. Various studies on EDCs relate to reproductive and developmental effects on aquatic animals, while much less information is well established about the effect of these compounds on terrestrial animals. Aquatic ecosystem often functions as a reservoir for pollutants originating from wastewater and atmospheric deposition, thereby posing significant risks to associated organisms.

A frequently cited example of the effect of EDCs is gonadal abnormality and reproductive impairment in fish. Reproductive impairment including reduced fertility, masculinization of females, and feminization of males have been documented in recent years in the vicinity of pollution sources worldwide **(Munkittrick *et al.*, 1998; Damstra *et al.*, 2002; Matthiessen, 2006)**.

Research on wild fish populations have reported intersex conditions as well as testicular abnormalities in a significant proportion of males from estuaries, rivers and coasts **(Lye *et al.*, 1997; Jobling *et al.*, 1998; Allen *et al.*, 1999)**. Such feminizing effects are linked to exposure to estrogens present in the aquatic habitats, and xenoestrogens in the environment. Synthetic estrogens are pharmaceutical chemical compounds developed to replicate the activity of natural estrogens, including agents such as ethinylestradiol

(EE2), a common component of oral contraceptives, and diethylstilbestrol. In contrast, xenoestrogens are environmental and industrial contaminants that, although not intended for estrogenic purposes, they exhibit estrogen-like activity, and can nonetheless elicit effects through the estrogen receptor signaling pathway.

Classically, estrogens have traditionally been considered to act solely through genomic pathways, exerting agonistic steroid-like effects via receptor binding. However, substantial evidence indicates that certain estrogenic compounds exert effects independent of estrogen receptors (ERs), suggesting that non-genomic pathways also contribute to the action of EDCs. This highlights the need for developing diverse test methods to assess the EDC potential of commercially available compounds, since multiple biochemical pathways may serve as targets. **(Marty *et al.*, 2011)**.

Estrogen/xenoestrogen receptor

On a cellular scale, estrogens act by binding to intracellular proteins known as estrogen receptors (ERs). Six distinct regions have been identified within ER sequences; of these, regions E (ligand-binding) and C (DNA-binding) are relatively conserved among teleost ERs, whereas the remaining regions display greater divergence. Since the functions of these variable regions are not fully understood, the impact of interspecies heterogeneity on signal transduction remains unclear **(Nimrod & Benson, 1996)**. Binding of natural or xeno-estrogenic compounds to ERs triggers a cascade of molecular events that may eventually influence reproduction and development **(Vethaak *et al.*, 2005)**.

By binding to ERs, xenoestrogens are able to activate or inactivate a number of genes leading to altered production of proteins in target cells **(Rampel & Schlenk, 2008)**.

The xeno-estrogens are capable to disrupt the physiological pathways of endogenous estrogen actions in vertebrates and produce effects on reproductive phenotypes such as intersex and reproductive dysfunction, and also evoke non-reproductive responses as altered regulation of genes, unexpected protein synthesis and DNA damage **(Rempel & Schlenk, 2008)**. The early developmental stages show to be more susceptible to xenoestrogens, resulting in the direct impact on viability of the young animals and long-term effects that influence diverse functions in adults. These compounds, as they mimic gonadal steroids, have organizational actions on nervous system and behavior.

Exposing male fish to (xeno-) estrogens *in vitro* stimulate efficiently the production of the estrogen-inducible yolk precursor protein vitellogenin (VTG), while simultaneously inducing testicular abnormalities, including the development of mixed gonadal tissues **(Tyler *et al.*, 1998, Legler *et al.*, 2000)**. The emphasis on (xeno-) estrogens in aquatic environments stems from evidence that wastewater treatment plant (WTP) effluents contain estrogenic compounds—including the E2 & E1 (natural

estrogens), EE2 (synthetic estrogen), and xeno-estrogens (e.g: alkylphenol surfactants) — at concentrations high enough to elicit estrogenic responses in organisms **(Tyler *et al*., 1998, Legler *et al*., 2002)**. Having outlined the mechanisms of EDCs, we now focus on alkylphenols as a key class of these pollutants.

Alkylphenols

Alkylphenols (APs), alkylphenol ethoxylates (APEs), and their derivatives constitute a large group of organic compounds that have been reported to exhibit estrogenic activity. Laboratory investigations have demonstrated that these chemicals are capable of imitating estradiol's effects in both *in vitro* and *in vivo* models **(Nimrod & Benson, 1996)**. Although alkylphenolic compounds bind to the estrogen receptor with potencies 2,000–10,000 times lower than 17β-estradiol **(Routledge & Sumpter, 1997)**, nonylphenol (NP) and octylphenol (OP) have also been shown to interact with the androgen receptor at lower affinity. Nevertheless, both NP and OP exert estrogenic effects in fish **(Jobling *et al*., 1995)**.

For over four decades, alkylphenol ethoxylates (APEs) have been broadly employed as nonionic surfactants in a wide range of industrial, such as paper and pulp processing, textiles, coatings, metal treatment, plastics, elastomers, paints, and agriculture pesticides, lubricating, industrial detergent formulations, and cosmetics **(Ahel *et al*., 1994a)**. In addition, APEs are applied as offshore cleaners, emulsifiers, and wetting agents **(Blackburn *et al*., 1999)**. Industrial uses alone account for approximately 55% of the total APE market. Additional uses of APE include industrial and institutional cleaning products (30%), household cleaning products (15%) with less than 1% employed in minor uses, including raw materials for APE production, as well as the manufacture of phenolic resins, polymers, heat stabilizers, and antioxidants in aviation jet fuel, in addition to serving as curing agents **(Ying *et al*., 2002)**. In the plastics industry, APs are additionally used as antioxidants and have been reported to leach from plastics utilized in food processing and packaging **(Soto *et al*., 1991)**.

Alkylphenol ethoxylates (APEs) are synthesized by reacting branched-chain alkylphenols (APs) with ethylene oxide results in the formation of an ethoxylate chain. The principal APs used for this purpose are nonylphenol (NP) and octylphenol (OP). Of these, nonylphenol ethoxylates (NPnEOs) dominate the market, representing roughly 80% of global production, while octylphenol ethoxylates (OPnEOs) account for the remaining ~20% **(White *et al*., 1994)**. Concerns regarding APs and APEs have been heightened in environmental research because of their widespread distribution and the persistence of their metabolites in aquatic and terrestrial ecosystems **(Kolpin *et al*., 2002; Månsson *et al*., 2008)**. APs are among the most recalcitrant organic pollutants and are capable of bioaccumulation in living organisms **(Ahel *et al*., 1993)**. Microbial degradation of APEs during sewage treatment generates alkylphenols, particularly NP and related compounds **(Giger *et al*., 1984)**. In Europe, NP is the most commercially important AP, with an

estimated annual production of ~75,000 tons, about 60% of which is used in the manufacture of NPEs **(EU, 2000)**. Due to their toxicity and persistence, many EU countries began banning the use of alkylphenols as early as 1976.

Nonylphenol (NP) is more environmentally persistent than its parent compound, nonylphenol ethoxylates (NPEs) **(Maguire, 1999)**; this compound has been environmentally detected in surface waters, aquatic sediments, and groundwater indicating its widespread distribution across different environmental compartments **(Bennie, 1999)**. Recent research has recognized nonylphenol (NP) as the primary breakdown product of nonylphenol ethoxylates (NPEs), owing to its limited biodegradability, high potential for bioaccumulation in aquatic organisms, and significant toxicity and estrogenic effects **(Ahel *et al*., 1994a)**.

Environmental fate

The most commercially important APEs are nonylphenol ethoxylates (NPEs) and octylphenol ethoxylates (OPEs), with NPEs representing nearly 80% of total APE consumption. Global production is estimated at approximately 500,000 tons annually, of which about 60% ultimately enters aquatic environments **(Sole *et al*., 2000)**. To properly evaluate their environmental risk, it is essential to understand the environmental behavior, transformation pathways, and fate of APEs. Behavior and fate are significantly affected by degradation and sorption once these substances enter the environment **(Ying *et al*., 2002)**.

In land-applied biosolids, APEs generally undergo rapid degradation, with up to 90% biodegraded within approximately three months. However, this process can be considerably slower in waterlogged soils with limited oxygen availability. Since APEs tend to adsorb strongly to soil particles, their migration from treated land is unlikely unless heavy rainfall induces erosion and sediment transport. When best management practices (BMPs) for erosion and runoff control, as mandated by state and federal regulations, are implemented, land application sites are typically not significant sources of APEs to surface waters **(Environment Canada, 2001)**.

Microbial metabolism

Microbial degradation of APEs typically begins via initial attack on the ethoxylate chain instead of an aromatic ring or hydrophobic alkyl chain **(Ahel *et al*., 1994b, El-Sayed Ali, 2023a, b)**. The degradation of ethoxylate chains occurs progressively, either through ether bond cleavage or by terminal alcohol oxidation which is subsequently followed by cleavage of the resulting carboxylic acid. According to **Bell *et al*. (1989)**, the biotransformation of octylphenol polyethoxylates was assessed in sewage-derived bacterial cultures under aerobic as well as anaerobic conditions. Similarly, **Ahel *et al*. (1994b)** reported comparable results while examining the degradation of octyl- and

nonylphenol ethoxylates using bacterial strains isolated from forest soils, wastewater, and river water. These studies consistently demonstrate that bacteria convert APEOs into short-chain APEOs, alkylphenol carboxylic acids (APECs), and alkylphenols (APs). The metabolism of these degradation products seems to proceed at a limited rate **(El-Sayed Ali *et al*., 2017; El-Saye Ali *et al*., 2018)** .

Photochemical breakdown

Apart from microbial degradation, **Ahel *et al*. (1994c)** demonstrated that NP is also liable to photochemical breakdown. Experiments conducted in natural lake water revealed that NP exhibted a half-life that ranges from 10–15 hours under continuous midday summer sunlight in the surface of the water layer, while degradation at depths of 20–25 cm occurred at a rate about 1.5 times slower. The degradation products were not identified in this study. In contrast, photolysis of NPnEO was found to proceed much more slowly, suggesting that it is unlikely to represent a significant removal pathway in aquatic environments.

Breakdown during sewage treatment

APEs are frequently transported to sewage treatment plants, where they undergo only partial degradation, yielding alkylphenolic by-products that are subsequently discharged into rivers and coastal waters with treated effluent. A modeling study estimated that in the UK, approximately 83% of NPE production is released into the environment, with about 37% entering aquatic systems **(CES, 1993)**.

Exposure routes to fish

Chemicals can enter animal bodies through multiple pathways. In fish, the primary routes of entry are generally considered to be via the gills and the gastrointestinal tract through both food and water intake. Additionally, in smaller fish, where the surface area-to-volume ratio is relatively high, dermal absorption through the skin represents an important route of exposure to toxicants **(Lien & McKim, 1993)**.

When toxicants affect aquatic organisms, the precise pathways of entry are often uncertain. The degree of toxicity is influenced not only by the uptake, metabolism and distribution of the compound but also through its molecular interactions at the site of action. Hydrophobic chemicals including 4-NP, when absorbed through the gills or skin, may elicit stronger estrogenic effects compared to ingestion via the diet. This is because dietary uptake subjects the compound to first-pass metabolism through intestine and liver before systemic circulation, where metabolic transformation may reduce the estrogenic activity of 4-NP before it reaches sensitive tissues such as the gonads or liver. In contrast, the gills provide a highly efficient route for chemical transfer between water and bloodstream **(Rand, 1995)**. Moreover, the oxygenated blood leaving the gills in fish, is rapidly distributed through major organs, including gonads, allowing toxicants to reach target tissues directly, prior to hepatic degradation. Similarly, chemicals absorbed through the skin are presumed to bypass immediate metabolism and may therefore reach internal organs in their intact form.

To assess the potency of 4-NP by several exposure routes, male fathead minnows (*Pimephales promelas*) were exposed for two weeks either through waterborne exposure or via the diet. Liver and blood samples were collected for analysis of vitellogenin mRNA expression and plasma vitellogenin concentrations, respectively. Results obtained from this study demonstrate that 4-NP exhibits a markedly greater estrogenic potency—approximately ten times higher—when taken up directly into the bloodstream via the gills or skin of fish, comparing with dietary exposure **(Pickford *et al*., 2003, Barber *et al*., 2007)**.

Bioaccumulation and biotransformation

Bioaccumulation: Aquatic organisms, including both flora and fauna, can serve as repositories for lipophilic environmental xenobiotics, accumulating these compounds from water, sediments, and food sources. Numerous investigations have shown the bioaccumulation of NP in algae, fish, ducks, and marine organisms from freshwater systems **(Ahel *et al*., 1993; Lewis & Lech, 1996; Staples *et al*., 1998; Heinis *et al*., 1999; Dasmahapatra *et al*., 2023)**. Algae, in particular, exhibit a strong capacity for NP accumulation, with reported bioconcentration factors (BCFs) reaching as high as 10,000. In fish tissues, BCFs were estimated to range from 13–410 for NP, 3–300 for NPE1, and 3–330 for NPE2. Comparable concentrations have also been detected in the tissues of wild ducks. The relatively lower NP levels found in some higher organisms may represent outcomes shaped by tissue metabolism and elimination processes. However, current knowledge of the metabolic fate of alkylphenols in aquatic organisms remains limited **(Vazquez-Duhalt *et al*., 2005)**.

Biotransformation: Tissue distribution and elimination of NP residues were determined in tissues of he rainbow trout (*Oncorhynchus mykiss*) by injecting the caudal vessel with a single dose of 0.375mg NP labeled with 37.5 µCi ^{3}H. The order of residue concentration was examined in different tissues at 1, 2, 4, 24, 48, 72 and 144h after dosing and showed the following order: bile > faeces > liver > pyloric caeca > kdney > brain, gill, gonad, heart, plasma, skeletal muscle and skin. Despite rapid metabolism and excretion, a substantial depot of parent compound remained in muscle which could have implications for maintenance of NP residues and associated biological activity **(Coldham *et al*. 1998)**. Once absorbed, the lipophilic properties of these persistent contaminants favor their accumulation, while their hormonal activity can be altered through biotransformation. Under physiological conditions, NP is likely to be stored in adipose tissue and subsequently metabolized in the liver, which may promote its distribution to hormonally sensitive organs. **(Vazquez-Duhalt *et al*., 2005)**.

Moreover, **Pedersen and Hill (2002)** examined *in vivo* the accumulation and distribution of OP in the soft tissues of rudd (*Scardinius erythrophthalmus*) during ten days. Extraction of radioactive residues of 14C-OP revealed that OP was the predominant

compound detected in muscle, ovary, and testis, while in kidney, liver, bile blood and gill, it was extensive metabolic transformation. During a 10-day depuration period, a rapid decline of soluble residues from tissues was observed.

Numerous studies have investigated the metabolism of NP and tissue distribution in juvenile Atlantic salmon (*Salmo salar*) using both *in vivo* and *in vitro* approaches **(Meucci & Arukwe, 2006). Arukwe *et al*. (2000a)** evaluated in vivo metabolism by administering a single oral dose of (^3H) 4NP to juvenile fish and collecting samples 24, 48, and 72 hours post-exposure. Findings indicated that 4NP was primarily biotransformed into its glucuronide conjugate, with smaller amounts converted into hydroxylated and oxidized derivatives. The estimated half-life of carcass and muscle tissue residues was between 24 and 48 hours post-exposure. The half-life of residues in muscle tissue was estimated to be between 24 and 48 hours after exposure. More metabolites were present in urinary samples compared with biliary samples. **Arukwe *et al*. (2000b)** extended their work to include studies on the *in vivo* and *in vitro* metabolism as well as tissue distribution of NP in salmon. *In vivo* experiments involved exposing fish either to waterborne (^3H)-4NP for 72 hours or to a single oral dose. *In vitro* assays used hepatocytes isolated from farmed salmon treated with (^3H)-4NP was also realized. The results demonstrated that 4NP was predominantly metabolized *in vivo* into glucuronide conjugates and hydroxylated metabolites, with bile serving as the primary excretion pathway. Residual levels in carcass and muscle showed a half-life of 24–48 hours for both exposure methods. Whole-body autoradiography indicated that intragastric administration led to accumulation mainly in the gastrointestinal tract and bile, whereas waterborne exposure produced a more uniform distribution across organs, including intestinal contents, brain, gills, liver, kidney, skin, and abdominal fat. *In vitro* experiments indicated that βNF pretreatment did not affect either the speed or the profile of NP biotransformation in hepatocytes. By exposing fathead minnows (*Pimephales promelas*) to 0.33, 0.93, 2.36 µg/l of NP for 42 days, there was a great accumulation of NP in fish tissue, presenting bioconcentration factors (BFs) ranging from 245 to 380 **(Snyder *et al*., 2001)**. In addition, **Ferreira-Leach and Hill (2001)** detected radioactive residues of ^{14}C OP in all tissues of the rainbow trout (*Onchorynchus mykiss*) after 1 day of exposure reaching a steady state after 4 days of exposure. The highest concentration was found in bile, followed by faeces, pyloric caeca, liver and intestine, accumulated in fat with BCFs 1180 and in muscle, brain, gills, eye and bone with bioconcentration factor of BCFs between 100 and 260.

In a study on Atlantic cod (*Gadus morhua*), **Jonsson *et al*. (2008)** administered AP (10 mg/kg fish) for 4 and 16 days and demonstrated that bile metabolites could be characterized after enzymatic deconjugation.

Estrogenic activities of nonylphenol (ER-mediated)

Environmental problems associated with estrogenic compounds are often first observed in aquatic ecosystems, manifesting as reproductive malfunction such as inter-

sex conditions and male feminization **(Jobling *et al.*, 1998)**. Potential impacts of estrogenic pollutants on reproduction in wild fish have been evaluated using biomarkers such as vitellogenin (VTG) stimulation in plasma of male and histopathological changes in gonadal tissues **(Vethaak *et al.*, 2005)**. VTG, a yolk protein precursor, is normally synthesized in the female liver under the influence of 17β-estradiol and circulates in the blood **(Mommsen & Walsh, 1988)**. Notably, male levels are typically negligible **(Kinnberg *et al.*, 2000)**. Nevertheless, exogenous estrogens can induce its synthesis, making male plasma VTG a well-established biomarker of estrogenic exposure **(Sumpter & Jobling, 1995)**.

In addition to VTG induction, natural estrogens and NP have been shown to cause structural and functional disturbances in the testes of teleosts **(Christensen *et al.*, 1999; Kinnberg *et al.*, 2000)**. Evidence also confirms that nonylphenol is capable of impairing reproductive health across vertebrate groups. For example, **Lukáčová *et al.* (2013)** demonstrated adverse impacts on fish and mammals, including testicular disruption, Sertoli and Leydig cells damage, and diminished sperm production and motility.

The mode of action of NP and OP is thought to involve binding to estrogen receptors (ERs), thereby mimicking or disrupting the normal effects of endogenous 17β-estradiol **(Nimrod & Benson, 1996)**. Estrogen normally initiates VTG synthesis through ER activation **(Rasmussen *et al.*, 2005)**. The ER itself is composed of several domains: A variable N-terminal A/B domain; a conserved DNA-binding C domain; a hinge D domain; a moderately conserved E domain for ligand binding; and a short, F domain at the carboxy terminus that are poorly conserved **(Pakdel *et al.*, 1989)**.

ERs have been identified in various vertebrates, including fish, and multiple receptor isoforms have been reported in both fish and humans **(Hawkins *et al.*, 2000)**. Although ERs from closely related species typically display similar binding affinities for both endogenous and exogenous estrogens **(Tollefsen *et al.*, 2002, 2008)**, pronounced variations in ligand binding have been reported among receptors from more distantly related species **(Harris *et al.*, 2002)**. For instance, the estrogen receptors of the rainbow trout (rtER) exhibit considerable divergence in the amino acid sequence of the ligand-binding domain (E domain), sharing only ~60% similarity with human ERα (hER) **(Pakdel *et al.*, 1989)**. This divergence suggests potential differences in ligand-binding specificity and/or affinity between fish and human receptors, which may influence how endogenous and exogenous estrogens interact across species **(Olsen *et al.*, 2005)**.

Several investigations have shown that NP exhibits estrogenic activity in teleost fish, particularly in freshwater species, through both *in vivo* and *in vitro* experiments **(White *et al.*, 1994; Routledge & Sumpter, 1996, 1997; Toppari *et al.*, 1996; Kinnberg *et al.*, 2000)**. *In vivo* exposures have consistently demonstrated elevated plasma VTG concentrations in NP-treated fish **(Christensen *et al.*, 1999)**. In species such as the sheepshead minnow (*Cyprinodon variegatus*), rainbow trout (*Oncorhynchus*

mykiss), barbs (*Barbus graellsi*), medaka (*Oryzias latipes*), tilapia (*Oreochromis niloticus*), and common carp (*Cyprinus carpio*), the dynamics of hepatic VTG mRNA expression, plasma VTG accumulation, and VTG clearance have been thoroughly characterized **(Thibaut *et al*., 1999; Hemmer *et al*., 2002; Holth *et al*., 2008; El-Sayed Ali *et al*., 2014a, b; Shaaban *et al*., 2021; Shaaban *et al*., 2022a, b)**. Similar responses have been observed in Atlantic salmon, flounder, common carp, and eelpout **(Madsen *et al*., 2002; Yadetie & Male, 2002; Rasmussen *et al*., 2005; Barse *et al*., 2006)**.

Fish embryonic gonads are bipotential, capable of differentiating into either ovaries or testes **(Demska-Zakęś & Zakęś, 2002)**. This process is regulated by genetic, hormonal, and in some cases environmental factors. Consequently, exposure to exogenous hormones or hormone-like chemicals during critical developmental windows can interfere with normal sexual differentiation. Numerous investigations have assessed the reproductive risks posed by NP in adult fish, revealing disruptions that include altered testicular morphology, impaired spermatogenesis, and changes in reproductive behavior **(Kinnberg *et al*., 2000; Weber *et al*., 2002, Miura *et al*., 2005)**. Numerous authors examined the effects of NP on zebrafish at various stages of life including embryos/larvae **(El-Sayed Ali, 2010;** El-**Sayed Ali & Legler, 2011, Ghanem, 2021)** and adults. Different aspects have been reported as the reduction in egg production **(Zoller, 2006)**, increase in bile pyrene metabolites **(Holth *et al*., 2008)**, inhibition in testicular growth and sex differentiation that lead to development of ova-testis and inhibition in testicular growth following exposure to AP **(Andersen *et al*., 2003)**. Stimulation of gonadal intersex and secondary sexual characteristics were assessed in Japanese medaka when exposed to NP. Exposure to this chemical resulted in pronounced reproductive abnormalities, including gonadal intersex in 80% of males, the presence of mixed sexual characters in more than 45% of exposed individuals, and complete inhibition of anal fin papillae in all males **(Balch & Metcalfe, 2006).**

Exposure to elevated concentrations of water PW released from off-shore oil industry operations, which contained alkylphenols (APs), has been shown to disrupt reproductive physiology in Atlantic cod (*Gadus morhua*). Female cod subjected to produced water (PW) exhibited elevated plasma VTG compared to controls, along with disrupted development of oocytes and decreased levels of estrogens. In respect to males, alteration in testis maturity was altered, with an accumulation of spermatogonia and primary spermatocytes accompanied by a decline in mature sperm production **(Sundt & Björkblom, 2011)**.

Similarly, **Del Giudice *et al*. (2012)** investigated NP-induced VTG synthesis in adult male spotted ray (*Torpedo marmorata*) using *in situ* hybridization and immune-histochemistry. Following NP injection, VTG mRNA and protein were detected in liver. These findings suggest that in *T. marmorata*, NP stimulates hepatic VTG production, with subsequent protein transport to other tissues, while testicular synthesis remains unaffected.

In a different context, **Gassel *et al*. (2013)** examined juveniles of the yellowtail fish (*Seriola lalandi*) for persistent organic pollutants and nonionic surfactants from the North Pacific Central Gyre. NP was detected in approximately one-third of the samples, with mean concentrations of 52.8 ± 88.5 ng/g ww overall and 167 ± 72.3 ng/g ww when excluding non-detects. Given NP's strong association with wastewater effluents and its limited potential for long-range transport, coupled with prior detection of NP in gyre plastics, the authors concluded that plastic-mediated exposure was the most reasonable source of NP contamination for that species.

Moreover, when hermaphrodite fish, *Mangrove rivulus* was subjected to NP, no testicular tissue was formed **(Tanaka & Grizzle, 2002)**. Moreover, a clear reduction in gonado-somatic index (GSI) was detected in platyfish **(Kinnberg *et al*., 2000)** when exposed to such class of chemicals.

Cathepsin D (CAT D) has been considered as a biomarker of xenobiotic exposure **(Carnevali & Maradonna, 2003)**. In addition to plasma VTG, eggshell proteins such as zona radiata proteins (Zrp) or their hepatic mRNA expression in juvenile male fish have also been suggested as biomarkers of endocrine disruption, since they play key roles in preventing polyspermy during fertilization and in protecting embryos from mechanical damage **(Walther, 1993)**. **Jobling *et al*. (1996)**, **Arukwe *et al*. (1997)** and **Oppen-Berntsen *et al*. (1999)** also supported their use as biomarkers. Based on these considerations, **Yang *et al*. (2006)** exposed adult zebrafish (both sexes) to NP at levels of 0.1, 1, 10, 50, 100, and 500μg/ L for twenty- one days. Gonadosomatic index (GSI) in males and females, and vitellogenin (VTG) induction in males were applied as biomarkers of parental impairment, and the study identified 50μg/ L as the no observed effect concentration (NOEC) for both endpoints. Afterward, NP-exposed females and males (50μg/ L) were crossed with control partners in clean water for 1 week to assess reproductive outcomes. The evaluated endpoints comprised embryonic CAT D activity, fecundity, thickness of eggshell, hatching success, and vertebral malformations. Whereas, no significant changes were observed in groups with NP-exposed males, exposure of females to 50μg/ L NP negatively impacted reproduction, as indicated by decreased CAT D activity ($P< 0.05$), a 23.6% reduction in eggshell thickness, and a significantly higher malformation rate ($P< 0.001$). These findings indicate that NP can cause reproductive damage in zebrafish at the parental NOEC and further suggest that CAT D activity and eggshell thickness may represent more sensitive biomarkers for assessing the reproductive effects of endocrine-disrupting chemicals.

Novel effects of nonylphenol (non ER-mediated)

Beside the previously described estrogenic impacts of these chemicals on the liver of various aquatic species, **Vetillard and Bailhache (2006)** explored the molecular

effects of 4-NP on additional organs, including the brain. Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to 4-NP concentrations ranging from 2.2µg/ L to 2.2mg/ L, and after 3 and 6 days, changes in the mRNA expression of gonadotropin-releasing hormone (GnRH) and estrogen receptor (ER) isoforms in the brain, as well as ER isoforms and vitellogenin (VTG) in the liver, were analyzed. In the liver, exposure to the highest 4-NP concentrations significantly induced VTG and ER long-isoform mRNA expression, while transcription of the ER short-isoform remained unchanged. In the brain, 4-NP caused a dose-dependent reduction in sGnRH2 gene expression, while sGnRH1 and ER mRNA levels were unchanged. Furthermore, significant alterations in body weight and fork length were observed in the rainbow trout exposed to phenolic compounds for 22 days **(Ashfield *et al*., 1998)**. In hepatocytes, 96h exposure to alkylphenols (AP) caused loss of membrane integrity, indicative of cytotoxicity **(Tollefsen *et al*., 2008)**. Metabolic inhibition was identified as a more sensitive marker of acute toxicity. In addition, epidermal structure was disrupted following NP exposure, showing irregular tissue organization, detachment of pavement cells, cytoplasmic vacuolation, severe nuclear deformation, and altered mucous cell granulation patterns **(Burkhardt-Holm *et al*., 2000)**.

Depletion of total glutathione (tGSH), CYP1A and CYP3A levels and also EROD activities in Atlantic cod (*Gadus morhua*) were also associated with phenolic compounds. However, the expression of the CYP19A2 gene was markedly enhanced in a dose-dependent manner. The expression of CYP19A1 was basically resistant to these compounds in zebrafish in 3 days **(Sturve *et al*., 2006)**. For the same species, very low concentrations (0.02, 2, 20, 40 & 80 ppm) of AP inhibited also the hepatic CYP1A enzyme activity and CYP3A protein **(Hasselberg *et al*., 2004)**.

Hepatic mRNAs expression was analyzed in zebrafish subjected to elevated concentrations of AP (200, 500, 3000, 7000, and 7500ng/ L). In males, 31% of the genes showed up-regulation after one week of exposure, with 19% still up-regulated after seven weeks. In females, gene up-regulation was limited, affecting only 8% after 1 week and 3% after seven weeks. Other effects included a decreased condition factor in males, spinal deformities in the F1 generation, and elevated bile pyrene metabolites was also detected after 7 weeks of exposure. Additionally, **Barse *et al*. (2006)** demonstrated a significant decrease in alkaline phosphatase and asprtate aminotransferase activity, alanine aminotransferase and acid phosphatase. In common carp, hepatic- and reno-somatic indices were elevated relative to controls, whereas the testicular-somatic index was decreased, accompanied by histo-architectural alterations in both testicular and hepatic tissues.

Process of parr-smolt transformation in salmonids is controlled by endocrine system and could be interrupted by such chemicals **(Boeuf, 1993)**. Exposure of Atlantic salmon (*Salmo salar* L.) smolts to environmentally relevant concentrations of the estrogenic compound of 4-NP (5–20 µg/L) during peak migration did not significantly

affect gill Na^+/K^+-ATPase activity. In contrast, exposure to combined mix of 4-NP and atrazine (5.0/1.0 & 10.0/2.0µg/ L, respectively) resulted in pronounced changes, including lowered plasma Cl^- and Na^+ concentrations, altered gill Na^+/K^+-ATPase activity, and elevated mortality following seawater transfer **(Moore *et al.*, 2003)**. Consistent results were also recorded by **Madsen *et al*. (2004).**

Example of the action of NP on parr-smolt transformation

Exposure of *Salmo salar* L. during the critical developmental stage known as parr-smolt transformation (PST) is reported to contribute significantly to population decline **(Fairchild *et al.*, 1999)**. PST is characterized by complex hormonal changes and rapid growth **(Høgåsen, 1998)**. Growth hormone (GH) plays a pivotal role during this process, both through its direct stimulation of somatic growth and its indirect role in enhancing seawater adaptation **(Ágústsson *et al.*, 2001)**. Many of the physiological actions of growth hormone (GH) are exerted indirectly through insulin like growth factor I (IGF-I) **(Green *et al.*, 1985)**. Specifically, GH promotes IGF-I synthesis through interaction with GH receptors in various tissues, with the liver being the principal site of production **(Björnsson, 1997)**. Smolts of *Salmo salar* were subjected to treatments of 20µg/ L waterborne 4-nonylphenol (4-NP). Tagged fish were individually sampled 3 times annually to assess subsequent seawater growth and plasma IGF-I levels. Both body mass and circulating IGF-I concentrations were significantly influenced by 4-nonylphenol exposure, indicating the disruption of normal hormonal functions **(Arsenault *et al.*, 2004)**. Moreover, the effects of OP on reducing both physiological and behavioral components of smoltification were investigated by **Bangsgaard *et al*. (2006)**. **Luo *et al*. (2005)** examined the roles of 4-nonylphenol on ERα gene expression in both liver and gills of sockeye salmon to clarify its molecular function during smoltification. Fish received treatments twice weekly with either 15 or 150mg NP/kg body weight at three developmental stages: Pre-, early and late smolting. Quantification of ERα mRNA levels was carried out using real-time PCR. Results showed that basal ERα mRNA expression reached its highest levels during early smolting in the liver, gill, and pituitary. At this stage, estradiol (E2) levels were increased in the liver significantly, while 4-nonylphenol exposure did not produce any detectable changes. No notable alterations were recorded in the brain. These findings indicate that basal ERα mRNA expression varies with developmental stage, suggesting that tissue sensitivity to estrogen shifts during smolting. In addition, 4-nonylphenol influenced ERα gene expression differently in gills and liver, with effects dependent on the stage of smolt development.

Endocrinologically, the pathways of estrogenic substances which affect smoltification and salt water adaptation, **McCormick *et al*. (2005)** treated Atlantic salmon with graded doses of 4-nonylphenol (0.5– 150µg/ g) for periods of 7 and 14 days. After 14 days, fish were challenged with seawater transfer for 24h to estimate their

tolerance. Increases in plasma GH occurred only at intermediate NP doses (10 and 40µg/ g), while plasma thyroxine (T4) levels declined in a dose-dependent manner, though this inhibitory effect was evident only at 150µg/ g dose after 14 days. These findings suggest that smoltification and salinity tolerance may be impaired by exposing to estrogens. These compounds impair hypo-osmoregulatory function, while NP-induced suppression of thyroid hormone levels may further disrupt the regular transformation of parr-smolt in Atlantic salmon.

Lerner *et al*. (2007) examined the long term impacts of waterborne nonylphenol (NP; 10 or 100µg/ L) on Atlantic salmon yolk-sac larvae by exposing them for 21 days and monitoring outcomes up to one year later. NP treatment leads to nearly 50% mortality during exposure, with additional deaths occurring 30 and 60 days post-treatment. After one year, fish exposed as yolk-sac larvae exhibited a reduction in sodium-potassium adenosine triphosphatase (Na^+, K^+-ATPase) activity in the gill and diminished seawater tolerance during smoltification. Behavioral assessments demonstrated a two- fold increase in latency to enter seawater and a five-fold reduction in seawater preference. Endocrine measurements showed a 20% reduction in plasma insulin-like growth factor I (IGF-I) and a 35% decrease in triiodothyronine (T3) in NP-exposed fish, whereas plasma growth hormone (GH) and thyroxine (T4) were unchanged. Additionally, NP-exposed groups displayed higher plasma cortisol levels and impaired osmoregulatory performance following handling stress. These findings suggest that the early exposure to environmentally realistic NP concentrations may cause both acute and late mortality and may impose lasting "organizational" impacts on salmonid life-history traits.

Conclusions and future directions

Evidence demonstrates that alkylphenols exert severe impacts on various fish species, affecting both endocrine and non-endocrine pathways. Despite the wealth of knowledge gained from existing literature on the actions of phenolic compounds, further studies are needed across different fish species and life stages. In particular, *in vivo* investigations at environmentally relevant concentrations are recommended to better understand ecological risks. Moreover, remediation remains a critical area of focus, as alkylphenols are difficult to remove through conventional physicochemical methods once they diffuse into leachates and wastewaters. Bioremediation, employing organisms with high alkylphenol-degrading capacity, represents a promising alternative strategy.

Declarations

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Availability of data and material (all data and materials as well as software application support their published claims and comply with field standards).

Code availability (software application).
Ethics approval (All ethics including dealing with animals are approved).
Consent to participate (I agree).
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