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Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: Influence of hydrodynamics and physical–chemical properties

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ABSTRACT

The fate and exposure risks of pharmaceutically active compounds (PhACs) and endocrine disrupting chemicals (EDCs) in marine environments are not well-understood. In this study we developed a multi-residue analytical method for quantifying concentrations of forty target compounds in seawater from Singapore. Analyses of samples ($n = 24$) from eight sites showed the occurrence of several compounds, including gemfibrozil (<0.09–19.8 ng/L), triclosan (<0.55–10.5 ng/L), carbamazepine (<0.28–10.9 ng/L) and ibuprofen (<2.2–9.1 ng/L). A 3D hydrodynamic model for Singapore was used to predict residence time (t_R). Principal Components Analysis revealed a strong relationship between t_R and contaminant concentrations. While source emissions are undoubtedly important, proximate distance to a wastewater treatment plant had little influence on concentrations. The site with the greatest t_R, which exhibited the highest concentrations, is adjacent to Singapore's largest protected wetland reserve. The results highlight an important linkage between hydrodynamic behavior and contaminant exposure risks in complex coastal marine ecosystems.

1. Introduction

In recent years, the World Health Organization and regulatory authorities across Europe, North America and elsewhere have highlighted concerns regarding potential ecological and human health impacts related to contaminants of emerging concern, including pharmaceutically active compounds (PhACs) and various endocrine disrupting chemicals (EDCs), (EC, 2011; USEPA, 2012; WHO, 2012). Many of these compounds are widely used and produced, with annual production volumes in the kilotons to megatons range. For example, according to a recent review by Howard and Muir (2011), carbamazepine, ibuprofen or diclofenac are amongst the top selling pharmaceutical compounds. Also, the global production capacity for bisphenol A (BPA) was 5.2 million metric tonnes in 2008 (Dow Chemical Company, 2012).

The discharge of PhACs and EDCs into the environment is mostly associated with household, municipal and industrial waste streams (Schwarzenbach et al., 2006). These compounds have been consistently observed in wastewater effluents and freshwater systems (Metcalfe et al., 2010; Schwab et al., 2005; Vanderford and Snyder, 2006). Since organisms in receiving waters experience more direct exposure to wastewater contaminants, PhAC and EDC residues in the environment may pose greater risks to wildlife and ecological health, compared to human health (Morley, 2009). The environmental behavior of micropollutants such as PhACs and EDCs remains very much unknown (Schwarzenbach et al., 2006). This is particularly true for coastal marine systems with complex ecological and hydrodynamic profiles, especially in tropical regions. Investigations of PhACs and EDCs in seawater is limited to a few studies of temperate coastal waters (Benotti and Brownawell, 2007; Jia et al., 2011; Magner et al., 2010; Nakada et al., 2008; Togola and Budzinski, 2008; Weigel et al., 2004; Wille et al., 2010). Given that a substantial fraction of the world's population lives in large, coastal cities, there is a current need to better understand the fate and exposure risks of PhACs and EDCs in coastal marine ecosystems.

PhACs encompass several families of compounds with a wide range of physical–chemical properties. They are often water soluble compounds and relatively non-bioaccumulative. However, a number of high-production volume pharmaceuticals qualify, based on an assessment of their structure, as probably persistent and

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bioaccumulative (Howard and Muir, 2011). Natural and synthetic EDCs have inherent endocrine-disrupting properties and may interfere with the natural functions of hormones and potentially modify sexual development and reproductive function in organisms (Oberdorster and Cheek, 2001; Scholz and Kluver, 2009; Soin and Smagghe, 2007). PhACs consist of a variety of human and veterinary drugs which contain biologically active ingredients designed for a variety of chemico-biological interaction at a target site. Such biologically active compounds can elicit physiological and behavioral effects, even at relatively low exposure concentrations in organisms (Brausch and Rand, 2011; Dann and Hontela, 2011; Santos et al., 2010).

Singapore is a highly urbanized city state in Southeast Asia, 120 km north of the equator (Fig. 1). This tropical island city has a current population of approximately 5.2 million, linked across the Straits of Johor to the second most populous city in Malaysia, Johor Bahru (population 1.4 million). Singapore is home to one of the world's busiest shipping ports and a variety of industrial activities, including oil and gas, electronics, textiles and fine chemicals manufacturing. Singapore is a world leader in water treatment and water resource management (Luan, 2010). Like many other coastal world cities, a fraction of the treated domestic and industrial wastewater in Singapore is ultimately discharged to coastal waters. Effective monitoring of chemical contaminants is crucial for water quality management and environmental risk assessment initiatives. There is a current need to better assess the occurrence and behavior of PhACs and EDCs in coastal marine environments.

The objectives of the present study are threefold, including to (i) develop and evaluate a multi-residue analytical method for quantitative determination of a suite of forty PhACs and EDCs in coastal seawater samples, (ii) conduct a field study to generate a comprehensive dataset for those substances in Singapore's coastal marine environment and (iii) investigate the influence of coastal hydrodynamics and physical–chemical properties on the distribution and exposure risks of PhACs and EDCs. Chemicals such as atrazine, bisphenol A, linuron, nonylphenol, tetrabromobisphenol A and thiabendazole were included in the study due to their endocrine disruption potential (Bonefeld-Jorgensen et al., 2007; California EPA, 2001; Friedmann, 2002; Hogan et al., 2012; Huang et al., 2012; Manabe et al., 2006; Preuss et al., 2006; Van der Ven et al., 2008).

2. Material and methods

2.1. Chemicals

High-purity standards of the native compounds were supplied by Merck (USA), Wako Pure Chemicals (Japan), Sigma–Aldrich (USA) and Tokyo Chemical Industry (Japan). Isotopically labeled compounds used as internal standards were purchased from Cambridge Isotope Laboratories (USA) and C/D/N Isotopes (Canada) (Table S1, See Supporting Information). Although not present in technical mixtures and often used as reference (Preuss et al., 2006), 4-*n*-nonylphenol was used in the present study to assess the performance of the analytical method for this compound structure. Primary stock solutions of all individual analytes were prepared in methanol and were stored at −20 °C in the dark. HPLC-grade solvents were obtained from Fisher Scientific (UK) and Tedia (USA). All equipment was washed with laboratory detergent and rinsed with MilliQ water (Millipore, USA). Glass equipment (e.g. sample bottles, glass fiber filters) was baked at 300 °C overnight, and rinsed consecutively with dichloromethane, acetone and methanol before use. All reusable polymerware (e.g. SPE manifold parts) was rinsed with methanol.

2.2. Field sampling

Triplicate water samples were collected from a boat at 8 locations around Singapore in June–July 2011 (Fig. 1). Sampling occurred during the relatively dry phase of Singapore's Southwest Monsoon Season, characterized by mean daily rainfall typically <150 mm (Figs. S1–S2, See Supporting Information). Seawater samples were collected below surface (3 m depth) in a solvent rinsed amber glass bottle. Details on the water quality (pH, Total and Dissolved Organic Carbon, Total Suspended Solids) are given in Table S2 (See Supporting Information). Samples were transported on ice in the dark to the laboratory and processed immediately.

2.3. Solid phase extraction

The majority of investigations of PhACs and EDCs have utilized solid phase extraction (SPE) coupled with liquid-chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) for identification and trace quantification of PhAC and EDC residues in environmental samples (Metcalfe et al., 2010; Schwab et al., 2005; Vanderford and Snyder, 2006). While USEPA method 1694 provides protocols for extraction and analysis of PhACs and EDCs in water and biosolids (USEPA, 2007), relatively few methods for the quantitative determination of PhACs and EDCs in seawater have been reported (Benotti and Brownawell, 2007; Togola and Budzinski, 2008; Wu et al., 2010; Zhang and Zhou, 2007). In the present study, seawater samples (1 L) were filtered through a glass fiber filter (diameter 47 mm, particle retention 1 μm) and pH was adjusted (either pH 2.00 or 7.00, ±0.05) using diluted hydrochloric acid (1 M). Prior to extraction, samples were spiked with deuterated and ^{13}C-labeled analogs and equilibrated for 14 h in the dark at 4 °C. Two hours prior to extraction, 500 mg tetrasodium ethylenediamine-tetraacetate dehydrate was added in the sample (USEPA, 2007). Samples were extracted using HLB cartridges (60 mg, 3 mL; Phenomenex). Cartridges were preconditioned with 5 mL methanol, 5 mL Milli-Q water and 5 mL Milli-Q water with pH matching the sample (either pH 2.00 or 7.00). After extraction, the cartridges were rinsed with MilliQ water, and then dried under vacuum for 15 min. Analytes were eluted with 12 mL methanol followed by 6 mL methanol/acetone (1:1). Combined extracts were concentrated to approximately 1mL under a gentle flow of nitrogen at 40 °C. Injection internal standards (400 pg atrazine-d_5, 50 pg $^{13}C_6$-2,4,5-trichlorophenoxyacetic acid) were added just prior to LC-ESI-MS/MS analysis.

2.4. Identification and quantification of target analytes using LC-ESI-MS/MS

Extracts were analyzed by LC-ESI-MS/MS, using an Agilent 1100 HPLC coupled with Applied Biosystems Q-TRAP 4000 MS/MS. Chromatographic separation was achieved on a Poroshell 120 SB-C18 column (2.1 mm; 150 mm; 2.7 micron; Agilent

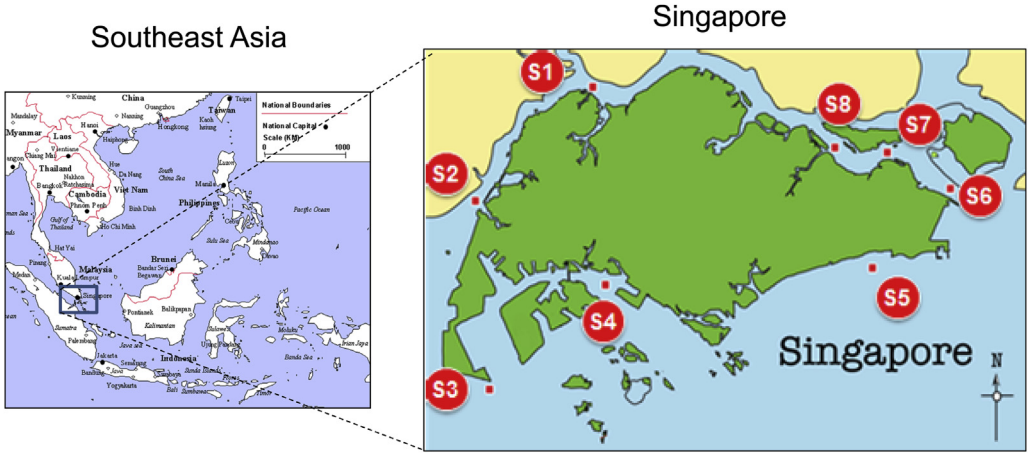

Fig. 1. Map showing study area and the different sampling locations in Singapore's coastal waters.