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Analysis of environmental endocrine disrupting chemicals using the E-screen method and stir bar sorptive extraction in wastewater treatment plant effluents

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ABSTRACT

Endocrine disrupting chemicals (EDCs) have become a major issue in the field of environmental science due to their ability to interfere with the endocrine system. Recent studies show that surface water is contaminated with EDCs, many released from wastewater treatment plants (WWTP). This pilot study used biological (E-screen assay) and chemical (stir bar sorptive extraction–GC–MS) analyses to quantify estrogenic activity in effluent water samples from a municipal WWTP and in water samples of the recipient river, upstream and downstream of the plant.

The E-screen assay was performed on samples after solid phase extraction (SPE) to determine total estrogenic activity; the presence of estrogenic substances can be evaluated by measuring the 17-β-estradiol equivalency quantity (EEQ). Untreated samples were also assayed with an acute toxicity test (*Vibrio fischeri*) to study the correlation between toxicity and estrogenic disruption activity.

Mean EEQs were 4.7 ng/L (±2.7 ng/L) upstream and 4.4 ng/L (±3.7 ng/L) downstream of the plant, and 11.1 ng/L (±11.7 ng/L) in the effluent. In general the WWTP effluent had little impact on estrogenicity nor on the concentration of EDCs in the river water. The samples upstream and downstream of the plant were non-toxic or weakly toxic ($0<TU<0.9$) while the effluent was weakly toxic or toxic ($0.4<TU<7.6$). Toxicity and estrogenic activity were not correlated.

At most sites, industrial mimics, such as the alkylphenols and phthalates, were present in higher concentrations than natural hormones. Although the concentrations of the detected xenoestrogens were generally higher than those of the steroids, they accounted for only a small fraction of the EEQ because of their low estrogenic potency. The EEQs resulting from the E-screen assay and those calculated from the results of chemical analyses using estradiol equivalency factors were comparable for all samples and closely correlated.

1. Introduction

In recent years endocrine disrupting chemicals (EDCs) have become a major issue in the field of environmental science due to their ability to interact with human estrogenic receptors, thus interfering with the endocrine system (Colborn et al., 1993; Crews et al., 2000). An EDC is defined as "an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine functions" (EU Commission, 1996); the European Union includes EDCs in the list of so-called emerging contaminants (European Commission Report, 2001). EDCs are

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ubiquitous in the environment because of their seemingly endless number of uses and origins in the residential, industrial and agricultural fields. These substances are derived from both anthropogenic and natural sources; it is suggested that industrial and municipal effluents as well as urban and agricultural runoff are the major sources of EDCs discharged into the aquatic environment (Dresbrow et al., 1998; Snyder et al., 1999; Boyd et al., 2003). In particular, several research groups have studied the effect on aquatic ecosystems of wastewater treatment plant (WWTP) effluents continuously discharged into the recipient bodies of water (Jobling et al., 1998; Routledge et al., 1998; Tilton et al., 2002).

During the last few years, several compounds originally considered harmless have been suggested to have estrogenic effects. Recent studies on EDCs present in the environment have indicated that they include plastic softeners (bisphenol-A) or detergents (4-nonylphenol or 4-octylphenol), heavy metals (cadmium and copper), natural and synthetic compounds (17-β-estradiol (E2) and 17-alpha-ethinyl estradiol (EE2)) (Ying et al., 2002; Basheer et al., 2004; Garcia-Morales et al., 1994; Jiang et al., 2005; Sarmah et al., 2006; Tan et al., 2007). Many other compounds are also suspected of interacting with estrogen receptors (ERs), making it difficult to predict the estrogenicity of xenobiotics only on a chemical basis. Several studies have targeted the estrogenic EDCs, i.e. those interacting with the human estrogen receptor alpha (hERa) (Kortenkamp, 2007; Salste et al., 2007). Since the ligand-binding domain gap is larger than 17-β-estradiol requires, a variety of other molecules can interact with hERa. As the structures of estrogenic receptors are very similar for different vertebrates, EDCs can affect the endocrine functions of many species in the ecosystem (Brzozowski et al., 1997).

There is an increasing concern that the release of EDCs from WWTP discharges affects reproductive processes in exposed freshwater and marine organisms (Purdom et al., 1994; Roux, 1994; APHA, 1998; Rodgers-Gray et al., 2000; Vermeirssen et al., 2005). Reports of feminized male fish and abnormal sexual development of reptiles and birds have suggested that many chemicals, both natural and synthetic, exhibit estrogenic activity (White et al., 1994; Sumpter, 1995; Goksøyr, 2006; Orlando and Guillette, 2007). It has also been claimed that the increasing incidence of breast and testicular cancer in humans might be caused by EDCs acting as estrogens (Carlsen et al., 1995; Safe, 2005).

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 methods have been developed to successfully determine ultra-traces of target EDCs in the aquatic environment (Dresbrow et al., 1998; Johnson et al., 2000) by gas chromatography coupled to mass spectrometry (GC–MS) or gas chromatography–tandem mass spectrometry (GC–MS–MS) as well as detecting estrogens in different matrices by liquid chromatography–tandem mass spectrometry (LC–MS–MS) (Draisci et al., 1998). Recently, Van Hoeck et al. (2008) introduced a new screening method for EDCs employing stir bar sorptive extraction (SBSE) (Baltussen et al., 1999). This technique is based on the analyte accumulation on a thick film of polydimethylsiloxane (PDMS) coated on a magnetic stir bar and is characterized by highly effective sampling capability and recoveries, enabling several analytes to be simultaneously detected and quantified at the ultra-trace level. Since the low polarity of PDMS makes it difficult to extract polar and medium-polarity analytes, the recovery of different classes of polar compounds has been improved by derivatizing them with ad hoc reagents that decrease their polarity and increase their volatility thus facilitating their GC analysis. By this method, different aliquots of a water sample are treated with derivatizing agents specific to different classes of compounds and then submitted to SBSE; after sampling, the stir bars from each aliquot are submitted to simultaneous thermal desorption, and the recovered analytes on-line transferred to a GC column for GC–MS analysis.

Although chemical analyses can successfully reveal the presence of EDCs in the aquatic environment, they are generally focused to determining target substances in the matrices of interest. Because of the large number of EDCs that can be present in a complex environmental sample, target chemical analyses are not always sufficient to determine all EDCs it may contain. Moreover, chemical analyses do not consider mutual and synergic interactions and the biological effects of the whole sample (Nelson et al., 2007). On the contrary, *in vitro* bioassays are based on the interaction between EDCs and estrogenic receptors and can measure the total estrogenic EDC activity of a sample (Legler et al., 1999; Körner et al., 2001). *In vitro* assays are therefore useful to determine estrogenic activity in environmental samples containing complex mixtures of contaminants and to evaluate the total biological activity of all its components that act through the same mode of action. Several *in vitro* assays have been developed to assess the estrogenic activity of individual compounds or complex mixtures (Zacharewski, 1997; Kinnberg, 2003).

This study aimed to monitor the total estrogenic activity, calculated on the human breast cancer cell line (MCF-7) proliferation or by the E-screen assay, and to measure the concentrations of supposed EDCs representative of different classes of compounds (phenols, acids and amines, organotin and highly apolar compounds) by multi shot SBSE with *in situ* derivatization followed by thermal desorption (TD) and chromatographic analysis (GC–MS), in both WWTP effluent (OUT) and samples of the water body, upstream (US) and downstream (DS) of the plant. The toxicity of the water samples was also determined with the Microtox™ test, to correlate different biological endpoints with the impact of the municipal WWTP effluent on the recipient water body. Last but not least, the complementarity of chemical and biological assay results in determining the contribution of selected EDCs to the total estrogenic activity in WWTP effluent and in the water body was also estimated.

2. Materials and methods

2.1. Characteristics of the municipal wastewater treatment plant

The WWTP involved in this study is a consortium plant serving four towns of a metropolitan area in northern Italy, for a total of about 250,000 population equivalent. The mean treated flow is around 42,000 m^3/day. The plant includes a