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Naproxen as an emerging pollutant in the aquatic environment - a review of toxicological effects on fish

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Abstract

Naproxen (NPX) is a widely used nonsteroidal anti-inflammatory drug frequently detected in surface waters, generally at concentrations below acute thresholds, although substantially higher levels may occur in polluted wastewater discharges. Its continuous release into aquatic environments raises concerns regarding chronic exposure and its potential adverse effects on fish. This study aimed to synthesize the available evidence on NPX toxicity in fish, with particular emphasis on developmental alterations, morphological abnormalities, histopathological damage, endocrine disruption, and oxidative stress responses across different species and life stages.

The review was conducted using a structured search of international databases to identify studies on NPX occurrence and toxicity in fish. Selected publications were critically evaluated for the main biological endpoints and toxicological findings. The analysed studies revealed that early-life exposure to NPX can delay hatching and induce structural abnormalities, including pericardial and yolk sac edema, scoliosis, spina bifida, and paravertebral haemorrhage in *Danio rerio* and *Cyprinus carpio* embryos and larvae. Exposure to higher NPX concentrations was associated with thyroid dysfunction, male-biased sex ratios, reduced juvenile survival, and marked changes in estrogen receptor gene expression. Histopathological findings indicated concentration-dependent damage to the gill and kidney tissues, impairing gas exchange and osmoregulatory functions. Also, oxidative stress has been identified as a common toxicological endpoint occurring at environmentally comparable concentrations (≥1 µg/L) in juvenile *Labeo rohita* and adult *Danio rerio*. These findings support the recognition of NPX as an aquatic contaminant of concern and provide a scientific basis for its inclusion in environmental monitoring and pharmaceutical risk-assessment frameworks.

Keywords: Pharmaceutical contamination, naproxen, developmental toxicity, endocrine disruption, fish early life stages

Introduction

Naproxen (NPX; (S)-2-(6-methoxynaphthalen-2-yl)propanoic acid) is a non-selective nonsteroidal anti-inflammatory drug (NSAID) that exerts its pharmacological action by inhibiting both isoforms of the cyclooxygenase enzyme (COX-1 and COX-2), thereby blocking the synthesis of prostaglandins responsible for inflammation, pain and fever (Bereketoglu et al., 2020). Because of its well-established efficacy, NPX has become one of the most widely consumed NSAIDs worldwide, available both by prescription and over the counter for the management of arthritis, dysmenorrhea, musculoskeletal pain and other inflammatory conditions (Gheorghe et al., 2016; Wojcieszyńska and Guzik, 2020; Beldean-Galea et al., 2025). Even if NPX ranks among the most frequently detected pharmaceutical compounds in European aquatic environments, including drinking water sources (Grenni et al., 2013, Przedpelska et al. 2025), still it remains broadly classified as an emerging contaminant with not a primary target status on the current active EU Surface Water Watch List. In surface water, naproxen's degradation rates are highly condition-dependent, ranging from several days to months depending on water chemistry, depth, and season (Tixier et al, 2003; Challis et al. 2014; Avetta et al, 2016). NPX mineralisation in aquatic sediment systems also varies markedly. Although Kunkel and Radke (2008) reported efficient NPX removal in deeper, anoxic bottom-sediment layers, this finding should not be generalised across all sediment types or redox conditions. In contrast, Koumaki et al. (2017) found that NPX and ketoprofen degradation was approximately twofold slower under oxygen-depleted conditions compared to aerobic conditions. Even if it lacks individual legislative restrictions, NPX is an important research target because it occurs in complex pharmaceutical mixtures and may contribute to combined effects at environmentally relevant concentrations, known as a "cocktail effect" causing additive or synergistic toxicity. So, experimental evidence on the toxicological effects of NPX in fish encompasses controlled laboratory studies examining both individual exposure and co-exposure to other pharmaceuticals (Sehonova et al., 2017; Kwak et al., 2018). The interpretation of findings across these studies requires consideration of differences in susceptibility associated with species, life stage, and exposure route, as well as the toxicokinetic processes regarding the absorption, distribution, metabolism, and excretion of the compound (Matthee et al., 2023).

The present review focuses on the occurrence and toxicological effects of NPX in fish, acting alone or in complex mixture with other water contaminants, with particular emphasis on early-life-stage exposure and developmental toxicity (Medkova et al., 2023; Kim and An, 2025). Exposure regimes range from environmentally relevant concentrations to levels exceeding those typically encountered in surface waters, enabling the assessment of diverse outcomes across biological scales. Evidence from morphological, histopathological, biochemical, molecular, endocrine, behavioral, and reproductive endpoints were integrated to explain species- and life-stage-specific responses.

1. Occurrence of naproxen in the aquatic environment

The primary route through which NPX enters aquatic environments is human and animal excretion following therapeutic use for pain management and inflammation reduction. After oral administration, a substantial fraction of the ingested dose is eliminated from the body either unchanged or as conjugated metabolites and subsequently enters municipal sewage systems. Incomplete removal during conventional wastewater treatment has mainly

contributed to the continuous accumulation of NPX in aquatic environments (Tixier et al., 2003).

In their study, Loss et al. (2009) detected NPX in 69% of the 122 river water samples analysed across 27 European countries, with concentrations reaching up to 2.027 μg/L in highly populated river basins. Grenni et al. (2013) detected NPX in the River Tiber at a concentration of 264 ± 5 ng/L, exceeding the 10 ng/L threshold established by the European Medicines Agency (EMA) for initiating a quantitative environmental risk assessment. Concentrations of NPX in surface waters across the European Union and Italy have been found to exceed the recommended environmental threshold by factors ranging from ten to five hundred (Tixier et al., 2003; Grenni et al., 2013; Liška et al., 2021).

More recent data from the Hungarian section of the Danube revealed pronounced variability in NPX concentrations both among sampling sites and, within individual sites, across distances from the riverbank (József et al., 2023). At one site, mean concentrations ranged from 177.1 ± 16.8 ng/L at 5 m to 737.1 ± 142.7 ng/L at 15 m from the bank. Also, at Békásmegyer sampling point, NPX concentrations ranged from 249.9 ± 55.6 to 486.9 ± 99.1 ng/L, in Budapest District XI sampling point ranged from 359.3 ± 80.2 ng/L to 1125.4 ± 356.0 , and in Budapest District XX varied from 3848.9 ± 733.2 to 12,029.3 ± 1772.9 ng/L. The latter represents the highest NPX concentration reported to date for the Danube (József et al., 2023). This micro-scale spatial heterogeneity suggests that NPX residues in the Danube are not uniformly distributed even within a single cross-section, likely reflecting the proximity to point-source discharges and/or incomplete mixing across the river's width.

In a three-month monitoring study of three municipal wastewater-treatment plants, two rivers, and Lake Greifensee in Switzerland, Tixier et al. (2003) found that NPX reached concentrations of up to 2.6 μg/L in plant effluents, the highest reported maximum among the six pharmaceutical compounds investigated. They determined that the average daily NPX load from the three plants was 6.06 g/day, with an additional approximately 5.6 g/day entering the lake from the two monitored rivers. River concentrations ranged from compound-specific detection limits of 1.5–10 ng/L to 400 ng/L, with NPX ranking among the highest at every sampling time. Lake modelling estimated an approximately 14-day half-life for NPX in the epilimnion, while biodegradation and photo-transformation were identified as possible elimination processes (Tixier et al., 2003).

A similar pattern is reflected in Romanian monitoring data, where different authors (Gheorghe et al., 2016; Beldean-Galea et al., 2025) reported that NPX was consistently detected at all sampling locations. The average concentrations found in Romanian surface waters, including the Argeș and Mureș rivers, the Danube, and the Danube Delta, were all below 1 μg/L. Gheorghe et al. (2016) found that the average removal efficiency of NPX in municipal wastewater treatment plants in Pitești, Brașov, and Târgu Mureș was only 57%, the lowest among the eight pharmaceutical substances monitored in that study.

A combination of biotic and abiotic processes governs the fate of NPX in aquatic environments. Despite degrading relatively rapidly, NPX is best described as a pseudo-persistent compound, meaning that its continuous reintroduction into aquatic systems through widespread anthropogenic use maintains detectable and ecotoxicologically relevant concentrations in surface waters (Grenni et al., 2013). It should also be noted that NPX degradation does not necessarily result in complete mineralisation, and the transformation products are potentially more persistent or toxic than the original compound (DellaGreca.,

2004; Cory et al., 2019). The presence of NPX in many river samples, even at low to moderate concentrations, raises concerns mostly about chronic exposure to aquatic organisms and potential cumulative effects.

2. Naproxen's bioaccumulation and bioconcentration

NPX does not show one universal accumulation pattern across aquatic animals. Beyond its presence in water, NPX has been shown to undergo bioconcentration in aquatic organisms (Brozinski et al., 2011), but its uptake and accumulation in fish are influenced by biotransformation and excretion, with substantial differences between whole-body burdens and concentrations measured in specific biological compartments (Lahti et al., 2011; Brozinski et al., 2013; Cory et al., 2019)

In rainbow trout (*Oncorhynchus mykiss*) exposed to 1.6 µg/L NPX, the parent compound and its metabolites were detected in bile, with total biliary bioconcentration factors (BCFtotal-bile) ranging from 500 to 2,300. The predominant metabolites were acyl glucuronides of NPX and 6-O-desmethylnaproxen, demonstrating uptake from water followed by metabolic conversion and biliary excretion (Brozinski et al., 2011). These values represent the combined enrichment of NPX and its metabolites in bile and should therefore be distinguished from whole-body bioconcentration factors for the unchanged compound. Consistent with these findings, Lahti et al. (2011) reported extensive NPX biotransformation in rainbow trout, with glucuronide conjugates predominating, highlighting the importance of the biological compartment and analytical inclusion of metabolites when interpreting accumulation. Field observations further support environmental uptake, as NPX-related residues were detected in the bile of wild bream (*Abramis brama*) and roach (*Rutilus rutilus*) collected downstream of a wastewater treatment plant (Brozinski et al., 2013). Although these findings demonstrate internal exposure under environmental conditions, they do not independently establish whole-body bioaccumulation or trophic biomagnification. Evidence from chronic laboratory exposure also indicates that metabolic changes may influence NPX accumulation: following 60-day exposure of zebrafish (*Danio rerio*) to 0.1–100 µg/L NPX contributes to bioconcentration, alongside thyroid disruption (Xu et al., 2019). Also, in zebrafish embryos exposed to NPX at 1 mg/L, whole-body bioconcentration factors of 1.9–9.3 were reported, indicating that even early life stages can accumulate this compound from the surrounding water (Molina-Fernandez et al., 2016). However, low whole-body bioconcentration was reported in the three-spined stickleback (*Gasterosteus aculeatus*), despite the occurrence of organ-level adverse effects (Näslund et al., 2020). Additionally, the authors report that NPX exhibits lower bioaccumulation potential, with bioconcentration factors 3-4 times lower than diclofenac. Huynh (2014) observed a similar pattern in yellow perch, with uptake occurring primarily during the first three days of exposure, followed by slow depuration, with reported elimination half-lives of 23–100 days and a whole-fish bioconcentration factor of 5.8.

These studies support NPX uptake and compartment-specific accumulation in fish, while indicating that its bioaccumulation potential requires interpretation in relation to species, exposure conditions, metabolic capacity, and the distinction between parent-compound residues and metabolite enrichment.

3. Effects of naproxen on aquatic organisms

3.1. Acute toxicity and mortality

Across standard acute toxicity endpoints, NPX consistently ranks among the least toxic pharmaceuticals tested. Guideline-based studies summarised in AstraZeneca's environmental risk assessment provide additional evidence on the acute and prolonged toxicity of naproxen to fish. In rainbow trout (*Oncorhynchus mykiss*), a 96-h exposure to naproxen acid yielded a mortality LC_{50} of 52 mg/L and a no observed effect concentration (NOEC) of 32 mg/L. In a 32-day early-life-stage test with fathead minnow (*Pimephales promelas*), the NOEC for hatching, survival, and growth was 1.0 mg/L, while the lowest observed effect concentration (LOEC) was reported as exceeding this concentration (AstraZeneca, 2025).

Li et al. (2016) reported 96 hours median lethal concentrations (LC_{50}) of 115.2 mg/L for zebrafish (*Danio rerio*) embryos and 147.6 mg/L for larvae, indicating greater embryonic sensitivity under the tested conditions. In *Cyprinus carpio*, the 96 h LC50 value of 269.15 ± 3.2 mg/L indicated the least toxicity among the eight pharmaceutical compounds evaluated in Romanian surface waters, corresponding to a low acute ecological risk quotient under Danube-relevant exposure concentrations (Gheorghe et al., 2016).

Despite this low acute toxicity, chronic exposure at sub-lethal concentrations of NPX did produce mortality in fish. Kwak et al. (2018) observed significantly reduced survival in juvenile Japanese medaka (*Oryzias latipes*) exposed to 0.5 mg/L NPX in a chronic toxicity study. Similarly, Näslund et al. (2020) reported mortality of at least 25% in each replicate aquarium of three-spined stickleback (*Gasterosteus aculeatus*) exposed to 1,232 µg/L by day 21, necessitating termination of that treatment. Mortality was also significantly elevated at 299 µg/L after 27 days.

In a 32-day common carp (*Cyprinus carpio*) embryo-larval test, Sehonova et al. (2017) examined the toxicity of NPX sodium alone and its mixture with tramadol hydrochloride. The reported lowest-observed-effect concentration was 10 µg/L for NPX sodium and for the NPX - tramadol mixture, and effects on mortality were observed in the mixture treatment, not in the NPX-only treatment.

SanJuan-Reyes et al. (2020) provided evidence of embryotoxicity in fertilised common carp (*Cyprinus carpio*) eggs exposed to six dilutions of an industrial effluent containing, undiluted, 1.09 ± 0.02 mg/L of NPX. They reported that the effluent LC_{50} and malformation EC_{50} were 0.275% and 0.133%, respectively, after 96 hours post-fertilisation. However, because the experimental exposures were conducted in complex mixtures that also contained other contaminants, these findings cannot be attributed specifically to NPX.

3.2. Developmental and morphological effects

The early-life findings are consistent with broader evidence that fish responses to pharmaceuticals depend on life stage and species-specific physiology (Matthee et al., 2023).

Much of the available evidence on the developmental and morphological effects of NPX in fish derives from studies involving co-exposure to other NSAIDs, pharmaceuticals, or contaminants present in aquatic environments. Such exposure conditions complicate the attribution of observed effects specifically to NPX, as these responses may reflect the individual or combined actions of multiple substances.

In their study regarding the subchronic toxicity of naproxen sodium alone and in combination with tramadol hydrochloride during early development in common carp

(*Cyprinus carpio*), Sehonová et al. (2017) demonstrated that both naproxen sodium alone and the naproxen-sodium/tramadol-hydrochloride mixture affected early-life-stage endpoints but with different profiles. Hatching was retarded from day 3 post-fertilization onward at all tested concentrations (10–200 mg/L), and morphometric parameters (body weight, total length, condition factor) were altered in a concentration- and time-dependent manner across the 32-day test.

In zebrafish, in a separate full-life-cycle study, Barros et al. (2024) found that NPX exposure at 0.1–5.0 µg/L decreased zebrafish growth and reproduction, with effects detected at concentrations as low as 0.1 µg/L. Bereketoglu et al. (2020) reported concentration-dependent embryonic toxicity, including mortality and delayed hatching, following acute exposure of zebrafish to a panel of NSAIDs at 0.1–50 mg/L, including NPX.

In fathead minnows (*Pimephales promelas*) exposed throughout their life cycle to a mixture of six pharmaceuticals and triclosan, with a measured NPX concentration of 793 ± 120 ng/L in the highest treatment, Parrott and Bennie (2009) reported no significant impairment of survival, growth, or reproduction in the exposed generation. Instead, more frequently abnormalities like cardiac oedema, spinal deformities and yolk-sac oedema occurred in F1 offspring. Less frequent in F1 findings included subcutaneous oedema, craniofacial and eye deformities, missing fins and haemorrhages. Because the exposure was a formulated mixture, these effects cannot be attributed only to NPX.

A comparable pattern of enhanced mixture embryo-toxicity for a hospital effluent containing 1.83 ± 0.30 µg/L NPX was reported by Luja-Mondragon et al. (2019). At 3% effluent dilution, malformations affected 41.6% of embryos; at 6% dilution, this value increased to 88.3%. The main structural abnormalities were yolk deformation, scoliosis, altered chordae structure, tail malformation, fin deformity, and mouth hyperplasia. A high rate of delayed hatching was also observed. However, the concurrent presence of other pharmaceuticals, metals, and disinfectants limits attribution of these effects specifically to naproxen.

Similarly, the industrial effluent containing 1.09 ± 0.02 mg/L NPX tested by SanJuan-Reyes et al. (2020) induced developmental abnormalities at 12 hours post-fertilisation across all 0.1–1.1% tested effluent dilutions. The authors observed concentration-dependent increases in malformations, including fin deformities, rachischisis, scoliosis, yolk deformation, and yolk-sac oedema. Severe abnormalities, including spina bifida and paravertebral haemorrhage, occurred at the 1.1% highest effluent dilution tested. The authors also reported delayed hatching at 72 hours post-fertilisation. Nevertheless, the association with other contaminants complicates addressing the observed effects only to NPX.

The differences in experimental set-ups related to species, developmental stage, exposure duration, and chemical formulation influenced the observed toxic responses, making difficult comparisons among these reported results.

3.3. Oxidative stress and molecular/gene-expression effects

As previously mentioned, oxidative stress and changes in gene expression are closely linked to the other biological responses to NPX exposure discussed in this review. These responses can therefore help to elucidate the underlying mechanisms of toxicity. The reviewed studies indicate that NPX induces context-dependent changes in antioxidant defences, oxidative damage, and cytotoxic outcomes in fish. The direction and magnitude of the response vary with exposure period, concentration, fish species, life stage, analysed tissue, and whether NPX is administered alone or as part of a mixture or industrial effluent, as well.

Stancová et al. (2015a) investigated oxidative-stress responses in adult zebrafish (*Danio rerio*) exposed to NPX at 0.001, 0.1, and 5 mg/L for 14 days. The lowest concentration was intended to represent environmental exposure. Oxidative-stress endpoints included the activities of glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S-transferase (GST), and catalase (CAT), measured after 3, 7, and 14 days, together with lipid peroxidation measured at the end of the exposure using thiobarbituric acid reactive substances (TBARS). After 3 days, GPx increased at 0.001 mg/L and GST at 0.1 mg/L. After 7 days, GPx and GR showed stronger responses, whereas GST decreased after its earlier induction. By day 14, most enzyme activities approached control values, although CAT remained elevated even at 0.001 mg/L concentration. TBARS values were 37.18±4.42 nmol/g wet tissue in controls, 49.27±7.04 at 0.001 mg/L, 53.65±5.25 at 0.1 mg/L, and 59.23±8.90 at 5 mg/L but the differences were not statistically significant.

In a complementary analysis of the same experiment, Stancová et al. (2015b) examined additional responses to NPX exposure in zebrafish exposed to 1 and 100 µg/L of NPX for two weeks, focusing on oxidative-stress-related mRNA transcripts measured in liver and intestine. The endpoints were CYP1A1, GST p2, GPx 4b, Ucp-2, and CAT. No significant transcriptional changes occurred in liver. In intestine, Ucp-2 decreased at 1 µg/L, GST p2 increased at 100 µg/L, and CAT increased at both concentrations, indicating a moderate, tissue-specific antioxidant and redox response.

Juvenile common carp exposed by Shaalan and Sayed (2025) to 14.40 µg/L naproxen for two weeks showed increased SOD (superoxide dismutase), CAT, and GPx activities in serum. The reported means values in NPX group were 29.310 U/mL for SOD, 22.380 U/mL for CAT, and 56.840 U/mL for GPx, compared with 24.630 U/mL, 16.030 U/mL, and 47.850 U/mL in controls, respectively, indicating antioxidant-defense activation.

In contrast, African catfish (*Clarias gariepinus*) exposed to 1.73–3.46 mg/L NPX for 21 days followed by 7 days of depuration showed significantly reduced SOD and CAT activities in a dose-dependent manner, whereas LPO, GR, and GPx increased in NPX s dose- and duration-dependent patterns (Melefa et al., 2025).

Another fish species investigated was *Labeo rohita,* as fingerlings, exposed to 1 µg/L and 50 µg/L NPX for 35 days (Priyadarshinee et al. 2023). The authors investigated the oxidative stress in the liver induced by NPX, with sampling on days 7, 14, 21, 28, and 35. The oxidative-stress panel included hepatic reactive oxygen species (ROS), LPO, CAT, SOD, GSH, GPx, and GST. Hepatic ROS increased significantly at both concentrations throughout the exposure period, while LPO was significantly higher in both NPX groups at all time points except day 7 at 1 µg/L, indicating dose- and time-dependent oxidative damage. In contrast, CAT activity was significantly reduced on most sampling days, SOD activity decreased significantly at most time points, and GSH levels declined significantly in exposed fish, with non-significant exceptions mainly in the low-dose group at days 7 and 14. GPx activity also decreased significantly in most treatment-time combinations, whereas GST activity increased significantly at both concentrations. Overall, Priyadarshinee et al., (2023) demonstrated that the environmentally relevant levels of NPX exposure increased ROS and lipid peroxidation while depleting or inhibiting major antioxidant defenses in *L. rohita*.

Furthermore, extensive evidence of oxidative stress was more featured in exposures of the fish to complex mixtures of NPX with other water contaminants. For example, the experiment conducted by Sehonova et al. (2017) highlighted the fact that mixture exposure modified the response to naproxen only. In common-carp embryos and larvae exposed for

32 days days to naproxen sodium alone or to a 1:1 NPX-Na–tramadol hydrochloride mixture at 10, 50, 100, and 200 mg/L, the profiles of the outcomes were different. The NPX-Na alone reduced GR at 100 mg/L, did not significantly change GPx, produced a bidirectional GST response, and reduced TBARS at 10 mg/L rather than increasing lipid peroxidation. In contrast, the 1:1 naproxen sodium–tramadol hydrochloride mixture decreased GST at 100 mg/L and GPx at 50–200 mg/L, GR was unchanged while TBARS increased at 50 and 100 mg/L.

Also, San Juan-Reyes et al. (2013) examined the effects of exposure to effluent from an NSAID-manufacturing plant on common carp. The exposure times were 12, 24, 48, 72 and 96 hours. Data showed increased hydroperoxides, MDA-associated lipid peroxidation, and protein carbonyls, especially in gill. SOD increased in gill, liver, and blood but decreased in brain, while CAT and GPx increased in several tissues. In this study, gill showed the greatest overall response, identifying it as the principal target tissue for this effluent-induced oxidative injury. In 2015, SanJuan-Reyes et al. reported related outcomes based on ROS/RNS-mediated biomolecular damage for the anterior experiment. This time, the endpoints were: comet-assay DNA damage, micronucleus frequency, caspase-3 activity, and TUNEL-positive apoptotic cells. DNA damage increased significantly relative to controls from 24 h and reached its maximum at 48 h; micronuclei increased significantly from 24 h, with the highest frequency at 72 h. Caspase-3 activity increased significantly at 24, 48, and 96 h, peaking at 48 h, while TUNEL-positive cells increased from 24 h and reached their maximum at 72 h. NPX concentration showed strong positive correlations with comet-assay DNA damage at 12 hours, micronuclei at 48 hours and 72 hours, and caspase-3 activity at 48 hours. The authors concluded that the industrial effluent induced geno- and cytotoxicity in carp, with oxidative stress proposed as an important pathway linking NSAID exposure to DNA damage and apoptosis (SanJuan-Reyes et al., 2015). Because the effluent also contained other contaminates, these findings cannot be assigned specifically to naproxen.

3.4. Histopathological effects

Evidence from the analysed studies in this review suggests that NPX affects multiple organs in fish, with lesion features shaped by species, developmental stage, exposure duration, and concentration.

In adult zebrafish exposed for 14 days to 0.001, 0.1, or 5 mg/L naproxen, Stancová et al. (2015) reported no apparent treatment-related changes in the kidney, skin, or intestine but gill and liver tissues responded to environmentally relevant NPX exposure. Thus, gills showed hyperemia, widening of the leaflet apex, and epithelial desquamation even at the environmental concentration of 0.001 mg/L. Hepatic alterations included separation of hepatocytic trabeculae, hyperemia, and edema at 0.001 mg/L, steatosis at 0.1 mg/L, and mixed steatosis and glycogen-type dystrophy at 5 mg/L.

Gill's tissue structural abnormalities were also reported by Sehonova et al. (2017) in common carp embryo and larvae after 32 days of exposure to NPX-Na alone or a 1:1 NPX-Na –tramadol mixture at 10–200 mg/L. At 10 mg/L NPX-Na only, gill leaflets were deformed. The mixture produced a broader change, including increased skin mucous cells, gill-lamella deformation, and pericardial effusion.

Hepatic lesions were observed by Priyadarshinee et al. (2023) in *Labeo rohita* exposed to 1 or 50 µg/L for up to 35 days. The response was dose- and time-dependent. At 1 µg/L, pyknotic nuclei and necrosis occurred by day 7, followed by hepatocyte and cytoplasmic

degeneration by day 35. At 50 µg/L, lesions progressed from intercellular spaces and hepatocyte aggregation to pyknosis, inflammatory infiltrates, necrosis, vacuolation and hepatocyte degeneration (Priyadarshinee et al., 2023).

Moreover, early life stages appeared particularly susceptible to liver structural injury. Li et al. (2016) exposed zebrafish embryos and larvae to naproxen and examined larval liver after eight days. Five-day exposure to 75 and 100 mg/L caused hepatocyte swelling, vacuolar degeneration, indistinct cell borders, and, at 100 mg/L, nuclear pyknosis. After eight days, slight vacuolar degeneration and pyknosis were observed at 10 mg/L, more pronounced lesions at 50 mg/L, and severe injury at 75 mg/L, including pyknosis, hepatocyte necrosis, and cytolysis. The severity of hepatic injury therefore increased with both concentration and exposure duration. Other changes like hatching inhibition, pericardial edema, and malformations were also considered signs of developmental toxicity (Li et al., 2016).

Renal toxicity represents another significant endpoint in NPX's toxicity profile. The kidney is essential for maintaining osmotic and acid-base homeostasis, processes that are metabolically expensive and especially critical for larval survival. In the experiments conducted by Naslund (2020, 2026) on juveniles of three-spined stickleback (*Gasterosteus aculeatus*) exposed to 0, 18, 70, 299 or 1232 µg/L of NPX. The concentrations of 299 and 1232 µg/L induced renal hematopoietic hyperplasia (45% and 78% incidence, respectively) in fish. This pathological response indicates stress-induced proliferation of hematopoietic tissue within the kidney, suggesting either impaired oxygen delivery (prompting compensatory hematopoiesis) or direct chemical stimulation of hematopoietic lineages. Altered hepatocellular vacuolation occurred at 299 µg/L but decreased at 1232 µg/L, a change the authors considered potentially related to reduced food intake rather than direct hepatic injury. The study found no tubular necrosis and jaw lesions occurred mainly at the higher concentrations (Naslund, 2026).

Barros et al. (2024) reported gonadal alterations in zebrafish exposed across the full life cycle to 0.1–5.0 µg/L naproxen, with changes detected at 0.1 µg/L alongside reduced growth and reproduction.

In conclusion, qualitative histopathological responses were demonstrable across gill, liver, kidney, and gonadal tissues, with inconsistent quantitative assessment. Also, evidence for severe lesions were strongest at elevated or prolonged exposures, while claims of effects at environmental concentrations require context interpretation and independent replication.

3.5. Reproductive and endocrine-disrupting effects

Direct NPX evidence for endocrine disruption and reproductive impairment in fish was less extensively characterised endpoints of NPX exposure in fish. Experimental studies indicate that naproxen can impair fish reproduction and alter endocrine-related molecular pathways, although the magnitude of these effects depend on species, life stage, exposure duration, and concentration.

Kwak et al. (2018) tested the fish species medaka (*Oryzias latipes*) and measured steroidogenic responses in vitro using human adrenocortical carcinoma cell line H295R. At 0.5 mg/L, NPX significantly decreased juvenile medaka survival and significantly increased transcription of the estrogen-receptor gene, erβ2. At 10 mg/L in H295R cells, NPX significantly increased 17β-estradiol level and the estradiol to testosterone ratio, suggesting that it can modulate sex-hormone production and altered transcription of steroidogenesis-

related genes (vtg1, erb2, cyp17). The same study reported that detected NPX levels in ambient Korean rivers were far below the effective concentrations measured in the experiment, while warning that adverse effects, including endocrine disruption, were biologically plausible and could not be excluded in highly polluted areas (Kwak et al., 2018).

The strongest reproduction-specific evidence comes from a full-life-cycle zebrafish study. Barros et al. (2024) exposed *Danio rerio* to 0.1–5.0 µg/L naproxen and observed reduced growth and reproduction, together with gonadal histopathological alterations, at concentrations as low as 0.1 µg/L. At 0.7 µg/L, naproxen altered gonadal transcription of genes associated with reproduction, particularly steroid-hormone biosynthesis and epigenetic or epitranscriptomic processes. Because this study integrated the whole organism, gonadal, and transcriptomic endpoints across the life cycle, its findings provide stronger evidence for reproductive toxicity than studies restricted to molecular biomarkers (Barros et al., 2024).

In zebrafish, long-term exposure (15 to 40 days post-fertilization) to an environmentally relevant concentration of NSAIDs (100 µg/L), including NPX, resulted in a male-biased sex ratio (18 % increase in male population relative to controls), a shift attributed to NPX's non-selective inhibition of cyclooxygenase activity and possible induction of apoptosis in developing gonadal tissue (Bereketoglu et al., 2020). Also, in zebrafish, Ji et al. (2013) found that naproxen was among the NSAIDs capable of modifying hormone-related gene expression in adult stage, with sex-dependent transcriptional responses in the hypothalamic-pituitary-gonad axis.

Taken together, the available studies support describing naproxen as endocrine-active and potentially reproductive-toxic to fish under laboratory conditions, rather than establishing that environmentally typical NPX concentrations could cause reproductive decline in wild fish.

4. Mitigation strategies for reducing naproxen pollution in aquatic environment

Reducing the impact of NPX on aquatic ecosystems requires an integrated approach acting at three complementary levels: (i) limiting the release of NPX into surface waters through improved wastewater treatment; (ii) increasing the capacity of exposed fish, particularly farmed species, to counteract the adverse effects of pharmaceutical residues through functional feeds; and (iii) reducing NPX loading at the source through more rational consumption and proper disposal.

Improved wastewater treatment remains the most practical way to limit the environmental release of NPX. As reported by Gheorghe et al. (2016), conventional activated sludge treatment achieves only partial NPX removal, at approximately 50–60%, with significant residual concentrations persisting in the treated effluent. Various studies have proposed advanced treatment technologies, such as ozonation coupled with biological methods through constructed wetlands (Lancheros et al., 2019), activated carbon adsorption (Lach and Szymonik, 2019), photocatalytic and advanced oxidation (Sepulveda et al., 2024), and enzymatic biodegradation (Zdarta et al., 2019) to achieve substantially higher removal efficiencies.

Combining a horizontal subsurface flow constructed wetland planted with *Cyperus ligularis* with ozonation Lancheros et al. (2019) achieved naproxen removal of at least 97.3% at an ozone dose of 15 mg/L. Removal did not differ significantly among the three ozone doses

tested (10, 15, and 21 mg/L), but the combined system significantly outperformed either treatment alone, supporting its potential for reducing naproxen in water. This type of system is a relevant option for lower-energy polishing or decentralised treatment because it successfully combines microbial biodegradation, sorption to media and biofilms, and plant-associated processes with hydraulic retention to remove more pharmaceuticals, not just NPX.

Under laboratory conditions, Lach and Szymonik (2019) removed dissolved NPX using commercial activated carbons. The authors established that higher temperatures increased adsorption, while pH increasing determined a decrease in adsorption. Thus, for an initial naproxen sodium concentration of 252 mg/L, a carbon dose of 3 g/L achieved more than 95% removal.

Zdarta et al. (2019) conducted another laboratory-scale experiment involving immobilised laccase on a tailor-made support of polymer nanofibers by adsorption and encapsulation, to improve the stability and reusability of the enzyme. This experiment was conducted under optimised batch conditions (pH 5 and a temperature of 25°C) and was successful in removing over 90% of NPX from a 1 mg/L solution. Additionally, the biocatalytic systems demonstrated sustained operational stability. Encapsulated laccase retained an NPX removal efficiency above 60% after five consecutive degradation cycles, highlighting the systems' potential for practical application.

Sepúlveda et al. (2024) used photocatalysis by light-activated TiO_2 single-tube powders with and without magnetite nanoparticles to degrade NPX at approximately 94% within 1 hour and complete removal within 2 hours under simulated sunlight. Under blue light, magnetite-modified nanotube powders achieved approximately 92% NPX degradation within 3 h, compared with approximately 60% for unmodified nanotubes. Under green light, both modified and unmodified powders exhibited only a 20% NPX removal after 3 hours. A main limitation of the experiment was that the disappearance of the parent compound did not confirm complete mineralisation or eliminate the possibility of residual toxicity associated with the transformation products.

Even with improved treatment, fish inhabiting surface waters, and farmed fish supplied with such water, may still be chronically exposed to low concentrations of NPX and other pharmaceutical residues. A complementary strategy is therefore to develop functional feeds that strengthen the physiological capacity of fish to counteract these effects. This is particularly relevant in aquaculture, where diet composition can be directly controlled.

Research in this area is still emerging, and a first line of evidence concerns dietary probiotics. Ebrahim et al. (2023) reported that exposure of zebrafish (*Danio rerio*) to 100 µg/L NPX for 60 days reduced growth and altered thyroid-related endpoints, including decreased T4 levels and increased TSH gene expression. Supplementation with 1.5×10^8 CFU/g *Lactobacillus reuteri* improved weight gain and moderated several thyroid-related responses, suggesting its potential role in alleviating NPX-associated effects.

Reducing NPX loading at the source is equally important, because it limits the quantity of the compound that reaches wastewater treatment plants and, ultimately, surface waters. Responsible prescription and use of NSAIDs, including NPX, can reduce environmental loading. In healthcare settings, as well as in households, proper disposal of unused or expired pharmaceuticals prevents the inadvertent release of active ingredients into sanitary sewers (OECD, 2019).

Conclusions

Unlike many conventional pollutants, pharmaceutical compounds such as NPX are intentionally designed to produce biological effects at low concentrations. Their environmental release is therefore concerning even when they occur below concentrations associated with chronic toxicity.

Our paper highlights the diversity of experimental conditions, which provide complementary insights into NPX toxicity. However, the findings require careful consideration when interpreting environmental relevance. Studies at elevated concentrations contribute to hazard characterisation and the elucidation of concentration-response relationships, whereas chronic exposure at environmentally relevant levels informs, more directly, the assessment of potential effects under realistic exposure scenarios. Accordingly, evidence from individual and mixture exposures should be assessed independently, taking into account exposure duration, life stage, and endpoint sensitivity. Variation in the evaluated endpoints complicates further comparisons across studies, even if NPX concentration could reach acute lethal concentrations or induce sublethal effects under prolonged exposure, reflecting different temporal patterns of toxicity.

NPX showed a broader concentration threshold for observable effects (approximately 70–299 μg/L) that substantially exceeds typical surface water concentrations, suggesting that environmental risk posed by current usage patterns is limited. However, this assessment must be tempered by recognition that contaminated aquatic systems and industrial discharge zones may exceed critical thresholds by orders of magnitude.

While NPX represents a substantially less hazardous NSAID alternative compared to diclofenac, with lower bioaccumulation potential and higher effect thresholds, the existence of developmental hazards argues for continued efforts to minimise aquatic pharmaceutical contamination. These efforts should prioritise wastewater treatment improvements, stewardship in pharmaceutical prescribing, and implementation of water quality standards incorporating early life stage toxicity data.

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