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Revealing ecological risks of priority endocrine disrupting chemicals in four marine protected areas in Hong Kong through an integrative approach☆

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ABSTRACT

Marine Protected Areas (MPAs) in Hong Kong are situated in close proximity to urbanized areas, and inevitably influenced by wastewater discharges and antifouling biocides leached from vessels. Hence, marine organisms inhabiting these MPAs are probably at risk. Here an integrative approach was employed to comprehensively assess ecological risks of eight priority endocrine disrupting chemicals (EDCs) in four MPAs of Hong Kong. We quantified their concentrations in environmental and biota samples collected in different seasons during 2013–2014, while mussels (*Septifer virgatus*) and semi-permeable membrane devices were deployed to determine the extent of accumulation of the EDCs. Extracts from the environmental samples were subjected to the yeast estrogen screen and a novel human cell-based catechol-*O*-methyltransferase ELISA to evaluate their estrogenic activities. The results indicated ecological risks of EDCs in the Cape d'Aguilar Marine Reserve. This integrated approach can effectively evaluate ecological risks of EDCs through linking their concentrations to biological effects.

1. Introduction

Marine protected areas (MPAs) shield marine organisms within a well-defined boundary, but they do not work well in protecting the organisms from outside threats (Allison et al., 1998). Many chemical pollutants can be transported from pollution sources to an MPA by water currents or surface runoff, and thus adversely impact the marine organisms living there, and thereby undermine the effectiveness of the MPA as a conservation measure (Terlizzi et al., 2004; Xu et al., 2015a). It is, therefore, important to implement effective monitoring, risk assessment and control of chemical contamination at MPAs, in particular those MPAs located within or near urbanized areas.

The Government of the Hong Kong Special Administrative Region (HKSAR) has set up three marine parks and one marine reserve to protect corals and their associated ecosystems since the mid-1990s'; these are the Hoi Ha Wan Marine Park (HHW), Yan Chau Tong Marine Park (YCT), Tung Ping Chau Marine Park (TPC) and Cape d' Aguilar Marine Reserve (MR). These MPAs are, however, located near to urbanized areas and are inevitably affected by chemical pollutants, including those released from shipping activities, partially-treated sewage effluent and contaminated surface runoff (Xu et al., 2015a). Chemical pollutants have been identified in the marine environment of Hong Kong (Chan, 1995; Fang et al., 2009), but investigations regarding these chemical pollutants in the MPAs are scarce.

There has been an increasing global concern about endocrine disrupting chemicals (EDCs) due to their widespread distribution, persistence, bioaccumulative tendency and toxicological effects to wildlife at trace levels (Kamata et al., 2010). Estrogens (e.g., steroid hormones) and xenoestrogens (e.g., surfactants) can induce vitellogenesis and intersex gonads in male fish (Harris et al., 1997; Lech et al., 1996), suppress sexual behaviour in male fish (Oshima et al., 2003) and reduce spawning success and growth rate of female fish (Lech et al., 1996). EDCs can cause disruptive endocrine effects

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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). Aromatase, estrogen receptor and vitellogenin genes were significantly up-regulated in the marine medaka fish (*Oryzias melastigma*) after exposure to nonylphenols and bisphenol A as well as EDC-contaminated seawater collected from the MR in Hong Kong (Xu et al., 2015b).

Tributyltin (TBT) and triphenyltin (TPT), acting as xenoandrogens, are highly toxic to many aquatic species, and able to trigger imposex in females of marine gastropods at concentrations as low as 1 ng/L in seawater (Hoch, 2001; Fromme et al., 2005). TBT and TPT remain persistent in Hong Kong waters, and elevated levels of these compounds, in particular TPT can be detected in various marine mollusc and fish species (Ho and Leung, 2014a,b). However, there were limited documented studies determining the ambient concentrations of organotins in the marine environment of Hong Kong. Kueh and Lam (2008) surveyed the occurrences of selected EDCs in coastal waters, rivers, sediments and biota in Hong Kong, and indicated that partially treated sewage effluents acted as primary sources for an array of EDCs such as TBT and nonylphenols (NPs). We also demonstrated the presence of high concentrations of NPs and bisphenol A (BPA) in the treated sewage effluents discharged from different sewage treatment plants in Hong Kong, as well as in natural seawater and sediment samples collected from the MR of Hong Kong. Both the diluted sewage effluents and natural seawater contaminated with the EDCs in this reserve can trigger high levels of estrogenicity as shown by the yeast estrogen screen (YES) bioassay and gene expression responses in marine medaka fish (Xu et al., 2014, 2015a, 2015b).

Since the conventional chemical analytical monitoring of ambient concentrations of these EDCs in seawater and sediment samples only provides snapshots of their contamination profiles, other more ecologically relevant monitoring approaches should be adopted to more accurately evaluate the bioavailability and impacts of the EDCs to organisms living in the MPAs. For example, transplantation of passive samplers and/or biomonitors like mussels can offer a time-integrated picture of the bioavailable EDC levels and their bioaccumulation extent (Karr, 1981). Mussels and other bivalve molluscs have been widely utilized as biomonitors due to their ability to accumulate a wide variety of EDCs (Arditsoglou and Voutsa, 2012). Artificial passive sampling devices have been commonly applied to supplement the monitoring of trace organic pollutants in many studies (e.g., David et al., 2010) as these passive samplers have a clean baseline and their uptake follows a predictable manner. One of the most widely used ones is the semipermeable membrane device (SPMD) which consists of a thin film of a neutral lipid enclosed in thin-walled tubing made of low-density nonporous polymers (Huckins et al., 1993). In general, the uptake and release of organic pollutants by the SPMD are less affected than biomonitors by salinity and temperature, and thus alleviate the confounding problems of growth, reproduction and geographic limitation in the use of biomonitors (Richardson et al., 2001).

Despite the highly efficient and accurate detection and quantification of target EDCs by such advanced instrumental analyses, this chemical analysis does not consider additive or synergistic effects of structurally different EDCs present in complex mixtures to a living organism. In contrast, bioassays integrate effects of overall estrogenic potencies, including antagonistic and synergistic effects (Ma et al., 2007). To investigate the integrated estrogenic effects of the sewage effluents as well as the receiving waters and sediments, the use of effective bioassays can provide complementary results to the chemical analyses. Currently, the YES assay is the most widely employed assay for assessing estrogenicity in environmental samples worldwide (Arnold et al., 1996). However, the YES is simply a gene reporter system being cloned in yeast, and hence it cannot reflect how animal cells naturally respond to the EDCs. A human cell-based *in vitro* assay has been developed by our co-authors (Prof. S.L. Ho and Dr. P.W.L. Ho) and such a novel assay could more accurately reflect how mammalian cells respond to the challenge of EDCs. Recently, it was reported that catechol-*O*-methyltransferase (COMT, an important enzyme for the metabolism of carcinogenic catechols and catecholamines) in human cells can be powerfully down-regulated by different EDCs (e.g. estradiol, BPA, octylphenol) via the estrogen-response-element (ERE) in the gene promoter region (Ho et al., 2008a, 2008b). Based on these observations, a novel cell-based COMT ELISA has been developed to assess quantitatively estrogenic activity in biota and environmental samples using a human cell line (European patent EP2328909/WO20100034183) (Ho et al., 2013). In contrast to the YES assay which measures the proliferation of the activation of the estrogen responsive element in the genetically modified yeasts, the COMT assay elucidated reduction of native protein expression in a human cell in response to either a pure testing compound or an extract with a mixture of unknown components. The present study was also designed to apply this COMT ELISA for extracts of seawater and sediment samples collected from the four MPAs of Hong Kong, and the results were compared with those obtained from the YES assay as an indirect validation of the COMT ELISA.

This study describes a pragmatic integrated approach for effectively evaluating the ecological risks posed by eight priority EDCs through linking their concentrations in various environmental matrices of interest to the biological effects as reflected by the YES and COMT ELISA bioassays (Fig. 2). First, this study investigated the occurrence and distribution of eight priority EDCs (i.e., NP, BPA, TBT, TPT, octylphenol (OP), estrone (E1), estradiol (E2), and ethinylestradiol (EE2)) in seawater, sediment and marine organisms collected from the four MPAs of Hong Kong (i.e., HHW, YCT, TPC and MR; Fig. 1). Secondly, this study also examined the extent of accumulation of these EDCs in the transplanted black mussel (*Septifer virgatus*) and SPMDs at the four MPAs. Thirdly, estrogenic activities in seawater and sediment samples collected from the MPAs were further evaluated by both YES assay and COMT ELISA. The adoption of these two bioassays was mainly due to the fact that our previous study revealed high estrogenic activities in the seawater and sediment samples collected from the MR of Hong Kong (Xu et al., 2015b). Based on the overall risk assessment results, the MPAs at ecological risk from EDCs were identified, and appropriate recommendations for risk reduction management were made.

2. Materials and methods

2.1. Water, sediment and biota sampling

Seawater and sediment samples were collected at the four MPAs (i.e., HHW, YCT, TPC and MR) in July 2013 and Jan 2014, respectively (Fig. 1). HHW is a sheltered bay located north of the Sai Kung West Country Park in northeast of Hong Kong. YCT is located on the northeast coast of Plover Cove Country Park. TPC encloses the small island Ping Chau in the Mirs Bay on the northeastern water of Hong Kong. MR lies on the southeastern tip of Hong Kong Island. Water samples were taken from 1 m below the water surface and triplicate samples were taken from each MPA. About 50 mL of methanol was immediately added to each 1 L water sample to suppress biotic