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Occurrence of estrogens in the Scheldt estuary: A 2-year survey

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Abstract

Despite the increased research and regulatory interest in numerous bioactive agents, including natural hormones, xeno-hormones and pharmacological agents, little is known about the presence of these compounds in the estuarine and marine environment. In this study, the results of a 2-year survey on the occurrence of the natural female sex hormones, estradiol (E2) and estrone (E1) and the synthetic steroid, ethinylestradiol (EE2) in the Scheldt estuary (Belgium-The Netherlands) are presented. Chemical analysis of the water samples was performed using Speedisk^{TM} extraction. Suspended matter samples were analyzed with accelerated solvent extraction (ASE) and detection was performed with gas chromatography coupled to multiple ion trap mass spectrometry. Detected concentrations were in the low ng L^{-1} range. E1 and βE2 (β-isomer of E2) were detected in water and suspended matter, whereas concentrations of EE2 were below the limit of quantification (LOQ). E1 was observed most frequently and at concentrations up to 10 ng L^{-1} in water and up to 0.84 ng g^{-1} in suspended matter samples.

Keywords: Scheldt estuary; Estrogens; Steroids; Endocrine disruptors

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1. Introduction

Concern about the presence of natural and synthetic estrogens in the environment has increased in recent years due to the observation that environmental concentrations in the ng L^{-1} range can induce vitellogenesis in fish (induction of vitellogenin, an egg yolk protein in plasma usually associated with adult females) (Jobling et al., 1998), cause intersex and feminization in male fish (Williams et al., 2001, 2003) and influence human reproduction (Young et al., 2004). Recently, it has been suggested that in addition to effects on sexual differentiation and reproduction, sex hormones appear to influence the human immune system (Bouman et al., 2005).

The main input of natural steroid estrogens into the aquatic environment is through human and animal excreta (through waste water treatment plants, WWTPs). The quantity of excreted steroids depends on sex, race, hormonal status, stage of menstruation, use of contraceptives and pregnancy (Vandenbergh, 2000; Young et al., 2004). Synthetic steroids used in contraceptives, originate mainly from humans. Another potential source of aquatic hormonal contamination is cattle feedlot effluent and agricultural run-off as sewage and manure is used as fertilizer in certain countries (Vandenbergh, 2000; Lintelmann et al., 2003; Orlando et al., 2004; Soto et al., 2004; Young et al., 2004). Moreover, hormone supplements are used in animal husbandry and aquaculture (Kuster et al., 2004; Orlando et al., 2004, 96/22/EC; 2003/74/EC). Finally,

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other important sources of both natural and synthetic estrogens are domestic effluents that are indirectly or directly discharged into the aquatic environment (Kuster et al., 2004).

A significant number of these compounds are excreted and released into the environment as inactive conjugates (mainly glucuronates and sulfates). However, deconjugation by bacterial enzymes in WWTPs or in the aquatic environment, re-activates these conjugates to the biologically active parent compounds (Johnson et al., 2000; D'Ascenzo et al., 2003; Young et al., 2004). In the aquatic environment, E2 is rapidly biodegraded to E1 which in turn is degraded to E3 (Jürgens et al., 1999). EE2 which is designed to resist degradation (in order to be effective as an oral contraceptive) is degraded at a lower rate (Young et al., 2004).

In order to understand the potential threats of these compounds to aquatic ecosystems, their occurrence, transport and transformation has to be understood. The occurrence of steroid hormones in European waste water effluents (Baronti et al., 2000; Johnson et al., 2000; Vethaak et al., 2002; Aerni et al., 2004; Carballa et al., 2004; Johnson et al., 2004) and freshwater systems has been documented in detail (Belfroid et al., 1999; Williams et al., 2001; Wenzel et al., 2003; Young et al., 2004). Most studies suggest that both natural and synthetic estrogens commonly enter freshwater systems through sewage treatment effluents (Williams et al., 2003). However, very few studies have documented the occurrence of these compounds in estuarine and marine environments (Belfroid et al., 1999; Thomas et al., 2001; Vethaak et al., 2002; Noppe et al., 2005). Our study is not aimed at detecting these substances in effluents or at a specific freshwater site like most of the above cited studies report. Indeed the major goal of this study was to establish the overall, 'background' environmental concentrations of specific estrogens in an estuarine system like the Scheldt estuary (Belgium-The Netherlands). Monitoring of the environmental concentration of substances, not related to specific point sources, is important in the context of the global assessment of the potential impact of these chemicals on the environment.

From an ecological point of view, the Scheldt estuary is an important tidal river system in Europe as it is an important breeding and hibernating area for coastal birds and an important nursery for juvenile fish and many crustaceans (Baeyens et al., 1998; Salomons et al., 1998). Moreover, the Western Scheldt (the Dutch Part of the Scheldt estuary), excluding the shipping channels, is recognized as a protection zone under the EU Habitats directive (92/43/EC).

In this study, the natural estrogens estradiol ((17α or β)-estra-1,3,5(10)-triene-3,17-diol) (αE2 or βE2), estrone (3-hydroxyestra-1,3,5(10)-trien-17-one) (E1), estriol ((16α, 17β)-estra-1,3,5(1)-triene-3,16,17-triol) (E3), and the synthetic estrogen ethinyl estradiol ((17α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol) (EE2), all listed on the OSPAR (treaty of Oslo and Paris) list of substances of possible concern, were monitored in water and suspended matter samples collected in the Scheldt estuary. This paper presents data of these estrogens during the first 2 years (2002–2004) of the ENDIS-RISKS project (www.vliz.be/projects/endis), a 4-year project focusing on distribution, exposure and effects of endocrine disruptors in the Scheldt estuary. The results are discussed in the context of their distribution in different environmental matrices (dissolved and particulate) and their occurrence in the estuary. Additionally, the fate and potential risk for this estuarine ecosystem are evaluated.

2. Materials and methods

2.1. Standards and reagents

All used solvents were of analytical grade quality and purchased from Across Organics (Fairlawn, NI, USA) or VWR (Merck, Darmstadt, Germany). αE2, βE2, E1 and EE2 were provided by Sigma–Aldrich Corp. (St. Louis, MO, USA), E1-D4 (estrone-2,4,16,16d$_4$) by CDN Isotopes (Pointe-Claire, Quebec, Canada) and equilinine (EQ) (d-1,3,5(10),5,8-1,3,510,5,8-estrapentaen-3-ol-17-one) by Steraloids Inc. (Newport, RI, USA). The derivatization reagent (MSTFA^{++}) was prepared using MSTFA (*N*-methyl-*N*-trimethylsilyl-trifluoracetamid, FilterService, Eupen, Belgium), ammonium iodide (Sigma–Aldrich Corp., St. Louis, MO, USA) and ethanethiol (Acros Organics, Fairlawn, NU, USA). Individual and composite working standards were prepared by appropriate dilution of the standard stock solutions in ethanol. All solutions were stored at 4 °C in the dark. For the accelerated solvent extraction (ASE), purified sea sand was used to reduce the void volume of the extraction cells (Merck, Darmstadt, Germany).

2.2. Study area

The river Scheldt originates in northern France (Saint-Quentin) at about 350 km upstream of Vlissingen in the Netherlands where the river discharges into the North Sea (Fig. 1). The estuarine zone of the tidal system is about 70 km long and extends from the North Sea to the Dutch-Belgian border near Bath (S09 see Fig. 1, Baeyens et al., 1998; Steen et al., 2001). The downstream stretch from the city of Ghent (Belgium, see Fig. 1) to the North Sea is under tidal influence and is named the Sea Scheldt. The Dutch part of the estuary is called the Western Scheldt. The Scheldt estuary covers one of the most heavily populated regions of Europe, with the cities of Ghent (B) and Antwerp (B) (see Fig. 1.) with harboring and highly diversified industrial activities. Consequently, the estuary is among the most polluted estuaries in the world and dramatically affected by man's activities, as a large amount of domestic and industrial waste is released into the river (Steen et al., 2001). Four aspects make the Scheldt estuary distinct from other estuaries: (1) the Scheldt has a tide-governed estuary due to the low river flow resulting in long residence times; (2) the upper estuary receives large inputs of biodegradable organic matter inducing anoxic conditions in the water column during summer; (3) a considerable number (and direct supply) of contaminants occur in the upper estuary as a result of the diverse industrial activities around Antwerp and upstream activities around Ghent; (4) the anoxic zone, the area of maximum contaminant input and the zone of maximum turbidity coincide geographically, making it very difficult to distinguish between their individual effects on the chemical distribution and behavior (Baeyens et al., 1998; Salomons et al., 1998).

2.3. Sampling

Samples from the Scheldt estuary were collected using the *RV Belgi*ca. Three times a year (spring, summer, autumn) from 2002 until 2004, water,