Distribution, behavior and fate of azole antifungals during mechanical, biological, and chemical treatments in sewage treatment plants in China

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ABSTRACT

Residue of azole antifungals in the environment is of concern due to the environmental risks and persistence. Distribution, behavior, and fate of frequently used azole antifungal pharmaceuticals were investigated in wastewater at two sewage treatment plants (STPs) in China. Fluconazole, clotrimazole, econazole, ketoconazole, and miconazole were constantly detected at 1–1834 ng L^{-1} in the wastewater. The latter four were also ubiquitously detected in sewage sludge. Fluconazole passed through treatment in the STPs and largely remained in the final effluent. On the contrary, biotransformation and sorption to sludge occurred to the other azoles. Ketoconazole was more readily bio-transformed, whereas clotrimazole, econazole, and miconazole were more likely to be adsorbed onto and persisted in sewage sludge. Lipophilicity plays the governing role on adsorption. The highest concentrations in the raw wastewater were observed in winter for the azole pharmaceuticals except for fluconazole. The seasonal difference was smoothed out after treatment in the STPs.

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1. Introduction

Azole substances are widely used as antifungal agents in human and veterinary pharmaceuticals, agricultural fungicides, and biocides in various products (Zarn et al., 2003). In China, around 10 azole compounds are frequently used as antifungal pharmaceuticals, e.g., ketoconazole, clotrimazole, and miconazole. These azole pharmaceuticals are administered topically (e.g. cream and suppository) and orally, or are injected intravenously, depending on substance. Topical application of pharmaceuticals generally results in a higher emission of the active ingredient due to relatively small absorption of 5–10% via skin, which means that 90–95% of the remaining ingredient may be directly removed from treated skin by washing and will subsequently be emitted into sewage (Letzel et al., 2009). The massive usage and higher emissions may therefore lead to substantial amounts of azole residues in the environment.

Azole antifungals are suspected to potentially affect endocrine systems of aquatic vertebrates because they interact with several cytochrome P-450 enzymes and inhibit CYP19 that takes part in hormone conversions (Ankley et al., 2005; Gyllenhammar et al., 2009; Hasselberg et al., 2008; Kenneke et al., 2009; McKinlay et al., 2008; Zarn et al., 2003). Clotrimazole was found to interfere with aromatase activity in gonads and brain of larvae of *Xenopus tropicalis* (Gyllenhammar et al., 2009). Some azole compounds, e.g.,

clotrimazole and ketoconazole, are quite persistent and are likely to partition into solid environmental matrices (Huang et al., 2010; Lindberg et al., 2010; Kahle et al., 2008; Thomas and Hilton, 2004). Solid matrices, e.g., sediment and soil, may subsequently become secondary sources of contamination of azole substances in the environment. In addition, potential bioaccumulation of these azole compounds is also a concern although considering the lipophilicity indicated by the high Log$K$ow values (Kahle et al., 2008).

Azole antifungals have been detected in wastewater and surface water in Europe (Berenzen et al., 2005; Gasperi et al., 2008; Kahle et al., 2008; Lindberg et al., 2010; Peschka et al., 2007; Roberts and Thomas, 2006; Stamatis et al., 2010; Thomas and Hilton, 2004; Van De Steene and Lambert, 2008). Persistence of clotrimazole, fluconazole, tebuconazole, and propiconazole has also been reported (Kahle et al., 2008; Lindberg et al., 2010).

STPs have been demonstrated to be one of the most important point sources of various pharmaceutical residues in the environment due to incomplete elimination of these substances (McArdell et al., 2003). Fate (e.g., adsorption, bio- and chemical transformation) and transport of several groups of antibacterials and other pharmaceuticals during wastewater treatment have been well studied through bench-scale laboratory tests as well as in full-scale STPs (Behera et al., 2011; Gobel et al., 2005; Junker et al., 2006; Lindberg et al., 2006; McArdell et al., 2003; Plosz et al., 2010; Radjenovic et al., 2009; Zorita et al., 2009). In contrast, in-depth studies about behavior of the azole antifungals during various treatments in STPs, such as mechanical, biological, and chemical processes, are still lacking in spite of some works about distribution of these compounds in liquid phase

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(Berenzen et al., 2005; Kahle et al., 2008; Roberts and Thomas, 2006). Therefore, much is yet to be done to get insight into fate of azole antifungals in the environment.

This work aimed at investigating the occurrence and behaviors of frequently used azole antifungals in municipal wastewater in China. Concentrations and mass loads of the azole substances were determined in raw sewage, in effluents after major treatment steps, and in sludge at two STPs located in Guangzhou, a metropolis of Southern China, in order to elucidate their distribution, transport, and fate in wastewater of China. Since climatic conditions may affect environmental occurrence and fate of pharmaceuticals (Choi et al., 2008; Loraine and Pettigrove, 2006; McArdell et al., 2003; Vieno et al., 2005), samplings were performed in different seasons to see the seasonal variations. The results would also allow us to trace pathways of the azole antifungals into the environment.

2. Materials and methods

2.1. Chemicals

Clotrimazole, econazole nitrate, fluconazole, ketoconazole, miconazole nitrate, propiconazole, and tebuconazole were purchased from Sigma-Aldrich (St. Louis, MO, USA). Clotrimazole-d_5, fluconazole-d_4, and carbamazepine-d_{10} were bought from C/D/N isotopes (Pointe-Claire, Quebec, Canada). Miconazole-d_5 Nitrate and ketoconazole-d_8 were purchased from Toronto Research Chemicals (North York, ON, Canada) and Campro Scientific (Veenendaal, The Netherlands), respectively. HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). High purity water was generated by a Milli-Q ultra-pure water system (Millipore, Billerica, MA, USA).

2.2. Sampling

The two STPs, named as STP A and STP B, have been detailed previously (Peng et al., 2011, Fig. S1). They can be representative of typical municipal wastewater sources and treatment processes in China. Briefly, STP A handles a mixture of domestic and industrial wastewater with a treatment capacity of 30,000 $m^3 d^{-1}$ and uses conventional activated sludge treatment consisting successively of aerated grit filtering, primary sedimentation, aerated active sludge treatment, and secondary clarification, serving a population of about 400,000. STP B is a large scale STP with three parallel-operated treatment lines and serves a population of 1,500,000. The first (STP B1) and second lines treat predominantly domestic wastewater (>90%) with a total treatment capacity of 330,000 $m^3 d^{-1}$ and use identical treatment process including a aerated grit chamber, a bioreactor comprised successively of anaerobic, anoxic, and aerobic processes, and a secondary clarifier. The third line (STP B3) also accepts a certain amount of industrial wastewater and landfill leachate (about 300 $m^3 d^{-1}$ during dry season and ≥1800 $m^3 d^{-1}$ during wet season) besides domestic wastewater, with a treatment capacity of 22000 $m^3 d^{-1}$. Its bioreactor consists successively of anoxic, anaerobic, and aerobic processes. Ultraviolet disinfection (UV) and chlorination were performed in STP A and STP B, respectively before final discharge. The sludge retention age is 10 d in both STPs and the production of dewatered sludge is 25 and 400 t per day in STP A and STP B, respectively.

Samplings were performed in May (spring) and November (fall) 2008 in both STPs. Additional samplings were performed in STP B in July 2010 (summer) and February 2011 (winter) to fully investigate the seasonal variations. In order to investigate in-depth the behavior of the azole antifungals, raw wastewater and effluents after each major treatment, i.e., effluents after primary sedimentation, secondary clarification, and UV disinfection in STP A, and effluents after anaerobic and anoxic biological processes, secondary clarification, and chlorination in STP B1, were collected. The influent and final effluent samples were also collected along STP B3 in order to estimate the mass loads. Furthermore, influent and final effluent samples were collected every day over 1-week period in STP B1 and STP B3 from 15 to 21 February 2011 to illustrate the daily variations of the targets in wastewater. Dewatered sludge samples were collected in all seasons. In addition, untreated solid was also collected in summer, fall, and winter from the grit chambers.

Wastewater was collected hourly from 8:00 to 12:00 to build a 40-L composite sample because it was not allowed to set composite samplers in the STPs. Untreated solid and dewatered sludge were collected as grab samples. The wastewater was sampled in amber glass bottles without headspace. Sodium azide was added immediately to suppress bioactivity (Benotti et al., 2009). The untreated solid and dewatered sludge were wrapped with prebaked (450 °C) aluminum foil and sealed in zip lock polyethylene bags. Samples were kept on ice during transport to the laboratory. The wastewater samples were stored in darkness at 4 °C until treatment within 48 h and sludge samples were stored at −20 °C.

2.3. Analysis and quality control

The analytical details were provided previously (Huang et al., 2010). Briefly, wastewater samples were filtered with prebaked 0.7-μm glass fiber filters (GF/F, Whatman, Maidstone, England) (Behera et al., 2011; Huang et al., 2010). The filtrate and suspended particulate matter retained on GF/F were analyzed separately.

The filtrate was spiked with the internal standards (Table S1) at 100 ng L^{-1} for each compound and concentrated by solid phase extraction with an HLB cartridge (Waters, Massachusetts, USA). The analytes were determined with ultra-high performance liquid chromatography–tandem mass spectrometry in multi-reaction monitoring mode. The LC-MS/MS conditions were provided in Supplementary material (Tables S2, S3).

The suspended particulate matter and sludge samples were lyophilized, homogenized, spiked with the internal standards, and extracted by ultrasonic-assisted extraction (USE) with methanol containing 0.1% formic acid. The USE extract was diluted with high purity water to bring the methanol content to <2% and subsequently treated and analyzed as described above for the filtrates.

Detailed QA/QC procedure was described previously, which included procedural blanks (high purity water for liquid and clean quartz sand for solid samples), recovery tests, laboratory spikes (high purity water fortified with the analytes), and duplicate analysis. Recoveries and limits of quantification of the analytes were 41–110% and 0.5–6 ng L^{-1}, respectively. No quantifiable analytes were detected in the blanks. Relative standard deviations for duplicate analysis of environmental samples were always within 16%. Potential impact of sodium azide on the analytes in samples was investigated by adding sodium azide into the laboratory spikes. No obvious difference was observed in the concentrations between the fortified high purity water with and without sodium azide over 48 h storage at 4 °C.

2.4. Mass loads

Mass loads (ML) were calculated for each azole antifungal using the following equation:

$$ML\left(mg\ day^{-1}\right) = Ci \times Qj$$

where Ci is the measured concentration of the azole compound and Qj is the flow rate of the wastewater or production of dewatered sludge.

3. Results and discussions

3.1. Concentrations and mass loads

Fluconazole was only analyzed in winter samples of STP B. The concentrations were 22–170 ng L^{-1} and 50–139 ng L^{-1} in the influent and final effluent, respectively (Figs. 1 and 2), falling in the range reported for wastewater of Switzerland (Kahle et al., 2008) and Sweden (Lindberg et al., 2010). Fluconazole can be prescribed as oral pills and capsules (Table S1). Unused pharmaceuticals may be discarded into garbage and subsequently enter landfill leachate. This may explain the higher fluconazole concentrations in STP B3 (77–170 ng L^{-1}) than those in STP B1 (22–101 ng L^{-1}). The mean influent mass loads of fluconazole normalized by the served population were 0.0253–0.0472 mg d^{-1} person^{-1} (Table 1), also comparable to those in Swiss wastewater (Kahle et al., 2008).

The other four azole pharmaceuticals were analyzed in all samples. Ketoconazole was the only azole pharmaceutical detected in dissolved phase (filtrate) of the wastewater in STP A (<MQL-45 ng L^{-1}). Whereas in the dissolved phase of wastewater in STP B, clotrimazole (<MQL-35 ng L^{-1}), miconazole (6–26 ng L^{-1}), and ketoconazole (not detected-85 ng L^{-1}) were frequently detected. However, concentration of dissolved econazole was always ≤1 ng L^{-1} (Fig. 1). Kahle et al. (2008) reported same range of dissolved concentration for clotrimazole (12–78 ng L^{-1}) in Swiss wastewater. Nevertheless, clotrimazole, econazole, and miconazole were not quantitatively detected in dissolved phase of wastewaters in Sweden (Lindberg et al., 2010).

On the other hand, the four azole pharmaceuticals were ubiquitously detected in suspended particulate matter of the wastewater samples. The overall concentrations combining dissolved and particulate phases of the wastewater samples are shown in Fig. 1.

Ketoconazole was the most abundant in the influent of STP A (136–260 ng L^{-1}). In the raw wastewater of STP B1, clotrimazole was the most abundant (256–1834 ng L^{-1}), followed by miconazole (241–1086 ng L^{-1}) and ketoconazole (41–384 ng L^{-1}). However, in the influent of STP B3, the highest total concentrations were generally found for ketoconazole (66–856 ng L^{-1}), followed by clotrimazole and miconazole (76–227 ng L^{-1}, Fig. 2). As mentioned above, STP A treats a mixture of domestic and industrial wastewaters. STP B1 treats predominantly domestic wastewater while STP B3 accepts a certain amount of domestic wastewater and municipal landfill leachate. The difference in distribution pattern of the azoles between the influent samples maybe ascribed to the different sources of wastewater received by the STPs. Econazole was the lowest in all wastewater samples (6–82 ng L^{-1}). The population-normalized influent mass loads (0.0013–0.2778 mg d^{-1} person^{-1}) of the azoles obtained in this work (Table 1) are obviously higher than those in wastewaters of Switzerland (Kahle et al., 2008). Except for fluconazole, concentrations of the azole pharmaceuticals decreased significantly after treatment (Figs. 1 and 2).

Concentrations of the azole pharmaceuticals in the raw wastewater of STP B1 were relatively higher on Tuesday and Wednesday and were fairly constant in the other days. In the influent of STP B3, the azole concentrations were quite constant from Tuesday to Friday while showed slight fluctuation from Saturday to Monday. However, the fluctuation was smoothed out after treatment in the STP, resulting in similar effluent concentrations in the whole week (Fig. 2). Nevertheless, more data are needed to clarify the reason for the concentration fluctuation in the influent samples.

Fluconazole was not analyzed in sludge samples considering that it was not detected in sludge in previous report (Lindberg et al., 2010). The other four azole pharmaceuticals were detected in all
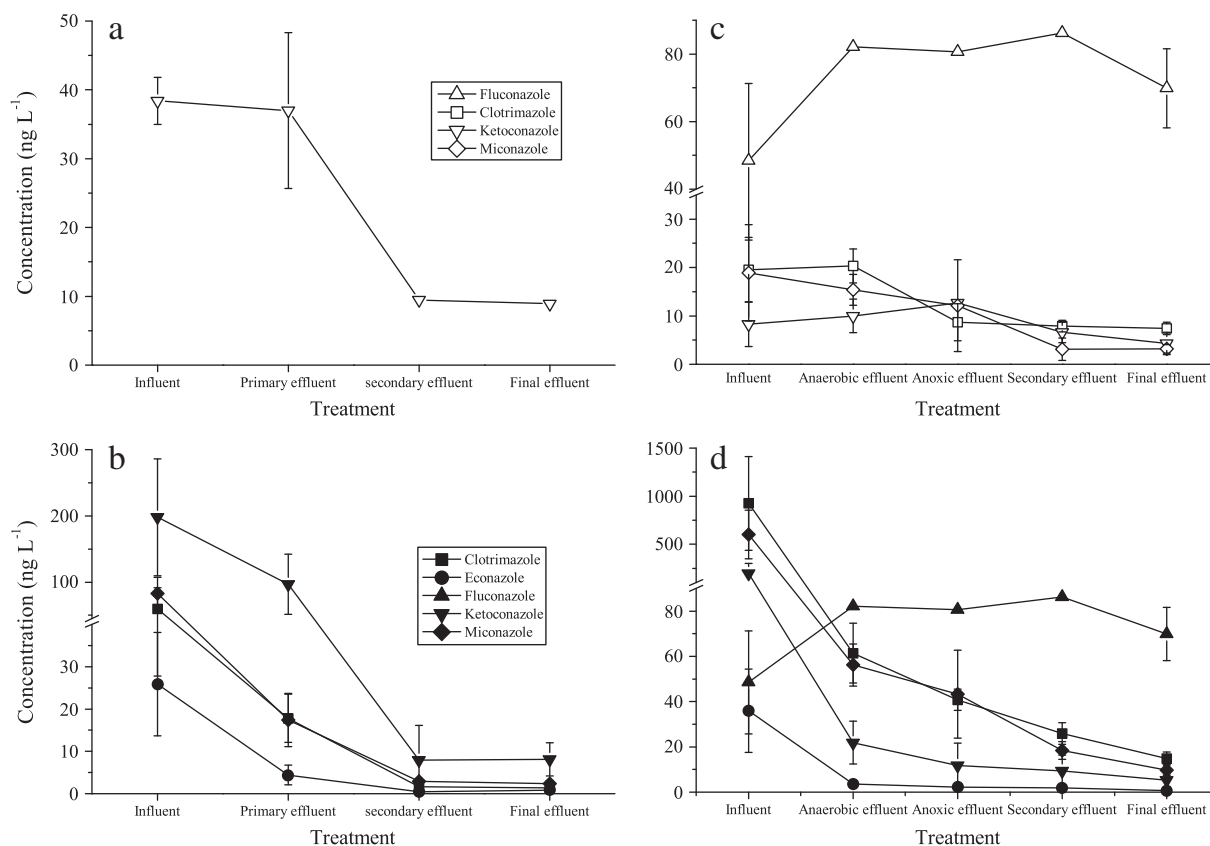
Fig. 1. Concentrations of the azole antifungals in wastewater samples during treatment in STP A (a, b) and STP B1 (c, d). Open symbols represent the concentrations in liquid phase and filled symbols represent the total concentrations combining those in liquid and particulate phases. Error bars represent standard deviations (n≥2). Fluconazole was not analyzed in STP A.
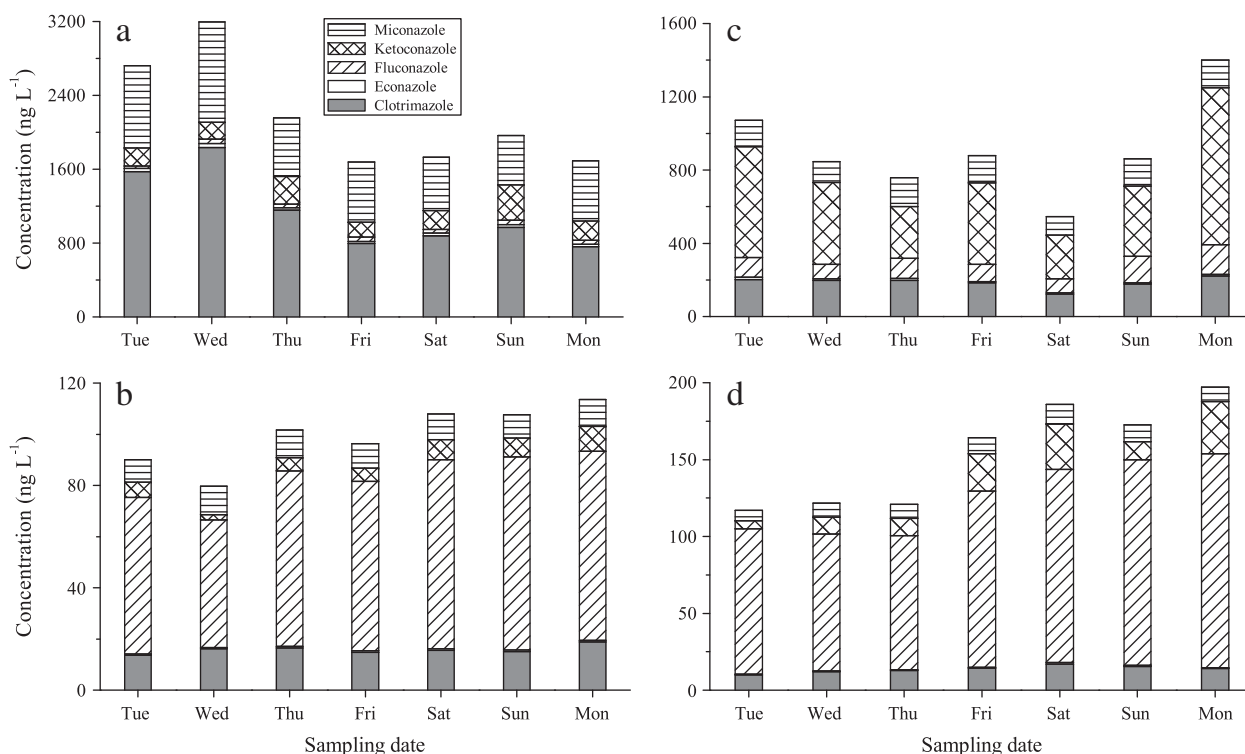
Fig. 2. Daily variations of the azole antifungals in the wastewater during a week. (a) Influent of STP B1; (b) Final effluent of STP B1; (c) Influent of STP B3; (d) Final effluent of STP B3.

sludge samples, with the lowest in the untreated solid (5–268 ng g^{-1} dw). The distribution patterns in sludge agreed well with those in the raw wastewater, with ketoconazole and clotrimazole being the most abundant in STP A and STP B, respectively. Econazole had the lowest concentrations (Table 2). This result is different from that reported for Swedish sludge in which concentrations were in the order of ketoconazole > econazole > clotrimazole and miconazole (Lindberg et al., 2010), probably reflecting different usage of these pharmaceuticals between countries.

Propiconazole and tebuconazole were only detected occasionally at trace level (≤10 ng L^{-1}) in wastewater and not detected in sludge

samples, and therefore will not be included in the following detailed discussion.

3.2. Fate and transport in the STPs

Transport and behaviors of the azole antifungal pharmaceuticals in the STPs were illustrated in Fig. 1 and Table 2. The concentrations/ mass loads of fluconazole did not change significantly during STP treatment, indicating its persistence, which is consistent with the results of previous research (Kahle et al., 2008; Lindberg et al., 2010). On the contrary, both the dissolved and total concentrations of the

Table 1
Mass loads of the azole antifungals normalized by the served population (mg/person/d) in the sewage treatment plant, Guangzhou, China.[a]

	Fluconazole		Clotrimazole		Econazole		Ketoconazole		Miconazole	
	Inflow	Outflow[b]	Inflow	Outflow[b]	Inflow	Outflow[b]	Inflow	Outflow[b]	Inflow	Outflow[b]
GZSTPA										
May 2008 (spring)	NA	NA	0.0062	0.0021	0.0026	0.0007	0.0195	0.0050	0.0075	0.0028
November 2008 (fall)	NA	NA	0.0028	0.0036	0.0013	0.0009	0.0102	0.0050	0.0049	0.0044
GZSTPB										
May 2008 (spring)	NA	NA	0.1442	0.1022	0.0191	0.0099	0.0661	0.0147	0.1079	0.1025
November 2008 (fall)	NA	NA	0.0698	0.1115	0.0061	0.0107	0.1870	0.0088	0.0748	0.0933
July 2010 (summer)	0.0472	0.0393	0.1320	0.0944	0.0130	0.0084	0.1269	0.0179	0.0820	0.0869
February 2011 (winter)	0.0253 ± 0.0053 [c]	0.0312 ± 0.0049[c]	0.2778 ± 0.0925[c]	0.1575	0.0080 ± 0.0018[c]	0.0092	0.1198 ± 0.0305[c]	0.0331	0.1784 ± 0.0426[c]	0.1278

[a] Propiconazole and tebuconazole were not included in this table because they were not detected at appreciable amount in the wastewater samples.
[b] Outflow is sum of mass loads in final effluent and dewatered sludge.
[c] Mean ± standard deviation (n = 7).

Table 2
The azole antifungals in sewage sludge (ng/g dry weight).[a]

	Clotrimazole		Econazole		Ketoconazole		Miconazole	
	Untreated solid	Dewatered sludge	Untreated solid	Dewatered sludge	Untreated solid	Dewatered sludge	Untreated solid	Dewatered sludge
GZSTPA								
May 2008 (spring) [b]	NA	190 ± 28	NA	54 ± 8	NA	437 ± 137	NA	240 ± 41
November 2008 (fall)	18	327	11	81	268	454	40	398
GZSTPB								
May 2008 (spring) [b]	NA	1442 ± 107	NA	140 ± 4	NA	194 ± 3	NA	1405 ± 96
November 2008 (fall)	73	1583	17	153	35	126	190	1307
July 2010 (summer)	59	1352	8	120	231	232	88	1258
February 2011 (winter)	39	2547	5	135	116	490	64	2069

[a] Fluconazole, propiconazole and tebuconazole were not included in this table because fluconazole was not analyzed and the latter two were not quantifiable in any sludge sample.
[b] Mean ± absolute deviation of duplicate analyses.

other azole pharmaceuticals reduced along the treatment lines. Decline of the dissolved azole concentration occurred in biological processes. Neither primary sedimentation nor UV irradiation/chlorination causes obvious variations of the dissolved azole concentrations. In contrast, the total concentrations decreased on average by 40–80% after primary sedimentation and only 1–14% of these azoles remained in the final effluent (Fig. 1). Biodegradation and sorption have been postulated as the major elimination processes of pharmaceuticals during STP treatment (Vieno et al., 2005). The obtained result suggests that both biotransformation and adsorption to solids of the azole pharmaceuticals except fluconazole have occurred during treatment in the STPs. Previous research has reported significant reduction of clotrimazole (Kahle et al., 2008; Roberts and Thomas, 2006), ketoconazole, and miconazole (Van De Steene and Lambert, 2008) in wastewater after treatment. However, the authors did not differentiate the roles of each process on the decrease of the concentrations. Lindberg et al. (2010) revealed significance of adsorption in the fate of ketoconazole, econazole, and miconazole.

In contrast to fluconazole that transport predominantly in aqueous phase, averagely more than 95% of the other four azole pharmaceuticals entering the STPs were adsorbed onto suspended particulate matters of the influent. The particulate-associated portions decreased along treatment in the STPs and reached $27 \pm 19\%$ (ketoconazole) to $82 \pm 25\%$ (econazole) in the final effluent. The partition coefficients (LogPCs) between the particulate and dissolved phases were estimated for the four azoles based on the measured concentrations, which ranged from 4.9 to 5.0 in the raw wastewater and from 4.9 to 5.7 in the final effluent (Fig. 3), confirming the strong

tendency of these compounds to be adsorbed to solids. Furthermore, the calculated LogPCs were quite close to the reported LogKow, probably suggesting the governing role of the lipophilicity on adsorption of these azole pharmaceuticals to solids. This is obviously different from typical antibacterials, e.g., fluoroquinolones that also showed strong adsorption to solids but have very low LogKow values (Lindberg et al., 2006; Peng et al., 2011) and have been revealed to be adsorbed onto solids via mechanisms such as ion exchange/bridging and surface complexation (such as H-bonding) rather than hydrophobicity (Carrasquillo et al., 2008).

Many pharmaceuticals are readily subject to photolysis (Pereira et al., 2007). Concentrations of clotrimazole and other pharmaceutical were found to decrease slightly after UV treatment in British STPs (Roberts and Thomas, 2006). Some pharmaceuticals also demonstrate appreciable reactivity with chlorine (Dodd et al., 2005). Nevertheless, the azole concentrations did not change significantly after either UV disinfection or chlorination in the STPs investigated in this work as mentioned above. This may also be associated with the inefficiency of chlorine dose, UV strength, and/or hydraulic retention time. Further work is needed to clarify the fate of the azole antifungals during these chemical processes.

Moderate to significant relationships ($R^2 = 0.40$–0.86) were found between the dissolved concentrations of the azole pharmaceuticals and the contents of dissolved organic carbon except for fluconazole (Fig. S2), suggesting that the presence and transformation of dissolved organic matter can have a significant impact on the fate and transport of these azole pharmaceuticals in wastewater.

A close relationship was found between the concentrations of clotrimazole and miconazole, and econazole in the wastewater and
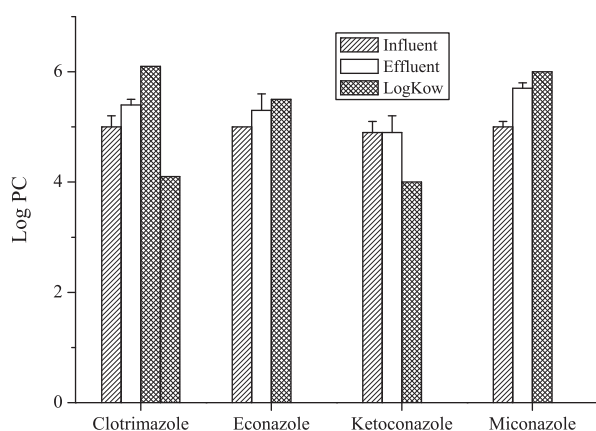
Fig. 3. Comparison of the estimated partition coefficients (Log PCs) for the azole pharmaceuticals with the LogKow. Error bars represent standard deviations (n = 20). Clotrimazole has two reported LogKow values.
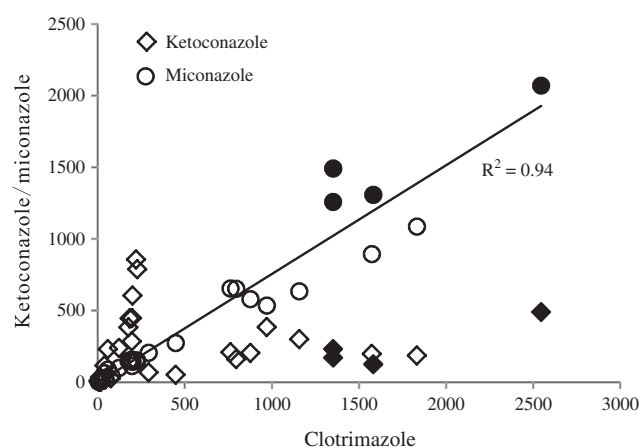
Fig. 4. Correlation between the azole antifungal concentrations in the wastewater (open symbols) and sludge (filled symbols) samples.

sludge samples (Fig. 4), suggesting a similar fate for these compounds. However, ketoconazole concentrations showed no obvious correlation with those of the other azole compounds, indicating a potentially different fate for ketoconazole from the other azoles. Lindberg et al. (2010) reported that in Sweden, 53% of the purchased fluconazole eventually appeared in the final effluents, while 35–209% of the other four compounds ended up in the digested dewatered sludge, also inferring varying behaviors of the azole pharmaceuticals in the wastewater.

In summary, the investigated azole antifungal pharmaceuticals in wastewater were fairly persistent during treatment in the STPs. However, their fate varied depending on the compound. Fluconazole passed through the STP treatment and averagely 95% of fluconazole in the raw wastewater remained in the final effluent. On the contrary, averagely 70%, 80%, 20%, and 90% of clotrimazole, econazole, ketoconazole, and miconazole, respectively, ended up and persisted in the dewatered sludge (Fig. 5). In addition, a minor fraction could have been removed by grit filtering based on the measured concentrations in the untreated solid (Table 2). The results suggest that biodegradation and adsorption occurred to the azole pharmaceuticals except fluconazole in the STPs. Ketoconazole was more readily bio-transformed. For clotrimazole, econazole, and miconazole, however, biotransformation was less prominent and adsorption to sludge was the principal fate.

3.3. Seasonal variations

The mass loads of influent and treated effluent for the azole pharmaceuticals in different seasons were shown in Table 1. In STP A, influent mass loads were slightly higher in spring than in fall. In STP B, influent mass load of fluconazole was higher in summer than in winter. However, for the other four azoles, the highest mass loads were seen in winter, followed by spring, summer (except for ketoconazole), and fall. The lower mass loads in fall are likely due to less discharge rather than to dilution effect by precipitation because November generally has the lowest rainfall and relative humidity over a year in Guangzhou (http://www.gzstats.gov.cn/). The dry climate may be less favorable for growth and propagation of fungi, possibly leading to less usage and consequently smaller discharge of the antifungal pharmaceuticals. McArdell et al. (2003) observed higher winter season loads of macrolide antibacterials in wastewater of Canada, which correlated well with sales volumes. Regretfully, the sales statistics of these azole pharmaceuticals are not available in China and therefore it is impossible to correlate the consumption to the occurrence of these compounds in wastewater yet.

In contrast, the total outflows from the STPs varied relatively narrowly (Table 1). Besides, the outflows of clotrimazole, econazole, and miconazole were even higher than the inflows in fall, probably indicating poorer transformation. Vieno et al. (2005) reported a potentially lower biodegradation rate of non-steroidal anti-inflammatory drugs in winter due to low water temperature in a Finnish STP. McArdell et al. (2003) suggested that lower elimination of macrolides in winter was probably due to lower biological activity. Nevertheless, the year-round mild climate (average temperature from 11 to 29 °C) is not likely to cause significant differences in microbiological activities. Azole antifungal pharmaceuticals enter municipal wastewater in the form of both parent compounds and metabolites (Kahle et al., 2008). As a result, re-transformation of the metabolites back to parent compounds during STP treatment might result in increase of the outflows totaling mass loads in effluent and sludge. In addition, the influent, effluent, and sludge samples were taken during the same time periods and, thus, did not correspond exactly to the same sewage package considering that the hydraulic retention time is 12–15 h and solid retention time is 10 days in the STPs, which may also result in bias in estimation of mass balance. Unfortunately, composite samplers were not allowed to set in the STPs during implementation of this work. In addition, samplings performed in different years may also impact the seasonal variations.
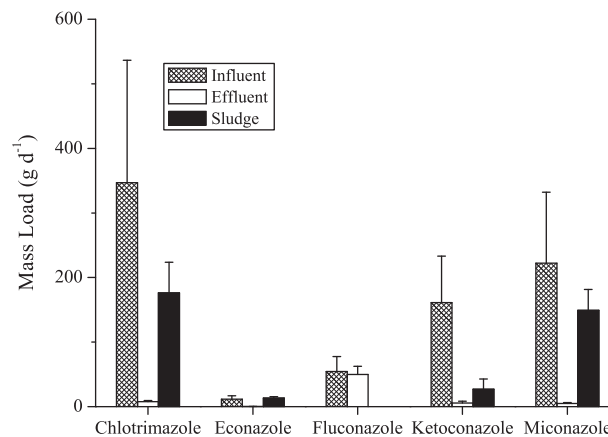

Fig. 5. Mean mass loads of the azole antifungals in STP B. Error bars represent standard deviations (n = 2 for fluconazole and n = 4 for the other azole compounds).

Therefore, further work is needed to fully elucidate the seasonal effects on the occurrence and behavior of the azole pharmaceuticals in wastewater.

4. Conclusions

Azole antifungal pharmaceuticals were widely present in wastewater and sludge and were fairly persistent during treatment in the STPs with varied fate depending on the compound. Fluconazole remained largely in the final effluent. On the contrary, biotransformation and adsorption to sewage sludge occurred to the other four azole pharmaceuticals. Ketoconazole was more readily bio-transformed, whereas for clotrimazole, econazole, and miconazole, biotransformation was less prominent and most of them tended to end up and to persist in sewage sludge. Lipophilicity plays a governing role on the adsorption of these azole compounds to sludge. The inflows of the azole pharmaceuticals in the STPs appeared the highest in winter and the lowest in fall except for fluconazole, while no seasonal variations were observed for the outflows. The azole pharmaceuticals in municipal wastewater may find their way to the environment via discharge of treated wastewater and disposal of sewage sludge. Therefore, the occurrence and behavior of these substances in the environment warrant further research concerning their persistence and potential ecological risks. To the best of our knowledge, this is the first attempt to investigate azole antifungal pharmaceuticals in the environment in China.

Acknowledgments

Appendix A. Supplementary data

Detailed description of the analytes, internal standards, LC–MS/MS parameters, schematics of the investigated sewage treatment plants with sampling points and sample types, and other necessary information were provided in the Supplementary material. Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2012.03.067.

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