OCCURRENCE OF SELECTED PHARMACEUTICALS IN A RECEIVING RIVER

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Introduction

Pharmaceuticals are a large and diverse group of compounds, which were designed to prevent, cure and treat diseases and improve health. Most of the pharmaceuticals and their metabolites are excreted in human feces and urine after use and eventually enter sewage systems. With increasing consumption, wastewater treatment plants (WWTPs) receive wastewater containing various pharmaceuticals residues at concentrations from ng/L to μg/L. Current WWTPs are not designed to remove these emerging pollutants and their capability for removing pharmaceuticals is therefore not efficient. As a result, many pharmaceuticals have been detected in both WWTP effluents and natural waters. This has lead to an increasing concern regarding possible risks because of pharmaceuticals released into the aqueous environment.

In this study, we aim to investigate the occurrence of selected pharmaceuticals in an urban WWTP receiving river. In addition, potential risks of pharmaceuticals on the aquatic organisms in the WWTP receiving river were also evaluated.

Materials and methods

Chemicals. Ibuprofen, naproxen, ketoprofen, diclofenac, and clofibric acid were obtained from Sigma-Aldrich (St.Louis, MO, USA). HPLC grade methanol, acetone, as well as formic acid were purchased from Tedia Company, Inc (USA), and ultra-pure water was produced by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Sampling. River water samples were collected from the river receiving a WWTP effluent. Sampling sites were selected at WWTP effluent outfall, the upstream (200m) of the WWTP effluent outfall and downstream (200m, 500m, 800m, 1.6km, 3.2 km) of the WWTP effluent outfall, respectively. Four sampling campaigns were conducted on December 22nd, 2010, January 4th, 8th, 2011, and March12 th, 2011, respectively. Grab water samples were collected in 4 L clean amber glass bottles from 0.5-1 m below the surface. Before sample collection, each bottle was pre-rinsed with river water for three times. Then the collected water samples were transported in a cooler to the laboratory. Sediments were collected in prewashed jars and kept in a cooler during transport to the laboratory.

Immediately after delivery to the laboratory, surface water samples were filtered through pre-baked (400 °C, > 4 h) 0.7μm glass fiber filters (GF/F, Whatman, Mainstone, England). The filtrate was stored at 4 °C to avoid any degradation until extraction. The suspended particles collected on glass fiber filters and sediments were wrapped with pre-baked (450 °C) aluminum foil, sealed in Ziplock bags, and stored at −18 °C until further treatment.

Sample extraction and analysis. Water samples were extracted using a solid-phase extraction (SPE) system. The pH of the samples was adjusted to 4.0 with 2-mol/L HCl. A known amount (200 ng) of fenoprop was added as surrogate standard. After the cartridges (ENVI-18, 500 mg, 3 mL) were conditioned by applying 2 mL of methanol and 2 mL of Milli-Q water (pH = 2), the water samples were introduced to the cartridges by means of PTFE tubes at a flow rate of approximately 5-8 mL/min. After washing by 5 mL of 5.0% methanol solution, the cartridges were dried under vacuum for 2 h and eluted with 3 mL of acetone. The extracts were then evaporated to approximately 500μL under a gentle nitrogen stream and 500 μL of methanol was added. Evaporation continued until the final volume of the extracts was 500μL. Suspended particles and sediments were freeze-dried and were accurately weighed before being extracted. Then dried sediment or suspended particles was extracted with 6 mL and 2 mL of methanol in series and twice with 2 mL acetone. 200 ng of surrogate standard (fenoprop) was spiked into the slurry in the first extraction step. In all extraction steps, the slurry was ultrasonicated for 10 min and then centrifuged at 5000 rpm for 5 min. Supernatants from all of the extraction

steps were combined and evaporated to 1 mL under a stream of nitrogen. This concentrates were redissolved in 100 mL of distilled water (pH = 4), and the SPE was carried out in the same manner as that described above.

All samples were analyzed by TSQ Quantum high performance liquid chromatography coupled with mass spectrometry (Thermo Fisher Scientific, San Jose, CA, USA). The separation was performed on an Agilent Eclipse XDB C18 reversed phase column (150 mm × 2.1 mm, 5μm), with the flow rate of 350 μL/min. Methanol and water with 0.1% (v/v) acetic acid were used for separation. The injection volume was 10μL, and the column temperature was 30 °C. The gradient was held at 75% of methanol for 5 min, and increased to 90% of methanol within 5 min and held for 5 min, and then reset to initial conditions of 75 % of methanol in 5 min and held for 5 min. The mass spectrometer detection of pharmaceuticals was operated in selected reaction monitoring (SRM) mode. Analyses were performed in the negative ion mode. Quantification of the target compounds was performed using an external standard method. The linear range was established between 1 and 500 μg/L with a correlation coefficient (R2) of 0.9997. The limit of detection (LOD) were 1 pg for clofibric acid and 5 pg for the other tested compounds, respectively. The limit of quantitation (LOQ) was 50 pg for all target compounds.

Quality assurance/quality control (QA/QC). For each batch of 6 samples, a procedural blank, a spiked blank, a matrix spiking sample, and a matrix spiking duplicate were processed. No quantifiable analytes were detected in the blank samples. The recoveries of five acidic pharmaceuticals and surrogate compound were 89.7±8.2% for water samples and 82.5±9.3% for solid samples, respectively. The concentrations of targets were not recoveries corrected.

Results and discussion

Occurrence of pharmaceuticals in sediments. The relatively low concentrations of selected pharmaceuticals were detected in sediments. The concentrations of five pharmaceutical compounds ranged from not detected to 12.9 ng/g on a dry weight basis. It is noticeable that diclofenac was detected in almost all sediment samples at concentrations ranging from 4.89 to 12.9 ng/g. This may be due to relatively high logKow (4.51), which tends to associate with particles and consequently being detected in sediments[1]. Clofibric acid was the next most frequently detected (93%) with a maximum concentration of 1.96 ng/g. Ibuprofen and ketoprofen were also detected, but with low frequencies of 50% and 67%, and with the mean levels less than limit of detection. No naproxen was found in all the sediment samples. It is also noted that the occurrence pattern of the five pharmaceuticals in each sampling site was similar, indicating a uniform source of these pharmaceuticals. TOC showed a good correlation to total pharmaceutical concentrations expect naproxen ($r^2 = 0.42$, $p < 0.01$) in all sediment samples, which indicates that TOC may be one of the dominant sorbents for pharmaceuticals occurring in sediments.

Occurrence of pharmaceuticals in water. As shown in Figure. 1, all of the selected pharmaceuticals were detected in water samples with ibuprofen showing the highest concentrations (up to 173.5 ng/L). Diclofenac and clofibric acid were other two dominant pharmaceuticals in water samples. Diclofenac and clofibric acid has been identified as refractory contaminants in several investigations of municipal sewage influents and effluents [2-5].It was noted that pharmaceutical concentrations in WWTP discharge point are always obviously higher than those in the upstream and downstream in the river. For example, the mean concentration of ibuprofen was found to be 60.6 ng/L in upstream sample of the outfall, which had risen to 173.5 ng/L at the outfall indicating a input of this compound through WWTP effluent, and it subsequently fell to 57.5 ng/L at the downstream 200 m of the outfall, a clear sign of reduction in concentration. The same trend is observed for other pharmaceuticals. This is not unexpected, as it has previously been stated that the main sources of such pharmaceuticals to river systems is through WWTP effluents [6-7].

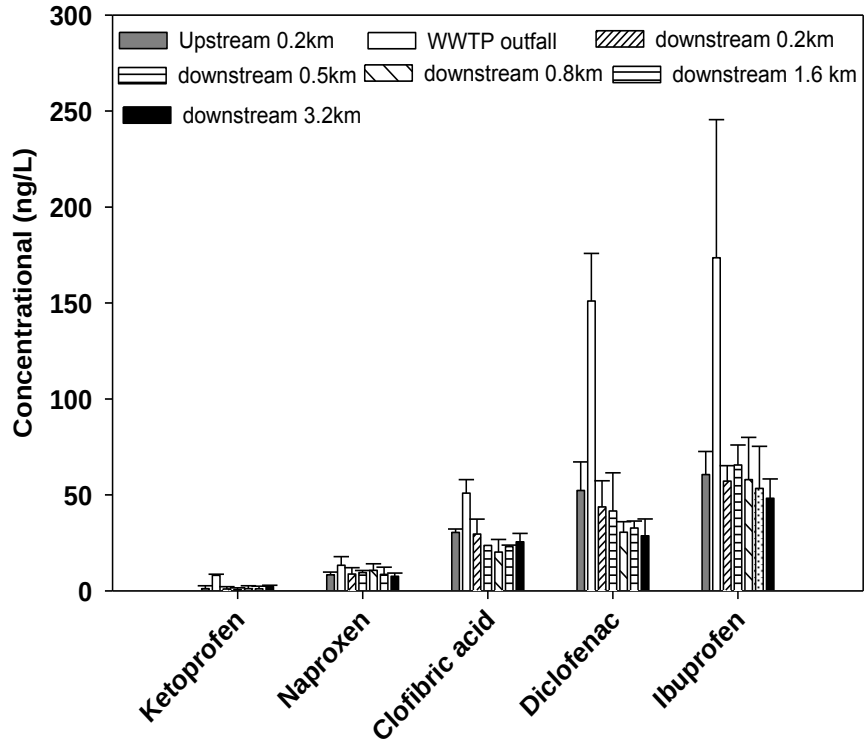

Figure 1. Distribution of selected pharmaceuticals in water samples.

Occurrence of pharmaceuticals in particulate matter. Diclofenac and clofibric acid were found in all of the particulate matter samples. Ibuprofen, naproxen and ketoprofen were detected with low frequencies of 90%. Moreover, particulate matter samples showed approximately 1-3 times higher concentrations of some pharmaceuticals compared to sediment samples. This may be due to higher total organic carbon content in particulate matter phase (1.3%-4.1%). A positive correlation was found between pharmaceutical concentrations and total organic carbon ($r^2 = 0.63$, $p < 0.05$), again suggesting that total organic carbon is a significant adsorbent in particulate matter for pharmaceuticals.The highest concentrations were found for diclofenac (5.83-18.9 ng/g) and clofibric acid (1.7-6.3 ng/g).The spatial distribution pattern was similar to the corresponding sediments with the highest concentrations at the WWTP outfall. Consistent with water samples, particulate matter from WWTP outfall displayed the highest pharmaceuticals concentrations indicating the importance of WWTP as a source of pharmaceuticals to the river concerned.

Environmental risk. The environmental risk quotient (RQ) can be calculated by dividing the measured environmental concentration (MEC) by the Predicted No-Effect Concentrations (PNEC) of the compound. If RQ is higher or equal to 1, there is a risk for adverse effects in the aquatic environment [8]. The PNECs for the acidic pharmaceuticals were adopted from the literatures [9-10]. Based on the worst-case scenario, only the RQ value for diclofenac in river water was higher than 1, indicating it poses a potential risk to aquatic organisms. Nevertheless, theses evaluation is only focused on the risk that individual compounds may cause to aquatic organisms, it must be noted that the impact of a mixture of these chemicals could prove more toxic than the individual compounds alone.

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