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EFFECT OF SOME WATERBORNE PHARMACEUTICALS ON FISH HEALTH

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Abstract

Pharmaceutically active compounds (PhACs) occurred in aquatic environments and have the potential to adversely affect the homeostasis of the endocrine axis leading to immunological, developmental, neurological and reproductive disarray at the organism level. These compounds are frequently discharged or run-off into the water stream and consequently is an increasing concern in research for the threat posed by PhACs on aquatic fauna. Fishes are vulnerable to different compounds, but the effects depend on specie, toxic concentration and exposure time. The concentrations of PhACs detected in the aquatic environment are relatively low but depending on the compound, they can reach up to a few hundreds of ng/g. The detection of pharmaceuticals in the aquatic environment has predominantly been reported in the developed world (USA, EU, Japan, and Australia) which are dominant countries in global pharmaceutical sales, but their presence tends to become ubiquitous. The aim of this paper is to highlight the main adverse effects of most common PhACs found in Danube river on fish health in order to assess the PhAC with the higher risk for fishes.

Key words*: Endocrine disruptors, fish health, pharmaceutically active compounds.*

INTRODUCTION

In the aquatic environment, pharmaceutically active compounds (PhACs) are increasingly present and are expected to rise over time. PhACs include human pharmaceuticals, veterinary medicines and aquaculture drugs. There are worldwide studies in the aquatic environment to determine the concentrations for some pharmaceuticals, but not all of them are considered a real risk for the environment (Corcoran et al., 2010). Depending on the predominant human and animal diseases there are categories of pharmaceuticals more often used and more common in the aquatic environment. The most used classes of pharmaceuticals are antibiotics, nonsteroidal anti-inflammatory drugs, beta-blockers, antiepileptics, antihypertensives, antidepressant (Corcoran et al., 2010; Yang et al., 2014; Ebele et al., 2017). Pharmaceuticals are ingested directly by fish through specific treatments, applied in aquaculture industry to control diseases, or indirectly due to the discharge of insufficiently treated wastewater to eliminate medicines for human and animal use (Kibenge et al., 2020). The main condition for restoring the affected aquatic environment or protecting it is to observe the traceability and monitoring these medicines, to assess the risks and identify the sources of pollution (Bobrowska-Korczak et al., 2021). Most of these pharmaceuticals reaching the aquatic environment act as endocrine disruptors (ED) on fish populations (Biswas et al., 2021). Pharmaceuticals are generally nonpolar and are able to pass through biological membranes by diffusion, to targets within specific cells and tissues (Khetan & Collins, 2007).

The most spread pharmaceuticals in aquatic ecosystem are anti-inflammatories (paracetamol, diclofenac, ibuprofen), antibiotics (trimethoprim, sulfamethoxazole, erythromycin, clarithromycin), anticonvulsants (carbamazepine), beta-blockers (metoprolol) (Kibenge et al., 2020). In the last two decades, a lot of experiments and researchers have been carried out to find which are the common PhACs in the aquatic environment and to evaluate their effects on fish. The concentrations of the most PhACs detected in the aquatic environment are relatively low, in the range ng/L to µg/L (Corcoran et al., 2010).

The legal status of PhACs vary at global level as there are countries with well-developed legislation where PhACs in the aquatic environment are observed for their biological effects (Europe, Japan, North America) (EMEA, 2006), and countries where the legislation is more permissive or poorly developed (India, China) (Corcoran et al., 2010).

In the last 20 years, the occurrence of some pharmaceuticals has been periodically reported also in the Lower Danube basin, in special in the last years, due to the increased sensitivity of the used analytical methods (Patel et al., 2019). Therefore, the most frequently founded pharmaceuticals were carbamazepine (3.94–945 ng/L), diclofenac (0.8–255 ng/L), sulfamethoxazole (30-204 ng/L), trimethoprim (0.8-223 ng/L) and ibuprofen (3.32-346 ng/L) (Chitescu et al., 2021).

Diclofenac (DCF) is an important nonsteroidal anti-inflammatory drug (NSAID) of high consumption suspected of causing damage in nontarget organisms (Parolini, 2020). It has a well-known safety and efficacy profile. In aquatic environment are found high concentrations of diclofenac that indicate an unacceptable risk in terms of environmental risk assessment (Schwaiger et al., 2004).

Ibuprofen (IBU) is the third most popular drug in the world with a relatively high therapeutic dose of 600 to 1200 mg/day (USEPA 2009). Paracetamol (PRM) is one of the most commonly used analgesic and antipyretic drug around the world available alone and also in combination with other medicines (Nunes et al., 2015). Toxic effects induced by IBU and PRM has been emphasized for different aquatic organisms such as invertebrates (Parolini, 2020; Muniz-Gonzales, 2020) and fish (Stancova et al., 2017).

Carbamazepine (CBZ) is frequently measured in river and seawater, even though it is not a pharmaceutical of high consumption. Due to low elimination rate of 4-8% (Clara et al., 2004) the most prevalent drugs are antiepileptics and antidepressants like CBZ and venlafaxine (Huerta et al., 2018). In aquatic animals CBZ affected homeostasis and physiological systems in various ways modifying reproduction, feeding behaviour and even growth and survival (Baali & Cosio, 2022).

Sulfamethoxazole (SMX) is a bacteriostatic sulphonamide antibiotic that is commonly used in human and veterinary medicine used across the globe, first produced in 1962. Sulphonamides competitively inhibit bacterial enzyme dihydropteroate synthase (DHPS) (Corcoran et al., 2010).

Although the occurrence of various antibiotics in the aquatic environment, are frequently reported, toxic effects of these drugs in fish are poorly studied.

Due to its wide use and low biodegradability, sulfamethoxazole (SMX), has been detected in aquatic environment and the toxic effect has been reported for different aquatic organisms (Zhang et al., 2021; Yang et al., 2014).

The monitoring of antibiotic residues including sulfamethoxazole in catfish Pangasius imported in Thailand (Jansomboon et al., 2017) evidenced sulfamethoxazole maximum concentrations of 245.91 µg/kg fish.

Metoprolol (MTP) is prescribed for hypertension, relieve angina and prevent heart attacks (Martinez-Rodriguez et al., 2018). Metoprolol is one of the most useful beta-blockers (Triebskorn et al., 2007 being frequently detected in aquatic systems, surface water and municipal water (Kümmerer, 2010).

MATERIALS AND METHODS

The present study is based on a literature survey on publications acquired for the information related to the target pharmaceuticals and their impact on fish physiology. The platformed used included mainly Google Scholar and Web of Science and the most used key terms were “fish health”, “fish toxicity”, “toxicologic impact” “pharmaceutically active compounds”, “paracetamol”, “carbamazepine”, “diclofenac”, “metoprolol”, “ibuprofen”. It has been selected and reviewed a number of 54 published articles focusing on targeted pharmaceuticals from the perspective of their impact on freshwater and marine fish species.

RESULTS AND DISCUSSIONS

Impacts of DCF on fish health

Diclofenac is NSAID and is one of the most detected compounds in aquatic environment. 28 days experiments on 2 years old rainbow trout

exposed to diclofenac in concentrations between 1 µg/L and 500 µg/L highlighted histopathological changes in the kidney and the gills (Schwaiger et al., 2004; Triebskorn et al., 2004). In the kidney, it was observed a distinct proliferation of the renal interstitial tissue, a severe hyaline droplet degeneration, accumulation of proteinaceous material and necrosis of tubular epithelial cells. In the gills, changes were represented by a degenerative and necrotic of the pillar cells, a dilation of the capillary walls. The researchers concluded that the damage increased significantly after exposure at 5 µg/L. As a threshold for no observed effect concentration (NOEC) could be established to be 1 µg/L. The general health of the fish can be affected at a concentration of 20 µg/L because it has been observed changes in respiratory epithelial cells, which could interfere with normal respiratory function, plus renal changes. (Schwaiger et al., 2004).

The histological research was followed by the cytological research of rainbow trout exposed to different concentrations of diclofenac (Triebskorn et al., 2004). The conclusions were that ultrastructural studies provide more detailed information. For example, diclofenac induced cellular reactions indicating activated hepatic metabolism had already become visible in the trout liver after exposure to 1 µg/L (Triebskorn et al., 2004). To these are added 50% effect concentration (EC_{50}). EC_{50} (*Daphnia magna*, motility) for diclofenac was shown to be 22.43 mg/L (Triebskorn et al., 2007).

Of interest to some researchers were the feeding performance and swimming speed, which were studied on 3-month-old medaka fish under the action of 1 mg/L diclofenac for 9 days (Nassef et al., 2010). As a result, feeding behaviour was altered by exposure to DCF.

In contrast to other experiments performed on specimens of about 3-month-old or 2 years, it was performed on zebrafish embryos exposed for 24, 48 and 72 h (van den Brandhof & Montforts, 2010). The aim of this experiment was to highlight the effects on embryonic mortality, yolk deformity, heartbeat, head malformation, otoliths and heart, tail movement and detachment, pigmentation, scoliosis, and successful hatching. After 72 h, NOEC was 1.5 mg/L. Above this concentration appeared specific effects on hatching, yolk sac and tail deformation. The EC_{50} for 72 h was 5.3 mg/L.

There were also experiments performed over a longer period of time. For example, to quantify the chronic toxicity and bio-concentration of DCF there were realised early life stages (ELS) test for 95 days exposure rainbow trout and 35 days for zebrafish (Memmert et al., 2013).

Following these experiments, it is concluded that concentrations of DCF above 320 µg/L endanger the two species of fish.

Given the experiments with contradictory conclusions regarding the effects of diclofenac on fish at different stages of life, there have been experiments to clarify this (Schwarz et al., 2017). Thus, fertilized brown trout eggs and 6-month-old brown trout were subjected to different concentrations of diclofenac at temperatures of 7°C. It was concluded that the early life stages of brown trout were not affected while the brown trout brood was affected by concentrations of up to 10 µg/L diclofenac (Schwarz et al., 2017).

These experiments showed that exposure time is a very important parameter. Thus, for a relatively short exposure time, a higher concentration of diclofenac is required to see harmful effects, while for a long period exposure time, the effects occur at lower concentrations.

Impacts of IBU on fish health

Studies on fish have shown that ibuprofen can induce changes in various tissues and systems at both sublethal and aquatic environment concentrations. Thus, studies performed on model fish *Danio rerio* exposed to concentrations up to 25 mg/L for 28 days showed the installation of oxidative stress (Bartoskova et al., 2013), while in embryos neurotoxicity and locomotor changes were observed in the context of 6 to 96 hours post-fertilization exposure to concentrations up to 500 µg/L (Xia et al., 2017). In fact, similar studies on zebrafish embryos have shown that ibuprofen is metabolized and excreted in a similar way to mammals (Jones et al., 2012) and that it affects organogenesis, larval growth and survival (David & Pancharatna, 2009). Oxidative stress in liver, blood, and gill was also observed in other fish species such as common carp, when exposed to ibuprofen between 12 and 96 hours at 17.6 mg/L (Islas-Flores et al., 2013).

Moreover, histopathological deformities in the gills, liver, and kidney were described for african catfish exposed at 3.78 mg/L for 30 days (Ogunwole et al., 2021).
The toxic effect of ibuprofen was also evaluated for much lower concentrations found in aquatic ecosystems. Thus, the exposure of freshwater species *Rhamdia quelen* for 14 days to ibuprofen concentrations up to 10 µg/L induced immuno suppressive effect and nephrotoxicity (Mathias et al., 2018) while for zebrafish impaired the cardiovascular development at concentrations ranging between 0.91 and 21.9 µg/L (Zhang et al., 2020).

Impacts of CBZ on fish health

Due to its resistance to degradation, even in the most modern wastewater treatment plants, CBZ is considered a persistent contaminant in the environment (Saggioro et al., 2018). In short-term studies (96 h) the exposure of zebrafish (*D. rerio*) at low concentrations of 1 µg/L carbamazepine resulted in significant morphological effects: accelerated yolk sac absorption, greater body length, increased swim bladder appearance (Qiang et al., 2016). On the other hand, chronic exposure of zebrafish to CBZ (10 µg/L for 63 days) caused alterations in the gonad's follicular stages, increasing enzymatic activity in some organs (acetylcholinesterase (AChE) activity in the head and muscle, glutathione S-transferase (GST) activity in gills and lactate dehydrogenase (LDH) activity in the liver) or inhibiting enzymatic activity in other structures (GST activity in the intestine) (Silva Santos et al., 2018). Similar studies revealed that exposure of adult zebrafish to 10 µg/L CBZ for 6 weeks decreases not only reproductive output of zebrafish but also have transgenerational effects impacting as well unexposed progeny up to the F4 generations (Fraz et al., 2019).
In other species, such as common carp, sublethal concentration of 5.97 mg/L (1/10th of LC_{50} value) induced alterations in the activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and LDH in various organs (Malarvizhi et al., 2010). Exposure of rainbow trout at sublethal concentrations of CBZ (up to 2.0 mg/L) for 42 days induced antioxidant responses (thiobarbituric acid reactive substances (TBARS), carbonyl proteins (CP), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx)) and inhibition of energy metabolic parameters ($RNA - DNA$ ratio, adenosine triphosphatases ($Na^+ - K^+ - ATPase$)) in muscle (Li et al., 2010).
Besides enzymatic and metabolic effects, CBZ acts also as endocrine disruptor inducing significant decrease of vitellogenin (VTG) concentration in striped catfish females exposed at concentrations of 25 µg/L and 50 µg/L for 4 months (Ortiz et al., 2021). Recent study emphasized that CBZ, even in low environmentally relevant concentrations, induces DNA damage and apoptosis in Chinese rare minnow (Yan et al., 2021).
However, even though short-term exposure at CBZ low environmental concentrations induced reversible oxidative damage in the liver of *Carassius carassius* unrecoverable neurotoxicity potential was also observed (Nkoom et al., 2020) underlining the risk associated with this residue in the environment.
Regarding the metabolization of CBZ by fish, a study measuring the accumulation and biotransformation of CBZ in *Jenynsia multidentate* (Valdes et al., 2016) showed that the brain and liver accumulated the highest quantity while gills, intestine and muscle the lowest. The identified metabolites were 2-hydroxycarbamazepine (2-OH-CBZ) and carbamazepine-10,11-epoxide (CBZ-EP).

Impacts of PRM on fish health

The paracetamol effects on a neotropical fish species (guaru), after 96 h of exposure to concentrations ranging from 0.008, to 80 mg/L, were observed only at the highest concentration and just at behavioural level (Matus et al., 2018). This observation is partially in line with the results reported by other researchers (Nunes et al., 2014), after exposing eel, *Anguilla Anguilla,* 96 hours to paracetamol concentrations ranging from 5 to 3125 µg/L. This experiment indicated that exposure to paracetamol was not capable of inducing a response in liver and gills and did not cause oxidative stress. However, at higher concentrations signs of neurotoxicity were observed.
On the other hand exposure of embryos and larvae of *Danio rerio* to paracetamol at

concentrations of 0.005-3.125 mg/L affected CAT, GST, cholinesterase, GPOx, and LPO activities (Nogueira et al., 2019).
Testing much lower environmental concentrations of paracetamol (0, 0.25, 2.5 µg/L) on male fish of *Rhamdia quelen* held in a semi-static bioassay for 21 days other authors (Guiloski et al., 2017) emphasized haematological, enzymatic (hepatotoxicity) and hormonal alterations (disrupted the hypothalamic-pituitary-gonadal) in special at the highest tested concentration.
This set of results shows that the paracetamol toxicity is multilevel exerted on fish impairing several key physiological functions and relationships among them.

Impacts of SMX on fish health

Most of the studies conducted to evaluate the impact of sulfamethoxazole on fish were emphasized, using zebrafish, alteration of growth, development and reproduction function. Thus, in a chronic exposure of 150 days using SMX concentrations of 2, 20, and 200 µg/L an increase in ethoxyresorufin-O-deethylase (EROD) activity was observed (Yan et al., in 2016). In terms of reproduction, egg production has declined, and the hatching success rates of embryos were reduced. Other signs of abnormal development like weak pigmentation, pericardial edema, yolk sac edema, blood clotting, lordosis was observed. Environmentally relevant levels of SMX (5.6 µg/L) tested on male zebra fish for 21 days revealed thyroid dysfunctions, an increase in thyroxin (T4) hormone concentration, an increase in expression of thyrotropin-releasing hormone receptor (trhr1) and thyroid-stimulating hormone (Kwon et al., 2016). Other acute trail testing a larger range of SMX levels (0.1, 1, 10, 100 µg/L for 120 h) showed that the zebrafish larvae's antioxidant system was affected through up-regulating the activities of antioxidant enzymes and down-regulating the reactive oxygen species (ROS) and that the ability of zebrafish to resist pathogen was suppressed (Liu et al., 2019). It is noteworthy to mention that, in some fish species like catfish Pangasius, imported in Thailand, the concentrations of sulfamethoxazole reached the maximum level of 245.91 µg/kg fish (Jansomboon et al., 2017).
In much higher doses, SMX delivered by diet (20 up to 1000 mg/kg) to Nile tilapia negatively affected growth performance, reduced intestinal biological diversity and changed the structure of the intestinal flora (Fang et al., 2021).

Impacts of MTP on fish health

Experiments realised on Nile tilapia fish from fertilized egg until 80 days post-hatch to 0.12 µg/L, 1.20 µg/L, 11.61 µg/L and 116.86 µg/L of MTP (Groner et al., 2017) highlighted changes consisted in alteration in gene expression patterns of pituitary gonadotropins luteinizing hormone (lh), follicle stimulating hormone (fsh) and increase in expression of VTG. It was observed an increased occurrence of hypertrophy, infiltration by leucocytes (at highest metoprolol concentration), proliferation of mucous and chloride cells, epithelial lifting, hyperplasia and hypertrophy of mucus and chloride cells.
Experiments performed on zebrafish in order to evaluate the effects of MTP on embryo mortality, tail movement, malformation of head, pigmentation, heartbeat, deformity of yolk, scoliosis, and hatching success at 24, 48 and 72h evidenced scoliosis at 25.3 mg/L MTP (van den Brandhof et al., 2010). At higher concentrations the effects were represented also by growth retardation and heart abnormalities.
In other experiment performed on freshwater teleost *Common carp* to highlight the toxicity induced by MTP in concentrations of 10 ng/L, 10 µg/L and 10 mg/L for the following exposure times 12, 24, 48, 72 and 96 h (Martinez-Rodriguez, 2018) SOD and CAT activity increased significantly in all organs. The most affected organs were gills and liver, while the least susceptible to these compounds was the brain (Table1).

Table 1. The effects of some PhACs on fish health

PhACs	Autors and year	Fish species	Experimental conditions	Effects on fish health
Diclofenac (DCF)	Schwaiger et al., 2004	rainbow trout	concentrations ranging from 1 µg/L to 500 µg/L	✓ kidney: a distinct proliferation of the renal interstitial tissue, a severe hyaline droplet degeneration, accumulation of proteinaceous material and necrosis of tubular epithelial cells ✓ gills: a degenerative and necrotic of the pillar cells, a dilation of the capillary walls.
	Hoeger et al., 2005	brown trout	concentrations of 0.5-50 µg/L	✓ head kidney: hinders the stimulation of prostaglandin synthesis
	Triebskorn et al., 2004;	rainbow trout	concentrations of 1-5 µg/L	✓ kidney: induction of glomeruloneophritis, necrosis of endothelial cells, and hyaline droplet degeneration ✓ gills: pillar cell necrosis, epithelial lifting, hyperplasia, and hypertrophy of epithelial chloride cells
	Memmert et al., 2013	rainbow trout	concentrations of 3.2, 10, 32, 100, 320, and 1000 mg/L for 95 days	✓ kidney: hyaline droplet degeneration of the tubular epithelial cells and the occurrence of an interstitial nephritis
		zebrafish	concentrations of 10, 32, 100, 320, 1000, and 3200 mg/L for 34 days	✓ gills: a necrosis of pillar cells leading to damage of the capillary wall within the secondary lamellae
	Nassef et al., 2010	medaka	concentrations of 1 mg/L for 9 days	✓ effects on feeding behavior and swimming speed
	Brandhof et al., 2010	zebrafish	2.9 - 5.8 - 11.6 -23.3 - 46.5 mg/L for 72h	✓ 2.9 mg/L and upwards hatching and growth retardation was recorded together with a more profound pigmentation of the yolk sac
	Guiloski et al., 2017	silver catfish	0.2-20 µg/L for 21 days	✓ 5.8 mg/L effects were recorded for heartbeat
	Triebskorn et al., 2007	rainbow trout common carp	1, 5, 20, 100, or 500 µg/L diclofenac, for 28 days under flow-through conditions (water flow rate 9 L/h)	✓ oxidative stress ✓ testosterone levels in male fish ✓ liver: collapse of cellular compartmentation, glycogen reduction, membrane material, dilation and vesiculation of ER, increased amount of macrophages ✓ kidney: glomerulonephritis with thickened basal lamina, shortening of pedicels and retraction from basal lamina, necrosis of endothelial cells, hyaline droplet degeneration ✓ gills: epithelial lifting, pillar cell necrosis, hyperplasia and hypertrophy of chloride cells

PhACs	Autors and year	Fish species	Experimental conditions	Effects on fish health
Paracetamol (PRM)	Nunes et al., 2014	Anguilla anguilla	5, 25, 125, 625 and 3125 µg/L for 96 hours	✓ the paracetamol did not cause oxidative stress
	Guiloski et al., 2017	catfish	0.25, and 2.5µg/L	✓ anti-androgenic effect, a decrease in testosterone levels of male fish and an increase in the levels of estradiol on silver catfish
	Matus et al., 2018	guaru	0.008, 0.08, 0.8, 8.0 and 80 mg/L	✓ the paracetamol effects observed were not in terms of swimming patterns of fish
Carbamazepine (CMZ)	Nassef et al., 2010	medaka	6.15 mg/L for 9 days	✓ effects on feeding behavior
	Malarvizhi et al., 2011	common carp	0.2, 2, and 20 mg/L concentrations for 2 h	✓ oxidative stress in spermatozoa and decreases sperm motility and velocity
	Hampel et al., 2014	atlantic salmon	7.85 µg/L for 5 days	✓ upregulation of mRNA expression level of pituitary hormones like somatolactin, prolactin, and growth hormone somatotropin
	Triebskorn et al., 2007	rainbow trout, common carp	1, 5, 20, 50, or 100 µg/L carbamazepine for 28 days under flow-through conditions (water flow rate 9 L/h)	✓ liver: increased amount of macrophages, membrane material ✓ kidney: vesiculation and dilation of endoplasmic reticulum (ER) in posterior portions (PII) and distal tubules (DI), enlarged mitochondria in DI, increased amount of macrophages in PII and DI, increased amount of cellular debris in intercellular spaces and secondary lysosomes in basal portions of cells ✓ gills: epithelial lifting, hyperplasia and hypertrophy of mucus cells
	Qiang et al., 2016	zebrafish larvae and embryos	1, 2, 5 µ/L of carbamazepine for 72 h and 96 h	✓ accelerated the development of embryos zebrafish and perturbed their behaviours in larvae and embryos zebrafish
Metoprolol (MET)	Triebskorn et al., 2007	rainbow trout, common carp	1, 5, 20, 50, or 100 µg/L for 28 days under flow-through conditions (water flow rate 9 L/h)	✓ liver: at 1 µg/L collapse of cellular compartmentation, glycogen reduction, membrane material, dilation and vesiculation of ER, increased amount of macrophages, cellular disintegration at the spaces of Disse ✓ trunk kidney: slight thickening of the basal membrane in the RC, slightly elongated and more branched endocytotic channels in the PI, and an increased amount of macrophages in all investigated kidney portions ✓ gills: epithelial lifting, hyperplasia and hypertrophy of mucus and chloride cells, dilation of ER in chloride cells with electron dense-lumen
	Brandhof et al., 2010	zebrafish	6.25, 12.5, 25, 50, 100 mg/L for 72 h	✓ 25.3 mg/L with scoliosis as the sensitive effect, and growth retardation and heart abnormalities at higher concentrations

PhACs	Autors and year	Fish species	Experimental conditions	Effects on fish health
Metoprolol (MET)	Groner et al., 2017	nile tilapia	0.12-116.86 µg/L 80 days post-hatch	✓ alteration in gene expression patterns of pituitary gonadotropins (lh, fsh) and increase in expression of VTG ✓ increase of hypertrophy, proliferation of mucous and chloride cells, as well as infiltration by leucocytes (at highest metoprolol concentration) ✓ epithelial lifting, hyperplasia and hypertrophy of mucus and chloride cells
	Martinez-Rodriguez et al., 2018	common carp	10 ng/L, 10 µg/L and 10 mg/L for 12, 24, 48, 72 and 96 h	✓ SOD and CAT activity increased in all organs. The most affected organs were gill and liver, while the least susceptible to these compounds was brain.
Sulfamethoxazole (SMX)	Kwon et al., 2016	male zebra fish	5.6 µg/L for 21 days	✓ thyroid dysfunctions, an increase in thyroxin (T4) hormone concentrationan ✓ increase of thyrotropin-releasing hormone rece ptor (trhr1) and thyroid-stimulating hormone, beta subunit (tshb) mRNA in the zebra fish brain
	Yan et al., 2016	zebra fish	2, 20, and 200 µg/L for 150 days	✓ increase in EROD activity, depressed the egg production of the parental fish by 13.2 to 26.4 % by the highest concentration ✓ the hatching success rates of embryos were reduced by 15.7 to 26.7% ✓ lower survival rates of the larvae were observed after the parents exposed to 200 µg/L SMX ✓ induced a higher development abnormality rate: pericardial edema, weak pigmentation, blood clotting, yolk sac edema, underdeveloped eye and ear, and lordosis
	Liu et al., 2019	zebrafish larvae	0.1, 1, 10, 100 µg/L for 120 h	✓ affect the young fish's antioxidant system through up-regulating the activities of antioxidant enzymes and down-regulating the ROS ✓ suppresses the ability of zebrafish to resist pathogen
	Fang et al., 2021	nile tilapia	LS - 20 mg/kg day; MS - 200 mg/kg day; HS - 1000 mg/kg day) for 4 weeks and then fed with normal feed for 4 weeks	✓ reduced the biological diversity, changed the structure of the intestinal flora, affected the number and proportion of dominant bacteria, without changing the main dominant bacteria at the phylum leve ✓ changed the growth performance of nile tilapia MS>LS>NS, while HS antibiotics significantly retarded growth the structure of intestines flora

CONCLUSIONS

The problem concerning the presence of PhACs in the aquatic environment is significant. The experiments performed in the last decades showed that the PhACs may alter aquatic ecosystem equilibria by the negative effects on fish populations. It should be noted though those experimental trials analysed here were performed during short periods of time from a few hours, days to weeks. However, the effect of analysed products was significant even at low environmental concentrations estimating deeper effects in fish species with longer life cycle. This illustrates the need for more long-term chronic studies to evaluate the effect of different PhACs in fish, either individually or in combinations found in the aquatic environment. These studies are relevant not only for environmental risk assessment but also from the perspective of aquaculture industry, in special for open production systems.

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