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Short-term exposure to pharmaceuticals negatively impacts marine flatfish species: Histological, biochemical and molecular clues for an integrated ecosystem risk assessment

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ABSTRACT

The marine habitat and its biodiversity can be impacted by released pharmaceuticals. The short-term (7 days) effect of 3 commonly used drugs – warfarin, dexamethasone and imidazole – on Senegalese sole (*Solea senegalensis*) juveniles was investigated. Occurrence of hemorrhages, histopathological alterations, antioxidant status, activity of antioxidant enzymes and expression of genes involved in the xenobiotic response (*pxr*, *abcb1* and *cyp1a*), were evaluated. The results showed a time and drug-dependent effect. Warfarin exposure induced hemorrhages, hepatocyte vacuolar degeneration, and altered the activity of glutathione peroxidase (GPx) and the expression of all the studied genes. Dexamethasone exposure increased liver glycogen content, altered antioxidant status, GPx and superoxide dismutase activities, as well as *abcb1* and *cyp1a* expression. Imidazole induced hepatocyte vacuolar degeneration and ballooning, and altered the antioxidant status and expression of the tested genes. The present work anticipates a deeper impact of pharmaceuticals on the aquatic environment than previously reported, thus underlining the urgent need for an integrated risk assessment.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are paramount for human health and well-being, but their uncontrolled release into the environment represents a hazard for the entire ecosystem. They are considered a class of emerging contaminants that need to be better studied and more closely monitored, with the implementation of effective regulations (Boxall et al., 2012; Fawell and Ong, 2012; Birch et al., 2015; Klatte et al., 2017). Although most wastewater treatment plants (WWTPs) are designed to eliminate degradable organic compounds, many of them are in fact unable to completely remove all of these pollutants (Patel et al., 2019). Consequently, when some of them (metabolized or not) are released into inland and marine water bodies, they can be absorbed by aquatic organisms (reviewed by Arpin-Pont et al., 2016 and Branchet et al., 2021). Despite the fact that most of the reported environmental concentrations of PPCPs are in the range of ng L^{-1} to μg L^{-1}, pharmaceutical monitoring efforts have been limited to specific areas, in particular seasons and for a specific set of pharmaceuticals (Patel et al., 2019). Furthermore, their bioaccumulation and biomagnification throughout the food web also represent a risk, resulting in toxicity for aquatic organisms and habitat loss (Sánchez-Avila et al., 2012; Meyer et al., 2019).

Warfarin can be used to treat human blood disorders or for pest control (rodenticide), due to its anticoagulant action (Bevans et al., 2013), affecting different biological processes (Granadeiro et al., 2019; Sanyaolu et al., 2019; Beato et al., 2020). Direct and indirect episodes of warfarin toxicity have previously been reported in non-targeted species (Primus et al., 2005; Masuda et al., 2015; Pitt et al., 2015), and its toxic effect on aquatic organisms (e.g., fish) tested (Weigt et al., 2012; Fernández et al., 2014; Marques et al., 2017; Granadeiro et al., 2019).

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However, to date, toxicity has only been found at concentrations higher than those reported in the environment (2595 ng L^{-1}; Patel et al., 2019). Nevertheless, warfarin accumulation (from 1.8 to 8.7 ng g^{-1} dry weight) in two fish species (*Gerres oyena* and *Chanos chanos*) inhabiting the coastal waters of Saudi Arabia polluted with urban effluents (Ali et al., 2018), evidenced its capacity to bioaccumulate.

Dexamethasone is a commonly used corticosteroid (Kostich and Lazorchak, 2008), employed in both medical and veterinary therapies (Burkina et al., 2015; LaLone et al., 2012), exerting an anti-inflammatory and anti-allergic effect via the glucocorticoid receptors (Rhen and Cidlowski et al., 2005). Current WWTPs cannot remove dexamethasone efficiently from wastewater (Chang et al., 2007; Liu et al., 2011; Herrero et al., 2013). To the best of our knowledge, the highest reported concentrations of dexamethasone in the environment range from 11 to 180 μg L^{-1} (Creusot et al., 2014). Liver hepatomegaly and steatosis, growth reduction, reproductive alterations and oxidative stress, have been reported in fish upon exposure to dexamethasone (Chen et al., 2016; Guiloski et al., 2015; LaLone et al., 2012; Salas-Leiton et al., 2012; Yin et al., 2017).

Imidazole is a heterocyclic aromatic compound with different applications. A large variety of pharmaceuticals contain imidazole as it improves their solubility and bioavailability (reviewed in Zhang et al., 2014). While the imidazole mode of action is still poorly understood, its toxic effects and related compounds have been reported in several mammalian species (Pagella et al., 1983; Kuemmerle et al., 1987; Noseda et al., 1988). Little is known about imidazole removal by WWTPs, but related compounds such as clotrimazole have been frequently detected in effluents and receiving waters of WWTPs (reviewed in Corcoran et al., 2010). Indeed, its presence in aquatic environments and bioaccumulation in non-target species has been demonstrated (Eglo et al., 1994; Castillo et al., 1997; Corcoran et al., 2014). Nevertheless, our knowledge of the potential toxic effects of imidazole in aquatic organisms is mainly restricted to lethal doses established for a reduced number of species (BASF AG, 1977, 1988).

Animals can respond to the presence of exogenous compounds. After their absorption from the gastrointestinal tract, xenobiotic detoxification and protection against chemical toxicity occurs at the liver, the principal site of xenobiotic metabolism (Gu and Manatou, 2012). There, the pregnane X receptor (PXR) – among other nuclear receptors – is recognized as the master regulator of the transcription of genes encoding some enzymes (e.g. CYPs including CYP1a) as well as membrane transporters (e.g. ABCB1) involved in this process (Chen et al., 2012). Xenobiotic metabolism will lead, in turn, to the presence of oxidized compounds, more easily excreted from the body, but resulting in the production of reactive oxygen species (ROS). In order to avoid oxidative stress and altering total antioxidant status (TAS), the organism needs to counteract the production of ROS through the action of antioxidant compounds and enzymes from the redox system, including glutathione reductase (GR), glutathione peroxidase (GPx) and/or superoxide dismutase (SOD; Regoli et al., 2014). Since pharmaceutical release into the natural environment might impose a threat to aquatic organisms inhabiting coastal areas, known to be more prone to acting as a sink for released PPCPs (Yang et al., 2020), we evaluated how the flatfish species Senegalese sole (*Solea senegalensis*) – a species used in marine ecotoxicology studies (Oliva et al., 2012; Solé et al., 2016; among others) – responds to warfarin, dexamethasone and imidazole exposure. Recently, Pes et al. (2021) showed how Mediterranean mussel (*Mytilus galloprovincialis*) responded to short-term (7 days) exposure to the above-mentioned drugs. Senegalese sole juveniles also inhabit coastal areas, but while the Mediterranean mussel inhabits the tidal interphase, the benthic behavior of Senegalese sole makes it a suitable sentinel for marine sediment pollution (Gonçalves et al., 2014). Thus, the present study provides a more integrated risk assessment of the presence of PPCPs in the marine coast.

2. Material and methods

2.1. Ethical statement

Fish facilities (permit 0421/2013), personnel and experiments (permit 0421/2020) were accredited and approved by the Portuguese National Authority for Animal Health (DGAV), and conducted following the ARRIVE guidelines (Percie du Sert et al., 2020), according to major international and national regulations for animal experimentation and welfare: The Code of Ethics of the World Medical Association (Declaration of Helsinki; http://europa.eu.int/scadplus/leg/en/s23000.htm) and guidelines 2010/63/EU of the European Parliament and Council, 86/609/EU of the European Union Council, and the Decreto-Lei 113/2013 from the Portuguese government.

2.2. Rearing and sampling procedures

Senegalese sole juveniles bred at the Aquaculture Research Station of the Portuguese Institute of the Sea and the Atmosphere (EPPO/IPMA, Olhão, Portugal), were transferred to the Centre of Marine Sciences (CCMAR, Faro, Portugal) and acclimated for 15 days to laboratory conditions. 252 specimens (mean weight 0.83 ± 0.21 g) were selected and randomly distributed into 21 flat bottom 1-L tanks (12 individuals per tank). Experimental conditions were as follows: temperature of 19.5 ± 1.8 °C, salinity of 30 ± 1 practical salinity units (PSU), dissolved oxygen levels > 4 mg L^{-1} and a 12:12 h light/dark photoperiod. In order to maintain water quality and drug exposure levels, 80% of the water (and drug) was renewed every two days. Levels of ammonium, nitrite and nitrates were checked to confirm water quality throughout the experiments. The Senegalese sole were daily fed with commercial inert diets formulated by Sparos Lda (Olhão, Portugal).

The fish were exposed to drugs for 3 and 7 days, then euthanized with an overdose of MS-222 (300 mg mL^{-1}) and sampled. Total fish body wet weight (BWW) and liver weight (LVW) were assessed with a WA80 precision (± 0.1 mg) scale (Adam Equipment). The presence of haemorrhages was determined by visual inspection at each sampling time and results were expressed as a percentage of incidence. At 3- and 7-days post-exposure (dpe), livers from 2 specimens from each biological replicate (tank) were isolated and washed in DNase/RNase-free water, pooled and preserved in 500 μL of TRIzol Reagent (Ambion) at −80 °C until RNA extraction was conducted. At 7 dpe, another 2 livers from each tank replicate were isolated and washed with PBS (pH 7.4), pooled and homogenized with a T 10 basic Ultra-Turrax (IKA). After centrifugation at 10,000*g* and 4 °C for 30 min in a CT15RE HIMAC centrifuge, the supernatant was aliquoted and kept at −80 °C until enzyme activity was assessed. Finally, livers from 3 specimens from each replicate were sampled at 7 dpe and stored in 4% paraformaldehyde (PFA; pH 7.4) for histological analysis.

2.3. Drug working concentrations

The effect of warfarin (CAS number 129-06-6; Sigma Aldrich, Spain) was evaluated at a low dose (LW) of 1 mg L^{-1} (3.03 μM) and a high dose (HW) of 10 mg L^{-1} (30.27 μM). These concentrations were lower than those previously reported to have a negative effect on fish species (Fernández et al., 2014; Marques et al., 2017; Granadeiro et al., 2019), but equal to those recently tested in the Mediterranean mussel (Pes et al., 2021). Although these concentrations are higher than those reported in the environment to date (up to 2595 ng L^{-1}; Patel et al., 2019), they represent an attempt to model the potential effect of different anticoagulants used as rodenticides (brodifacoum, bromadiolone, difenacoum, flocoumafen, difethialone, chlorophacinone, coumatetralyl and warfarin) recently reported to be present in water samples and fish livers (Regnery et al., 2020).

Dexamethasone (CAS number 50-02-2; Sigma Aldrich, Spain) was used at a low dose (LD) of 0.392 mg L^{-1} (1 μM) and a high dose (HD) of

3.92 mg L^{-1} (10 μM). The lower concentration here tested was the same as that used in fish *in vitro* systems by Wassmur et al. (2010) and reported to have some effect. It is only twice the highest concentration found in the environment (180 μg L^{-1}; Creusot et al., 2014), so close to be environmentally relevant.

Imidazole (CAS number 288-32-4; Sigma Aldrich, Spain) was evaluated at a low dose (LI) of 0.013 mg L^{-1} (0.191 μM) and a high dose (HI) of 0.13 mg L^{-1} (1.91 μM). Both concentrations were selected according to the safety data available for sea and fresh water environments (0.013 and 0.13 mg L^{-1}, respectively; Imidazole, ULTROL® Grade – CAS 288–32–4 – Calbiochem MSDS – 4015 – Merck (merckmillipore.com)). Furthermore, several imidazole-related compounds have been found at 37.7 μg L^{-1} in some water bodies (Patel et al., 2019).

Stock solutions (12,500 mg L^{-1} for warfarin, 1000 mg L^{-1} for dexamethasone and 50,000 mg L^{-1} for imidazole) were prepared in seawater and aliquots were kept at −20 °C until used. The Control fish were not exposed to any drug.

2.4. Histological analysis

Liver samples fixed in PFA were washed and embedded in paraffin following a standardized protocol, and then sectioned using a microtome (Microm) to achieve 5–6 μm serial histological sections. Deparaffinized slides were stained with the Hematoxylin-Eosin histomorphological staining technique to assess the presence of histopathological alterations caused by toxicant exposure. Periodic Acid-Schiff (PAS) and diastase-PAS were used to study differences in carbohydrate distribution (glycogen content) between treatments. Both techniques were performed according to Pearse (1985). Images of representative histological sections were taken using a Leitz Wetzlar microscope equipped with a built-in SPOT Insight Color camera (Ernst Leitz Wetzlar GmbH, Germany). The results were manually recorded using a semi-quantitative assessment scoring (percentage of individuals presenting the histological lesion) from four independent observers, comparing the sections of the control with the experimental treatments.

2.5. Oxidative stress determination

All enzymatic activities were assessed using a BioTek Gen 5 microplate reader at room temperature. Total protein content was measured according to the method by Bradford (1976), using Bio-Rad Protein Assay reagent and bovine serum albumin (BSA) as standard. Absorbance at 595 nm, after 30 s of mixing and 5 min incubation, was recorded. Reactions were performed using technical triplicates. Total antioxidant status (TAS), and glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were assessed using Cayman kits (ref. NX2332, GR2368, 703102 and SD125, respectively). TAS (in mmol g^{-1}) was measured at 600 nm (Miller et al., 1993). GR and GPx activities (in U g^{-1}) were determined at 340 nm (Melissinos et al., 1981; Arthur and Boyne, 1985) and SOD activity (in U μg^{-1}) was assayed at 505 nm (Arthur and Boyne, 1985). TAS, GPx, GR and SOD activities were determined with technical duplicates in three biological replicates.

2.6. Gene expression analysis

Total RNA was extracted using TRIzol Reagent, according to the manufacturer's protocol. RNA quantity and purity were determined from 260/280 and 260/230 absorbance ratios using a Nano-Photometer (UV-Vis Spectrophotometer, Implen). Samples with values < 1.8 and < 1.6, respectively, were re-purified (Walker and Lorsch, 2013). Traces of genomic DNA were removed from RNA samples using RQ1 RNase-Free DNase (Promega) and cDNA was synthetized using M-MLV reverse transcriptase (Invitrogen) for 1 h at 37 °C. Reaction was stopped at 95 °C for 5 min, and cDNA was kept at −20 °C until used. For each assay, a negative control (without RNA) was performed.

Semi-quantitative PCR (qPCR) reactions were conducted on a StepOnePlus Real-Time PCR system (Applied Biosystems) in triplicate using: 10 μL of SensiFAST SYBR Hi-ROX kit (Bioline), 0.5 μL of forward and reverse gene-specific primers (10 μM; Table 1), 7 μL of DNase/RNase-free water and 2 μL of diluted (1:10) cDNA. For calibration purposes, one specific sample was run in each qPCR plate (Derveaux et al., 2010). DNA amplification was achieved as follows: 2 min at 95 °C, followed by 40 cycles of 10 s at 95 °C and 20 s at 65 °C. At the end of each amplification, a melting curve was conducted as follows: 95 °C for 15 s, 70 °C for 1 min, and 15 s of 0.5 °C increments until reaching 95 °C. Gene expression levels were determined according to Pfaffl (2001). A normalization procedure using *ubiquitin* (*ubq*) as reference gene was done (Infante et al., 2008).

2.7. Statistical analysis

Results are presented as mean ± standard deviation. Shapiro-Wilk's test and Bartlett's test were conducted to confirm data normal distribution and homoscedasticity of variance, respectively. When a one-way ANOVA analysis detected significant differences, Tukey's multiple-comparison test was used to identify the experimental groups showing significant differences. In case of high variability among experimental groups, Student's t-test was performed to unveil potential significant differences. In both cases, significance was set at $P < 0.05$. Statistical analyses were done with Prism 5.0 (GraphPad Software, Inc).

3. Results and discussion

3.1. Drug impact at organism and organ level

Among all the drugs and concentrations tested, only exposure to the highest concentration of warfarin (HW) for 7 days induced mortality, although at a low rate (5.5 ± 9.6%). Increased mortality may be related to the higher percentage of fish showing bleeding events upon treatment with warfarin (Fig. 1), as previously suggested by Fernández et al. (2014) in zebrafish (*Danio rerio*) larvae exposed to warfarin. The present results evidenced higher warfarin toxicity (higher sensitivity of the Senegalese sole to warfarin toxicity) than that reported in previous studies of fish species (Weigt et al., 2012; Fernández et al., 2014; Marques et al., 2017) and marine invertebrates (*M. galloprovincialis*; Pes et al., 2021). Differences related to increased mortality and bleeding disorders between vertebrates and invertebrates may be the consequence of a different requirement for vitamin K. Indeed, although vitamin K reductases are present in both vertebrates and invertebrates, their activity is critical to control blood coagulation in vertebrates, but not in invertebrates (Oldenburg et al., 2015).

Biometric indexes, histopathological, biochemical and gene expression responses after drug exposure were evaluated at the liver as it has a key role in xenobiotic detoxification (Gu and Manatou, 2012). Body wet weight (BWW), liver weight (LVW) and hepatosomatic index (HSI) were not significantly affected by warfarin exposure in any of the experimental groups when compared to the Control group (Table 2). A similar effect – i.e. no alteration of BWW, LVW and HSI – was reported in Mediterranean mussels exposed to warfarin for the same period (7 days) and at the same concentrations (Pes et al., 2021). Nevertheless, histopathological lesions were observed in the liver of warfarin-exposed fish. While the liver of the Control fish showed a typical disposition of hepatic parenchyma with cords of polygonal hepatocytes surrounding the sinusoids (Fig. 2a, b), degenerative changes in liver cells, including vacuolar degeneration of hepatic cells and nuclear pyknosis, were observed in warfarin-exposed fish. Furthermore, sinusoidal dilatation and congestion were also detected, especially in fish exposed to HW (Fig. 2c, d; Table 3). Similar hepatotoxic effects were found by Granadeiro et al. (2019) in zebrafish larvae exposed to higher concentrations (125 mg L^{-1}) of warfarin. Most side effects reported in patients under chronic therapy with warfarin are bleeding, swelling, bruising, articular

Table 1
Primers used to determine relative gene expression in flatfish liver exposed to warfarin, dexamethasone and imidazole. Gene name, accession numbers (GenBank, or Sequence Read Archive (SRA)), primers and amplicon size used to determine relative gene expression quantification in the Senegalese sole (*Solea senegalensis*) exposed to different drugs.

Gene name	Accession number	Component	5′ to 3′ nucleotide sequence	Amplicon size (bp)
ATP binding cassette subfamily B member 1 – abcb1	47816[a]	Forward	CGTCACAGGGAGGGAAAGAGG	179
		Reverse	GCGCAGATGAGCCCCACTACA	
Cytochrome P450, family 1, subfamily A – cyp1a	GU946412	Forward	CGAGGGGGATTTTTCGGGCA	132
		Reverse	AGGGCACTGTAGGCCAGCTTTCTG	
Pregnane X receptor – pxr	KC108909	Forward	GACGGTGTACGAGCGGGTTTC	118
		Reverse	GGGACATGGCTTGCATGAGAACA	
Ubiquitin – ubq	AB291588	Forward	AGCTGGCCCAGAAATATAACTGCGACA	93
		Reverse	ACTTCTTCTTGCGGCAGTTGACAGCAC	

[a] Unigene number assigned from Solea Data Base (http://www.juntadeandalucia.es/agriculturaypesca/ifapa/aquagenet/soleaDB)

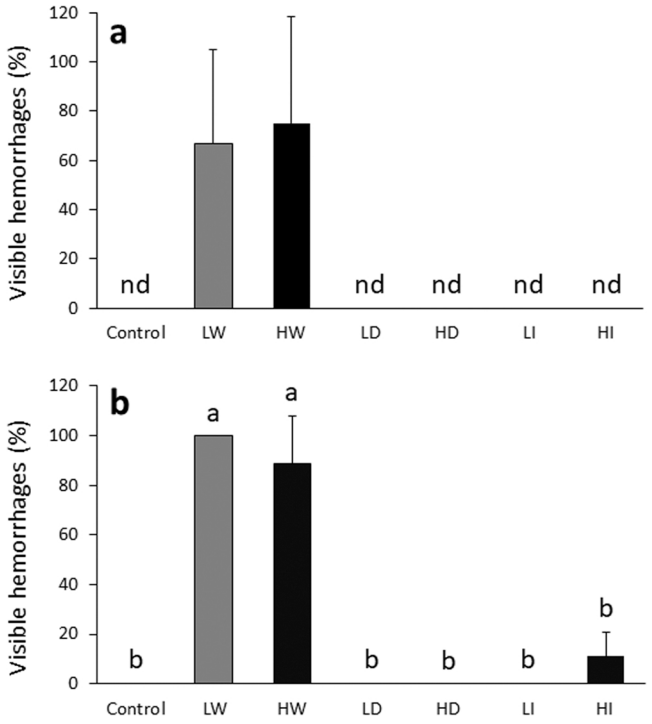

Fig. 1. Incidence of hemorrhages in percentage (mean and standard deviation) in *Solea senegalensis* at 3 (a) and 7 (b) days after drug exposure. *Control*, fish not exposed; *LW*, fish upon 1 mg L^{-1} warfarin exposure; *HW*, fish exposed to 10 mg L^{-1} of warfarin; *LD*, fish exposed to 0.392 mg L^{-1} of dexamethasone; *HD*, fish exposed to 3.92 mg L^{-1} of dexamethasone; *LI*, fish exposed to 0.013 mg L^{-1} of imidazole; *HI*, fish exposed to 0.131 mg L^{-1} of imidazole; *nd*, non-detected. Significant differences among experimental groups are indicated with different letters at the top of each bar (one-way ANOVA, $P < 0.05$, N = 3).

pain or the calcification of the vascular system (Chatrou et al., 2012; Elango et al., 2021), but not liver injury. Although rarely reported, acute liver failure has been observed in some patients treated with warfarin, or any other anticoagulant working as a vitamin K antagonist (Maura et al., 2020). Warfarin has also been associated with reduced risk of transaminase elevation (Nipun and Goldhaber, 2006; Maura et al., 2020), one of the most commonly used indicators of hepatic damage. While warfarin clinical trials are performed in adult patients, our studies were conducted in developing organisms, resulting in a more severe pathological condition. Reduced γ-carboxylation of vitamin K-dependent proteins other than blood clotting factors may account for the vacuolar degeneration and nuclear pyknosis of hepatic cells, sinusoidal dilatation and congestion observed in histological sections of Senegalese sole juveniles.

Dexamethasone has been shown to be effective in the treatment of liver injury, such as the induced through bile duct ligation (a model for biliary obstruction), blocking the release of pro-inflammatory cytokines, reducing the release of anti-fibrotic mediators and/or protecting the levels of antioxidant enzymes (Eken et al., 2006). Nevertheless, dexamethasone exposure to healthy rats led to swelling and/or vacuolization of hepatocytes (Ejiri et al., 2003), as well as hepatocyte apoptosis and the consequent increase in transaminases (Huang et al., 2016). Discrete nuclear pyknosis of hepatocytes was also observed in Senegalese sole exposed to the highest concentration of dexamethasone (HD), in line with data reported by Zhong et al. (2021) in the mosquitofish (*Gambusia affinis*), where hepatocyte degeneration, focal necrosis and pyknotic nucleus were observed in specimens exposed to dexamethasone (50 μg L^{-1}) for 60 days. An increase in glycogen content (PAS-deep magenta staining; Diastase/PAS-light pink staining) was also observed in soles exposed to both the low (LD) and high (HD) concentrations of dexamethasone when compared to Control fish (Fig. 2e, f; Table 3). Similar effects have been reported in mammalian species in which exposure to lower concentrations than those here tested led to an increase in glycogen content (Zheng et al., 2009; Niu et al., 2018; Divari et al., 2020). This effect may be related to the metabolic action of glucocorticoids, known to promote hepatic gluconeogenesis and increased glycogen storage as a metabolic adaptation during stress conditions (Niu et al., 2018; Divari et al., 2020).

Fish exposed to low and high concentrations of imidazole (LI and HI, respectively) showed vacuolar degeneration and nuclear pyknosis of hepatocytes. Furthermore, capillary hyperemia and ballooning of hepatocytes were also detected especially in fish exposed to HI (Fig. 2g, h

Table 2
Mean ± standard deviation values for body wet weight (BWW), liver weight (LVW) and hepatosomatic index (HSI) of the Senegalese sole (*Solea senegalensis*) juveniles exposed to drugs for 3 and 7 days.

Treatment		BWW (mg)	LVW (mg)	HSI (%)
Control	Day 3	840 ± 150	20 ± 2.0	1.88 ± 1.42
LW		950 ± 80	16.9 ± 9.8	1.99 ± 1.52
HW		890 ± 50	11.4 ± 3	1.29 ± 0.28
LD		860 ± 70	11.3 ± 2.7	1.42 ± 0.42
HD		930 ± 40	11.6 ± 1.6	1.25 ± 0.12
LI		910 ± 100	16.9 ± 9.7	1.75 ± 0.73
HI		800 ± 140	22 ± 12.3	2.84 ± 1.49
Control	Day 7	840 ± 50	9.8 ± 2.5	1.17 ± 0.24
LW		770 ± 150	9.5 ± 0.4	1.24 ± 0.19
HW		750 ± 40	8.8 ± 1.6	1.12 ± 0.16
LD		770 ± 60	7.6 ± 0.8	0.99 ± 0.11
HD		810 ± 110	8.4 ± 0.3	1.08 ± 0.11
LI		790 ± 50	9.8 ± 0.6	1.27 ± 0.09
HI		790 ± 40	8.0 ± 0.6	1 ± 0.09

Control, flatfish not exposed to any drug; *LW*, specimens exposed to a low dose of warfarin (1 mg L^{-1}); *HW*, specimens exposed to a high dose of warfarin (10 mg L^{-1}); *LD*, specimens exposed to a low dose of dexamethasone (0.392 mg L^{-1}); *HD*, specimens exposed to a high dose of dexamethasone (3.92 mg L^{-1}); *LI*, specimens exposed to a low dose of imidazole (0.013 mg L^{-1}); *HI*, flatfish exposed to a high dose of imidazole (0.131 mg L^{-1}). N = 3.

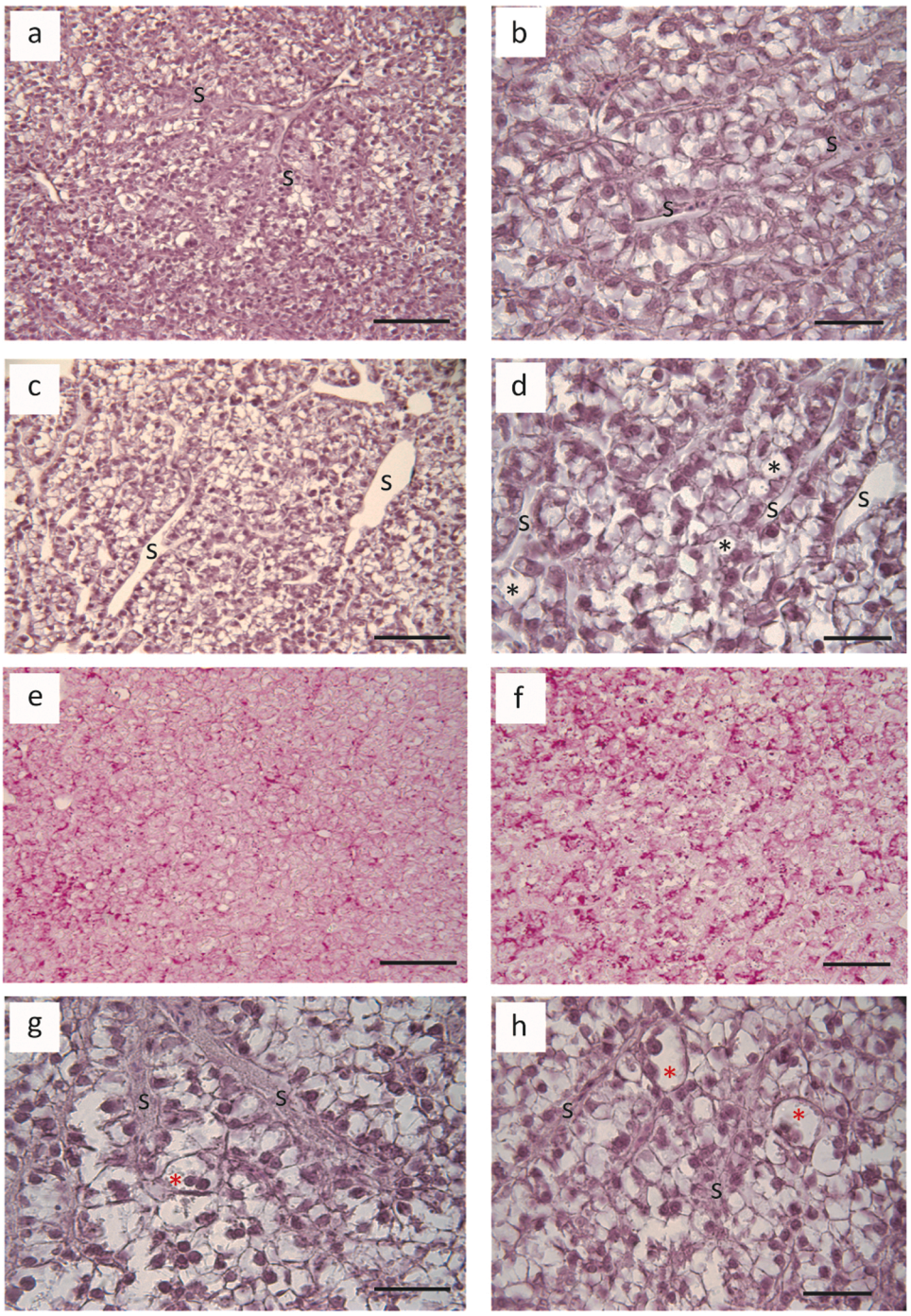

Fig. 2. Microphotographs of liver sections of Senegalese sole (*Solea senegalensis*) at 7 days after drug exposure. Hematoxylin-eosin (a, b, c, d, g, h) stain and Periodic Acid-Schiff (PAS) (e and f) reaction. Liver from Control specimens (a and b). Liver from Senegalese sole exposed to a low dose of warfarin (1 mg L^{-1}) (c and d). Note vasculature dilatation (S) and hepatocytes vacuolar degeneration (*black asterisks*). Glycogen content in Control (e) and dexamethasone (3.92 mg L^{-1}) exposed Senegalese sole (f). Magenta particles represent glycogen, note increased glycogen content in exposed fish (f) compared with the Control (e). Liver sections from Senegalese sole exposed to imidazole (0.13 mg L^{-1}) (g and h). Note capillary (S) hyperemia and hepatocytes ballooning (*red asterisks*). *Black asterisks*, vacuolar degeneration of hepatocytes; *red asterisks*, hepatocyte ballooning; S, sinusoids. Scale bars represent 50 μm (a, e and f) or 25 μm (b, d, g and h).

and Table 3). The treatment of mycoses with imidazole compounds (the triazoles) as antifungal has been declined recently due to their toxic effect on liver and hormones (Mourad and Perfect, 2018), probably as a result of decreased mitochondrial membrane potential, impaired electron transport (Haegler et al., 2017) and elevated transaminases (Gupta et al., 2015). The effects observed here at cellular level in fish liver are in line with liver injury reported in humans by imidazole compounds, and raise further concerns about the use and release of imidazole in aquatic environments.

The present results evidenced how exposure to these drugs altered the physiological status of the liver, most likely impairing the redox system due to the activation of drug metabolism through pregnane X receptor (PXR) signaling in a drug- and concentration-dependent manner (see below). Although it may be difficult to obtain an ecosystem perspective when using organisms from different taxonomic groups (marine vertebrates *versus* marine invertebrates) and with different biological features, the reported toxic effects of the three tested drugs (at tissue and/or organismal level) clearly raise concerns and unveil the ecological implications that their release might have for aquatic organisms (DellaGreca et al., 2004; Webb, 2004; Corcoran et al., 2014; Bal et al., 2017; Pes et al., 2021), in particular those inhabiting marine coastal areas.

3.2. Biochemical responses to drug exposure

All aerobic organisms produce ROS due to cellular respiration and

Table 3
Prevalence (in %) of histopathological lesions in liver of Senegalese sole (*Solea senegalensis*) specimens exposed to drugs for 7 days.

Treatment	Control	LW	HW	LD	HD	LI	HI
Capillary hyperemia	0	0	0	0	0	33.3	100
Vasculature dilation	0	33.3	100	0	0	0	0
Pyknotic nuclei	0	33.3	66.7	0	33.3	33.3	66.7
Vacuolar degeneration	0	33.3	100	0	0	33.3	66.7
Ballooning of hepatocytes	0	0	0	0	0	33.3	100
Increase of glycogen content	0	0	0	33.3	100	0	0

Control, flatfish not exposed to any drug; *LW*, specimens exposed to a low dose of warfarin (1 mg L^{-1}); *HW*, specimens exposed to a high dose of warfarin (10 mg L^{-1}); *LD*, specimens exposed to a low dose of dexamethasone (0.392 mg L^{-1}); *HD*, specimens exposed to a high dose of dexamethasone (3.92 mg L^{-1}); *LI*, specimens exposed to a low dose of imidazole (0.013 mg L-1); *HI*, flatfish exposed to a high dose of imidazole (0.131 mg L^{-1}). N = 3.

metabolism, but to prevent cellular damage, there is a balance between ROS production and their scavenging by antioxidant defenses (Regoli et al., 2014). Any disequilibrium in this balance towards an over production of ROS may lead to oxidative stress. Among the different antioxidant responses, our study focused on the drug effect on TAS and GR, GPx and SOD enzyme activities at 7 dpe (Fig. 3). TAS was significantly altered in fish exposed to LD, HD and LI (Fig. 3a' and a''). While GR activity remained unaltered in all fish, regardless of the drug and concentration tested (Fig. 3b, b' and b''), GPx activity increased in fish treated with HW and HD, but not with HI (Fig. 3c, c' and c''). Furthermore, only HD exposure led to increased SOD activity when compared to the Control fish (Fig. 3d, d' and d'').

The molecular mechanisms underlying warfarin effects on the oxidative status of aquatic organisms are still poorly understood. Granadeiro et al. (2019) found that exposure of zebrafish to warfarin during embryogenesis altered the expression of genes involved in the redox system and oxidative stress response. Using the same experimental design, Pes et al. (2021) also reported that both warfarin concentrations (LW and HW) increased GPx activity in the Mediterranean mussel, in line with higher GPx activity in Senegalese sole juveniles exposed to HW.

The histopathological effects observed in the liver of animals exposed to dexamethasone (steatosis and apoptosis) are due to increased oxidative stress (Huang et al., 2016). Our data showed that a lower concentration of dexamethasone and a shorter exposure time than those previously tested (Costantini et al., 2011; Guiloski et al., 2015) led to an increase in TAS in Senegalese sole, probably due to the increase in GPx and SOD activity in exposed animals. Increased GPx activity reported in fish under HD exposure is also in line with the above-cited literature in fish species, as well as in marine invertebrates (Pes et al., 2021). In contrast, increased SOD activity in Senegalese sole exposed to dexamethasone is in contradiction with data reported by Guiloski et al. (2015). Differences in fish species, fish size and experimental approach (water exposure *versus* nutritional exposure) may account for these discrepancies.

Imidazole compounds impaired the redox balance in exposed

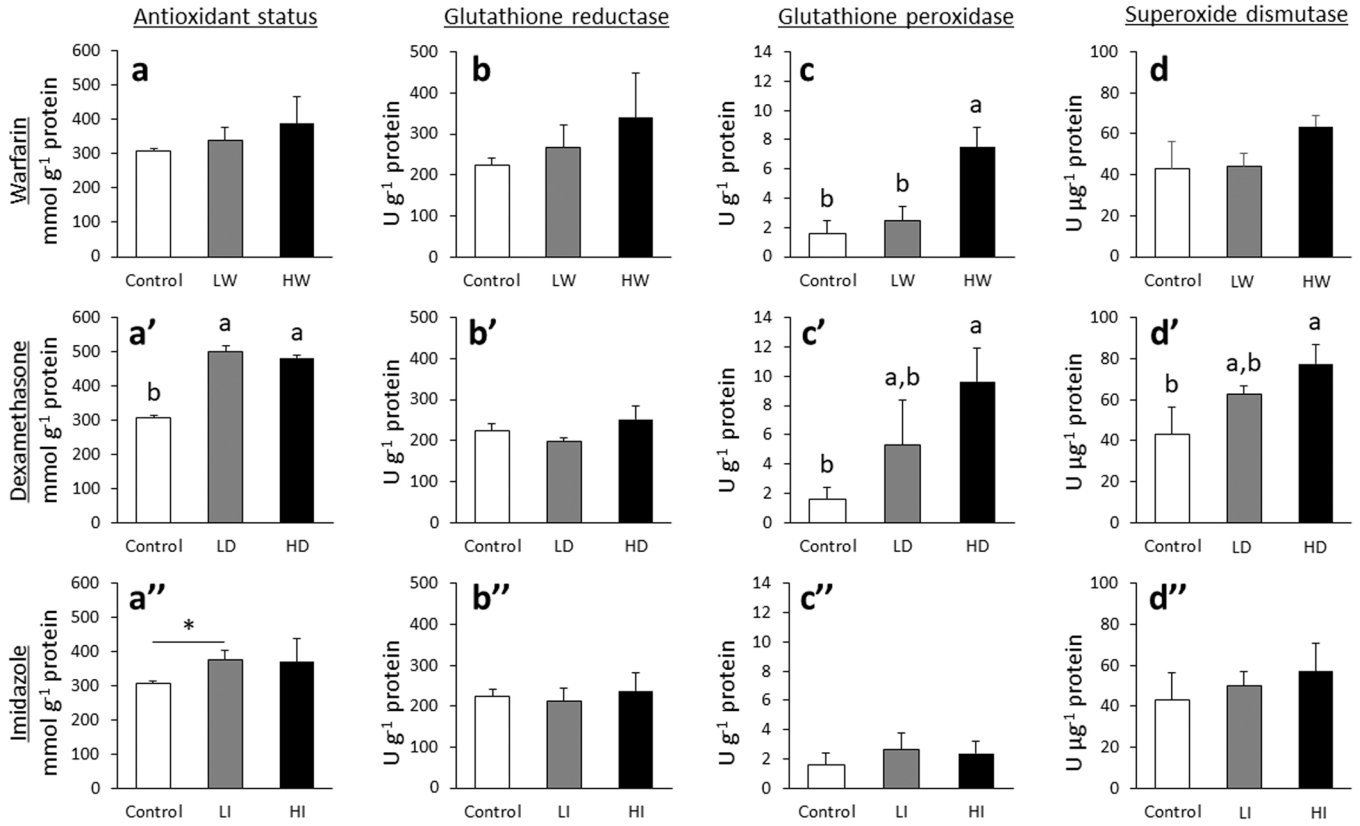

Fig. 3. Total antioxidant status (a, a' and a''), glutathione reductase (b, b' and b''), glutathione peroxidase (c, c' and c'') and superoxide dismutase (d, d' and d'') activities (expressed as mean ± standard deviation) in Senegalese sole (*Solea senegalensis*) after 7 days exposure to warfarin, dexamethasone or imidazole. *Control*, fish not exposed; *LW*, fish upon 1 mg L^{-1} warfarin exposure; *HW*, fish exposed to 10 mg L^{-1} of warfarin; *LD*, fish exposed to 0.392 mg L^{-1} of dexamethasone; *HD*, fish exposed to 3.92 mg L^{-1} of dexamethasone; *LI*, fish exposed to 0.013 mg L^{-1} of imidazole; *HI*, fish exposed to 0.131 mg L^{-1} of imidazole. Significant differences among experimental groups are indicated with different letters or with an asterisk at the top of each bar (one-way ANOVA or Student's t-test, respectively; $P < 0.05$, N = 3).

organisms, for instance by increasing mRNA and protein levels of SOD (Adeyemi et al., 2020; Haegler et al., 2017). Although we observed a tendency for imidazole exposure to increase GR, GPx and SOD activities, only TAS measured in fish exposed to LI was significantly different from the Control group. Contradictory results in aquatic organisms have been reported regarding the response of the antioxidant system to imidazole compounds. Lushchak (2016) found that compounds derived from imidazole induced oxidative stress in several fish species, while Solé et al. (2014) reported unaltered antioxidant enzyme activities in Senegalese sole exposed to ketoconazole, an imidazole antifungal agent. Structure differences between ketoconazole and imidazole may lead to different capacities in inducing oxidative stress. Furthermore, the response of Senegalese sole to imidazole exposure here reported is in contrast to that described by Pes et al. (2021), where Mediterranean mussel exposed to both LI and HI showed increased GPx activity, but no alteration in TAS, GR and/or SOD activities.

In general, increased redox enzyme activities might be related with the drug detoxification process, where sequential rounds of oxidative reactions will inactivate and/or metabolize these drugs in the liver. A comparison of the antioxidant defense response in Senegalese sole juveniles and Mediterranean mussels to the drugs here tested, suggests that they are similar depending on the drug considered, regardless of differences existing in the functioning of vertebrate liver and invertebrate hepatopancreas (Rőszer et al., 2014). In this regard, all drugs increased GPx activity in the Mediterranean mussel, while warfarin and dexamethasone also increased GPx activity in Senegalese sole. Considering antioxidant activities in Mediterranean mussel, GPx was the most active enzyme (with activities ranging from 200 to 800 U g^{-1} protein; Pes et al., 2021). Dexamethasone altered SOD activity in Senegalese sole but not in mussels, which may be related to a lower amount of SOD in invertebrates (Felton, 1995).

3.3. Molecular pathways of drug-sensing and metabolism are differentially regulated upon short exposure to pharmaceuticals

Warfarin is a mixture of enantiomers (S- and R-warfarin), the R-enantiomer being a ligand for the PXR (Rulcova et al., 2010). In this study, *pxr* up-regulation in Senegalese sole juveniles exposed to LW for 3 days (Fig. 4a), is in agreement with previous studies performed in fish (Fernández et al., 2014; Marques et al., 2017). Noticeably, a higher dose of warfarin and/or a longer exposure time (7 days) did not translate into greater up-regulation of *pxr* expression. The recently reported feedback regulation of *pxr* expression through its own ligands (Smutny et al., 2020) may explain the absence of a greater effect. ABCB1 is an energy-dependent pump responsible for xenobiotic efflux from cells (Ambudkar et al., 2006), whose expression is under the control of PXR in humans (Haerian et al., 2011). In our work, exposure to LW was also translated into a higher expression of *abcb1* when compared to that of non-exposed soles at 3 dpe, in line with the dose- and time-dependent stimulation of *pxr* expression. However, a tendency towards down-regulation of *abcb1* expression was observed at 7 dpe, only being significant in HW-exposed juveniles (Fig. 4b and b'). Cytochrome P4501A (CYP1A) is an enzymatic system able to oxidize structurally unrelated compounds such as fatty acids, steroids and xenobiotics like polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs; Jönsson et al., 2009). Previous studies showed that transcription of CYP genes is governed by PXR activation in mammals (Ihunnah et al., 2011). However, our results suggest that warfarin exposure did not induce *cyp1a* gene expression in sole and even decreased it in fish exposed to LW for 7 days (Fig. 4c and c').

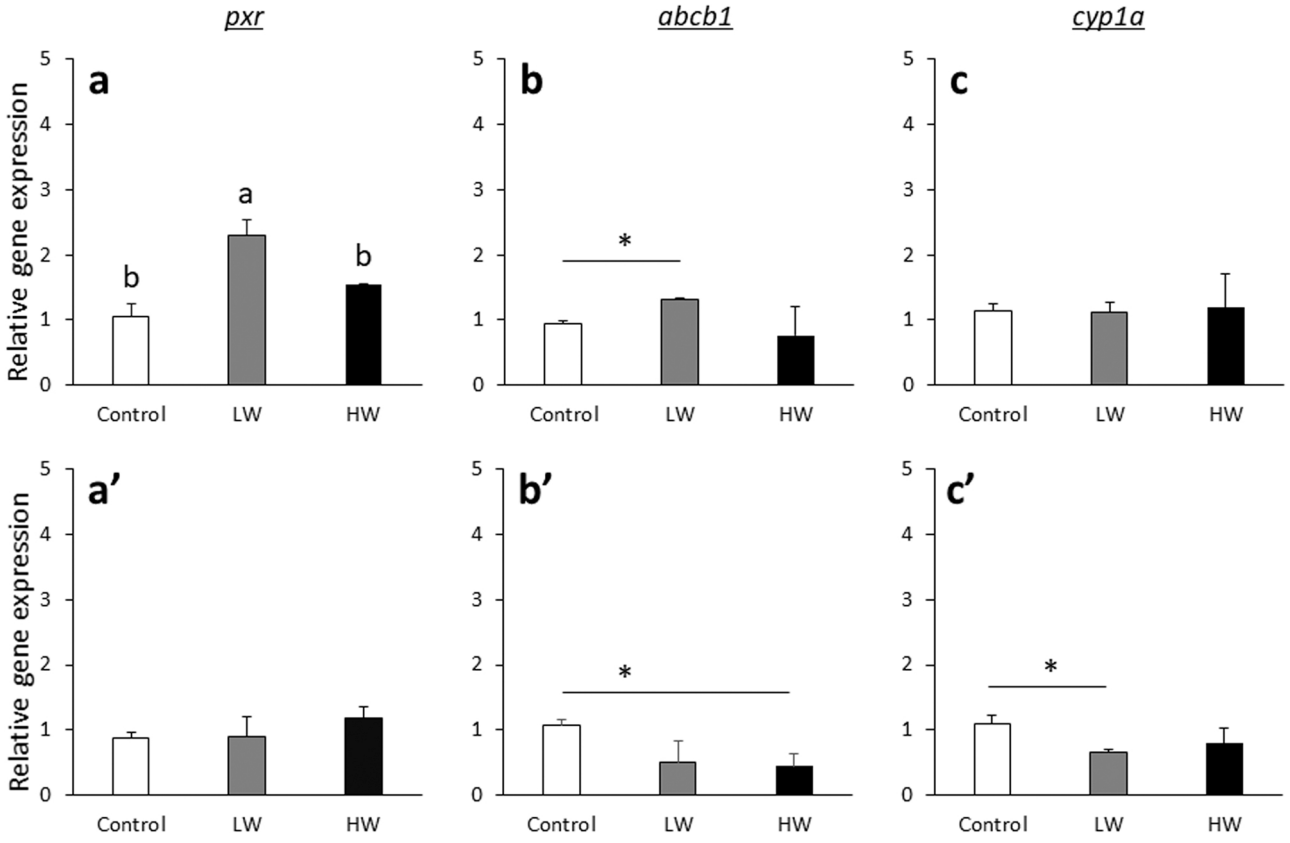

Fig. 4. Relative gene expression (expressed as mean ± standard deviation) of *pxr* (a and a'), *abcb1* (b and b') and *cyp1a* (c and c') in Senegalese sole (*Solea senegalensis*) exposed to warfarin for 3 (a–c) and 7 (a'–c') days. *Control*, fish not exposed; *LW*, fish upon 1 mg L^{-1} warfarin exposure; *HW*, fish exposed to 10 mg L^{-1} of warfarin. Significant differences among experimental groups are indicated with different letters or with asterisk at the top of each bar (one-way ANOVA or Student's t-test, respectively; $P < 0.05$, N = 3).

Dexamethasone exposure significantly decreased *pxr* expression in fish exposed to LD for 3 days (Fig. 5a), but not in fish exposed to a higher dose (HD) or for longer time (7 days). As for warfarin, a feedback regulation may explain such differences. Although *pxr* up-regulation has already been reported in humans and rats treated with dexamethasone (Lehmann et al., 1998; Pascussi et al., 2000; Shi et al., 2010; Hunter et al., 2017), data in rainbow trout showed that hepatocytes exposed to 1 μM of dexamethasone (same concentration as the lower concentration tested in our study) consistently exhibited a decreased expression of *pxr* (Wassmur et al., 2010). In our work, an up-regulation of *abcb1* expression at 3 days in fish exposed to HD (Fig. 5b), and a down-regulation at 7 days in both LD- and HD-treated fish (Fig. 5b') was observed. Variation in *abcb1* expression may be a consequence of the dynamic regulation of genes controlled by PXR (including *abcb1*) and their transcriptional activation through glucocorticoid receptors, known to mediate *abcb1* gene induction (Wang and LeCluyse, 2003). Indeed, contradictory results have been reported for the regulation of the expression and activity of the ATP-binding cassette efflux pumps by drugs, in particular by glucocorticoids (Hirano et al., 2004; Nishimura et al., 2004; Richaud-Patin et al., 2004; Martin et al., 2008; Tanaka et al., 2009; Wassmur et al., 2010; Manceau et al., 2012). In sole, *cyp1a* expression increased in fish exposed to HD for 3 days (Fig. 5c) but decreased in fish exposed to both dexamethasone concentrations (LD and HD) at 7 days (Fig. 5c'). While Burkina et al. (2013) and Zhang et al. (2006) showed that dexamethasone exposure did not change *cyp1a* expression in rats and dogs, Monostory et al. (2005) found *cyp1a* expression increased in rats but decreased in humans. As for other genes, a negative-feedback autoregulatory loop of *cyp1a* gene expression has been demonstrated (Morel et al., 1999).

Regarding imidazoles, Takeshita et al. (2002) and Venkatesh et al. (2011) described ketoconazole as a PXR antagonist in humans, while clotrimazole is a known agonist of PXR in fish (Moore et al., 2002; Milnes et al., 2008). In our study, both high and low concentrations of imidazole up-regulated *pxr* expression at 3 dpe, but not at 7 dpe (Fig. 6a, a'). As for warfarin exposure, *abcb1* expression was increased in fish treated with LI for 3 days (Fig. 6b) but reduced in fish exposed to LI or HI for 7 days (Fig. 6b'). In fish, imidazole-related compounds have been shown to regulate the CYP1A system, activating or inhibiting CYP1A protein and *cyp1a* mRNA levels (Snegaroff and Bach, 1989; Levine and Oris, 1999; Hegelund et al., 2004; Navas et al., 2004; Babin et al., 2005; Hasselberg et al., 2005). Here, Senegalese sole juveniles exposed to imidazole showed up-regulated *cyp1a* expression at both concentrations upon exposure for 3 days (Fig. 6c) but down-regulation in fish exposed to HI for 7 days (Fig. 5c').

In general, warfarin, dexamethasone and imidazole exposure led to a dynamic regulation of *pxr* expression in Senegalese sole similar to that of *nr1j* genes observed in mussels exposed to the same drug concentrations (Pes et al., 2021). Although the expression of *cyp1-like* genes was not explored by Pes et al. (2021) in the Mediterranean mussel exposed to the three drugs, Zanette et al. (2013) reported no significant changes in gene expression upon exposure to various agonists of the aryl hydrocarbon receptor (AHR), suggesting that a great divergence in recognizing and metabolizing xenobiotics might exist with vertebrate homologues.

In the present research study, the potential effects of pharmaceuticals were explored using environmentally relevant levels (regarding imidazole and related products) or close to those environmentally reported (dexamethasone). In the case of warfarin, levels are higher than those measured in the environment but represent an attempt to model the potential effects of the several related compounds now used in the industry as anticoagulants (see Regnery et al., 2020). In addition,

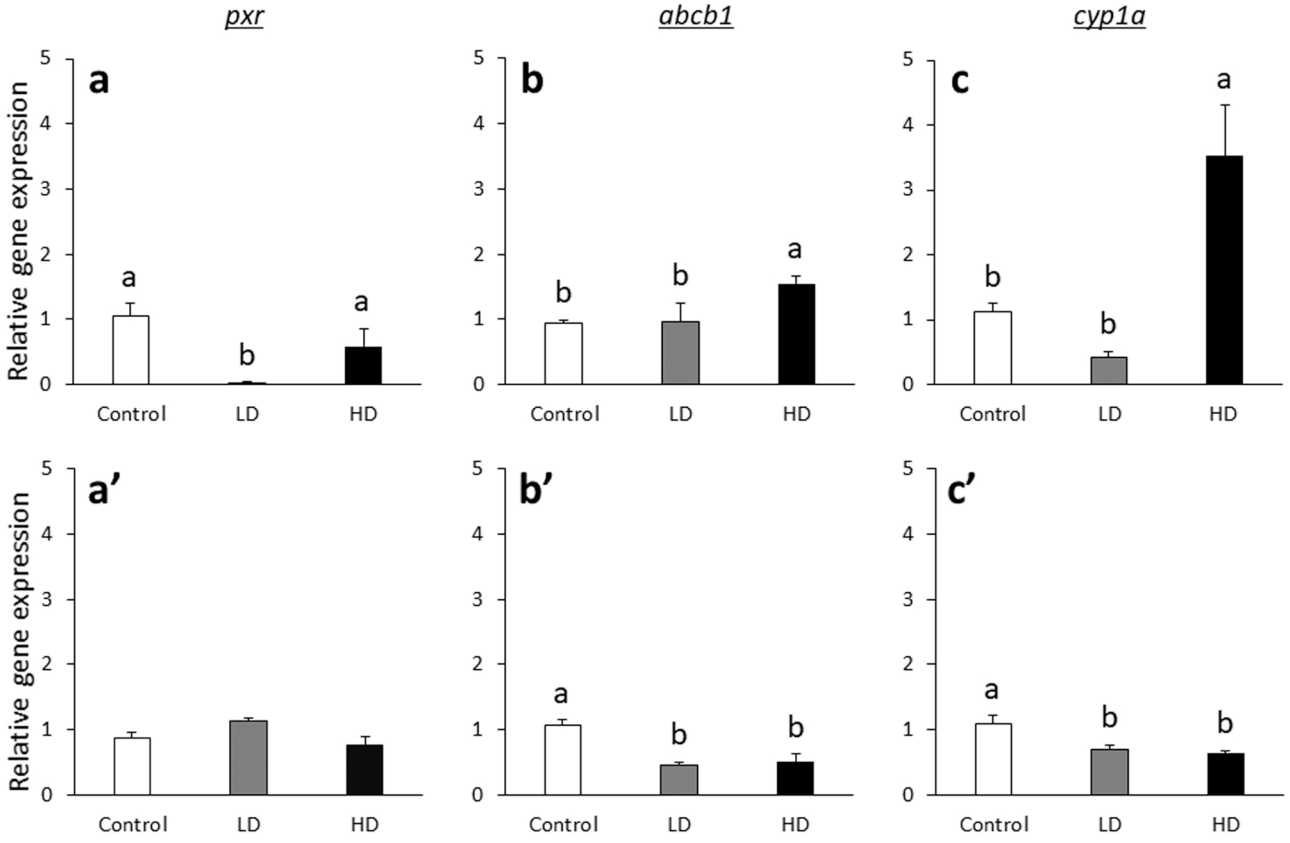

Fig. 5. Relative gene expression (expressed as mean ± standard deviation) of *pxr* (a and a'), *abcb1* (b and b') and *cyp1a* (c and c') in Senegalese sole (*Solea senegalensis*) exposed to dexamethasone for 3 (a–c) and 7 (a'–c') days. *Control*, fish not exposed; *LD*, fish exposed to 0.392 mg L^{-1} of dexamethasone; *HD*, fish exposed to 3.92 mg L^{-1} of dexamethasone. Significant differences among experimental groups are indicated with different letters at the top of each bar (one-way ANOVA, $P < 0.05$, N = 3).

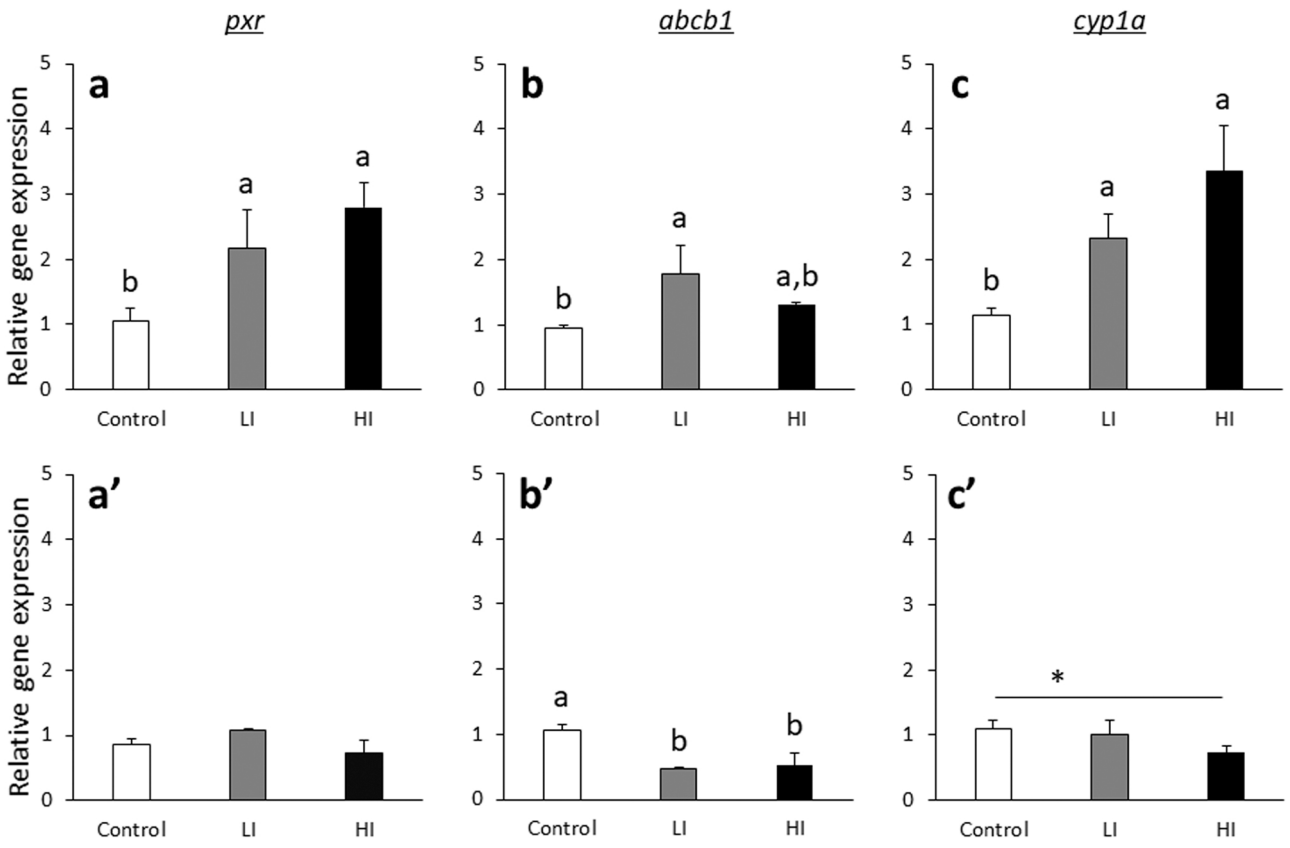

Fig. 6. Relative gene expression (expressed as mean ± standard deviation) of *pxr* (a and a'), *abcb1* (b and b') and *cyp1a* (c and c') in Senegalese sole (*Solea senegalensis*) exposed to imidazole for 3 (a–c) and 7 (a'–c') days. *Control*, fish not exposed; *LI*, fish exposed to 0.013 mg L^{-1} of imidazole; *HI*, fish exposed to 0.131 mg L^{-1} of imidazole. Significant differences among experimental groups are indicated with different letters or with an asterisk at the top of each bar (one-way ANOVA or Student's t-test, respectively; $P < 0.05$, N = 3).

because pharmaceutical monitoring efforts have been limited to specific areas and particular seasons, it is possible that environmental levels reported in the literature may be underestimated. Indeed, the most recent and extensive review of the presence of pharmaceuticals in the ecosystem (Patel et al., 2019) concluded that (i) advanced methods for accurate and continuous detection of pharmaceuticals should be developed and applied, and (ii) more attention should be paid to monitoring them in rapidly developing industrial nations. Furthermore, monitoring in regions with low or no implementation of WWT systems should be urgently conducted in order to have a more realistic and worldwide picture. Nevertheless, the present results indicate that a benthic fish (Senegalese sole) inhabiting the marine coast is sensitive to the release of PPCPs, and that studies at organism, tissue and molecular levels should be conducted to unveil their potential effects as well as an integrated risk assessment of their presence in aquatic environments.

4. Conclusion

In the present study, we characterized the short-term toxic effects of three commonly used pharmaceuticals (at organism and tissue level) in Senegalese sole juveniles, a species inhabiting the coastal benthos. Vasculature dilatation, pyknotic nuclei and vacuolar degeneration were observed in the liver of Senegalese sole exposed to lower concentrations (1 mg L^{-1}) of warfarin than previously tested. New insights into the toxic effects of dexamethasone (increased glycogen content) and imidazole (liver capillary hyperemia, pyknotic nuclei, vacuolar degeneration and ballooning of hepatocytes) were also collected. Furthermore, we observed that the same doses of warfarin, dexamethasone and imidazole and the same exposure times, exerted partially similar biochemical and molecular responses in a marine flatfish (the Senegalese sole; this study) and a marine invertebrate species (the Mediterranean mussel; Pes et al., 2021). Based on the data collected in marine organisms from different trophic/taxonomic levels, we propose that toxic effects of pharmaceuticals released in the aquatic environment may have a wider impact on coastal ecosystems. Thus, a more integrated environmental risk assessment should be conducted to gain knowledge on ecological implications, particularly in aquatic organisms, as well as to define and implement effective regulatory decisions.

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CRediT authorship contribution statement

Katia Pes: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Juan B. Ortiz-Delgado:** Formal analysis, Investigation, Writing – review & editing. **Carmen Sarasquete:** Formal analysis, Investigation, Writing – review & editing. **Vincent Laizé:** Supervision, Resources, Writing – review & editing, Founding acquisition. **Ignacio Fernández:** Conceptualization, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. All authors have read and agreed with the published version of the

manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Adeyemi, O.S., Eseola, A.O., Plass, W., Otuechere, C.A., Elebiyo, T.C., 2020. New imidazoles cause cellular toxicity by impairing redox balance, mitochondrial membrane potential, and modulation of HIF-1α expression. Biochem. Biophys. Res. Commun. 529, 23–27. https://doi.org/10.1016/j.bbrc.2020.05.059.

Ali, A.M., Rønning, H.T., Sydnes, L.K., Alarif, W.M., Kallenborn, R., Al-Lihaibi, S.S., 2018. Detection of PPCPs in marine organisms from contaminated coastal waters of the Saudi Red Sea. Sci. Total. Environ. 621, 654–662. https://doi.org/10.1016/j.scitotenv.2017.11.298.

Ambudkar, S.V., Kim, I.W., Sauna, Z.E., 2006. The power of the pump: mechanisms of action of P-glycoprotein (ABCB1). Eur. J. Pharm. Sci. 27 (5), 392–400. https://doi.org/10.1016/j.ejps.2005.10.010.

Arpin-Pont, L., Bueno, M.J.M., Gomez, E., Fenet, H., 2016. Occurrence of PPCPs in the marine environment: a review. Environ. Sci. Pollut. Res. Int. 23, 4978–4991. https://doi.org/10.1007/s11356-014-3617-x.

Arthur, J.R., Boyne, R., 1985. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci. 36, 1569–1575. https://doi.org/10.1016/0024-3205(85)90381-9.

Babin, M., Casado, S., Chana, A., Herradon, B., Segner, H., Tarazona, J.V., Navas, J.M., 2005. Cytochrome P4501A induction caused by the imidazole derivative Prochloraz in a rainbow trout cell line. Toxicol. Vitr. 19 (7), 899–902. https://doi.org/10.1016/j.tiv.2005.06.037.

Bal, N., Kumar, A., Du, J., Nugegoda, D., 2017. Multigenerational effects of two glucocorticoids (prednisolone and dexamethasone) on life-history parameters of crustacean *Ceriodaphnia dubia* (Cladocera). Environ. Pollut. 225, 569–578. https://doi.org/10.1016/j.envpol.2017.03.024.

BASF AG, 1977. BASF AG, Department of Toxicology, unpublished Screening Test, PF94, 15.03.1977.

BASF AG, 1988. BASF AG, Department of Ecology, Bestimmung der akuten Wirkung von Imidazol auf den Wasserfloh Daphnia magna Staus, unpublished study, 1/0948/2/88-0948/88, 26.10.1988.

Beato, S., Toledo-Solís, F.J., Fernández, I., 2020. Vitamin K in vertebrates' reproduction: further puzzling pieces of evidence from teleost fish species. Biomolecules 10 (9), 1303. https://doi.org/10.3390/biom10091303.

Bevans, C.G., Krettler, C., Reinhart, C., Tran, H., Koßmann, K., Watzka, M., Oldenburg, J., 2013. Determination of the warfarin inhibition constant Ki for vitamin K 2,-3-epoxide reductase complex subunit-1 (VKORC1) using an in vitro DTT-driven assay. BBA-Gen. Subj. 1830 (8), 4202–4210. https://doi.org/10.1016/j.bbagen.2013.04.018.

Birch, G.F., Drage, D.S., Thompson, K., Eaglesham, G., Mueller, J.F., 2015. Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary, Australia. Mar. Pollut. Bull. 97 (1–2), 56–66. https://doi.org/10.1016/j.marpolbul.2015.06.038.

Boxall, A.B., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., Ankley, G.T., Beazley, K.F., Belanger, S.E., Berninger, J.P., Carriquiriborde, P., Coors, A., Deleo, P.C., Dyer, S.D., Ericson, J.F., Gagné, F., Giesy, J.P., Gouin, T., Hallstrom, L., Karlsson, M.V., Larsson, D.G., Lazorchak, J.M., Mastrocco, F., McLaughlin, A., McMaster, M.E., Meyerhoff, R.D., Moore, R., Parrott, J.L., Snape, J.R., Murray-Smith, R., Servos, M.R., Sibley, P.K., Straub, J.O., Szabo, N.D., Topp, E., Tetreault, G.R., Trudeau, V.L., Van Der Kraak, G., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ. Health Perspect. 120 (9), 1221–1229. https://doi.org/10.1289/ehp.1104477.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Branchet, P., Arpin-Pont, L., Piram, A., Boissery, P., Wong-Wah-Chung, P., Doumenq, P., 2021. Pharmaceuticals in the marine environment: what are the present challenges in their monitoring? Sci. Total Environ. 766, 142644 https://doi.org/10.1016/j.scitotenv.2020.142644.

Burkina, V., Zlabek, V., Zamaratskaia, G., 2013. Clotrimazole, but not dexamethasone, is a potent in vitro inhibitor of cytochrome P450 isoforms CYP1A and CYP3A in rainbow trout. Chemosphere 92 (9), 1099–1104. https://doi.org/10.1016/j.chemosphere.2013.01.050.

Burkina, V., Sakalli, S., Rasmussen, M.K., Zamaratskaia, G., Koba, O., Thai, G.P., Grabic, R., Randak, T., Zlabek, V., 2015. Does dexamethasone affect hepatic CYP450 system of fish? Semi-static in-vivo experiment on juvenile rainbow trout. Chemosphere 139, 155–162. https://doi.org/10.1016/j.chemosphere.2015.06.031.

Castillo, L.E., de la Cruz, E., Ruepert, C., 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. Environ. Toxicol. Chem. 16 (1), 41–51. https://doi.org/10.1002/etc.5620160104.

Chang, H., Hu, J., Shao, B., 2007. Occurrence of natural and synthetic glucocorticoids in sewage treatment plants and receiving river waters. Environ. Sci. Technol.. 41 (10), 3462–3468. https://doi.org/10.1021/es062746o.

Chatrou, M.L., Winckers, K., Hackeng, T.M., Reutelingsperger, C.P., Schurgers, L.J., 2012. Vascular calcification: the price to pay for anticoagulation therapy with vitamin K antagonists. Blood Rev. 26, 155–166. https://doi.org/10.1016/j.blre.2012.03.002.

Chen, Y., Tang, Y., Guo, C., Wang, J., Boral, D., Nie, D., 2012. Nuclear receptors in the multidrug resistance through the regulation of drug-metabolizing enzymes and drug transporters. Biochem. Pharmacol. 83, 1112–1126. https://doi.org/10.1016/j.bcp.2012.01.030.

Chen, Q., Jia, A., Snyder, S.A., Gong, Z., Lam, S.H., 2016. Glucocorticoid activity detected by in vivo zebrafish assay and in vitro glucocorticoid receptor bioassay at environmental relevant concentrations. Chemosphere 144, 1162–1169. https://doi.org/10.1016/j.chemosphere.2015.09.089.

Corcoran, J., Winter, M.J., Tyler, C.R., 2010. Pharmaceuticals in the aquatic environment: a critical review of the evidence for health effects in fish. Crit. Rev. Toxicol. 40, 287–304. https://doi.org/10.3109/10408440903373590.

Corcoran, J., Lange, A., Cumming, R.I., Owen, S.F., Ball, J.S., Tyler, C.R., Winter, M.J., 2014. Bioavailability of the imidazole antifungal agent clotrimazole and its effects on key biotransformation genes in the common carp (*Cyprinus carpio*). Aquat. Toxicol. 152, 57–65. https://doi.org/10.1016/j.aquatox.2014.03.016.

Costantini, D., Marasco, V., Møller, A.P., 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. J. Comp. Physiol. B. 181 (4), 447–456. https://doi.org/10.1007/s00360-011-0566-2.

Creusot, N., Aït-Aïssa, S., Tapie, N., Pardon, P., Brion, F., Sanchez, W., Thybaud, E., Porcher, J.M., Budzinski, H., 2014. Identification of synthetic steroids in river water downstream from pharmaceutical manufacture discharges based on a bioanalytical approach and passive sampling. Environ. Sci. Technol. 48 (7), 3649–3657. https://doi.org/10.1021/es405313r.

DellaGreca, M., Fiorentino, A., Isidori, M., Lavorgna, M., Previtera, L., Rubino, M., Temussi, F., 2004. Toxicity of prednisolone, dexamethasone and their photochemical derivatives on aquatic organisms. Chemosphere 54 (5), 629–637. https://doi.org/10.1016/j.chemosphere.2003.09.008.

Derveaux, S., Vandesompele, J., Hellemans, J., 2010. How to do successful gene expression analysis using real-time PCR. Methods 50 (4), 227–230. https://doi.org/10.1016/j.ymeth.2009.11.001.

Divari, S., De Lucia, F., Berio, E., Sereno, A., Biolatti, B., Cannizzo, F.T., 2020. Dexamethasone and prednisolone treatment in beef cattle: influence on glycogen deposition and gene expression in the liver. Domest. Anim. Endocrinol. 72, 106444 https://doi.org/10.1016/j.domaniend.2020.106444.

Eglo, O.M., Aspmi, R., Lode, O., 1994. Runoff and leaching experiments of dichlorprop, MCPA, propiconazole, dimethoate, and chlorsulfuron in outdoor lysimeters and field catchment areas. Norw. J. Agric. Sci. 13, 53–78.

Ejiri, N., Katayama, K., Doi, K., 2003. Induction of CYP3A1 by dexamethasone and pregnenolone-16 alpha-carbonitrile in pregnant rat and fetal livers and placenta. Exp. Toxic. Pathol. 54, 273–279. https://doi.org/10.1078/0940-2993-00263.

Eken, H., Ozturk, H., Ozturk, H., Buyukbayram, H., 2006. Dose-related effects of dexamethasone on liver damage due to bile duct ligation in rats. World J. Gastroenterol. 12, 5379–5383. https://doi.org/10.3748/wjg.v12.i33.5379.

Elango, K., Javaid, A., Khetarpal, B.K., Ramalingam, S., Kolandaivel, K.P., Gunasekaran, K., Ahsan, C., 2021. The effects of warfarin and direct oral anticoagulants on systemic vascular calcification: a review. Cells 10, 773. https://doi.org/10.3390/cells10040773.

Fawell, J., Ong, C.N., 2012. Emerging contaminants and the implications for drinking water. Int. J. Water Resour. Dev. 28 (2), 247–263. https://doi.org/10.1080/07900627.2012.672394.

Felton, G.W., 1995. Oxidative Stress of Vertebrates and Invertebrates. Oxidative Stress and Antioxidant Defenses in Biology. Springer, Boston, MA, pp. 356–434.

Fernández, I., Santos, A., Cancela, M.L., Laizé, V., Gavaia, P.J., 2014. Warfarin, a potential pollutant in aquatic environment acting through Pxr signaling pathway and γ-glutamyl carboxylation of vitamin K-dependent proteins. Environ. Pollut. 194, 86–95. https://doi.org/10.1016/j.envpol.2014.07.015.

Gonçalves, C., Martins, M., Diniz, M.S., Costa, M.H., Caeiro, S., Costa, P.M., 2014. May sediment contamination be xenoestrogenic to benthic fish? A case study with *Solea senegalensis*. Mar. Environ. Res. 99, 170–178. https://doi.org/10.1016/j.marenvres.2014.04.012.

Granadeiro, L., Dirks, R.P., Ortiz-Delgado, J.B., Gavaia, P.J., Sarasquete, C., Laizé, V., Cancela, M.L., Fernández, I., 2019. Warfarin-exposed zebrafish embryos resembles human warfarin embryopathy in a dose and developmental-time dependent manner–From molecular mechanisms to environmental concerns. Ecotoxicol. Environ. Saf. 181, 559–571. https://doi.org/10.1016/j.ecoenv.2019.06.042.

Guiloski, I.C., Ribas, J.L.C., da Silva Pereira, L., Neves, A.P.P., de Assis, H.C.S., 2015. Effects of trophic exposure to dexamethasone and diclofenac in freshwater fish. Ecotoxicol. Environ. Saf. 114, 204–211. https://doi.org/10.1016/j.ecoenv.2014.11.020.

Gu, X., Manatou, J.E., 2012. Molecular mechanisms underlying chemical liver injury. Expert Rev. Mol. Med. 14, e4 https://doi.org/10.1017/S1462399411002110.

Gupta, A.K., Daigle, D., Foley, K.A., 2015. Drug safety assessment of oral formulations of ketonazole. Expert Opin. Drug Saf. 14, 325–334. https://doi.org/10.1517/14740338.2015.983071.

Haegler, P., Joerin, L., Krähenbühl, S., Bouitbir, J., 2017. Hepatocellular toxicity of imidazole and triazole antimycotic agents. Toxicol. Sci. 157, 183–195. https://doi.org/10.1093/toxsci/kfx029.

Haerian, B.S., Lim, K.S., Mohamed, E.H.M., Tan, H.J., Tan, C.T., Raymond, A.A., Wong, C.P., Wong, S.W., Mohameda, Z., 2011. Lack of association of ABCB1 and PXR polymorphisms with response to treatment in epilepsy. Seizure 20 (5), 387–394. https://doi.org/10.1016/j.seizure.2011.01.008.

Hasselberg, L., Grøsvik, B.E., Goksøyr, A., Celander, M.C., 2005. Interactions between xenoestrogens and ketoconazole on hepatic CYP1A and CYP3A, in juvenile Atlantic cod (*Gadus morhua*). Comp. Hepatol. 4 (1), 2. https://doi.org/10.1186/1476-5926-4-2.

Hegelund, T., Ottosson, K., Rådinger, M., Tomberg, P., Celander, M.C., 2004. Effects of the antifungal imidazole ketoconazole on CYP1A and CYP3A in rainbow trout and killifish. Environ. Toxicol. Chem. 23 (5), 1326–1334. https://doi.org/10.1897/03-155.

Herrero, P., Borrull, F., Marcé, R.M., Pocurull, E., 2013. Pressurized liquid extraction and ultra-high-performance liquid chromatography-tandem mass spectrometry to determine endogenous and synthetic glucocorticoids in sewage sludge. Talanta 103, 186–193. https://doi.org/10.1016/j.talanta.2012.10.030.

Hirano, T., Onda, K., Toma, T., Miyaoka, M., Moriyasu, F., Oka, K., 2004. MDR1 mRNA expressions in peripheral blood mononuclear cells of patients with ulcerative colitis in relation to glucocorticoid administration. J. Clin. Pharmacol. 44 (5), 481–486. https://doi.org/10.1038/sj.bjp.0705982.

Huang, Y.-H., Chen, C.-J., Tang, K.-S., Sheen, J.-M., Tiao, M.-M., Tain, Y.-L., Chen, C.-C., Chu, E.-W., Li, S.-W., Yu, H.-R., Huang, L.-T., 2016. Postnatal high-fat diet increases liver steatosis and apoptosis threatened by prenatal dexamethasome through the oxidative effect. Int. J. Mol. Sci. 17, 369. https://doi.org/10.3390/ijms17030369.

Hunter, S.R., Vonk, A., Grey, A.K.M., Riddick, D.S., 2017. Role of glucocorticoid receptor and pregnane X receptor in dexamethasone induction of rat hepatic aryl hydrocarbon receptor nuclear translocator and NADPH-cytochrome P45 oxidoreductase. Drug Metab. Dispos. 45 (2), 118–129. https://doi.org/10.1210/en.2006-0658.

Ihunnah, C.A., Jiang, M., Xie, W., 2011. Nuclear receptor PXR, transcriptional circuits and metabolic relevance. BBA-Mol. Basis Dis. 1812 (8), 956–963. https://doi.org/10.1016/j.bbadis.2011.01.014.

Infante, C., Matsuoka, M.P., Asensio, E., Cañavate, J.P., Reith, M., Manchado, M., 2008. Selection of housekeeping genes for gene expression studies in larvae from flatfish using real-time PCR. BMC Mol. Biol. 9 (1), 28. https://doi.org/10.1186/1471-2199-9-28.

Jönsson, M.E., Brunström, B., Brandt, I., 2009. The zebrafish gill model: Induction of CYP1A, EROD and PAH adduct formation. Aquat. Toxicol. 91 (1), 62–70. https://doi.org/10.1016/j.aquatox.2008.10.010.

Klatte, S., Schaefer, H.C., Hempel, M., 2017. Pharmaceuticals in the environment–a short review on options to minimize the exposure of humans, animals and ecosystems. Sustain. Chem. Pharm. 5, 61–66. https://doi.org/10.1016/j.watres.2018.02.033.

Kostich, M.S., Lazorchak, J.M., 2008. Risks to aquatic organisms posed by human pharmaceutical use. Sci. Total Environ. 389 (2–3), 329–339. https://doi.org/10.1016/j.scitotenv.2007.09.008.

Kuemmerle, H.-P., Dominguez-Gil, A., Koepcke, K., Hitzenberger, G., 1987. Pharmacokinetic profile of imidazole 2-hydroxy-benzoate, a novel nonsteroidal anti-inflammatory agent. Int. J. Clin. Pharm. Ther. Toxicol. 24, 581–597.

LaLone, C.A., Villeneuve, D.L., Olmstead, A.W., Medlock, E.K., Kahl, M.D., Jensen, K.M., Durhan, E.J., Makynen, E.A., Blanksma, C.A., Cavallin, J.E., Thomas, L.M., Seidl, S. M., Skolness, S.Y., Wehmas, L.C., Johnson, R.D., Ankley, G.T., 2012. Effects of a glucocorticoid receptor agonist, dexamethasone, on fathead minnow reproduction, growth, and development. Environ. Toxicol. Chem. 31 (3), 611–622. https://doi.org/10.1002/etc.1729.

Lehmann, J.M., McKee, D.D., Watson, M.A., Willson, T.M., Moore, J.T., Kliewer, S.A., 1998. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. J. Clin. Investig. 102 (5), 1016–1023. https://doi.org/10.1172/JCI3703.

Levine, S.L., Oris, J.T., 1999. Noncompetitive mixed-type inhibition of rainbow trout CYP1A catalytic activity by clotrimazole. Comp. Biochem. Phys. C 122 (2), 205–210. https://doi.org/10.1016/S0742-8413(98)10108-1.

Liu, S., Ying, G.G., Zhao, J.L., Chen, F., Yang, B., Zhou, L.J., Lai, H.J., 2011. Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry. J. Chromatogr. A 1218 (10), 1367–1378. https://doi.org/10.1016/j.chroma.2011.01.014.

Lushchak, V.I., 2016. Contaminant-induced oxidative stress in fish: a mechanistic approach. Fish Physiol. Biochem. 42 (2), 711–747. https://doi.org/10.1007/s10695-015-0171-5.

Manceau, S., Giraud, C., Declèves, X., Batteux, F., Chéreau, C., Chouzenoux, S., Scherrmann, J.M., Weill, B., Perrot, J.Y., Tréluyer, J.M., 2012. Expression and induction by dexamethasone of ABC transporters and nuclear receptors in a human T-lymphocyte cell line. J. Chemother. 24 (1), 48–55. https://doi.org/10.1179/1120009×12Z.00000000010.

Marques, C., Roberto, V.P., Granadeiro, L., Trindade, M., Gavaia, P.J., Laizé, V., Cancela, M.L., Fernández, I., 2017. The xenobiotic sensor PXR in a marine flatfish species (*Solea senegalensis*): gene expression patterns and its regulation under different physiological conditions. Mar. Environ. Res. 130, 187–199. https://doi.org/10.1016/j.marenvres.2017.07.021.

Martin, P., Riley, R., Back, D.J., Owen, A., 2008. Comparison of the induction profile for drug disposition proteins by typical nuclear receptor activators in human hepatic and intestinal cells. Br. J. Pharmacol. 153 (4), 805–819. https://doi.org/10.1038/sj.bjp.0707601.

Masuda, B.M., Fisher, P., Beaven, B., 2015. Residue profiles of brodifacoum in coastal marine species following an island rodent eradication. Ecotoxicol. Environ. Saf. 113, 1–8. https://doi.org/10.1016/j.ecoenv.2014.11.013.

Maura, G., Bardou, M., Billionnet, C., Weill, A., Drouin, J., Neumann, A., 2020. Oral anticoagulants and risk of acute liver injury in patients with nonvavular atrial fibrillation: a propensity-weighted nationwide cohort study. Sci. Rep. 10, 1164. https://doi.org/10.1038/s41598-020-68304-8.

Melissinos, K.G., Delidov, A.Z., Varsov, A.G., Begietti, S.S., Drivas, G.J., 1981. Determination of glutathione reductase activity. Nephron 28, 76–79.

Meyer, M.F., Powers, S.M., Hampton, S.E., 2019. An evidence synthesis of pharmaceuticals and personal care products (PPCPs) in the environment: imbalances among compounds, sewage treatment techniques, and ecosystem types. Environ. Sci. Technol. 53 (22), 12961–12973. https://doi.org/10.1021/acs.est.9b02966.

Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V., Milner, A., 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 84, 407–412. https://doi.org/10.1042/cs0840407.

Milnes, M.R., Garcia, A., Grossman, E., Grün, F., Shiotsugu, J., Tabb, M.M., Kawashima, Y., Katsu, Y., Watanabe, H., Iguchi, T., Blumberg, B., 2008. Activation of steroid and xenobiotic receptor (SXR, NR1I2) and its orthologs in laboratory, toxicologic, and genome model species. Environ. Health Perspect. 116 (7), 880. https://doi.org/10.1289/ehp.10853.

Monostory, K., Kőhalmy, K., Prough, R.A., Kóbori, L., Vereczkey, L., 2005. The effect of synthetic glucocorticoid, dexamethasone on CYP1A1 inducibility in adult rat and human hepatocytes. FEBS Lett. 579 (1), 229–235. https://doi.org/10.1016/j.febslet.2004.11.080.

Moore, L.B., Maglich, J.M., McKee, D.D., Wisely, B., Willson, T.M., Kliewer, S.A., Lambert, M.H., Moore, J.T., 2002. Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors. Mol. Endocrinol. 16 (5), 977–986. https://doi.org/10.1210/mend.16.5.0828.

Morel, Y., Mermod, N., Barouki, R., 1999. An autoregulatory loop controlling CYP1A1 gene expression: role of H2O2 and NFI. Mol. Cell. Biol. 19 (10), 6825–6832. https://doi.org/10.1128/MCB.19.10.6825.

Mourad, A., Perfect, J.R., 2018. Tolerability profile of current antifungal armoury. J. Antimicrob. Chemother. 73, 26–32. https://doi.org/10.1093/jac/dkx446.

Navas, J.M., Chana, A., Herradón, B., Segner, H., 2004. Induction of cytochrome P4501A (CYP1A) by clotrimazole, a non-planar aromatic compound. Computational studies on structural features of clotrimazole and related imidazole derivatives. Life Sci. 76 (6), 699–714. https://doi.org/10.1016/j.lfs.2004.09.015.

Nipun, A., Goldhaber, S.Z., 2006. Anticoagulants and transaminase elevation. Circulation 113, 698–702. https://doi.org/10.1161/circulationaha.105.603100.

Nishimura, M., Naito, S., Yokoi, T., 2004. Tissue-specific mRNA expression profiles of human nuclear receptor subfamilies. Drug Metab. Pharmacokinet. 19 (2), 135–149. https://doi.org/10.2133/dmpk.19.135.

Niu, L., Chen, Q., Hua, C., Geng, Y., Cai, L., Tao, S., Ni, Y., Zhao, R., 2018. Effects of chronic dexamethasone administration on hyperglycemia and insulin release in goats. J. Anim. Sci. Biotechnol. 9 (1), 26. https://doi.org/10.1186/s40104-018-0242-4.

Noseda, G., Fragiacomo, C., Peruzzi, M., Cremonesi, P., Chinea, B., 1988. Comparative absorption kinetics of imidazole and salicylic acid in volunteers after administration of ITF 182 tablets and suppositories. Int. J. Clin. Pharm. Res. 8, 169–173.

Oldenburg, J., Watzka, M., Bevans, C.G., 2015. VKORC1 and VKORC1L1: why do vertebrates have two vitamin K 2,3-epoxide reductases? Nutrients 7 (8), 6250–6280. https://doi.org/10.3390/nu708528.

Oliva, M., Vicente, J.J., Gravato, C., Guilhermino, L., Galindo-Riaño, M.D., 2012. Oxidative stress biomarkers in Senegal sole, *Solea senegalensis*, to assess the impact of heavy metal pollution in a Huelva estuary (SW Spain): seasonal and spatial variation. Ecotox. Environ. Saf. 75, 151–162. https://doi.org/10.1016/j.ecoenv.2011.08.017.

Pagella, P.G., Bellavite, O., Agozzino, S., Dona, G.C., Cremonesi, P., De Santis, F., 1983. Pharmacological studies of imidazole 2-hydroxybenzoate (ITF 182), an antiinflammatory compound with an action on thromboxane A2 production. Arzneimittelforschung 33, 716–726.

Pascussi, J.M., Drocourt, L., Fabre, J.M., Maurel, P., Vilarem, M.J., 2000. Dexamethasone induces pregnane X receptor and retinoid X receptor-α expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. Mol. Pharmacol. 58 (2), 361–372. https://doi.org/10.1124/mol.58.2.361.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chem. Rev. 119 (6), 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299.

Pearse, A.G.E., 1985. Histochemistry. Theoretical and Applied In: Analytical Technology, fourth ed., Vol. 2. Churchill Livingstone, New York. NY.

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W. J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg D., S., Steckler, T., Wuerbel, H., 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol. 18 (7), e3000410 https://doi.org/10.1371/journal.pbio.3000410.

Pes, K., Friese, A., Cox, C.J., Laizé, V., Fernández, I., 2021. Biochemical and molecular responses of the Mediterranean mussel (*Mytilus galloprovincialis*) to short-term exposure to three commonly prescribed drugs. Mar. Environ. Res. 168, 105309 https://doi.org/10.1016/j.marenvres.2021.105309.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT PCR. Nucleic Acids Res. 29 (9), e45 https://doi.org/10.1093/nar/29.9.e45.

Pitt, W.C., Berentsen, A.R., Shiels, A.B., Volker, S.F., Eisemann, J.D., Wegmann, A.S., Howald, G.R., 2015. Non-target species mortality and the measurement of brodifacoum rodenticide residues after a rat (*Rattus rattus*) eradication on Palmyra Atoll, tropical Pacific. Biol. Conserv. 185, 36–46. https://doi.org/10.1016/j.biocon.2015.01.008.

Primus, T., Wright, G., Fisher, P., 2005. Accidental discharge of brodifacoum baits in a tidal marine environment: a case study. Bull. Environ. Contam. Toxicol. 74 (5), 913–919. https://doi.org/10.1007/s00128-005-0668-1.

Regnery, J., Schulz, R.S., Parrhysius, P., Bachtin, J., Brinke, M., Schäfer, S., Reifferscheid, G., Friesen, A., 2020. Heavy rainfall provokes anticoagulant rodenticides' release from baited sewer systems and outdoor surfaces into receiving streams. Sci. Total Environ. 740, 139905 https://doi.org/10.1016/j.scitotenv.2020.139905.

Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar. Environ. Res. 93, 106–117. https://doi.org/10.1016/j.ma renvres.2013.07.006.

Rhen, T., Cidlowski, J.A., 2005. Antiinflammatory action of glucocorticoids – new mechanisms for old drugs. N. Engl. J. Med.. 353 (16), 1711–1723. https://doi.org/10.1056/NEJMra050541.

Richaud-Patin, Y., Vega-Boada, F., Vidaller, A., Llorente, L., 2004. Multidrug resistance 1 (MDR-1) in autoimmune disorders IV. P-glycoprotein overfunction in lymphocytes from myasthenia gravis patients. Biomed. Pharmacother. 58 (5), 320–324. https://doi.org/10.1016/j.biopha.2004.04.008.

Rőszer, T., 2014. The invertebrate midintestinal gland ("hepatopancreas") is an evolutionary forerunner in the integration of immunity and metabolism. Cell Tissue Res. 358 (3), 685–695. https://doi.org/10.1007/s00441-014-1985-7.

Rulcova, A., Prokopova, I., Krausova, L., Bitman, M., Vrzal, R., Dvorak, Z., Blahos, J., Pavek, P., 2010. Stereoselective interactions of warfarin enantiomers with pregnane X nuclear receptor in gene regulation of major drug-metabolizing cytochrome P450 enzymes. J. Thromb. Haemost. 8, 2708–2717. https://doi.org/10.1111/j.1538-7836.2010.04036.x.

Salas-Leiton, E., Coste, O., Asensio, E., Infante, C., Cañavate, J.P., Manchado, M., 2012. Dexamethasone modulates expression of genes involved in the innate immune system, growth and stress and increases susceptibility to bacterial disease in Senegalese sole (*Solea senegalensis* Kaup, 1858). Fish Shellfish Immun. 32 (5), 769–778, 769-778.10.1016/j.fsi.2012.01.030.

Sánchez-Avila, J., Tauler, R., Lacorte, S., 2012. Organic micropollutants in coastal waters from NW Mediterranean Sea: sources distribution and potential risk. Environ. Int. 46, 50–62. https://doi.org/10.1016/j.envint.2012.04.013.

Sanyaolu, A.O., Oremosu, A.A., Osinubi, A.A., Vermeer, C., Daramola, A.O., 2019. Warfarin-induced vitamin K deficiency affects spermatogenesis in Sprague-Dawley rats. Andrologia 51 (10), e13416. https://doi.org/10.1111/and.13416.

Shi, D., Yang, D., Yan, B., 2010. Dexamethasone transcriptionally increases the expression of the pregnane X receptor and synergistically enhances pyrethroid esfenvalerate in the induction of cytochrome P450 3A23. Biochem. Pharmacol. 80 (8), 1274–1283. https://doi.org/10.1016/j.bcp.2010.06.043.

Smutny, T., Dusek, J., Hyrs ova, L., Nekvindova, J., Horvatova, A., Micuda, S., Gerbal-Chaloin, S., Pavek, P., 2020. The 3′-untranslated region contributes to the pregnane X receptor (PXR) expression down-regulation by PXR ligands and up-regulation by glucocorticoids. Acta Pharm. Sin. B 10, 136–152. https://doi.org/10.1016/j.apsb.2019.09.010.

Snegaroff, J., Bach, J., 1989. Effects of the fungicide prochloraz on xenobiotic metabolism in rainbow trout: inhibition in vitro and time course of induction in vivo. Xenobiotica 19 (3), 255–267. https://doi.org/10.3109/00498258909042271.

Solé, M., Fortuny, A., Mañanós, E., 2014. Effects of selected xenobiotics on hepatic and plasmatic biomarkers in juveniles of *Solea senegalensis*. Environ. Res. 135, 227–235. https://doi.org/10.1016/j.envres.2014.09.024.

Solé, M., Mañanós, E., Blázquez, M., 2016. Vitellogenin, sex steroid levels and gonadal biomarkers in wild Solea solea and Solea senegalensis from NW Mediterranean fishing grounds. Mar. Environ. Res. 117, 63–74. https://doi.org/10.1016/j.marenvres.2016.03.010.

Takeshita, A., Taguchi, M., Koibuchi, N., Ozawa, Y., 2002. Putative role of the orphan nuclear receptor SXR (steroid and xenobiotic receptor) in the mechanism of CYP3A4 inhibition by xenobiotics. J. Biol. Chem. 277 (36), 32453–32458. https://doi.org/10.1074/jbc.M111245200.

Tanaka, S., Masuda, M., Nakajima, K., Ido, N., Ohtsuka, T., Nishida, M., Utsumi, H., Hirano, T., 2009. P-glycoprotein function in peripheral T lymphocyte subsets of myasthenia gravis patients: clinical implications and influence of glucocorticoid administration. Int. Immunopharmacol. 9 (3), 284–290. https://doi.org/10.1016/j.intimp.2008.11.007.

Venkatesh, M., Wang, H., Cayer, J., Leroux, M., Salvail, D., Das, B., Wrobel, J.E., Mani, S., 2011. In vivo and in vitro characterization of a first-in-class novel azole analog that targets pregnane X receptor activation. Mol. Pharmacol. 80 (1), 124–135. https://doi.org/10.1124/mol.111.071787.

Walker, S.E., Lorsch, J., 2013. RNA purification–precipitation methods. Methods Enzymol. 530, 337–343. https://doi.org/10.1016/B978-0-12-420037-1.00019-1.

Wang, H., LeCluyse, E.L., 2003. Role of orphan nuclear receptors in the regulation of drug-metabolising enzymes. Clin. Pharmacokinet. 42 (15), 1331–1357. https://doi.org/10.2165/00003088-200342150-00003.

Wassmur, B., Gräns, J., Kling, P., Celander, M.C., 2010. Interactions of pharmaceuticals and other xenobiotics on hepatic pregnane X receptor and cytochrome P450 3A signaling pathway in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 100 (1), 91–100. https://doi.org/10.1016/j.aquatox.2010.07.013.

Webb, S., 2004. A Data-Based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals I – Collation of Available Ecotoxicity Data. Pharmaceuticals in the Environment. Springer, Berlin, Heidelberg, pp. 175–201. https://doi.org/10.1007/978-3-662-09259-0_24.

Weigt, S., Huebler, N., Strecker, R., Braunbeck, T., Broschard, T.H., 2012. Developmental effects of coumarin and the anticoagulant coumarin derivative warfarin on zebrafish (*Danio rerio*) embryos. Reprod. Toxicol. 33 (2), 133–141. https://doi.org/10.1016/j.reprotox.2011.07.001.

Yang, L., Zhou, Y., Shi, B., Meng, J., He, B., Yang, H., Yoon, S.J., Kim, T., Kwon, B., Khim, J.S., Wang, T., 2020. Anthropogenic impacts on the contamination of pharmaceuticals and personal care products (PPCPs) in the coastal environments of the Yellow and Bohai seas. Environ. Int. 135, 105306 https://doi.org/10.1016/j.envint.2019.105306.

Yin, G., Cao, L., Du, J., Jia, R., Kitazawa, T., Kubota, A., Teraoka, H., 2017. Dexamethasone-induced hepatomegaly and steatosis in larval zebrafish. J. Toxicol. Sci. 42 (4), 455–459. https://doi.org/10.2131/jts.42.455.

Zanette, J., Jenny, M.J., Goldstone, J.V., Parente, T., Woodin, B.R., Bainy, A.C., Stegeman, J.J., 2013. Identification and expression of multiple CYP1-like and CYP3-like genes in the bivalve mollusk *Mytilus edulis*. Aquat. Toxicol. 128, 101–112. https://doi.org/10.1016/j.aquatox.2012.11.017.

Zhang, K., Kuroha, M., Shibata, Y., Kokue, E., Shimoda, M., 2006. Effect of oral administration of clinically relevant doses of dexamethasone on regulation of cytochrome P450 subfamilies in hepatic microsomes from dogs and rats. Am. J. Vet. Res. 67 (2), 329–334. https://doi.org/10.2460/ajvr.67.2.329.

Zhang, L., Peng, X.M., Damu, G.L.V., Geng, R.X., Zhou, C.H., 2014. Comprehensive review in current developments of imidazole-based medicinal chemistry. Md. Res. Rev. 34, 340–437. https://doi.org/10.1002/med.21290.

Zheng, X.F., Liu, L., Zhou, J., Miao, M.Y., Zhou, J.R., Zhu, D., Xia, Z.F., Jiang, C.L., 2009. Biphasic effects of dexamethasone on glycogen metabolism in primary cultured rat hepatocytes. J. Endocrinol. Invest. 32 (9), 756–758. https://doi.org/10.1007/BF03346532.

Zhong, L., Liang, Y.Q., Lu, M., Pan, C.G., Dong, Z., Zhao, H., Chengyoung, L., Zhong, L., Yao, L., 2021. Effects of dexamethasone on the morphology, gene expression and hepatic histology in adult female mosquitofish (*Gambusia affinis*). Chemosphere 274, 129797. https://doi.org/10.1016/j.chemosphere.2021.129797.