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Critical Review

The potential for adverse effects in fish exposed to antidepressants in the aquatic environment

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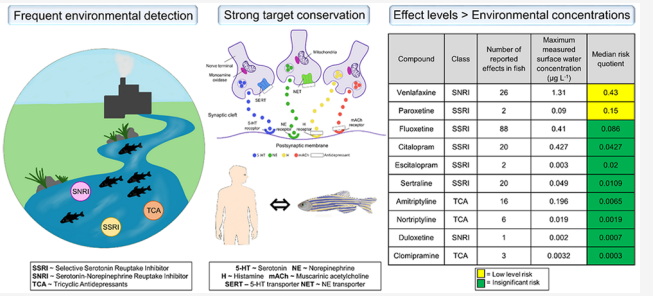
ABSTRACT: Antidepressants are one of the most commonly prescribed pharmaceutical classes for the treatment of psychiatric conditions. They act via modulation of brain monoaminergic signaling systems (predominantly serotonergic, adrenergic, dopaminergic) that show a high degree of structural conservation across diverse animal phyla. A reasonable assumption, therefore, is that exposed fish and other aquatic wildlife may be affected by antidepressants released into the natural environment. Indeed, there are substantial data reported for exposure effects in fish, albeit most are reported for exposure concentrations exceeding those occurring in natural environments. From a critical analysis of the available evidence for effects in fish, risk quotients (RQs) were derived from laboratory-based studies for a selection of antidepressants most commonly detected in the aquatic environment. We conclude that the likelihood for effects in fish on standard measured end points used in risk assessment (i.e., excluding effects on behavior) is low for levels of exposure occurring in the natural environment. Nevertheless, some effects on behavior have been reported for environmentally relevant exposures, and antidepressants can bioaccumulate in fish tissues. Limitations in the datasets used to calculate RQs revealed important gaps in which future research should be directed to more accurately assess the risks posed by antidepressants to fish. Developing greater certainty surrounding risk of antidepressants to fish requires more attention directed toward effects on behaviors relating to individual fitness, the employment of environmentally realistic exposure levels, on chronic exposure scenarios, and on mixtures analyses, especially given the wide range of similarly acting compounds released into the environment.



Compound	Class	Number of reported effects in fish	Maximum measured surface water concentration (μg L^{-1})	Median risk quotient
Venlafaxine	SNRI	26	1.31	0.43
Paroxetine	SSRI	2	0.09	0.15
Fluoxetine	SSRI	88	0.41	0.086
Citalopram	SSRI	20	0.427	0.0427
Escitalopram	SSRI	2	0.003	0.02
Sertraline	SSRI	20	0.049	0.0109
Amitriptyline	TCA	16	0.196	0.0065
Nortriptyline	TCA	6	0.019	0.0019
Duloxetine	SNRI	1	0.002	0.0007
Clomipramine	TCA	3	0.0032	0.0003

KEYWORDS: *ecotoxicology, SSRI, SNRI, tricyclic, monoamines, risk assessment, fish*

INTRODUCTION

The number of people suffering from mental health problems is rising, with anxiety and depression now the most commonly diagnosed psychiatric conditions. Antidepressant drugs are the primary treatment for these conditions, and globally they are one of the most commonly prescribed classes of human pharmaceuticals. To illustrate this, between 2008 and 2018, antidepressant prescriptions in England rose from 36 to 70.9 million per annum,[1] a figure that is likely to increase even further due to societal uncertainties challenging mental health. Three classes of antidepressants predominate global patient usage: tricyclic antidepressants (TCAs); selective serotonin reuptake inhibitors (SSRIs); and serotonin-norepinephrine reuptake inhibitors (SNRIs); with other classes such as the serotonin partial reuptake inhibitors (SPARI), norepinephrine-dopamine reuptake inhibitors, and newer multimodal action drugs forming the minority in terms of prescription numbers.[2−4] Although each class exhibits subtle differences in their primary mechanism of action, all rely on modulating brain serotonergic, adrenergic and dopaminergic systems in order to elevate patient mood. There are significant geographical variations in the specific antidepressant drugs used. In England, for example, in 2018 the top three prescribed drugs by weight were amitriptyline (10 149 kg), sertraline (7408 kg), and venlafaxine (4402 kg); whereas in the U.S. in 2018, these were sertraline (57 575 kg), venlafaxine (48 363 kg), and duloxetine (37 863 kg).[1,5,6] In both the UK and the U.S., however, sertraline was the most frequently prescribed (see Supporting Information (SI) Table S1 for a summary of the prescription rates and usage of antidepressants across the UK, U.S., Australia, Sweden, and Brazil).[1,5]

As with the majority of human pharmaceuticals, the primary route by which antidepressants enter the aquatic environment is through patient use and subsequent excretion. Antidepressants were first reported in the aquatic environment in 2002, when fluoxetine was detected in various U.S. water sources,[7] and since then, many studies have reported the presence of a range of antidepressants at ng L^{-1} levels in surface waters across the globe

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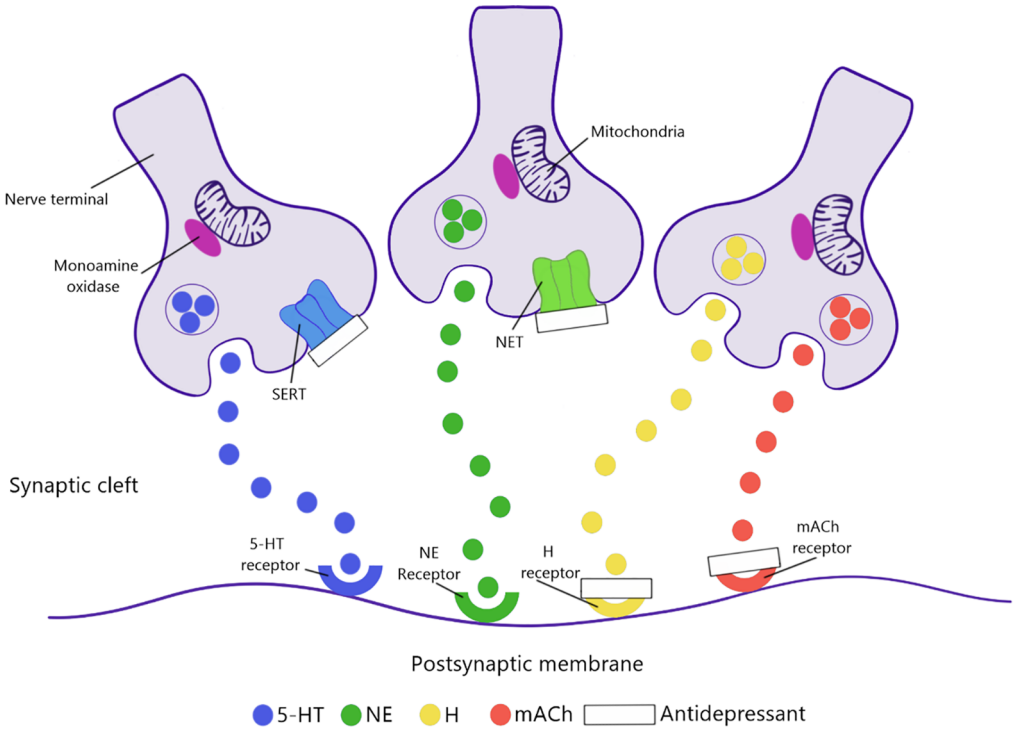

Figure 1. A schematic diagram showing the primary therapeutic mechanisms of action of the three major classes of antidepressant drugs, the TCAs, SSRIs, and SNRIs. SSRIs block the reuptake of 5-HT into presynaptic terminals via SERT inhibition, thus increasing the levels of 5-HT in the synaptic cleft. SNRIs and TCAs, in addition to inhibiting the reuptake of 5-HT, also prevent the reuptake of NE via NET inhibition. Furthermore, TCAs exhibit secondary pharmacological actions via postsynaptic muscarinic acetylcholine and histamine receptor antagonism. Abbreviations: serotonin (5-HT), norepinephrine (NE), histamine (H), and muscarinic acetylcholine (mACh).

(see SI Table S3). In most cases, but not all, measured levels of individual antidepressants are below those expected to have an adverse effect in fish; however, the primary targets for antidepressant action show high structural conservation between mammals and fish,[8] some of these drugs are known to bioaccumulate in fish,[9,10] and there are limited chronic exposure data (see SI Table S4). Furthermore, a number of recent studies have in fact reported specific physiological and behavioral effects in fish for environmentally realistic exposure scenarios to antidepressants (see SI Table S5) supporting the potential for effects on wild fish populations within the wider aquatic environment. Fish will also be exposed to mixtures of antidepressants with similar modes of action and thus there is a plausible likelihood for combined effects.[11,12]

Here we review the evidence supporting the potential risks of antidepressants to fish in the environment, and using published data on measured exposure concentrations and reported effects, estimate the risks posed to fish. Finally, we highlight existing data gaps and recommend further research to build a more comprehensive understanding of the risks of antidepressants in the aquatic environment.

■ ANTIDEPRESSANT MODES OF ACTION

TCAs were one of the first major classes of antidepressants prescribed for clinical use in the 1980s, but the development of safer and more effective drugs has subsequently led to a global decline in their use.[13] TCAs predominantly act via the inhibition of the noradrenaline (or norepinephrine, NE) and serotonin (5-hydroxytryptamine or 5-HT) transporters (SLC6A2, NET, and SLC6A4, SERT, respectively), which are located on the presynaptic membranes of the central nervous system as well as being found in the peripheral nervous system and in some other tissues. This action collectively serves to elevate local NE and 5-HT concentrations by decreasing their reuptake from the synaptic cleft. Different TCAs act on NET and SERT with differing levels of potency,[14] and they also differ in terms of their secondary pharmacology which includes in some cases action on muscarinic acetylcholine (mACh)[15] and histamine (H) receptors.[16] A schematic representation of the actions of the major classes of antidepressants is shown in Figure 1. Historically, the most highly prescribed TCA was amitriptyline which is still used in some countries for the treatment of severe depression. In the patient, amitriptyline is metabolized by *N*-demethylation to nortriptyline, which is also prescribed for the treatment of depression-related disorders and shares a common mechanism of therapeutic action with the parent compound.[17]

TCAs were largely superseded by the discovery of the SSRIs, which are now the most widely prescribed class of antidepressants due to their superior safety profiles.[18] SSRIs act by selectively blocking reuptake of 5-HT into presynaptic neurons through the inhibition of the SERT,[19,20] and in common with the TCAs, the level of potency for the SERT and the secondary pharmacology profile differs across individual drug molecules.[21] The first widely marketed SSRI, fluoxetine, has a high affinity for the SERT, with virtually no affinity for other neurotransmitters and receptors.[22] Fluoxetine is marketed as a racemic mixture of R (−) and S (+) enantiomers, both of which inhibit 5-HT reuptake. The two enantiomers of fluoxetine's major metabolite norfluoxetine, however, possess contrasting levels of potency; S-norfluoxetine is 14 times more potent and is therefore considered to be the main contributor to fluoxetine's 5-HT uptake inhibition.[23] Although fluoxetine was the first SSRI to be widely prescribed, in recent years newer SSRIs such as citalopram and sertraline have now become more

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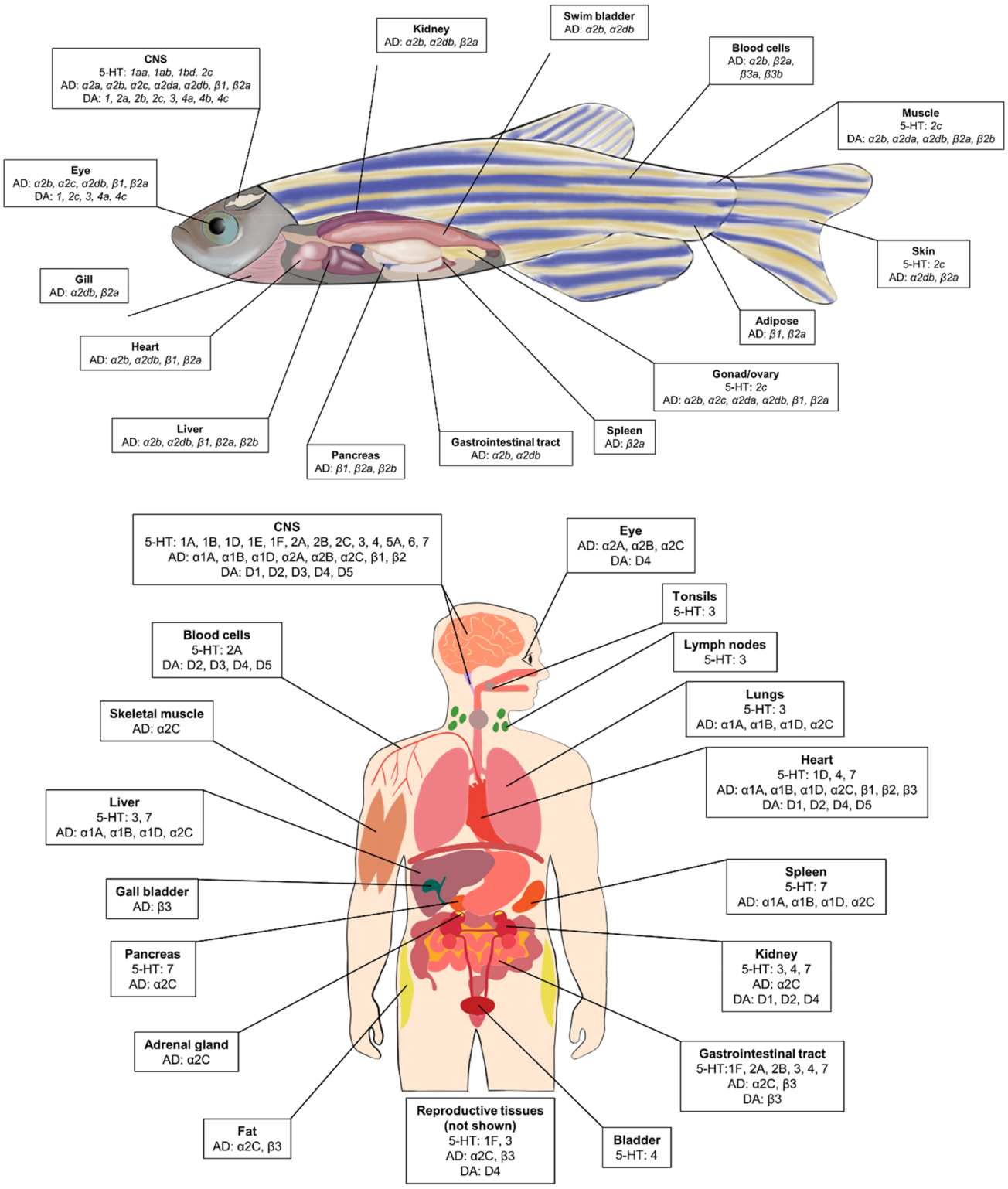

Figure 2. Comparative distribution of serotonergic, adrenergic and dopaminergic receptors in zebrafish and human tissues. Abbreviations: serotonergic receptors (5-HT), adrenoceptors (AD) and dopaminergic receptors (DA). Refer to SI Table S3 for a full list.

widely used due to improvements in efficacy and pharmacokinetics.[24]

The third main class of antidepressants developed, the SNRIs, were designed to target both NET and SERT,[25] recognizing that combination therapy was significantly more effective at treating depression.[26] The first marketed SNRI was venlafaxine, which is produced as a mixture of R (−) and S (+) enantiomers. The R-enantiomer inhibits both SERT and NET, whereas the S-enantiomer predominantly inhibits the SERT. As such, the inhibition of 5-HT uptake is around three times greater than that of NE.[27] Venlafaxine has a pharmacologically active major metabolite, O-desmethylvenlafaxine (ODV), which inhibits 5-HT and NE reuptake with similar efficacy to the parent compound.[25]

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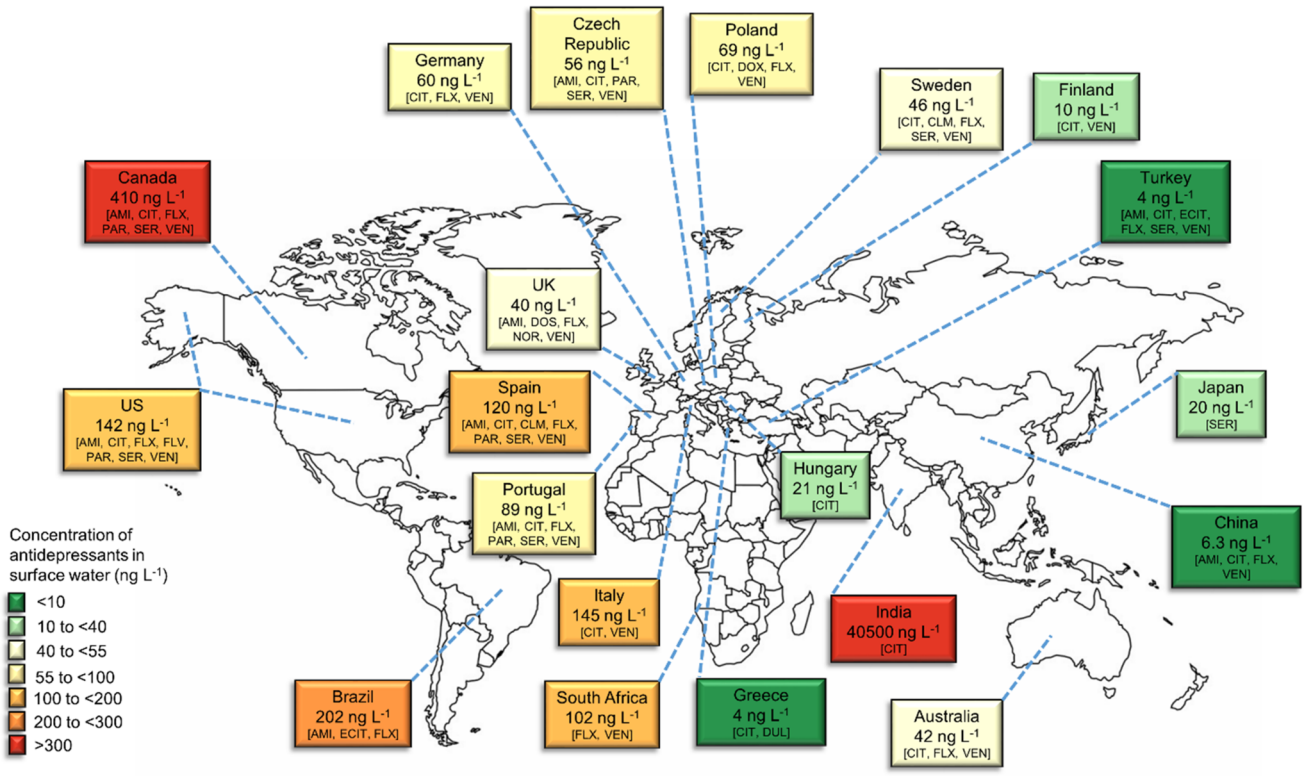

Figure 3. Occurrence of antidepressants in surface waters worldwide. Values shown were calculated by taking the average of all maximum reported concentrations (mean or median used where maximum was not reported) of antidepressants in that country to illustrate the highest possible risk. Only TCA, SSRI, and SNRI parent compounds were included unless metabolites are prescribed for the treatment of depressive disorders. See SI Table S3 for a full listing. Abbreviations: amitriptyline (AMI), clomipramine (CLM), citalopram (CIT), dosulepin (DOS), duloxetine (DUL), escitalopram (ECIT), fluoxetine (FLX), fluvoxamine (FLV), nortriptyline (NOR) paroxetine (PAR), sertraline (SER), and venlafaxine (VEN).

COMPARATIVE PHYSIOLOGY OF THE MONOAMINERGIC SYSTEMS OF MAMMALS AND FISH

5-HT is a commonly found neurotransmitter in the vertebrate central nervous system (CNS) and in mammals, 5-HT is involved in the regulation of a variety of basic functions including motor control, arousal, sleep, feeding, social behavior, learning, and memory.[28] In the mammalian CNS, the anterior and posterior raphe are the source of most serotonergic neurons and these comprise nine nuclei which project both anteriorly and posteriorly to multiple brain regions.[29] The organization of the serotonergic system in bony fish (teleosts) is similar to that in mammals, although teleost fish have only six raphe nuclei.[30] 5-HT positive cells have also been detected in the teleost retina, pretectum, hypothalamus/posterior tuberculum, vagal lobes and spinal cord,[31] although it is still not clear if these are distinctly serotonergic, and what there precise role is. The chemical neuroanatomy of the zebrafish (*Danio rerio*) serotonergic system is reviewed in Herculano and Maximino (2014).[32] Two isoforms of the human SERT (*slc6a4a* and *slc6a4b*) have been identified in zebrafish, which show 66−69% and 75% amino acid sequence homology, respectively, to other mammalian 5-HT transporters.[33] *Slc6a4a* is expressed widely across the zebrafish brain, most notably in the pretectum and raphe, while expression of *slc6a4b* is restricted to the hypothalamus, paraventricular organ and medulla oblongata.[34] Across fish species, three major 5-HT receptor subtypes have been identified, 5-HT1, 5-HT2, and 5-HT7 (see SI Table S2 for receptor distribution). In zebrafish 5-HT1 has been further subcategorised into three (5-HT1aa, 5-HT1ab, and 5-HT1bd)[34] and 5-HT2 into two further subgroups (5-HT2A and 5-HT2C).[35] The two encoded zebrafish proteins, *htr1aa* and *htr1ab*, are 69% and 76% identical to the human HTR1A protein, respectively,[34] and overall 66% of human 5-HT drugs targets have equivalent orthologs in zebrafish.[8] In common with mammals, teleost 5-HT plays a key role in motor activity,[36] feeding and appetite[37,38] and social behavior including aggression.[39] 5-HT also modulates fish reproductive processes via multiple pathways including reportedly stimulating the release of gonadotropin[40,41] and directly affecting oocyte maturation in Japanese medaka (*Oryzias latipes*).[42]

The noradrenergic system of mammals is involved in a wide range of physiological and behavioral processes including aggression,[43] memory,[44] pain regulation,[45] and anxiety/mood.[46] The primary therapeutic target of antidepressants, NET, is encoded by a single gene, SLC6A2, for which a single orthologue, *slc6a2*, has been identified in zebrafish.[47] The primary source of noradrenergic projects in mammals is the locus coeruleus (in excess of 45 000 neurons in humans)[48,49] and this circuitry is highly conserved across vertebrate lineages including teleost fish. However, only about 14 neurons in total have been reported in the zebrafish.[47] In teleosts, three main groups of adrenoreceptors have been identified (α_1, α_2 and ß) which correspond to the those known in humans[50−53] (see SI Table S2 for receptor distribution). Of these, there are a total of nine known subtypes across mammals[54] and although data in other species are limited, five distinct α_2 receptor genes have been identified in zebrafish.[50] Three of these are orthologues to the human α_{2A}, α_{2B}, α_{2C} receptors, and the remaining two are

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duplicated α_2 subtypes, $\alpha_{2\,Da}$ and α_{2Db}, with no human orthologues. Collectively these paralogues share between 80 and 87% similarity in their protein sequences compared with the mammalian genes.[50,51] In addition, zebrafish are known to possess five ß adrenoceptors (humans only have three), with one orthologue for ß1 and two for ß2 and ß3.[55] In teleosts, norepinephrine plays a role in the control of pigmentation,[55] blood pressure regulation[56] and in modulating a variety of behaviors including aggression levels. For example, in the Siamese fighting fish (*Betta splendens*), aqueous exposure to NE increases gill flaring suggesting the involvement of this monoamine in regulating levels of aggression.[57]

Although not considered the primary mechanism by which most antidepressant drugs exert their therapeutic effect, many of these compounds also modulate levels of dopamine in the CNS. In the larval zebrafish brain, the dopaminergic transporter (DAT, *slc6a3*) is expressed in the olfactory bulb, pretectum, retina, and locus coeruleus.[58] In common with the other monoamines, the dopaminergic system plays a role in modulating a variety of adaptive functions including memory,[44] reward,[59] cognition and attention[60] and motor control[61] in mammals. In teleosts, the dopaminergic system has been reported to have an involvement in a range of behavioral effects, including reducing anxiety-like behaviors[62] and increasing boldness,[63] as well as modifying locomotion[64,65] and associative learning performance (for zebrafish[66] and cleaner wrasse, *Labroides dimidiatus*[67]). Humans and zebrafish (among other teleost species) share similar receptor orthologues (see SI Table S2 for receptor classification and distribution) with relatively high homology,[68−70] but their distribution is variable (see Figure 2). The D5 receptor subtype present in humans has not been identified in fish.

ANTIDEPRESSANTS IN THE AQUATIC ENVIRONMENT

As is the case for all human pharmaceuticals, the concentrations of antidepressants detected in the environment are a function of patient usage, metabolism, and excretion rates, as well as the degree to which active drug residues are effectively removed during wastewater treatment plant (WWTP) processes.[71,72] Patient usage varies according to socioeconomic status, with most studies reporting antidepressants in the environment in developed countries in which they are most highly prescribed.[73,74] Here, they are typically detected at ng L^{-1} to low $\mu g\ L^{-1}$ levels in WWTP effluents and surface waters (environmental concentrations of various antidepressants are summarized in Figure 3 and SI Table S3). The maximum reported concentrations of the most widely used TCA, amitriptyline, reported in effluent and river water in the UK, are 0.243 $\mu g\ L^{-1}$[75] and 0.0716 $\mu g\ L^{-1}$,[76] respectively. Local hotspots of high antidepressant concentrations have, however, been reported in specific watercourses or effluent streams. For example, the SSRI citalopram has been detected in WWTP effluent and downstream river water from an area in India with a high density of drug manufacturing plants, at levels as high as 430 and 76 $\mu g\ L^{-1}$, respectively, and therefore excluded from Figure 3.[77] The most frequently prescribed SNRI, venlafaxine, has been recorded at 2.19 $\mu g\ L^{-1}$[78] and 1.31 $\mu g\ L^{-1}$[79] in WWTP effluent and downstream of a WWTP in the U.S. In some cases, metabolites of antidepressants have been measured in surface water samples at higher concentrations than the parent compound. The presence of metabolites is particularly important in the case of those that are biologically active. For example, venlafaxine's metabolite, O-desmethylvenlafaxine, has been measured at concentrations up to six times higher than the parent compound in Germany.[80]

BIOAVAILABILITY AND THE POTENTIAL FOR BIOACCUMULATION

Once in the aquatic environment, factors that determine the partitioning of drug residues into biological compartments include compound lipophilicity and features of water physiochemistry such as pH,[81] as well as the extent to which drugs are metabolized and excreted from organisms subjected to environmental exposure.[82] Antidepressants include ionizable organic compounds, and the water pH can alter their speciation, in turn affecting their bioavailability.[83,84] For example, the bioconcentration of fluoxetine in Japanese medaka has been shown to increase with increasing pH and as a result, this drug is more toxic at a higher pH.[81] Bioaccumulation can be expressed as the bioconcentration factor (or BCF = the ratio between internal and external concentrations) and this can differ both between species and also across different body tissues within an individual fish (data are summarized in SI Table S4). Illustrating this, exposure of rainbow trout (*Oncorhynchus mykiss*) to a WWTP effluent containing antidepressants over 13 days resulted in a higher accumulation of citalopram in the liver (BCF = 47) compared with in the brain (BCF = 9),[85] whereas for a longer term exposure (three months) of brook trout (*Salvelinus fontinalis*), the opposite pattern was observed.[86] Similarly, in a study on the round goby (*Neogobius melanostomus*), both bupropion and venlafaxine were shown to bioconcentrate in the brain, plasma, gonads, liver, and muscle, whereas citalopram was detected only in the plasma.[87] Bioavailability (bioaccumulation) alone, of course, does not determine the likelihood of biological effects in fish which is largely driven by the compound potency. Thus, even though venlafaxine has a lower bioavailability in fish compared with many other antidepressants,[88−91] for identical exposure regimes to fluoxetine, sertraline and venlafaxine, only venlafaxine was seen to alter the diurnal activity patterns of male mosquitofish (*Gambusia holbrooki*).[92]

Mathematical models provide a complementary tool to predict the bioaccumulation potential of toxicants in nontarget organisms. The fish plasma model utilizes the relationship between the target (i.e., human) and nontarget (i.e., fish) organisms internal concentrations to predict the likelihood of an effect occurring, assuming the evolutionary conservation of drug targets.[93,94] Margiotta-Casaluci *et al.* (2014), show that the FPM can be reliably applied to predict plasma concentrations of the antidepressant fluoxetine in fathead minnows (*Pimephales promelas*) under a chronic 28-day aqueous exposure, however, the lowest observed effect concentrations (or LOECs) for behavioral anxiety-related end points were just above that of human therapeutic levels.[95] However, the FPM is less reliable in this regard when considering ionizable drugs.[96]

ALTERATION IN NEUROTRANSMITTER LEVELS AND RECEPTOR/TRANSPORTER EXPRESSION IN FISH

A key indicator of induced biological activity of an antidepressant (including in a nontarget species) is alteration in monoaminergic levels in the CNS (summarized in SI Table S5). A wide range of studies have shown that antidepressant exposure can disrupt neurotransmitter levels in fish. In Demin *et al.* 2017, for example, they found that exposure of adult zebrafish

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to amitriptyline resulted in an elevation of 5-HT neurotransmission in the brain through an increase in the ratio of 5-hydroxyindoleacetic acid (5-HIAA) to 5-HT, indicating heightened turnover of this neurotransmitter at the synapse.[97] No effect of amitriptyline was been found on noradrenaline synthesis which is in agreement with the pharmacological profile of this drug.[98] In the majority of published studies, monoamine concentrations have been assessed in whole brain samples (using high-performance liquid chromatography) which is a rather crude approach given that effects will vary across different brain regions. To illustrate this, exposure of rainbow trout to venlafaxine (0.2 and 1 $\mu g\ L^{-1}$ for 7 days) resulted in 5-HT, NE, and DA only being elevated in the midbrain.[99] Thus, application of methods that distinguish regional monoamine concentrations in the brain are an essential step for more precise interpretation of the likely effects of antidepressant exposure in fish.

Antidepressants have also been shown to affect the expression of genes coding for relevant transporters and receptors in fish, although reported effects are variable.[100,101] For example, exposure of zebrafish to fluoxetine has been shown to down-regulate *htr1aa*, *htr1ab*, *htr1b*, *htr2a*, and *htr5a*[102] and to have inhibitory effects on the adrenergic and dopaminergic systems more generally, as well as inhibitory effects on the serotonergic pathway in zebrafish.[103] In contrast, however, other studies on the effects of fluoxetine in zebrafish embryos and larvae have reported upregulation of genes coding for the 5-HT transporters *slc6a4a* and *slc6a4b* in response to environmentally relevant exposure concentrations.[104]

DEVELOPMENT, PHYSIOLOGICAL, AND BEHAVIORAL EFFECTS OF ANTIDEPRESSANTS IN FISH

Given the evidence for altered neurotransmitter levels in the brains of fish exposed to antidepressants, downstream physiological and behavioral effects would be expected.[20,105] In support of this, there are a range of physiological and behavioral effects reported in fish exposed to antidepressants (these are summarized in SI Table S5). In terms of morphological and developmental effects, they include acceleration of embryonic hatching, inhibition of growth, and effects on bone development. Zebrafish embryos exposed to amitriptyline, for example, have been shown to exhibit an acceleration in hatching in a dose-dependent manner with a low effect concentration of 0.01 $\mu g\ L^{-1}$,[106] a concentration measured in some surface waters. Various TCAs and SSRIs (but interestingly not SNRIs), have been shown to inhibit growth in a wide variety of fish species, and exposure contexts. For example, in the brackish-water fish species, meagre (*Argrosomus regius*), exposure to fluoxetine (3 $\mu g\ L^{-1}$ for 15 days) resulted in a reduction in both body length and weight which were correlated with serotonin-mediated appetite suppression.[107] Similarly, exposure of zebrafish to amitriptyline and fluoxetine (at concentrations as low as 0.1 $\mu g\ L^{-1}$) have been linked to the inhibition of embryo-larval growth, where there was down-regulation of the early growth response-related genes (*egr1* and *egr4*) and dual-specificity phosphatase (*dusp5*), a gene involved in cell growth and differentiation.[108] Citalopram and sertraline have also both been shown to affect bone development in zebrafish, although only at concentrations several orders of magnitude above those detected in the environment.[109]

There is little published data on the implications of antidepressant on mammalian reproduction,[110,111] but considerably more for the effects of antidepressants on fish reproduction and its associated behaviors. Examples include reduced egg production in zebrafish exposed to venlafaxine (six week exposure to 10 $\mu g\ L^{-1}$)[112] and fluoxetine (32 $\mu g\ L^{-1}$ for seven days).[113] For fluoxetine (100 $\mu g\ L^{-1}$ for four weeks) this was also the case for fathead minnows, where male reproductive behavior (nest care and mate aggression) was also altered.[114] Antidepressants have also been reported to affect precopulatory mating behaviors. For example, in the Eastern mosquitofish, exposure to fluoxetine (at 31 and 374 ng L^{-1} for 35-days) resulted in males spending significantly longer periods of time associated with females compared with controls.[115] Similarly, in the presence of a competitor, males exposed to fluoxetine (478.50 ng L^{-1} for 30 days) have been show to attempt to mate with females more frequently than for control males.[116] These authors also reported an increase in sperm count in Eastern mosquitofish that had been exposed to fluoxetine (29.51 and 379.5 ng L^{-1} over a 30-day period).[116] Collectively, these data would suggest an enhancement in individual male reproductive performance.

In natural environments, food availability is often limited and also intermittent and consequently, any modification of feeding activity could have significant effects on survival and reproductive success. Antidepressants are well-known modulators of feeding and appetite in mammals, and these drugs have also been widely reported to affect food intake in a variety of fish species. Studying the effect of fluoxetine on appetite suppression in fathead minnows, Stanley *et al.* 2007 found that S-fluoxetine was 3.3-fold more potent in reducing appetite than R-fluoxetine, with LOECs of 51 and 170 $\mu g\ L^{-1}$, respectively and the racemic mixture of R- and S-fluoxetine had an intermediate potency (LOEC of 106 $\mu g\ L^{-1}$),[117] consistent with their relative potencies in mammals.[118] This appetite suppressive effect is supported with the findings of a reduction of Neuropeptide Y (NPY, an appetite stimulant widely distributed in the fish CNS)[119] in the telencephalon of goldfish (*Carassius auratus*) after repeated injection of fluoxetine.[120] Some studies, however, have reported the opposite effect of antidepressant treatment on appetite in fish. For example, in the three-spined stickleback (*Gasterosteus aculeatus*), exposure to citalopram (1.5 $\mu g\ L^{-1}$) led to them attacking the food provisioned more frequently.[121]

Given that antidepressants are generally prescribed to treat depression and related disorders such as anxiety, the effects of these drugs on measures of anxiety in fish has been relatively well studied. In fish, measures such as exploratory behavior including diving, thigmotaxis (wall-hugging) and scototaxis (light-dark preference) have been developed as surrogate measures for assessing anxiety related responses. The novel tank diving test is a well-established and commonly used proxy for assessing anxiety in fish treated with pharmaceuticals.[122,123] In this test, a fish is introduced to a novel environment following drug exposure, and a variety of parameters are assessed including latency to enter the top of the water column, the amount of time spent there and the number of transitions between the tank top and bottom.[122] Using this assay, the aforementioned anxiolytic effects have been observed across a range of species, antidepressant drugs and experimental regimes.[124−126] Similarly, thigmotaxis has also been used as a behavioral index of anxiety.[127] Given their anxiolytic effect, it would be expected that fish exposed to antidepressants would increase their boldness and exploratory behavior, and this indeed has been demonstrated for various antidepressant drugs. For example, Japanese medaka exposed to fluoxetine explore the environment more (100 $\mu g\ L^{-1}$ over 10 days).[124] In the assessment of

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scototaxis, the preference of an individual for a dark compartment is measured by recording the number of entries into and time spent in dark versus light zones.[128] Adult zebrafish, similar to several other species, exhibit a natural preference for the dark compartment,[128] and increased time spent in the light compartment is associated with an increase in boldness. Maximino *et al.* (2011) demonstrated that over a 2 week period, adult zebrafish spent a greater amount of time in the light compartment after daily injections of 10 mg kg^{-1} fluoxetine compared to the controls.[129] In an ecological context, an increase in boldness could leave individuals more vulnerable to predation and there is evidence to suggest that these changes in boldness may be manifested at the level of the hypothalamic-pituitary-interrenal axis. For example, adult zebrafish exposed to fluoxetine or nortriptyline (0.01 mg L^{-1} for 7 days) and subsequently challenged with an unpredictable chronic stressors, exhibited marked reductions in whole body cortisol levels compared with untreated (stressed) controls.[130] In contrast, exposure of zebrafish to a very high dose of fluoxetine (5000 $\mu g\ L^{-1}$ for 2 h) has been shown to result in an elevation in whole trunk cortisol levels.[131]

Somewhat paradoxically, other studies have reported anxiogenic effects in fish following antidepressant exposure. For example, Japanese medaka larvae were found to spend more time in the peripheral zone of a thigmotactic test arena following a 72 h exposure to either citalopram, sertraline or fluoxetine,[132] and wild guppies (*Poecilia reticulata*) subjected to a chronic 28-day fluoxetine exposure of just 16 ng L^{-1} showed prolonged freezing bouts and spent a significantly greater proportion of time under plant cover compared to control animals following a simulated bird strike.[133] It is not known whether these effects are due to the primary or secondary pharmacology of the compound in question, or differences in target functional homology between species. However, anxiolytic or anxiogenic phenotypes could be costly to an individual's fitness, affecting predator avoidance behaviors, foraging ability and mate acquisition.

The broader consequences of altered animal boldness includes a potential impact on social behaviors which in fish includes shoaling and aggression. Indeed, a reduced latency to shoal has been seen in crucian carp (*Carassius carassius*) exposed to sertraline (between 4.3 and 116 $\mu g\ L^{-1}$ for seven days)[134] and in juvenile meagre exposed to venlafaxine (20 $\mu g\ L^{-1}$ via the water or 160 $\mu g\ kg^{-1}$ via the diet for 28 days).[125] Conversely, another study found fewer transitions away from the shoal in male zebrafish when exposed to citalopram (at 0.1 $\mu g\ L^{-1}$ for 14 days).[135] This has important implications as shoaling in wild fish populations is often seen as a trade-off between the costs of increased foraging versus antipredator benefits.[136] Similarly, antidepressant exposure has been shown to reduce fish aggression levels: bluehead wrasse (*Thalassoma bifasciatum*);[137] rainbow trout;[138] Arabian killifish (*Aphanius dispar*);[139] round goby;[140] three-spine stickleback;[141] and zebrafish.[131,142] In contrast, some studies have also reported elevated levels of aggression in association with antidepressant exposure. For example, dominant male Gulf toadfish (*Opsanus beta*) exposed to fluoxetine (either 10 or 25 $\mu g\ g^{-1}$ via intraperitoneal injection),[143] and three-spined stickleback under chronic exposure to citalopram (1.5 $\mu g\ L^{-1}$ via the water for 30 days, with a 100-day depuration period in contaminant free water)[121] both showed increases in aggression. In the wild, a change in aggression in males may affect the ability to acquire mates (if a territory is lost to a more dominant rival) or increase the likelihood of injury if rivals fight under conditions where one would normally adopt a submissive role.[144]

It is increasingly recognized that the effects of some pollutants can be seen in subsequent generations, and this has recently become of interest in studies of antidepressant effects in fish. Transgenerational inheritance can involve epigenomic changes in the germline, which are transmitted to subsequent generations. In order for any environmentally induced phenotypic changes in the progeny of exposed adults to be classified as transgenerational inheritance mechanisms, effects must be present in at least the F3 generation as the F1 embryo and F2 generation germ line may have been inadvertently exposed.[145] A series of studies have been recently published on zebrafish investigating the transgenerational inheritance of various phenotypes following exposure to fluoxetine.[146−148] In this work, the F0 generation was exposed to fluoxetine at either 54 or 0.54 $\mu g\ L^{-1}$ from 0 to 6 days and then subsequent generations were bred in contaminant free water. Adult F0 whole-body cortisol levels were lower following acute fluoxetine exposure, and this persisted for three consecutive generations in the unexposed descendants (F1−F3). Transcriptomic profiling of the kidney from these animals indicated modifications to pathways closely associated with cortisol synthesis.[146] Both F0 and F3 larvae were found to have fluoxetine-induced alterations in certain steroidogenic pathways, including the down-regulation of a gene involved in cortisol activation.[147] Importantly, even with the removal of antidepressants, ancestral exposure could affect the biological responses of future generations if re-exposed. For example, exposure of larvae from the F4 generation (outlined above) to 5 $\mu g\ L^{-1}$ venlafaxine from 0 to 6 dpf found cortisol levels in the subsequent adult females to be lower in both the unstressed and stressed groups (standardized net stressor test).[148] This was not seen in males, suggesting a female-specific effect of heightened sensitivity to venlafaxine following a historic fluoxetine exposure. The potential for transgenerational inheritance effects where subsequent generations may be sensitized to the effect of drugs indicates a potential for greater health risks upon exposure to antidepressants than perhaps anticipated.

ESTIMATING THE POTENTIAL ENVIRONMENTAL RISK OF ANTIDEPRESSANTS

The ratio between the environmental concentration (EC) and the no observed effect concentration (NOEC ∼ the concentration below that at which a biological effect has been observed) can be used to provide an indication of the potential for ecological risk associated with a particular compound. When this ratio is below 0.1 the risk is considered "insignificant", and when it is above 1, this is a level for potential concern warranting further evaluation of the fate and effects of the drug.[149] Collating these data for fluoxetine from 22 European countries suggests an overall moderate environmental risk, however, there is considerable geographical variation in usage and for some countries the risk will be insignificant.[150] Generally, the predicted environmental concentration (PEC) in particular tends to overestimate the actual concentration of contaminants present in the environment (the measured environmental concentration or MEC). Consequently, the ratio of measured to the NOEC normally provides a more conservative estimate of risk.[151,152] Accordingly, here we calculated the ratio between the MEC (surface water), and NOEC, reported for a number of fish species using physiological and behavioral end points (the MEC:NOEC) to generate a risk quotient, or RQ (see SI Table S6 for

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the full list of studies used). In order to present the worst case scenario for the potential risk of fish to antidepressant exposure, the MECs used to derive the RQs are those that represent the highest reported concentration of each compound in surface waters across the globe. The data for these calculations were obtained through searches conducted via Web of Science, Scopus, and Google from the period July 2017 to February 2021. The search words included all of the antidepressants in the TCA, SSRI, and SNRI groupings, combined with the word "fish". In addition, if any of these papers included references to effect studies that were not found in these searches, these were sourced and incorporated also. Overall, 128 studies were sourced and used for these analyses, of which all were published in English. For our calculated RQs, all aqueous antidepressant fish exposure studies found in the literature were included, with exception of one study that reported an extremely high MEC for citalopram in the Isakavagu-Nakkavagu water body, India, was exluded.[77] Our rational here was that this is an extremely unusual case as the water body receives multiple pharmaceutical manufacturing plant inputs resulting in extremely high water concentrations. Collectively, our RQ calculations suggest that venlafaxine and fluoxetine pose a low potential risk to fish, and citalopram, escitalopram, amitriptyline, sertraline, nortriptyline, duloxetine, paroxetine, and clomipramine all have an insignificant risk (see Table 1), accepting the limitations of both our approach and the data available. These data limitations include first, the number of studies from which effect data were derived varied considerably between compounds; the RQ values for some antidepressants are from just a single or small number of studies. Second, data for most studies used were effects observed at the lowest tested concentration meaning that the actual NOECs were not as accurate as ideally needed. As a consequence of this, the RQs were often skewed by studies using dose ranges considerably higher than those with environmental relevance, and the classifications of antidepressants as being of low or insignificant risk is therefore likely to be conservative. Third, there was a lack of chronic exposure studies for many of the antidepressants. Finally, in our approach to calculating these RQ values, we took the decision to include all available studies to provide data sets for as broad a range of compounds as possible and elements of the experimental design were not considered as a means of exclusion or inclusion. Therefore, studies vary for example in their exposure durations, method of exposure (static-renewal versus flow-through), and the developmental stage of the species tested. These RQ calculations, however, are useful for providing broad indications of those antidepressants which pose the greater potential environmental risk.

Table 1. Risk Quotients Were Calculated by Dividing the Highest Reported Measured Environmental Concentration (MEC) with the No Observed Effect Concentration (NOEC) Reported for Fish (If a NOEC Was Not Available, The Lowest Concentration Reporting an Effect Was Used)[a]

Compound	Class	Log Dow	Number of reported effects	MEC (μg L^{-1})	Median RQ
Venlafaxine	SNRI	1.43	27	1.31[79]	0.66
Fluoxetine	SSRI	1.75	98	0.41[153]	0.41
Citalopram	SSRI	1.27	20	0.427[154]	0.0427
Escitalopram	SSRI	1.27	2	0.003[155]	0.02
Sertraline	SSRI	3.14	19	0.049[79]	0.0109
Amitriptyline	TCA	2.96	16	0.196[156]	0.0065
Nortriptyline	TCA	2.28	6	0.019[76]	0.0019
Duloxetine	SNRI	1.53	1	0.002[79]	0.0007
Paroxetine	SSRI	1.46	2	0.09[157]	0.0006
Clomipramine	TCA	3.31	3	0.0032[158]	0.0003

[a]The colours show the level of environmental risk, according to the European Medicines Agency: potential risk (MEC/NOEC > 0.1: yellow) and insignificant risk (MEC/NOEC < 0.1: green). Effects were classified as being either physiological or behavioural and if multiple effects from either grouping were reported in the study, the lowest NOEC was used to calculate the RQ. Log_{Dow} values of lipophilicity were taken from ACD/Labs, Chemspider (accessed 2021/10/03). See SI Table S6 for a full list.

THE POTENTIAL IMPORTANCE OF MIXTURES

In the aquatic environment, fish are likely to be exposed to mixtures of contaminants and arguably, this is particularly important in the case of pharmaceuticals where multiple chemical entities can act via the same specific molecular mechanism, and are often found within the same effluents and receiving waters. The antidepressants amitriptyline, nortriptyline, dosulepin, fluoxetine, norfluoxetine, and venlafaxine (spanning all three major antidepressant classes) have been detected in UK waters at a range of concentrations in the low ng L^{-1} range[76] and summing these levels up as a mixture gives a maximum concentration in river water and effluent as high as 207.9 ng L^{-1} and 727.7 ng L^{-1}, respectively. This takes exposure levels into the range reported to induce physiological and behavioral effects in fish.[99,106,159,160] The likelihood of additive effects for exposure to mixtures of antidepressants is supported by a study on a mixture of three TCAs (amitriptyline, nortriptyline, and clomipramine, for 30 days) in common carp measuring for effects on mortality, developmental retardation and antioxidant enzyme activity.[11] This was similarly the case for zebrafish larvae exposed to a mixture of sertraline, paroxetine, fluoxetine, and mianserin where survival and proliferation of hepatocytes in the liver was reduced.[12] Other nonantidepressant drugs and chemical contaminants present in surface waters may also interact to modify the effects of antidepressants, but little study has been done in this regard.

KNOWLEDGE GAPS AND CONCLUDING REMARKS

The high degree of structural conservation for antidepressant targets between mammals and fish raises the potential for biological effects in fish in the natural environment and laboratory-based studies have reported effects across a wide range of fish species. Generally, relatively low concentrations of these drugs are detected in the aquatic environment, and when compared with their effective concentrations in fish, this would suggest that currently, antidepressant drugs represent a low environmental risk to fish. However, there are a number of very recent studies that start to question this, which may reflect differences in some of the behavioral end points measured.[161−163] A systematic analyses of published studies applying the CRED (criteria for reporting and evaluating ecotoxicity data)[164] approach might be usefully applied to provide a more robust evaluation for differences between studies and their associated findings. Furthermore, there are few published studies investigating the impacts of antidepressant drugs on

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fish following chronic exposure,[11,165] their potential for bioaccumulation and food chain transfer, or for exposure to environmentally relevant mixtures, and these are areas of priority for research. Also, prescription rates of antidepressants are on the rise and thus concentrations are likely to increase in the natural environment. Emerging studies in mammals also support the need to investigate the transgenerational and epigenetic effects of antidepressants in fish. Critically, we also argue that far more studies in fish are needed focusing on the most relevant end points for antidepressant effects on behaviors as these are still relatively uncommon.

In the case of specific behaviors, robust measures are needed in order to ascertain exactly how a behavior is affected, and how these effects impact the ability of fish (and other wildlife) to react and respond to changing environmental conditions (e.g., predation, mating, foraging, social interaction, etc.). A change in an individual's behavior, for example mate choice, could alter population structure and subsequently lead to a variation in traits selected in the next generation. Gaining a greater understanding of which neural circuits are influenced by exposure to CNS-active drugs may help in directing the development of these behavioral tests. There are now methodologies that allow brain function to be assessed in response to chemical exposure, via general and specific neural pathways and processes in the CNS using fish transgenic models. Coupled with imaging methods,[166−168] these can provide powerful ways to potentially direct more targeted analyses of effects of antidepressants on fish behaviors affecting their fitness and survival in natural environments. A meta-analysis of published studies on the sensitivity, reliability and repeatability of the behavior end points measured would help direct where efforts are best applied in future studies to most effectively quantify the effects of antidepressants on behavior in fish. It is also the case that the vast majority of studies reporting on the effects of antidepressants in fish have done so under laboratory based conditions, and future research should therefore look to assess effects under more natural conditions which may differ, especially for behaviors, in those environments.

Changes in an individual's behavior as a consequence of exposure to contaminants may affect species interactions to (indirectly) affect ecological systems.[169,170] In the case of a range of antidepressant drugs, it has been shown that exposure can affect food consumption efficiency in various fish species,[117,121,134,171,172] which in natural systems could then affect species populations at higher or lower trophic levels, but very little attention has been directed to these potential effects. These studies are challenging, but would help to better understand the wider potential for effects on ecological community structure and function for exposure to antidepressants in the environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c04724.

Table S1: Global prescription statistics; Table S2: Comparative distribution of human and zebrafish serotonergic, adrenergic and dopaminergic receptors in neuronal and peripheral tissues; Table S3: Occurrence of antidepressants in the aquatic environment; Table S4: Bioaccumulation of antidepressants in fish tissues and biofluids; Table S5: Summary of *in vivo* data on sublethal effects reported in fish with aqueous antidepressant exposure; Table S6: Risk quotient calculations for assessing potential risk of antidepressant exposure in fish (PDF)

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Notes

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