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Hazard/Risk Assessment

Assessment of the Potential Ecotoxicological Effects of Pharmaceuticals in the World's Rivers

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Abstract: During their production, use, and disposal, active pharmaceutical ingredients (APIs) are released into aquatic systems. Because they are biologically active molecules, APIs have the potential to adversely affect nontarget organisms. We used the results of a global monitoring study of 61 APIs alongside available ecotoxicological and pharmacological data to assess the potential ecotoxicological effects of APIs in rivers across the world. Approximately 43.5% (461 sites) of the 1052 sampling locations monitored across 104 countries in a recent global study had concentrations of APIs of concern based on apical, nonapical, and mode of action–related endpoints. Approximately 34.1% of the 137 sampling campaigns had at least one location where concentrations were of ecotoxicological concern. Twenty-three APIs occurred at concentrations exceeding "safe" concentrations, including substances from the antidepressant, antimicrobial, antihistamine, β-blocker, anticonvulsant, antihyperglycemic, antimalarial, antifungal, calcium channel blocker, benzodiazepine, painkiller, progestin, and lifestyle compound classes. At the most polluted sites, effects are predicted on different trophic levels and on different endpoint types. Overall, the results show that API pollution is a global problem that is likely negatively affecting the health of the world's rivers. To meet the United Nations' Sustainable Development Goals, work is urgently needed to tackle the problem and bring concentrations down to an acceptable level. *Environ Toxicol Chem* 2022;00:1–13. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Pharmaceuticals; Mixtures; Ecotoxicology; Contaminants; Hazard/risk assessment; Surface waters

INTRODUCTION

Over 1900 active pharmaceutical ingredients (APIs) are used to treat and prevent disease in humans (Burns et al., 2018). It is inevitable that these substances will be emitted to the natural environment during their manufacture, use, and disposal (Boxall, 2004), so it is not surprising that a wide range of pharmaceuticals have been detected in surface waters in many regions of the world (aus der Beek et al., 2016; Wilkinson et al., 2022). There is a growing concern that exposure to these APIs can negatively affect the health of ecosystems because they are designed to interact with receptors and biochemical pathways in humans, many of which are conserved in nontarget organisms (Gunnarsson et al., 2019) and have the potential to cause toxicological side effects. For example, there is evidence from whole-lake studies that synthetic estrogens cause endocrine disruption at concentrations close to those seen in the environment (Kidd et al., 2007). The use of the nonsteroidal anti-inflammatory compound diclofenac resulted in a notable decline in vulture populations on the Indian subcontinent, leading to potential impacts on human health (Markanda et al., 2008; Oaks et al., 2004); and antidepressants have been shown to affect fish behavior, which could alter susceptibility to predation (Brodin et al., 2014; Weinberger & Klaper, 2014). There is also a growing concern that the presence of antimicrobial compounds in the environment is contributing to the selection of drug-resistant bacteria (Wellington et al., 2013), potentially contributing to the 1.2 M extra deaths in 2019 resulting from antimicrobial-resistant infections (Murray et al., 2022).

To fully understand the likely impacts of pharmaceuticals in the environment on ecosystem health, it is essential to understand the concentrations that occur in the environment. Available monitoring data for APIs have previously been compared with ecotoxicological thresholds to quantify potential impacts in riverine systems (see Bagnis et al., 2020; Boxall et al., 2012; Hossain et al., 2018; Kelly & Brooks, 2019; Schafhauser

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et al., 2018; Topaz et al., 2020; Zhou et al., 2016). These studies have, however, tended to either consider the potential effects of multiple APIs in a single country (see Bagnis et al., 2020; Boxall et al., 2012; Topaz et al., 2020; Zhou et al., 2016) or explored the potential effects of single compounds across multiple countries (see Kelly & Brooks, 2019; Schafhauser et al., 2018). The studies that have considered multiple countries have mainly relied on published monitoring data, which are not necessarily comparable. These studies have typically been done compound by compound, with limited attempts made to consider the potential effects arising from mixture interactions (Topaz et al., 2020). There is therefore a need to extend the existing work on the ecotoxicological effects of APIs in riverine systems to a wider range of countries and APIs and to consider the potential effects of API mixtures.

Recently, an extensive monitoring study to understand the levels of API contamination in rivers across the globe was published (Wilkinson et al., 2022). In that study, samples were taken from over 258 rivers across 104 countries covering all continents. These samples were analyzed for 61 pharmaceuticals from a range of classes (Wilkinson et al., 2019), for the first time providing a picture of the scale of contamination in the world's rivers by pharmaceuticals. In that study, a preliminary assessment of the impacts of the APIs on the aquatic systems was performed by comparing the measured data with predicted no-effect concentrations (PNECs) and critical environmental concentrations (CECs) reported in the literature. This ecotoxicological assessment work did not, however, consider the broader literature on the apical effects of APIs; the potential for nonapical effects, such as behavioral or biochemical effects; or the impacts of combinations of APIs. Because PNECs or CECs were not available for all APIs, it was not possible to assess the potential ecotoxicological impacts of all APIs that were detected. Therefore, we used these global monitoring data alongside published ecotoxicity data on both the apical and the nonapical effects of APIs and predictive models to perform a holistic assessment of the potential ecotoxicological effects of individual APIs and mixtures of APIs in river systems across the globe.

METHODS

Pharmaceutical concentration data

Data on the concentrations of APIs in surface waters were taken from Wilkinson et al. (2022). The data set contained concentration data for 61 APIs, representing 19 therapeutic classes used for human and veterinary medicine in surface water samples collected from 1056 locations on 258 rivers across 104 countries around the globe. Fifty-three APIs were detected in at least one sample, with the most frequently detected pharmaceuticals being carbamazepine (an anticonvulsant), metformin (a Type 2 diabetes treatment), and caffeine (a stimulant and lifestyle chemical), all of which were detected at over half the sites monitored. The highest cumulative API concentrations in surface waters were observed in sub-Saharan Africa, South Asia, and South America, with Lahore in Pakistan being the most polluted system (Wilkinson et al., 2022).

Ecotoxicity data

Median effect concentrations (EC50s) and no-observed-effect concentrations (NOECs) for the 53 compounds were obtained from apical effect studies (algae 72-h growth, *Daphnia* 48-h immobilization, fish 96-h mortality, *Daphnia* 21-day reproduction, and fish 28-day growth and reproduction studies) and *Daphnia* nonapical effects studies (e.g., behavior and cytotoxicity) from the published literature, online databases (see iPiE sum [Intelligence-Led Assessment of Pharmaceuticals in the Environment, 2021], the US Environmental Protection Agency's [USEPA's] ECOTOX [USEPA, 2021a, 2021b], the European Chemicals Agency [European Union, 2021]), and the company Material Safety Datasheets. If experimental data were not available for an API, ecotoxicity was predicted using a combination of the Ecological Structure Activity Relationships (USEPA, 2021a, 2021b), CompTox (USEPA, 2020), and the VEGA QSAR software (Ver 1.1.5). Predicted data were only used where the models indicated that the estimates were reliable and within respective applicability domains. All apical ecotoxicity data were scored using the Klimisch scoring system to assess their reliability. Data were entered into an Excel spreadsheet for subsequent use in the ecotoxicological effects characterization work.

Assessment of ecotoxicological effects at monitored locations

Apical effects of single compounds. The chronic NOEC or acute EC50 or median lethal concentration (LC50) apical data were used to derive PNECs for each API for each taxonomic group (fish, *Daphnia*, and algae) using an assessment factor of 1000 for the acute data and 10 for chronic data (Equation 1). Chronic PNECs were used in preference to acute data, and the lowest taxonomic PNEC was used in the calculations.

$$\mathrm{PNEC_{apical}} = \frac{\text{Lowest NOEC/EC50/LC50}}{\text{Assessment factor}} \quad (1)$$

Apical hazard quotients ($\mathrm{HQ_{apical}}$) were then calculated, based on the lowest PNEC for the different taxonomic groups, for each API for each sampling site, using Equation 2.

$$\mathrm{HQ_{apical}} = \frac{\mathrm{MEC}}{\mathrm{PNEC_{apical}}} \quad (2)$$

In Equation 2, MEC is the measured environmental concentration of the pharmaceutical at a sampled location. If the $\mathrm{HQ_{apical}} \geq 1$, it was concluded that the compound could have a negative effect on organisms at the sampling location.

Nonapical effects of single compounds. The lowest nonapical lowest-observed-effect concentration (LOEC) for an API was selected to obtain the nonapical HQs using Equation 3.

$$\mathrm{HQ_{nonapical}} = \frac{\mathrm{MEC}}{\mathrm{LOEC_{nonapical}}} \quad (3)$$

If the $\mathrm{HQ_{nonapical}} \geq 1$, it was concluded that nonapical effects such as impacts on organism behavior or on biochemistry could

be occurring at the sampled location. No assessment factors were used in this approach.

Mode of action–related effects of single compounds. The fish plasma modeling approach (Huggett et al., 2002) was used to identify pharmaceuticals with the potential to cause detrimental effects at measured concentrations, through mechanisms conserved between humans and aquatic vertebrates. The CECs for each API were calculated using an adaptation of an approach proposed by Fick et al. (2010). The octanol–water distribution coefficient (D_{OW}) for each API at a pH of 7.4 was obtained from ChemSpider (Royal Society of Chemistry, 2022) and used to estimate the theoretical plasma bioconcentration factor ($K_{plasma:water}$) using Equation 4.

$$\log K_{plasma:water} = 0.73 \times \log D_{OW} - 0.88 \quad (4)$$

To determine the CEC for each pharmaceutical, the therapeutic concentrations in human plasma (H_tPC) were acquired from the Mammalian Pharmacokinetic Prioritization for Aquatic Species Targeting database as the maximum human therapeutic plasma concentration (Berninger et al., 2016). Subsequently, the CECs for each API were calculated using Equation 5.

$$CEC = \frac{H_tPC}{CR \times K_{plasma:water}} \quad (5)$$

In Equation 5, CR is the concentration ratio between H_tPC and the fish steady-state plasma concentration and was considered to be 1.

The HQs for mode of action–related effects were then determined as the ratio of the CEC and the MEC for each pharmaceutical using Equation 6.

$$HQ_{CEC} = \frac{MEC}{CEC} \quad (6)$$

In Equation 6, where an $HQ_{CEC} \geq 1$ was obtained, it was concluded that effects related to the mode of action of the API could be occurring in organisms at the sampled location.

Apical effects of mixtures. An assessment of the potential ecotoxicological impacts of pharmaceutical mixtures at each location was performed based on the apical endpoint data only. The HQs for the mixture of APIs at each sampling site were calculated following Topaz et al. (2020). The PNECs for each pharmaceutical for each taxonomic group ($PNEC_{fish}$, $PNEC_{Daphnia}$, and $PNEC_{algae}$) were estimated using an assessment factor of 1000 for acute data and 10 for chronic data. The HQs for the mixture in a sample for the separate taxonomic groups were then calculated using the different PNECs using Equation 7.

$$HQ_{mixtg} = \left[\sum_{i=1}^{53}\left(\frac{MC_i}{PNEC_{i,tg}}\right)\right] \quad (7)$$

In Equation 7, HQ_{mixtg} is the HQ for the hazard of a pharmaceutical mixture to each taxonomic group and $PNEC_{i,tg}$ is the PNEC of each pharmaceutical for the taxonomic group. The highest HQ_{mixtg} for each location was then taken as the mixture HQ of the sampling site. Where an $HQ_{mixtg} \geq 1$ was obtained, it was concluded that the mixture of pharmaceuticals at the location assessed could be negatively affecting the ecosystem.

RESULTS

Of the 61 APIs monitored in the global study (Wilkinson et al., 2022), eight (cloxacillin, diphenhydramine, miconazole, norfluoxetine, oxazepam, oxytetracycline, raloxifene, and sertraline) were not detected in any water sample and so were not considered in the ecotoxicological analysis.

Assessment of ecotoxicological effects at monitored locations

Apical effects of single compounds. For the 53 detected APIs, either EC50/LC50 or NOEC data were available for 52 APIs for all taxonomic groups (Table 1). For 39 of these APIs, experimental NOEC data were available for at least one taxonomic group. For five APIs (cotinine, hydrocodone, lidocaine, salbutamol, and temazepam) PNECs were based on predicted ecotoxicological data. No experimental data were found for itraconazole, and quantitative structure–activity relationship predictions for itraconazole were also considered as not reliable, so a PNEC was not calculated for this molecule. Itraconazole was detected in only one sample in the global study (Wilkinson et al., 2022), so the lack of ecotoxicity data for this molecule has limited impact on the results of the analyses.

It was therefore possible to estimate PNECs for 52 APIs out of the 53 detected, with 58% of these based on NOEC data and 42% on EC50/LC50 values (Table 1). For 22 APIs, the lowest PNEC was derived from data on effects on algae, while the lowest PNECs for 14 APIs were based on *Daphnia* data and another 14 on fish data. The PNEC for gabapentin was based on all taxonomic groups given that they had equal ecotoxicity values, whereas the PNEC for oseltamivir was based on fish and *Daphnia* data (Table 1).

Generally, the concentrations observed in the river water samples were lower than apical PNEC values, with 918 (87.3%) of the 1052 sampling sites having no API concentrations above PNECs. The HQs ranged from 1.33×10^{-6}, which was observed for cimetidine in a sample obtained from Kuala Lumpur in Malaysia, to 28.3 for sulfamethoxazole in a sample obtained from Bukavu in the Democratic Republic of Congo (Figure 1). No sites in Antarctica and Oceania had concentrations of any API above the PNECs. For the other continents, Africa had the highest percentage (26.9%) of sites where measured API concentrations exceeded PNECs, while North America had the lowest (2.5%).

Ten APIs had concentrations above the apical PNECs (Figure 1). The compound with the highest percentage of sampling locations where concentrations were above the PNEC was sulfamethoxazole, where measured concentrations exceeded the PNEC at 83 sites (7.9%). Other APIs with an $HQ \geq 1$ were nicotine (3.3% of sites, 35 sites), clarithromycin

TABLE 1: Apical predicted-no-effect concentrations, nonapical endpoints, and critical environmental concentrations for each of the study pharmaceuticals that were used to assess the potential for ecotoxicological effects at each of the monitored locations in the Wilkinson et al. (2022) global monitoring study

Compound	$PNEC_{apical}$ (ng/L)	$PNEC_{fish}$ (ng/L)	$PNEC_{daphnia}$ (ng/L)	$PNEC_{algae}$ (ng/L)	Nonapical endpoint type	Nonapical endpoint (ng/L)	CEC (ng/L)
Amitriptyline	310^{UD}	310^{UD}	1080^{N}	$29{,}900^{N}$	Changes in the activity of nitric oxide synthase in fish	1	3248
Artemisinin	240^{E}	$12{,}050^{UD}$	$22{,}880^{UD}$	240^{E}	ND	ND	ND
Atenolol	$148{,}000^{N}$	$320{,}000^{N}$	$148{,}000^{N}$	$1{,}000{,}000^{N}$	Changes in microcystin content in algae	20,000	17,002,000
Caffeine	$12{,}000^{N}$	$30{,}000^{N}$	$12{,}000^{N}$	$10{,}000{,}000^{N}$	Variation in acetylcholinesterase in fish	16,000	18,952,216
Carbamazepine	2500^{N}	2500^{N}	2600^{N}	$50{,}000^{N}$	Changes in activities of antioxidant enzymes in fish	1000	328,572
Cetirizine	$10{,}000^{E}$	$10{,}000^{E}$	$1{,}880{,}000^{N}$	$35{,}800{,}000^{N}$	ND	ND	382,411
Cimetidine	$880{,}000^{N}$	$10{,}000{,}000^{N}$	$880{,}000^{N}$	$10{,}500{,}000^{N}$	ND	ND	968,144
Ciprofloxacin	470^{E}	$60{,}000^{E}$	$1{,}090{,}000^{N}$	470^{N}	Alterations in antioxidant activity in algae	500,000	805,081,800
Citalopram	1600^{E}	9136^{E}	3900^{E}	1600^{E}	Changes in behavior in fish	200	35,530
Clarithromycin	250^{N}	$100{,}000^{E}$	$210{,}000^{N}$	250^{N}	ND	ND	27,773
Clotrimazole	1000^{N}	2500^{N}	1000^{N}	1700^{N}	Variation in cell viability in fish	345,000	35
Codeine	$101{,}000^{E}$	$238{,}239^{UD}$	$1{,}080{,}000^{N}$	$101{,}000^{E}$	ND	ND	142,142
Cotinine	$64{,}500^{PE}$	$973{,}000^{PE}$	$1{,}220{,}000^{PE}$	$64{,}500^{PE}$	ND	ND	125,407
Desvenlafaxine	$32,200^{E}$	$210{,}000^{N}$	$820{,}000^{N}$	$32{,}200^{E}$	ND	ND	272
Diazepam	$27{,}300^{N}$	$27{,}300^{N}$	$80{,}000^{N}$	$61{,}000^{N}$	Changes in behavior in fish	260,000	5603
Diltiazem	8200^{E}	$15{,}000^{E}$	8200^{E}	$2{,}500{,}000^{E}$	Alterations in brain aromatase (CYP19A2) in fish	1	7134
Enrofloxacin	1910^{N}	$79{,}500^{UD}$	$500{,}000^{N}$	1910^{N}	ND	ND	ND
Erythromycin	200^{N}	$10{,}000{,}000^{N}$	$1{,}110{,}000^{N}$	200^{N}	Variations in antioxidant responses in algae (glutathione)	60,000	221,447
Fexofenadine	$1{,}000{,}000^{N}$	$1{,}000{,}000^{N}$	$2{,}500{,}000^{N}$	$2{,}500{,}000^{N}$	ND	ND	16,725
Fluconazole	$50{,}000^{UD}$	$50{,}000^{UD}$	$2{,}000{,}000^{N}$	$306{,}300^{N}$	ND	ND	2,338,837
Fluoxetine	320^{N}	320^{N}	8900^{N}	740^{N}	Changes in behavior of fish	250	5165
Gabapentin	$100{,}000^{E}$	$100{,}000^{E}$	$100{,}000^{E}$	$100{,}000^{E}$	ND	ND	39,899,734
Hydrocodone	939^{PE}	939^{PE}	5320^{PE}	4200^{PE}	ND	ND	57,003
Itraconazole	ND	ND	ND	ND	ND	ND	727
Ketoconazole	50^{N}	600^{N}	$25{,}000^{N}$	50^{N}	Changes in cytochrome P450 side chain cleavage (CYP11A) in fish	30,000	90,701
Ketotifen	1800^{N}	$419{,}000^{PE}$	1800^{N}	$155{,}000^{N}$	ND	ND	94
Lidocaine	3590^{PE}	$23{,}000^{E}$	5400^{PE}	3590^{PE}	ND	ND	1,368,646
Lincomycin	7800^{N}	$420{,}000^{N}$	$7{,}650{,}000^{N}$	7800^{N}	Behavioral changes in fish	15,000,000	184,356,303
Loratadine	5300^{N}	8400^{N}	7800^{N}	5300^{N}	ND	ND	1
Metformin	$100{,}000^{N}$	$220{,}000^{N}$	$100{,}000^{N}$	$9{,}900{,}000^{N}$	Alterations in gonadotropin releasing hormone 3 mRNA in fish	1000	215,179,057
Metronidazole	$100{,}000^{UD}$	$100{,}000^{UD}$	$25{,}000{,}000^{N}$	$203{,}000^{N}$	ND	ND	20,922,870
Naproxen	$15{,}000^{N}$	$100{,}000^{N}$	$15{,}000^{N}$	$620{,}000^{N}$	Changes in induction of ethoxyresorufin *O*-deethylase in fish	2,303,000	346,783,924
Nevirapine	$43{,}000^{E}$	$65{,}000^{E}$	$76{,}900^{E}$	$43{,}000^{E}$	ND	ND	444,662
Nicotine	1390^{E}	4000^{UD}	1390^{E}	$320{,}000^{N}$	Behavioral changes in fish	4,200,000	70,643
Norethisterone	37^{N}	37^{N}	4400^{E}	500^{E}	Variations in thyroid hormones in fish	7	304
Oseltamivir	$100{,}000^{N}$	$100{,}000^{N}$	$100{,}000^{N}$	$1{,}000{,}000^{N}$	ND	ND	171,007
Paracetamol	$572{,}000^{N}$	$9{,}500{,}000^{N}$	$572{,}000^{N}$	$13{,}400{,}000^{N}$	Behavioral changes in fish	500	38,725,764
Pregabalin	$100{,}000^{N}$	$100{,}000^{N}$	$480{,}000^{N}$	$3{,}200{,}000^{N}$	ND	ND	219,882,887
Propranolol	100^{N}	100^{N}	200^{N}	9000^{N}	Changes in estradiol levels in fish	1000	21,955
Ranitidine	$31{,}000^{N}$	$112{,}000^{UD}$	$31{,}000^{N}$	$15{,}000{,}000^{N}$	ND	ND	1,093,629
Salbutamol	7000^{PE}	7000^{PE}	$100{,}000^{E}$	$57{,}200^{E}$	ND	ND	390,535
Sitagliptin	$390{,}000^{N}$	$920{,}000^{N}$	$980{,}000^{N}$	$390{,}000^{N}$	ND	ND	469,906

(*Continued*)

TABLE 1: (*Continued*)

Compound	$PNEC_{apical}$ (ng/L)	$PNEC_{fish}$ (ng/L)	$PNEC_{daphnia}$ (ng/L)	$PNEC_{algae}$ (ng/L)	Nonapical endpoint type	Nonapical endpoint (ng/L)	CEC (ng/L)
Sulfadiazine	13,000[N]	103,000[E]	212,000[E]	13,000[N]	Variations of chlorophyll A concentrations in algae	1,000,000	286,220,015
Sulfamethoxazole	590[N]	800,000[N]	173,000[N]	590[N]	Modifications in acetylcholinesterase activity in fish	16,000	583,339,325
Temazepam	2280[PE]	70,230[PE]	72,180[PE]	2280[PE]	Changes in vitellogenin concentrations in fish	708	4373
Tetracycline	310[N]	220,000[E]	340,000[E]	310[N]	Modification of catalase levels in fish	5	1,563,507,612
Thiabendazole	309[E]	390[UD]	309[E]	9000[UD]	Changes in glutathione S-transferase activity in fish	45,000	ND
Tramadol	6200[UD]	6200[UD]	69,690[E]	58,660[E]	Behavioral changes in fish	658,000	316,519
Triamterene	10,000[E]	13,000[E]	10,000[E]	14,000[E]	ND	ND	1,135,795
Trimethoprim	310,000[N]	10,000,000[N]	312,000[N]	310,000[N]	Behavioral changes in fish	50,000,000	78,630,540
Tylosin	300,000[UD]	300,000[UD]	4,500,000[N]	825,000[N]	ND	ND	563,532
Venlafaxine	4800[E]	100,000[E]	38,000[UD]	4800[E]	Behavioral changes in fish	5000	68,565
Verapamil	4010[E]	30,000[N]	21,660[E]	4010[E]	Changes in glucose levels	470	2777

PNEC = predicted-no-effect concentration; CEC = critical environmental concentration; UD = undefined; N = no-observed-effect concentration; E = median effect or lethal concentration (EC50/LC50); ND = no data; PE = predicted EC50/LC50; CYP19A2/CYP11A = cytochrome P450 19A2/11A; mRNA = messenger RNA.

(2.9%, 30 sites), caffeine (2.3%, 24 sites), erythromycin (1.7%, 18 sites), propanol (1.1%, 11 sites), artemisinin (0.7%, 7 sites), amitriptyline (0.5%, 5 sites), ciprofloxacin (0.4%, 4 sites), and carbamazepine (0.3%, 3 sites). Although metformin was detected in over half of the sites monitored in the global study (Wilkinson et al., 2022) and paracetamol, metformin, and metronidazole had some of the highest concentrations, none of these APIs were found to have concentrations greater than their respective PNECs. This was due to the low apical ecotoxicity of these molecules, with PNECs for these APIs being 100,000 ng/L (metformin and metronidazole) and 572,000 ng/L (paracetamol).

Sulfamethoxazole was the API where concentrations most frequently exceeded PNECs for locations in Africa and South America, with 23.8% and 12% of the sampling sites exceeding the "safe" concentration, respectively. For sampling locations in Asia, nicotine was the API with the highest number of sites (10.3%) with an HQ ≥ 1. For Europe, propranolol was the molecule with the greatest frequency of PNEC exceedances (2.9%) at sampling locations. For North America, sulfamethoxazole and caffeine were the APIs where measured concentrations most frequently exceeded PNEC values, with 1.7% of locations having HQ ≥ 1 for both compounds (Supporting Information, Figure S1A).

Nonapical effects of single compounds. Data on nonapical effects were obtained for 28 APIs, with fish being the taxonomic group with the most data (Table 1). These data covered 18 endpoints including effects on enzyme activity, microcystin content, chlorophyll concentrations, vitellogenin expression, messenger RNA (mRNA) transcripts, hormone levels, and fish behavior (Table 1).

Eleven compounds (amitriptyline, caffeine, citalopram, diltiazem, metformin, norethisterone, paracetamol, sulfamethoxazole, temazepam, tetracycline, and verapamil) were found to have concentrations in surface waters above the nonapical LOEC values (Figure 2). Three of these (amitriptyline, caffeine, and sulfamethoxazole) were also identified as being of ecotoxicological concern based on the apical PNECs. The HQs ranged from 6.48×10^{-8} to 1180, with trimethoprim having the lowest HQ at a site in Baghdad, Iraq, and amitriptyline having the highest HQ in a sampling location in London, United Kingdom, situated 250 m downstream from a wastewater-treatment plant (WWTP; Figure 2).

A greater proportion of the monitored sites had measured concentrations above levels of concern based on nonapical effects compared with the apical effects. In the present study, 42.5% (447 locations) had concentrations of at least one pharmaceutical greater than a nonapical LOEC. Similar to the single-compound apical assessment, no sites in Antarctica and Oceania had concentrations of any API greater than nonapical LOECs, whereas for the other continents, Europe had the lowest percentage of sites where concentrations exceeded LOEC values (37.8%) and Asia had the highest percentage of sites where concentrations exceeded LOEC values (56.8%).

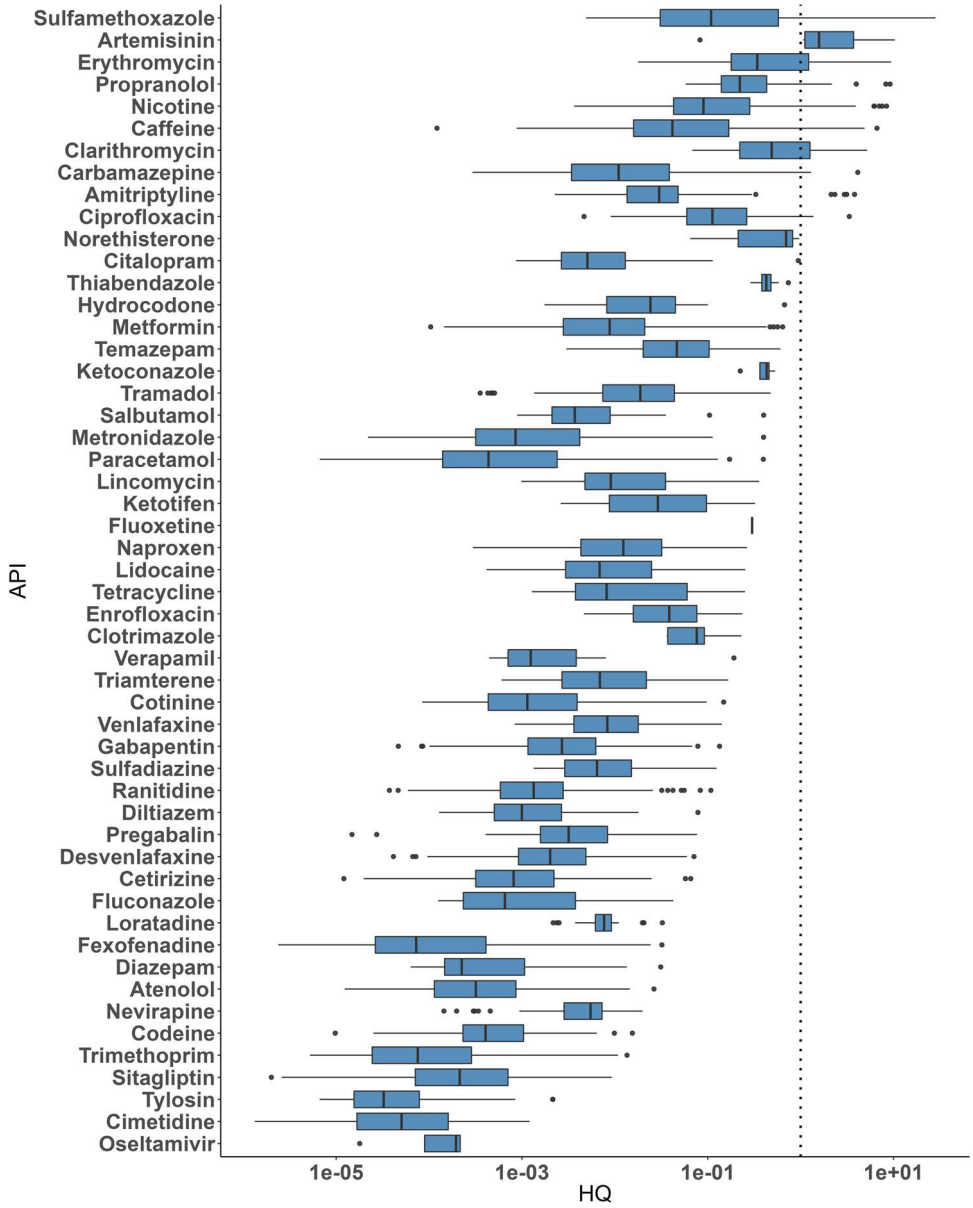

FIGURE 1: Box and whisker plot of hazard quotients (HQs) obtained for the 1052 different locations monitored in the Wilkinson et al. (2022) global study based on apical endpoint data. Dotted line represents HQ = 1. Boxes show the mean and upper and lower quartile HQs, while whiskers represent the maximum and minimum HQ values. API = active pharmaceutical ingredient.

The antihyperglycemic metformin, which presented some of the highest concentrations in the global monitoring study (Wilkinson et al., 2022), was the compound where measured concentrations most frequently exceeded LOECs. For the nonapical effects of metformin, 27.4% (288) of sites had concentrations above the LOEC obtained from a study demonstrating alteration of gonadotropin-releasing hormone 3 mRNA in fish as an endpoint (Crago & Klaper, 2018). Caffeine, diltiazem, paracetamol, amitriptyline, and tetracycline had concentrations above their nonapical LOEC for 20.6% (217 sites), 15% (158 sites), 14.6% (154 sites), 9.3% (98 sites), and 1.3% (14 sites) of the sites, respectively. Concentrations of citalopram, norethisterone, sulfamethoxazole, temazepam, and verapamil exceeded nonapical NOECs for <1% of the sampling sites (see Supporting Information, Figure S1B).

Mode of action–related effects of single compounds. With the exception of artemisinin, enrofloxacin, and thiabendazole (where no data were found on their H_tPC), it was possible to calculate CECs for all of the detected APIs. These CECs ranged from 1.42 ng/L (loratadine) to 1.6 mg/L (tetracycline; see Supporting Information, Table S3).

Five APIs had measured concentrations exceeding CECs for at least one site in the global monitoring study (Wilkinson

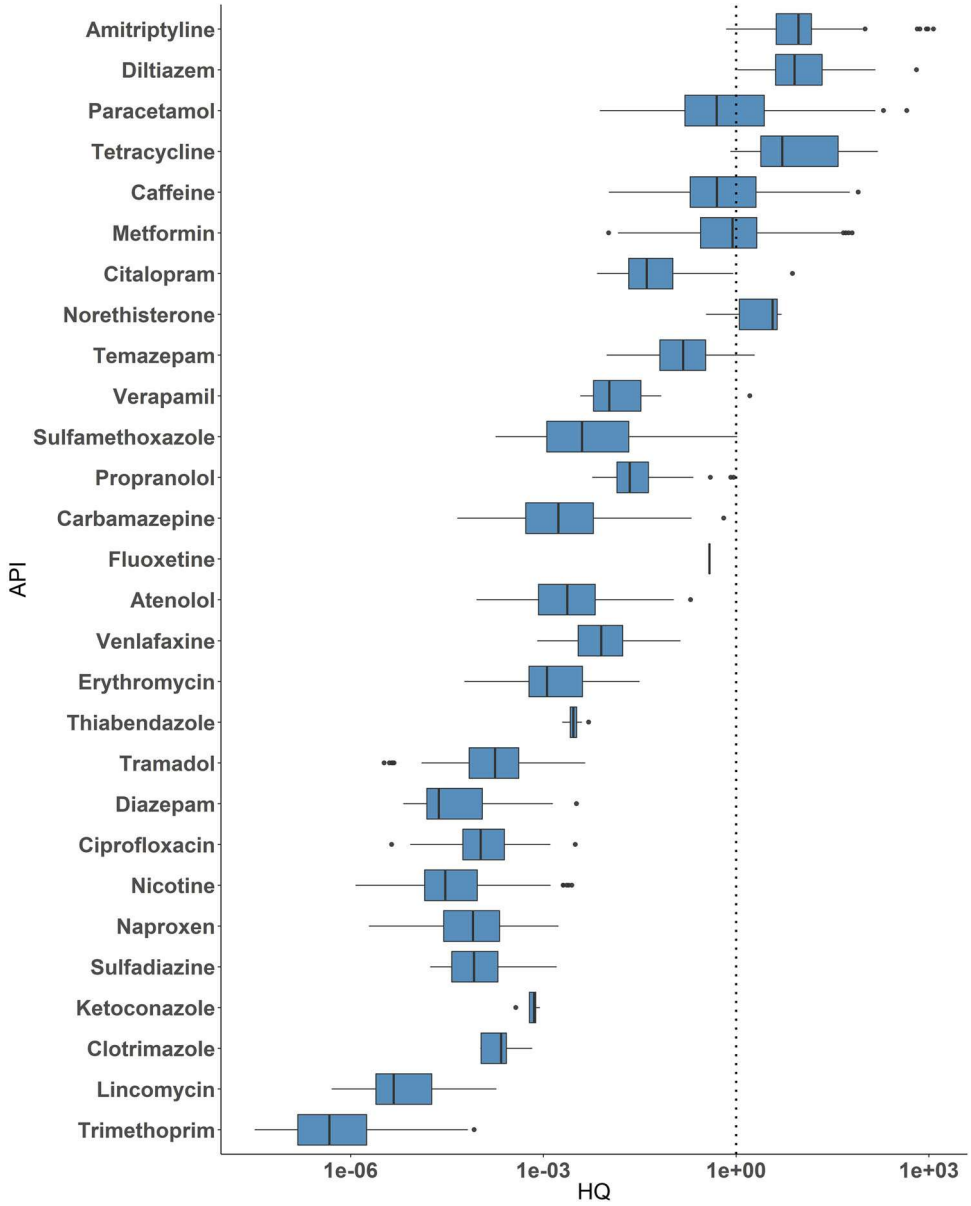

FIGURE 2: Box and whisker plot of the nonapical hazard quotients (HQs) obtained for the 1052 different locations monitored in the Wilkinson et al. (2022) global study. Dotted line represents HQ = 1. Boxes show the mean and upper and lower quartile HQs, while whiskers represent the maximum and minimum HQ values. API = active pharmaceutical ingredient.

et al., 2022; Figure 3), with desvenlafaxine most frequently (4.8% of sites) exceeding the CEC, followed by loratadine (2.4% of sites), fluoxetine and clotrimazole (0.5% of sites for each), and ketotifen (0.1% of sites).

The HQs calculated using the CECs ranged from 2.61×10^{-6} for tetracycline to 121.3 for loratadine, with the highest HQ found in a sampling location in Juba, South Sudan, where the sample was collected at a seasonal stream flowing through Juba town (Figure 3). Only 7.5% (79 sites) of sites had concentrations of one or more API above their CECs, with North America having the highest percentage (21.2%) of sites where CEC values were exceeded. Similar to the other single-compound approaches, no mode of action–related impact was identified in any of the sampling sites in Antarctica and Oceania (Supporting Information, Figure S1C).

Apical effects of mixtures. For the mixture assessment, taxon-specific PNECs were available for fish, *Daphnia*, and algae for 52 APIs (no data found for itraconazole; Table 1). A map of the mean cumulative HQ for each of the 137 campaigns in the global monitoring study (Wilkinson et al., 2022) is shown in Figure 4, and the proportion of sites within a campaign with an HQ ≥ 1 is shown in Supporting Information, Figure S2. When the potential effects of mixtures were considered at the different monitoring sites, 15.7% (165 sites) were found to have a cumulative HQ ≥ 1. Africa (27.3%), Asia (22.6%), and South America (18.5%) had the highest percentages of sites with a cumulative HQ ≥ 1. Meanwhile, Europe (7.3%) and North America (6.8%) had the lowest percentage of sites with a mixture HQ ≥ 1. No sites in Antarctica or Oceania had a mixture HQ ≥ 1.

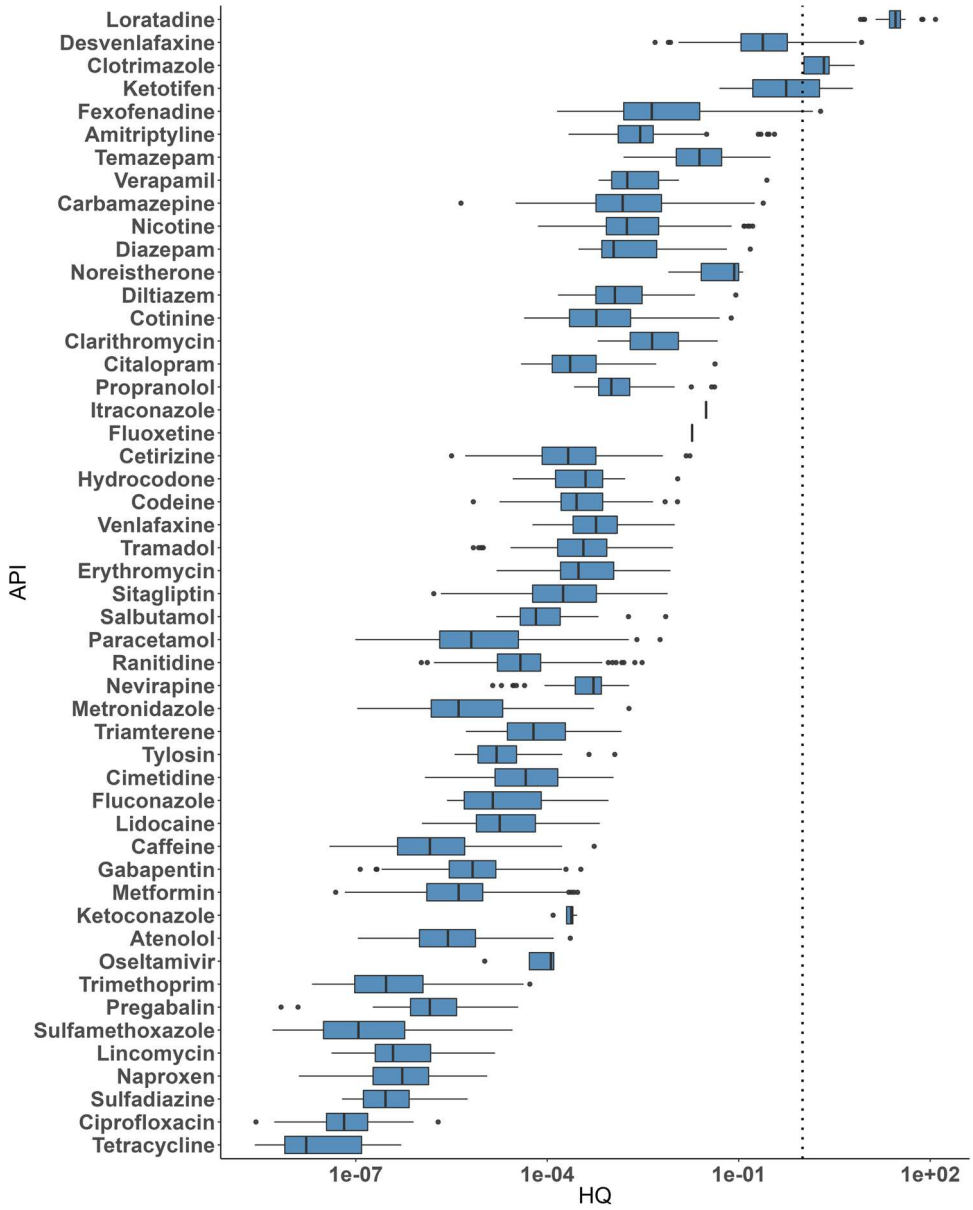

FIGURE 3: Box and whisker plot of hazard quotients (HQs) obtained for the 1052 different locations monitored in the Wilkinson et al. (2022) global study derived using critical environmental concentrations. Dotted line represents HQ = 1. Boxes show the mean and upper and lower quartile HQs, while whiskers represent the maximum and minimum HQ values. API = active pharmaceutical ingredient.

Twenty-one sampling locations were found to have mixture HQs ≥ 10, and these (Figure 4) were located in Africa (15 sites), Asia (three sites), South America (two sites), and Europe (one site). The locations with the highest mixture HQs were situated in Africa and were primarily associated with three sampling campaigns (Lagos in Nigeria, Nairobi in Kenya, and Bukavu in the Democratic Republic of the Congo) where garbage disposal, sewage discharge points, dumping of raw sewage by exhauster trucks, and pharmaceutical manufacturing activities were observed. The highest HQ for Asia was observed in Lahore, Pakistan. For South America, the site with the highest HQ was located in La Paz, Bolivia, where a dumping site for septic tank extractors was reported to be located. The highest HQ in Europe (Tubingen, Germany) was situated in a small stream downstream of a WWTP. Overall, the site with the highest mixture HQ (34.9) was in Nairobi, Kenya, where significant disposal of garbage on the riverbank was observed. This high HQ used the HQ of algae and was driven mainly by two antimicrobial compounds, sulfamethoxazole and erythromycin.

DISCUSSION

Although numerous previous studies have characterized the potential ecotoxicological effects of APIs in aquatic systems, these have either focused on characterizing the risks of a selection of APIs across a single country or on single APIs across multiple countries. They have often relied on published

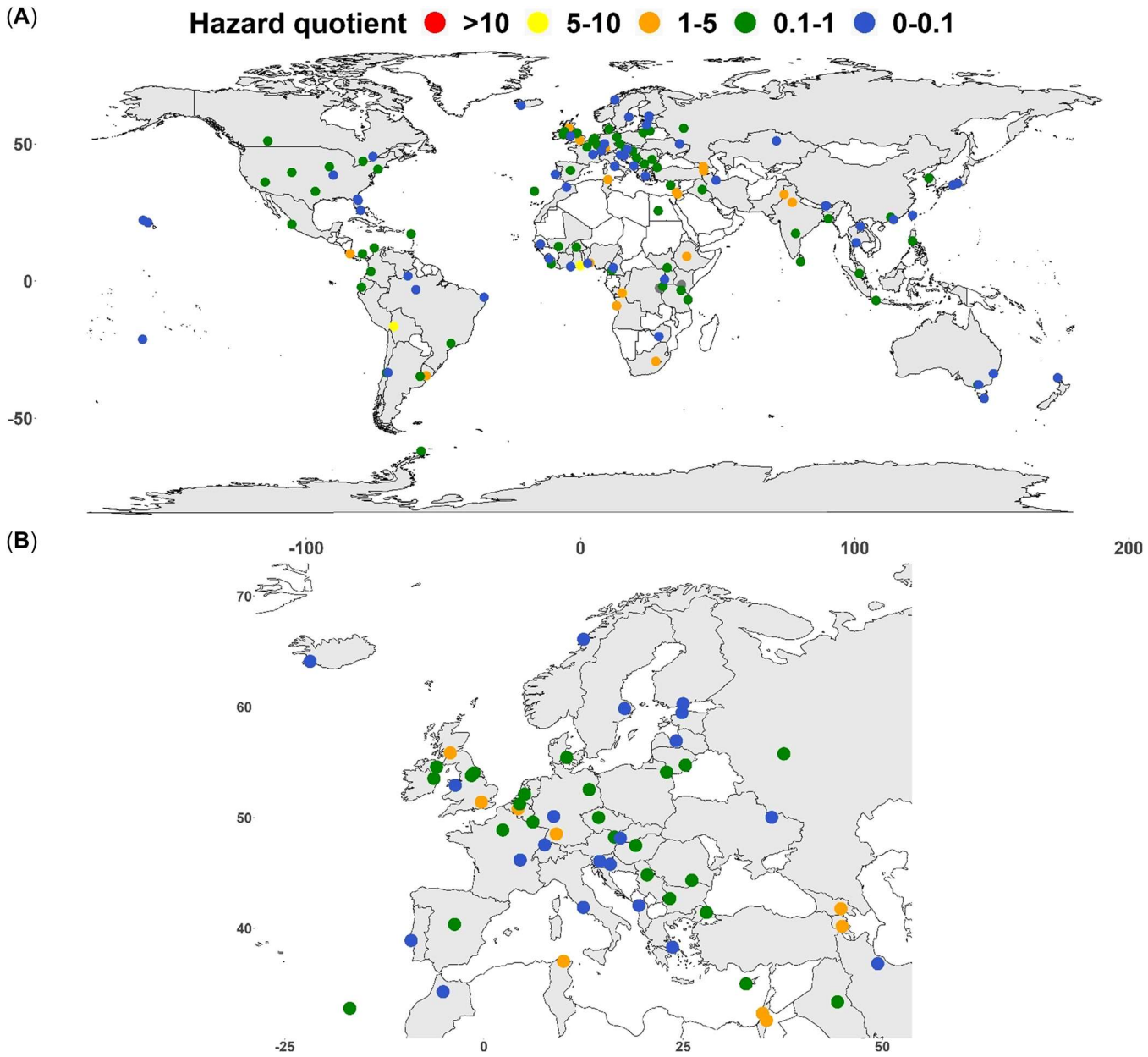

FIGURE 4: World (**A**) and European (**B**) map indicating the average mixture hazard quotients (HQs) for each sampling campaign in the Wilkinson et al. (2022) global monitoring project. Blue = HQ between 0 and 0.1, green = HQ between 0.1 and 1, orange = HQ between 1 and 5, yellow = HQ between 5 and 10, and red = HQ > 10. Campaigns in Iceland and Venezuela did not detect any compounds.

exposure data from studies employing different sampling regimes and analytical methodologies, making it challenging to interpret and compare the results. The lack of global API monitoring data means that for many regions of the world we have no idea of the level of potential impacts. Therefore, we used a unique data set on the concentrations of 61 high-use APIs in rivers from 104 countries to perform the first truly global holistic assessment of their potential ecotoxicological effects.

The availability of ecotoxicity data on APIs has increased significantly in recent years as a result of, for example, the pharmaceutical industry becoming more transparent and making data more readily available (see Vestel et al., 2016). Using a combination of industry data and data from the literature, it was possible to derive PNECs for 52 of the 53 APIs detected in the global monitoring study (Wilkinson et al., 2022). The reliability of these PNECs is likely to be highly variable because of differences in the types and number of data points available. Even for some of the most commonly detected substances (e.g., gabapentin, nicotine, lidocaine, and cotinine) in the Wilkinson et al. (2022) study, there is an absence of chronic data in the literature. This makes the resulting quantification of potential ecotoxicological effects uncertain. Moving forward, we should therefore be working to develop open-access, comprehensive, and robust data sets for APIs and other substances whose environmental occurrence we know is widespread.

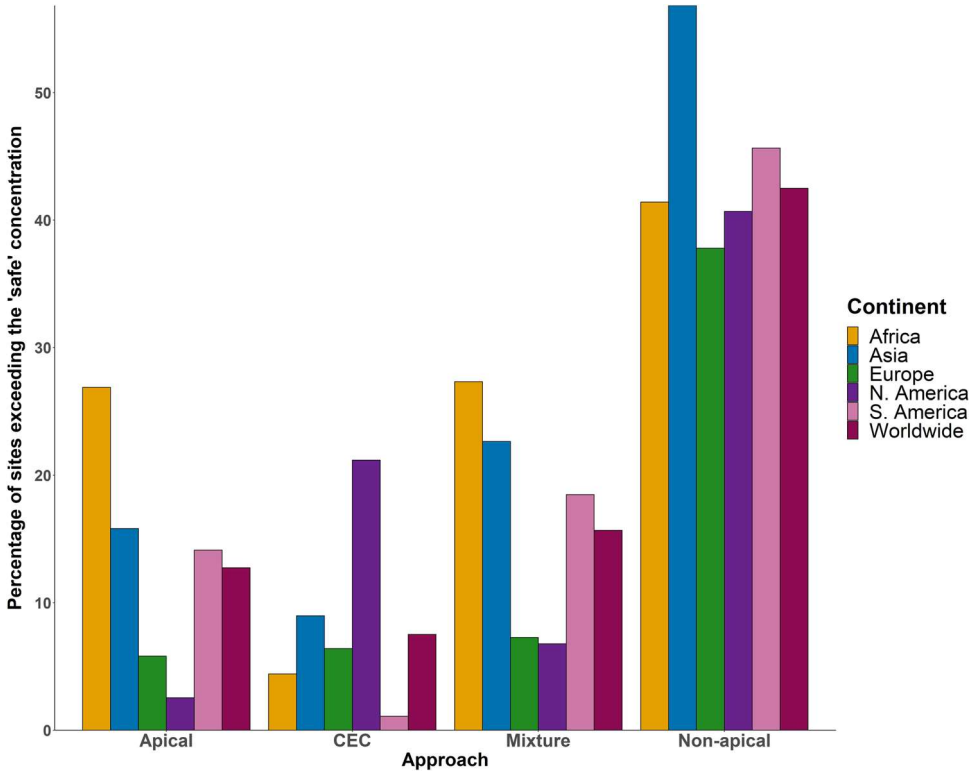

FIGURE 5: Percentage of sites in the Wilkinson et al. (2022) global monitoring study where concentrations exceed "safe" limits based on apical, critical environmental concentration, mixture, and nonapical ecotoxicological endpoints of the pharmaceuticals for at least one sampling site. Oceania and Antarctica had no sites exceeding the "safe" limits. CEC = critical environmental concentration.

When PNECs, apical data, nonapical data, and CECs were used for the single-compound assessments of potential ecotoxicological effects in rivers across the world, 43.8% of locations (i.e., 461 sites) were identified with concentrations of ecotoxicological concern (Figure 5). For apical effects, 12.7% of locations were found to have concentrations exceeding the PNEC (Figure 5). In their preliminary risk-assessment work, Wilkinson et al. (2022) reported that one in four sites had API concentrations above apical PNECs. The discrepancy between the present study and the Wilkinson et al. research (Wilkinson et al., 2022) is due to the large uncertainty factors used for sulfamethoxazole and propranolol in the PNECs which were available in the published literature at the time of their assessment. This further strengthens our call to work toward large, open-access and robust data sets for API toxicity data.

When the nonapical effects were taken into consideration, 42.5% (447 locations) of the sampling locations were found to have concentrations greater than LOECs for effects such as behavior and physiological processes (Figure 5). Nonapical ecotoxicological data were found for only 28 APIs, suggesting that if data were available for all 53 detected APIs, the number of locations with API concentrations of concern would be greater still, although the ecological relevance of many of these nonapical endpoints is still uncertain (Boxall et al., 2012). Only 7.5% (79 sites) of sites had concentrations of one or more API above their CECs.

Twenty-three APIs had concentrations for at least one sampling location above concentrations where an effect on organisms might be expected (Supporting Information, Figure S3). Ten of the 23 APIs identified, including molecules used to treat depression, bacterial infections, epilepsy, and anxiety, as well as hormone treatments and stimulants, were found to be at concentrations of ecotoxicological concern based on apical endpoints. For the nonapical assessments, 11 APIs, including hormonal treatments and treatments for pain, insomnia, diabetes, and depression, were identified as of concern. The APIs identified where concentrations exceeded the CEC included substances used to treat depression, asthma, allergies, and fungal infections. Only three APIs (amitriptyline, caffeine, and sulfamethoxazole) were identified as posing ecotoxicological concern based on more than one endpoint type (i.e., exceeding "safe" values derived from both apical and nonapical endpoints). This shows the value in applying a range of endpoints in studies of this type. Although the apical endpoint–based assessments provide an indication of risks to the traditional environmental protection goals used in ecotoxicological risk assessment (i.e., death, growth, and reproduction), the use of nonapical data and pharmacological data will likely identify the potential for other effects, such as impacts on behavior, histological changes, biochemical changes, or up-/down-regulation of genes. Although the ecological relevance of these effects is unclear (Boxall et al., 2012), many argue that they should be considered in the regulatory assessment process for chemical impacts within the environment (see Ford et al., 2021). The mismatch between the APIs identified as of concern using the apical and nonapical data

and those identified as of concern based on the use of CECs is intriguing, with many more compounds being found of concern based on the apical and nonapical data. The use of pharmacological data, such as those used in the derivation of CECs, has been suggested as an early-warning approach to identify substances of concern in the environment. Our findings indicate that predictions using current approaches employing pharmacological data to predict environmental impacts may in fact be inaccurate and misleading.

Several of the APIs for which environmental concentrations were greater than concentrations where ecotoxicological effects are expected have previously been highlighted as molecules of concern in surface waters in various parts of the world. For example, concentrations of antibiotics in surface waters have been shown to exceed PNEC values in European and Chinese surface waters (Guo et al., 2016; X. Liu et al., 2018; Wang et al., 2017; Yao et al., 2017). The anticonvulsant carbamazepine has been reported to occur at concentrations of concern for acute and chronic effects in fish, *Daphnia*, and algae in Africa (Bagnis et al., 2020), China (N. Liu et al., 2020), and Israel (Topaz et al., 2020). Stimulants have been highlighted as a group of concern in terms of aquatic impacts in Poland (Styszko et al., 2021), Israel (Topaz et al., 2020), China (N. Liu et al., 2020), and Italy (Riva et al., 2019).

The use of a single compound–based approach to estimate the impacts of pharmaceuticals could underestimate real impacts because the natural environment will be exposed to mixtures of substances. In the Wilkinson et al. (2022) study, for example, up to 34 different APIs were detected in single river water samples. To address this, apical data were used in a concentration addition model to estimate the potential effects of a mixture of APIs at each of the monitoring locations. The concentration addition model used in the present study assumes that the APIs in a mixture are acting in an additive manner and on the same endpoint. While there is a possibility that pharmaceutical mixtures could interact antagonistically or synergistically (Backhaus & Faust, 2012), several studies (see Backhaus et al., 2000; Bain & Kumar, 2014; Cleuvers, 2003; Godoy et al., 2019; Topaz et al., 2020) have demonstrated the applicability of the model for the characterization of mixture impacts in the environment. When the mixture HQs for each sampling site were considered, the proportion of sites exceeding the PNEC increased from 12.7% obtained from the single-compound apical approach to 15.7%, and the maximum HQ increased from 28.3 to 34.9. For the locations with the highest mixture HQs, the predicted effect was primarily driven by three antibiotics (sulfamethoxazole, erythromycin, and clarithromycin).

Large differences were seen in the proportion of sites where concentrations exceeded the "safe" levels for the different continents. Africa had the highest proportion of sites (27.3%) with mixture HQs ≥ 1, whereas North America had the lowest proportion of sites (6.8%). As noted in Wilkinson et al. (2022), these continental differences are likely explained by differences in the affordability and ease of access to certain medicines in regions with fewer regulations, differences in wastewater-treatment and waste-management infrastructure, and the presence of pharmaceutical manufacturing. Based on the information provided by sampling teams in the global monitoring study (Wilkinson et al., 2022), of the 165 sites where an HQ ≥ 1 was observed, 15.8% were associated with WWTP inputs, whereas 15.2% were reported to have sewage or wastewater present. Only 10.1% and 2.8% out of the 887 sites where all HQs were <1 were described by sampling teams as being associated with a WWTP and sewage and/or wastewater, respectively (Supporting Information, Table S5).

As well as acting in combination to affect a single organism type, the mixtures will also be acting on different components of the food web and affecting multiple endpoints. To demonstrate the different impacts that might be expected at a particular location, we pooled the mixture HQ results with the HQ results for the different nonapical and mode of action–related effects for the top 10 "riskiest" sites. The results of this analysis are shown in Figure 6 and indicate that at these contaminated sites unacceptable impacts on algal growth, *Daphnia* reproduction, and the biochemistry and physiology of fish are expected. It is likely that these multi–trophic level effects will exacerbate impacts on the ecological communities in these systems. Moving forward, we should be working not only to improve the characterization of effects of mixtures on single organisms but also to explore mixture effects on multiple species and multiple endpoints. Only then will we be able to understand the impacts of these real-world exposures on global aquatic ecosystems.

While the present study provides a major step forward in our understanding of the potential global ecotoxicological impacts of pharmaceuticals, there is still much to do. Given that over 1900 APIs are currently in use to treat and prevent human diseases (Burns et al., 2018), the ecotoxicological effects characterization of the 61 APIs in the present study may well be underestimating the actual impacts on aquatic systems around the world. This is particularly so given that the suite of compounds monitored in the global study (Wilkinson et al., 2022), for analytical reasons, omitted some APIs of environmental concern such as the synthetic estrogen ethinylestradiol and the nonsteroidal anti-inflammatory compound diclofenac. The rivers that were monitored will contain not only APIs but also other pollutants such as industrial chemicals, pesticides, and metals. While the global monitoring study (Wilkinson

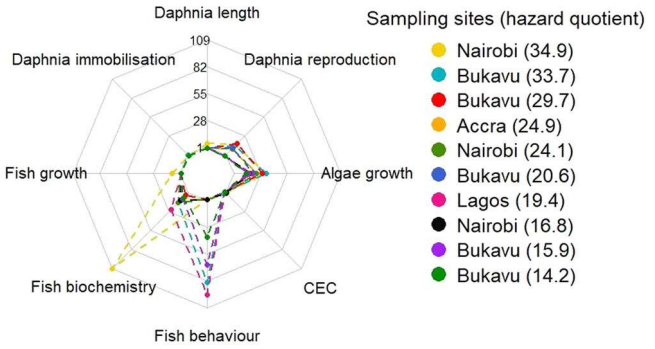

FIGURE 6: Spider diagram illustrating the potential hazard of pharmaceuticals to different endpoints identified for the top 10 sampling locations of the highest mixture hazard quotients.

et al., 2022) is probably the largest initiative of its kind to date, it still only provides a snapshot understanding of levels of exposure in a subset of the world's rivers. In the future, we should build on the Wilkinson et al. (2022) work to extend environmental monitoring to a wider range of locations, sampling time points, and contaminant classes to gain a fuller picture of the impacts of the global pollutome on the health of rivers across the world.

CONCLUSION

Riverine systems are constantly exposed to APIs introduced into the aquatic environment through their production, use, and disposal. Currently, ecotoxicological risk assessments have mainly been carried out using a single-compound approach based on apical effects. We, for the first time, present a global assessment of the potential ecotoxicological impacts of APIs on aquatic ecosystems. We demonstrate that approximately 43.5% of river locations globally have concentrations where ecotoxicological effects might be expected, with some locations expected to suffer effects on multiple trophic levels and on multiple endpoints. If we are to fulfill the United Nations' 17 Sustainable Development Goals, particularly Goal 6, "Clean Water and Sanitation" (United Nations, 2015), we urgently need to tackle the global problem of pharmaceutical pollution.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5355.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (abm538@york.ac.uk).

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