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RESEARCH ARTICLE

Chiral profiling of azole antifungals in municipal wastewater and recipient rivers of the Pearl River Delta, China

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Abstract Enantiomeric compositions and fractions (EFs) of three chiral imidazole (econazole, ketoconazole, and miconazole) and one chiral triazole (tebuconazole) antifungals were investigated in wastewater, river water, and bed sediment of the Pearl River Delta, South China. The imidazole pharmaceuticals in the untreated wastewater were racemic to weakly nonracemic (EFs of 0.450–0.530) and showed weak enantioselectivity during treatment in the sewage treatment plant. The EFs of the dissolved azole antifungals were usually different from those of the sorbed azoles in the suspended particulate matter, suggesting different behaviors for the enantiomers of the chiral azole antifungals in the dissolved and particulate phases of the wastewater. The azole antifungals were widely present in the rivers. The bed sediment was a sink for the imidazole antifungals. The imidazoles were prevalently racemic, whereas tebuconazole was widely nonracemic in the rivers. Seasonal effects were observed on distribution and chirality of the azole antifungals. Concentrations of the azole antifungals in the river water were relatively higher in winter than in spring and summer while the EF of miconazole in the river water was higher in summer. The mechanism of enantiomeric behavior of the chiral azole antifungals in the environment warrants further research.

Keywords Enantiomeric composition · Enantiomeric fraction · Chiral azole antifungals · The Pearl River · Water · Bed sediment · Wastewater

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Introduction

Chirality of pharmaceuticals in the environment has attracted increasing concerns (Wong 2006). Enantiomers of a chiral pharmaceutical generally have identical physicochemical properties but may be different in bioactivities (Stanley et al. 2007; Huhnerfuss and Shah 2009), implying that the pharmaceutical possibly experiences enantiomer-specific metabolism in organisms and/or enantiomer-specific bioreactions in the environment, leading to enrichment of one enantiomer and/or depletion of the other (Wong 2006; Perez and Barcelo 2008; Huhnerfuss and Shah 2009; Garrison et al. 2011), which may provide an insight into sources and fate of the chiral pharmaceutical in the environment. Enantiomeric fraction of propranolol has been applied to identify wastewater discharge in the environment and to make apportionment of contamination sources (Fono and Sedlak 2005). Besides, chirality of nonsteroid anti-inflammatory drugs (e.g., ibuprofen and naproxen), β-blockers (e.g., atenolol, metoprolol, and propranolol), and some illicit amphetamine-like drugs has been reported in wastewater and receiving waters (Bagnall et al. 2012; Buser et al. 1999; Kasprzyk-Hordern et al. 2010; Kasprzyk-Hordern and Baker 2012a, b; MacLeod et al. 2007; MacLeod and Wong 2010; Matamoros et al. 2009; Nikolai et al. 2006). Nevertheless, enantiomeric compositions and fate of chiral pharmaceuticals in the environment are far from well documented considering their huge family and wide detection.

As the largest class of synthetic antimycotics, azole antifungals have been detected worldwide in aquatic environment (Huang et al. 2010; Kahle et al. 2008; Lindberg et al. 2010; Peng et al. 2012; Van De Steene and Lamber 2008), causing ecological concerns due to their persistence (Kahle et al. 2008; Peng et al. 2012) and endocrine-disrupting potency (Baudiffier et al. 2013; Dilmaghanian et al. 2004; Hasselberg et al. 2008; Norgaard 2010; Trosken et al. 2004). The estimated half-lives of difenoconazole stereoisomers ranged from 169.0 to 238.9 days and from 177.7 to 315.0 days under aerobic and anaerobic conditions, respectively (Dong et al. 2013). Many azole antifungals contain one or more chiral centers in their structures and consequently have one or more enantiomer pairs. Chiral triazole fungicide difenoconazole was found to be stereoselective in both fungicidal activity and toxicity towards nontarget organisms (Dong et al. 2013). Fipronil was enantioselectively transformed by rainbow trout indicated by changes of its enantiomeric fraction over time (Knowick et al. 2006). Enantioselectivity was observed in biodegradation of chiral triazole fungicide triadimefon in soil (Li et al. 2011). Degradation of fenbuconazole and its chiral metabolites was observed to be enantioselective in soil under both aerobic and anaerobic conditions (Li et al. 2012). Difenoconazole showed moderately and slightly enantioselective degradation in soil under aerobic and anaerobic conditions, with enrichment of (2*S*,4*S*)- and (2*S*,4*R*)-difenoconazole, respectively (Dong et al. 2013). Therefore, it is necessary to reveal the enantiomer-specific fate of the azole antifungals in the environment in order to assess accurately their ecological effects as well as provide guidance for future application of these antifungals (Li et al. 2012; Dong et al. 2013).

In a previous work, we investigated the occurrence and behavior of commonly used azole antifungals in wastewater of China. Five azole antifungal pharmaceuticals (clotrimazole, econazole, fluconazole, ketoconazole, and miconazole) and two azole fungicides (propiconazole and tebuconazole) were detected at nanograms per liter in the wastewater and low micrograms per liter level in the sewage sludge (Peng et al. 2012). These antifungals may find their way into the environment through the discharge of wastewater and disposal of sludge. Econazole, ketoconazole, miconazole, and the two fungicides are chiral compounds and are generally marketed as racemic mixtures (Huang et al. 2012). However, there are scarce researches so far to characterize the chirality of these antifungals in the environment.

Thus, this work aimed to (1) elucidate the enantiomer-specific behavior of the chiral azole antifungals in wastewater during treatment in a typical municipal sewage treatment plant, (2) delineate chiral and achiral profiles of the commonly used azole antifungals in water and bed sediment of the Pearl River and its tributaries, South China, and (3) illustrate seasonal effects on the chiral and achiral profiles of the azole antifungals.

Material and experiments

Study area and sample collection

Located in the tropical/subtropical region of South China, the Pearl River Delta (PRD) is subjected to typical Asian monsoon climate—mild but rainy in spring, hot and humid in summer, and mild and dry in fall and winter, with an annual mean ambient temperature of 21–23 °C and annual relative humidity of about 75 %. It is also one of the most densely populated areas in China. High consumption of antifungal drugs in the PRD is expected due to the mild and relatively wet weather and large population. The treatment rates of municipal wastewater and industrial wastewater are around 70 and 95 %, respectively (http://www.gdstats.gov.cn/tjnj/2012/ml1.htm). All the treated and the rest of untreated wastewater are discharged into the Pearl River and the tributaries which merge into the South China Sea via the Pearl River Estuary (Fig. 1).

The investigated sewage treatment plant (STP) is located in Guangzhou (Fig. 1), a metropolis of the PRD, which has been detailed previously (Peng et al. 2012). A brief description of the STP and a sketch map of its treatment processes along with sampling points were provided in the Supporting Information (SI hereafter) and Fig. S1. Sample collection in the STP has also been depicted in detail elsewhere (Peng et al. 2012). Briefly, samplings were conducted in July 2010 (summer) and February 2011 (winter). In summer, only influent and final effluent samples were collected along the first and third treatment lines of the STP. Whereas in winter, the samples were collected at the inlet, the outlets of anaerobic tank, anoxic tank, secondary clarifier, and the final outlet along the first line in order to gain an insight into the enantiomer-specific behavior of the antifungals in various processes. Wastewater was collected hourly from 8:00 a.m. to 12:00 p.m. in amber glass bottles without headspace. Untreated solid from the grid chamber and dewatered sludge were collected in both seasons, whereas thickened sludge and recycled sludge were only collected in winter.

Fourteen sampling sites were set along the Pearl River watershed, eight in the mainstream Pearl River (PR1–PR8), three in Liuxi River (LX1–LX3), and one in each of the three major tributary rivers, Beijiang (BJ), Dongjiang (DJ), and Xijiang (XJ) near their confluences with the mainstream (Fig. 1). Water samples (40 L each) were collected in July 2010 (summer), December 2010 (winter), and October 2011 (fall) with a polymethyl methacrylate zone sampler. Samplings were conducted always during ebb period in order to reduce the influence of inflowing seawater. Sediment was collected with a stainless steel grab bucket (15 cm depth), wrapped with prebaked (450 °C) aluminum foil, and sealed in ziplock polyethylene bags.

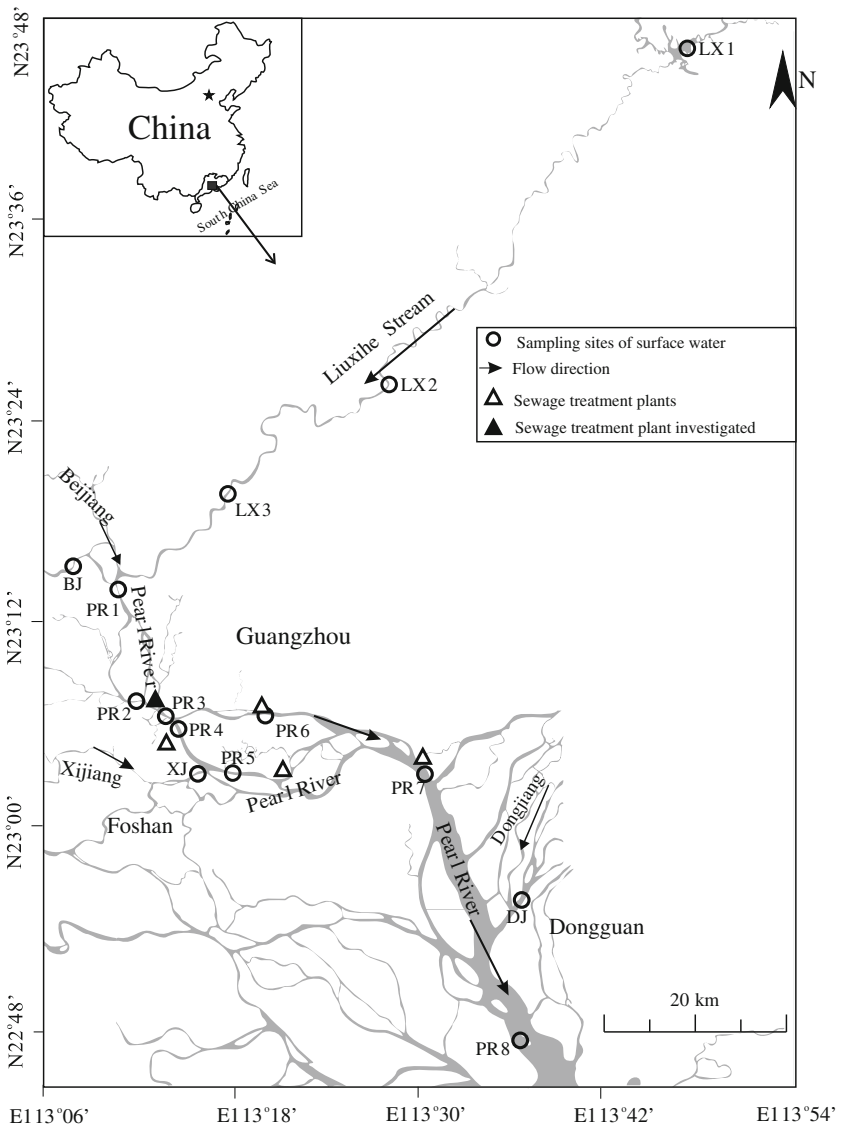

Fig. 1 Sketch map of the sampling sites in the Pearl River Delta

Sodium azide (0.5 g L^{-1}) was added into the water samples immediately after sampling to suppress potential bioactivities. All samples were kept in ice packs during transport to the laboratory. The hourly collected wastewater samples were then combined to obtain composite samples. All the water samples were stored in darkness at 4 °C until treatment within 48 h while sludge and the sediment samples were stored at −20 °C.

Sample treatment and analysis

The investigated azole antifungals and their major physicochemical properties have been described previously (Huang et al. 2010) and were also provided in Table S1. Propiconazole was excluded in this work because its two enantiomer pairs could not be identified due to lack of individual enantiomer standards (Huang et al. 2012). Details about sample preparation and analysis have been depicted elsewhere (Huang et al. 2010, 2012). Briefly, the water samples were filtered with 0.7 μm glass fiber filters (GFFs, Whatman, Buckinghamshire, UK). The filtrates were spiked with internal standards and enriched on Oasis HLB cartridges (Waters, Milford, MA, USA) using a Syncore Polyvap system with a solid-phase extraction (SPE) unit (Buchi, Flawil, Switzerland). Sediment, suspended particulate matter of the wastewater retained on GFFs, and sludge samples were lyophilized and homogenized, spiked with internal standards, and extracted by ultrasonic-assisted extraction. The extracts were diluted with high-purity water and treated by SPE as mentioned above.

Analysis was performed by an Agilent LC 1200 system coupled to an Agilent 6410 triple quadrupole MS with electrospray ionization in positive mode (Agilent, Palo Alto, CA, USA). The LC-MS/MS conditions have been detailed previously (Huang et al. 2010, 2012) and also were provided in the SI. Data acquisition was performed in multiple

Table 1 Partition coefficients (Log K_d) of the azole antifungals between water and bed sediment in the Pearl River watershed

	Log K_d			Log Kow[b]
	Water/bed sediment	Influent[a]	Final effluent[a]	
Clotrimazole	3.8±0.5	5.0±0.2	5.4±0.1	4.1/6.3
Econazole	3.6	5.0±0.0	5.3±0.3	5.5
Ketoconazole	3.7±1.1	4.9±0.2	4.9±0.3	4.3[c]
Miconazole	3.3±1.0	5.0±0.1	5.7±0.1	6.1

[a]Peng et al. (2012)
[b]Kahle et al. (2008)
[c]http://www.drugbank.ca/

reaction monitoring (MRM) mode. Ion transitions for individual compounds and MS parameters were provided in Table S2. Quantification was performed using internal standard method.

For achiral determination, the analytes were separated on an Agilent ZORBAX Eclipse XDB C18 rapid resolution high throughput narrow column (2.1×50 mm, 1.8 μm particle size).

Enantioseparation of econazole, miconazole, and tebuconazole was achieved on a 4×100-mm α_1-acid glycoprotein column (5.0 μm particle size, Chiral Technologies Europe, Illkirch, France). Ketoconazole enantiomers were separated on a 2×100-mm human serum albumin column (5.0 μm particle size, Chiral Technologies Europe, Illkirch, France). Typical MRM chromatograms were provided in Fig. S2.

For MS operation, the source temperature was 100 °C and the capillary voltage was 3.0 kV. Nitrogen was used as the desolvation gas at a temperature of 350 °C and a flow of 10 L min^{-1}. Nitrogen was also used as the nebulizer at a pressure of 40 psi.

Quality assurance and quality control

Quality assurance and quality control followed the procedures detailed previously (Huang et al. 2010, 2012). Recoveries were 75–103 % and limits of quantification were 0.5–7 ng L^{-1} in river water and 0.3–3 ng g^{-1} in sediment (Table S3). Duplicate analysis of randomly selected samples showed relative standard deviations within 20 % for the analytes (Table S4–S10). Concentrations of the analytes and enantiomers were presented only when signal-to-noise ratios (s/n) were ≥10 for quantitative ion transition and ≥3 for qualitative ion transition. The EF values for racemic standards (n=18) were 0.500±0.006, 0.500±0.007, 0.503±0.007, and 0.500±0.007 for econazole, ketoconazole, miconazole, and tebuconazole, respectively, independent on concentration (Huang et al. 2012; Table S3). Currently marketed cream drugs of econazole (Xi'an Janssen Pharmaceutical N.V., Xi'an, China), ketoconazole (Guangdong Shunfeng Pharmaceutical Co. Ltd, Foshan, China), and miconazole (Xi'an Janssen Pharmaceutical N.V., Xi'an, China) in China were determined to be racemic with EFs of 0.489, 0.511, and 0.500, respectively.

Statistical analysis

Data were processed with Origin 7.5 (OriginLab, Northampton, MA, USA). An azole in a sample was considered racemic when its EF fell within $X\pm3\sigma$ (n=18) of the EF of racemic standard, which means that the chiral azole antifungals in the samples were deemed racemic when the EFs fell in the range of 0.479–0.524, and otherwise were considered nonracemic.

Results and discussion

Chiral profiles of the azole antifungals in the wastewater and sludge

In the dissolved phase (filtrate) of the wastewater, enantiomers of miconazole were quantified at 0.5–9.0 ng L^{-1}. However, ketoconazole enantiomers were only quantifiable in the wastewater from the third line, ranging from 7 to 45 ng L^{-1}, whereas econazole enantiomers were not quantifiable in any sample (Table S4). On the contrary, the enantiomers of econazole, ketoconazole, and miconazole were widely detected in the suspended particulate matter of the wastewater from unquantifiable to 2,423 ng g^{-1} dry weight (Table S5). Both the dissolved and sorbed concentrations of ketoconazole enantiomers were significantly lower in the influent from the first line than from the third line (Tables S4 and S5), agreeing well with the previous result of the achiral analysis which was ascribed to addition of industrial wastewater and landfill leachate in the third line (Peng et al. 2012). The dissolved concentrations of all the enantiomers decreased after treatment in the STP (Table S4). In contrast, the sorbed concentrations of ketoconazole enantiomers decreased significantly after treatment, especially in the third line, whereas the sorbed concentrations of the enantiomers of econazole and miconazole showed only moderate to negligible variations (Table S5). Enantiomers of econazole, ketoconazole, and miconazole were detected in sludge samples at 4–1,194 ng g^{-1} dry weight (Table S6). Tebuconazole enantiomers were not quantitatively detected in either wastewater or sludge samples.

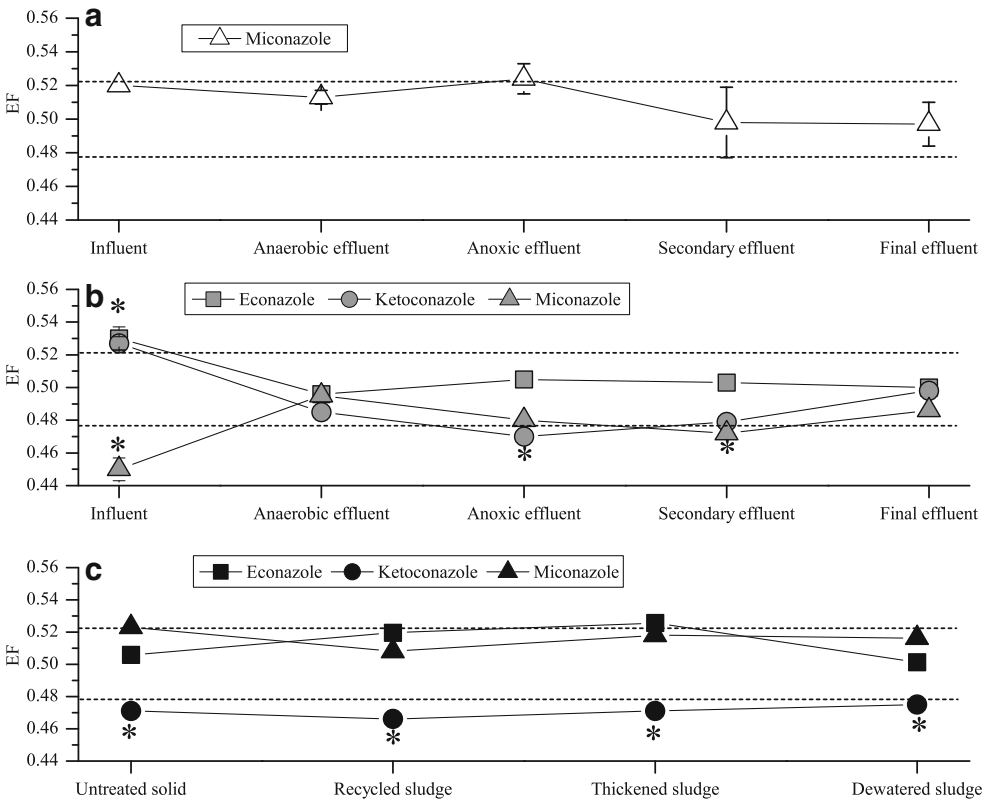

Fig. 2 Enantiomeric fraction of the azole antifungals in **a** dissolved phase, **b** suspended particulate matter of the wastewater, and **c** the sludge. *Error bar* represents absolute deviation (n=2). The azole antifungals with EFs between *dotted lines* are racemic. *Asterisk*: nonracemic

The EF of econazole in the dissolved phase of the wastewater could not be calculated due to unquantifiable enantiomers (Table S4). In summer (July 2010), EF of the sorbed econazole changed from 0.493 and 0.485 in the influent to 0.501 and 0.494 in the final effluent in the first and third treatment lines, respectively, while in winter (February 2011), the EF decreased from 0.530 in the influent to 0.496 after anaerobic bio-process and kept rather constant (0.500–0.505) till the final effluent (Fig. 2b and Table S5) in the first line and increased from 0.501 in the influent to 0.520 in the final effluent in the third line. The EF of econazole in sewage sludge fluctuated between 0.501 and 0.526 (Fig. 2c and Table S6) without seasonal difference (p=0.618).

EF of the dissolved ketoconazole in the wastewater remained constant (0.484–0.485) in summer and decreased from 0.484 in the influent to 0.477 in the effluent in winter (Table S4). In the suspended particulate matter of the wastewater, EF of the sorbed ketoconazole was also constant (0.513–0.515) in summer, whereas in winter, the EF decreased from 0.527 in the influent to 0.470 after anaerobic and anoxic processes and then increased to 0.497 in the final effluent in the first line and decreased from 0.511 in the influent to 0.474 in the final effluent in the third line (Fig. 2b and Table S5). Ketoconazole in the sewage sludge was racemic in summer and weakly nonracemic in winter (Fig. 2c and Table S6).

The EF of the dissolved miconazole in wastewater decreased from 0.497 in the influent to 0.468 in the final effluent in summer and fluctuated between 0.497 and 0.527 in winter (Fig. 2a and Table S4). In the suspended particulate of the wastewater, EF of the sorbed miconazole in summer increased from 0.485 and 0.470 to 0.493 and 0.505 in the first and third lines, respectively. In winter, the EF increased from 0.450 in the influent to 0.495 after anaerobic biodegradation and fluctuated between 0.472 and 0.486 thereafter in the first line, whereas it was constant in the third line (Fig. 2b and Table S5). Miconazole in the sludge was always racemic (Fig. 2c and Table S6).

The above results revealed that chiral azole antifungals in the influents were prominently racemic to weakly nonracemic. Hamdy and Brocks (2009) reported non-stereoselective metabolism of ketoconazole by rat liver microsomes. Enantioselective pharmacokinetics of econazole and miconazole was rarely reported. Nevertheless, they may experience similar pharmacokinetics as ketoconazole due to their similar structures and pharmacology. In addition, these drugs are mainly administered topically, which means that most of them may be directly removed from treated intact skins by washing and subsequently be emitted into sewage. Thus, racemic composition of the chiral azole antifungals in the raw wastewater is reasonable.

Previous researches have revealed enantioselective degradation of several chiral pharmaceuticals, e.g., ibuprofen, naproxen, and β-blockers under anaerobic, anoxic, and aerobic conditions (Buser et al. 1999; Fono and Sedlak 2005; MacLeod et al. 2007; Matamoros et al. 2009; Kasprzyk-Hordern and Baker 2012a). However, in this work, the EFs

of the chiral azoles in dissolved phase of the wastewater varied only slightly despite significant reduction of the concentrations (Fig. 2a and Table S4), suggesting very low enantioselective biodegradation.

In contrast, in the particulate phase of the wastewater, the chiral azoles changed from weakly/moderately nonracemic in the influent to racemic after anaerobic process in winter without obvious variations of the concentrations (Fig. 2b and Table S5). Hamdy and Brocks (2009) revealed that biomacromolecules, such as proteins and adipose, were able to enantioselectively react with chiral compounds. The biomacromolecules expectedly abundant in the suspended particulate matter of the influent are likely removed significantly after anaerobic biotreatment, possibly leading to change of the enantiomeric compositions of the sorbed chiral azoles.

In addition, in the third treatment line of the STP, the sorbed miconazole changed from slightly nonracemic (EF 0.470) to racemic (EF 0.505) in summer while the sorbed ketoconazole changed from racemic (EF 0.511) to slightly nonracemic (EF 0.474) in winter after treatment, both accompanying with significant reduction of the concentrations (Table S5), suggesting potential presence of weakly enantioselective transformation. Dong et al. (2013) observed only moderate enantioselective degradation of difenoconazole in soil after 120 days under aerobic condition and even lower under anaerobic conditions. Thus, weak enantioselectivity of the chiral azole pharmaceuticals in the suspended particulate matter of the wastewater during treatment is probably associated with the short hydraulic retention time in the STP (Fig. S1).

Enantiomerization, i.e., transformation of one enantiomer to the other, has been reported for some chiral pharmaceuticals (MacLeod and Wong 2010; Kasprzyk-Hordern and Baker 2012b), herbicide, and pesticides (Buerge et al. 2013; Buser and Muller 1997; Muller and Buser 1995, 1997; Li et al. 2011) in waters and soils. Nevertheless, Buerge et al. (2006) observed no enantiomerization for cyproconazole and epoxiconazole in soils. Dong et al. (2013) found no evidence of interconversion for stereoisomers of difenoconazole after 120 days of incubation under both aerobic and anaerobic conditions. However, it is not known whether enantiomerization occurred for the chiral azole antifungals in the wastewater based on available information.

It is also interesting to note that the EFs of the chiral azoles in the dissolved phase were different from those in the particulate phase of the wastewater ($p<0.01$). For instance, the EF of the sorbed miconazole was generally lower than that of the dissolved miconazole (Fig. 2 and Tables S4 and S5), suggesting that different behaviors, such as enantiomer-specific biodegradation and/or reactions, probably occurred to the chiral azoles in the dissolved and particulate phases of the wastewater.

The EFs of the chiral azoles in the sewage sludge were quite constant, corresponding well with the fairly constant concentrations (Fig. 2c and Table S6), indicating that the thickening and dewatering processes did not affect both the concentrations and enantiomeric compositions of the chiral azole antifungals.

The EFs of miconazole in the dissolved phase of the wastewater and sewage sludge were higher in winter than in summer (the differences were small but statistically significant with $p=0.04$ and 0.01, respectively, Tables S4 and S6), whereas the EF of ketoconazole in the sludge was higher in summer than in winter ($p=0.02$, Table S6). Kasprzyk-Hordern and Baker (2012a) reported seasonal differences in stereomeric compositions of illicit amphetamine-like drugs, venlafaxine, and atenolol in the wastewater of the UK and ascribed the difference to variation of usage pattern and microbial activities. However, detailed discussions cannot be made about the seasonal differences in the enantiomeric compositions of the azole antifungals based on available information.

Azole antifungals in the river water

The azole antifungals were widely detected in the river water. Fluconazole had the highest concentration (maximum and median concentrations of 109.6 and 27.9 ng L^{-1}, respectively), likely due to its strong hydrophilicity (Table S1) and poor elimination in wastewater that has been revealed previously (Peng et al. 2012). The second most abundant was miconazole, followed by propiconazole and tebuconazole. Clotrimazole was detected at low level, whereas ketoconazole and econazole were only occasionally detected at around 1 ng L^{-1} (Fig. 3a).

Spatially, Liuxi River usually had relatively lower concentrations. Both detection frequencies and concentrations of the azoles in the other three tributary rivers (sites BJ, XJ, and DJ) were in the same range as those in the mainstream Pearl River (Fig. S3a and Table S7), which is reasonable considering that Foshan and Dongguan where these tributaries flow through (Fig. 1), respectively, are also densely populated areas (http://www.gdstats.gov.cn/tjnj/2012/ml1.htm).

Clotrimazole, fluconazole, and miconazole in the river water showed relatively higher concentrations in winter than in spring and summer ($p<0.03$) (Fig. 3b and Table S7). Seasonal variations of the azole antifungals in the wastewater have been elucidated previously. Higher concentrations were observed in winter for clotrimazole and miconazole but in summer for fluconazole in the untreated wastewater. However, the seasonal differences were smoothed out in the final effluent prior to discharge (Peng et al. 2012). Taking into consideration the annual precipitation (Table S11) and wastewater treatment rate of >70 % in the studied area, dilution effect by rainfall was thus proposed as the governing factor affecting the seasonal pattern of these azole pharmaceuticals in the river waters. Previous researches have also revealed dilution effects by water flows on seasonal variations

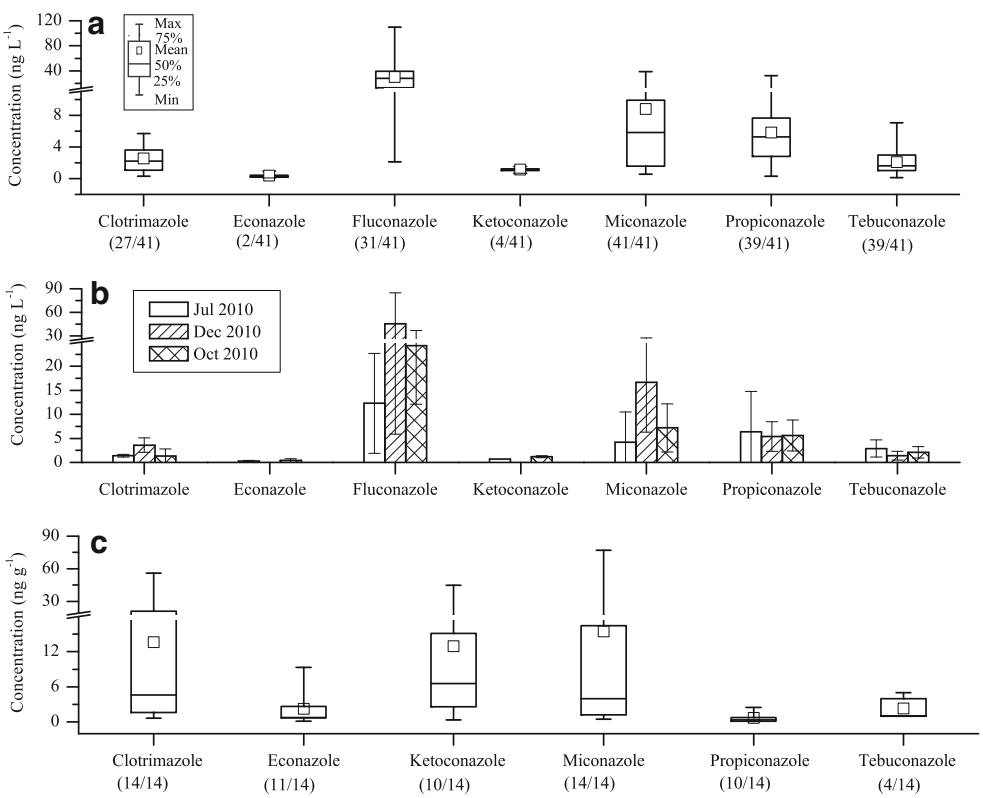

Fig. 3 The azole antifungals in the rivers: **a** distribution in water; **b** seasonal pattern in water; **c** distribution in bed sediment. The detection frequency is given in *parentheses* as number of quantifiable samples/number of analyzed samples. *Error bars* represent the standard deviation in the bar plot

for several pharmaceuticals in surface waters (Daneshvar et al. 2010; Huang et al. 2011; Vieno et al. 2005; Yu et al. 2011). On the other hand, the two azole pesticide concentrations usually showed no obvious seasonal difference ($p>0.08$), possibly associated with their usage. However, no further discussion can be made due to lack of relevant usage statistics. No seasonal

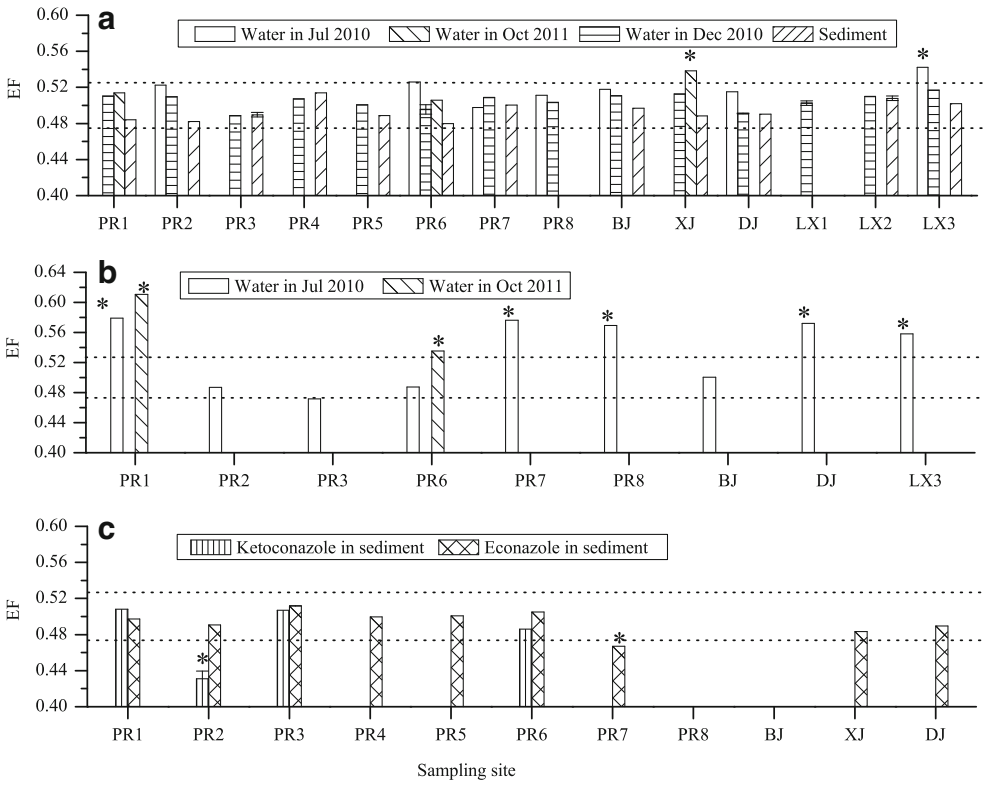

Fig. 4 Enantiomeric fraction of the chiral azole antifungals in the rivers: **a** Miconazole in water and bed sediment; **b** tebuconazole in water; **c** ketoconazole and econazole in bed sediment. For sampling sites, see Fig. 1. The azole antifungals with EFs between *dotted lines* are racemic. *Asterisk*: nonracemic. *Error bars* represent the standard deviation. EF values not calculated due to unquantifiable enantiomers are not shown in the figure

comparison could be reasonably made for econazole and ketoconazole due to limited detections and low concentrations.

Azole antifungals in the bed sediment

Fluconazole was not analyzed in the bed sediment. The two triazole biocides were always detected at around 1 ng g^{-1} dry weight. On the contrary, the four imidazole pharmaceuticals were widely detected with a maximum concentration of 76.9 ng g^{-1} dry weight. Miconazole was the most abundant, followed successively by clotrimazole, ketoconazole, and econazole (Fig. 3c), which was similar to the distribution pattern in sewage sludge reported previously (Peng et al. 2012). Spatially, sediment in the mainstream Pearl River had the highest concentration, followed in order of the three tributaries and Liuxi River (Fig. S3b and Table S8).

The sediment/water partition coefficients (Log K_d) were 3.3–3.8 for the imidazole pharmaceuticals (Table 1), lower than those between the dissolved and particulate phases of the wastewater (Peng et al. 2012), and the values of Log Kow, however, are still suggesting sediment as an important reservoir for the imidazole pharmaceuticals in the rivers. No obvious correlation was found between the azole concentrations and contents of total organic carbon (Fig. S4). Mechanism of the imidazole antifungals' absorption on sediment in the rivers needs further research. As in sewage sludge, concentrations of the imidazole pharmaceuticals in the river sediments showed closely positive correlations with each other (Fig. S5), demonstrating their similar sources and/or environmental fate in the sediment.

Chiral profiles of the azole antifungals in the rivers

Enantiomers of econazole and ketoconazole were not quantifiable in the river water. Miconazole in the river water was predominantly racemic with only a few exceptions at sites LX3 (EF 0.542) in summer and XJ (EF 0.538) in fall (Fig. 4a and Table S9). On the other hand, tebuconazole was widely nonracemic with EFs of 0.467–0.611 (Fig. 4b). Seasonally, miconazole in the river water showed higher EF in summer ($p<0.01$) than in the other seasons, which was contrary to that in the dissolved phase of wastewater mentioned above. Kasprzyk-Hordern and Baker (2012b) revealed disagreement in stereoisomeric compositions of some pharmaceuticals, e.g., ephedrine and atenolol, in the wastewater and the receiving waters of the UK and ascribed it to different consortia of microorganisms and/or environmental conditions involved in stereoselective degradation of the pharmaceuticals during wastewater treatment and in the receiving waters. Thus, the result in this work suggested that different enantioselective processes probably occurred to miconazole in the rivers and wastewater. However, extensive discussion cannot be made based on limited data available. Seasonal variations of enantiomeric composition of tebuconazole cannot be made because its enantiomers were only occasionally detected in fall and not detected in winter (Fig. 4b).

In sediment samples, the chiral imidazoles were mostly racemic (EF 0.482–0.514) except ketoconazole at site PR2 and econazole at site PR7 (Fig. 4 and Table S10). Econazole and miconazole have previously been found to be quite persistent in the wastewater during treatment in the STP, with transformation rate of <20 % (Peng et al. 2012). This result probably indicated persistence of the imidazoles antifungal pharmaceuticals in the river sediment. No statistically spatial differences were observed in the enantiomeric fractions of the chiral azoles (Fig. 4).

Conclusion

In summary, the imidazole antifungals were racemic to moderately nonracemic in the raw wastewater, with EFs of 0.484–0.527 and 0.450–0.530 in the dissolved and particulate phases, respectively. The EFs did not vary significantly after treatment in the STP even if the concentrations decreased significantly, suggesting weakly enantioselective degradation of the chiral azole antifungals. Different enantiomer-specific biodegradation and/or reactions probably occurred to the chiral azoles in the dissolved and particulate phases of the wastewater. Miconazole was generally racemic, whereas tebuconazole was widely nonracemic in the river water and all the chiral imidazoles were predominantly racemic in the bed sediment. Seasonal differences were observed for the chiral and achiral profiles of the azole antifungals in the wastewater and river water. The mechanism of enantiomeric behavior of the chiral azole antifungals in the environment warrants further research.

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References

Bagnall JP, Evans SE, Wort MT, Lubben AT, Kasprzyk-Hordern B (2012) Using chiral liquid chromatography quadrupole time-of-flight mass spectrometry for the analysis of pharmaceuticals and illicit drugs in surface and wastewater at the enantiomeric level. J Chromatogr A 1249:115–129

Baudiffier D, Hinfray N, Ravaud C, Creusot N, Chadili E, Porcher J-M, Schulz RW, Brion F (2013) Effect of in vivo chronic exposure to clotrimazole on zebrafish testis function. Environ Sci Pollut Res. doi:10.1007/s11356-013-1474-7

Buerge IJ, Poiger T, Muller MD, Buser HR (2006) Influence of pH on the stereoselective degradation of the fungicides epoxiconazole and cyproconazole in soils. Environ Sci Technol 40:5443–5450

Buerge IJ, Muller MD, Poiger T (2013) The chiral herbicide beflubutamid (II): enantioselective degradation and enantiomerization in soil, and formation/degradation of chiral metabolites. Environ Sci Technol. doi:10.1021/es301877n

Buser HR, Muller MD (1997) Conversion reactions of various phenoxyalkanoic acid herbicides in soil. 2. Elucidation of the enantiomerization process of chiral phenoxy acids from incubation in a D2O/soil system. Environ Sci Technol 31:1960–1967

Buser HR, Poiger T, Muller MD (1999) Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater. Environ Sci Technol 33:2529–2535

Daneshvar A, Svanfelt J, Kronberg L, Weyhenmeyer GA (2010) Winter accumulation of acidic pharmaceuticals in a Swedish river. Environ Sci Pollut Res 17:908–916

Dilmaghanian S, Gerber JG, Filler SG, Sanchez A, Gal J (2004) Enantioselectivity of inhibition of cytochrome P450 3A4 (CYP3A4) by ketoconazole: testosterone and methadone as substrates. Chirality 16:79–85

Dong F, Li J, Chankvetadze B, Cheng Y, Xu J, Liu X, Li Y, Chen X, Bertucci C, Tedesco D, Zanasi R, Zheng Y (2013) Chiral triazole fungicide difenoconazole: absolute stereochemistry, stereoselective bioactivity, aquatic toxicity, and environmental behavior in vegetables and soil. Environ Sci Technol 47(7):3386–3394

Fono LJ, Sedlak DL (2005) Use of the chiral pharmaceutical propranolol to identify sewage discharges into surface waters. Environ Sci Technol 39:9244–9252

Garrison AW, Avants JK, Jones WJ (2011) Microbial transformation of triadimefon to triadimenol in soils: selective production rates of triadimenol stereoisomers affect exposure and risk. Environ Sci Technol 45:2186–2193

Hamdy DA, Brocks DR (2009) Nonlinear stereoselective pharmacokinetics of ketoconazole in rat after administration of racemate. Chirality 21:704–712

Hasselberg L, Westerberg S, Wassmur B, Celander MC (2008) Ketoconazole, an antifungal imidazole, increases the sensitivity of rainbow trout to 17[alpha]-ethynylestradiol exposure. Aquat Toxicol 86:256–264

Huang Q, Yu Y, Tang C, Peng X (2010) Determination of commonly used azole antifungals in various waters and sewage sludge using ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 1217:3481–3488

Huang Q, Yu Y, Tang C, Zhang K, Cui J, Peng X (2011) Occurrence and behavior of non-steroidal anti-inflammatory drugs and lipid regulators in wastewater and urban river water of the Pearl River Delta, South China. J Environ Monit 13:855–862

Huang Q, Zhang K, Wang Z, Wang C, Peng X (2012) Enantiomeric determination of azole antifungals in wastewater and sludge by liquid chromatography–tandem mass spectrometry. Anal Bioanal Chem 403:1751–1760

Huhnerfuss H, Shah MR (2009) Enantioselective chromatography—a powerful tool for the discrimination of biotic and abiotic transformation processes of chiral environmental pollutants. J Chromatogr A 1216:481–502

Kahle M, Buerge IJ, Hauser A, Muller MD, Poiger T (2008) Azole fungicides: occurrence and fate in wastewater and surface waters. Environ Sci Technol 42:7193–7200

Kasprzyk-Hordern B, Baker DR (2012a) Enantiomeric profiling of chiral drugs in wastewater and receiving waters. Environ Sci Technol 46:1681–1691

Kasprzyk-Hordern B, Baker DR (2012b) Estimation of community-wide drugs use via stereoselective profiling of sewage. Sci Total Environ 423:142–150

Kasprzyk-Hordern B, Kondakal VVR, Baker DR (2010) Enantiomeric analysis of drugs of abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry. J Chromatogr A 1217:4575–4586

Konwick BJ, Garrison AW, Black MC, Avants JK, Fisk AT (2006) Bioaccumulation, biotransformation, and metabolite formation of fipronil and chiral legacy pesticides in rainbow trout. Environ Sci Technol 40:2930–2936

Li Z, Zhang Y, Li Q, Wang W, Li J (2011) Enantioselective degradation, abiotic racemization, and chiral transformation of triadimefon in soils. Environ Sci Technol 45:2797–2803

Li Y, Dong F, Liu X, Xu J, Li J, Kong Z, Chen X, Zheng Y (2012) Environmental behavior of the chiral triazole fungicide fenbuconazole and its chiral metabolites: enantioselective transformation and degradation in soils. Environ Sci Technol 46:2675–2683

Lindberg RH, Fick J, Tysklind M (2010) Screening of antimycotics in Swedish sewage treatment plants—waters and sludge. Water Res 44:649–657

MacLeod SL, Wong CS (2010) Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments. Water Res 44:533–544

MacLeod SL, Sudhir P, Wong CS (2007) Stereoisomer analysis of wastewater-derived [beta]-blockers, selective serotonin reuptake inhibitors, and salbutamol by high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 1170:23–33

Matamoros V, Hijosa M, Bayona JM (2009) Assessment of the pharmaceutical active compounds removal in wastewater treatment systems at enantiomeric level. Ibuprofen and naproxen. Chemosphere 75:200–205

Muller MD, Buser HR (1995) Environmental behavior of acetamide pesticide stereoisomers. 2. Stereoselective and enantioselective degradation in sewage-sludge and soil. Environ Sci Technol 29:2031–2037

Muller MD, Buser HR (1997) Conversion reactions of various phenoxyalkanoic acid herbicides in soil.1. Enantiomerization and enantioselective degradation of the chiral 2-phenoxypropionic acid herbicides. Environ Sci Technol 31:1953–1959

Nikolai LN, McClure EL, MacLeod SL, Wong CS (2006) Stereoisomer quantification of the beta-blocker drugs atenolol, metoprolol, and propranolol in wastewaters by chiral high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 1131:103–109

Norgaard KB, Cedergreen N (2010) Pesticide cocktails can interact synergistically on aquatic crustaceans. Environ Sci Pollut Res 17:957–967

Peng X, Huang Q, Zhang K, Yu Y, Wang Z, Wang C (2012) Distribution, behavior and fate of azole antifungals during mechanical, biological, and chemical treatments in sewage treatment plants in China. Sci Total Environ 426:311–317

Perez S, Barcelo D (2008) Applications of LC-MS to quantitation and evaluation of the environmental fate of chiral drugs and their metabolites. Trends Anal Chem 27:836–846

Stanley JK, Ramirez AJ, Chambliss CK, Brooks BW (2007) Enantiospecific sublethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. Chemosphere 69:9–16

Trosken ER, Scholz K, Lutz RW, Volkel W, Zarn JA, Lutz WK (2004) Comparative assessment of the inhibition of recombinant human CYP19 (aromatase) by azoles used in agriculture and as drugs for humans. Endocr Res 30:387–394

Van De Steene JC, Lamber WE (2008) Validation of a solid-phase extraction and liquid chromatography-electrospray tandem mass spectrometric method for the determination of nine basic pharmaceuticals in wastewater and surface water. J Chromatogr A 1182:153–160

Vieno NM, Tuhkanen TT, Kronberg L (2005) Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ Sci Technol 39:8220–8226

Wong CS (2006) Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. Anal Bioanal Chem 386:544–558

Yu Y, Huang Q, Wang Z, Zhang K, Tang C, Cui J, Feng J, Peng X (2011) Occurrence and behavior of pharmaceuticals, steroid hormones, and endocrine-disrupting personal care products in wastewater and the recipient river water of the Pearl River Delta, South China. J Environ Monit 13:871–878