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The use of *in vitro* bioassays to quantify endocrine disrupting chemicals in municipal wastewater treatment plant effluents

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Abstract

In vitro bioassays are widely used to detect and quantify endocrine disrupting chemicals (EDCs) in the influents and effluents of municipal wastewater treatment plants (WWTP). These assays have sometimes led to false positive or negative results, partly due to the low EDC concentrations in the samples. The objectives of the present study were: (a) to compare the estrogen screen (E-Screen) and the yeast estrogen screen (YES) bioassays using the 17β-estradiol (E2) or its equivalence and (b) to investigate if a combination of the E-Screen and YES assays can be used to improve the accuracy of EDC detection and quantification. The E-Screen bioassay was conducted with the MCF-7 (BOS) human breast cancer cell line while the YES bioassay employed two different types of recombinant yeast. The influent and effluent samples collected from the five WWTPs operated by the Greater Vancouver Regional District (GVRD) were analyzed by both the E-Screen and the YES bioassays. Since the results of the E-Screen and YES bioassays varied by up to 4-fold on the same split sample of a nominal E2 concentration, the mean value of the E-screen and YES bioassays was used to represent the EDC activity of a given WWTP sample. Results of these studies showed that the E2 equivalent concentration in each WWTP sample was consistently higher than 1 ng/L, a concentration that may potentially cause endocrine disruption in different aquatic species. The composition of selected EDCs in a subset of effluent samples was examined using a gas chromatograph–high resolution mass spectrometer (GC–HRMS). EDC composition in 10 WWTP samples correlated with the mean endocrine disrupting activities of the E-Screen and YES bioassays. Results also indicated that secondary treatment plants are comparable to the primary treatment plants in removing EDCs from the final effluents.

Keywords: MCF-7; YES; Endocrine; Wastewater; Treatment

1. Introduction

In the aquatic environment, exposure of organisms to endocrine disrupting chemicals (EDCs) has been linked to endocrine effects in male fish such as vitellogenin induction and feminized reproductive organs (Tyler et al., 1998; Purdom et al., 1994; Aherne and Briggs, 1989; Routeledge et al., 1998). It is suggested that industrial and municipal effluents as well as urban and agricultural runoff are the major sources of EDC discharged into the aquatic environment (Desbrow et al., 1998; Snyder et al., 1999; Boyd et al., 2003). Therefore, when rainbow trout (*Oncorhynchus mykiss*) were kept in cages close to the

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discharges of wastewater treatment plant (WWTP) effluents, vitellogenin synthesis was induced in the male fish (Harries et al., 1997). Elevated levels of vitellogenin and decreased serum testosterone were also reported in male carp (*Cyprinus carpio*) caught near WWTP discharges (Folmar et al., 1996). Vitellogenin elevation and gonadal intersex also were observed in roach (*Rutulis rutulis*) and flounder (*Platichthys flesus*) caught near WWTP discharge sites (Jobling et al., 1998; Allen et al., 1999). Among the fish sampled in watersheds receiving WWTP discharges, about 70% of the fish were female (Hansen et al., 1998). These observations are consistent with the hypothesis of chemically induced feminization of fish at sites near WWTP discharges.

In response to the potential hazard of EDCs in the aquatic environment, several screening programs have been implemented using a variety of chemical analyses, *in vitro* and *in vivo* bioassays. Analytical methodologies based on gas chromatography–mass spectrometry or gas chromatography–tandem mass spectrometry have been developed and used for the ultra-trace determination of target EDCs in the aquatic environment (Desbrow et al., 1998; Johnson et al., 2000). Analytical techniques based on liquid chromatography–tandem mass spectrometry have also been used successfully for the determination of estrogens in different matrices (Draisci et al., 1998). Although chemical analysis can reveal the presence of EDCs in the aquatic environment, most chemical analysis is focused towards the determination of target substances in the matrices of interest. Considering the large number of EDC substances that can be present in complex environmental matrices, target chemical analyses could be limited in providing a complete account of all EDCs present in a specific environmental matrix. Moreover, mixture interaction is not taken into consideration and the biological effects of the chemical mixture cannot be determined. In contrast, *in vitro* bioassays which are based on the interaction between the EDCs and the estrogenic receptors can determine the total estrogenic activity of EDCs in a mixture (Legler et al., 1999; Routledge and Sumpter, 1996).

The Greater Vancouver Regional District (GVRD) is committed to a receiving environment monitoring (REM) approach of managing its liquid waste discharges in its Liquid Waste Management Plan (GVRD, 2001). This monitoring approach for the receiving environment of all five of GVRD's Wastewater Treatment Plants includes the characterization of WWTP effluent to define the nature of the effluent and potential effects. Within the GVRD there are two primary WWTPs that discharge into the marine environment, and three secondary WWTPs that discharge into the Fraser River (Fig. 1). Primary

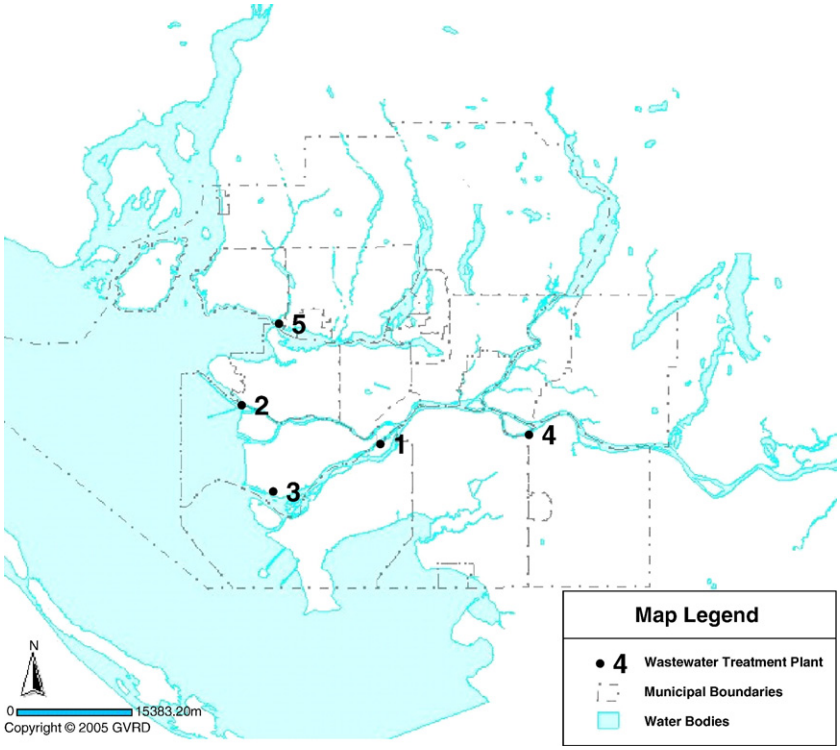

Fig. 1. Wastewater treatment plants of the Greater Vancouver Regional District (modified from GVRD, 2005).