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Determination and Source Apportionment of Five Classes of Steroid Hormones in Urban Rivers

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We developed an original analytical method for monitoring five estrogens, nine androgens, nine progestogens, six glucocorticoids, and one mineralocorticoid in one water sample using liquid chromatography-electrospray tandem mass spectrometry, and then 45 river, 13 discharging sites, and 4 composite effluent samples were analyzed to reveal their occurrence and sources in urban rivers. Of the 45 river samples, androgens were the dominant steroids detected (total concentrations up to 480 ng/L), followed by glucocorticoids (up to 52 ng/L), progestogens (up to 50 ng/L), and estrogens (up to 9.8 ng/L). The summed concentration for each class of detected hormones in 13 discharging site samples was higher than that in river samples, up to 1887 ng/L for androgens, 390 ng/L for glucocorticoids, 75 ng/L for progestogens, and 25 ng/L for estrogens. A principal component analyses with multiple linear regression based on the profiles of all target compounds was applied to identify the source apportionment and to predict the contribution from different sources. It was found that 62.7% of the mean summed hormones were contributed by freshly discharged untreated sewage, 29.4% by treated sewage and/or naturally attenuated untreated sewage, and 7.9% by an unknown source, possibly pharmaceutical manufacturing plants.

Introduction

Studies on endocrine disrupting chemicals in the aquatic environment have predominantly focused on the effects of estrogenic substances of which natural and synthetic steroid estrogens have received special attention due to their potency (*1–3*). However, in addition to estrogens, other classes of steroid hormones, including androgens, progestogens, glucocorticoids, and mineralocorticoids can be released into the aquatic environment and present a risk to aquatic organisms. Recent studies have linked exposure to androgens with masculinization of fish (*4–6*). There are studies showing that exposure to glucocorticoids such as cortisol impairs immune function, reproduction, and development (*7–9*). In addition, numerous steroid hormones, including progestogens and some androgens or estrogens, act as reproductive pheromones in fish. By interfering with pheromonal signaling, these hormones can elicit adverse effects on reproductive behavior in many fish species at extremely low concentrations

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(*10*). Therefore, the presence of any class of steroid hormones in the environment is an issue of concern.

Steroid hormones and their metabolites are naturally excreted by humans and livestock. The amount of excreted androgens and progestogens via urine can be 100–1000 times higher than that of estrogens (*11*). Many steroid hormone drugs, especially synthetic glucocorticoids and progestogens, are widely used in human and veterinary therapy. Synthetic glucocorticoids such as dexamethasone, prednisone, prednisolone, and 6α-methylprednisolone are used to treat a wide variety of inflammatory conditions such as arthritis, colitis, asthma, bronchitis, certain skin rashes, and allergic or inflammatory conditions of the nose and eyes. Synthetic progestogens such as megestrol acetate, medroxyprogesterone acetate, norethindrone, and norgestrel are used in contraceptive treatments for the promotion of menstrual cycles and treatment for abnormal uterine bleeding, symptoms of menopause, and certain types of cancer. Norethindrone and norgestrel are often associated with estrogens in contraceptive treatments at concentrations exceeding those of estrogens 5–10-fold, and megestrol acetate and medroxyprogesterone acetate are used even at higher concentrations than that of norethindrone and norgestrel (*12*). Synthetic androgens such as stanozolol, methyl testosterone, trenbolone, and nandrolone are not only often used for clinical therapy but also illegally used as growth promoters in cattle and calves and are abused in sports to improve the performance of athletes. All these natural and synthetic steroid hormones can be discharged into environmental waters via effluent from sewage treatment plants (STPs). Recently, we reported the presence of natural and synthetic glucocorticoids in environmental waters (*13*). Jenkins et al. (*14*) revealed the presence of androstenedione (an androgen) and progesterone (a progestogen) in a river receiving paper mill effluents. Yamamoto et al. (*15*) reported the occurrence of androstenedione and testosterone in urban rivers in Japan, and Kolpin et al. showed the occasional occurrence of two androgens (testosterone and *cis*-androsterone) and three progestogens (progesterone, 19-norethisterone, and mestranol) in water resources during one nationwide survey in 1999–2000 in the United States (*16*). While these studies have demonstrated that natural and synthetic steroid hormones are present in the environment, almost all of these studies targeted a narrow range of hormones (i.e., estrogens and occasionally a few selected androgens and progestogens such as testosterone, androstenedione, and progesterone). In order to better assess the environmental risks from steroid hormones, it is necessary to develop an analytical method that can simultaneously analyze a broad range of hormones in environmental matrices.

In this study, by optimizing the elution solvent, we developed a single solid-phase extraction (SPE) method that allows the analysis of members of the five classes of steroid hormones (nine androgens, nine progestogens, five estrogens, six glucocorticoids, and one mineralocorticoid; Figure S1 of the Supporting Information) in one environmental water sample. We then applied this method to investigate the occurrence of each compound in rivers of Beijing, China. The target natural and synthetic hormones were selected because they have been detected, are expected to be present in the environment, are important members of steroid hormone classes, are used in significant quantities, and/or can be accurately measured in environmental samples using available technologies. Finally, source apportionment analyses were carried out using principal component analyses with multiple linear regression based on the profiles of all
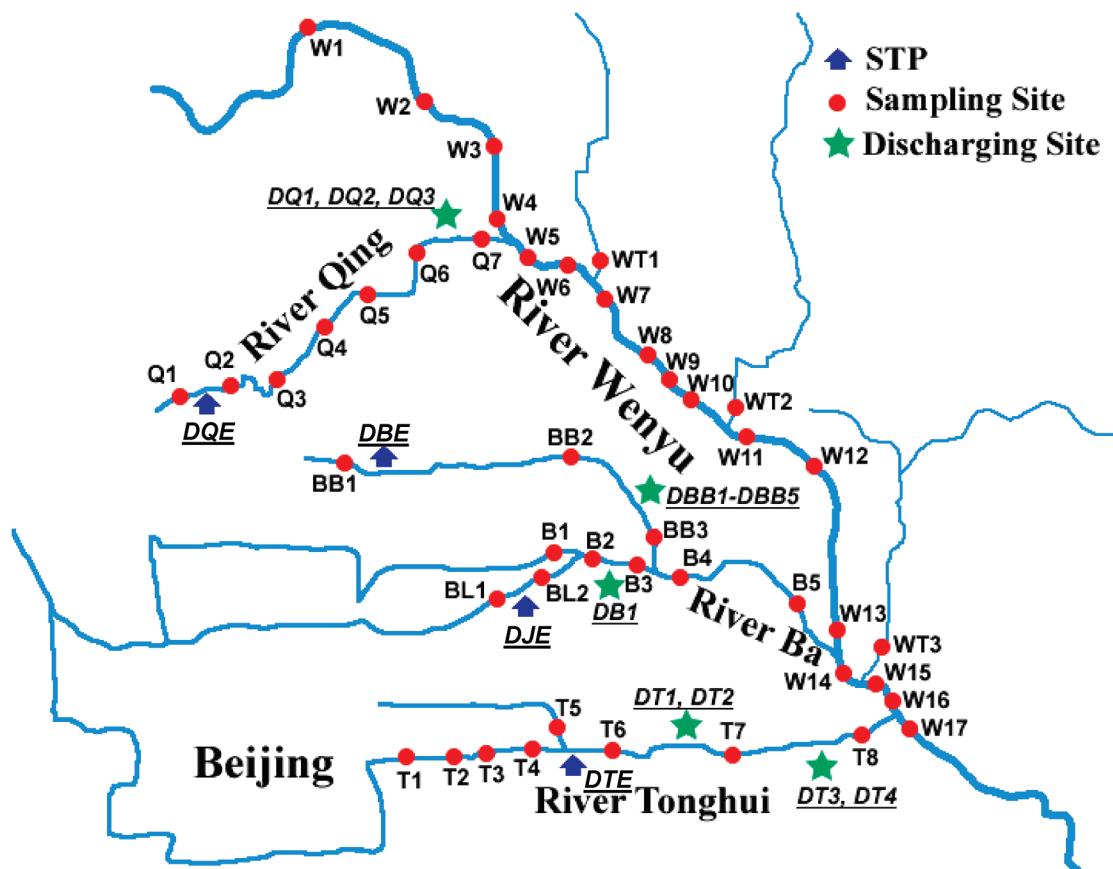
FIGURE 1. Map of sampling sites in rivers of Beijing.

target compounds to interpret the contribution from different sources to total hormones.

Experimental Section

Sample Collection. The study areas and sampling sites are shown in Figure 1. River Wenyu and its tributaries cover the most populated area of Beijing, China, and the land cover is principally urban land. No livestock farms are found near the investigated area. Four main STPs are located on the tributaries of River Wenyu, and their effluents are directly discharged into the tributaries of River Wenyu and finally enter the River Wenyu. By using flow proportional samplers, 24 h composite effluent samples were collected from the four STPs each day on July 17−23, 2006. Formaldehyde (final concentration 1%, v/v) was added immediately after collection, and filtration and extraction was carried out within 6 h. Daily 24 h composite samples were extracted, and then 7 day elutants were pooled as one composite sample for a complete week. On August 9−12, 2006, grab river water samples were collected from 45 sites on River Wenyu and its tributaries, and 13 grab water samples were taken from discharging sites occasionally found along the investigated rivers, where water was directly discharged into the rivers, but their sources were not very clear. There are several pharmaceutical manufacturing plants located in the investigated area such as near the sampling sites on River Qing. These plants produce products containing hormone components, but no obvious pharmaceutical plant effluents were discovered. All samples, including river water samples (n = 45), water samples from the discharging sites (n = 13), and STP effluents (n = 4), were used for source apportionment analysis. Samples were collected between 10 a.m. and 4 p.m. on four consecutive days from upstream to downstream and from its tributaries to River Wenyu to avoid potential artifacts related to the time of day. One replicate sample was collected for every 10 of all 62 samples. Samples were taken by using stainless bottles and transferred to glass bottles immediately. After collection, formaldehyde (final concentration 1%, v/v) was added to each sample immediately, and all samples were sent to the lab in the afternoon of each day. Samples were extracted on the same day after being filtered by a glass microfiber filter GF/C 1.2 μm (Whatman, Maidstone, U.K.), and the cartridges were kept at −80 °C for one week prior to analysis. Chemical information, sample preparation, and condition of LC-MS/MS analyses are provided in the Supporting Information.

Results and Discussion

Analytical Procedure and Method Performance. In order to optimize the simultaneous extraction of a broad range of steroid hormones, several solvent mixtures were tested. Ultimately, ethyl acetate followed by a mixture of ethyl acetate and acetonitrile (v/v, 1:1) was chosen for the elution from the HLB cartridge. The selected elution solvents also produced cleaner extracts compared to methanol with respect to the color of the extract when a real sample was used. After elution from the HLB cartridge, the extract was further purified by silica or with an additional florisil cartridge and then analyzed by LC-MS/MS.

The efficiency of the extraction and purification procedure was assessed by spiking the river sample 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. $^{13}C_2$-TTR, $^{13}C_2$-NTD, NGT-d_6, and PGT-d_9 were used as surrogate standards for androgens and progestogens; E1-d_2, EE2-d_4, and βE2-d_3 for estrogens; and CRL-d_2 for glucocorticoids and mineralocorticoids. Analyte addition was made

with the criterion of at least 3 times the original concentration that was determined prior to the fortification experiment. As shown in Table S2 of the Supporting Information, the absolute recoveries of the target standards and surrogate standards ranged from 75% to 100% with a relative standard deviation (RSD) lower than 10% ($n = 3$). During the recovery experiment, the spiked river samples were analyzed in a 10 day period, and the typical RSD was lower than 12% by day-by-day replicate determinations. The estimation of the method detection limits (MDLs) was based on the peak-to-peak noise of the baseline near the analyte peak (the selected precursor ion production–ion transition with lower sensitivity) obtained by analyzing field samples and on a minimal value of signal-to-noise of 3. The MDLs for all target steroid hormones in the river samples were in the range of 0.008–0.5 ng/L except for two androgens, ADR (5 ng/L) and EADR (12 ng/L), due to some isobaric interferences existing in the extract samples (Table S2 of the Supporting Information).

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. 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%. It should be noted that the matrix spike samples, including the samples collected at discharging sites DB1, DBB1, and DT4, probably had a much more complicated matrix than the river sample, and the absolute recovery results are listed in Table S2 of the Supporting Information. We also evaluated the extent of signal suppression and enhancement in LC-ESI/MS/MS detection by spiking extracts of one river sample and one sample from a discharging site with standard solution, and the signal effect observed with each analyte was calculated using the percentage of signal intensity in the sample matrix versus the signal of the same concentration in the pure solvent (methanol or acetonitrile). The results showed that the signal suppression for all target analytes was generally less than 15% (Table S3 of the Supporting Information). It should be noted that although we used extensive cleanup steps to treat the water samples, we still found severe signal suppression (up to 80%) by using LC-ESI-MS/MS in negative mode to analyze estrogens in a sample collected from discharging site DBB1 (Figure S2a of the Supporting Information), but the signal suppression was largely improved in positive mode (Figure S2b and Table S3 of the Supporting Information). Also, the absolute recoveries of estrogen surrogates in all samples ($n = 62$) were 70–82%, further confirming the low signal suppression of our methods. Every sample extract was duplicate analyzed, and the average was used for the detected compounds.

Levels of Steroid Hormones. Figure 2 shows the distribution of the four classes of steroid hormones detected in the river samples. Of the five classes of thirty steroid hormones, three estrogens (E1, βE2, and αE2), five androgens (ADD, NAD, ADR, EADR, and TTR), seven progestogens (PGT, 17-HPT, 21-HPT, MHPT, DPO, MTA, and MPA), and six glucocorticoids (CRL, CRN, DEX, MPREL, PREL, and PRE) were detected in the 45 river samples. Estrogens were most frequently detected among all steroid hormones with a detection frequency of 91–100% in river samples. The concentrations of E1, βE2, and αE2 were 0.38–8.0 ng/L, <0.10–1.6 ng/L, and <0.02–0.91 ng/L, respectively, of which the highest concentrations were comparable to those in surface water in The Netherlands (18) but lower than those in U.S. streams (16). E1 was the most abundant among the detected estrogens. Although αE2 was not excreted by

humans and there are no livestock farms near the investigated area, comparable concentrations and detection frequencies of βE2 and αE2 were still observed in the analyzed samples including STP effluents. This is possibly due to the biological conversion occurring in the environment or treatment process exemplified by the conversion of E1 (originally produced from βE2) into αE2 in anaerobic condition (19), and further research is necessary. The detection frequency of androgens was 100%, 2.2%, 36%, 16%, and 42% for ADD, NAD, ADR, EADR, and TTR, respectively, and the total concentration of androgens was the highest of the five classes of steroid compounds studied. In the few previous environmental investigations (15, 16, 20), ADD and TTR were the major androgens considered, and their concentrations were generally comparable to those of estrogens. In our studies, we first reported that ADR and EADR had comparable high detection frequency with ADD and TTR, and the highest concentrations for these two compounds (ADR, 390 ng/L; EADR, 110 ng/L) were even higher than those of ADD (99 ng/L) and TTR (8.6 ng/L), indicating that these pollutants should not be neglected in future investigations. Relatively low concentrations of progestogens and glucocorticoids were detected in the river water samples. Detected progestogens included five natural compounds (PGT, 17-HPT, 21-HPT, MHPT, and DPO) and two synthetic compounds (MTA and MPA) with detection frequencies of 93%, 6.7%, 16%, 33%, 22%, 53%, and 91%, respectively. The presence of PGT and NTD in river samples has been reported previously (16, 21), and the highest concentrations of PGT (199 ng/L) and NTD (872 ng/L) in U.S. streams were much higher than those in our studies (PGT, 26 ng/L; NTD, <0.30 ng/L). It should be noted that the two synthetic progestogens, MPA and MTA, were first detected in environmental samples, and their concentrations were 0.23–25 ng/L in 24 detected samples and 0.04–34 ng/L in 41 detected samples, respectively. For glucocorticoids, the detection frequencies of CRL, CRN, DEX, and PREL were very high (96–100%), followed by PRE (53%) and MPREL (6.7%). The concentrations of two natural compounds (CRL, 0.11–20 ng/L; CRN, 0.05–28 ng/L) in detected samples were higher than those of detected synthetic compounds (DEX, 0.05–8.0 ng/L; MPREL, 0.20–0.41 ng/L; PREL, 0.25–1.8 ng/L; and PRE, 0.04–2.4 ng/L).

In addition to the river samples, we also analyzed 4 STP composite effluents for 1 week and 13 water samples collected from discharging sites along the tributaries of River Wenyu, which possibly contribute to the occurrence of steroid hormones in river waters (Figure 3). As for these 13 water samples, sites DQ2, DQ3, and DBB4 were close to fish ponds, and sites DB1, DBB1, DBB3, DT3, and DT4 were located at the residential area and therefore were probably influenced by domestic sewage. The sources for other discharging sites were unclear. It is found that levels of steroid hormones in STP effluents (sites DQE, DJE, DBE, and DTE) and samples collected at sites DQ2, DQ3, and DBB4 (aquaculture) were much lower than those detected in their upstream and downstream river water samples, indicating that STP effluents and aquaculture were not the main sources of steroid hormones in this area, but the concentrations of steroid hormones at sites DB1, DBB1, DBB2, DBB3, DT1, DT3, and DT4 were higher than those in river water samples, showing that domestic sewage may be an important source for these sites. The detection frequency and highest concentrations of three estrogens (E1, βE2, and αE2), three progestogens (PGT, 17-HPT, and DPO), four androgens (ADD, ADR, EADR, and TTR) and four glucocorticoids (CRL, CRN, PREL, and PRE) in samples from discharging sites were much higher than those in river samples, especially for androgens and glucocorticoids, of which the highest concentrations were 3.3–3.7- and 5.2–9.6-fold higher than those in river samples, respectively. One synthetic androgen, SZL, and two synthetic
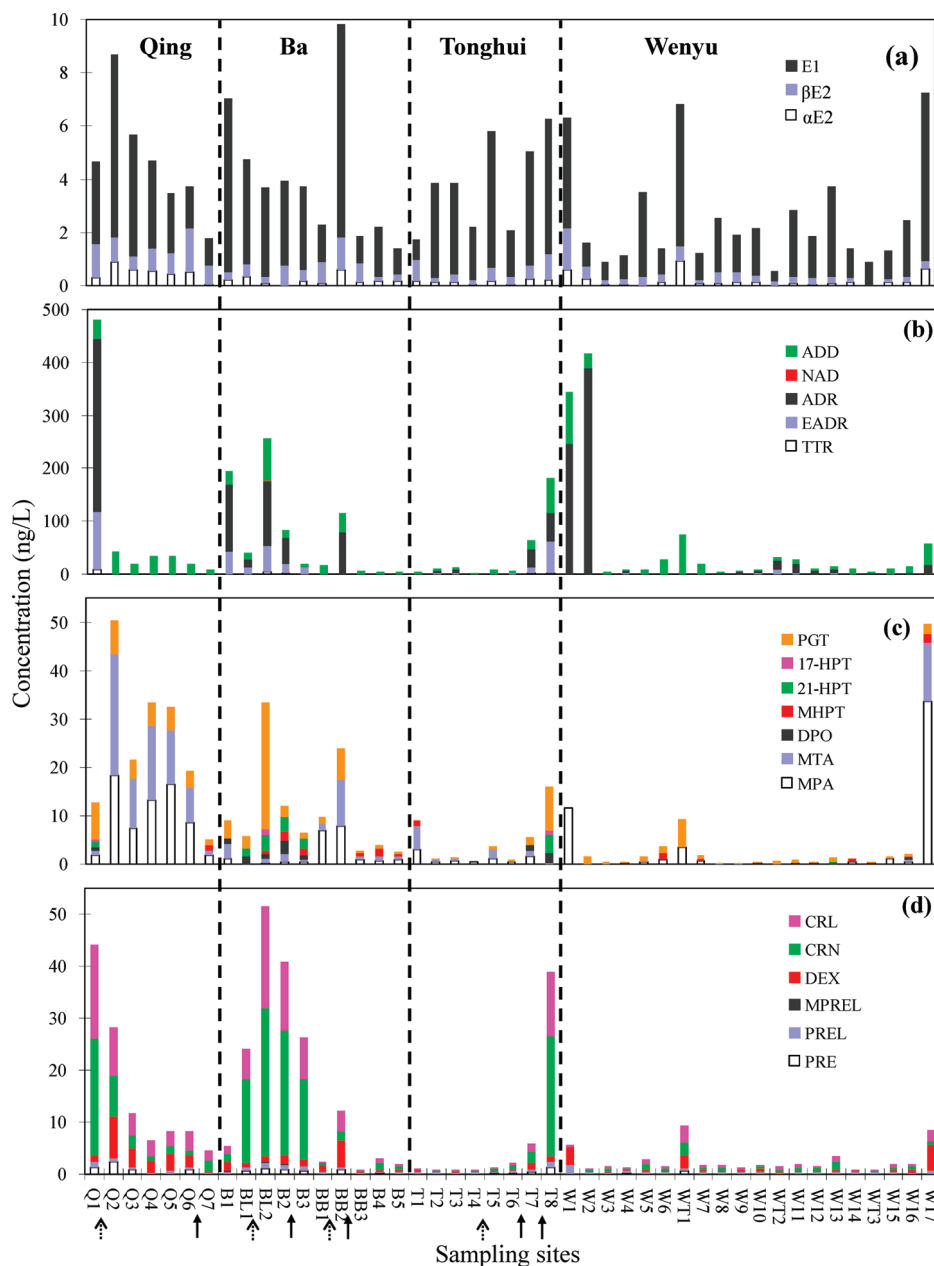
FIGURE 2. Distribution of the detected four classes of steroid hormones in 45 river water sampling sites in Beijing. Sampling sites are shown in Figure 1. (a) Estrogens. (b) Androgens. (c) Progestogens. (d) Glucocorticoids. Solid arrow, discharging sites. Dotted arrow, STP effluents.

progestogens, NTD and NGT, were detected in a few samples from discharging sites. The low detection frequency of the two synthetic progestogens was similar with other investigations (12, 16). It is interesting that the concentration proportion of two synthetic progestogens (MTA and MPA) and one synthetic glucocorticoid (DEX) at some sampling sites, especially on River Qing, were much higher than those at other river sampling sites, discharging sites, and STP effluents. In addition, we did find synthetic hormones such as DEX in the composite influent sample of STP on the River Qing (13), but the concentrations of DEX (0.62 ng/L) were much lower than that (8 ng/L) at 2 km downstream of the STP on the River Qing, and synthetic hormones MTA, MPA, and DEX can be easily removed in STPs as observed in our previous studies (13, 27), suggesting that domestic treated and untreated sewage was not the source of these synthetic hormones. Thus, although the discharging pipes from pharmaceutical manufacturing plants near those sites to rivers were not found, these plants could still be the possible

sources because they produce some medicines containing synthetic hormone components such as βE2, MTA, MPA, and DEX, and the relatively high concentrations of αE2 at corresponding sampling sites compared to other sites could also be attributed to the production activities in those plants and/or transformation from βE2 in the environment (19).

Among the target compounds, two synthetic estrogens (EE2 and DES), three synthetic androgens (MTTR, TBL, and NDL), and one mineralocorticoid (ADT) were not detected in any samples. The above results demonstrate that aquatic organisms in the rivers of Beijing are coexposed to four classes of steroid hormones.

Hormonal Profile and Source Apportionment. Of the five major classes of steroid hormones quantified in this study, androgens were the predominant steroid hormones in all samples collected from rivers, discharging sites, and STP effluents, with an average contribution of 59%, followed by glucocorticoids (15%), progestogens (15%), and estrogens (10%). To further identify the source apportionment based
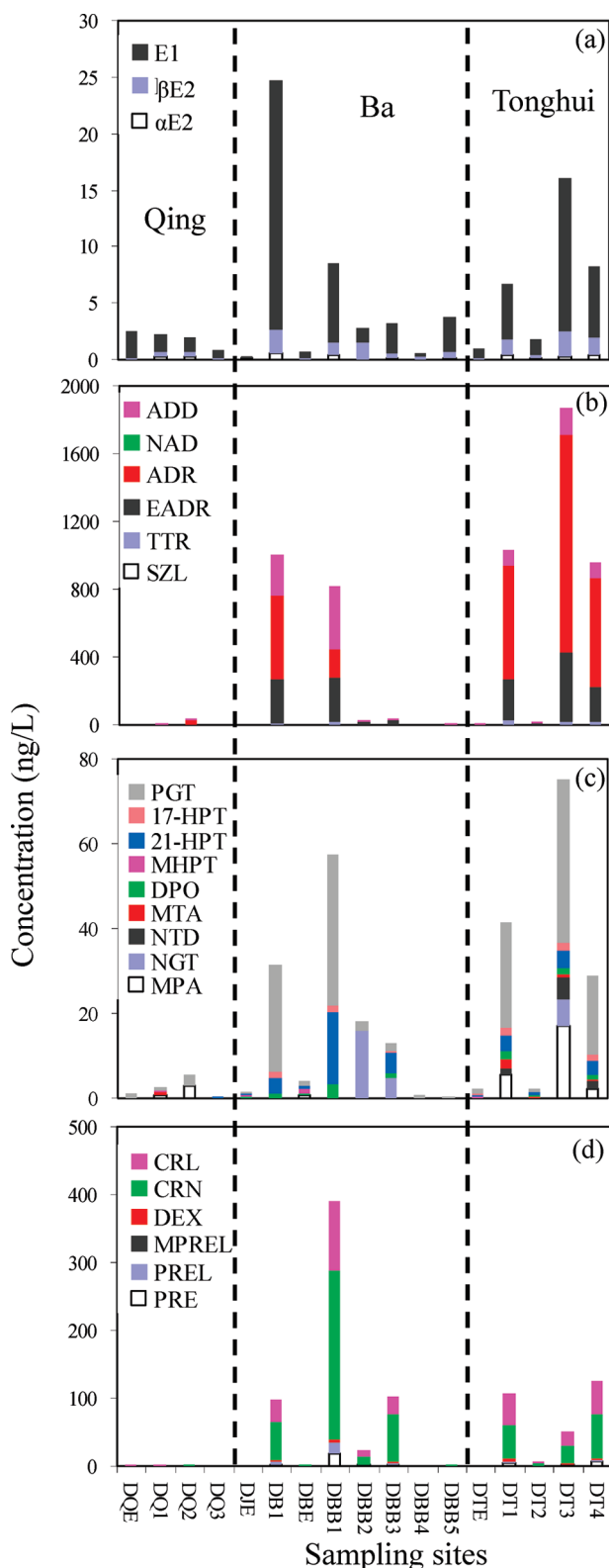
FIGURE 3. Distribution of the detected four classes of steroid hormones in 13 discharged samples and 4 STP composite effluents in Beijing. Sampling sites are shown in Figure 1. (a) Estrogens. (b) Androgens. (c) Progestogens. (d) Glucocorticoids.

on the profiles of all target compounds, we performed for all samples principal component analysis followed by multiple linear regression (PCA-MLR), which have been used for source identification of polycyclic aromatic hydrocarbons in sediment and atmosphere (22−24) and dioxins sediment cores (25, 26). The compounds used for multivariate analysis are

shown in Table 1, and chemicals without detection or with low detection frequency were not included. Four principal components (PC1, PC2, PC3, and PC4) were identified after varimax rotation, and these components accounted for 53.98%, 17.13%, 10.98%, and 5.56% of the total variance, respectively. The second rotated component (PC2) is highly associated with βE2, E1, ADR, EADR, TTR, PGT, and 17-HPT. We recently reported 90−100% removal rates of ADR, EADR, TTR, and PGT (very low concentrations, <1 ng/L, of these compounds are detected in effluents despite influents levels up to 1000 ng/L) by STPs that operate exclusively via aerobic treatment process (27), suggesting these compounds are prone to degradation in the river environment. Thus, PC2 can be highly indicative of the source due to freshly discharging untreated domestic sewage into the river environment. The third rotated component (PC3) was characterized by high loadings of αE2 and three synthetic hormones MTA, MPA, and DEX. This profile suggested an unknown source, while pharmaceutical manufacturing plants near those sites were speculated to be the potential source as discussed above. PC4 correlated only with MHPT. Because this compound was reported to be formed during the wastewater treatment process (27), PC4 would represent a bioconversion source from wastewater treatment processes or possibly in the environment. The profile in the first rotated component (PC1) had high loadings of ADD, 21-HPT, DPO, CRN, CRL, PREL, and PRE and moderate loadings of PC2 components (EADR, TTR, 17-HPT, and PGT). The persistence of glucocorticoids (CRN, CRL, and PREL) is higher than that of other hormones in environment, which is supported by their low removal percentages in aerobic unit of STP (2−36%) (12), compared with androgens and progestogens (90−100%) (27−29). Thus, high proportions of glucocorticoids in this component suggest a source from treated sewage or naturally attenuated untreated sewage. Moderate loadings of the PC2 components further indicate the presence of a small proportion of naturally attenuated untreated sewage. Therefore, PC1 would indicate treated sewage and/or naturally attenuated untreated sewage, which could be further clarified by local pollution information.

Multiple linear regression analysis of elements in the factor scores matrix (t_k) against the normal standard deviate of the SumHormone values ($\hat{Z}$) was performed on the PCA scores to determine the mass apportionment of the four components in all river samples. Stepwise modeling was also used to remove any insignificant parameters, and only parameters that were significant at the 0.10 significance level were retained as eq 1.

$$\hat{Z}_{\text{SumHormone}} = 0.41t_1 + 0.88t_2 + 0.10t_3 \quad (r^2 = 0.961, \\ p < 0.001) \quad (1)$$

where SumHormone is the total concentrations of target hormones, t_1 is defined as treated sewage and/or naturally attenuated untreated sewage, t_2 is freshly discharged untreated domestic sewage, and t_3 is the pharmaceutical manufacturing plant source. The analysis demonstrated that the first three components (PC1, PC2, and PC3) were retained, and the percentage contribution, defined as $B_k/\Sigma B_k$ (B_k is the coefficients for the three components in the regression equation) were 29.4% for t_1, 62.7% for t_2, and 7.9% for t_3. Overall, more than 60% of the hormone burden in this area was from freshly discharged untreated sewage. The contribution of each source k to the SumHormone can be calculated by eq 2.

Contribution of source k (ng/L) =
$$\text{mean}_{\text{SumHormone}} \times (B_k / \sum B_k) + B_k o'_{\text{SumHormone}} t_k \quad (2)$$

where $\text{mean}_{\text{SumHormone}}$ is mean concentrations of total hormone concentrations (185.7 ng/L), $o'_{\text{SumHormone}}$ is the standard devia-

TABLE 1. Varimax-Rotated Component Matrix Following Principal Component Analysis of All Water Samples[a]

variable	variable abbreviation	rotated component number			
		1	2	3	4
estrone	E1	0.208	**0.689**	0.374	−0.124
estradiol	βE2	0.188	**0.721**	0.366	−0.145
α-estradiol	αE2	0.104	0.205	**0.864**	−0.162
androstenedione	ADD	**0.740**	**0.520**	0.218	−0.092
androsterone	ADR	0.073	**0.947**	0.048	−0.026
epiandrosterone	EADR	0.436	**0.878**	0.035	−0.026
testosterone	TTR	0.423	**0.801**	0.008	−0.018
progesterone	PGT	**0.536**	**0.773**	0.146	−0.026
17α-hydroxyprogesterone	17-HPT	0.431	**0.859**	−0.011	−0.019
21α-hydroxyprogesterone	21-HPT	**0.934**	0.313	−0.024	0.029
methylhydroxyprogesterone	MHPT	−0.032	−0.134	0.039	**0.965**
17α, 20β-dihydroxy-4-progegnen-3-one	DPO	**0.686**	0.424	−0.070	0.166
megestrol acetate	MTA	−0.073	−0.121	**0.908**	0.028
medroxyprogesterone acetate	MPA	−0.139	0.185	**0.847**	0.227
cortisol	CRL	**0.888**	0.406	0.078	−0.034
cortisone	CRN	**0.963**	0.236	0.023	−0.039
dexamethasone	DEX	0.373	0.207	**0.866**	−0.030
prednisolone	PREL	**0.961**	0.108	0.060	−0.071
prednisone	PRE	**0.935**	0.241	0.110	−0.087
percentage variance explained (%)		53.98	17.13	10.98	5.56

[a] Samples include river samples, samples from discharged sites, and STP composite effluents (values > 0.5 are highlighted).
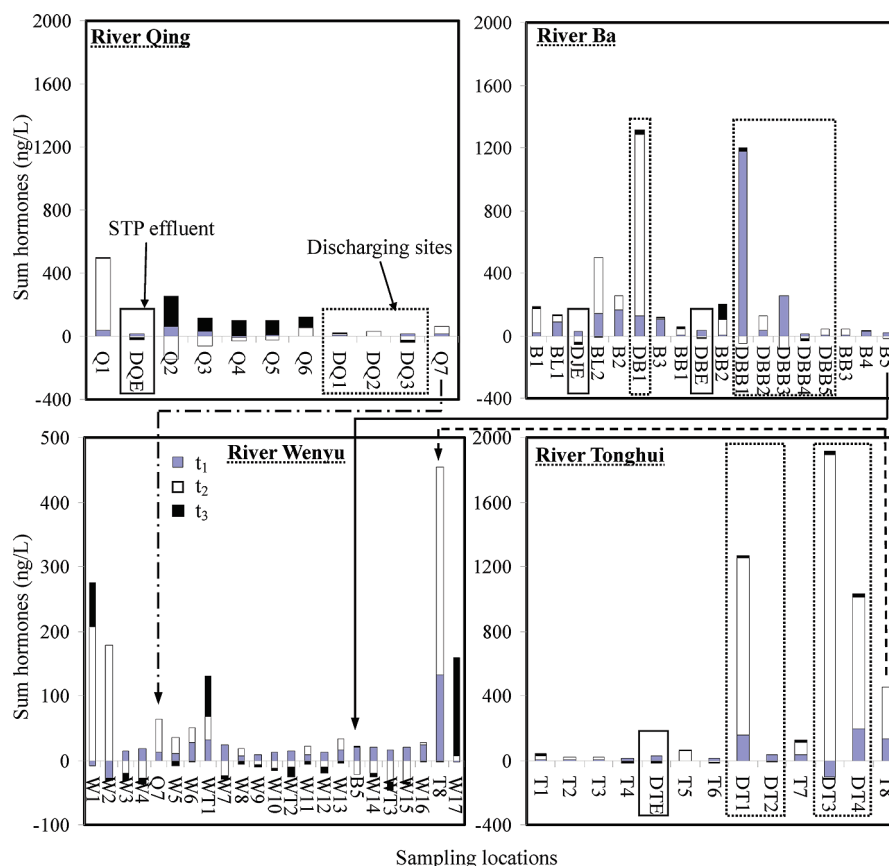

FIGURE 4. PCA-MLR source contribution plots for all water samples including river, discharged, and STP composite effluent samples. Sampling sites are shown in FIGURE 1. t_1: treated sewage and/or naturally attenuated untreated sewage. t_2: freshly discharged untreated sewage. t_3: a different source from domestic sewage.

tion of SumHormone for all samples (378.5 ng/L), and B_k is the coefficients for the three components in the regression equation. Figure 4 shows the estimated contributions for each source in all samples. The positive contributions explain the variations of the source contributions in all rivers, and the negative contributions indicate the outcome of improper variable scaling inherent in PCA methods as described previously (23, 24).

As shown in Figure 4, the hormone concentrations in STP effluents were very low and mainly contributed by t_1 (i.e., treated sewage), further supporting that STP effluents were not an important source in the investigated area. For discharging sites DBB1 and DBB3, the hormone concentrations (totally up to 1400 ng/L) were much higher than those (totally less than 12 ng/L) in STP effluents, and their locations

were at the residential area without STP nearby. However, glucocorticoids are relatively difficult to degrade in the aerobic condition compared with androgens and progestogens (such as ADR, EADR, and PGT). Thus, the high concentration proportions of gucocorticoids suggested that the hormone contamination at sampling sites DBB1 and DBB3 was not mainly contributed by t_2 (freshly discharged untreated sewage) but by t_1 (i.e., naturally attenuated untreated sewage), which corresponded to the PCA-MLR results. This also led us to conclude that the hormone profile should be considered in distinguishing between freshly discharged and naturally attenuated untreated sewage. As for different rivers, the results clearly show that the hormone concentrations originating from each source in the River Wenyu are lower than those in the three tributaries, but the contributions from t_1 are relatively high in River Wenyu. This can be explained by the fact that this river is far from human activities, and most sewage is first discharged into the tributaries before entering into River Wenyu. The contributions from t_2 were very high at the discharging sites (DT1, DT3, and DT4) and downstream in River Tonghui, suggesting that the discharging of untreated fresh sewage is the main source of steroid hormones in this river. The discharge of untreated fresh sewage was also found upstream of Rivers Wenyu, Ba, and Qing. It should be noted that the contributions from t_3 are high in most locations at River Qing, indicating there is an unknown source, possibly the discharge from pharmaceutical manufacturing plants. This phenomenon was also found in three locations at River Wenyu and two locations at River Ba.

In the PCA-MLA analysis, negative source contributions can be found in some samples, particularly in the samples from River Wenyu with low concentrations (Figure 4). This phenomenon is due to the improper variable scaling inherent in eigenvalue-based (e.g., PCA) methods, which was also reported in other study (24). Thus, more uncertainties exist in the source apportionment for samples with low concentrations. In our previous study, the ratio between the combined concentrations of two natural glucocorticoids (CRL and CRN) and the concentration of one synthetic glucocorticoid, PREL, was found to be a potential index to distinguish the treated sewage discharging from untreated ones (13). Because this ratio is independent of concentration level, it would be helpful to qualitatively indicate the contamination sources such as treated or untreated sewage for the samples even with low hormone concentrations. In the samples from River Wenyu with negative source contribution (Figure 4), the ratios of glucocorticods were 1−4 except for the sample at site W13 (7), indicating that the treated sewage was the main source of their hormone contamination, while untreated sewage partly contributed to the hormone contamination at site W13. Overall, this work reported the simultaneous occurrence of 23 natural and synthetic steroid hormones based on an analytical method for simultaneously determining five classes of steroid hormones and gave the first impression of the profile and source apportionment of these five classes of steroid hormones in the rivers.

Acknowledgments

Supporting Information Available

Detailed descriptions of target steroid hormones, materials, sample preparation, LC-MS/MS analysis, method validation, and concentrations of steroid hormones in river samples, discharging site samples, and STP effluents. This material is available free of charge via the Internet at http://pubs.acs.org.

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