Behaviors of Glucocorticoids, Androgens and Progestogens in a Municipal Sewage Treatment Plant: Comparison to Estrogens

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ABSTRACT:

This work investigated the behaviors of seven glucocorticoids, eight androgens, and nine progestogens compared to six estrogens in a municipal sewage treatment plant (STP) in Beijing, China. Among all of the hormones considered, androgens were the dominant steroids detected in all samples (total concentrations up to 10 \( \times \) 216 \( \times \) 912 ng/L for influents, 171 \( \pm \) 10 ng/L for effluents, and 647 \( \pm \) 52 ng/g for dehydrated sludge samples), followed by estrogens (102 \( \pm \) 8 ng/L, 14 \( \pm \) 2 ng/L, and 14 \( \pm \) 1 ng/g), progestogens (57 \( \pm \) 6 ng/L, 8 \( \pm \) 2 ng/L, and 13 \( \pm \) 3 ng/g), and glucocorticoids (42 \( \pm \) 2 ng/L, 0.7 \( \pm \) 0.1 ng/L, and 1.2 \( \pm \) 0.3 ng/g). With the exception of 19-nor-4-androstene-3,17-diol (NAD, 67%), removal rates for androgens were relatively high (98/0 \( \times \) 99%), while those for glucocorticoids, estrogens, and all progestogens except 6\( - \)methylhydroxyprogesterone (MHPT) were 85/0 \( \times \) 99%, 78/0 \( \times \) 99%, and 73/0 \( \times \) 96%, respectively. Glucocorticoids, androgens, and progestogens were mainly removed by degradation as with estrogens, while different behaviors were observed in the aerated grit chamber, anaerobic tank, anoxic tank, and aerobic tank units. Many of the detected glucocorticoids, androgens, and progestogens were eliminated in the anaerobic tank, but estrogens were largely degraded in the aerobic one. Significant increases in the mass of 21\( \alpha \)-hydroxyprogesterone (21-HPT) and MHPT in the anaerobic tank and anoxic tank, respectively, were due to deconjugation.

INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are a category of environmental contaminants that interfere with the function of the endocrine system. Among EDCs, estrogenic steroid hormones have attracted considerable attention, as they are a potential cause for enhanced feminization of fish exposed to treated wastewater. Recent studies have documented that other steroid hormones, including glucocorticoids, androgens, and progestogens, also present a risk to exposed organisms and have been found to induce masculinization and impair immune function, reproduction, and development of aquatic organisms. For example, long-term treatment (diet) of glucocorticoid cortisol to rainbow trout was reported to inhibit locomotion, aggressive behavior, and immunological response. Studies have documented the masculinization of fish exposed to androgens in mill effluent-contaminated river. Additionally, laboratory experiments showed that endocrine-disruptive effects might occur in fish exposed to complex mixtures of androgens and/or estrogens, even when each chemical is present at low doses that do not induce observable effects individually. Therefore, the occurrence and fate

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of steroid hormones besides estrogens in the environment deserve greater attention.

Due to their therapeutic properties, natural and more potent synthetic steroid hormones are extensively used in medication and animal farming. As for glucocorticoids, natural cortisol, cortisone, and synthetic prednisone, prednisolone, dexamethasone, and 6α-methylprednisolone are widely used against human diseases like severe allergies, skin problems, asthma, and arthritis. Androgens, such as synthetic trenbolone, are illegally used as growth promoters. In addition, a broad number of natural and synthetic androgens and progestogens are applied in human and veterinary therapy. These steroid hormones are mainly excreted by human and livestock as initial compounds, conjugates, and metabolites. Most of them had been detected in both influent and effluent of sewage treatment plants (STPs). Since residual steroids can enter the aquatic environment with STP effluents and potentially contaminate soil and ground/surface water when dehydrated sludge is applied to agricultural fields, the elimination of steroid hormones in STPs plays an essential role in contamination control. An aqueous concentration survey of glucocorticoids in several Beijing STPs and of androgens and progestogens in two Japanese STPs demonstrated that the removal efficiencies of glucocorticoids, androgens, and progestogens were mostly above 75%. There is, however, a difference in the removal efficiencies of chemicals such as natural progestogen 17α, 20β-dihydroxy-4-progengnen-3-one (DPO). While <60% DPO was removed in the two Japanese STPs with conventional activated sludge treatment (aerobic tank only), the compound was eliminated by almost 100% in several Chinese STPs with anaerobic-anoxic-oxic process. Additionally, synthetic progestogen, 6α-methyl-17α-hydroxyprogesterone (MHPT), appeared in higher concentrations in effluents than in influents in all investigated STPs. Thus, understanding the fate of these steroids could contribute to a significant improvement in STP treatment processes. However, except for several estrogens, there is still a lack of understanding as to which processes in the STP are effecting their removal. In addition, the concentrations of glucocorticoids, androgens, and progestogens in activated sludge, which are crucial to the analysis of behaviors of these compounds in STP, have not been reported previously, possibly due to lack of a analytical method. The aim of this work was first to develop a UPLC-ESI-MS/MS analysis for a valid sample preparation method to extract and clean up glucocorticoids, androgens, progestogens, and estrogens in sewage sludge. The second objective was to study the behaviors of glucocorticoids, androgens, and progestogens in STP treatment processes by mass balance analysis, and the results were compared to estrogens.

## MATERIALS AND METHODS

### Reagents and Materials

The targeted 31 hormones including seven glucocorticoids, eight androgens, nine progestogens and six estrogens are shown in Supporting Information Figure S1. The purities of all of the analytical standards used in this study were ≥95%. Corticosterone (CORT), cortisol (CRL), cortisone (CRN), prednisone (PRE), prednisolone (PREL), dexamethasone (DEX), 6α-methylprednisolone (MPREL), androstenedione (ADD), testosterone (TTR), 19-nor-4-androstene-3,17-diol (NAD), trenbolone (TBL), nandrolone (NDL), methyl testosterone (MTTR), epitandrosterone (EADR), androsterone (ADR), progesterone (PGT), 17α-hydroxyprogesterone (17-HPT), 21α-hydroxyprogesterone (21-HPT), norethindrone (NTD), 17α,20β-dihydroxy-4-progengnen-3-one (DPO), norgestrel (NGT), 6α-methylhydroxyprogesterone (MHPT), megestrol acetate (MTA), medroxypregesterone acetate (MPA), estradiol (E3), and hexestrol (HEX) were purchased from Sigma (St Louis, MO, USA). Estrone (E1), 17β-estradiol (E2β), 17α-estradiol (E2α), and ethinylestradiol (EE2) were purchased as powders from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Surrogate standards including CRN-d2, NTD-13C2, PGT-d6, NGT-d6, NAD-d6, TTR-13C3, E1-d2, E2β-d2, and EE2-d2 were supplied by C/D/N Isotope (Montreal, Canada). Formic and acetic acids, methanol, acetonitrile, ethyl acetate, hexane, and dichloromethane were all HPLC grade purchased from Fisher Chemical Co. (Beijing, China). HPLC-grade water was prepared using a Milli-Q RC apparatus (Millipore, Bedford, MA, USA). Oasis HLB (500 mg, 6 mL), Silica (3 mL, 500 mg) and Florisil (1 g, 6 mL) solid phase extraction cartridges were purchased from Waters (U.S.). LC-NH3 cartriges (3 mL) were purchased from Supelco, USA.

### Flow Scheme of Plant and Sample Collection

The plant investigated in this work serves approximately 814,000 people and is designed to treat 400,000 t/d of wastewater, which is from domestic sources. The mechanical treatment includes a screen and an aerated grit chamber. The primary sludge was pumped into the dehydrating house, while the primary effluent was directed to the activated sludge system. The hydraulic retention times in aerated grit chamber, anaerobic tank, anoxic tank and aerobic tank were 15.3, 1.5, 3, and 10.8 h, respectively. After settling in the secondary clarifier, part of the activated sludge was returned to the anaerobic tank, and the rest was conveyed to be dehydrated. The total solid retention time was 20–25 d. The scheme of the municipal STP and the sampling locations are shown in Figure 1. Samples from each sampling location were taken on 7, 8, and 11 July, 2008, respectively. Single 24 h composite water samples of raw wastewater, outlet of the aerated grit chamber and secondary effluent were collected each day by using flow proportional samplers (cooled at 4 °C) with a sampling interval of 2 h. The sewage was not accessible for sampling before the aerated grit chamber. Other sludge-liquid and sludge samples were generally taken at the outlet of every treatment step randomly between 9 and 11 a.m. The target analytes were measured separately in sludge and water of samples from the activated sludge process. The average results of mass balance analysis based on concentration data of each sampling day were adopted for discussion. During the sampling period, the sewage inflow to the STP kept around 200,000 m3 in dry weather, and water temperatures were 23–25 °C in biological treatment units. Several regularly measured parameters (e.g., BOD5, COD, and DO) were displayed in Table S5 (Supporting Information).

The average nitrogen concentrations in the secondary effluent were 0.67 mg/L for NH4-N and 10.8 mg/L for NO3-N. The average removal efficiencies of BOD5, COD, total phosphate and suspended solid ranged from 91 to 98%.

### Sample Preparation

The water samples were analyzed according to the method reported in our previous paper. Briefly, the analytes were extracted from filtered water samples onto HLB cartridges. Extracts were purified with a Silica column and an additional Florisil cartridge for estrogens before analyzing with UPLC-MS/MS. More details are provided in Supporting Information.

The method used for measuring steroid hormones in sludge was developed in this work. A 0.5-g freeze-dried sludge was extracted with mixture of methanol and acetone (1:1, v/v) by ultrasonication of 10 min. Then the slurry was centrifuged at

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6000 r/s for 10 min and the supernatants were decanted into an eggplant-shaped flask to be combined. This process was carried out three times, and the collected extract was concentrated to below 10 mL using a rotary evaporator. The concentrated extract was mixed with 200 mL ultrapure water to pass through a HLB cartridge, which had been previously conditioned with 6 mL ethyl acetate, 6 mL acetonitrile, and 12 mL distilled water. The cartridge was washed with 10 mL of distilled water, and dried under a flow of nitrogen. Fifteen mL of ethyl acetate and 6 mL of ethyl acetate/acetonitrile (1:1, v/v) were used to elute the analytes. Then the extracts were dried and redissolved in 5 mL methanol to pass through preconditioned NH2-SPE cartridge. The extracts were dried and redissolved in 5 mL methanol to pass through preconditioned NH2-SPE cartridge. The filtrate was collected and further purified by silica or with an additional Florisil cartridge for UPLC-MS/MS analysis as applied to the analysis of water samples.

**UPLC-ESI-MS-MS Analysis.** The UPLC apparatus was an Acquity Ultra Performance LC (Waters, Milford, MA, U.S.). Androgens, progestogens, and glucocorticoids were separated using a Waters Acuity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm particle size) (USA). Estrogens were separated by a Waters Acuity UPLC BEH C8 column (100 × 2.1 mm, 1.7 μm particle size). Each of the columns was maintained at 40 °C at a flow rate of 0.3 mL/min and the injection volume was 5 μL. The gradient conditions of mobile phase were displayed in Supporting Information Table S1. Mass spectrometry was performed using a Premier XE tandem quadruple mass spectrometer (Waters) equipped with a Z-Spray ionization (ESI) source. ESI-MS/MS detections were performed in the negative ion mode for glucocorticoids and estrogens, and in the positive ion mode for androgens and progestogens. In the analysis of androgens and progestogens, [M+H]+ was selected as the precursor ion. For glucocorticoids, [M + acetate]+, the adducts with CH3COOH, were selected as the precursor ions, and the aldehyde group removed molecule ([M − H−CH2O]−) was chosen as the most abundant product ion. Concerning estrogens, [M − H]− was used as the precursor ions. The two most abundant multiselectected reaction monitoring (MRM) transitions, cone voltages, and collision energies were optimized for each steroid by infusing standard solutions in the mass spectrometer. Of the two MRM transitions, the first transition was selected for quantitation, and the other one was used for confirmation. The target compounds and their corresponding surrogate standards were shown in Supporting Information Table S2. Common MS parameters were as follows: capillary voltage, 3.0 kV (ESI−)/3.5 kV (ESI+); source temperature, 120 °C; desolvation temperature, 450 °C; source gas flow, 50 L/h; and desolvation gas flow, 800 L/h.

**Quantification and Quality Control.** 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 production-ion transition with those of standards. And the quantification was accomplished using the multiselectected reaction monitoring (MRM) transitions that were most abundant or accompanied with least background interference. One distilled water blank was analyzed per 10 samples to assess the potential contamination. Through the whole determination procedure, no contamination of distilled water blanks was detected. Immediately following a standard injection of 500 μg/L (higher than the maximum concentration in sample extracts), pure methanol blank was injected and showed no carryover of target analytes. Recoveries of target analytes in water and sludge samples were calculated and used to assess the accuracy of the method, and relative standard deviation (RSD) was used to evaluate the precision. To automatically correct the losses of analytes during extraction or sample preparation, and to compensate for variations in instrument response from injection to injection, 13C2-TTR, 13C2-NTD, NGT-d6, and PGT-d9 were used as surrogate standards for androgens and progestogens; CRL-d2 for glucocorticoids, and E1-d2, EE2-d4, and E2-d3 for estrogens.

**Mass Balance Calculation.** Mass balances were performed by multiplying concentrations of each target analyte by average daily flow rates (eq 1).

\[
W = C_{\text{dissolved}} \times Q + C_{\text{adsorbed}} \times Q \times C_{\text{TSS}}
\]

where \(W\) is the total mass of one target analyte in aqueous phase and adsorbed to sludge; \(C_{\text{dissolved}}\) and \(C_{\text{adsorbed}}\) represent the dissolved and adsorbed concentrations, respectively; \(Q\) is water flow; \(C_{\text{TSS}}\) represents the concentration of total suspended solids.

In order to assess the contribution of sorption and degradation of the steroid hormones in the STP, the mass load of steroid hormones that was lost due to sum of all transformation processes \(W_{\text{Lost}}(g/d)\) was calculated using the following equation:

\[
W_{\text{Lost}} = W_{\text{Inf}} - W_{\text{Eff}} - W_{\text{Sludge}}
\]
where \( W_{\text{Inf}} \) and \( W_{\text{Eff}} \) respectively, represent the mass load of one target analyte in influent and effluent (g/d); \( W_{\text{Sludge}} \) is the mass output in dehydrated sludge (g/d).

The mass change percentages for each steroid hormone in each treatment unit was calculated as \((W_{\text{Outflow}} - W_{\text{Inflow}})/W_{\text{Inflow}} \times 100\%\), where \( W_{\text{Outflow}} \) and \( W_{\text{Inflow}} \) represent the mass load of a steroid hormone in outflow and inflow, respectively. When the actual concentrations were below corresponding MDLs, half the method detection limits were used for calculation. A t test (95% confidence interval) was performed to assess whether the mean removal efficiency value of the three days was statistically significant. To evaluate the mass change percentage in aerated grit chamber, at first we should estimate the mass load of each steroid hormone in primary sludge (\( W_{\text{PriSludge}} \)) since the primary sludge was not accessible for sampling. According to the flow scheme of STP, \( W_{\text{PriSludge}} \) can be calculated by eq 3.

\[
W_{\text{PriSludge}} = W_{\text{Inf}} - W_{\text{PriLost}} - W_{\text{PriEff}}
\]

(3)

where \( W_{\text{PriLost}} \) and \( W_{\text{PriEff}} \) represent the mass loss in this unit and the mass load in primary effluent of one target analyte, respectively. Assuming that steroid hormones are not degraded during the dehydration, \( W_{\text{PriLost}} \) can be expressed as eq 4.

\[
W_{\text{PriLost}} = W_{\text{Lost}} - W_{\text{SubsLost}}
\]

(4)

where \( W_{\text{SubsLost}} \) is the total mass loss in other treatment units including anaerobic, anoxic, aerobic tank, and secondary clarifier. Thus, \( W_{\text{SubsLost}} \) can be estimated by eq 5.

\[
W_{\text{SubsLost}} = W_{\text{Eff}} - W_{\text{ExSludge}} - W_{\text{Eff}}
\]

(5)

where \( W_{\text{ExSludge}} \) is the mass load in excess sludge.

**Enzyme Treatment.** A mixed enzyme from *Helix pomatia* (Roche Diagnostics GmbH, Mannheim, Germany) containing \( \beta \)-glucuronidase and \( \beta \)-arylsulfatase was used to simultaneously deconjugate glucuronide and sulfate conjugates of 21-HPT and MHPT. The details for the deconjugation method were provided in Supporting Information.

## RESULTS AND DISCUSSION

**Matrix Effects and Method Performance.** For activated sludge sample analysis, the cleanup method was optimized in consideration of the heavy matrix effects in the LC-MS(/MS) analysis of steroid hormones, especially estrogens. We simultaneously extracted seven glucocorticoids, eight androgens, nine progestogens, and six estrogens, and their surrogate standards from sludge samples by ultrasonic extraction, and then used a HLB cartridge followed by silica cartridge to purify the extracts. Figure S2 (Supporting Information) shows the typical MRM LC-MS/MS chromatograms obtained from field samples. Among the four classes of steroid hormones detected in the STP influents (Supporting Information Table S4), the average concentration of total androgens (10,216 ± 912 ng/L) was much higher than those of total progestogens (57 ± 6 ng/L), glucocorticoids (42 ± 2 ng/L), and estrogens (102 ± 8 ng/L), which is similar to earlier investigations in several Beijing STPs. The removal efficiencies of glucocorticoids ranged from 85% (MPREL) to 99% (CRL). Except NAD, 21-HPT and MHTP, high removal efficiencies (85–99%) were found for most androgens and progestogens. Similar high removal efficiencies (>90%) were also reported for four natural androgens (ADR, EADR, TTR, and ADD) and one progestogen (PGT) in two Japanese STPs. It should be noted that the removal efficiencies of synthetic androgen NAD (67%) and natural progestogen 21-HPT (73%) were relatively low, and that the concentration of synthetic progestogen MHPT in effluent was 0.73 ng/L, which was 28% higher than that in influent. Previous investigations have also reported the occurrence of MHPT in effluents although the compound was undetectable in influents. The apparent removal efficiencies of estrogens from our study were 78% for E1, 93% for E2β, 84% for E2α, and 99% for E3, which were in range of previously reported studies, although poor removal of E1 (59%) was also reported. This STP was operated under comparatively high temperatures during sampling period (23–25 °C), long solid retention time (20–25 d) and low food to micro-organisms ratio (F:M) of 0.06 mg BOD mg MLVSS d\(^{-1}\) which would be favorable to the removal of organic chemicals.

As shown in Table S4 of the Supporting Information, the profiles of each class of the detected steroid hormones in different sludge samples were similar, since large amounts of sludge in the STP were cycled as internal recirculation and return sludge. In the dehydrated sludge, the total concentration of androgens (647 ± 52 ng/g) was much higher than glucocorticoids (1.2 ± 0.3 ng/g), progestogens (13 ± 3 ng/g), and estrogens (14 ± 1 ng/g) due to the abundance of androgens in the influent (Figure 3). It should be noted that the total concentration of glucocorticoids was particularly low although the total concentration of glucocorticoids was comparable with progestogens and estrogens in influent. This likely resulted from the relatively low estimated logarithm of octanol–water partition coefficients (1.24–2.18, ACD/ChemSketch 10.0) compared to other steroids (2.42–4.13, ACD/ChemSketch 10.0). The most abundant compounds among androgens, glucocorticoids,
progestogens, and estrogens were EADR, PREL, PGT, and E1, respectively.

The mass balance results for each target analyte (Table S5 of the Supporting Information) were expressed in chemical mass fractions (%) detected in (i) effluent, (ii) dehydrated sludge, and (iii) lost or transformed relative to the calculated initial loading (100%) (Figure 4). Consistent with results of previous research, the calculated fractions of mass losses due to degradation for estrogens accounted for 75–99% of initial loadings, while the contribution of sorption and output of dehydrated sludge was much less (<5%). With the exception of synthetic progestogen MHPT, the other three classes of steroids were also mainly removed by degradation (63–>99%), and the removal through sludge wastage was less than 10%. This result is consistent with the conclusion of a recently conducted aerobic degradation test for androgens and progestogens. In our study, the calculated \( W_{\text{Lost}} \) of MHPT was \(-0.04 \) g/d, indicating that MHPT could be produced in the STP.

**Behaviors Along Treatment Processes.** To better understand the degradation/transformation behavior of steroid hormones in STPs, we calculated the mass flux and mass change percentages of detected analytes in the four basic treatment units, including the aerated grit chamber and activated sludge system (Figure 5).

**Estrogens.** The mass flux of E3 was eliminated by 52% in the aerated grit chamber, while the mass of E1 increased by 33% and \( E_2^\beta \) and \( E_2^R \) showed no significant changes in mass. The mass increase of E1, which led to the relatively low apparent removal efficiency of the compound, has been attributed to the transformation of E3 and/or \( E_2^\beta \) to E1 and the deconjugation of sulfates and glucuronides of E1 as documented by many studies. In our study, in the anaerobic tank, the aqueous levels of E3, E1, and \( E_2^\beta \)
 obviously fell due to the dilution of return sludge (Supporting Information Figure S3). However, the total mass of E2β and E2α calculated by eq 1 were 32% and 47% more than their mass load in inflow, respectively (details see Supporting Information Figures S3 and S4), which could be due to the cleavage of E2β and E2α conjugates and the conversions from E1 under anaerobic conditions. Similar with results of previous research, the outputs of E2β and E2α were 21% and 19% less than inputs in the anoxic tank, respectively, and E1, E2β, and E2α were significantly degraded with elimination rates of 22%, 30%, and 38% in the aerobic tank, respectively.

**Glucocorticoids.** In the aerated grit chamber, 32% of synthetic glucocorticoid DEX was removed in mass flux, while the input and output of the other detected glucocorticoids were approximately equal (Figure 5). In contrast to estrogens E2β and E2α, significant degradation of natural CRL, CRN, and synthetic PRE (87%, 70% and 74% removed, respectively) was observed in the anaerobic tank. The degradation mechanisms might include mineralization as carbon sources as well as cometabolism exemplified by the conversion of PRE into androgens or progestogens under anaerobic conditions by human intestinal bacteria. In the anoxic tank, half of the MDLs were used to estimate the mass change percentages of the glucocorticoids with concentrations below detection limits, only synthetic MPREL showed a decrease of mass flux (details see Supporting Information Figure S5). While in the aerobic tank, the mass of CRN and CRL were further eliminated by 45% and 52%, respectively. The final concentration of CRL was 0.11 ng/L in the outflow of
the aerobic tank and the concentration of CRN was below its detection limit (0.1 ng/L). According to mass balance calculation (Supporting Information Figures S6 and S7), no statistically significant change in mass flux for PREL and CORT were observed due to their wide variation among three days’ calculation results as shown in Figure 5. Among the seven detected glucocorticoids, MPREL showed a relatively low aqueous removal rate (85%), which would be attributed to its especially low concentration in influent (0.2 (0.02 ng/L). Thus, the high elimination rate in both the anaerobic tank and aerobic tank of CRN and CRL led to their high total removal compared with other glucocorticoids. Overall, the anaerobic stage positively contributed to the degradation of glucocorticoids, while the HRT (1.5 h) of anaerobic treatment was shorter than those of aerobic tank (10.8 h) and anoxic tank (3.8 h).

Androgens. Natural androgens ADD, TTR, and EADR were significantly degraded by 34%, 69%, and 43%, respectively, in the aerated grit chamber. ADD and ADR was eliminated by 77% and 80%, respectively, in the anaerobic tank. The mass of EADR decreased 30% in the subsequent anoxic tank. While no significant mass change of any androgen was observed in the aerobic tank, indicating that androgens at low levels (0.16–16 ng/L in aqueous phase) can hardly be biodegraded by aerobic sludge. Among the five detected androgens, NAD showed relatively low aqueous removal rate (67%), which is possibly due to its low level in influent (1.4 ± 0.4 ng/L).

Progestogens. In the aerated grit chamber, only synthetic progestogen MPA and natural progestogen DPO showed significant mass reduction (52% and 37%, respectively). The mass of three natural progestogens, 17-HPT, DPO, and PGT, and one synthetic progestogen, MTA, significantly decreased by 48%, 20%, 19%, and 47% in the anaerobic tank, respectively. In the anoxic tank, MPA and MTA decreased in mass by 11% and 8%, respectively. It should be noted that the mass of natural 21-HPT significantly increased by 46% (corresponding to 0.54 g/d) in the anaerobic tank and the mass of synthetic progestogen MHPT increased by 18% (corresponding to 0.27 g/d) in the anoxic tank, which contributed to the relatively low removal efficiencies of these two compounds. No progestogen was significantly eliminated in the aerobic tank, which was similar to the results for androgens but different from estrogens and glucocorticoids.

A glucuronidation experiment has suggested that 21-HPT can exist in human serum as glucuronides, and some progestogens can be partly excreted as conjugates in feces by separating conjugated and unconjugated steroids. Thus, the increase of 21-HPT in the anaerobic tank and MHPT in the anoxic tank might be due to the cleavage of conjugates such as glucuronides and sulfates of 21-HPT and MHPT, although information on the concentrations of conjugated progestogens in urine and serum has not been reported until now. To support this hypothesis, we attempted to deconjugate both the glucuronide and sulfate conjugates in several water and sludge samples collected from the treatment process and calculate the mass flux of free and conjugated 21-HPT and MHPT. The ratio of the total mass of 21-HPT conjugates to the mass of free 21-HPT (0.24) in the
anaerobic tank outflow was less than that in its inflow (2.52). The ratio of the mass of MHPT conjugates to the mass of free MHPT in the anoxic tank outflow (0.10) was also lower than the inflow (0.33). These results indicate that deconjugation of 21-HPT and MHPT conjugates occurred in the anaerobic tank and anoxic tank, respectively. In addition to deconjugation, biotransformation from other chemicals would also contribute to the mass increase of MHPT and 21-HPT. For MHPT, biotransformation from MPA via ester hydrolysis is a possible pathway, as exemplified by other ester chemicals in biological treatments. In fact, in the anoxic tank, the MHPT mass increased, and vice versa for MPA (Figure 5). The mass increase of 21-HPT would be due to the 21-hydroxylation of PGT which has been observed in the fermentation of Acremonium strictum. To clarify the biotransformation pathways in STP treatment processes as mentioned above, further studies are necessary.

Thus, this work provides the first description of the behaviors of three classes of steroid hormones in an STP. In the effluent, E1, EADR, CRN, and PGT were the predominant components of estrogens, androgens, glucocorticoids, and progestogens, respectively. Since various behaviors were observed for target steroid hormones along the basic STP treatment processes, it is important to simultaneously include the anaerobic-anoxic-oxic process for the treatment of removing hormones. Additionally, according to previous study, the level of E1 was 0.1–10 ng/L in tile drainage water after the application of manure slurry which contained E1 as much as 866 ng/g. Considering the low concentrations of steroid hormones in dehydrated sludge (e.g., E1 11.7 ± 0.8 ng/g), the potential risk of sewage sludge application might be very low compared to animal wastes, and direct studies on the fate of steroid hormones in land treated by sewage sludge are still needed for risk analysis.

**ASSOCIATED CONTENT**

**Supporting Information.** Detailed descriptions of target steroid hormones, water sample preparations, method validation, enzyme treatment, and concentrations and mass flux of four classes of steroid hormones along treatment processes of the STP. This material is available free of charge via the Internet at http://pubs.acs.org.

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**REFERENCES**


