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Widespread monitoring of chiral pharmaceuticals in urban rivers reveals stereospecific occurrence and transformation

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ABSTRACT

The present work aimed to discuss the enantiomeric occurrence of chiral pharmaceuticals including 5 parent compounds (PCs) metoprolol, propranolol, atenolol, venlafaxine and fluoxetine as well as 6 of their transformation products (TPs) in surface water in Beijing. Among which, 9 out of 11 were detected during the two sampling campaigns with *N*-*O*-Didesmethylvenlafaxine (NODDV) and α-hydroxymetoprolol confirmed in the catchment for the first time. Metoprolol acid (MTPA) was the most abundant up to 1508 ng L^{-1}

enantioselective environmental analysis of pharmaceuticals is an emerging area of great importance, nevertheless, the concern about the stereochemistry on its environmental fate is more recent with much gaps in knowledge.

Wastewater treatment plants (WWTPs) were often reported for poor removal rates of pharmaceuticals (Patel et al., 2019). What's more, it is important to bear in mind that the breakdown or removal of the parent compounds during wastewater treatments does not necessarily mean the removal of toxicity, because a great number of transformation products could be formed at high yields, while many of them are more stable in environment and exert higher toxicity to non-target organisms than their parent compounds (Huntscha et al., 2013). Some research groups have made significant contribution to monitor various PhACs in the aquatic environment such as analgesic (Caballo et al., 2015), β-blockers and antidepressants (Carpenter and Helbling, 2018; Miller et al., 2019), and illicit drugs (Archer et al., 2017). But still, only limited data is available regarding the role of chirality during the transformation of PhACs at any stage in the environment. In order to better understand the environmental fate of these chemicals of concern, the transformation products should also be simultaneously taken into account in environmental monitoring.

China has become one of the biggest pharmaceuticals producer and consumer in the world (Bu et al., 2013). The pharmaceutical production doubled from 2003 to 2011(Liu and Wong, 2013; Rehman et al., 2015). On the other hand, this rapid growth is also leading to an elevated risk of environmental contamination. Until now, the available research works undertaken regarding the stereo-occurrence of PhACs in freshwater, such as the lakes and rivers in Switzerland (Buser et al., 1999), Guadalquivir River basin in Spain (López-Serna et al., 2013), River Avon in England (Bagnall et al., 2012), Douro River estuary in Portugal (Coelho et al., 2019) as well as Arc River in southern France (Li et al., 2013), were mainly concentrated on Europe and US, while the scenario in developing countries is far less documented. Given this, special attention on the pharmaceuticals contamination in different areas, especially in China is required to be paid.

Beijing is one of the world's most densely populated cities with huge pharmaceutical consumption and large quantities of domestic sewage produced (about 3.3 million tons per day). Besides, the wastewater treatment capacity as well as municipal drainage infrastructure is not quite sufficient. As a consequence, the urban surface waters are expected to suffer considerable contamination contributed from non-point sources such as landfills, livestock breeding and unregulated discharge of domestic wastewater (Liu et al., 2017; Sui et al., 2011). As the capital of China, Beijing provides an ideal place of economic developed regions for investigating the characteristics of PhACs pollution in highly urbanized areas at large scale. In previous studies, our research group ascertained the significant presence of dozens of PhACs in aquatic environment in Beijing (Duan et al., 2018; Ma et al., 2019; Ma et al., 2017). But until now, no studies have reported the occurrence of chiral pharmaceuticals and transformation products at enantiomeric level in surface waters in China.

Beiyun river basin (including the main stream and its tributaries) was selected as a representative hotspot since it receives over 80% of effluents from WWTPs in urban area of Beijing, and about 90% of the river flow is considered WWTP effluent (Ma et al., 2017; Zhang et al., 2016). In present study, 11 chiral PhACs, including 5 drugs belonging to two therapeutic categories (β-blockers and antidepressants) and 6 of the chiral transformation products were simultaneously determined at enantiomeric level along the main stream and several tributaries during wet season and dry season. The objectives of the research are (1) to investigate the occurrence and spatiotemporal variation in this catchment, (2) to elucidate the relationships between the concentrations and enantiomeric characteristics, and (3) further evaluate the potential risks to aquatic systems. To our best knowledge, this is the first to demonstrate enantioselective occurrence of chiral pharmaceuticals and metabolites in surface waters in China. The combined profiling approach would yield unique insights and draw outlines of the stereoselectivity of PhACs in environment. This study also aims to provide a new perspective for future studies on predicting the fate of chiral micropollutants in environment and explore to what extent the presence of enantioenrichment can be explained by the attenuation of their parent compounds.

2. Materials and methods

2.1. Chemicals and reagents

Target PhACs was selected based on their consumption pattern, ubiquitous occurrence and possibility of chiral biotransformation. The chemical structures and physicochemical properties of the studied compounds were shown in Table S1 in Supporting information. The analytical racemate standards of atenolol (ATN), metoprolol tartrate (MTP), propranolol hydrochloride (PHO), fluoxetine hydrochloride (FLX) and venlafaxine (VFX) as well as six major chiral transformation products, including metoprolol acid (MTPA), α-hydroxymetoprolol (a mixture of diastereomers and enantiomers, α-HMTP), 4-hydroxypropranolol (4-OH-PHO), norfluoxetine (NFLX), *O*-desmethylvenlafaxine (ODV), and *N,O*-didesmethylvenlafaxine (NODDV) were obtained from Sigma-Aldrich (Steinheim, Germany). Enantiopure standards of (S)-norfluoxetine ((S)-NFLX) was purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany).

Surrogatete/internal standards of isotopically labelled (±)-atenolol-d7, (±)-metoprolol-d7, (±)-propranolol-d7, (±)-fluoxetine-d5 and (±)-venlafaxine-d6 were obtained from Toronto Research Chemicals Inc (Toronto, Canada). SPE cartridges of Oasis HLB (200 mg/6 mL) were procured from Waters Corporation (Milford, MA, USA). HPLC-grade methanol, LC-MS grade formic acid and ammonium acetate were purchased Fluka (Buchs, Switzerland). Ultrapure water of 18.2 MΩ cm^{-1} was prepared by the Milli-Q purification system (Millipore, Milford, MA, USA).

Individual stock solutions of racemate standards and internal standards (IS) were dissolved in methanol (1 mg mL^{-1}) stored at −18 °C. Working standard solutions (1 mg L^{-1}) for calibration curve containing mixture of the 11 chiral compounds of 2–500 μg L^{-1} (1–250 μg L^{-1} for each enantiomer) were prepared freshly by serial dilution and stored at 4 °C in the dark.

2.2. The catchment sampling campaign

The samples were taken along the Beiyun rivers with 90% of the river flow composed by WWTPs effluents in urban area of Beijing, covering approximately 15 million inhabitants. Considerable load of micropollutants was expected due to the significant influence of WWTP effluents and other potential non-point source pollution in this area.

A total of 68 surface water samples were collected by grab sampling at 0.5 m under the surface of rivers in July (wet season) and November (dry season) 2016 along its main stream and six small- to medium-sized tributaries. Information on sampling sites were described in Supporting information S1 and Tables S2 and S3. The two sampling campaigns were conducted in wet and dry seasons in order to understand the impact that precipitation might have on pollution load, as well as enantiomer-specific fate of drugs resulting from different microbial activity in hot and cold weather. Grab sampling was chosen as opposed to composite sampling due to the possibility of enantiomer-specific degradation of drugs occurring during 24 h composite sampling time. All samples were stored in 2.5 L amber, trace clean glass bottles with 0.02% (w/v) NaN_3 solution added to prevent microbial degradation, then transported refrigerated at 4 °C to the laboratory and pretreated within 48 h.

2.3. Sample preparation and instrumental analysis

After filtration through glass fiber filters (GF/F. 47 mm, Whatman, Kent, UK), a 500 mL portion of sample was adjusted to pH 4 by 1 mmol/L HCl and 1 mmol/L NaOH solution, and spiked with mixed internal standard (50 ng L^{-1} of each compound) before SPE. Detailed pretreatment procedures were described in Supporting information S2.

Chiral determination was performed on an ultra-high performance liquid chromatography (Ultimate3000 HPLC system, Dionex, USA) coupled to tandem mass spectrometry (ESI-MS/MS, API4000, ABSciex, USA) operated in positive ionization mode. Enantioseparations were carried out on a Chirobiotic V column by SUPELCO Analytical (vancomycin, 250 × 4.6 mm, I.D. 5 μm, Sigma-Aldrich, Steinhein, Germany) with a mobile phase containing 10 mM NH_4OAc buffer (pH = 4, adjusted by formic acid) in H_2O: MeOH (10: 90) under isocratic flow rate of 0.6 mL/min. The injection volume was 10 μL, the column temperature was 20 °C. Acquisition was performed in a multiple reaction monitoring (MRM) mode. Tuning and optimization of the MS/MS parameters (declustering potential (DP), collision energy (CE), entrance potential (EP), collision cell exit potential (CXP)) were confirmed by direct infusion of standards in solvent. The Optimized MRM condition for each analyte was given in Table S4 (Supporting Information).

2.4. Quality assurance and quality control

During sequence analysis, procedural blanks (pure methanol) were injected every five samples to wash the column, in case of sample contamination. Matrix spiked and sample duplicates were injected randomly throughout sample batches to monitor instrumental performance. The IS method was used for quantification. Concentrations above the method detection limit (MDL) but below the method quantification limit (MQL) were assigned a value of half the MQL, and those at or below the MDL were assigned a value of zero. The method performance were described in Supporting Information S3 and full validation presented in Table S4 (Supporting Information).

2.5. Calculations

2.5.1. Enantiomeric fraction (EF)

Enantiomeric fraction (EF) was calculated using the following equation

$$\mathrm{EF} = \frac{E1}{E1 + E2} \text{ or } \frac{\mathrm{E}(S)}{\mathrm{E}(S) + \mathrm{E}(R)} \quad (1)$$

where E1 and E2 are concentrations of the first and the second eluting enantiomer, respectively, and E(*R*) and E(*S*) are R-and S-enantiomers respectively if the elution order is known. The EF values can range from 0 to 1, with EF = 0.5 representing the racemate.

Elution order was determined by the injection of enantiopure standards or according to previous researches. For beta-blockers, atenolol, metoprolol and propranolol, elution order of E1 and E2 enantiomers were identified as S-(−)- and R-(+)-enantiomers, respectively using the same type of chiral column. For antidepressents venlafaxine and fluoxetine with their metabolites *O*-desmethylvenlafaxine (ODV), N,O-didesmethylvenlafaxine (NODDV) and norfl

Table 1
Concentrations (ng L^{-1}), detection frequency (DF, %) and EFs of target substances in two sampling campaigns.

	Wet season (July)						Dry season (November)					
	Median	Mean	25th percentile	75th percentile	DF	EF values (mean ± SD)	Median	Mean	25th percentile	75th percentile	DF	EF values (mean ± SD)
ATN	1.0	1.4 [0.9, 1.9][a]	0.7	1.7	76.5 (26)[b]	0.57 ± 0.11	6.7	6.2 [4.7, 7.6]	4.4	8.1	58.8 (20)	0.48 ± 0.03
MTPA	434.7	414.1 [358.0, 475.0]	310.4	527.5	100	0.49 ± 0.02	375.9	589.9 [416.9, 762.8]	131.5	999.1	100	0.49 ± 0.01
MTP	275.6	270.9 [220.5,317.6]	160.6	324.8	100	0.45 ± 0.03	269.2	290.5 [243.9, 343.0]	194.9	375.9	100	0.42 ± 0.09
α-HMTP	31.2	34.9 [28.3,41.5]	19.5	47.6	100	/	9.7	32.4 [19.9, 44.9]	5.4	40.0	100	/
PHO	1.1	1.5 [1.0, 2.0]	0.4	2.2	52.9 (18)	0.52 ± 0.13	3.3	3.5 [3.0, 3.9]	2.5	4.5	91.2 (31)	0.36 ± 0.03
4-OH-PHO	ND	ND	ND	ND	ND	/	ND	ND	ND	ND	ND	/
VFX	25.4	23.9 [20.0, 27.6]	12.5	30.9	100	0.54 ± 0.05	22.7	21.9 [18.2, 25.6]	16.1	26.7	100	0.55 ± 0.03
ODV	63.4	56.8 [45.1, 68.2]	32.5	79.2	88.2 (30)	0.48 ± 0.07	42.9	62.8 [46.6, 79.0]	21.5	96.2	100	0.48 ± 0.07
NODDV	11.6	10.0 [8.1, 11.8]	5.9	13.7	82.4 (28)	0.41 ± 0.05	13.7	13.0 [10.1, 15.8]	5.2	20.1	94.1 (32)	0.40 ± 0.09
FLX	0.9	2.0 [0.5, 3.5]	0.7	1.2	52.9 (18)	0.51 ± 0.11	ND	ND	ND	ND	ND	/
NFLX	ND	ND	ND	ND	ND	/	ND	ND	ND	ND	ND	/
ΣPhACs	834.1	807.1 [700.0, 914.0]	557.2	991.1	/	/	750.9	1019.4 [775.4, 1263.3]	371.6	1580.0	/	/

(ND, not detected; ATN, atenolol; MTPA, metoprolol acid; MTP, metoprolol; α-HMTP, α-hydroxymetoprolol; PHO, propranolol; 4-OH-PHO, 4-hydroxypropranolol; VFX, venlafaxine; ODV, O-desmethylvenlafaxine; NODDV, N-O-Didesmethylvenlafaxine; FLX, fluoxetine; NFLX, norfluoxetine).

[a] Brackets indicates the statistical uncertainties: 95% confidence intervals.

[b] Parentheses indicate the number of samples detected above MDL

higher in dry season. In comparison, metoprolol was the dominant β-blocker with average/median concentrations reached hundreds of ng L^{-1} (49.0–680.1 ng L^{-1}), about two orders of magnitude higher than atenolol or propranolol. This was much higher than previous reports (Bagnall et al., 2012; López-Serna et al., 2012; Petrović et al., 2014), but agreed well with those measured nationwide (Yao et al., 2018) as well as observations at WWTPs in this area (Duan et al., 2018), probably due to the fact that the studied rivers had been receiving a large proportion of effluents from WWTPs alongside. The level of atenolol was similar to that in the Dal river, Sweden (3.5–8.5 ng L^{-1}) (Lindim et al., 2016) and below that observed in Spain (nd.-179.0 ng L^{-1}) (López-Serna et al., 2013). The different traits of atenolol and metoprolol were probably ascribed to their differences in consumption rates in China, and also related to their stability, as atenolol usually has a short half-life (< 10 days) in a natural setting (Xu et al., 2019). α-HMTP, as one of the main TPs of metoprolol, was found to be omnipresent in both sampling campaigns and peaked in dry season at a concentration of 123.8 ng L^{-1}, similar with the results in Oslofjord, Norway (Langford and Thomas, 2011). Another transformation product metoprolol acid (MTPA), also a common metabolite of both metoprolol and atenolol (Godbillon and Duval, 1984), was the most abundant with the maximum concentration up to 1.5 mg L^{-1}, which was very likely resulted from metoprolol considering the consumption pattern in China (Xu et al., 2019). The median concentration of atenolol in dry season (6.7 ng L^{-1}) was about 8-folds higher than that in summer, whereas α-HMTP was outstanding in summer (31.2 ng L^{-1}). Analogously, higher median concentrations in wet season were also observed for MTPA, venlafaxine and ODV, implying little dilute effects in flood period was exerted on their occurrence.

Venlafaxine was another persistent substance. Its concentration varied from a few to 46.4 ng L^{-1}, the peak value was lower than that in River Ruhr (180 ng L^{-1}), Germany (Schlüsener et al., 2015), but comparable with the determinations in St. Lawrence River, Canada (Lajeunesse et al., 2008). For the two TPs of venlafaxine, the less frequently detected *N,O*-Didesmethylvenlafaxine (NODDV), which was investigated the first time in domestic aquatic environment, presented the lowest median concentration of the three. This was in accordance with the findings for Arc River in France (Li et al., 2013), whilst the median concentrations of the active metabolite, *O*-desmethylvenlafaxine (ODV), were 1.9–2.5 folds higher than its parent compound during both sampling campaigns, probably due to its limited removal rate during wastewater treatment previously reported in Germany and UK (Schlüsener et al., 2015; Evans et al., 2017). It is believed the risk of cardiovascular diseases could be higher in cold season, thus, higher occurrence of β-blockers would be expected, however, in our study, although some of the substances showed statistical differences between two sampling campaigns, the median/average concentrations did not vary much, especially for metoprolol and venlafaxine, which may imply the reason for season-associated consumption rates was not predominant.

3.2. Spatial distribution

Variations were observed among rivers (Fig. 2). The total concentrations in main stream ranged from 445.2 to 1480.1 ng L^{-1} in wet season, and from 212.7 to 1860.8 ng L^{-1} in dry season. The spatial variation was more significant than seasonal variation. For the average total concentrations, Liangshui river showed the highest loads (1090.3 ng L^{-1} in wet season and 1787.2 ng L^{-1} in dry season), followed by Qinghe river (811.1 ng L^{-1} in wet season and 1458.5 ng L^{-1} in dry season), whereas Tonghui river (654.9 ng L^{-1} in wet season and 597.6 ng L^{-1} in dry season) and Bahe river (460.7 ng L^{-1} in wet season and 295.6 ng L^{-1} in dry season) were much lower. This was on one hand related to the quantities of inhabitants in the watershed area (see Table S2 in SI). For another, the lower burden of Bahe river could be attributed to the relatively higher removal efficiency of WWTP located

upstream, which was applying a treatment process of anaerobic/anoxic/oxic-membrane process (A/A/O-MBR) followed by ozone and sodium hypochlorite disinfection and showing good performance for MTP elimination (average removal rate of 98%) (Zhang et al., 2018). In contrast, six out of 9 quantifiable compounds (metoprolol, MTPA, α-HMTP, VFX, ODV and NODDV) showed its respective peak concentration in Liangshui river. This could be explained by its composition of large proportion of WWTP effluent (up to 90% under dry conditions) and the fact that these sites are in urban area where the population is large. A large WWTP (daily flow of 600,000 m^3/d, corresponding to the WWTP8 in our previous study (Duan et al., 2018) is located at upstream near site L1, but employed conventional activated sludge treatment process, leading to a considerable level of pharmaceuticals residue along the river. When comparing with other sites, the total concentrations sharply increased at sites W6 (in wet season) and W7 (in dry season), showing the impacts of effluent discharge as there are a big WWTP and the Airport nearby. Generally, concentrations found in downstream river water were higher than those in upstream river water, but the trend was less obvious in wet season. In dry season, concentrations in upstream (sites: W1-W4) were at lower level compared with those at sites downstream (W9-W13), while in wet season, the loads at W1-W4 got significantly elevated. Similar phenomenon was observed for Tonghui river and Bahe river. One explanation is that during the flood season, the massive rainfall could increase the flow thus reduce the removal efficiency as a consequence. Another contributing factor might be due to the insufficient sewage collection system leading to a WWTP overloading.

For individual substances, the concentrations of venlafaxine, metoprolol, MTPA and NODDV were significantly different among tributaries. In contrast, no significant spatial variation ($p \geq 0.05$) was observed for ODV and α-HMTP in the two sampling periods. Higher concentrations of TPs measured downstream and near WWTP outfalls revealed they were to a large extent emitted from the WWTPs, and could be transported substantial distances in surface water without significant natural attenuation. Heavy loads at sites of densely populated and industrialized areas were also supported by the concentration ratios of TPs to their corresponding parent compounds, which will be discussed below, and further indicated the impacts of human activities on PhACs contamination were at different levels and region specific.

3.3. Composition pattern and contribution of TPs

total residues, in consequence, TPs of this therapeutic class, that is α-HMTP and MTPA, made up the vast majority of the sum of all four TPs (59–95%). The compositions were quite different between two sampling periods and among each tributaries. As seen in Fig. 3, the proportions of β-blockers TPs (α-HMTP + MTPA) varying from 32.2 to 71.3%, was predominant in most cases except for Tonghui River, which might be attributed to its relatively lower burdens than other rivers. This was also reflected in the lower ratios of TPs *vs.* parent compounds (eg. ODV/VFX, NODDV/ VFX, and α-HMTP/MTP) as well as in Bahe river (see Table S7).

In humans, 90% of the total venlafaxine elimination is resulted from *O*-desmethylation through cytochrome P450 enzymes leading to the active metabolite *O*-desmethylvenlafaxine (ODV), and then further was metabolized to *N,O*-didesmethylvenlafaxine (NODDV) (Eap et al., 2003). The excretion of venlafaxine and its metabolites is primarily by the renal route, with about 5% and 29% appearing as unchanged form for VFX and ODV, respectively (Howell et al., 1993). Owing to the relatively lower consumption rates compared with β-blockers in China, the total antidepressants (VFX, ODV and NODDV) made minor contribution to the overall residues (10% on average). The TPs of venlafaxine displayed a significant correlation with the total antidepressants load during both sampling campaigns, with higher R^2 (0.982) in cold season. ODV was found surpassed the concentration of its parent compound VFX in almost all samples. The concentration ratios of ODV/VFX (2.7 ± 1.3) and NODDV/VFX (0.57 ± 0.24) were equal to those detected in Ruhr River (Schlüsener et al., 2015). Besides, although the total antidepressants load showed significant difference (p = 0.029) between two samplings, probably ascribed to the elevated flow rates leading to lower concentrations in wet season, the proportion of TPs (ODV + NODDV) did not vary significantly (average of 7.5% in wet season and 7.3% in dry season, respectively), suggesting the concentration ratios of the metabolites/venlafaxine could be potential indicators for biodegradation assessment.

The presence of metabolites in the environment is not surprising since a high percentage of the dose is excreted as metabolites for most of the pharmaceuticals. It could originate from the untreated domestic sewage input and/or from the input of microorganism metabolism during wastewater treatments and the environmental transformation. In present study, the concentrations of the antidepressants are directly linked to the proportion of treated wastewater. The varying compositions may lay in several aspects. On one hand, the theoretical percentages of excretions can vary for individuals since the metabolism may work differently in them. In addition, the consumption rate for particular therapeutic class of pharmaceuticals might be season-associated driven. What's more, the removal efficiency of WWTPs, and the transformations in the treatment process as well as in the environment do not affect all compounds to the same extent. The dramatic occurrence of TPs in receiving water indicated a change of priority pollutants, suggesting concentrations of metabolites/transformation products may exert significant impact on the estimation of pharmaceuticals burden and consumption based on sewage epidemiology. As reported in many cases, the removal rates of the metabolites were lower than those of their respective parent compound (Rúa-Gómez and Püttmann, 2012; Schlüsener et al., 2015), a higher ratio of metabolite *vs.* parent pharmaceutical in the effluents would be expected. To further elucidate whether biotransformation of parent compounds in WWTPs occurred, a more comprehensive survey covering WWTPs influents and effluents are required. All these showed the importance of monitoring transformation products of pharmaceuticals for subsequent environmental risk assessment.

3.4. Enantiospecific profiling of chiral drugs and their metabolites

The enantiomeric fraction were calculated for samples with concentrations of both enantiomers above the MQL. Chiral analysis of β-blockers and antidepressants have been applied in water samples using vancomycin-based chiral analytical columns (Kingbäck et al., 2010; Souchier et al., 2016). As α-HMTP has two chiral centers, four isomers were expected to be eluted. However, under the chromatographic condition, only two peaks of, what were most probably, the diastereoisomers were enabled to be separated with an R_s of 1.2 (Fig. S2), hence it was not presented in Fig. 4. Nevertheless, this is, to the authors' knowledge, the first time that simultaneously stereospecific monitoring of chiral drugs and their major TPs was conducted in environmental waters nationwide.

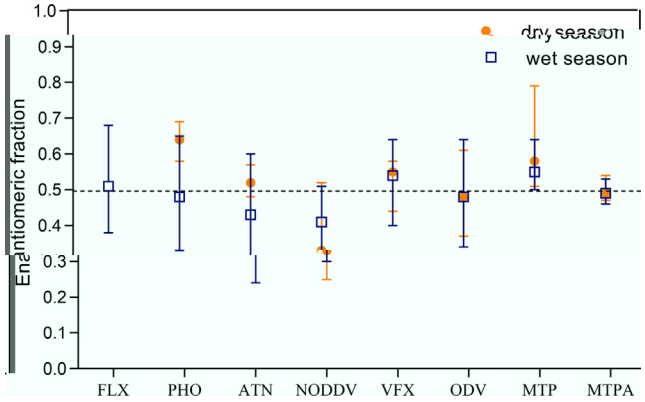

Fig. 4. The enantiomeric fractions (EFs) of detected pharmaceuticals in both seasons. Symbols denote the mean value, and bars denote the variation ranges. (ATN, atenolol; MTPA, metoprolol acid; MTP, metoprolol; PHO, propranolol; VFX, venlafaxine; FLX, fluoxetine; ODV, O-desmethylvenlafaxine; NODDV, N-O-Didesmethylvenlafaxine).

The variation of enantiomeric fraction did not show significant seasonal difference (p > 0.05), of which, ATN, PHO and FLX were to a large extent due to their lower detected level and frequency. The EFs of MTP were between 0.79 and 0.50 with the (S)-form enriched, while MTPA was composed of almost equal amounts of enantiomers (0.49 ± 0.02). Similar findings of enrichment of (S)-MTP were also observed in surface water in UK (Evans et al., 2017). For VFX, the EFs of 0.54 ± 0.06 in wet season and 0.55 ± 0.03 in dry season were not significantly altered within or among rivers, including at the sites near WWTP outfalls (p > 0.05). in contrast, ODV existed close to a racemate with EFs slightly lower than 0.5 (0.48 on average) during the two sampling periods, which was also in agreement with our previous study for wastewater samples (Duan et al., 2018). NODDV was the compound found mainly enriched with its (R)-form, giving an EF < 0.5.

To further elucidate how EF was changing during transformation, Fig. 5 exhibits the relationship between parent compound and its metabolite for the (R)- and (S)-enantiomer, respectively. Linear regression with R^2 > 0.6 suggested a major common mechanism of metabolites formation was involved. Take VFX as an example, stereoselective metabolism of VFX into ODV was assumed for the biotransformation of (R)-VFX into (R)-ODV and (S)-VFX into (S)-ODV (Li et al., 2013). The transformation rate could be obtained by the slope of the linear regression. To further confirm whether the two enantiomers biotransformed with different kinetic rates, ANCOVA was performed on data displayed in Fig. 5 to test the difference of slopes from 1.0. The results showed that the slopes of linear regression for ODV/VFX in wet season was significantly different from 1.0 (p = 0.04), suggesting the two enantiomers might biotransform through specific minor alternative pathways that favor the (R)-enantiomer, as reported in human metabolism (Kingbäck et al., 2010).

Analogously, slightly increasing EFs of VFX showing a preferential attenuation of (R)-enantiomers during wastewater treatment was also observed in (Kasprzyk-Hordern and Baker, 2012) This may explain the seemingly decreasing EFs of ODV and NODDV in present study compared to their parent compound VFX, supposing the (R)-enantiomer is more readily biotransformed than the (S)-enantiomer. In addition, the

difference of correlation coefficients (R^2) between two sampling campaigns may to some extent indicate a complex environmental process or a post-release of raw sewage in the catchment, since very limited removal rates were found in WWTPs for ODV and NODDV (Schlüsener et al., 2015). Lab-scale simulation with VFX has indicated the extent of EF variations was proportional to the fractional conversion of the contaminant (Qu et al., 2019). Hence, the chiral signature of VFX could be regarded as a potential tool to estimate the degree of biodegradation, however, extending the findings from lab studies to field conditions is of challenge and warrants more verification.

3.5. Risk assessment and environmental implication

Toxicity data were collected from literature focusing on aquatic organisms at different trophic levels (Tables S8 and S9 in SI). Unfortunately, ecotoxicity information of TPs was not available for risk assessment. Regarding to the worst case scenarios, the environmental risks of the pharmaceuticals were estimated by Hazard quotient (HQ) using the maximum measured concentration of each compound and the calculated PNECs for each trophic level based on the most sensitive values of EC_{50} (Fig. 6). Due to the lack of enantiospecific toxicity data, HQs were calculated only with the sum of the two enantiomers. The risk was classified based on the criteria: no risk ($HQ < 0.01$), low risk ($0.01 \leq HQ < 0.1$), medium risk ($0.1 \leq HQ < 1$), and high risk ($HQ \geq 1$) (Hernando et al., 2006). As to atenolol, a PNEC value of 20.0 ng L^{-1} for fish was employed according to (Steinbach et al., 2014). The resulting HQ ranged from < 0.01 to 5.62. Atenolol posed a medium risk to fish, while metoprolol exhibited low to medium harm to aquatic organisms. The ecotoxicity of propranolol was noticeable, however, the HQ was on the edge of low level thanks to its relatively low concentration in the surface water. In contrast, despite the low detection level of fluoxetine, high risk was found due to its high toxicity on algae, similar to the situation in freshwater lake in China (Ma et al., 2016). Venlafaxine posed lower risks in present study, compared to medium or high risks found in (Fernández-Rubio et al., 2019; Ruan et al., 2019), where a much lower PNEC value derived from chronic test was employed.

Transformation products can retain the biological activity or may even possess more severe ecotoxic effects than the parent compound (Li et al., 2016). In spite of earlier efforts monitoring micropollutants in domestic waters (Mei et al., 2018; Yuan et al., 2013; Zhang et al., 2019), the data is far from sufficient for environment management. What's more, knowledge about enantiospecific responses of aquatic species to different pharmaceutical during long-term exposure has been barely understood. Considering their prevalence and resistance to attenuation in aquatic systems, these emerging contaminants could contribute substantially to the risks, therefore, more Yuanto-387.59(The)-264.6(ec

outfalls indicated the major source from WWTPs effluents. The detected TPs could be transported substantial distances in surface water without significant attenuation. Enantioselectivity was observed with an enrichment of S-metoprolol, S-venlafaxine and R-NODDV. For venlafaxine, a significant association between ratios of TP/PC for each enantiomer suggested the (R)-enantiomer is more readily biotransformed than the (S)-enantiomer. Therefore, EF variations could possibly be interpreted independently of the environmental conditions and the contaminant concentration. This study provided a new perspective towards profiling the enantiospecific environmental behavior of emerging contaminants in river catchment level. It was worthy of concern that chiral transformation products tend to be enriched with enantiomers of opposite configuration to their parent compounds, which might lead to unexpected environmental impacts.

CRediT authorship contribution statement

Ruixue Ma: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Han Qu:** Data curation, Formal analysis. **Bin Wang:** Funding acquisition, Project administration, Writing - review & editing. **Fang Wang:** Validation, Software. **Gang Yu:** Resources, Supervision.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105657.

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