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Chiral Pharmaceuticals: A Review on Their Environmental Occurrence and Fate Processes

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Abstract

More than 50% of pharmaceuticals in current use are chiral compounds. Enantiomers of the same pharmaceutical have identical physicochemical properties, but may exhibit differences in pharmacokinetics, pharmacodynamics and toxicity. The advancement in separation and detection methods has made it possible to analyze trace amounts of chiral compounds in environmental media. As a result, interest on chiral analysis and evaluation of stereoselectivity in environmental occurrence, phase distribution and degradation of chiral pharmaceuticals has grown substantially in recent years. Here we review recent studies on the analysis, occurrence, and fate of chiral pharmaceuticals in engineered and natural environments. Monitoring studies have shown ubiquitous presence of chiral pharmaceuticals in wastewater, surface waters, sediments, and sludge, particularly β -receptor antagonists, analgesics, antifungals, and antidepressants. Selective sorption and microbial degradation have been demonstrated to result in enrichment of one enantiomer over the other. The changes in enantiomer composition may also be caused by biologically catalyzed chiral inversion. However, accurate evaluation of chiral pharmaceuticals as trace environmental pollutants is often hampered by the lack of identification of the stereoconfiguration of enantiomers. Furthermore, a systematic approach including occurrence, fate and transport in various environmental matrices is needed to minimize uncertainties in risk assessment of chiral pharmaceuticals as emerging environmental contaminants.

Keywords

Chiral pharmaceuticals, stereoselective degradation, plant uptake, wastewater, chiral chromatography

1 Introduction

Pharmaceuticals are ubiquitous in aquatic environments impacted by wastewater effluent and are often detected at concentrations ranging from ng/L to $\mu\text{g/L}$ (Gómez et al., 2010; López-Serna et al., 2013; Pal et al., 2010). However, pharmaceuticals are pollutants of major concern because they are biologically active compounds that can potentially alter the physiology and behavior of a non-target organism at low doses (Arnold et al., 2013; Daughton and Ternes, 1999; Halling-Sørensen et al., 1998). For example, exposing Eastern mosquitofish to fluoxetine for 28 days at a sublethal concentrations altered their antipredator behavior (Martin et al., 2016). Furthermore, in a different study, the aggression behavior essential for survival decreased when crayfish were exposed to naproxen for 23 h (Neal and Moore, 2017). Therefore, understanding the distribution, fate, and transport of pharmaceuticals in the aquatic environment is critical for their environmental risk assessment.

At least 50% of pharmaceuticals in current use are chiral compounds often sold as racemates (Nikolai et al., 2006; Ribeiro et al., 2012). However, enantiomers of chiral pharmaceuticals often exhibit stereoselectivity in environmental occurrence (Kasprzyk-Hordern and Baker, 2012; Ma et al., 2016; MacLeod and Wong, 2010), fate (Amorim et al., 2016; Bagnall et al., 2013; Ribeiro et al., 2013), and toxicity (Stanley et al., 2007; Sun et al., 2014). For example, a recent study on occurrence of pharmaceuticals in Dongting Lake in China showed that the concentration of (*S*)-fluoxetine was higher than that of (*R*)-fluoxetine (Ma et al., 2016). Stereoselectivity was observed in the biodegradation of amphetamine in river water and activated sludge microcosm with (*S*)-enantiomer preferentially degraded (Evans et al., 2016). Furthermore, exposing fathead minnow to fluoxetine showed that (*S*)-fluoxetine was more potent to sublethal standardized and behavioral endpoints than its antipode (Stanley et al., 2007). However, despite

the growing knowledge on stereoselectivity in environmental distribution, fate and toxicity of pharmaceuticals, chirality has not been consistently incorporated in the environmental risk assessment of chiral pharmaceuticals.

Reviews on chiral pharmaceuticals in the environment show that the body of knowledge on stereoselectivity in the environment has steadily grown over the past two decades. Initial challenges in separating and detecting pharmaceuticals in complex matrices contributed to the poor understanding of the environmental behavior of chiral pharmaceuticals (Wong, 2006). However, the development of versatile chiral columns in liquid chromatography (LC) such as Chirobiotic V and application of chromatographic instruments coupled to a mass spectrometer solved much of the analytical problem (Evans and Kasprzyk-Hordern, 2014; Pérez and Barceló, 2008). Other reviews explored the application of stereoselectivity in distribution and fate as a tool for source apportionment studies (Hashim et al., 2010; Kasprzyk-Hordern, 2010; Ribeiro et al., 2012). The purpose of this review is to summarize up-to-date progress in evaluation of chiral pharmaceuticals as environmental contaminants, and identify knowledge gaps for future research. We also consider several aspects that were inadequately addressed in the previous reviews, including enantioselectivity in adsorption, the importance of exact stereoconfiguration in environmental monitoring, and chiral inversion as a factor influencing the enantiomer composition of chiral pharmaceuticals in the environment. We further make recommendations on research opportunities, including development of multi-dimensional chromatographic methods for better enantiomer resolution, evaluation of enantioselectivity in processes such as biodegradation in soil, accumulation into plants, and metabolism in aquatic and terrestrial organisms, and better use of enantiomer profiles as predictive tools in elucidating enantioselectivity in fate and transport of pharmaceuticals as environmental contaminants.

1.1 Terminology

Chiral compounds are molecules with one or more stereogenic centers. The enantiomers of a chiral compound have identical chemical structures, but different spatial arrangements of the atoms around the stereogenic center (Ribeiro et al., 2012). Enantiomers are non-superimposable stereoisomeric pairs of chiral compounds, while diastereomers are stereoisomers that are not mirror images (Figure 1). The physicochemical properties of diastereomers are not identical, while those of enantiomers are similar except for their ability to rotate a polarized light. For example, *R*- and *S*-ibuprofen are enantiomers, but 17 α - and 17- β estradiol are diastereomers. Enantiomers are commonly named using the *R/S* convention, where the Cahn–Ingold–Prelog rules are used to prioritize substituent groups around the stereogenic center (Smith, 2009). Alternatively, enantiomers are also identified according to the direction they rotate a polarized light, with (+) rotating the polarized light clockwise and (–) anticlockwise. A racemate ($\pm$) does not rotate polarized light, as it contains an equimolar mixture of enantiomers.

1.2 Chirality in Biological Systems

Enantiomers characteristically engage in stereoselective interactions in a chiral environment such as a biological system, due to their three-dimensional structures. The stereoselective interactions may result in enantiomer-specific binding, catalysis, or stabilization and this may result in the observed differences in therapeutic properties, fate, or human health and environmental effects (Wong, 2006; Wong and Warner, 2010). An eutomer is an isomer that has a high affinity for the receptor, while a distomer has less affinity (Ariens et al., 1988; Ariëns, 1984). For example, ($\pm$)-atenolol is a β -receptor antagonist used for treating cardiovascular diseases. Pharmacological studies showed that (*S*)-atenolol is the eutomer whereas (*R*)-atenolol is

the distomer (Stoschitzky et al., 1993). However, most studies on ecotoxicity of pharmaceuticals overlooked chiral selectivity in toxicity by assuming that enantiomers exhibit identical biological activity.

1.3 Chiral Pharmaceuticals as Regulatory Dilemma

Several studies between 1960s and early 1990s showed that distomers often had low affinity for the primary drug target but could be an eutomer at a different receptor (Ariëns, 1986, 1984). Hence, Ariëns et al. (1986) argued that distomers might be ‘harmful pollutants’ that could be injurious to the patient or environment. From 1992 onwards, the regulatory authorities in the United States, Europe, Japan and China recommended that pharmaceutical companies separate the enantiomers, evaluate their therapeutic activity, and if possible, market the eutomer instead of the racemate (Agranat et al., 2002; Shindo and Caldwell, 1995; U.S. Food and Drug Administration, 1992). Consequently, the number of racemic drugs approved by the U.S. Food and Drug Administration has declined (Figure 2). For instance, the U.S. FDA approved only 14 and then 4 racemic mixtures from 2005 to 2010 and 2011 to 2015, respectively. However, despite the current trends in usage, there has not been a corresponding interest in understanding the implications of chirality in the environmental risk of pharmaceuticals as contaminants (Figure S1).

The European Commission and national regulatory agencies in Europe recognized pharmaceuticals as contaminants of emerging environmental concern (Küster and Adler, 2014). This led to the development of regulatory frameworks for the environmental risk assessment of pharmaceuticals. However, the regulatory frameworks overlook the stereochemistry of pharmaceuticals, despite the need to treat enantiomers as chemical mixtures (Küster and Adler,

2014; Stanley and Brooks, 2009). Daughton (2015) attributed the dilemma in regulating chiral environmental pollutants to challenges in stereoselective analysis. The past two decades has seen significant advancements in development of chiral separation and stereoselective synthesis methods, thus making it possible to detect trace levels of chiral pharmaceuticals in environmental media (Emke et al., 2014; Pérez and Barceló, 2008). Environmental evaluation of pharmaceuticals was previously prioritized according to their sales, physicochemical properties, or occurrence in the environment (Carlsen and Walker, 2003; Mansour et al., 2016; Sanderson et al., 2003). Unfortunately, such prioritization does not specifically consider chirality. Additional criteria recently stipulated in prioritization of pharmaceuticals, e.g., the use of quantitative structural activity relationships to predict toxicity (Carlsen and Walker, 2003; Christen et al., 2010; Grisoni et al., 2016), and leveraging pharmacological data (Berninger et al., 2016; Huggett et al., 2003; Lalone et al., 2014; LaLone et al., 2013) and WWTP removal efficiency (Fick et al., 2010), may present a potential for incorporating chirality. Therefore, there is an urgent need for the development of prioritization strategies for monitoring and regulating pharmaceuticals as environmental contaminants that is cognizant of their stereoselective behaviors.

2 Analysis of Chiral Pharmaceuticals in Environmental Samples

The goal of chiral separation and detection in environmental analysis is to 1) establish analytical conditions enabling a resolution of the enantiomers, 2) identify the stereoconfiguration of the analytes, and 3) determine the enantiomer composition of the analyte in the environmental compartment under investigation. The separation, identification and quantification of enantiomers depend on the ability to consistently attain baseline separation. Therefore, enantioresolution (R_s) (Eq. 1) and enantioselectivity (α) (Eq. 2) are the most critical parameters to be considered in the development and optimization of a chiral analytical method:

$$R_S = 2 \times \frac{t_2 - t_1}{w_1 + w_2} \quad (1)$$

$$\alpha = \frac{(t_1 - t_0)}{(t_2 - t_0)} \quad (2)$$

where t_1 and t_2 are the retention times of the first (*E1*) and second (*E2*) eluted enantiomers, respectively, t_0 is the column void time found by recording the first baseline perturbation, and w_1 and w_2 are the base peak widths of the first and second eluted enantiomers, respectively. For environmental analysis, an R_S value greater than 1.5 is desirable, since the enantiomers under such conditions are essentially separated at the baseline. Enantiomer fraction (*EF*) (Eq. 3) in chiral analysis depicts the enantiomer composition of the chiral compound in a sample:

$$EF = \frac{[R]}{[R] + [S]} \quad (3)$$

where $[R]$ and $[S]$ are the fractions of *R* and *S*-enantiomers, respectively. When the stereoconfiguration is unknown, concentrations of the (+) and (-) or *E1* and *E2* are often used instead. The *EF* value is 0.5 for a racemate, and 1.0 for a single enantiomer.

2.1 Liquid Chromatography

Analysis of chiral pharmaceuticals in environmental matrices is often achieved using gas (GC), liquid (LC), and recently supercritical fluid chromatography (SFC). However, LC is the most commonly used technique in analysis of chiral pharmaceuticals likely because of the ready availability of chiral columns. In LC, enantiomer pairs may be separated by indirect or direct methods. Indirect methods involve formation of diastereomers by derivatization with chiral reagents. The diastereomers are then separated on a conventional achiral column. Derivatization is often used when the functional groups close to the chiral center limit the interactions with a

stationary phase (Ribeiro et al., 2012). For example, recently 2,3,4,6-tetra-O-acetyl- β -glucopyranosyl isothiocyanate was used a chiral derivatization reagent in the chiral analysis of ammuxetine in biological samples (Yu et al., 2017). The derivatization reaction can be accelerated using a solid support, microwave-assisted derivatization and ultrasound-assisted derivatization (Zhou et al., 2017).

Direct methods are usually preferred in environmental chiral LC analysis because derivatization adds an additional sample preparation step that can introduce artefacts due to racemization and impurities. Derivatization requires the chiral chemical to possess a labile group that can be derivatized (Cavazzini et al., 2011). Furthermore, an additional sample preparation step may be required when the chiral derivatization reagents is not 100% enantiopure. In direct enantioseparation, the chiral analytes undergo stereospecific interactions to form diastereoisomeric complexes with either the chiral stationary phase (CSP) or a chiral selector in the mobile phase (Hashim and Khan, 2011; Ribeiro et al., 2012). Since the selector and CSP are chiral, their interactions with the enantiomers are stereospecific. However, chiral selectors are expensive and are often not compatible with mass spectrometers; hence, CSPs are often the most desirable option in environmental analysis. Direct separation is often challenging because it requires the use of volatile mobile phase additives, which may contribute artefacts to the analysis (Yu et al., 2017). Oftentimes, the mobile phase additives and impurities from the complex environmental matrices adsorb to CSPs, shortening their life-span while decreasing reproducibility (Yu et al., 2017). In contrast, derivatization offers an advantage since it normally does not require mobile phase additives and provides offline sample pretreatment. Hence, there is a need for continued development in sample pretreatment techniques, including derivatization.

2.1.1 Chiral Stationary Phases

The precise mechanisms of chiral separations in chromatographic analysis of pharmaceuticals is not fully understood. Chiral recognition of enantiomers by chiral selectors or CSPs is often explained by a three-point model (Figure 3). Due to the stereoconfiguration of CSP, three functional groups on only one of the enantiomers undergo molecular interactions with three sites on the CSP to form a diastereomeric complex. The differences in the binding energy of the transient diastereomeric complexes result in the differential elution of the enantiomers (Berthod, 2006; Davankov, 1997; Evans and Kasprzyk-Hordern, 2014). This model assumes that the main molecular interaction is attraction, but repulsion or hydrophobic interactions may also contribute to chiral recognition. The three-point model is not applicable when chiral recognition is due to an analyte undergoing steric fitting in a cleft on the CSP or when the analyte interacts with the CSP involving four configuration dependent points (Berthod, 2009).

Since the development of a commercial CSP by Pirkle et al. in 1981, several materials have been employed as CSPs (Fernandes et al., 2013; Pérez and Barceló, 2007; Pirkle et al., 1981). The most commonly used CSPs in environmental analysis of chiral pharmaceuticals are polysaccharides (Ali et al., 2009a; Bagnall et al., 2012; Barclay et al., 2012; Mskhiladze et al., 2013), proteins (Ardakani et al., 2008; Barclay et al., 2012, 2011), and macrocyclic antibiotics (Aboul-Enein and Ali, 2002; Bagnall et al., 2012; Barclay et al., 2012; MacLeod et al., 2007; Ribeiro et al., 2013). Macrocyclic glycopeptides developed by Armstrong et al. (1994), particularly vancomycin and teicoplanin, are the most commonly used CSPs in the determination of chiral pharmaceuticals in the environment (Bagnall et al., 2012; Barclay et al., 2012; MacLeod et al., 2007; Ribeiro et al., 2012, 2013, 2014). For example, Wong and coworkers employed reverse phase LC coupled to a tandem MS in their investigation of chiral pharmaceuticals in

wastewater and surface water (MacLeod et al., 2007; MacLeod and Wong, 2010; Nikolai et al., 2006).

2.1.2 Polarity of Mobile Phase

Enantioseparation may be achieved in either normal or reverse phase mode. In normal phase, hydrophobic analytes are separated using a mobile phase comprised of primarily a mixture of alkanes and alcohols such as hexane - methanol, hexane - ethanol, hexane - 2-propanol and heptane - ethanol (Ali et al., 2009, Nie et al., 2013). However, in reverse phase, the mobile phase normally comprises of polar solvents such as water-methanol and water-ethanol systems (Ali et al., 2009a; Tang, 1996; Younes et al., 2011). Normal phase LC is often employed in preparative experiments because it offers high selectivity, high sensitivity, and easy sample recovery. However, normal phase is often not compatible with atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) in LC-MS systems due to the high risk of explosion (Nie et al., 2013). Furthermore, lowering the nebulizer temperature or increasing the fraction of polar organic solvent to reduce the explosion risk may result in poor sensitivity (Nie et al., 2013). As a result, reverse phase mode is preferred in environmental analysis since it has a better compatibility with MS detectors.

The most widely used technique in chiral pharmaceutical analysis is polar ionic mode where the mobile phase comprises of organic solvent such as methanol, liophilic salts, and an acid or base. Since most chiral pharmaceuticals are ionic compounds, their ionization depends on the pH of the mobile phase, thus ultimately affecting method performance. The mobile phase pH is often adjusted using formic acid (e.g., for basic compounds) or liophilic ions such as ammonium acetate and ammonium trifluoroacetate (Evans et al., 2015; MacLeod et al., 2007).

Liophilic ions play an additional role of promoting interactions between the enantiomers and the stationary phase by lowering the surface potential of the column (Sanganyado et al., 2014; Smuts et al., 2014). Several researchers used polar ionic mode in the determination of enantiomer compositions in environmental matrices where the mobile phase consisted of an organic solvent and mobile phase additives (Evans and Kasprzyk-Hordern, 2014). For example, Bagnall et al. (2012, 2013) measured the enantiomer compositions of illicit and prescription drugs in rivers using 4 mmol/L ammonium acetate in methanol and 0.005% formic acid. However, ammonium acetate and formic acid may cause ion suppression and diminish signal strength in an LC-MS/MS system when the detector is in the ESI (+) mode. Desai and Armstrong (2004) showed that the sensitivity of a chiral LC-MS/MS method was improved when ammonium acetate was replaced with ammonium trifluoroacetate. However, ammonium trifluoroacetate can cause the deterioration of chiral columns (Hashem et al., 2011). Hence, the selection of the most appropriate mobile phase remains a challenge in the analysis of chiral pharmaceuticals in environmental media.

2.2 Gas Chromatography

In recent years, there has been a rapid increase in development of chiral GC methods because they offer fast analysis and high sensitivity, reproducibility and selectivity (Xie and Yuan, 2017). Furthermore, chiral GC avoids the use of liquid mobile phases that often comprise of toxic solvents and additives. However, the utility of chiral GC in environmental monitoring is often limited to the analysis of volatile compounds that possess high thermal stability. For non-volatile analytes, derivatization using a chiral derivatization reagent is used to facilitate chiral separation while improving the thermal stability and volatility of the pollutant (Awad and El-aneid, 2013). For example, the chiral analysis of profens, a group of non-steroidal anti-

inflammatory drugs (NSAIDs) including ibuprofen, ketoprofen and naproxen, by GC employed derivatization using a chiral ionic liquid, (*R*)-1-phenylethylamine (Hashim et al., 2011; Hashim and Khan, 2011; Khan et al., 2013). Direct separation can be achieved using CSPs, namely amino acid derivatives and diamides, chiral metal complexes, and cyclodextrin (Awad and El-aneid, 2013). Application of CSPs in chiral GC was recently reviewed by Xie and Yuan (2017) and Menestrina et al. (2016).

2.3 Importance of Stereochemical Configuration for Risk Assessment

Stereochemical configurations are essential for unambiguously understanding enantioselective distribution, fate and toxicity of chiral pharmaceuticals in the environment. Most of the chiral pharmaceuticals studied to date are commercially available only as racemic mixtures. Therefore, it is important to identify the stereoconfiguration of the eluted enantiomers before reporting enantiomer-specific findings. Identifying enantiomers using their order of elution (*E1* and *E2*) is sufficient in revealing the phenomenon of enantioselectivity, but it is inadequate for environmental risk assessment. Several researchers reported that enantiomers undergo reversal in the order of elution on polysaccharide or macrocyclic glycopeptide-based columns. For example, Gyllenhaal and Stefansson (2008) observed solvent-induced reversal of the enantiomer elution order after changing the organic modifier from 2-propanol to methanol/ethanol (2:1, v/v). The elution order of enantiomers is influenced by the types of stationary phase, temperature, pH, mobile phase additives, and mobile phase modifiers (Okamoto, 2002; Xiang et al., 2011). Therefore, identifying enantiomers using only the order of elution is not desirable for promoting further research, identifying trends through meta-analysis of available data, or applying chirality in environmental risk assessment. Since the FDA recommendation on drug stereochemistry, the number of studies elucidating absolute

configurations of pharmaceuticals has increased (He et al., 2011). For example, the absolute configurations of enantiomers of oxadiazol-3-one, a compound with myocardial calcium entry channel blocking activity, were identified using vibrational circular dichroism and optical rotation (Stephens et al., 2007), where the enantiomers were labeled as (*S*)-(+)-oxadiazol-3-one and (*R*)-(-)-oxadiazol-3-one.

Chiral molecules reflect, absorb, refract and scatter polarized light in different ways. Chiroptical techniques measure these variations to establish the absolute configuration of an enantiomer. Vibrational optical activity is the most widely used chiroptical technique in pharmaceutical analysis, and it involves spectroscopic determination of their differences in response to left and right circularly polarized light by vibrating molecules (He et al., 2011). In vibrational circular dichroism, the incident polarized light is infrared and the enantiomer pairs produce spectra that are mirror images and yet equal in intensity. The absolute configuration of the enantiomers is then determined by comparing the experimental and the predicted vibrational circular dichroism spectra. Although sample preparation in vibrational circular dichroism is easy and does not require derivatization, it requires a relatively large sample (between 5 to 10 mg) and is only applicable for small and rigid samples (Gabrieli et al., 2016). In comparison, X-ray crystallography requires only a single crystal and offers absolute configurations with higher confidence. In X-ray crystallography, the absolute configuration is established from the differences in resonant scattering of X-ray by a crystal formed by co-crystallization of the enantiomer and a reference chiral molecule (Flack and Bernardinelli, 2008). Nuclear magnetic resonance (NMR) may be used in establishing the absolute configuration of chiral pharmaceuticals. Determination of absolute configuration of an enantiomer in NMR involves the formation of a diastereomer using a chiral derivatization agent and establishing their differences

in magnetic anisotropy effects (Allenmark and Gawronski, 2008). However, NMR may also require a relatively large amount of analyte, especially when it employs a large magnet, limiting its application potential.

3 Chiral Selectivity in Environmental Occurrence and Fate Processes

The main sources of chiral pharmaceuticals in the environment are wastewater effluents from wastewater treatment plants (WWTPs) and manufacturing facilities, biosolids, animal wastes, and seepage from landfills and households (Kümmerer, 2008, 2009, 2010). Biotic processes such as microbial degradation may alter the enantiomer composition of a chiral pharmaceutical due to selective transformations, whereas abiotic processes are not expected to elicit any selective effect (Baker and Kasprzyk-Hordern, 2013; Kasprzyk-Hordern et al., 2010; Kasprzyk-Hordern and Baker, 2012; MacLeod et al., 2007; MacLeod and Wong, 2010; Moreira et al., 2014; Nikolai et al., 2006; Ribeiro et al., 2012a; Wong and MacLeod, 2009). Unfortunately, most studies on the fate and effects of chiral pharmaceuticals in the environment overlooked the role of chirality (Ariëns, 1984; Kasprzyk-Hordern, 2010).

3.1 Environmental Occurrence of Chiral Pharmaceuticals

3.1.1 Analgesics

Analgesics are the most frequently detected class of pharmaceuticals in the environment (Table 1) (Ali et al., 2009b; Buser et al., 1999; Hashim and Khan, 2011; Suzuki et al., 2014). A number of studies considered the enantiomer profiles of analgesics in wastewater and surface water using GC-MS/MS (Khan et al., 2013) or LC-MS/MS (Camacho-Muñoz and Kasprzyk-Hordern, 2015; Suzuki et al., 2014). The enantiomers of ibuprofen were found in surface water at

concentrations from 0.02 $\mu\text{g/L}$ to 1.02 $\mu\text{g/L}$ (Camacho-Muñoz and Kasprzyk-Hordern, 2015; Khan et al., 2013). Both *R*- and *S*-enrichment was observed in studies conducted in Switzerland (Buser et al., 1999), Spain (Camacho-Muñoz and Kasprzyk-Hordern, 2015) and Australia (Khan et al., 2013), with *EF* values ranging from 0.41 to 0.77. The observed variations were attributed to differences in operating conditions of the WWTPs.

The *EF* values of chiral pharmaceuticals in wastewater influent are often influenced by the original enantiomer composition of the prescribed drugs. For example, naproxen is sold as a single enantiomer, and hence wastewater influent samples had *EF* values of approximately 1.0 (Caballo et al., 2015; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Khan et al., 2013). However, it was found that the *EF* value of naproxen decreased to 0.7–0.9 following the wastewater treatment process (Khan et al., 2013). This observation indicated that (*R*)-naproxen was formed during wastewater treatment, likely due to chiral inversion. The enantiomer composition may be also influenced by the stereoselectivity in human pharmacokinetics and pharmacodynamics of pharmaceuticals. For example, ketoprofen is prescribed as a racemic drug like ibuprofen. However, Caballo et al. (2015) found that the *EF* values of ketoprofen in the wastewater influent ranged from 0.54 to 0.68. In an earlier study, Grubb et al. (1999) observed that the elimination rate of (*R*)-ketoprofen was greater than its antipode after human consumption. Therefore, the deviation of *EF* values from 0.50 could be due to several processes spanning from human metabolism and elimination, and microbial transformations during wastewater treatment.

3.1.2 β -receptor antagonists

Beta-blockers are one of the most prescribed pharmaceuticals; they are commonly used to treat cardiovascular diseases. Fono and Sedlak (2005) detected enantiomers of propranolol in a wastewater effluent-dominated river in Southern California. Following surface water and lake water incubation experiments, it was concluded that stereoselectivity in the occurrence of propranolol was due to microbial degradation (Fono et al., 2006; Fono and Sedlak, 2005). Several studies showed the concentration of atenolol, metoprolol and propranolol in surface water to range from <1.0 ng/L to 0.02 μ g/L (Bagnall et al., 2012; Kunkel and Radke, 2012), and the corresponding *EF* values between 0.47 and 0.50 (Tables 1 and 2).

The *EF* values of atenolol in different wastewater influent samples were close to 0.50 (Evans et al., 2015; López-Serna et al., 2013; Vazquez-Roig et al., 2014). However, the *EF* values of atenolol in wastewater influents exhibited significant inter-day variations (Vazquez-Roig et al., 2014). The beta-blocking activity of atenolol is mainly associated with the *S* enantiomer (Mehvar and Brocks, 2001). The elimination rate of the (*R*)-enantiomer is often greater than that of its antipode. Hence, at certain times *R*-enrichment in the domestic sewage occurs. There was little to no apparent change in *EF* values between the wastewater influent and effluent for atenolol (Table 1). However, *S*-enrichment in wastewater effluent was reported for atenolol and metoprolol with *EF* values >0.54 (Bagnall et al., 2012), whereas *R*-enrichment was observed for propranolol with *EF* values <0.45 (Bagnall et al., 2012; Evans et al., 2015). Therefore, stereoselective distribution of chiral pharmaceuticals varied even within the same chemical group, as well as in different environmental settings.

3.1.3 Antidepressants

Together with β -receptor antagonists, antidepressants make up more than 20% of all pharmaceuticals prescribed in Europe and the U.S. The *EF* values of antidepressants in wastewater influent range from 0.50 to 0.60 (Table S1). For example, fluoxetine and venlafaxine had an *EF* value of 0.50, whereas citalopram had an *EF* value of 0.60 (Li et al., 2013; Liu et al., 2007; MacLeod et al., 2007), likely since citalopram products are marketed as both a racemic mixture and (*S*)-citalopram. Therefore, the observed (*S*)-enrichment was likely due to chiral inversion at the stage of manufacturing (Agranat et al., 2002). The *EF* values for citalopram and fluoxetine were measured to be 0.70 for both compounds in wastewater effluent (Evans et al., 2015). However, studies from the United Kingdom and Portugal showed the *EF* value of venlafaxine to vary from 0.43 to 0.55 (Bagnall et al., 2012; Castrignanò et al., 2016; Evans et al., 2015; Ribeiro et al., 2014).

3.2 Stereoselectivity in Fate Processes

Table 2 summarizes recent biodegradation studies of chiral pharmaceuticals in the environment. Abiotic environmental processes such as volatilization, sorption and abiotic transformations are generally non-selective for enantiomers because of their identical physicochemical properties (Wong, 2006). However, biotic degradation is often enantioselective due to the stereospecificity in the interactions of a chiral compound with proteins, enzymes and receptors in microbial degraders. Therefore, enantioselective analysis of enantiomers may serve as an indicator for microbially mediated degradation (Hashim et al., 2010; Kasprzyk-Hordern, 2010). Chiral pharmaceuticals such as ibuprofen, propranol and venlafaxine have been used as chiral signatures to infer the role of biological attenuation and to distinguish between abiotic and

biotic processes in surface water (Fono et al., 2006; Fono and Sedlak, 2005; Kasprzyk-Hordern and Baker, 2012; Li et al., 2013).

3.2.1 Biodegradation in Wastewater Microcosms

Wastewater treatment plants are engineered systems that serve as the main source of entry of chiral pharmaceuticals into both aquatic and terrestrial environments. Microbial degradation is the main removal pathway of chiral pharmaceuticals at secondary WWTPs (Khan et al., 2013; Martín et al., 2012; Subedi and Kannan, 2015). Several field and laboratory studies showed that microbial degradation of chiral pharmaceuticals in wastewater was stereoselective (Table 1). For example, the *EF* values of atenolol in different wastewater influent samples were close to 0.50 (Evans et al., 2015; López-Serna et al., 2013; Vazquez-Roig et al., 2014). However, *S*-enrichment was reported for atenolol and metoprolol in wastewater effluent, with *EF* values > 0.54 (Bagnall et al., 2012), whereas *R*-enrichment was observed for propranolol with *EF* values < 0.45 (Bagnall et al., 2012; Evans et al., 2015). Therefore, stereoselective removal of chiral pharmaceuticals varied even within the same therapeutic group. This was corroborated by Evans et al. (2015) who observed that the *EF* value of citalopram increased from 0.60 to 0.70, but there was no stereoselectivity in the removal of venlafaxine and fluoxetine (Evans et al., 2015). Therefore, a systematic understanding of the mechanism of removal of chiral pharmaceuticals in WWTPs is critical in both exposure assessment and developing mitigation efforts.

To better understand stereoselectivity in WWTPs, researchers often use laboratory setups where control of various parameters is possible. The most commonly used approach is a wastewater microcosm where mixed liquor from an aeration basin is diluted to total suspended solids ranging from 1 to 5 g/L. Adding an additional source of nutrients such as acetate to the

activated sludge inoculum was shown to have no effect on the stereoselectivity in degradation of alprenolol or propranolol (Ribeiro et al., 2013). Other studies on biodegradation of chiral pharmaceuticals did not introduce additional sources of nutrients (Fono and Sedlak, 2005; Gasser et al., 2012). A slight *R*-enrichment was observed in microbial degradation of both alprenolol and propranolol (Ribeiro et al., 2013). In a previous study, Ribeiro et al. (2012) also found that degradation of *S*-metoprolol was faster than its *R* counterpart. However, other studies on metoprolol and atenolol showed that *R*-enantiomers dissipated at a rate greater than or similar to the antipode (Ribeiro et al., 2012). The differences in enantiomer composition following biodegradation may be attributed to differences in microbial population and diversity and operating conditions of the WWTPs where the activated sludge was sampled. Future research is needed to determine the effects of operating conditions on stereoselectivity during degradation of chiral pharmaceuticals at WWTPs.

3.2.2 Degradation in Surface Water

Stereoselectivity in degradation of chiral pharmaceuticals in surface water has been reported in several field and laboratory studies. In a natural attenuation experiment lasting 92 d, temporal variations in the *EF* value of venlafaxine were observed (Li et al., 2013). When the flow in the river was low, a slight *R*-enrichment was detected, whereas an apparent *S*-enrichment occurred under normal or high flow conditions (Li et al., 2013). The temporal variation was probably caused by changes in microbial diversity due to changes in water chemistry over time. However, in a laboratory study, *S*-enrichment was more pronounced with the *EF* value increasing from 0.50 to 0.80. A lake water microcosm study showed that (*S*)-ibuprofen degraded faster than the *R*-enantiomer (Buser et al., 1999). In contrast, Winkler et al. (2001) found that (*R*)-ibuprofen degraded more rapidly in river water samples. Similarly, *R*-enrichment occurred

when ibuprofen was incubated in a constructed wetland under aerobic conditions, whereas no stereoselectivity was found under anaerobic conditions (Matamoros et al., 2009). Due to the complex nature of stereoselectivity, variations in microbial communities in different environments, and different chemical structures of chiral pharmaceuticals, the results highlighted above may not be extrapolated to other compounds or environmental systems. The fact that inconsistent stereoselectivity occurred under different conditions points to the need for a more systematic approach in elucidating the effects of environmental conditions such as pH, co-pollutants, dissolved organic matter content, biological oxygen demand and temperature on chiral selective degradation.

3.2.3 Chiral Inversion in the Environment

Chiral inversion is a process whereby the three-dimensional structure of a compound is changed by enzymes through the conversion of one enantiomer to its antipode (Khan, 2014). When isomerization results in formation of a racemic mixture, it is called racemization. Therefore, chiral inversion affects drug stability in drug discovery and development. Drug stability affects dosage, shelf-life, and biological activity or toxicity of the compound (Wsól et al., 2004). Hence, pharmaceuticals usually display a high stability under normal conditions. Consequently, isomerization or racemization of chiral pharmaceuticals has been seldom observed in the environment.

Previous studies on the fate of chiral pharmaceuticals in wastewater and the environment often overlooked chiral inversion and assumed that the observed differences in *EF* were caused solely by differences in the rate of degradation of enantiomers. However, several pharmacological studies demonstrated that certain pharmaceuticals were able to undergo chiral

inversion (Suzuki et al., 2014). For example, in humans, ibuprofen underwent unidirectional chiral inversion, where the (*R*)-enantiomer became (*S*)-ibuprofen, but the (*S*)-enantiomer did not invert to the (*R*)-enantiomer (Hao et al., 2005). The phenomenon is common among the profens in general. For example, enzymes in the human body stereospecifically change (*R*)-fenoprofen and (*R*)-benoxaprofen to their respective (*S*)-enantiomers (Khan, 2014). The degree and/or direction of chiral inversion may vary in different organisms. However, some drugs such as 2-phenylpropionic acid have been shown to undergo bidirectional chiral inversion (Khan, 2014). Although carbon stereogenic centers are known to be stable to chiral inversion, replacing one of the substitution groups with hydrogen increases its susceptibility to chiral inversion (Testa et al., 2016). Chemically- or biologically-mediated deprotonation of the stereogenic center may result in transition complex comprising of a planar carbanion (Testa et al., 2016). A subsequent protonation would result in chiral inversion. Bacteria such as *Nocordia diaphanozonaria* and *Nocardia corallina* have been shown to produce enzymes that chirally invert (*S*)-2-phenylpropionic acid and (*R*)-ibuprofen (Khan, 2014). The biologically-mediated chiral inversion of (*S*)- 2-phenylpropionic acid is thought to follow the following pathway (Khan, 2014): (*S*)-2-phenylpropionic acid reacts with acyl-CoA-synthetase to form an 'activated' coenzyme A derivative; enzymatic deprotonation of the stereogenic center to form a planar intermediate; enzymatic protonation to yield (*R*)-acyl-coenzyme A ester; and hydrolysis of (*R*)-acyl-coenzyme A ester resulting in (*R*)- 2-phenylpropionic acid (Khan, 2014).

Chiral inversion may complicate the interpretation of changes in *EF* values of chiral pharmaceuticals in the environment. For example, changes in *EF* values of ibuprofen may be caused by 1) chiral inversion of (*R*)-ibuprofen concurrent with non-stereoselective degradation, 2) enantioselective degradation, or 3) chiral inversion with concurrent enantioselective

degradation (Khan, 2014). In a study on the occurrence of naproxen and ibuprofen in wastewater, Khan et al. (2014) did not detect (*R*)-naproxen in the influent, suggesting 1) and 2) were less likely to be the source of the (*R*)-enantiomer. By comparing the *EF* values of naproxen in the influent and effluent, Khan et al. (2014) observed that the *EF* value decreased from 1.0 to between 0.7 and 0.9. Thus, (*S*)-naproxen underwent chiral inversion to form (*R*)-naproxen at the WWTP.

Suzuki et al. (2014) confirmed the role of chiral inversion in the fate of naproxen using single enantiomers, where single enantiomers were incubated in activated sludge and river water. It was further observed that there was a seasonal variation in the *EF* value due to enantioselective degradation and possibly chiral inversion. The occurrence of (*R*)-naproxen in the aquatic environment may be thus attributed to chiral inversion during wastewater treatment. Therefore, when chiral inversion is suspected, a more accurate risk assessment should treat the individual enantiomers as single compounds. Moreover, the assumption that enantioselective degradation exclusively causes enantiomer enrichment may be invalid for some chiral pharmaceuticals, and the role of chiral inversion in the fate of chiral pharmaceuticals needs to be better understood through future research.

3.2.4 Stereoselective Sorption

There is generally a poor understanding of stereoselectivity in adsorption of chiral pharmaceuticals. This is likely due to the assumption that adsorption, as an abiotic process, is considered non-stereoselective. However, environmental solid matrices such as minerals and organic matter are chiral structures (Celis et al., 2015, 2013; Gámiz et al., 2016; Oravec et al., 2010). Given that enantiomers behave differently in a chiral environment, there is reason to

believe that chiral minerals and organic matter may serve as a chiral environment to potentially foster stereoselectivity in sorption (Gámiz et al., 2016; Hazen and Sholl, 2003). The difference in *EF* values between the freely dissolved fraction and the sorbed fraction may be measured to indicate stereoselectivity in sorption. Stereoselectivity in sorption may result in one enantiomer becoming less available for transport, microbial degradation or uptake by non-target organisms. Such knowledge is essential in environmental risk assessment since availability of enantiomers affects their environmental fate and effects (Celis et al., 2013).

Experimental evidence for stereoselective adsorption of chiral pharmaceuticals in the environment remains scarce. Stereoselective adsorption of pesticides in agricultural soils has been demonstrated in several studies (Celis et al., 2013, 2015; Gámiz et al., 2013). For pharmaceuticals, the implications of chirality in sorption are likely more important in WWTPs than in soils, because adsorption is a key removal pathway of pharmaceuticals during wastewater treatment. Evans et al. (2015) found that there was enantiomer enrichment in chiral pharmaceuticals adsorbed to sludge. For example, the *EF* values of alprenolol and atenolol in wastewater influent and effluent were both 0.5 ± 0.0 whereas their *EF* values in sludge were 0.7 ± 0.2 and 0.4 ± 0.0 , respectively. This suggests that adsorption of alprenolol and atenolol may be stereoselective. However, since the sludge was sampled from the microbially active aeration basins, the observed differences in *EF* values could be due to biodegradation. Hence, it is difficult to unequivocally demonstrate stereoselective adsorption solely from field observations.

Several studies to discern stereoselectivity in adsorption employed batch equilibration with sterilized soil, sediment or sludge samples. Sterilization is often achieved using autoclaving to minimize biodegradation (Khan et al., 2009; Mashtare et al., 2011; Sanganyado et al., 2016). Stereoselective adsorption in pharmaceuticals was first demonstrated in the adsorption of

diastereomers of trenbolone, a synthetic growth hormone used in beef production, to soil (Khan et al., 2009). Khan et al. (2009) found that the adsorption of 17β -trenbolone to soil was twice that of 17α -trenbolone. In a similar study, the adsorption of 17β -estradiol to 7 different types of soil was found to be twice that of 17α -estradiol (Mashtare et al., 2011). Qiao et al. (2011) observed that octanol-water partition coefficients of the estradiol enantiomers were the same, indicating that hydrophobic interactions were not stereoselective. However, hexane-water and toluene-water partition coefficients exhibited stereoselectivity even though they were an order of magnitude lower than K_{ow} (Qiao et al., 2011). The authors suggested that although adsorption of estradiol was predominantly due to hydrophobic interactions, the observed stereoselectivity was probably due to polar interactions (Qiao et al., 2011). However, stereoselectivity may be expected in diastereomers since they have different physicochemical properties. The role of polar interactions in stereoselective adsorption was observed in adsorption of chiral pharmaceuticals to sludge (Sanganyado et al., 2016). Using batch experiments, stereoselectivity in the adsorption of enantiomers of acebutolol, atenolol, metoprolol, pindolol and propranolol on sludge was shown to increase with a decrease in hydrophobicity of the adsorbate. The EF values of acebutolol, atenolol and metoprolol adsorbed on the sludge were 0.27, 0.55 and 0.32, respectively, whereas for propranolol and pindolol it was about 0.50 (Sanganyado et al., 2016). These results suggested that polar interactions, rather than hydrophobic interactions, increased stereoselectivity in adsorption (Sanganyado et al., 2016). It was postulated that the selectivity in adsorption was likely due to the presence of chiral centers in natural organic matter. Hence, stereoselective adsorption may result in enrichment of the more toxic enantiomer in the wastewater effluent that is discharged into the aquatic environment. There is a need for a comprehensive understanding of

mechanisms contributing to chiral selectivity in adsorption since sorption affects the bioavailability of chiral pharmaceuticals in the environment.

4 Knowledge Gaps and Future Research Directions

4.1 Analytical Techniques

4.1.1 High Efficiency Columns in Liquid Chromatography

Although marked progress has been made in enantioseparation and identification of chiral pharmaceuticals, methods of analysis still limit the further advancement in this field. For example, conventional HPLC columns (4.6 or 5.0 μm particle diameter) are usually used for chiral separation of pharmaceuticals in environmental samples, and no use of ultra-high-performance LC (UPLC) columns (sub-2 μm particle diameters) has been reported, although UPLC-MS/MS is often adopted in analysis of pharmaceuticals as environmental contaminants (e.g., Evans et al., 2015; Bagnall et al., 2012; Vazquez-Roig et al., 2014). It is well known that decreasing the particle size will greatly increasing the plate numbers and improving resolution. At present, 3.0 and 3.5 μm fully porous particle-based chiral stationary phases are commercially available from major LC column suppliers, such as Phenomenex, Waters, Chiral Technologies, Supelco, and AZYP (Patel et al., 2016). The first practically useful sub-2 μm CSP was developed by Gasparrini and co-workers (2010). Various sub-2 μm CSPs using Whelk-O, vancomycin, teicoplanin, and teicoplanin aglycone as chiral selectors have been developed recently, and the plate numbers reached as high as 200,000 plates per meter (Patel et al., 2016). Therefore, these new columns may significantly improve the separation efficiency if applied to environmental analysis of chiral pharmaceuticals.

4.1.2 Multidimensional Achiral-Chiral Liquid Chromatography

Chiral separation usually does not reach the same efficacy as achiral separation, and thus a straightforward option is to couple achiral and chiral columns. The two-dimensional LC (2D-LC) with achiral-chiral coupling combines completely different separation mechanisms, and has been recently applied to analysis of pharmaceuticals and pesticides in human and animal samples (Leon-Gonzalez et al., 2014). Barreiro et al. (2011) first applied 2D achiral-chiral LC to wastewater analysis. A restricted access media of bovine serum albumin octyl column was used in the first dimension for the exclusion of the humic substances, and a polysaccharide-based chiral column was used in the second dimension for the enantioseparation of pantoprazole and lansoprazole enantiomers. Since organic matter was excluded in the first column, no sample extraction and clean-up were necessary. Although the detection limit was very low (200 and 150 ng/L for pantoprazole and lansoprazole, respectively), no enantiomers were detected (Barreiro et al., 2011). Considering the matrix complexity and the co-occurrence of various pharmaceuticals and other organic contaminants in the environment, the 2D-LC system may be a useful approach that needs to be further explored.

4.1.3 Combining Chiral Analysis with Other Modern Analytical Techniques

As discussed above, the *EF* values alone are often insufficient to make exclusive conclusions. Therefore, combinations with other modern techniques may be valuable. The isotope effect describes the selectivity of isotopologues in degradation and fractioning. Similar to *EF* values, the compound specific isotopic ratio has been used to elucidate degradation mechanism and to track sources. Jammer et al. (2014) combined these two techniques to assess the enzymatic hydrolysis of several pesticides. This 2D approach improved pathway

identification. Although GC-isotopic ratio MS (IRMS) was used in Jammer et al. (2014), LC-IRMS has been recently commercialized, and may offer a greater proficiency for similar analysis of chiral pharmaceuticals.

4.1.4 Gas Chromatography

The numbers of commercially available chiral columns for GC are relatively fewer compared with HPLC. Therefore, considering the analytical benefits of chiral GC, development of highly sensitive and selective chiral columns for GC applications is crucial. Future research should develop CSP materials with a higher universality in chiral recognition since environmental matrices contain multiresidues (Menestrina et al., 2016). Development of multidimensional GC is important since it offers higher selectivity and sensitivity when used for analyzing complex matrices (Xie and Yuan, 2017). Furthermore, development of fast analysis precolumn derivatization techniques should remain a priority considering the cost-effectiveness of using chiral derivatization reagents compared to CSPs.

4.2 Overlooked Fate Processes

4.2.1 Stereoselective Uptake, Translocation, and Metabolism

To date, most studies on environmental fate of chiral pharmaceuticals focused on degradation in wastewater and surface water, and a few other important processes have been largely ignored, such as wetland removal, soil degradation, and plant uptake and metabolism. Pharmaceuticals are introduced into soil through reclaimed water irrigation and sludge application (Bartrons and Peñuelas, 2017; Christou et al., 2017; Dodgen et al., 2014). Numerous studies showed that biodegradation in soil is an important pathway in the overall environmental

fate of pharmaceuticals and regulates secondary pollution including runoff, leaching, and transfer to terrestrial organisms (Dodgen et al., 2014; Grossberger et al., 2014; Koba et al., 2016; Schaffer et al., 2015). Once in soil, pharmaceuticals may be taken up into plant tissues, including edible parts, imposing unintended effects on food safety. For example, naproxen was detected at trace levels in cabbage and bell pepper when irrigated with treated wastewater under field conditions (Wu et al., 2014). Therefore, future research should consider enantioselectivity of chiral pharmaceuticals in these and other environmental processes.

4.2.2 Stereoselective Partitioning

Stereoselective partitioning in environmental compartments has not received adequate attention. Although a few studies suggested that the selective sorption to soil and sludge does happen with chiral chemicals, mechanisms and influencing factors are mostly unknown. Organic matter, with chiral structures, is proposed to be the cause for stereoselective sorption in soil or sludge. However, the importance of chiral surfaces of common soil-forming minerals should not be neglected. Previous studies showed that sorption of aspartate on the surfaces of calcite was enantioselective (Hazen et al., 2001). Other common chiral mineral surfaces, such as quartz, alkali feldspar, and clinopyroxene may also contribute to the stereoselectivity (Hazen and Sverjensky, 2010). Sorption of estrogens to Aldrich humic acid and leonardite was found to be stereoselective (Qiao et al., 2011). However, selectivity of sorption to organic matter from soil and sediment is unknown and merits further investigation.

Moreover, changes in environmental conditions or the presence of environmental components that affect the polarity of the chiral pharmaceuticals may potentially cause changes in stereoselective adsorption. Hence, stereoselective sorption to sludge, sediments or soil may be

affected by changes in pH, temperature, ionic strength and the presence of co-solutes. Therefore, systematic studies on the effects of these factors on stereoselectivity in adsorption are necessary for understanding the mechanism of stereoselective adsorption and to predict the distribution of chiral pharmaceuticals among different environmental media.

4.2.3 Stereoselective Occurrence

There are relatively few studies on stereoselective distribution of chiral pharmaceuticals in the environment (Evans and Kasprzyk-Hordern, 2014; Pérez and Barceló, 2007; Ribeiro et al., 2012). Only 22 pharmaceuticals were measured in all published studies to date (Table 1 and Table S1), which represents only a very small fraction of the total number of chiral pharmaceuticals in current use. Moreover, only one or a few compounds were monitored in each study. Therefore, further research should use multiresidue methods and target multiple chiral pharmaceutical contaminants in analysis. On the other hand, most previous studies focused on wastewater and little attention has been paid to surface water, sludge, soil, sediment or biological samples. Many of the chiral pharmaceuticals have high mobility because of their high water-solubility and low sorption to solids. Therefore, further research should consider other environmental compartments, including groundwater, source and treated drinking water, food produce, among others.

4.3 Quantitative Assessment of Chiral Degradation

Changes in enantiomer composition with time or distance are frequently used for source tracking and understanding the mechanism of degradation of chiral pharmaceuticals (Fono and Sedlak, 2005; Gasser et al., 2012). There are two methods commonly used to determine shifts in enantiomer composition of pharmaceuticals in the environment: 1) determination of *EF* values or

enantiomer ratios, which may exhibit concentration-dependent relationships, and 2) concentration-time profiles and EF -time profiles. However, both methods may be simplified and merged by using the Rayleigh equation (Jammer et al., 2014).

According to the Rayleigh equation ($\ln ER_t/ER_0 = \varepsilon_{ER} + \ln C_t/C_0$), the degree of biodegradation ($\ln ER_t/ER_0$) is directly proportional to the degree of enantiomer enrichment ($\ln C_t/C_0$) with the enantiomer enrichment factor (ε_{ER}) as the constant of proportionality. Recent studies have shown that biodegradation of venlafaxine and metoprolol exhibit Rayleigh dependency (Gasser et al., 2012; Souchier et al., 2016). Thus, ε_{ER} , a compound specific parameter, can be used as a qualitative and quantitative tool for assessing stereoselective degradation of chiral pharmaceuticals in the environment (Souchier et al., 2016). Jammer et al. (2015) further demonstrated that ε_{ER} may be predicted using molecular descriptors. Thus, the Rayleigh equation may be used to predict ε_{ER} for chiral transformation products and metabolites of pharmaceuticals with known Rayleigh equation parameters. Future studies may focus on the applicability of the Rayleigh equation as a quantitative assessment tool for stereoselective degradation and as a predictive model in environmental risk assessment.

4.4 Enantiospecific Toxicity

Data on the enantiospecific toxicity of chiral pharmaceuticals are valuable for improving the accuracy of their environmental risk assessment. However, studies on enantiospecific toxicity of chiral pharmaceuticals in the environment remain scarce although pharmacological properties of chiral pharmaceuticals are known to vary between enantiomers. No stereospecific toxicity was observed in *Daphnia magna* (De Andrés et al., 2009; Stanley et al., 2007), while the growth of *Daphnia magna* was inhibited more by (*S*)-atenolol than (*R*)-atenolol (De Andrés et al., 2009).

Since environmental concentrations are often below their acute aquatic toxicity levels, pharmaceuticals may cause chronic effects, rather than acute toxicity. Following a battery of traditional toxicity assessment, Brooks et al. (2003) observed that the environmental risk of fluoxetine was low, however they postulated that fluoxetine could elicit adverse effects at non-traditional endpoints. A subsequent chronic toxicity study demonstrated that fluoxetine elicited sublethal and behavioral effects stereoselectively on *Pimephales promelas* with (*S*)-fluoxetine more adversely affecting feeding behavior and growth than (*R*)-fluoxetine (Stanley et al., 2007). Therefore, there is a need to investigate enantiospecific toxicity of chiral pharmaceuticals at non-traditional endpoints. Furthermore, data on transport, fate and toxicity of individual enantiomers are also needed since enantiomers may display differences in bioaccumulation, persistence and toxicity in the environment. Molecular interactions of enantiomers with the primary drug targets may be additive, antagonistic or synergistic, and thus future ecotoxicological studies should treat chiral pharmaceuticals as mixtures (Stanley and Brooks, 2009). There is also a need for the development of a prioritization technique that incorporates chirality. Recently, leveraging pharmacological data and using techniques such as fish plasma model, has been proposed for predicting the environmental risk of pharmaceuticals (Berninger et al., 2016). Since pharmacological data often include biological activities of the enantiomers, such data could be used to predict enantiospecific toxicity.

5 Conclusions

Chiral pharmaceuticals pose a unique challenge to environmental monitoring and characterization given that their enantiomers exhibit differences in aquatic toxicity although their physicochemical properties are similar in achiral environments. Advances in chiral separation and detection resulted in a steady increase in studies on stereoselectivity in the occurrence and

biodegradation of pharmaceuticals in the environment. However, studies on stereoselectivity in the environmental toxicity of chiral pharmaceuticals remain scarce.

According to the critical analysis in this review, to understand the full implication of chirality on environmental risk of polar micropollutants, the following conclusions and perspectives regarding the analysis and environmental fate processes of chiral pharmaceuticals may be drawn:

- i. Development of strategies for prioritizing pharmaceuticals for environmental monitoring and characterization should incorporate their stereoselective behaviors.
- ii. Since identification of enantiomers according to their elution order in chromatography is not adequate as a source for developing environmental regulations, there is need for determination of exact stereoconfiguration of enantiomers in environmental analysis.
- iii. Current studies on chiral pharmaceuticals in the environment are primarily focused on analgesics, β -receptor antagonists and antidepressants, and there is need for expanding research to include other pharmaceutical groups such as antibiotics.
- iv. Occurrence and fate of chiral pharmaceuticals have been extensively studied in WWTPs. However, very few studies explored the role of operating conditions and presence of co-pollutants on stereoselective degradation of chiral pharmaceuticals at WWTPs.

- v. Since sediments, soil and sludge contain organic matter that offers a chiral environment, there is need for systematic research to establish the mechanisms of chiral adsorption of pharmaceuticals to these matrices.
- vi. Application of high efficiency columns, multidimensional LC and other modern analytical techniques such as GC-isotopic ratio MS in occurrence, fate and transport of chiral pharmaceuticals in the environment offers an opportunity for efficient analysis of complex matrices.
- vii. There is a need for understanding the plant uptake and metabolism of chiral pharmaceuticals given that the use of reclaimed water, biosolids and animal wastes in agricultural fields continues to increase globally.

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7 Supporting information

Additional information on occurrence and fate of chiral pharmaceuticals in the environment.

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1132

1133 Table 1 Occurrence and analysis of chiral pharmaceuticals in wastewater and surface water.

Pharmaceuticals	Country	Chiral analysis		Initial concentration			Final Concentration			Ref.
		Matrix	Detection	E1, µg/L	E2, µg/L	EF	E1 ^a , µg/L	E2 ^b , µg/L	EF ^c	
Fexofenadine	UK	Wastewater	LC-MS/MS				0.25±0.04	0.21±0.03	0.54	(Camacho-Muñoz and Kasprzyk-Hordern, 2015)
Ibuprofen	Switzerland	Wastewater	GC-MS		0.99-3.30	0.85-0.89		0.002-0.08 ^d	0.47-0.67	(Buser et al., 1999)
Ibuprofen	Switzerland	Surface water	GC-MS					n.d.- 0.008 ^d	0.41-0.60	(Buser et al., 1999)
Ibuprofen	Australia	Wastewater	GC-MS/MS				0.07	0.18	0.73	(Khan et al., 2013)
Ibuprofen	Australia	Creek ^e	GC-MS/MS	0.05	0.12	0.71	0.03	0.09	0.77	(Khan et al., 2013)
Ibuprofen	Australia	Creek, downstream ^f	GC-MS/MS				0.02	0.02	0.60	(Khan et al., 2013)
Ibuprofen	UK	Wastewater	LC-MS/MS				0.24±0.04	0.46±0.06	0.66	(Camacho-Muñoz and Kasprzyk-Hordern, 2015)

Pharmaceuti	Country	Chiral analysis	Initial concentration	Final Concentration	Ref.
Ibuprofen	Spain	Wastewater	LC-MS/MS	0.42±0.0 1.64±0.05 0.80 0.08±0.0 0.17±0.01 0.67	(Caballo et al., 2015)
Ibuprofen	Spain	Wastewater	LC-MS/MS	0.25±0.0 0.96±0.01 0.79 0.04±0.0 0.07±0.0 0.62	(Caballo et al., 2015)
Ibuprofen	Spain	Wastewater	LC-MS/MS	0.25±0.0 1.54±0.05 0.86 0.10±0.0 0.21±0.01 0.68	(Caballo et al., 2015)
Ibuprofen	Spain	Surface water ^h	LC-MS/MS	0.09 0.11 0.48 0.77 1.02 0.46	
Ketoprofen	Spain	Wastewater	LC-MS/MS	0.19±0.0 0.22±0.0 0.54 0.03±0.0 0.06±0.0 0.68	(Caballo et al., 2015)
Ketoprofen	Spain	Wastewater	LC-MS/MS	0.24±0.0 0.51±0.01 0.68 0.08±0.0 0.18±0.0 0.67	(Caballo et al., 2015)
Ketoprofen	Spain	Wastewater	LC-MS/MS	0.09±0.0 0.12±0.0 0.56 0.02±0.0 0.04±0.0 0.61	(Caballo et al., 2015)
Naproxen	Australia	Wastewater	GC-MS/MS	n.d. 0.02 1.0	(Khan et al., 2013)
Naproxen	Japan	Wastewater		n.d. 0.03-0.43 1.0 n.r. 0.01-0.11 0.88-0.91	(Suzuki et al., 2014)
Naproxen	Australia	Creek ^e	GC-MS/MS	n.d. 0.03 1.0 n.d. 0.60 1.0	(Khan et al., 2013)
Naproxen	Australia	Creek, downstream ^f	GC-MS/MS	n.d. 0.06 1.0	(Khan et al., 2013)

Pharmaceuti	Country	Chiral analysis		Initial concentration			Final Concentration			Ref.
Naproxen	UK	Wastewater	LC-MS/MS				0.09±0.02	1.33±0.50	0.94	(Camacho-Muñoz and Kasprzyk-Hordern, 2015)
Naproxen	UK	Surface water	LC-MS/MS				n.d.	0.136±n.a.	1	(Camacho-Muñoz and Kasprzyk-Hordern, 2015)
Naproxen	Japan	Surface water						0.08 ^d	0.84-0.98	(Suzuki et al., 2014)
Naproxen	Spain	Wastewater	LC-MS/MS	0.03±0.0	3.17±0.04	0.99	0.02±0.0	0.48±0.01	0.96	(Caballo et al., 2015)
Naproxen	Spain	Wastewater	LC-MS/MS	0.02±0.0	1.05±0.01	0.98	0.01±0.0	0.18±0.0	0.93	(Caballo et al., 2015)
Naproxen	Spain	Wastewater	LC-MS/MS	0.02±0.0	1.75±0.0	0.99	0.01±0.0	0.24±0.01	0.96	(Caballo et al., 2015)
Tetramisole	UK	Wastewater	LC-MS/MS				0.02±0.003	0.02±0.002	0.50	(Camacho-Muñoz and Kasprzyk-Hordern, 2015)
Alprenolol	UK	Wastewater	LC-MS/MS		0.22±0.002	0.50		0.20±0.005 ^d	0.50	(Evans et al., 2015)
Atenolol	UK	Surface	LC-MS/MS				0.01±0.001	0.02±0.001	0.47	(Bagnall et al., 2012)

Pharmaceuti	Country	Chiral analysis		Initial concentration		Final Concentration				Ref.
		water								
Atenolol	Spain	Surface	LC-MS/MS	0.002	0.002	0.50	<0.001	n.d.	-	(López-Serna et al., 2013)
Atenolol	UK	Wastewater	LC-MS/MS				0.59±0.02	0.50±0.01	0.55	(Bagnall et al., 2012)
Atenolol	Spain	Wastewater ^g	LC-MS/MS	0.63-0.81	0.68-0.93	0.41-0.51	0.32-0.63	0.35-0.62	0.48-0.51	(Vazquez-Roig et al., 2014)
Atenolol	Spain	Wastewater ^g	LC-MS/MS	0.80-1.32	0.80-1.13	0.48-0.53	0.39-0.95	0.41-1.08	0.42-0.50	(Vazquez-Roig et al., 2014)
Atenolol	Spain	Wastewater ^g	LC-MS/MS	0.54-0.95	0.91-1.23	0.34-0.44	0.30-0.67	0.31-0.79	0.46-0.51	(Vazquez-Roig et al., 2014)
Atenolol	UK	Wastewater	LC-MS/MS		0.44±0.018 ^d	0.50		0.07±0.005 ^d	0.50	(Evans et al., 2015)
Citalopram	UK	Wastewater	LC-MS/MS		0.65±0.05 ^d	0.60		0.05±0.004 ^d	0.70	(Evans et al., 2015)
Desmethylopram	UK	Wastewater	LC-MS/MS		0.002±0.0 ^d	1.0		nd	nd	(Evans et al., 2015)
Metoprolol	UK	Wastewater	LC-MS/MS				0.002±0.0	0.002±0.0	0.54	(Bagnall et al., 2012)
Venlafaxine	UK	Surface	LC-MS/MS				0.008±0.0	0.01±0.0	0.58	(Bagnall et al., 2012)

Pharmaceuti	Country	Chiral analysis		Initial concentration			Final Concentration			Ref.
.		water								
Venlafaxine	UK	Wastewater	LC-MS/MS				0.06±0.0	0.04±0.0	0.43	(Bagnall et al., 2012)
Venlafaxine	Portugal	Wastewater	LC-MS/MS				0.11±0.01	0.13±0.01	0.54	(Ribeiro et al., 2014)
Venlafaxine	Portugal	Wastewater	LC-MS/MS				0.10±0.01	0.13±0.01	0.55	(Ribeiro et al., 2014)
Venlafaxine	Portugal	Wastewater	LC-MS/MS				0.04±0.0	0.05±0.0	0.55	(Ribeiro et al., 2014)
Venlafaxine	UK	Wastewater	LC-MS/MS	0.35±0.03 ^d	0.50		0.22±0.02 ^d	0.50		(Evans et al., 2015)
Venlafaxine	UK	Wastewater ^f	LC-MS/MS				0.07-0.11	0.09-0.12	0.44-0.49	(Castrignanò et al., 2016)
Desmethylvenlafaxine	UK	Wastewater ^f	LC-MS/MS				0.29-0.32	0.19-0.25	0.53-0.60	(Castrignanò et al., 2016)
Desmethylvenlafaxine	UK	Wastewater	LC-MS/MS	0.70±0.02 ^d	0.50		0.29±0.01 ^d	0.50		(Evans et al., 2015)
Ephedrine	Spain	Wastewater ^g	LC-MS/MS	0.22-0.39	0.24-0.39	0.43-0.55	0.03-0.06	0.005-0.025	0.65-0.89	(Vazquez-Roig et al., 2014)
Ephedrine	Spain	Wastewater ^g	LC-MS/MS	0.15-0.36	0.14-0.34	0.41-0.60	0.03-0.04	0.02-0.05	0.42-0.56	(Vazquez-Roig et al., 2014)

Pharmaceuti	Country	Chiral analysis	Initial concentration			Final Concentration			Ref.
Ephedrine	Spain	Wastewater ^b	LC-MS/MS	0.11-0.48	0.11-0.40	0.36-0.62	0.02-0.03	0.004-0.009	0.75-0.85 (Vazquez-Roig et al., 2014)
Fluoxetine	UK	Wastewater	LC-MS/MS		0.05±0.004 ^d	0.70		0.03±0.0 ^d	0.70 (Evans et al., 2015)
Propranolol	UK	Surface water	LC-MS/MS				0.001±0.0	0.001±0.0	0.45 (Bagnall et al., 2012)
Propranolol	UK	Wastewater	LC-MS/MS				0.03±0.0	0.05±0.0	0.43 (Bagnall et al., 2012)
Propranolol	UK	Wastewater	LC-MS/MS		0.11±0.0 ^d	0.40		0.04±0.0 ^d	0.40 (Evans et al., 2015)
Salbutamol	UK	Wastewater	LC-MS/MS		0.28±0.03 ^d	0.50		0.46±0.01 ^d	0.50 (Evans et al., 2015)
Sotalol	UK	Wastewater	LC-MS/MS		0.25±0.02 ^d	0.50		0.15±0.009 ^d	0.50 (Evans et al., 2015)
Tramadol	UK	Wastewater ^f	LC-MS/MS				0.69-0.80	0.60-0.68	0.51-0.57 (Castrignanò et al., 2016)
Tramadol	UK	Wastewater	LC-MS/MS		1.32±0.06 ^d	0.70		0.51±0.05 ^d	0.70 (Evans et al., 2015)

1134 ^a first eluted or (*R*)-enantiomer

1135 ^b second eluted or (*S*)-enantiomer

1136 ^c Enantiomer Fraction, $EF = [S]/([S] + [R])$ or $EF = [E2]/([E1] + [E2])$

1137 ^d concentration of racemic mixture

1138 ^e during wet conditions

1139 ^f during dry conditions

1140 n.r. not reported

1141 n.d. not detected

1142 ^g inter-day variation

1143 Table 2 Degradation of chiral pharmaceuticals in wastewater and the environment.

Pharmaceuticals	Incubation			Observation	Reference
	Matrix	Initial conc., $\mu\text{g/L}$	Period, d		
Alprenolol	Activated sludge inoculum	10,000 and 1000	15	Slight <i>R</i> -enrichment	(Ribeiro et al., 2013)
Atenolol	Activated sludge inoculum	-	15	No enantioselectivity observed	(Ribeiro et al., 2012a)
Metoprolol	River water	-	14	No enantioselectivity observed	(Fono et al., 2006)
Metoprolol	Activated sludge inoculum	-	15	<i>R</i> -enrichment	(Ribeiro et al., 2012a)
Metoprolol	Effluent-sediment	10	65	<i>S</i> -enrichment	(Souchier et al., 2016)
Propranolol	Activated sludge inoculum	10,000 and 1000	15	Slight <i>R</i> -enrichment with half-lives of 9.68 and 10.13 for <i>R</i> - propranolol and <i>S</i> -propranolol, respectively	(Ribeiro et al., 2013)

Pharmaceuticals	Incubation			Observation	Reference
	Matrix	Initial conc., $\mu\text{g/L}$	Period, d		
Propranolol	Wastewater microcosm	1.00 ^c	6	EF ^a decreased from 0.5 to 0.43 as the compound underwent biodegradation	(Fono and Sedlak, 2005)
Propranolol	River water	1.00 ^c	20	No enantioselectivity observed	(Fono and Sedlak, 2005)
Ibuprofen	Wastewater microcosm	0.99	0.33	No dissipation in first 2 h, then ER ^b changed from 5.7 to ER of 2.7 after 8 h.	(Buser et al., 1999)
Ibuprofen	Lake water	0.20	37	<i>R</i> -enrichment	(Buser et al., 1999)
Ibuprofen	Constructed wetlands	25	20	<i>R</i> -enrichment occurred under aerobic conditions, whereas no stereoselectivity was observed under anaerobic conditions	(Matamoros et al., 2009)
Ibuprofen	Membrane bioreactor	6–43	1	<i>EF</i> ranged from 0.88 to 0.94 in the influent and 0.38 to 0.40 in the	(Hashim et al., 2013)

Pharmaceuticals	Incubation			Observation	Reference
	Matrix	Initial conc., $\mu\text{g/L}$	Period, d		
				effluent.	
Ibuprofen	Membrane bioreactor	2 ^d	1	<i>R</i> -enrichment occurred with an average decrease in <i>EF</i> from 0.52 to 0.39	(Hashim et al., 2011)
Naproxen	Membrane bioreactor	0.8–69	1	<i>EF</i> value in the influent decreased from 0.99 to between 0.86 to 0.94 in effluent.	(Hashim et al., 2013)
Naproxen	Membrane bioreactor	2 ^d	1	Chiral inversion was observed with <i>EF</i> decreasing from 0.99 to 0.65	(Hashim et al., 2011)
Naproxen	Constructed wetlands	25	20	<i>EF</i> decreased from between 0.88 and 0.90 to between 0.71 and 0.86.	(Matamoros et al., 2009)
Ketoprofen	Membrane bioreactor	0.1 to 27	1	No enantioselectivity observed	(Hashim et al., 2013)
Ketoprofen	Membrane	2 ^d	1	<i>S</i> -enrichment occurred with an	(Hashim et al., 2011)

Pharmaceuticals	Incubation			Observation	Reference
	Matrix	Initial conc., $\mu\text{g/L}$	Period, d		
	bioreactor			average increase in <i>EF</i> from 0.52 to 0.63	
Fluoxetine	Activated sludge inoculum	-	15	No enantioselectivity observed	(Ribeiro et al., 2012a)
Fluoxetine	<i>Labrys portucalensis</i> F11	618	30	<i>S</i> -enrichment.	(Moreira et al., 2014)
Venlafaxine	Wastewater microcosm	15	90	ER did not change significantly, but <i>S</i> - to <i>R</i> -enrichment under aerobic conditions but none under anaerobic was observed for O-desmethylvenlafaxine.	(Gasser et al., 2012)
Venlafaxine	River water	0.20	28	<i>R</i> -enantiomer degraded faster with <i>EF</i> values increasing from 0.5 to 0.8	(Li et al., 2013)

Pharmaceuticals	Incubation			Observation	Reference
	Matrix	Initial conc., $\mu\text{g/L}$	Period, d		
Venlafaxine	Natural attenuation, river	-	92	<i>EF</i> values were 0.62, 0.74 and 0.46 in rainfall, normal flow and low flow conditions, respectively	(Li et al., 2013)

1144 ^a $EF = E1/(E1 + E2)$ where *E1* and *E2* are concentration of first and second eluted enantiomers.

1145 ^b $ER = (\text{concentration of } R - \text{enantiomer}) / (\text{concentration of } S - \text{enantiomer}).$

1146 ^cSpiking concentration, initial concentration in microcosm not available.

1147 ^dAnalyte was continuously introduced

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Figure 1 A schematic representation of enantiomers and diastereomers of sertraline.

Figure 2 Number of new drugs approved by US Food and Drug Administration (FDA) for the period 2002 to 2015.

Figure 3 Chiral molecular interaction indicating left enantiomer having a stronger affinity for the active site than the right enantiomer.

Highlights

- Critical review of environmental fate process of chiral pharmaceuticals
- Summary of developments in chiral separation and detection of pharmaceuticals
- Chiral inversion and sorption noted as critical in environmental fate
- Studies on stereoselectivity in fate of pharmaceuticals in plants recommended