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Occurrence of Natural and Synthetic Glucocorticoids in Sewage Treatment Plants and Receiving River Waters

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This paper first reports the occurrence of six glucocorticoids (prednisone, prednisolone, cortisone, cortisol, dexamethasone, and 6α-methylprednisolone) in sewage treatment plants (STPs) and receiving rivers by establishing a method for analyzing glucocorticoids in complex environmental waters. For the various types of aqueous matrices considered, the absolute recoveries were from 73 to 99%, and limits of quantification were below 0.2 ng/L. Among the seven STPs studied, the average concentrations of prednisone, prednisolone, cortisone, cortisol, dexamethasone, and 6α-methylprednisolone in influents were, respectively, 2.6 ± 2.1, 3.0 ± 1.6, 30 ± 21, 39 ± 26, 1.2 ± 0.70, and 0.62 ± 0.65 ng/L, and their percent removals were 99 ± 3.1, 78 ± 8.8, 99 ± 1.2, 98 ± 2.5, 99 ± 1.8, and 100 ± 0%, respectively. The lower removal of prednisolone was found to be due to its relatively low efficiency of biodegradation, especially in anoxic and aerobic units. The frequently detected glucocorticoids in effluents were prednisolone, cortisol, and cortisone with average concentrations 0.56 ± 0.06, 0.50 ± 0.33, and 0.26 ± 0.10 ng/L. In the receiving waters, the Tonghui and Qing Rivers, the concentrations of these compounds in some samples were much higher than those in their corresponding STP effluents; these differences depended on the sampling date, suggesting that there was random discharging of untreated wastewaters into these rivers. In addition, the ratio between the combined concentrations of two natural glucocorticoids (cortisol and cortisone) and the concentration of one synthetic glucocorticoid, prednisolone, was found to be a potential index to reflect the wastewater discharging.

Introduction

The presence of pharmaceuticals, personal care products (PPCPs), and endocrine disrupting compounds (EDCs) in the environment has attracted increasing attention due to their potential hazards (*1*, *2*). Steroid hormones, including sex hormones, rank among the most important EDC/PPCPs with regard to their potency. Studies have revealed the adverse effects of sex hormones on aquatic organisms such as decreased fertility, feminization, and hermaphroditism (*3*−*5*), and thus, there have been increasing investigations on the occurrence and fate of estrogens, androgens, and progestogens in the environment (*6*−*12*).

Glucocorticoids are one group of steroids having important physiological functions. For fish and all other vertebrates, natural glucocorticoids such as cortisol regulate development and aging, and are a critical factor for successful adaptation to stress (*13*, *14*). Recent studies have demonstrated the potential ecotoxicological effects of these compounds on fish. It has been reported that long-term cortisol treatment (diet) not only inhibited locomotion and aggressive behavior of fish (*15*), but also influenced the immunological response for starry flounder (*Platyichthys stellatus*) (*16*). A recent study also indicated that rainbow trout treated with cortisol were significantly more likely to become subordinate in paired encounters with smaller untreated conspecifics (*17*).

Natural glucocorticoids, such as cortisol and cortisone, are excreted by the adrenal cortex, and they control energy supply through gluconeogenesis and suppress the responses to inflammation and infection (*18*). Such therapeutic properties have led to the usage of natural as well as more potent synthetic glucocorticoids against a great number of human diseases such as severe allergies, skin problems, asthma, and arthritis (*19*). Glucocorticoids are also widely applied in veterinary medicine to restore muscle strength and as growth promoters to increase muscle size in animals (*20*). Thus, a quantity of glucocorticoids, mainly excreted in the urine of mammals, is thought to be released into the aquatic environment through the effluent of sewage treatment plants (STPs) or wet-weather runoff, and become potential contaminants in aquatic environments (*21*).

To the best of our knowledge, no studies on the analysis and occurrence of glucocorticoids in municipal wastewater and environmental water have been reported in the literature. Only one analytical method has been developed for measuring cortisol in water by radioimmunoassay (RIA) during a lab exposure experiment (*22*). However, due to the lack of a cleanup procedure to remove matrix interference from samples such as municipal wastewater and environmental water, measured data based on the RIA technique might be overestimated because of cross-reactions (*23*).

In this study, we developed a valid sample preparation method to extract and clean up trace glucocorticoids from sewage and surface water, and the detection method was based on liquid chromatography−electrospray ionization tandem mass spectrometry (LC−ESI−MS/MS). Finally, we applied the developed method to investigate the occurrence of six natural and synthetic glucocorticoids in six STPs and receiving river waters in Beijing, China. As far as we are aware, this is the first report about the occurrence of glucocorticoids in STPs and environmental waters, and will direct future studies on the fate of glucocorticoids in aquatic environments.

Experimental Section

Chemicals. Cortisol, cortisone, prednisone, prednisolone, dexamethasone, and 6α-methylprednisolone (Table 1) were obtained from Sigma (St Louis, MO). Deuterated cortisol (cortisol-d_2) was used as surrogate standard, and was purchased from C/D/N Isotopes (Montreal, Canada). Formic acid was analytical grade (Beijing Chemicals, China). Methanol, acetonitrile, ethyl acetate, hexane, and dichloromethane were all HPLC grade purchased from Fisher Chemical Co. (China). Ultrapure water was prepared using an Easypure UV Compact Ultrapure System (Fisher Chemical Co., China) under a conductivity of 18.2 Ω·cm^{-1}.

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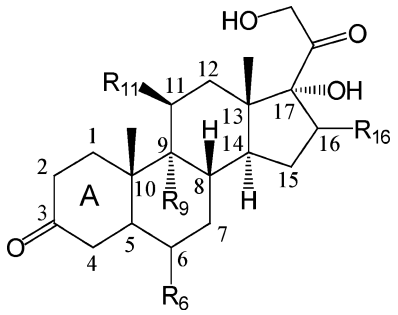
TABLE 1. Glucocorticoids Investigated in This Study



compound	A[a]	MW[b]	structure R_6	R_9	R_{11}	R_{16}
cortisol	Δ^4	362.5			−OH	
cortisone	Δ^4	360.4			=O	
dexamethasone	$\Delta^{1,4}$	392.5		-F	−OH	$-CH_3$
6α-methylprednisolone	$\Delta^{1,4}$	374.5	$-CH_3$		−OH	
prednisolone	$\Delta^{1,4}$	360.5			−OH	
prednisone	$\Delta^{1,4}$	358.4			=O	

[a] A: A ring. Δ^4: double bond carbon was assigned ring A location # 4; $\Delta^{1,4}$: double bond carbons were assigned ring A locations #1 and # 4. [b] MW: molecular weight.

Sample Collection. By using flow proportional samplers, 24-h composite samples of the influents and effluents were collected each day during the 4-week period studied (June 26 to July 23, 2006) from seven STPs, the main operating STPs in Beijing, China. These seven STPs are all operated with primary and secondary treatment processes without any post disinfection or additional filtration step. All of the plants mainly receive domestic waters; detailed information on the STPs and sampling dates are summarized in Table S1 (Supporting Information). Also, we collected water samples from Tonghui River and Qing River on the same bank (their width is between 15 and 25 m) once a week; these two rivers receive the effluents from the Gaobeidian and Qinghe STPs, respectively. The sampling stations along the Tonghui River were 2 km upstream, and 0.5, 0.55, and 2.5 km downstream, from the discharge point of Gaobeidian STP. And the sampling sites for the Qing River were situated at 4 and 2 km upstream, and 2 and 4 km downstream, of the Qinghe STP. All samples were filtered and extracted within 6 h from the time of collection.

Sample Preparation. To avoid SPE cartridge plugging, suspended materials were removed by filtration with a 1.2 μm pore size Whatman GF/C glass fiber pad (Maidstone, UK). After filtration, 70 mL of influents, 200 mL of effluents, and 2 L of river water respectively spiked with 7, 10, and 50 ng of surrogate standard were extracted through an Oasis HLB cartridge (6 mL, 60 mg, or 500 mg, Waters, USA), previously conditioned with 6 mL of ethyl acetate, 6 mL of acetonitrile and 12 mL of distilled water at a flow rate of 5−10 mL/min. The cartridge was washed with 10 mL of distilled water, and then was dried under a flow of nitrogen. Ethyl acetate/acetonitrile (1:1, v/v; 6 mL) was used to elute the analytes. For the influent and effluent samples, daily 24-hr composites were extracted, and then the 7-day elutants were pooled as composite samples for a complete week. The extracts were dried under a gentle nitrogen stream. The dry residues were redissolved in 0.2 mL of ethyl acetate, and then 1.8 mL of hexane was added. The mixed solutions were applied to silica cartridges (3 mL, 500 mg, Waters, USA), which had been preconditioned with 4 mL of water-saturated ethyl acetate and 4 mL of hexane/ethyl acetate (90:10, v/v). After the cartridges were rinsed with 3 mL of hexane/ethyl acetate (90:10, v/v), 3 mL of hexane/dichloromethane (10:30, v/v), and 3 mL of hexane/ethyl acetate (38:62, v/v), the analytes were eluted with 3 mL of water-saturated ethyl acetate. The solution was evaporated to dryness under a gentle stream of nitrogen, and reconstituted with methanol (0.49 mL for influent, 0.7 mL for effluent and 0.5 mL for river water) for LC−ESI−MS/MS analysis.

LC−ESI−MS/MS Analysis. The LC apparatus was an ACQUITY Ultra Performance LC (Waters, Milford, MA). Separation was accomplished with a Waters ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size) (USA). The column was maintained at 40 °C at a flow rate of 0.3 mL/min. Solvent A was 0.1% formic acid in ultrapure water, and solvent B was methanol. The gradient started at 35% B, was brought to 40% B in 6 min, to 80% B in the next 6 min, and to 95% B in another 3 min. Finally, the gradient was brought down to 35% B in 0.1 min, and this percentage was kept for 4 min until the next injection. The injection volume was 10 μL. Mass spectrometry was performed using a Quattro Ultima Pt tandem quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a Z-Spray ionization (ESI) source that was operated in negative-ion mode. The capillary voltage, cone voltage, and multiplier voltage were set at 3.0 kV, 45 V, and 650 V, respectively. The nebulizing, desolvation, and cone gas were supplied with ultrahigh purity nitrogen. The flow of desolvation gas and cone gas were set to 600 and 0 L/h, respectively. The source temperature and desolvation gas temperature were held at 100 and 350 °C, respectively. The radio frequency (RF) lens 1 and RF lens 2 were set as 27 and 0 V, respectively. The collision gradient was 2.0 eV.

Quantitation and Quality Control. Quantitative analysis of the glucocorticoids was performed using LC−ESI−MS/MS in multi-selected reaction monitoring (MRM). Figure 1 shows the MS spectra for the six glucocorticoids in the full-scan product-ion experiments at the corresponding collision energy. The $[M + formate]^-$ ions sequentially lost the neutral formic acid and formaldehyde, eventually yielding $[M - H - CH_2O]^-$ ions with the negative charge on the 17α-hydroxyl function as shown in Figure 1 (*25*). For each glucocorticoid, the $[M + formate]^-$ to $[M - H - CH_2O]^-$ transition was selected for quantitation, and the ratio of the quantitation transition and identification transition ($[M + formate]^-$ to $[M - H]^-$) was used for confirmation in the environmental samples. To automatically correct the losses of analytes during extraction or sample preparation, and to compensate for variations in instrument response from injection to injection, surrogate standard was used in this study. Considering that a gradient elution was applied, and thus the ionization conditions are different for each of the analytes eluting at different retention times, deuterated glucocorticoids for each corresponding glucocorticoid would be preferable to monitor the analytes. However, only deuterated cortisol could be obtained commercially. In this study, cortisol-d_2 was used as a surrogate standard for all glucocorticoids. Fortunately, these compounds show similar

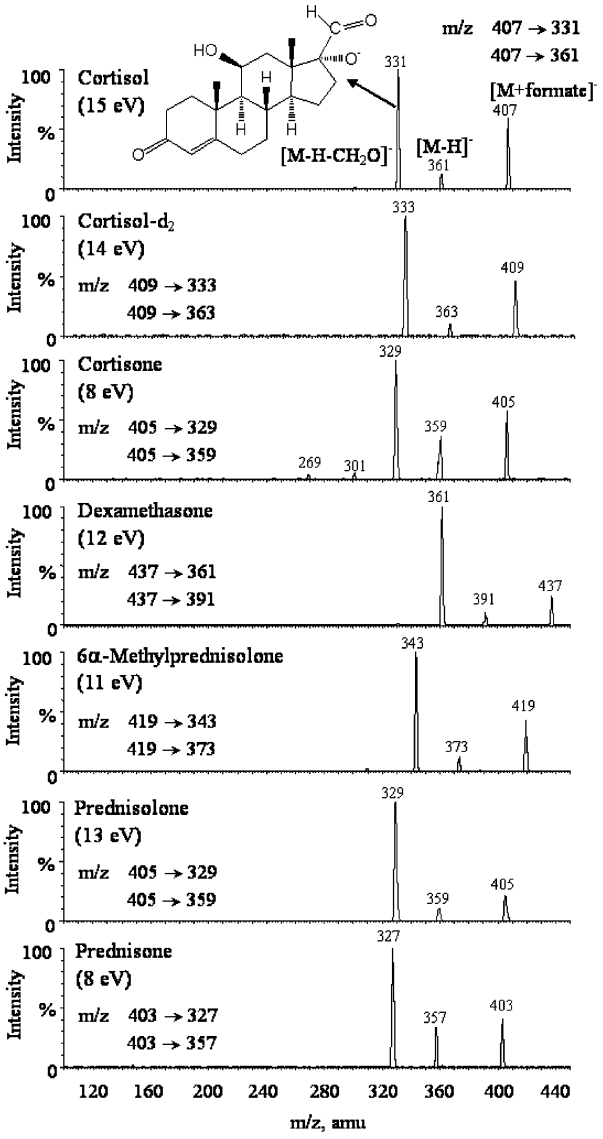


FIGURE 1. MS spectra of 100 μg/L of six glucocorticoids and surrogate standard with their respective formic acid adducts as precursor ions.

physicochemical properties such as logarithm octanol−water partition coefficient (1.27 for cortisol, and 1.16−1.66 for others) (*25*), and in the HPLC conditions used in this study, all glucocorticoids were eluted at similar retention times (7.63−9.96 min) which corresponds to the range of ca. 51−67% methanol in mobile phase. As shown in Figure S1 (Supporting Information), while the increasing proportion of methanol produces an increasing response, the response for cotisol-d_2 in the range of ca. 51−67% methanol in mobile phase is appropriately approximate to that for cotisol-d_2 eluting at ca. 56% methanol in the gradient elution used in this study (the response difference is below 12%).

All equipment rinses were done with methanol to avoid sample contamination, and one laboratory blank was analyzed every day to assess potential sample contamination. Duplicate analyses were carried out for each sample. Recovery experiments were done by spiking standard solutions to an influent and an effluent sample from Gaobeidian STP and a river water sample from the Tonghui River. Considering that individual STPs have different influent composition, the recovery experiments were also carried out for the other six STP influents. Analyte addition was made with the criterion of at least three times the original concentration that was determined prior to the fortification experiment. The overall recovery was used to considerate the accuracy of the method and matrix effects on LC−MS/MS analysis, and the RSD was used to evaluate the precision.

TABLE 2. Recoveries of the Six Glucocorticoids Spiked into the Various Types of Aqueous Matrices Considered

	recovery[a] % ±RSD		
compound	river water[b] (2 L)	STP effluent[c] (0.5 L)	STP influent[d] (0.2 L)
cortisol	86 ± 2.1	92 ± 11	87 ± 4.2
cortisol-d_2	79 ± 1.2	90 ± 4.9	85 ± 6.6
cortisone	78 ± 3.5	84 ± 6.7	81 ± 15
dexamethasone	84 ± 2.6	89 ± 10	81 ± 7.6
6α-methylprednisolone	75 ± 3.6	85 ± 8.9	73 ± 2.1
prednisolone	77 ± 3.5	73 ± 7.9	79 ± 1.7
prednisone	76 ± 3.8	87 ± 11	99 ± 4.4

[a] Mean values from three determinations by external standard quantification procedures. [b] Spiked concentration at 2 ng/L. [c] Spiked concentration in the range of 5−12 ng/L. [d] Spiked concentration in the range of 20−120 ng/L in the seven STPs considered.

Results and Discussion

Quantitation and Quality Control. Throughout the whole determination procedure, no contamination of blanks was detected. In the recovery experiments, the overall mean recoveries of the surrogate standard and six glucocorticoids using the samples from Gaobeidian STP and the Tonghui River ranged between 73 and 99% with an RSD lower than 15% (Table 2), and no significant ionization suppression was observed in present analysis. The recoveries of the surrogate standard and the six glucocorticoids for the other six STP influents were not significantly different from those for the Gaobeidian STP influent (Supporting Information, Table S2). To check for interfering peaks, the MS spectra together with the ratio of the monitored ions (by abundance) of the surrogate standard and the six glucocorticoids spiked in different water matrices were observed in the recovery studies (Supporting Information, Figure S2). The confirmation ratios for the spiked samples were all within 20% of that of the standards, indicating good purification performance of the proposed method.

Since all the target analytes were detected in three types of water matrices, the estimation of the limit of quantification (LOQ) was based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyzing field samples and on a minimal value of signal-to-noise of 9 (*26*, *27*). It was found that the LOQ for each glucocorticoid in the influent samples from the seven STPs was identical, which was supported by their similar recoveries as described above. Thus, the LOQs of the six glucocorticoids for the influent samples were between 0.08 and 0.2 ng/L, and their LOQs were between 0.02 and 0.04 ng/L in the effluent samples, and 0.01 and 0.02 ng/L in the river water samples (Table 3 and Supporting Information Tables S4 and S5).

Occurrence of Glucocorticoids in STPs. Six natural and synthetic glucocorticoids were analyzed in the influents and effluents collected from seven STPs in 2006. Figure 2 shows typical MRM LC−MS/MS chromatograms obtained from a composite influent and corresponding effluent sample, and the analytical results are shown in Table 3. Over a period of four weeks, the total average influent concentration of the six glucocorticoids, 171 ± 28 ng/L, was measured at Fangzhuang STP, which only treated domestic wastewater, and had no industrial influence. In comparison, the total average influent concentrations were 104 ± 20 ng/L for Gaobeidian STP, 78 ± 9 ng/L for Qinghe STP, 70 ± 4 ng/L for Xiaohongmen STP, 56 ± 17 ng/L for Wujiacun STP, 32 ± 14 ng/L for

TABLE 3. Concentrations (ng/L) of Six Glucocorticoids Entering and Leaving Seven Beijing Sewage Treatment Plants in June and July 2006[a]

		cortisol		cortisone		dexamethasone		6α-methyl prednisolone		prednisolone		prednisone		total		ratio[b]	
STP	date	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	out
Beixiaohe	2006/6/26−7/2	23	1.9	12	0.58	1.0	0.09	<0.08	<0.02	1.7	0.65	1.1	0.18	39	3.4	20	4
	2006/7/3−7/9	14	1.0	16	0.26	1.7	<0.02	<0.08	<0.02	1.8	0.48	1.3	<0.02	34	1.7	16	3
	2006/7/10−7/16	15	0.94	8.8	0.19	0.56	<0.02	<0.08	<0.02	1.6	0.58	0.95	<0.02	27	1.7	15	2
	2006/7/17−7/23	9.2	0.88	4.6	0.24	0.30	<0.02	<0.08	<0.02	1.5	0.51	0.73	<0.02	16	1.6	10	2
Fangzhuang	2006/6/26−7/2	120	0.43	68	0.25	1.8	<0.02	0.47	<0.02	6.6	0.59	8.4	<0.02	205	1.3	28	1
	2006/7/3−7/9	73	0.36	86	0.37	3.4	0.04	0.36	<0.02	7.5	0.62	7.0	<0.02	177	1.4	21	1
	2006/7/10−7/16	77	0.46	46	0.20	2.1	<0.02	<0.08	<0.02	5.0	0.52	5.5	<0.02	136	1.2	25	1
	2006/7/17−7/23	69	0.35	81	0.29	1.9	<0.02	0.11	<0.02	6.0	0.64	8.0	<0.02	166	1.3	25	1
Gaobeidian	2006/6/26−7/2	73	0.43	36	0.25	1.1	<0.02	1.7	<0.02	4.0	0.58	2.8	<0.02	119	1.3	28	1
	2006/7/3−7/9	55	0.40	56	0.46	2.1	<0.02	1.9	<0.02	3.6	0.58	3.2	<0.02	121	1.4	31	1
	2006/7/10−7/16	40	0.36	32	0.31	0.77	<0.02	1.0	<0.02	3.0	0.50	2.4	<0.02	79	1.2	24	1
	2006/7/17−7/23	61	0.31	27	0.19	0.34	<0.02	2.0	<0.02	3.6	0.47	2.7	<0.02	96	1.0	24	1
Jiuxianqiao	2006/6/26−7/2	22	0.30	24	0.24	0.86	<0.02	0.78	<0.02	2.3	0.57	1.8	<0.02	52	1.1	20	1
	2006/7/3−7/9	7.6	0.26	13	0.17	2.1	<0.02	0.35	<0.02	1.7	0.49	0.81	<0.02	25	0.9	12	1
	2006/7/10−7/16	18	0.25	11	0.15	1.2	<0.02	0.49	<0.02	1.7	0.47	0.80	<0.02	33	0.9	17	1
	2006/7/17−7/23	10	0.26	6.6	0.21	0.42	<0.02	0.32	<0.02	1.5	0.60	0.44	<0.02	20	1.1	12	1
Qinghe	2006/6/26−7/2	34	0.58	26	0.32	1.0	<0.02	<0.08	<0.02	2.5	0.56	1.8	<0.02	65	1.5	24	2
	2006/7/3−7/9	34	0.60	38	0.15	1.6	<0.02	0.27	<0.02	2.5	0.52	1.9	<0.02	78	1.3	29	1
	2006/7/10−7/16	47	0.40	31	0.20	1.3	0.04	0.48	<0.02	3.3	0.72	2.0	<0.02	85	1.4	24	1
	2006/7/17−7/23	48	0.59	29	0.17	0.62	<0.02	0.28	<0.02	2.2	0.56	2.2	<0.02	82	1.3	35	1
Wujiacun	2006/6/26−7/2	26	0.54	22	0.23	0.87	0.02	0.34	<0.02	2.4	0.59	2.0	<0.02	54	1.4	20	1
	2006/7/3−7/9	32	0.52	39	0.46	1.9	0.03	<0.08	<0.02	2.7	0.54	2.7	<0.02	79	1.5	26	2
	2006/7/10−7/16	20	0.36	14	0.27	0.58	<0.02	0.49	<0.02	1.9	0.59	1.5	<0.02	38	1.2	18	1
	2006/7/17−7/23	26	0.39	23	0.27	0.69	<0.02	0.34	<0.02	2.3	0.55	2.0	<0.02	54	1.2	21	1
Xiaohongmen	2006/6/26−7/2	39	0.35	26	0.21	1.2	<0.02	1.5	<0.02	3.3	0.59	2.6	<0.02	73	1.1	19	1
	2006/7/3−7/9	34	0.31	27	0.26	1.5	<0.02	0.89	<0.02	2.7	0.48	1.7	<0.02	67	1.1	23	1
	2006/7/10−7/16	34	0.30	24	0.20	0.86	<0.02	1.7	<0.02	2.9	0.51	2.4	<0.02	67	1.0	20	1
	2006/7/17−7/23	39	0.33	28	0.13	1.3	<0.02	1.6	<0.02	2.8	0.53	2.5	<0.02	74	1.0	24	1

[a] Average of duplicate injections. LOQ is 0.2 ng/L for cortisone, 0.08 ng/L for 6α-methylprednisolone, and 0.1 ng/L for the other four glucocorticoids in the STP influents, and 0.04 ng/L for cortisone and 0.02 ng/L for the other five glucocorticoids in the STP effluents. [b] Between the combined concentrations of two natural glucocorticoids (cortisol and cortisone) and the concentration of synthetic prednisolone.

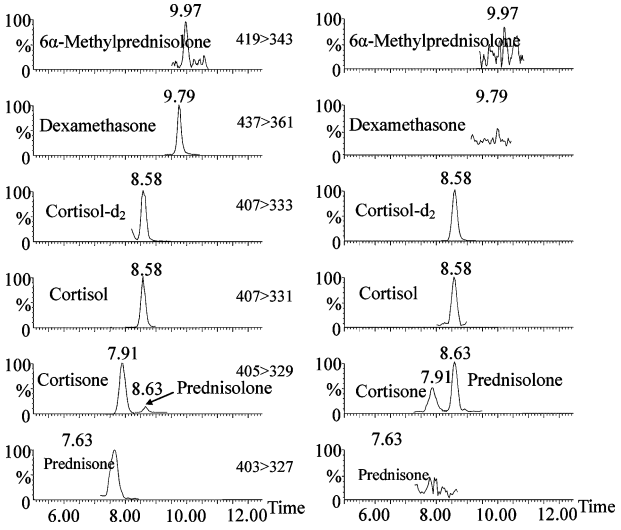


FIGURE 2. LC−MS/MS chromatograms of a composite influent (Qinghe, 2006/6/26−7/2) (left panels) and corresponding effluent (right panels) for six glucocorticoids as well as a surrogate standard, cortisol-d_2.

Jiuxianqiao STP, and 29 ± 10 ng/L for Beixiaohe STP, which treated both domestic and industrial wastewater. Among the detected glucocorticoids, cortisol and cortisone were always the dominant compounds in influents, with concentrations ranging from 9.2 to 120 ng/L and from 4.6 to 86 ng/L, respectively, while the concentrations of the other four synthetic glucocorticoids in the STP influents were from 0.44 to 8.4 ng/L for prednisone, 1.5 to 7.5 ng/L for prednisolone, 0.30 to 3.4 ng/L for dexamethasone, and <0.08 to 2.0 ng/L for 6α-methylprednisolone. This finding was expected considering that cortisol and cortisone are natural glucocorticoids excreted by humans rather than synthetic pharmaceutical substances like the other four glucocorticoids.

As shown in Table 3, the STP effluents still contained glucocorticoids that were therefore discharged into the receiving waters. Prednisolone, cortisone, and cortisol were detectable in all the effluents analyzed, with concentrations ranging from 0.47 to 0.72 ng/L, 0.13 to 0.58 ng/L, and 0.25 to 1.9 ng/L, respectively. It should be noted that in the seven STP influents, prednisolone accounted for 4% of the total average glucocorticoid concentrations, while in the effluent, its proportion increased to 42%. As for dexamethasone and prednisone, the detected frequencies were relatively low: dexamethasone was detected in 5 of 28 effluent samples analyzed, at concentrations ranging from 0.02 to 0.09 ng/L, and prednisone (0.18 ng/L) was only found once, in the Beixiaohe STP effluent. 6α-Methylprednisolone was not detected in any of the effluent samples in this sampling campaign.

Removal of Glucocorticoids in STPs. The percent removals of the six glucocorticoids were calculated by comparing the concentrations of each glucocorticoid in the influents and effluents from each plant (Figure 3). It was interesting to find that of the six glucocorticoids, five (prednisone, cortisone, cortisol, dexamethasone, and 6α-methylprednisolone) experienced relatively high and stable removal ranging from 92 to 100% among the seven STPs; however, the removal of prednisolone was lower than those

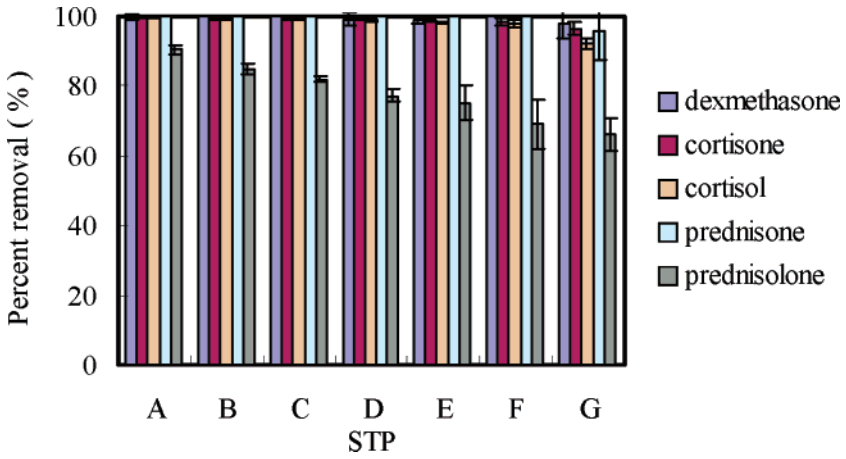


FIGURE 3. Percent removals of glucocorticoids in the seven STPs. The values for 6α-methylprednisolone are not shown because it was not detected in the effluents from any of the STPs. A: Fangzhuang; B: Gaobeidian; C: Xiaohongmen; D: Qinghe; E: Wujiacun; F: Jiuxianqiao; G: Beixiaohe.

of the other five glucocorticoids in all seven STPs considered (from 66% in Beixiaohe STP to 90% in Fangzhuang STP). Although the exact mechanism for the removal of the glucocorticoids in STPs is not known, the removal of a compound in a STP could be affected by at least two major factors: biodegradation and sorption to sludge. Because the calculated logarithm value of the octanol−water partition coefficient (log K_{ow}) of prednisolone (1.44) is similar to those calculated for the other five glucocorticoids (1.16−1.66) (*25*), it is difficult to explain its low removal from the view of sorption. Therefore, the removal of glucocorticoids during each individual biological process was estimated by comparing the entering and leaving concentration of each glucocorticoid, exemplified by the Gaobeidian STP (Supporting Information Table S3). According to the results, the removal of prednisolone during the anaerobic process was 72%, lower than those of prednisone (94%), cortisone (90%), cortisol (91%), dexamethasone (87%), and 6α-methylprednisolone (89%). It should be noted that in the subsequent anoxic unit, while 92−100% of each glucocorticoid other than prednisolone was removed, only 35% of prednisolone was removed, suggesting that the anoxic degradation of glucocorticoids was largely dependent on their structures. For the three residual glucocorticoids (prednisolone, cortisone, and cortisol) in the effluents after the anoxic unit, 2%, 6%, and 36%, respectively, were removed by the subsequent aerobic unit. Thus, the relatively low biodegradation efficiency of prednisolone would result in its occurrence in all seven STP effluents as one of three dominant compounds, even if its proportion in the influent was much lower than that of the other two compounds (cortisol and cortisone). It can also be concluded that for all glucocorticoids, the aerobic process was less efficient than the anaerobic and anoxic processes. As shown in Figure 3, of the seven STPs, the percent removal of each glucocorticoid in Beixiaohe STP was relatively low, especially for prednisolone. This could be explained by the fact that the biological treatment processes of the other six STPs consisted of anaerobic, anoxic, and aerobic units, but Beixiaohe's only included anoxic and aerobic units (Table S1). In addition, the shorter solid residence time (5.2 days) and hydraulic residence time (7 h) in Beixiaohe as shown in Table S1 would also contribute to the relatively low removal in Beixiaohe STP. It should be noted that the conjugated glucocorticoids would affect the percent removals of glucocorticoids as exemplified by the deconjugation occurrence of steroid glucuronidates and sulfates in STP (*24*), and thus the percent removals of glucocorticoids may be even better than that based on the free glucocorticoid concentrations.

Residual Glucocorticoids in River Waters. To study the occurrences of glucocorticoids in environmental waters due to discharge from STPs, in July 2006 we analyzed the samples taken from the Tonghui and Qing Rivers, which receive the effluent from Gaobeidian and Qinghe STPs, respectively. As shown in Tables S3 and S4 (Supporting Information), all six glucocorticoids were detected in the river water samples. The concentrations of cortisol, cortisone, prednisolone, prednisone, dexamethasone, and 6α-methylprednisolone were, respectively, 0.08−3.4, 0.06−4.2, 0.03−0.64, 0.12−0.86, 0.02−0.31, and 0.04−0.08 ng/L, depending on the sampling location and date. It should be noted that dexamethasone, which was only occasionally detected in STP effluents, was very frequently detected in the river samples (30 of 32) from both upstream and downstream of the two rivers, although at relatively low levels. In addition, 6α-methylprednisolone (3 of 16 river samples) and prednisone (9 of 16 river samples), which were never detected in the corresponding STP effluents, were also observed in the Tonghui River. The above results suggested untreated wastewater was discharged into the river.

Figure 4 shows the concentration variations along the two rivers. It was found that in some samples, the total concentrations were higher than those in the corresponding STP effluent samples, and similar to the corresponding influents; the concentration levels of cortisol and cortisone were significantly higher than those of the other four glucocorticoids. In fact, at a point near 0.55 km downstream of the Tonghui River, we did find a wastewater discharging pipe, and the total concentration of detected glucocorticoids was up to 9.0 ng/L, 8.2 and 12.5 times the corresponding STP effluent and the sample taken at 0.5 km downstream at the last sampling date, respectively. In the Qing River, the total concentration in the sample at 2 km downstream was 3.2 times the corresponding STP effluent at the first sampling date. These results supported the hypothesis that untreated wastewaters were being discharged into the river, and this discharge seemed to be random based on the fact that the high concentration levels of glucocorticoids in the downstream samples from the two rivers varied greatly in this sampling campaign, as shown in Figure 4.

From the above results, it was clear that cortisol, cortisone, and prednisolone were ubiquitous in STP influents, effluents, and river waters, and the removal of one synthetic compound, prednisolone, in wastewater treatment process was lower than that of two natural glucocorticoids (cortisol and cortisone) in STP, indicating that the glucocorticoids can provide information on the status of sewage discharging.

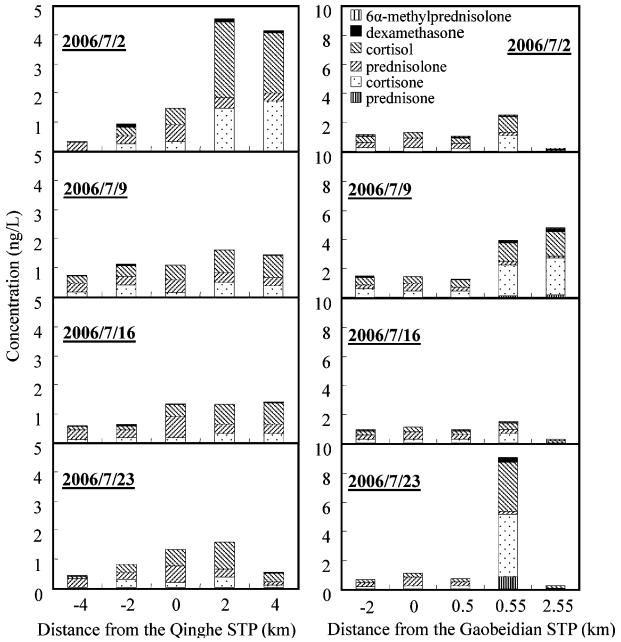


FIGURE 4. Concentrations of glucocorticoids in the Qing (left panels) and Tonghui (right panels) Rivers at different distances from the corresponding STPs. 0 distance refers to the STP effluent.

Thus, we estimated the ratios between the combined concentrations of cortisol and cortisone and the concentrations of prednisolone in influents and effluents from seven STPs. As shown in Table 3, the ratios in STP influents were from 10 to 35 which were larger than those in STP effluents (1 to 4). Furthermore, we also estimated the ratio in river samples to analyze whether there was untreated wastewater discharging into the two rivers. Of 32 river samples, the ratios in 5 samples with the relatively high total glucocorticoid concentrations compared to those of effluents ranged from 11 to 39, clearly indicating the inputs of untreated domestic waste; the ratios in 25 samples with the total concentrations relatively lower than those in effluents were from 1 to 4, indicating the consequence of the treated sewage coverage; and the ratios for the other two were 5 and 8, respectively, suggesting that there would be less input of sewage and/or dilution by large mass of river water (Supporting Information Tables S4 and S5). It should be noted that this paper provided a primary work on the potential of glucocorticoids used as an indicator of domestic waste pollution, and further work is necessary to clarify the effectiveness of glucocorticoids as a tracer by comparing them with other tracers such as linear alkylbenzenes (LABs) (*28*).

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Supporting Information Available

Additional figures, spectra, and tables of data. This material is available free of charge via the Internet at http://pubs.acs.org.

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