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The occurrence and ecological risks of endocrine disrupting chemicals in sewage effluents from three different sewage treatment plants, and in natural seawater from a marine reserve of Hong Kong

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ABSTRACT

We determined the concentrations of 12 endocrine disrupting chemicals (EDCs) in sewage effluents collected from three different sewage treatment plants (STPs) in Hong Kong, and found 4-nonylphenol (NP) and bisphenol A (BPA) were the most abundant EDCs. Effluent concentrations of NP and BPA were higher in dry season than in wet season, but opposite seasonal changes of NP were observed in receiving waters, probably due to the surface runoff. The two secondary STPs showed higher removal efficiency for these compounds than the preliminary STP, while having higher removal efficiency in wet season. Therefore, it is necessary to upgrade the preliminary STP and improve the EDC removal efficiency in dry season. Seawaters from the Cape D' Aguilar Marine Reserve adjacent to these STPs also exhibited elevated NP levels with a hazard quotient >1. Furthermore, diluted effluents from the STPs elicited significant transcriptional responses of EDC-related genes in the marine medaka fish.

1. Introduction

Endocrine disrupting chemicals (EDCs) are of global concern and broadly defined as chemicals that interfere with the normal function of endocrine systems in wildlife and humans (Burkhardt-Holm, 2010). Numerous laboratory experiments indicate that EDCs can cause negative health effects (e.g. growth, behaviour, reproduction and immune function) in fishes through disrupting their endocrine systems (Mills and Chichester, 2005). Estrogenic EDCs can adversely affect male fishes through induction of vitellogenin and inhibition of the development of secondary sexual characteristics at very low exposure concentrations (Länge et al., 2001).

Most EDCs are man-made organic chemicals being introduced to the marine environment through anthropogenic inputs such as contaminated sewage effluents and surface runoff. Typical representatives of synthetic EDCs, 4-nonylphenol (NP) and bisphenol A (BPA) are the major contributors to the endocrine-disrupting activities in aquatic environments (Auriol et al., 2006). NPs are the main degradation products of alkylphenols polyethoxylates which have been widely used as surfactants in household, agriculture, and industrial processes (White et al., 1994). At an exposure concentration as low as 10 ppb, NP can cause an increase of vitellogenin mRNA and a decrease in the growth rate of testes in male rainbow trout (Lech et al., 1996). BPA is an industrial raw material mainly used in plastic, rubber, adhesive, and cable industries, and known to cause a decrease in sperm production in mice (Von Saal et al., 1998), and lead to a delay in hatching of eggs and a suppression of growth in juvenile rainbow trout (Aluru et al., 2010). It has been widely recognized that effluent discharges from sewage treatment plants (STPs) are the major source of the EDCs to aquatic environments (Zhang and Zhou, 2008). A growing body of research has indicated that sewage effluents and even their receiving waters can introduce estrogen-like effects in fishes (Gibson et al., 2005).

There are limited documented studies examining the composition and concentrations of EDCs in sewage effluents and natural seawaters of Hong Kong (Li et al., 2007; Kueh and Lam, 2008). Li et al. (2007) discovered that concentrations of NP ranged from 29 to 2591 ng/L in surface water samples collected from Mai Po Marshes Nature Reserve, northwest of Hong Kong and its levels were higher in winter (dry season; November and December) than in late summer (moderately wet season; September and October).

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Kueh and Lam (2008) surveyed the ambient occurrences of selected EDCs, such as nonylphenol and nonlyphenol ethoxylates (NPEO), in coastal waters, rivers, sediments and biota, and their results suggested that sewage effluents acted as primary sources for these chemical contaminants. However, little is known about (1) the composition of EDCs in sewage effluents, and (2) the removal efficacy of EDCs from raw sewage by different types of STPs in Hong Kong. Since sewage effluents often comprise of a complex mixture of EDCs, it is essential to examine the composition of EDCs in local sewage effluents and identify the dominated chemical contaminants. Furthermore, the seasonal variability of EDC concentrations in STPs and receiving waters in sub-tropical Hong Kong are still largely unknown. This knowledge is important to decide appropriate measures for minimizing ecological risks from EDC emissions to sensitive receivers such as marine reserves in the marine environment.

EDCs can alter the expression of estrogenic-related (ER) genes, such as cyp19a and cyp19b, which may result in developmental and reproductive abnormalities in fishes (Kortner et al., 2009). EDCs can also cause disruptive endocrine effects through aryl hydrocarbon receptors (AHRs) and the peroxisome proliferator-activated receptors (PPARs) in fishes. The AHR pathway mainly regulates the activation of several genes that encode phase I and phase II xenobiotic metabolism enzymes, while the PPAR pathway intermediates receptors and genes involved in the regulation of energy homeostasis, cell proliferation, differentiation and survival (Fang et al., 2012). In this study, we used embryos and larvae of the marine medaka fish (*Oryzias melastigma*) to assess their transcriptional response to sewage effluents and receiving waters, involving 13 genes in the ER, AHR and PPAR signalling pathways.

There were three main objectives in this study. First, an attempt was made to quantify, for the first time, the concentrations of 33 common EDCs and identify the most abundant ones in sewage effluents collected from the three STPs which are located at south of Hong Kong Island and close to the Cape D' Aguilar Marine Reserve (Fig. 1). The 33 EDCs include natural and synthetic estrogens, androgens, progestagens and glucocorticoids. Second, since both NP and BPA were identified as the most abundant EDCs in our study, we further monitored the concentrations of NP and BPA in influents and effluents, as well as in the receiving waters from the Cape D' Aguilar Marine Reserve during both dry and wet seasons. Based on the measured concentrations of NP and BPA in the sewage effluents and receiving waters and their corresponding predicted no effect concentrations, ecological risks of these two compounds were assessed. Third, we investigated the effect of diluted sewage effluents and natural seawaters from the marine reserve on the mortality, hatching success, and gene expression in embryos of the marine medaka *O. melastigma* through a laboratory study. The results would provide supplementary information for evaluating the current ecological risk of NP and BPA to marine organisms in the marine reserve.

2. Materials and methods

2.1. Sampling

The three Sewage Treatment Plants (i.e., Shek O, Stanley, and SWIMS STP) are located in south of Hong Kong Island, Hong Kong, within 3 km distance to the Cape D' Aguilar Marine Reserve (Fig. 1). Shek O STP is a preliminary treatment facility (i.e., screening plant) designed for removal of suspended matters with a diameter larger than 6 mm and this plant only treats about 864 m^3/day of raw sewage from Shek O Village District (Table 1; Fig. 2). Stanley STP is a secondary treatment plant and daily treats about 8100 m^3 of raw sewage from Stanley and Tai Tam Districts. It has the largest treatment capacity among the three STPs. Its primary treatment consists of a screen and a grit chambers, and its secondary treatment includes an aerobic bioreactor followed by a secondary sedimentation and disinfection (Table 1; Fig. 2). The sludge is returned from the secondary sedimentation chamber for biological treatment, while the surplus activated sludge is dried before being taken to landfill. The SWIMS STP, located within the Cape D' Aguilar Marine Reserve, is designed to mainly treat the wastewater generated from the Swire Institute of Marine Science (SWIMS) within the marine reserve (Table 1; Fig. 2). As a trickling biofilter treatment plant, SWIMS STP consists of septic tank, biofilter tank, sand leach tank, disinfection tank and sedimentation chamber. The three STPs represent different levels of treatment efficiency: a screening treatment plant (Shek O STP), a secondary biological treatment plant (Stanley STP), and a trickling filter plant (SWIMS STP).

We sampled both influents and effluents from the three STPs, and receiving seawaters within the marine reserve three times during wet season (April, May, and June 2012) and three times during dry season (December 2012, January and February 2013). Hence, three replicates were used for each STP in each season. For both influents and effluents, 15-min wastewater samples were composited at the Stanley STP to prepare 24-h flow-weighted samples, and the 8-h composite samples with 1-h interval were collected at the SWIMS STP. For Shek O STP, influent and effluent samples were grabbed in triplicates between 11:00 and 13:00, with a 0.5 h interval between each influent or effluent sample. Natural seawater samples were collected at 0.5 m below sea surface in the Cape D' Aguilar Marine Reserve. In investigation of EDC composition in effluents and bioassay, we grabbed effluent samples in duplicates from each STP with a pre-cleaned stainless-steel bucket in April 2012. After collection, samples were transported on ice to laboratory and immediately filtered through GF/C glass fiber filter papers, and then stored at 4 °C. All samples were extracted for EDCs within a week. Blank water was taken to field as control to monitor any contamination during the transport.

2.2. Chemical analysis

Thirty-three phenolic EDCs, including androgens, estrogens, xenoestrogens, glucocorticoids, and progestagens, were analysed with RRLC–MS/MS as described by Liu et al. (2011). For each of the effluent samples, 1 L of water sample was filtered through a glass fiber filter (Whatman GF/F, 0.7 μm, UK). 100 μL each of 1 mg/L of E1-d4, E2-d4, T-d3, S-d3, CRL-d2 and P-d9 were added as the internal standards. Solid Phase Extraction (SPE) cartridges (Oasis HLB, 6 mL and 500 mg each) were preconditioned with methanol and HPLC water. The filtered water samples passed through the SPE cartridges at 5–10 mL/min. The target compounds were eluted using ethyl acetate. The extracts were dried and re-dissolved in 1 mL of methanol for clean-up. The glass cartridge was filled with glass wool, silica gel and anhydrous sodium sulphate from bottom to top. Each extract was added to the cartridge, which was preconditioned with methanol, ethyl acetate/methanol (90:10, v/v), and hexane. After the cartridge was rinsed with hexane, the target compounds were eluted with ethyl acetate/methanol. The eluate was then dried and reconstituted. The target compounds were analysed by RRLC–MS/MS with EI. Liquid chromatography was performed on an Agilent 1200 series RRLC system (Agilent Technologies). The chromatographic separation was performed on an Agilent Zorbax SB-C18 (100 mm × 3 mm, 1.8 μm) column with pre-column filter (2.1 mm, 0.2 μm). The column oven temperature was 40 °C and the injection volume was 10 μL. Mass spectrometry was performed using an Agilent 6460 Triple Quadrupole detector with ESI in both negative and positive modes (Agilent Corporation, USA). The quantitative analysis was performed in MRM mode.

The analytical procedure for NP and BPA was based on Zhao et al. (2009). Briefly, 1 L of each of the influent, effluent or seawater

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