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Occurrence and estrogenic activity of steroid hormones in Chinese streams: A nationwide study based on a combination of chemical and biological tools

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ABSTRACT

Steroid hormones (SHs) are continuously released into the aquatic environment through various pathways after being excreted by humans and animals, interfere with the normal function of the endocrine system and may affect the physiology and reproduction of exposed aquatic life. To conduct a nationwide investigation of the occurrence and biological effects of SHs in surface river/steam water in China, we quantitated 27 selected SHs in 217 surface water samples by solid-phase extraction (SPE) tandem LC-MS/MS and used a recombinant yeast estrogen assay to screen extracts of the water samples for estrogenic activities. SHs were commonly found in the surface water samples, and their levels were typically in the ng L^{-1} range. Estrone (E1) and estriol (E3) were normally present in several to dozens of times higher concentrations than estradiol (E2) and 17-a-Ethinylestradiol (EE2). The high concentrations (mean $>$ 1 μg L^{-1}) of Sum_{SHs} were primarily obtained in areas under extreme water stress, specifically the eastern coastal areas. Source apportionment based on the profiles of the target compounds indicated that 54.5% of the SHs in target samples came from freshly discharged untreated sewage. The estrogen equivalent ($EEQ_{(bio)}$) values ranged from 0.01 to 40.27 ng L^{-1}, and the calculated EEQ ($EEQ_{(cal)}$) values were generally lower than the corresponding $EEQ_{(bio)}$ values for all samples. E2 was the main contributor to the estrogenicity among the three estrogens, with a contribution ratio of 82.8%. The risk quotient values of E2 were highest and ranged from 1.55 to 782.95, and 76.0% of the target surface samples displayed the greatest environmental risk. We concluded that the impacts of SHs on humans in Chinese surface waters should not be ignored and that certain actions should be taken to decrease the levels of SHs in source waters, especially measures targeting SHs in untreated wastewater from the vast rural areas.

1. Introduction

Steroid hormones (SHs), one group of the important endocrine-disrupting compounds (EDCs), are contaminants of growing concern since they present potential adverse ecological effects and risks. These emerging contaminates are commonly present in water environments in extremely low concentrations, ranging from ng L^{-1} to μg L^{-1} (Yin et al., 2002; Zhang et al., 2015). SHs may influence the normal function of the endocrine system and present a potential threat to aquatic life (Colborn et al., 1993). Furthermore, prolonged exposure to estrogen, especially through drinking water, is a major cause of the increased incidence of reproductive diseases and cancer in humans (Carlsen et al., 1995; Wee and Aris, 2017). SHs are transported to aquatic environments through several pathways, including the excreta of humans and animals, directly discharged raw sewage, and from veterinary industries, which widely use SHs to prevent disease and maximize the size of livestock, poultry, and fish. As a result, the occurrence and distribution of SHs have been extensively found in aquatic environments, but a very limited number of studies concentrate on the other steroid hormones, including androgens, progestogens, glucocorticoids, and mineralocorticoids, especially in Chinese streams.

SHs widely occur in surface water across the globe, in concentrations up to the μg L^{-1} level. As early as 2002, Kolpin et al. (2002) employed GC–MS methods to quantify the levels of hormones in water samples from a network of 139 streams across 30 states. In their study, reproductive hormones (17α-estradiol, 17β-estradiol, estriol, and estrone) were detected in 40% of the streams sampled, with frequencies of detection similar to those of antibiotic pharmaceuticals. However, when toxicity is considered, the concentrations of reproductive hormones may have greater implications for the health of aquatic

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organisms than pharmaceuticals. Several analogous studies have since aimed at investigating SHs on national scales (Benotti et al., 2009; Kostich et al., 2013; Labadie and Budzinski, 2005; Salgueiro-Gonzalez et al., 2015). Comprehensive studies in this area of research have been conducted in the United States (Focazio et al., 2008; Kolpin et al., 2002; Kostich et al., 2013), Europe (Labadie and Budzinski, 2005; Salgueiro-Gonzalez et al., 2015), South Korea (Duong et al., 2010; Kim et al., 2007), Japan (Hashimoto et al., 2005) etc. Several studies of SHs in Chinese streams have focused on local watersheds, such as the Haihe (Luo et al., 2011) Liao (Bai et al., 2014), Yangtze River (Jiang et al., 2011; Wu et al., 2014) and Pearl River Basins (Diao et al., 2017). However, no single study has reported the occurrence of SHs in Chinese streams on a national level. Recently, Zhang et al. (2014) studied the national consumption, emission, and various fates of 7 natural SHs in China based on the quantitative research of the excretion of each steroid by humans and animals. Most of the steroids (62% of the total emissions) were emitted by livestock. Contributions from the excretion of livestock accounted for 93% of the total excretion of E1, 94% of that of E2, and 98% of that of E3. Finally, over 80% of the total excreted steroids were discharged into the surroundings, with 83 and 17% of that amount being received by water and soil compartments. A model was used to predict the environmental levels of 7 SHs in 58 river watersheds; higher concentrations of steroids were found in the rivers in east China, which were affected by the large quantities of steroids emitted by high densities of humans and animals (Zhang et al., 2014). No experimental data are available on the spread of SH contamination on a national scale.

Many research papers concerned with pollutants such as SHs have been published as the quantities of contaminants directly and indirectly emitted into the streams increases with the rapid development of industry and agriculture. The total excretion of hormones by humans and animals in China was assessed to be 3069 t/yr (Zhang et al., 2014). China therefore provides a perfect location to study the occurrence and spatial distribution of SHs in areas with high urbanization on a large scale. Furthermore, few studies have overall considered the occurrence and bioactivity of the SHs currently existing on a national scale. Evaluating the contamination levels of SHs in these streams using a combination of chemical and biological analysis tools is therefore important.

Combinations of chemical and biological tools can provide additional information for pollution evaluation. Instrumental monitoring, such as the use of liquid chromatography tandem mass spectrometry (LC-MS/MS), provides quantitative and semi-quantitative data about the trace pollutants in the aquatic environment, while biological analysis provides methods to identify the accumulation risks of target pollutants. Bioanalysis is commonly used as a supplement to chemical analysis in ecological risk assessment to solve complex environmental problems. A combination of in vitro bioassays and instrumental analysis better assesses the causal relationships between biological effects observed in the water samples and instrumental analysis results.

The primary aim of this study was to therefore conduct the nationwide investigation of the occurrence of SHs in surface waters in China using a combination of chemical and biological methods. The selected target SHs (including estrogens, androgens, progestogens, glucocorticoids, and mineralocorticoids) in Chinese surface waters and the spatial variations in their occurrence were revealed. The aims of this study thus include (a) determining the concentrations of the SHs in 217 river water samples, (b) discussing the estrogenic effect of organic extracts in 217 river water samples based on a yeast estrogen screen (YES) bioassay and (c) determining the source apportionment of the total SHs.

2. Materials and methods

2.1. Chemicals and reagents

All SH standards were purchased from Tokyo Chemical Industry (Tokyo, Japan) or Steraloids (Newport, RI). Carbamazepine-d_{10} was purchased from Cambridge Isotope Laboratories (Wellesley, MA), and estrone-d_4 was purchased from Toronto Research Chemicals (Toronto, Canada). Strata-X (500 mg, 6 mL) cartridges were purchased from Phenomenex (Torrance, CA).

2.2. Site description and sample collection

In this study, the occurrence of 27 SHs in river water samples obtained in 2014 and 2015 from a network of 29 streams across 31 provinces was investigated. Surface waters were targeted for this study. Details of the site description and a map of sampling points were described in our previous research paper (Yao et al., 2018), and all the samples were collected in the same batch. All the sites were randomly chosen in 2014 and 2015 without trying to determine the seasonal variation trends in the SH levels. The occurrences and levels therefore only hint at overall behavior when the water samples were collected. Although sampling spot selection did not follow a design with statistically representative samples, 217 surface water samples were selected from 31 provinces, and this sampling network included a broad range of potentially harmful and important pollutants, source strengths, environmental settings, and variations in the population served. Samples were collected using pre-cleaned dark brown glass bottles. Once the samples reached the lab, they were filtered through a 0.8 μm fiberglass membrane purchased from Jinteng (Shanghai, China) and then immediately solid-phase extraction (SPE)-processed.

2.3. Analytical methods

River water samples were extracted by SPE using 500 mg Strata-X cartridges in an AutoTrace 280 automatic SPE instrument (Thermo®, Sunnyvale, CA). Before extraction, the samples (1000 mL) were spiked with 1 mL of 5% Na_2EDTA and acidified to pH 3.0 using sulfuric acid; 2 isotopically labeled internal standards were then added at concentrations of 50 ng L^{-1}. The SPE cartridges were first preconditioned by the addition of 10 mL of MeOH, followed by 5 mL of acidified MeOH (0.1% formic acid in MeOH) and 10 mL of deionized water. The samples were then passed through the polymeric cartridges at a flow rate of 10 mL min^{-1}, and the cartridges were subsequently rinsed with 10 mL of 5% MeOH and dried under nitrogen flow for 5 min. The target substances were then eluted by the addition of 5.0 mL of MeOH, followed by 5.0 mL of basified MeOH (0.1% ammonium hydroxide in MeOH). The extracts were evaporated to complete dryness under a gentle nitrogen flow, and the compounds were redissolved in a final volume of 1.0 mL by spiked 5% MeOH in water. The final extracts were moved into 2 mL sample vials and placed away from light at −20 °C until mass spectrometry analysis. For the bioassay, samples (2000 mL) were extracted using the abovementioned protocol and without being spike with isotopically labeled internal standards.

The SH were examined using an HPLC system interfaced to an Agilent 6495 tandem quadrupole MS system. Detailed chromatographic analyses and MS/MS identification information for all the SHs are reported in the Supporting Information (SI). Other method validation parameters (precision, sensitivity, accuracy, limit of detection (LOD), and limit of quantitation (LOQ)) are provided in Tables S1 and S2 of the SI.

2.4. YES bioassay

A recombinant YES was performed by employing the procedure published by Routledge and Sumpter (1996) that referred to a slightly optimized method previously developed by Rastall et al. (2004). Briefly, the employed recombinant yeast cells contain expression plasmids carrying strong promoter sequences, the lac-Z (encoded β-galactosidase) reporter gene, and stably integrated DNA sequences of the human estrogen receptor (hER). By measuring the activity of β-