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PAPER

Occurrence and fate of androgens, estrogens, glucocorticoids and progestagens in two different types of municipal wastewater treatment plants†

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The occurrence and fate of fourteen androgens, four estrogens, five glucocorticoids and five progestagens were investigated in two different types of wastewater treatment plants (Plant A: activated sludge with chlorination, and Plant B: oxidation ditch with UV) of Guangdong province, China. 14, 14, and 10 of 28 target compounds were detected in the influent, effluent and dewatered sludge samples with the concentrations ranging from below $1.2 \pm 0.0 \text{ ng L}^{-1}$ (stanozolol) to $1368 \pm 283 \text{ ng L}^{-1}$ (epiandrosterone), below $1.0 \pm 0.0 \text{ ng L}^{-1}$ (progesterone) to $23.1 \pm 1.0 \text{ ng L}^{-1}$ (5α -dihydrotestosterone), $1.0 \pm 0.1 \text{ ng g}^{-1}$ (estrone) to $460 \pm 4.4 \text{ ng g}^{-1}$ (5α -dihydrotestosterone), respectively. The concentrations of total androgens (1554 – 1778 ng L^{-1} in influent, 13.3 – 47.8 ng L^{-1} in effluent, 377 – 923 ng g^{-1} in dewatered sludge) were much higher than those of total estrogens (41.5 – 60.2 ng L^{-1} in influent, 5.6 – 13.5 ng L^{-1} in effluent, 13.9 – 57.8 ng g^{-1} in dewatered sludge), glucocorticoids (171 – 192 ng L^{-1} in influent, 2.2 – 6.3 ng L^{-1} in effluent, N.D.– 4.4 ng g^{-1} in dewatered sludge), and progestagens (39.6 – 40.5 ng L^{-1} in influent, 6.9 – 12.1 ng L^{-1} in effluent, N.D. in dewatered sludge) in these two WWTPs. According to mass balance analysis, the removal rates of most target steroids in Plant A had exceeded 90%, while those in Plant B for nearly half of detected target steroids were lower than 80%. It is obvious that the treatment capacity of the activated sludge system (Plant A) is superior to the oxidation ditch (Plant B) in the degradation of steroids in sewage treatment systems. Androgens, estrogens and progestagens were mainly removed by sorption and degradation, while the reduction of glucocorticoids was primarily due to degradation.

1. Introduction

Endocrine disrupting chemicals (EDCs) in the environment may pose potential risks to aquatic organisms and human health due

to their hormonal activity.^{1–6} Natural and synthetic steroids are one of the most important classes of EDCs that have drawn widespread concerns in recent years, but most of the studies focus on estrogens.^{7–11} However, the excretion masses by humans and livestock of other steroids, including androgens, glucocorticoids and progestagens, are several times or hundred times higher than estrogens.^{12–15} In addition to natural steroids, many synthetic drugs are commonly used in humans and livestock as well as aquaculture; for example, synthetic estrogen 17α -ethynyl

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Environmental impact

Natural and synthetic steroids are one of the most important classes of EDCs that have drawn widespread concerns in recent years, but most of the studies focus on estrogens. However, the excretion masses by humans and livestock of other steroids, including androgens, glucocorticoids and progestagens, are several times or hundred times higher than estrogens. The understanding of the occurrence and fate of various steroids in WWTPs is very limited; more research is required to investigate their removal processes in WWTPs with different treatment technologies. This study investigated the occurrence and fate of 28 natural and synthetic steroids in wastewater and sludge of different stages of two WWTPs. This study finds that the treatment capacity of the activated sludge system (Plant A) is superior to the oxidation ditch (Plant B) in the degradation of steroids in sewage treatment systems. Fourteen steroids were detected in wastewaters from the two WWTPs, but only 10 steroids were detected in dewatered sludge, and mainly androgens, with a few compounds belonging to other classes (estrone, 17β -estradiol, and prednisolone). Androgens, estrogens and progestagens were mainly removed by sorption and degradation, while the reduction of glucocorticoids was primarily due to degradation.

estradiol in combination with synthetic progestagen norgestrel is used in oral contraceptives.

Natural excretion and drug use of steroids by humans and animals could lead to contamination of the environment due to their incomplete removal and reactivation in wastewater treatment plants (WWTPs) and direct discharge of wastes. Some studies have shown that exposure of aqueous organisms in WWTP effluent or in the receiving waters could cause adverse effects, and fish reproductive anomalies in some rivers have been linked to the presence of various steroids in sewage effluents.^{16–18} Therefore, it is crucial to understand the fate of different steroids in WWTPs to reduce potential negative impacts on organisms.

Except estrogens, only limited studies have reported on the concentration levels of various steroids in surface water and effluents of WWTPs.^{7,19–21} The reported concentrations of testosterone, estrone, cortisol and progesterone ranged from 2.8 to 6.0 ng L⁻¹, N.D. to 3.5 ng L⁻¹, below LOD to 2.67 ng L⁻¹, and 1.7 to 4.1 ng L⁻¹ in surface water,^{7,21} 0.2 to 1.2 ng L⁻¹, 8.6 to 0.2 ng L⁻¹, N.D. to 95.4 ng L⁻¹, and 0.8–2.3 ng L⁻¹ in effluent,^{19,21} respectively. So far only two recent studies reported the removal of different classes of steroids in WWTPs.^{22,23} The understanding of the occurrence and fate of various steroids in WWTPs is very limited; more research is needed to investigate their removal processes in WWTPs with different treatment technologies.

The objective of this study was to investigate the occurrence and fate of 28 natural and synthetic steroids in wastewater and sludge at different stages of two WWTPs in Guangdong, China. These steroids belong to four classes: androgens, estrogens, glucocorticoids and progestagens. The two plants apply different treatment technologies: the activated sludge treatment technology followed by chlorination for Plant A and the oxidation ditch followed by UV disinfection for Plant B. In addition, we compared the measured concentrations of natural steroids in the influents of the two WWTPs with those predicated based on human urine excretion data to test the accuracy of the model prediction.

2. Materials and methods

2.1 Chemicals and sample collection

High purity standards of 28 natural and synthetic steroids were purchased from various chemical suppliers. These steroids include 14 androgens, 4 estrogens, 5 glucocorticoids and 5 progestagens (Table 1). For more information about chemicals and materials as well as suppliers, please see ESI†.

Two municipal wastewater treatment plants (Plant A and Plant B) in Guangdong province, southern China were chosen for the study of steroids. Plant A serves a population of 425 000 equivalent inhabitants and treats up to 70 000 m³ per day of municipal wastewater. The wastewater treatment process in Plant A consists of pre-treatment (screens), a grit chamber and an activated sludge system which includes an anoxic tank, an anaerobic tank and an aerobic tank (A2O process), followed by a secondary clarifier. Part of the activated sludge is returned to the anoxic tank from the aerobic tank. The secondary effluent is further treated with chlorination before discharge as final effluent. Plant B serves a population equivalent of around 380 000 inhabitants and treats around 100 000 m³ per day of municipal wastewater. The treatment processes in Plant B

includes pre-treatment (screens), a grit chamber, followed by an oxidation ditch and a secondary clarifier. The tertiary treatment in Plant B employs a Newland NLQ series UV C open channel water disinfection system (Newland Entech, Fujian). Basic information, process flow charts and sampling locations of the two WWTPs are shown in Table S1† and Fig. 1.

Wastewater samples were collected from the sampling points, shown in Fig. 1, while activated sludge samples were obtained from the anoxic, anaerobic, and aerobic tanks of Plant A, oxidation ditch of Plant B, and returned sludge only collected in Plant A. Composite wastewater and sludge samples from the two WWTPs were collected in 1 L amber glass bottles in two consecutive days on November 2–3, 2010 (dry season). During the 24 h period, 4 bottles (1 L each) of wastewater samples were sampled every 3 hours, and then mixed to make composite samples for analysis. All samples were transported in coolers back to the laboratory and stored in the dark at 4 °C, and then processed within 48 h.

2.2 Sample extraction and instrumental analysis

Sample extraction and instrumental analysis followed our previous analytical method,²³ with the detailed method information listed in the ESI (Tables S2 and S3†). Briefly, water samples were extracted by solid-phase extraction using Waters Oasis HLB cartridges (500 mg, 6 mL), while liquid sludge samples were centrifuged, freeze-dried, and extracted by ultrasonication using ethyl acetate. All extracts were further purified by silica gel columns before being analyzed by rapid resolution liquid chromatography-tandem mass spectrometry (RRLC-MS/MS).

An Agilent 1200 LC-Agilent 6460 QQQ (RRLC-MS/MS) with an electrospray ionization (ESI) source was applied to analyze the target compounds. The chromatographic separation was performed on an Agilent Zorbax SB-C18 (100 mm × 3 mm, 1.8 μm) column with its corresponding pre-column filter (2.1 mm, 0.2 μm). Two gradient elution programs were applied for two groups of steroids (Group I: estrogens; Group II: androgens, progestagens, and glucocorticoids), with a flow rate of 0.3 mL min⁻¹ (Group I) and 0.35 mL min⁻¹ (Group II), respectively. Mass spectrometry was performed in both negative and positive ionization modes (Agilent Corporation, USA). The quantitative analysis of the target compounds was performed in multiple reaction monitoring (MRM) mode (ESI†).

2.3 Mass balance analysis

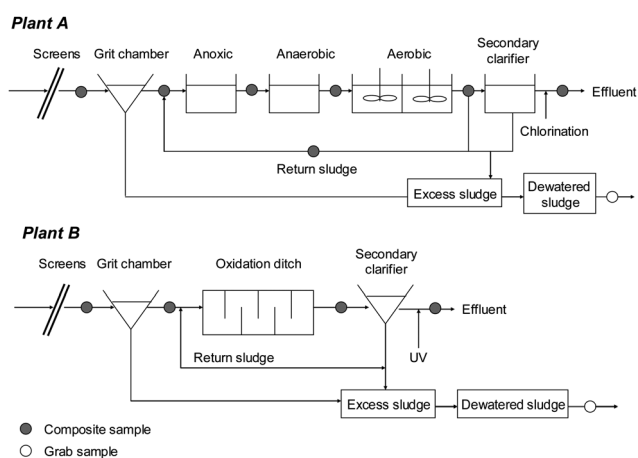
Mass balance analysis was applied to estimate mass flows of a target analyte entering and leaving a wastewater treatment plant both in treated wastewater and sludge forms. The aqueous phase removal rate was calculated to reflect the removal efficiency of a target analyte in each treatment process of WWTPs. The basic mass balance equation is given in the ESI†.

Estimation of steroids in influent based on serving population was also carried out for the selected natural steroids. Considering the detected concentrations, potencies in environment, and excretion data availability (Table S4†), ten natural steroids (five androgens (4-androstene-3,17-dione, androsterone, 5α-dihydrotestosterone, epi-androsterone, and testosterone), three estrogens (17β-estradiol, estrone and estriol), one glucocorticoid

Table 1 Physicochemical properties of estrogens, glucocorticoids, progestagens and androgens

Compound	Abbreviation	Log K_{ow}^b	S ^c /mg L ⁻¹	Log K_{oc}^d
<i>Estrogens</i>				
Estrone-2,4,16,16-d4 (I.S. ^a)	E1-d4			
Estrone	E1	3.43	147	3.02
17β-Estradiol-2,4,16,16-d4 (I.S.)	E2-d4			
17β-Estradiol	E2	3.94	82.0	2.90
17α-Ethinyl estradiol	EE2	4.12	116	2.70
Diethylstilbestrol	DES	5.64	3.3	4.06
<i>Glucocorticoids</i>				
Cortisol	CRL	1.62	220	1.38
Cortisol-d2 (I.S.)	CRL-d2			
Cortisone	CRN	1.81	297	1.31
Dexamethasone	DEX	1.72	75.1	1.57
Prednisolone	PREL	1.40	221	1.39
Prednisone	PRE	1.59	312	1.30
<i>Progestagens</i>				
Ethinyl testosterone	ET	3.44	74.2	2.43
Medroxyprogesterone	MP	3.50	22.2	2.84
19-Norethindrone	19-NTD	3.99	118	2.35
Norgestrel	NGT	3.48	35.8	2.63
Progesterone	P	3.67	5.0	3.46
Progesterone-d9 (I.S.)	P-d9			
<i>Androgens</i>				
Androsta-1,4-diene-3,17-dione	ADD	2.54	102	2.72
4-Androstene-3,17-dione	AED	2.76	66.0	2.84
Androsterone	ADR	3.07	31.9	2.75
17α-Boldenone	17α-BOL	N.A. ^e	N.A.	N.A.
17β-Boldenone	17β-BOL	3.05	117	2.40
5α-Dihydrotestosterone	5α-DHT	3.07	42.0	2.67
Epi-androsterone	EADR	3.07	31.9	2.75
4-Hydroxy-androst-4-ene-17-dione	4-OHA	2.66	N.A.	N.A.
Methyl testosterone	MT	3.72	51.9	2.57
19-Nortestosterone	19-NT	2.82	323	2.16
Testosterone	T	3.27	67.8	2.55
Testosterone-16,16,17-d3 (I.S.)	T-d3			
17α-Trenbolone	17α-TBL	N.A.	N.A.	2.77
17β-Trenbolone	17β-TBL	N.A.	N.A.	3.08
Stanozolol	S	4.42	1.41	3.36
Stanozolol-d3 (I.S.)	S-d3			

^a I.S., internal standard. ^b K_{ow} , octanol–water partition coefficient, calculated based on EPI Suite from U.S. EPA. ^c Solubility, calculated based on EPI Suite from U.S. EPA. ^d K_{oc} , the organic carbon partition coefficient, calculated based on EPI Suite from U.S. EPA. ^e Not available.

**Fig. 1** Flow schemes of the wastewater treatment Plants A and B.

(cortisol) and one progestagen (progesterone)) are selected as the representatives to estimate the mass loading into the WWTPs. For the detailed estimation method, please refer to ESI†.

3. Results

3.1 Occurrence of steroids in the two WWTPs

3.1.1 Concentrations of steroids in influents. Fourteen of 28 steroids were detected in the influents of the two plants, with concentrations ranging from 1.2 ± 0.0 ng L⁻¹ (stanozolol) to 1368 ± 283 ng L⁻¹ (epi-androsterone) (Tables S5 and S7†). Among the 14 detected steroids, 7 androgens (androsta-1,4-diene-3,17-dione, 4-androstene-3,17-dione, androsterone, 17β-boldenone, epi-androsterone, testosterone, and stanozolol), 2 estrogens (17β-estradiol and estrone), 2 glucocorticoids (cortisol and cortisone) and 2 progestagens (progesterone and norgestrel) were detected in all influent samples, while 5α-dihydrotestosterone was only quantified in Plant B. This compound was also detected in Plant A, but not quantified because one pair of qualitative ions did not meet the specified requirement. The total concentrations of different classes of steroids in influents between the two different WWTPs were similar, which is in good agreement with the unchanged nature of the main steroid source from humans. The average influent concentration of total androgens ($1554\text{--}1778$ ng L⁻¹) was much higher than those of total

estrogens (41.5–60.2 ng L⁻¹), glucocorticoids (171–192 ng L⁻¹), and progestagens (39.6–40.5 ng L⁻¹) in the two WWTPs.

Most natural androgens could be detected in influent of the two WWTPs with a wide concentration range from several ng L⁻¹ to thousand ng L⁻¹, while for synthetic androgens, only 17 β -boldenone and stanozolol were detected in each sampling of influents with the concentration range of 15.9 \pm 1.2 ng L⁻¹ to 18.8 \pm 3.6 ng L⁻¹, and 1.2 \pm 0.0 ng L⁻¹ to 1.3 \pm 0.0 ng L⁻¹, respectively. Only two natural estrogens (17 β -estradiol and estrone) could be determined in influents of these two plants. The synthetic estrogens diethylstilbestrol and 17 α -ethynyl estradiol had never been found in all samples. Two natural glucocorticoids (cortisol and cortisone) were detected in the influents with their concentration range 48.2–130 ng L⁻¹. Among the five progestagens analyzed, only one natural progestagen progesterone and one synthetic one norgestrel were detectable in the influents.

3.1.2 Concentrations of steroids in effluents. 14 of 28 targets were detected in the effluents with the concentrations ranging from below 1.0 \pm 0.0 ng L⁻¹ (progesterone) to 23.1 \pm 1.0 ng L⁻¹ (5 α -dihydrotestosterone) as shown in the ESI (Tables S5 and S7 \dagger). Androgens still dominated among the steroids analyzed in the effluents, whether at the number or the concentration level. It is worth noting that prednisolone was only detected in final effluent in Plant B but not in influent. Same as in the influent samples, only two estrogens (estrone and 17 β -estradiol) and two progestagens (progesterone and norgestrel) were detected in the effluent samples, with the concentrations ranging from 1.5 \pm 0.1 ng L⁻¹ to 8.7 \pm 0.3 ng L⁻¹, and from 1.0 \pm 0.0 to 11.0 \pm 0.9 ng L⁻¹, respectively. The concentrations of the detected estrogens were similar to those found in the previous studies,²⁴ while the concentrations of progestagens detected in the present study were slightly higher than those in previous studies.^{25,26}

3.1.3 Concentrations of steroids in dewatered sludge samples. Ten steroids were detected in sludge samples from the two plants (Tables S5 and S7 \dagger). These detected steroids were mainly androgens, with a few compounds belonging to other classes (estrone, 17 β -estradiol and prednisolone). The concentration of each steroid in the dewatered sludge varied greatly from N.D. to 460 \pm 4.4 ng g⁻¹. The mass percentages of most detected steroids in the dewatered sludge were below 20% when compared to the total mass in influent (Tables S6 and S8 \dagger). Among all steroids, two of natural androgens epi-androsterone and 5 α -dihydrotestosterone had the highest concentrations, ranging from 173 \pm 0.3 ng g⁻¹ to 460 \pm 4.4 ng g⁻¹. Natural estrogens (estrone and 17 β -estradiol) and one synthetic glucocorticoid prednisolone were also detected in dewatered sludge samples, with the concentration range of 1.0 \pm 0.1 ng g⁻¹ to 48.9 \pm 11.4 ng g⁻¹. None of the progestagens was detected in dewatered sludge samples.

3.1.4 Removal rates of steroids in different treatment stages of Plant A and Plant B. The aqueous phase removal rates for each steroid in both plants are listed in Table 2. For some steroids, the removal rates were negative after primary sedimentation, indicating that the concentrations of these compounds increased slightly after primary treatment *via* a grit chamber. This could be caused by the deconjugation of steroid conjugates (such as

glucuronides and sulfates) in the aqueous phase by the fecal bacteria *Escherichia coli*.^{27,28}

In Plant A, the removal of all detected steroids by primary sedimentation treatment was mostly not significant, and some of their removal rates were negative implying that the concentrations of those steroids had some increases after grit chamber effluent (Table 2). Following the anoxic treatment, the total removal of most androgens and glucocorticoids exceeded 80%, except for stanozolol with only 10%. Estrogens and progestagens had relatively lower removal rates between 46.9% and 77.9%. The removal rates of detected steroids in anaerobic, aerobic and chlorination treatment were below 23.7%, except for stanozolol with 70% and 17 β -estradiol with 61.8% in chlorination treatment. In general, the anoxic treatment process in Plant A had a dominant role in the removal of most steroids, while primary treatment and chlorination played a minor role. But owing to different physicochemical properties and molecular structures of these steroids, their removal rates varied to some degree at each treatment stage of Plant A. Basically, most detected steroids were easily removed by the treatment processes in Plant A.

In Plant B, large variations in the removal rates were observed for these steroids in the grit chamber (primary treatment) (Table 2). Significant reduction after oxidation ditch treatment was found for each class of steroids. The removal rates were up to 88.3% for glucocorticoids and androgens (except testosterone 66.7%), and ranged between 46.0% and 94.7% for progestagens and estrogens (Table 2).

Basically, after oxidation ditch treatment, most steroids in aqueous phase had significantly been eliminated, mainly due to their sorption onto sludge and degradation by microorganisms in the oxidation ditch process in Plant B. It is worth noting that concentrations of some androgens such as epi-androsterone and 5 α -dihydrotestosterone in final effluents increased remarkably after UV disinfection in comparison with those in the oxidation ditch effluent. So far it is not clear whether these were just normal concentration variations in wastewater treatment plants.

3.2 Mass balance analysis of steroids in the two WWTPs

Based on the influent concentration data (Tables S5–S8 \dagger , Fig. 2), the total mass loads of androgens, estrogens, glucocorticoids and progestagens in influents of these two WWTPs were in the range of 132–149 g per day, 4.0–4.5 g per day, 14.3–16.4 g per day, and 3.0–3.8 g per day, respectively. After different treatment processes, the total mass of androgens, estrogens, glucocorticoids and progestagens in final effluents were reduced to 0.3–8.2 g per day, 0.3–1.0 g per day, 0.1–0.4 g per day, and 0.2–1.2 g per day, respectively.

For Plant A, the mass loss percentages for most target steroids exceeded 90% (Fig. 3), with final concentrations in effluent below 10 ng L⁻¹, except norgestrel (11.0 \pm 0.9 ng L⁻¹). However, some steroids (17 α -trenbolone, 5 α -dihydrotestosterone and methyl testosterone, prednisolone, and medroxyprogesterone) were not detected in influent or effluent but detected in dewatered sludge.

For Plant B, nearly half of the detected steroids had their mass loss percentages of <80% (Fig. 3), with final concentrations in effluent below 10 ng L⁻¹, except epi-androsterone (14.2 \pm 0.6 ng L⁻¹) and 5 α -dihydrotestosterone (23.1 \pm 1.0 ng L⁻¹). Among all steroids considered, glucocorticoids yielded the best mass

Table 2 Aqueous phase removal rates (%) of steroids in different stages of Plant A and Plant B

Compound	Plant A					Plant B			
	Grit chamber	Anoxic	Anaerobic	Aerobic	Secondary clarifier and NaClO	Grit chamber	Oxidation ditch	Secondary clarifier	UV
<i>Androgens</i>									
Androsta-1,4-diene-3,17-dione	17.3	79.2	0.2	0.4	2.0	3.2	95.3	0.5	-1.0
4-Androstene-3,17-dione	-6.5	91.3	-0.4	0.4	10.4	-5.6	100	1.2	-4.2
Androsterone	-13.5	114	0.0	0.0	0.0	4.0	96.0	0.0	0.0
17 β -Boldenone	7.9	84.3	-0.7	0.0	6.4	2.0	86.3	0.7	-9.2
5 α -Dihydrotestosterone	N.D. ^a	N.D.	N.A. ^b	N.D.	N.D.	-34.3	134	0.0	-63.9
Epi-androsterone	-4.5	103	0.7	0.1	0.6	2.6	97.4	0.0	-2.7
Testosterone	-18.2	102	1.5	0.0	10.6	-1.5	68.2	15.2	-30.3
Stanozolol	10.0	0.0	0.0	0.0	70.0	0.0	0.0	0.0	-66.7
<i>Estrogens</i>									
17 β -Estradiol	8.4	51.7	-2.2	-24.7	61.8	-111	157	-22.6	15.7
Estrone	-20.7	78.9	17.0	0.0	18.5	25.4	69.3	0.4	-4.2
<i>Glucocorticoids</i>									
Cortisol	-20.1	112	-4.3	4.6	6.6	-9.7	110	0.0	0.0
Cortisone	7.0	87.6	0.7	2.0	2.0	15.3	84.7	0.0	0.0
Prednisolone	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.A. ^b
<i>Progestagens</i>									
Norgestrel	2.8	44.1	9.5	9.5	23.7	10.9	74.9	2.4	-18.0
Progesterone	-23.1	101	11.0	3.3	5.5	-7.3	75.6	12.2	-19.5

^a N.D., not detected in aqueous phase. ^b N.A., not available (some of the steroids not detected in influent but detected in some unit treatment process).

removal (100%), followed by progestagens (61.0–70.2%), estrogens (16.5–90.1%) and androgens (-66.7–100%). Some natural androgens such as androsta-1,4-diene-3,17-dione, 4-androstene-3,17-dione and androsterone had the mass loss of >89.3%. However, as found in Plant A, the total mass for some androgens in effluent and dewatered sludge, such as 5 α -dihydrotestosterone and stanozolol, was also found higher than that in influent. This is most probably due to the conversion from other steroids with a similar structure, or hydrolysis of their conjugated forms (glucuronides and sulfates).^{27,29}

Overall, glucocorticoids had high degradation in each plant while androgens, estrogens and progestagens varied greatly between the two plants. The results from the present study showed that activated sludge treatment (Plant A) generally had a better capacity than oxidation ditch (Plant B) to degrade various steroids in sewage systems.

3.3 Estimation of steroids in influent by serving population

Concentrations of some natural steroids in influents of Plant A and Plant B were estimated based on the two plant information and human excretion data in Tables S1 and S4†. From Table 3, it can be seen that the estimation of concentrations for these steroids was quite good although there were some differences between the estimated concentrations and measured concentrations. The biggest deviations were found for androsterone and progesterone with much higher estimated concentrations than the measured concentrations. Estriol was estimated at a concentration of around 1000 ng L⁻¹, but was not determined in the influents of the two plants due to matrix influences.

4. Discussion

Among the 28 steroids analyzed, 14, 14 and 10 compounds from the four steroid classes (androgens, estrogens, glucocorticoids

and progestagens) were detected in influent, effluent and dewatered sludge samples from the two WWTPs, respectively. High removals were achieved for most steroids by the activated sludge treatment process in Plant A and the oxidation ditch treatment process in Plant B, but with the exception for a few androgens in Plant B (Fig. 3). Removal mechanisms involved in the whole process from human excretion to discharge of the final effluent are discussed as follows.

Most steroids are excreted from the human body in the conjugated forms (glucuronides and sulfates). Before entering into a WWTP, some conjugated steroids are transformed into free steroids along the sewer lines by β -glucuronidase and sulfatase enzymes, which are released by *Escherichia coli*.^{27,29} Deconjugation might also occur later within the WWTPs for some conjugated steroids. This may partially explain the higher concentrations of a few androgens in effluent than in influent, in addition to conversion from some other steroids with similar molecular structures.^{30–34}

The total concentrations of androgens in influents of the two plants were found much higher than those of the other three classes of steroids. The average concentrations of estrogens, glucocorticoids and progestagens in influents were similar to the earlier reported values,^{22,24,25,35} while that of total androgens was an order of magnitude lower than the previous results reported by Fan *et al.*²² In that study, the sum concentrations of natural androgens androsta-1,4-diene-3,17-dione (802 ng L⁻¹), androsterone (2264 ng L⁻¹) and epi-androsterone (6344 ng L⁻¹) in influent contributed 91.7% of total androgens (10216 ng L⁻¹). However, in the present study, the concentrations of androsta-1,4-diene-3,17-dione (121–248 ng L⁻¹), androsterone (224–229 ng L⁻¹) and epi-androsterone (934–1368 ng L⁻¹) in influents were much lower. It is probably due to the different property of the influents, weather conditions of sampling seasons, and proportion of the serving population. 17 β -Boldenone is an anabolic steroid developed for veterinary use. Comparing the



Fig. 2 Concentrations (ng L^{-1}) of androgens, estrogens, glucocorticoids and progestagens in influent, grit chamber effluent, anoxic effluent, anaerobic effluent, aerobic effluent and final effluent of Plant A and Plant B. For compound abbreviations, please see Table 1.

concentrations of 17 β -boldenone with the total ones of androgens detected in influents, it reveals that domestic wastewater is the primary source of selected WWTPs rather than farming wastewater. But the concentrations of norgestrel (28.3–35.3 ng

L^{-1}) detected in the influents of the two plants were much higher than that from a previous study.²⁵ Norgestrel is used as a major active ingredient in oral contraceptives. The results from the present study may imply that the usage of oral contraceptives

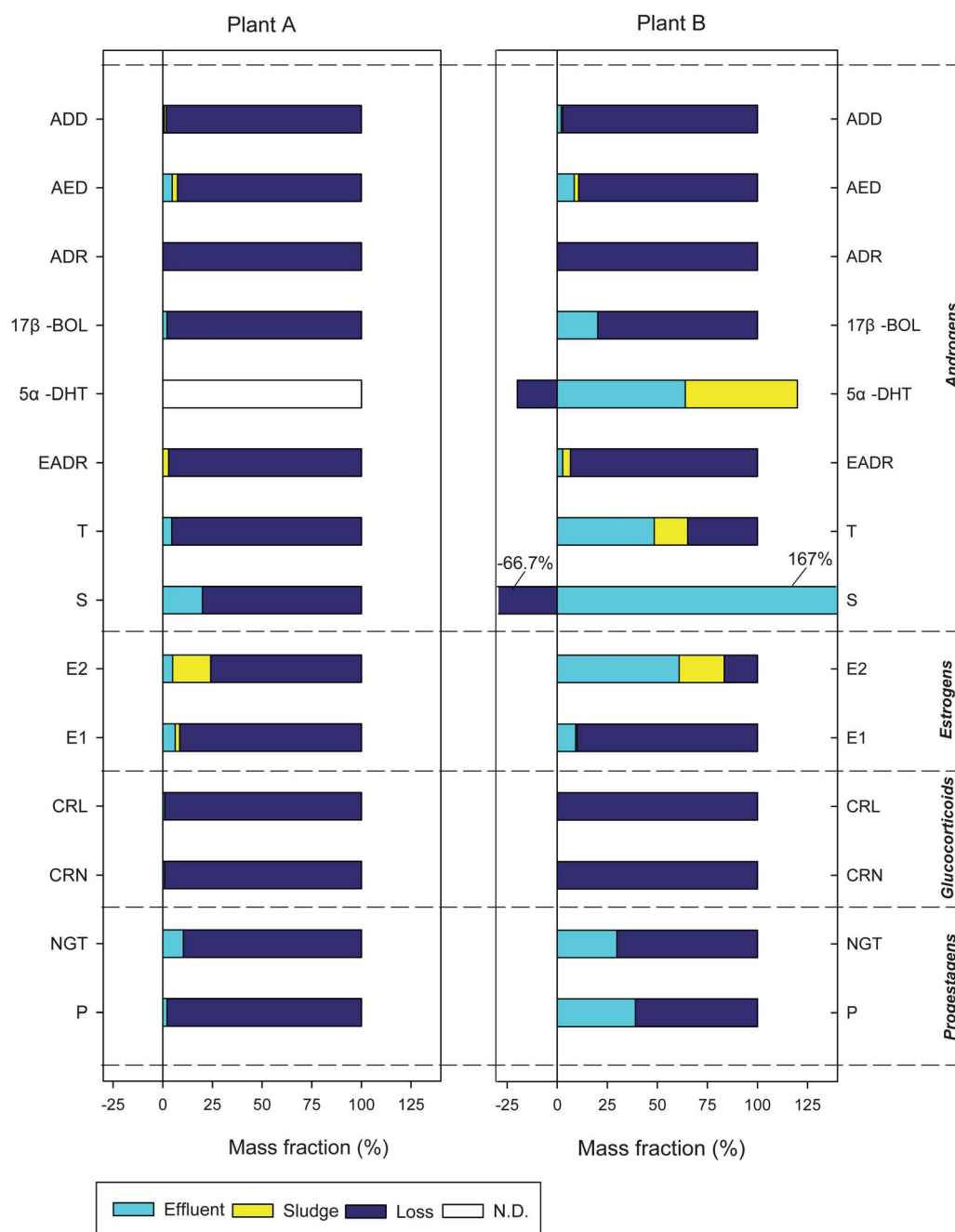


Fig. 3 Mass balance analysis results: the mass fractions (%) of androgens, estrogens, glucocorticoids and progestagens for effluent (cyan), dewatered sludge (yellow), loss by degradation or sorption (dark blue), and not-detected (N.D., white) in Plant A and Plant B. For compound abbreviations, please see Table 1.

containing norgestrel in this region is relatively higher due to the strict birth control policy in China.

Influent concentrations for 10 natural steroids in the two plants were also estimated based on human excretion data without consideration of degradation and transformation as well as animal source (Table 3). The estimated concentrations were generally good with the ratios of estimated concentration *versus* measured concentration being less than 10 for five steroids *i.e.* estrone, 17 β -estradiol, 4-androstene-3,17-dione, epi-androsterone, and cortisol. The estimated concentrations for the other 5 steroids (estriol, androsterone, 5 α -dihydrotestosterone,

testosterone, and progesterone) were found much higher than the measured concentrations or not detected in the plants. Many factors could cause this inconsistency, such as the uncertainty of selected estimation data from the literature, the incomplete deconjugation, sorption, degradation or transformation of steroids in the sewer line. For example, it is widely reported that 17 β -estradiol and estriol could be easily converted into estrone in sewerage systems.^{36,37}

From the physicochemical properties of these four classes of target steroids, loss from volatilization should be insignificant or negligible, and the main removal mechanisms for the steroids in

Table 3 Concentrations (ng L⁻¹) of selected steroids in influent by estimation and measurement in Plant A and B

Compound	Plant A			Plant B		
	Estimation	Measurement ^a	Estimation/ measurement ^b	Estimation	Measurement	Estimation/measurement
<i>Estrogens</i>						
17β-Estradiol	39.8	23.9	1.7	27.6	12.0	2.3
Estriol	1368	N.A. ^c	N.A.	948	N.A.	N.A.
Estrone	62.9	36.3	1.7	43.6	29.5	1.5
<i>Androgens</i>						
4-Androstene-3,17-dione	10.6	31.1	0.3	7.3	59.3	0.1
Androsterone	13 708	229	59.9	9499	224	42.4
5α-Dihydrotestosterone	40.3	N.D.	N.A.	27.9	64.4	0.4
Epi-androsterone	655	1368	0.5	454	934	0.5
Testosterone	180	8.9	20.2	125	6.9	18.1
<i>Progestagens</i>						
Progesterone	331	12.2	27.1	229	4.3	53.3
<i>Glucocorticoids</i>						
Cortisol	1157	130	8.9	802	123	6.5

^a The average concentration of each selected steroid by measurement in November. ^b The ratio of estimation and average measurement. ^c Not available (we did not determine this compound due to matrix interferences).

WWTPs include sorption and degradation processes. Based on the K_{oc} values (Table 1) of the selected steroids and total suspended solids of the two plants (Table S1†), theoretical distribution of these compounds in dissolved and adsorbed phases was calculated (Tables S9 and S10†). It can be seen that at the secondary biological treatment stage more than 95% (mass) of glucocorticoids could partition into the dissolved phase, while 12% to 52% (mass) of androgens, estrogens and progestagens could partition into the adsorbed phase. This is confirmed by measurement of these steroids in sludge samples from the two plants. Glucocorticoids except prednisolone were not found in sludge, but 13 compounds from classes of androgens, estrogens and progestagens were detected in sludge (Tables S9 and S10†). This could be explained by their hydrophobicities (K_{ow}) and sorption coefficients (K_{oc}) (Table 1). Glucocorticoids have lower tendency to adsorb onto sludge (log K_{oc} : 1.30–1.57) than the other three classes of steroids (log K_{oc} : 2.16–4.06). Therefore, sorption played little role in the removal of glucocorticoids, but it played a significant role in the removal of androgens, estrogens and progestagens in WWTPs.

The mass balance analysis results from the present study clearly demonstrated that degradation played a major role in the removal of steroids in Plant A and Plant B (Fig. 3). The treatment processes at different stages in the WWTPs involve a primary sedimentation process (screens and grit chamber), a secondary biological process (activated sludge or oxidation ditch) and a tertiary disinfection process (chlorination or UV), and each process contributed to the removal of these steroids to various degrees according to the treatment mechanism and its own capacity to interact with target compounds (Fig. 2). Degradation losses for most steroids by the primary treatment process were not significant, whereas the concentrations of some steroids such as 4-androstene-3,17-dione and progesterone in the primary effluent were even higher, which may be due to the deconjugation of these compounds in the primary stage. Significant losses for the steroids were observed in the secondary biological process stage, as demonstrated by rapid decreases in the concentrations of almost all target steroids in the secondary

effluents of the two plants (Fig. 2). The degradation losses were mainly attributed to the anoxic biological process in the A2O activated sludge system of Plant A, and to the aerobic biological process in the oxidation ditch of Plant B. Further degradation by other biological processes was also observed in Plant A.

Previous studies also demonstrated ready biodegradability of these steroids in the sewage treatment systems and in the environment.^{19,22,24,31,38–41} Estrogens (estrone and 17β-estradiol) have been well known to be degraded biologically in sewage treatment plants²⁴ and in the environment.^{38–41} Androgens, glucocorticoids and progestagens were also reported to be degraded in a sewage treatment plant with activated sludge treatment.²² High removals of androgens and progestagens in WWTPs through biodegradation processes were reported with half-lives of 0.6–3.3 h.¹⁹ Testosterone was degraded in soil–water slurries with half-lives of 0.3 to 7.3 days.³¹

Transformation has been reported for some steroids in the environmental media.^{30,32–34,42} Testosterone was found to be transformed to androstenedione (AED) in soil.³² Both trenbolone isomers (17α and 17β) could be transformed to trendione in soil under aerobic conditions.³⁰ Transformation of cortisol and cortisone into prednisolone and prednisone, respectively, and 4-androstene-3,17-dione (AED) to androsta-1,4-diene-3,17-dione (ADD) was observed in bovine faeces.^{34,42} Progesterone could be transformed biologically to 17α-hydroxyprogesterone, and subsequently into androstenedione, and other androgens.³³ The present and previous studies suggest that aerobic, anoxic and anaerobic biological processes could lead to degradation or transformation of these steroids in WWTPs.

Chlorination and UV disinfection processes could further oxidize residual steroids, but chlorination showed better oxidation performance than UV treatment based on the results from the present study (Tables 2 and 3). Hypochlorous acid (HOCl) in the chlorination process and hydroxyl radical in UV treatment could oxidize the phenol moiety in estrogens.⁴³ Chlorination of 17β-estradiol (E2) involves: (a) chlorine substitution reactions followed by dehydration, and (b) chlorine substitution reactions followed by cleavage of the C9–C10 bond.⁴⁴ The hydroxyl radical

can react with estrogens such as ethinyl estradiol by (a) addition to the phenolic ring, (b) abstraction of hydrogen in the aliphatic rings, or (c) addition to the ethinyl moiety.⁴³ However, progesterone did not show any reaction with chlorine at pH between 3.5 and 8.5 even in the presence of a large excess of chlorine.⁴⁵ In addition, prednisone was found photo-transformed into other compounds of androgen and pregnane series.⁴⁶ So far no studies have been reported on chlorination and UV treatment of other steroids. Further studies are required to explore the degradation mechanisms for these steroids by various oxidants used in sewage treatment plants.

Although the treatment technologies used in the WWTPs were very effective to degrade these steroids, trace amounts of some steroids could still be detected in the final effluents at concentrations up to 23.1 ng L⁻¹ for 5 α -dihydrotestosterone, 4.8 ng L⁻¹ for 17 β -estradiol, 11.0 ng L⁻¹ for norgestrel and 4.5 ng L⁻¹ for cortisol (Tables S5–S8[†]). Discharge of final effluents into rivers may affect aquatic organisms such as fish. Young *et al.*⁴⁷ proposed predicted no effect concentration (PNEC) values for estrogens: 3 ng L⁻¹ for estrone and 1 ng L⁻¹ for 17 β -estradiol;⁴³ while Zhao *et al.*⁸ obtained a PNEC value for 17 β -estradiol: 1.5 ng L⁻¹, which was derived from 77 *in vivo* no observed effect concentrations (NOECs). High risks may be expected for the aquatic organisms in the receiving rivers almost with 100% effluent from the two plants. Actually, fish feminization in UK rivers has been linked to the exposure of estrogens in effluent.¹⁸ Previous studies also reported that there is a connection between exposure of androgens and masculinization of fish.^{48,49} Since there have been no reported PNEC values for androgens, glucocorticoids and progestagens, proper risk assessment could not be made. Therefore, control measures should be applied to further remove these steroids in the final effluents in order to reduce potential risks to aquatic organisms.

5. Conclusions

Fourteen steroids were detected in wastewaters from the two WWTPs, but only 10 steroids were detected in dewatered sludge, and mainly androgens, with a few compounds belonging to other classes (estrone, 17 β -estradiol, and prednisolone). The aqueous phase removal rates for these steroids were quite good in the two plants, with Plant A (A2O activated sludge treatment) generally having better treatment capability than Plant B (oxidation ditch). Among the four classes of steroids, removal of glucocorticoids is mainly attributed to the degradation process. For androgens, progestagens and estrogens, both sorption and degradation play significant roles in their removal in wastewater treatment processes.

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