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Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: Comparison to estrogens

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ABSTRACT

Research has shown that exposure to androgens and progestogens can cause undesirable biological responses in the environment. To date, however, no detailed or direct study of their presence in wastewater treatment plants has been conducted. In this study, nine androgens, nine progestogens, and five estrogens were analyzed in influent and final effluent wastewaters in seven wastewater treatment plants (WWTPs) of Beijing, China. Over a period of three weeks, the average total hormone concentrations in influent wastewaters were 3562 (Wujiacun WWTP)–5400 ng/L (Fangzhuang WWTP). Androgens contributed 96% of the total hormone concentrations in all WWTP influents, with natural androgen (androsterone: 2977 ± 739 ng/L; epiandrosterone: 640 ± 263 ng/L; and androstenedione: 270 ± 132 ng/L) being the predominant compounds. The concentrations of synthetic progestogens (megestrol acetate: 41 ± 25 ng/L; norethindrone: 6.5 ± 3.3 ng/L; and medroxyprogesterone acetate: 6.0 ± 3.2 ng/L) were comparable to natural ones (progesterone: 66 ± 36 ng/L; 17α,20β-dihydroxy-4-progegnen-3-one: 4.9 ± 1.2 ng/L; 21α-hydroxyprogesterone: 8.5 ± 3.0 ng/L; and 17α-hydroxyprogesterone: 1.5 ± 0.95 ng/L), probably due to the wide and relatively large usage of synthetic progestogens in medical therapy. In WWTP effluents, androgens were still the dominant class accounting for 60% of total hormone concentrations, followed by progestogens (24%), and estrogens (16%). Androstenedione and testosterone were the main androgens detected in all effluents. High removal efficiency (91–100%) was found for androgens and progestogens compared with estrogens (67–80%), with biodegradation the major removal route in WWTPs. Different profiles of progestogens in the receiving rivers and WWTP effluents were observed, which could be explained by the discharge of a mixture of treated and untreated wastewater into the receiving rivers.

1. Introduction

Estrogenic substances have attracted significant interests in studies on reproductive endocrine disruption in aquatic environments (Purdom et al., 1994; Lye et al., 1997; Allen et al., 1999). Biological responses from exposure to androgenic substances have been associated with effluent from wastewater treatment plants (WWTPs) and paper mills (Howell et al., 1980; Bortone et al., 1989; Cody and Bortone, 1997; Larsson et al., 2000; Jenkins et al., 2001; Borg et al., 1993). *In vitro* androgenic activity (Jakobsson et al., 1999) and the masculinization of fish have been observed downstream from pulp mill effluent in Sweden and the United States (Howell et al., 1980; Bortone et al., 1989; Cody and Bortone, 1997; Larsson et al., 2000).

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Several studies have reported that some androgens and progestogens are important hormonal odorants and reproductive pheromones, which can affect the reproductive physiology and behavior of many fish species (Kolodziej et al., 2003).

The occurrence of estrogens in wastewaters and surface waters has been investigated in numerous studies (Belfroid et al., 1999; Baronti et al., 2000; Labadie and Budzinski, 2005; Kolpin et al., 2002). Natural estrogens (estrone and 17β-estradiol), as well as the synthetic estrogen 17α-ethynylestradiol, were identified as the compounds responsible for estrogenic activities in WWTP effluents and sewage runoff from agriculture and livestock (Hoffmann and Evers, 1986; Kolodziej et al., 2003; Orlando et al., 2004). The wide occurrence of trace level (ng/L) estrogens in wastewater and receiving waters has been well documented. Compared to estrogens, the environmental levels of androgens and progestogens should be much higher, since their excretion amount in human urine are 100–1000 times higher than those of estrogens (Shore and Shemesh, 2003). In addition, many hormone drugs, especially synthetic progestogens, are widely used in human and veterinary therapies. Synthetic progestogens such as megestrol acetate, medroxyprogesterone acetate, norethindrone, and norgestrel are used in contraceptive treatments for the promotion of menstrual cycles, correction of abnormal uterine bleeding, controlling the symptoms of menopause, and preventing certain types of cancer. In contraceptive treatments, norethindrone and norgestrel are often associated with estrogens at concentrations 5 to 10-fold of estrogens, and even higher concentrations of megestrol acetate and medroxyprogesterone acetate are often used (Labadie and Budzinski, 2005). However, only limited data on a narrow range of androgens and progestogens has been reported from surveys of pharmaceuticals and endocrine disruptor substances in wastewaters (Kolodziej et al., 2003; Vulliet et al., 2007; Fernandez et al., 2007; Batt et al., 2008) and surface waters (Jenkins et al., 2001; Yamamoto et al., 2006; Kolpin et al., 2002).

We recently developed an original analytical method for monitoring five classes of steroid hormones including estrogens, androgens, and progestogens from one water sample using liquid chromatography-electrospray tandem mass spectrometry. We found that androgens and progestogens were ubiquitously detected in urban rivers (Chang et al., 2009). To further explore the occurrence and removal of these compounds in WWTPs, nine androgens, nine progestogens, and five estrogens were analyzed in influent and final effluent wastewaters in seven WWTPs of Beijing, China. Degradation of androgens and progestogens in WWTP slurry was conducted to explore the removal mechanisms of androgens and progestogens. The contributions of WWTP effluents to the receiving rivers for all compounds were also assessed.

2. Experimental section

2.1. Materials

Twenty-three sex hormones as shown were targeted in this study: 19-nor-4-androstene-3,17-diol (NAD), trenbolone (TBL), nandrolone (NDL), androstenedione (ADD), norethindrone (NTD), 17α-hydroxyprogesterone (17-HPT), testosterone (TTR), 21α-hydroxyprogesterone (21-HPT), norgestrel (NGT), 17α, 20β-dihydroxy-4-progegnen-3-one (DPO), methyl testosterone (MTTR), epiandrosterone (EADR), stanozolol (SZL), 6α-methyl-hydroxyprogesterone (MHPT), megestrol acetate (MTA), medroxyprogesterone acetate (MPA), progesterone (PGT), androsterone (ADR), 17α-estradiol (αE2), $^{13}C_2$-NTD, $^{13}C_2$-TTR, NGT-d_6 and PGT-d_9 were purchased from Sigma (St Louis, MO, USA). Ethinylestradiol (EE2), 17β-estradiol (βE2), estrone (E1), diethylstilbestrol (DES), E2-d_3, E1-d_2, and EE2-d_4 were purchased as powders from Wako (Tokyo, Japan). Formic and acetic acids were analytical grade (Beijing Chemicals, China). Methanol, acetonitrile, ethyl acetate, hexane, and dichloromethane were all HPLC grade purchased from Fisher Chemical Co. (Beijing, China). Mercuric chloride ($HgCl_2$) was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade water was prepared using a Milli-Q RC apparatus (Millipore, Bedford, MA, USA).

2.2. Sample collection

By using flow proportional samplers, 24-h composite samples of the influents and effluents were collected each day during the 3-week study period (June 26–July 16, 2006) from seven operating WWTPs in Beijing, China. These WWTPs are operated with primary and secondary treatment processes with no post-disinfection or additional filtration step. All plants receive mainly domestic waters, and detailed information on the WWTPs and sampling dates are summarized in Table S1. We also collected water samples from Tonghui River and Qing River on the same bank (their width is between 15 and 25 m) once in a week as these two rivers receive the effluents from the Gaobeidian and Qinghe WWTPs, respectively. The sampling locations along the Tonghui River were 2 km upstream and 0.5, 0.55, and 2.5 km downstream from the discharge point of Gaobeidian WWTP. The sampling locations for Qing River were 4 and 2 km upstream, and 2 and 4 km downstream of the Qinghe WWTP. Formaldehyde (final concentration 1%, v/v) was added to each sample immediately after collection. Samples were extracted on the same day after being filtered by a glass microfiber filter GF/C 1.2 μm (Whatman, Maidstone, UK).

2.3. Degradation of androgens and progestogens in slurry

An aerobic degradation test was conducted to investigate the degree of biodegradation/adsorption of androgens and progestogens. Slurry was collected from the aeration basin of Qinghe WWTP and incubated within 4 h of collection. In the test, about 200 mL of slurry with a sludge concentration of 4 μg/L was incubated with mixtures of androgens and progestogens (10 μg/L for each compound in the suspension) in 250 mL flasks. The flasks were shaken horizontally (120 rpm) at 28 °C for 24 h. Three treatments performed in duplicate were included in the test: (I) slurry, (II) slurry + hormones, and (III) slurry + hormones + $HgCl_2$. Treatment I was used to monitor the target hormones in the slurry. For treatment II, biodegradation and sorption were the major removal routes, but biodegradation was excluded in treatment III due to the prevention of biological activities by the addition of $HgCl_2$ (Fu et al., 1996; De Weert et al., 2010). The incubated hormones included all target androgens and

progestogens except for NTD and NGT. These two compounds could not be detected in our preliminary experiments after $HgCl_2$ was added, probably due to the reaction between their methenyl groups and Hg^{2+}. Supernatant samples (10 mL) were removed from the flasks at 0, 1, 2, 5, 10, and 24 h and filtered by a glass microfiber filter GF/C 1.2 μm before analysis.

2.4. Sample extraction and cleanup

In this study, estrogens, androgens, and progestogens were simultaneously extracted by using one Oasis HLB cartridge (6 mL, 60 or 500 mg, Waters, USA). The cartridge was preconditioned with 6 mL of ethyl acetate, 6 mL of acetonitrile, and 12 mL of distilled water. 70 mL of influent, 200 mL of effluent, 2 L of river water, and 10 mL of incubated slurry water spiked with 7, 10, and 50 ng of E1-d_2, and 0.7, 2, and 10 ng of other surrogate standards were extracted using HLB cartridges at a flow rate of 5–10 mL/min. The cartridges were rinsed with 10 mL of distilled water, and were then dried under a flow of nitrogen. All hormones were eluted with 15 mL of ethyl acetate. The 7-day elutants of influents and effluents were then pooled as composite samples for a complete week. The extracts were dried and redissolved in 0.2 mL of ethyl acetate and 1.8 mL of hexane. The mixed solutions were applied to silica cartridges (3 mL, 500 mg, Waters), which had been preconditioned with 4 mL of water-saturated ethyl acetate and 4 mL of hexane/ethyl acetate (90:10, v/v). After the cartridges were rinsed with 3 mL of hexane/ethyl acetate (90:10, v/v), all hormones were eluted with 3 mL of hexane/ethyl acetate (38:62, v/v). The solution was evaporated to dryness under a gentle stream of nitrogen, and reconstituted with 0.5 mL of methanol to determine androgens and progestogens by LC-ESI-MS/MS. For estrogens, 0.2 mL of reconstituted methanol resolution was dried and redissolved with 1 mL of hexane–methylene chloride (DCM) (1:1, v/v), and then passed through the preconditioned Florisil cartridges (6 mL, 1 g, Waters). Ten millilitres of hexane–DCM (1:1, v/v) were discarded, and the fraction containing target estrogens was eluted with 6 mL of acetone–DCM (1:9, v/v). The solution was evaporated to dryness under a gentle stream of nitrogen, and reconstituted with 0.2 mL of acetonitrile.

2.5. LC-ESI-MS/MS analysis

The LC apparatus was an Acquity Ultra Performance LC (Waters). All hormones were separated using a Waters Acquity UPLC® BEH C18 column (100 × 2.1 mm, 1.7 μm particle size). The column was maintained at 40 °C at a flow rate of 0.3 mL/min, and the injection volume was 5 μL. Acetonitrile and 0.1% acetic acid in water were used for estrogen analysis. Gradient conditions were increased linearly from 20% to 80% acetonitrile in 4.5 min, and then to 100% acetonitrile in 0.1 min (held for 1 min). For the separation of androgens and progestogens, methanol and water containing 0.1% formic acid were chosen as mobile phases. Gradient conditions were initiated with 60% methanol followed by a linear increase to 65% methanol in 2.5 min. After being increased to 70% in 3.5 min, methanol was increased sharply to 100% in 0.1 min and then held for 1 min.

Mass spectrometry was performed using a Premier XE tandem quadrupole mass spectrometer (Waters) equipped with a Z-Spray ionization (ESI) source and operated in the positive ion (PI) mode. The following instrument conditions were used: capillary voltage, 2.5 kV; source temperature, 120 °C; desolvation temperature, 450 °C; source gas flow, 50 L/h; and desolvation gas flow, 900 L/h. Data acquisition was performed by multiple reaction monitoring (MRM). Table S2 summarizes the optimized ESI-MS/MS conditions for analysis of target hormones. For androgens and progestogens $[M+H]^+$ was selected as the precursor ion. For estrogens $[M+H-H_2O]^+$ was chosen as the precursor ion for E2, αE2, and EE2, while $[M+H]^+$ was chosen for E1, DES.

2.6. Analytical procedure and method performance

The efficiency of the extraction and purification procedure was assessed by spiking all samples with standard solutions of target analytes and surrogate standards. The surrogate standards were used to automatically correct for the loss of analytes during sample preparation and the matrix induced change in ionization and to compensate for variations in instrumental response from injection to injection. E1-d_2, EE2-d_4 and βE2-d_3 were used as surrogate standards for estrogens; and $^{13}C_2$-TTR, $^{13}C_2$-ethyl-NTD, NGT-d_6 and PGT-d_9 for androgens and progestogens. Analyte addition was at least three times the original concentration determined prior to the fortification experiment. The mean overall recoveries of the surrogate standards and target steroids ranged between 78 and 100% with an RSD lower than 15% ($n=3$). During the recovery experiment, the spiked influent samples were analyzed in a 10-day period, and the typical RSD was lower than 12% for day-by-day replicate determinations. No significant ionization suppression was observed from this analysis. Since many target steroids were expected to occur in influent and effluent samples, estimation of method detection limits (MDLs) was based on peak-to-peak noise of the baseline near the analyte peak (selected precursor ion production-ion transition with lower sensitivity) obtained by analyzing field samples and also on a minimum value of 3 for signal-to-noise. For the non-contaminated samples, target steroids were spiked at a concentration range of 0.005–100 ng/L using mixtures of standard solution. Table 1 lists the MDLs of each steroid in aqueous matrices considered.

Identification of the target steroids was accomplished by comparing the retention time (within 2%) and the ratio (within 20%) of the two selected precursor ion–product ion transitions with those of standards. Quality control also included at least one distilled water blank, one duplicate sample, and one matrix spike sample with a mixture of target analytes and surrogate standards per 10 samples. Throughout the whole determination procedure, contamination of blanks was never detected as indicated by the distilled water blanks. The standard deviations of the field duplicates were within ±10%.

3. Results and discussion

3.1. Occurrence of sex hormones in WWTPs

Table 2 shows the concentrations of nine androgens, nine progestogens, and five estrogens in the influent and effluent samples collected from seven Beijing WWTPs in 2006.

Table 1 – Method detection limits (MDLs, ng/L) for target sex hormones.

Hormone	Influent	Effluent	River water
Estrogens			
DES	1	0.35	0.25
E1	1	0.30	0.20
αE2	0.1	0.03	0.02
βE2	0.5	0.14	0.10
EE2	0.5	0.14	0.10
Androgens			
ADD	2.2	0.90	0.63
ADR	20	7.0	5.0
EADR	40	15	12
NAD	0.8	0.3	0.20
NDL	2.4	0.85	0.60
MTTR	0.8	0.3	0.20
SZL	0.24	0.09	0.06
TBL	0. 5	0.2	0.15
TTR	0.1	0.04	0.03
Progestogens			
DPO	2.0	0.7	0.5
17-HPT	0.3	0.14	0.10
21-HPT	0.3	0.14	0.10
MHPT	0.2	0.07	0.05
MTA	0.12	0.04	0.03
MPA	0.1	0.03	0.02
NGT	0.3	0.1	0.08
NTD	1.2	0.4	0.30
PGT	0.5	0.19	0.13

Typical MRM LC–MS/MS chromatograms for a composite influent and corresponding effluent are shown in Figs. S1–S3. Over three weeks, the average total hormone concentrations in influent wastewaters were highest in Fangzhuang WWTP (5400 ± 1544 ng/L), followed by Qinghe WWTP (4206 ± 904 ng/L), Jiuxianqiao WWTP (3912 ± 680 ng/L), Beixiaohe WWTP (3759 ± 1018 ng/L), Gaobeidian WWTP (3753 ± 583 ng/L), Xiaohongmen WWTP (3882 ± 677 ng/L), and Wujiacun WWTP (3562 ± 816 ng/L). The relatively high concentrations in Fangzhuang WWTP could be due to the large portion of domestic wastewater, since all other WWTPs have additional industrial influence.

As shown in Table 2, the concentrations of estrogens were lower than other two groups of hormones, and the contribution to total hormone concentrations was only 0.3 ± 0.1% in WWTP influents. Of the five estrogens analyzed, DES and EE2 were both under detection limits. But the three natural compounds, βE2 (1.5 ± 1.5 ng/L), αE2 (0.76 ± 0.49 ng/L), and E1 (8.7 ± 7.5 ng/L), were detected in almost all influent samples with concentrations in the range of previous investigations (Baronti et al., 2000; Johnson et al., 2000; Ternes et al., 1999). There is well-established evidence of the dominance of E1 in estrogens (Baronti et al., 2000; Johnson et al., 2000; Ternes et al., 1999), but αE2 has seldom been investigated due to lower excretion from the human body and/or lower estrogenic activity compared to βE2 (Hutchins et al., 2007). In the present study, ubiquitous occurrence of αE2 was found, which could be due to the conversion of E1 to αE2 under anaerobic conditions (Hutchins et al., 2007). Further research is required to clarify this hypothesis.

Among the three classes of sex hormones detected, androgens accounted for 96.5 ± 0.5% of the total hormone concentrations in all WWTP influents. Natural androgens, including ADR (2977 ± 739 ng/L), EADR (640 ± 263 ng/L), and ADD (270 ± 132 ng/L), were the most abundant compounds. These results are consistent with the high excretion of androgens in humans compared to other hormones (Shore and Shemesh, 2003). Thus, androgens are an important group of environmental hormones for future studies. To the best of our knowledge, androgen levels have only been reported in one unique investigation in Canadian WWTPs (Fernandez et al., 2007). The only androgen investigated, TTR, had average levels of 0–46 ng/L from three WWTPs, which are similar to those of this study (34 ± 23 ng/L). The concentrations of natural androgens (ADR, EADR, and ADD), however, were significantly higher (up to a thousand times) than those of TTR in the present study. The relatively high concentrations of ADR, EADR, and ADD have also been found in natural rivers of Japan in our previous study (Chang et al., 2008), suggesting that the presence of androgens in the environment, especially natural androgens, should receive more research attention. Besides natural androgens, lower levels of synthetic androgens (NAD and SZL) were also detected in influent wastewaters with concentrations of 1.8 ± 1.2 ng/L and 0.54 ± 0.17 ng/L, respectively. The hormones MTTR, TBL, and NDL were all under the detection limit.

For progestogens, seven compounds were detected in the influents of all WWTPs with average contributions of 3.3 ± 0.4% of the total hormone concentrations. Natural progestogens (PGT, DPO, 21-HPT, and 17-HPT) were detected with concentrations of 66 ± 36 ng/L, 4.9 ± 1.2 ng/L, 8.5 ± 3.0 ng/L, and 1.5 ± 0.95 ng/L, respectively. In a previous study, PGT was the predominant progestogen (Pauwels et al., 2008), but the concentrations (4.8–33 ng/L) were lower than those from the current study. Different from the findings of estrogens and androgens, synthetic progestogens (MTA: 41 ± 25 ng/L, NTD: 6.5 ± 3.3 ng/L, and MPA: 6.0 ± 3.2 ng/L) were detected with comparable levels to natural progestogens. This could be due to the wide and relatively large usage of synthetic progestogens in medical therapies. For example, NTD, MTA, and MPA are often associated with estrogens in contraceptive treatment at concentrations >5-fold exceeding those of estrogens (Labadie and Budzinski, 2005). In previous research, NTD has been detected with concentrations of 0–92 ng/L (Fernandez et al., 2007), much higher than those of the present study. Both MPA and MTA have also been detected in urban rivers with concentrations up to 25 ng/L and 34 ng/L, respectively (Chang et al., 2009). These findings indicate that the presence of these progestogens in the environment, especially synthetic ones, requires future study.

As shown in Table 2 and Fig. 1, the WWTP effluents discharging into the receiving waters still contained all three classes of sex hormones. Androgens were still dominant in effluents, with concentration contributions of 60 ± 14%, followed by progestogens (24 ± 8.3%), and then estrogens (16 ± 13%). The average concentrations were 0.10 (αE2)–8.6 (E1) ng/L for estrogens, 0.20 (TTR)–12 (ADD) ng/L for androgens, and 0.10 (17-HPT)–2.3 (PGT) ng/L for progestogens. While E1 was still the dominant estrogen compound in the effluents, the concentrations of βE2 became comparable to those of αE2. Both ADR and EADR, which occurred in WWTP

Table 2 – Average concentrations (ng/L) of steroid hormones entering and leaving seven Beijing WWTPs in June and July 2006.

	Beixiaohe		Fangzhuang		Gaobeidian		Jiuxianqiao		Qinghe		Wujiacun		Xiaohongmen	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Estrogens														
E1	9.1 ± 2.5	1.3 ± 0.8	11.8 ± 5.3	0.2 ± 0.1	8.8 ± 1.3	0.5 ± 0.2	7.5 ± 1.9	1.0 ± 1.2	19.1 ± 21.6	8.6 ± 1.0	6.5 ± 1.1	0.6 ± 0.6	6.9 ± 2.6	0.9 ± 0.3
βE2	2.1 ± 1.5	0.4 ± 0.5	3.8 ± 3.4	0.3 ± 0.4	0.9 ± 0.4	0.2 ± 0.2	1.1 ± 1.0	0.3 ± 0.2	1.6 ± 1.7	0.8 ± 0.7	1.0 ± 0.5	0.2 ± 0.3	1.5 ± 0.9	0.5 ± 0.7
αE2	1.0 ± 0.6	0.5 ± 0.7	1.1 ± 0.6	0.7 ± 1.1	0.9 ± 0.4	0.5 ± 0.8	0.7 ± 0.5	0.3 ± 0.4	1.1 ± 1.0	0.4 ± 0.5	0.7 ± 0.2	0.1 ± 0.2	0.7 ± 0.1	0.6 ± 0.9
Androgens														
ADD	177 ± 38	4.5 ± 1.2	330 ± 104	11.2 ± 5.9	220 ± 91.7	4.9 ± 1.7	203 ± 59	4.8 ± 4.0	267 ± 68	8.9 ± 1.0	293 ± 123	6.8 ± 2.6	157 ± 55	12 ± 3.4
ADR	2767 ± 945	ND	3700 ± 1539	ND	2700 ± 300	ND	2800 ± 436	ND	2867 ± 611	ND	2667 ± 751	4.3 ± 7.5	2767 ± 404	ND
EADR	763 ± 210	ND	977 ± 411	ND	407 ± 131	ND	537 ± 56.9	ND	553 ± 129	ND	357 ± 139	ND	577 ± 110	ND
NAD	1.4 ± 0.9	ND	1.8 ± 0.7	0.6 ± 0.5	1.7 ± 1.8	ND	3.0 ± 2.7	ND	2.1 ± 0.6	ND	1.2 ± 0.1	ND	1.4 ± 0.4	ND
SZL	ND	ND	0.2 ± 0.4	ND	ND	ND	0.1 ± 0.2	ND	ND	ND	ND	ND	ND	ND
TTR	26 ± 11	0.7 ± 0.4	76.7 ± 20	1.0 ± 0.6	22 ± 21	1.1 ± 0.8	27 ± 13	0.5 ± 0.1	27 ± 9.2	0.2 ± 0.4	21 ± 10	0.8 ± 0.2	25 ± 11	1.2 ± 0.8
Progestogens														
DPO	3.1 ± 0.8	ND	8.4 ± 1.0	ND	3.9 ± 1.2	ND	4.9 ± 1.6	ND	4.8 ± 1.4	ND	3.1 ± 1.4	ND	4.5 ± 2.2	ND
17-HPT	1.4 ± 1.0	ND	1.8 ± 1.5	0.1 ± 0.1	0.9 ± 0.6	0.1 ± 0.3	1.3 ± 1.0	0.1 ± 0.1	1.5 ± 0.5	ND	1.7 ± 1.8	0.1 ± 0.1	1.0 ± 0.5	0.1 ± 0.2
21-HPT	5.8 ± 0.6	0.9 ± 1.1	13 ± 3.1	0.6 ± 0.2	6.7 ± 4.8	0.1 ± 0.1	8.0 ± 1.8	0.7 ± 0.3	10 ± 3.3	1.0 ± 0.3	7.5 ± 2.3	0.7 ± 0.5	7.3 ± 1.1	1.1 ± 0.4
MHPT	ND	1.2 ± 0.4	ND	1.2 ± 0.4	ND	0.4 ± 0.2	ND	0.9 ± 0.5	ND	0.7 ± 0.5	ND	0.3 ± 0.1	ND	1.8 ± 0.7
MPA	40 ± 30	1.1 ± 0.9	58 ± 17	0.1 ± 0.2	30 ± 25	ND	38 ± 30	ND	18 ± 13	0.7 ± 0.6	34 ± 40	0.2 ± 0.2	35 ± 24	0.2 ± 0.2
MTA	3.4 ± 1.0	0.7 ± 0.7	7.8 ± 3.8	ND	9.3 ± 3.3	ND	7.8 ± 4.5	0.1 ± 0.1	5.1 ± 1.2	0.4 ± 0.3	1.9 ± 1.6	ND	5.3 ± 0.9	0.1 ± 0.2
NTD	7.0 ± 2.6	ND	12 ± 0.6	ND	4.6 ± 4.7	ND	7.0 ± 3.5	ND	5.3 ± 1.7	ND	6.1 ± 6.0	ND	7.3 ± 1.2	ND
PGT	35 ± 14	1.4 ± 0.2	57 ± 47	1.3 ± 0.3	69 ± 27	1.0 ± 0.6	58 ± 24.6	0.8 ± 0.1	108 ± 89	1.2 ± 0.3	62 ± 31	2.3 ± 0.5	61 ± 8.7	1.8 ± 0.3

ND: under detection.

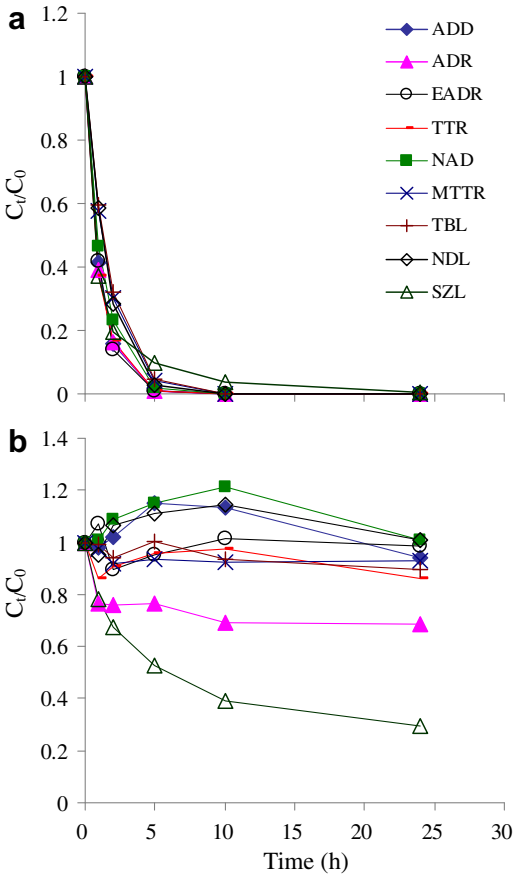

Fig. 1 – Degradation of androgens in activated sludge during 24 h incubation. (a) Slurry + androgens; and (b) slurry + androgens + $HgCl_2$.

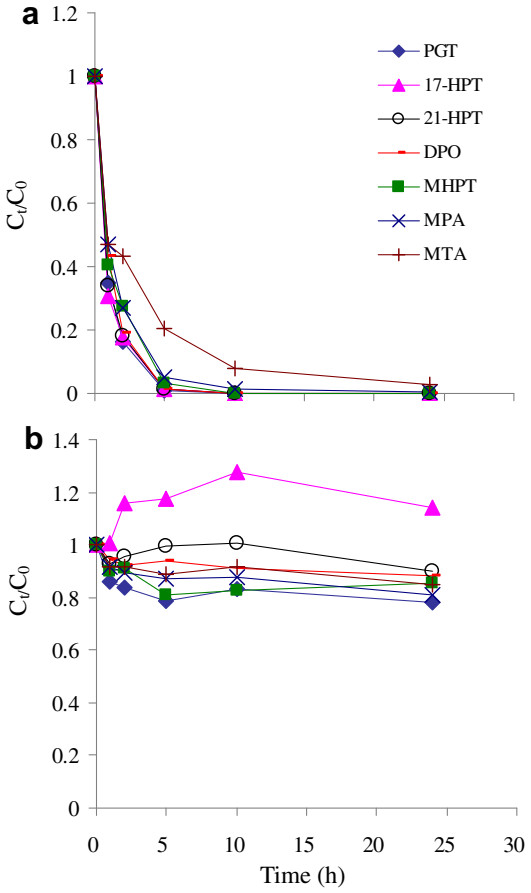

Fig. 2 – Degradation of progestogens in activated sludge during 24 h incubation. (a) Slurry + progestogens; and (b) slurry + progestogens + $HgCl_2$.

influents at very high levels, were under the detection limits in all effluents. Both ADD and TTR were the main androgens detected in all effluents, with the proportion of ADD in androgens increasing from 6% in influents to 85% in effluents. As for progestogens, PGT was the dominant compound in effluents, with the proportion of 21-HPT in progestogens increasing from 6% (influents) to 19% (effluents). It is interesting to note that MHPT was detected in all effluent samples despite not being detected in influents, suggesting possible biological conversion during wastewater treatment.

3.2. *Removal of sex hormones in WWTPs*

Removal efficiency of all target hormones was calculated by comparing influent and effluent concentrations. High removal efficiency of androgens and progestogens were found during the WWTP process: 100 ± 0% for ADR and EADR, 97 ± 3.0% for ADD, 96 ± 7.9% for TTR, 90 ± 22% for NAD, 100 ± 0% for SZL, 96 ± 9.4% for MTA, 98 ± 6.5% for MPA, 97 ± 1.7% for PGT, 96 ± 11% for 17-HPT, 91 ± 7.1% for 21-HPT, 100 ± 0% for DPO and NTD, and 100 ± 0% for MHPT. Similar high removal efficiency (100%) has also been reported for two androgens (TTR and ADD) and one progestogen (PGT) (Esperanza et al., 2004, 2007) in two pilot-scale WWTPs. But ADD was the dominant compound of all hormones in effluent despite its high removal efficiency in the present study. This can be explained by the significantly higher

Table 3 – Degradation parameters of first-order kinetics model.

Hormones	First-order kinetics			
	C_0 (μg/L)	k_1 (h^{-1})	$t_{1/2}$ (h)	r^2
ADD	15.4	0.93	0.7	0.9997
ADR	11.2	0.71	1.0	0.9768
EADR	10.9	0.64	1.1	0.9322
TTR	13.3	1.24	0.6	0.981
NAD	17.5	0.81	0.9	0.9994
MTTR	12.2	0.67	1.0	0.9993
TBL	7.5	1.03	0.7	0.9583
NDL	8.8	0.9	0.8	0.99
SZL	10.6	0.21	3.3	0.9405
PGT	11.8	0.69	1.0	0.9728
17-HPT	20.4	0.86	0.8	0.9984
21-HPT	13.9	0.84	0.8	0.9964
DPO	17.3	0.86	0.8	0.9998
MHPT	18.4	0.66	1.1	0.9982
NTD	11.4	0.57	1.2	0.9783
NGT	14.0	0.47	1.5	0.9983
MPA	13.7	0.42	1.7	0.9545
MTA	11.8	0.23	3.0	0.9558

First-order kinetic model was applied to fit the degradation results. The equation was $C_t = C_0 \times e^{-k_1 \cdot t}$, where, C_0 is initial concentration of androgens and progestogens; C_t is the concentrations of compounds at time t; and k_1 is the first-order rate constant; $t_{1/2}$ can be calculated as $0.693/k_1$.

influent concentrations of ADD, and its relatively low removal efficiency (97%) compared to ADR and EADR (100%). Estrogen removal was 80 ± 19% for βE2, 67 ± 51% for αE2, and 76 ± 46% for E1, which was in the range reported in a previous investigation (Baronti et al., 2000). The relatively low removal efficiency of estrogens compared to androgens and progestogens were also found by Labadie and Budzinski (2005), possibly due to the fact that estrogens with benzene rings are more resistant to degradation during WWTP processes (Labadie and Budzinski, 2005).

Generally, biodegradation and adsorption on sludge were the two main processes used to remove pollutants in WWTPs. Complete information on estrogens in WWTPs has been presented in previous studies, and this is invaluable when trying to understand the removal process of other hormones. Slow sorption kinetics of estrogens was observed in WWTPs due to their relatively low logKow (3.43–3.94, Lai et al., 2000; Andersen et al., 2003), thus similar logKow values of androgens and progestogens (2.55–4.09; KowWin Program, 1999, used in October 2008) may indicate a low tendency for adsorption on sludge particles (Fürhacker et al., 1999). The rapid degradation of PGT in spiked effluent wastewaters (Labadie and Budzinski, 2005) may suggest the high removal of androgens and progestogens in WWTPs through biodegradation processes. To test the removal mechanism hypothesis for androgens and progestogens, aerobic degradation tests were conducted with fresh slurry collected from the WWTPs. As shown in Figs. 1(a) and 2(a), target androgens and progestogens were significantly (>99%) removed within 24 h. The degradation half-lives calculated by first-order kinetics model were 0.6–3.3 h for all test compounds (Table 3). Conversely, in the treatment where biological activity of the sludge was inhibited by $HgCl_2$, 78–110% of the test compounds, except for SZL (29%), were still detected in the slurry after 24 h incubation (Figs. 1(b) and 2(b)). These results imply that elimination of most androgens and progestogens was achieved by biodegradation in WWTP, which is in accordance with our hypothesis. As for SZL, adsorption or reaction with $HgCl_2$ may be the major removal routes and further studies are needed.

3.3. *Occurrences of sex hormones in receiving river waters*

The contribution of sex hormones in WWTP effluents to its receiving river waters was analyzed from samples taken in

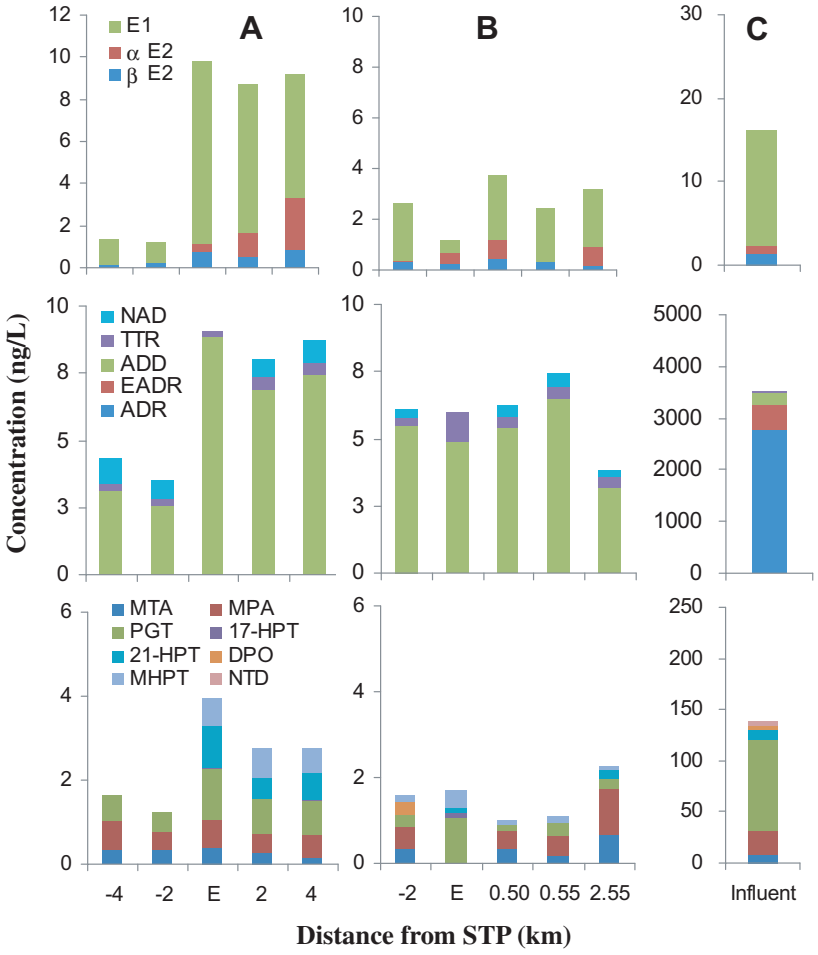

Fig. 3 – Concentrations of sex hormones (androgens, progestogens and estrogens) in the River Qing (A) and River Tonghui (B) at different distances from the WWTPs in the rivers. E: STP effluent. The levels and profiles of sex hormones in influents of Qing and Gaobeidian WWTPs were similar (C), thus the average concentrations were showed.

Tonghui River and Qing River, which receive the effluents from Gaobeidian and Qinghe WWTPs, respectively. Fig. 3 shows the levels and profiles of sex hormones detected in WWTP influents, effluents, and river water samples (Tables S3 and S4). In river samples, ADD was still the dominant androgen. The ubiquitous occurrence of ADD in the river environment was also reported by Yamamoto et al. (2006) and our recent publication (Chang et al., 2009). In addition, toxicity identification and evaluation studies indicated that ADD could be responsible for *in vitro* androgenic activity as well as the masculinization of female fish downstream of pulp mill effluent discharges (Jenkins et al., 2001; Thomas et al., 2002). These results imply that ADD could be one of the most important androgens in the environment. Although it was not detected in corresponding effluents, NAD (0.44–1.6 ng/L, 21 of 32 river samples) was present upstream and downstream of Qing and Tonghui rivers. Two progestogens (MPA and MTA) were dominant in rivers samples from Tonghui River, which was different from the progestogen profiles in WWTP effluents. The different profiles between river water and WWTP effluents indicate that sex hormones at the sampling locations of Qing River could be contributed by the discharge of a mixture of treated wastewater and naturally attenuated untreated wastewater during the study period. This agrees with our recent report (Chang et al., 2009) that about 29.4% of sex hormones in several Beijing Rivers was estimated to be contributed by treated wastewater and naturally attenuated untreated wastewater.

4. Conclusion

The presence of androgens and progestogens were investigated in seven WWTPs and two receiving rivers. Significantly higher levels of androgens and progestogens compared to estrogens occurred in all samples. Natural androgens were ubiquitous and dominant in the WWTPs and receiving rivers, while synthetic progestogens were present in WWTP influents at comparable levels of natural ones. Biodegradation was the major removal route for the high removal efficiencies of most androgens and progestogens in WWTPs. The presence of target hormones in the receiving rivers was mainly attributed to the discharge of a mixture of both treated and untreated wastewater.

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Appendix A. Supplementary information

Supplementary information associated with this article can be found in the online version at doi:10.1016/j.watres.2010.08.046.

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