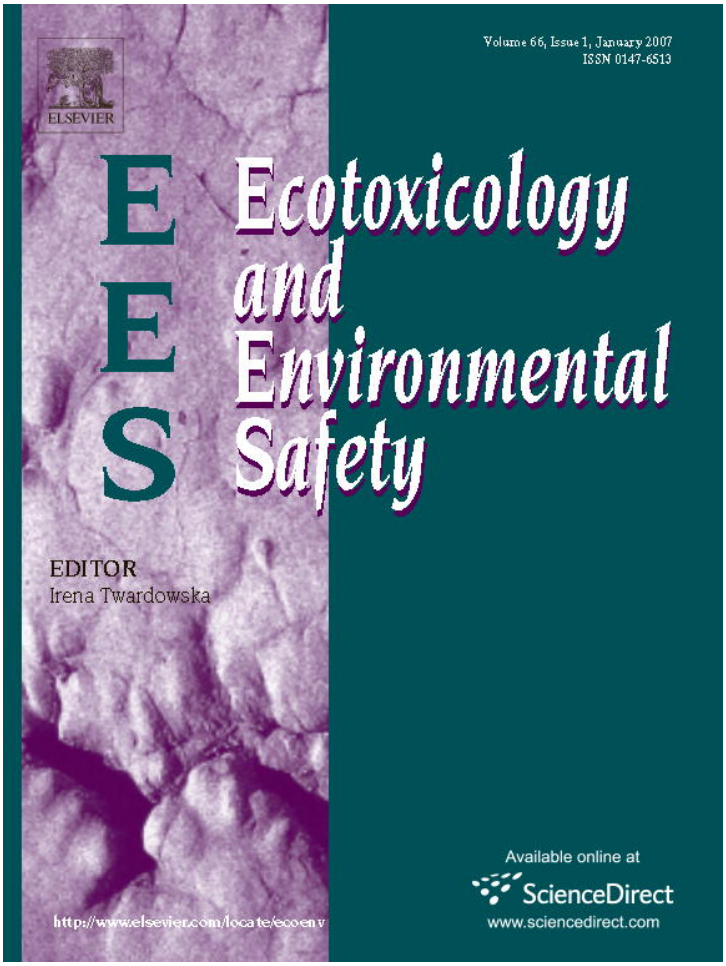

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Occurrence of estrogens in the Scheldt estuary: A 2-year survey

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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

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 Belgica*. Three times a year (spring, summer, autumn) from 2002 until 2004, water,

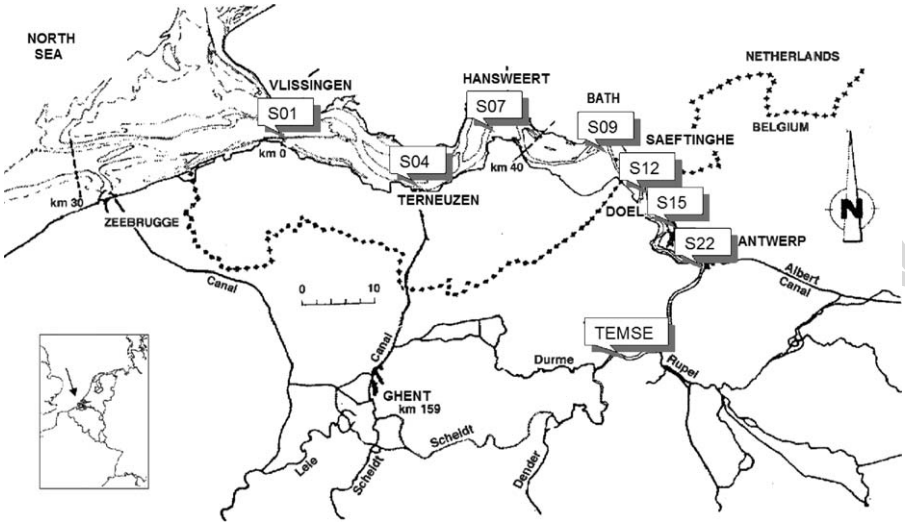

Fig. 1. Map of the Scheldt estuary with location of the different sampling sites: Vlissingen (S01), Terneuzen (S04), Hansweert (S07), Bath (S09), Saefthinghe (S12), Doel (S15), Antwerp (S22) and Temse (http://www.vliz.be/projects/endis).

Table 1
Quality assurance data for the analysis of α-, β E2 (β-estradiol), E1 (estrone) and EE2 (ethinylestradiol) in water and suspended matter

Matrix	Spiked range		αE2	βE2	E1	EE2
Water (2 L)	0.25–5 ng L^{-1}	Recovery (%)	105 ± 20	104 ± 25	108 ± 21	102 ± 21
		R^2	0.96 ± 0.04	0.95 ± 0.03	0.94 ± 0.06	0.96 ± 0.03
Suspended matter (5 g)	0.2–1 ng g^{-1}	Recovery (%)	110 ± 14	127 ± 35	124 ± 44	87 ± 26
		R^2	0.96	0.91	0.98	0.99

sediment and suspended matter samples were taken at different locations selected in accordance to national and international sampling and monitoring programs (Fig. 1). Campaigns were performed in December 2002, March, June 2003 and February, May, September and November 2004. Teflon-coated Go-Flo water samplers (General Oceanics Inc., Miami, Florida, USA) were used to avoid contamination with surface water.

Water samples were (if possible) taken at a depth of 4–5 m (Table 1) and 2 L was extracted immediately on board in order to prevent degradation during transport and storage. The extracts were stored in the dark at 4 °C. Clean-up and chromatographic analysis were performed upon return to the laboratory. Water samples, used for quality control of the analysis, were stored in amber bottles and acidified to pH 2 to prevent microbial degradation. These samples were extracted later or stored in the laboratory.

Suspended matter samples were collected using a flow-through centrifuge (Alfa Laval type MMB 304-S-11, Separator Spares International BV, The Netherlands) on board of the research vessel, transferred to amber jars and stored at −20 °C in the dark.

ASE was performed on the freeze-dried (Christ LMC-2, Germany) and homogenized (Pulverisette 5 Fritsch GmbH, Idar-Oberstein, Germany) suspended matter samples.

2.4. Extraction

Extraction of the water samples was performed as previously described (Noppe et al., 2005). In short, extraction of 2 L water was performed using Bakerbond Speedisk™ Octadecyl-bonded silica (C_{18}XF), 50 mm (J.T. Baker, Deventer, The Netherlands).

Suspended matter samples were extracted by ASE using an ASE 200 system (Dionex, Sunyvale, CA, USA) equipped with 11 mL stainless steel extraction cells. Prior to extraction, an aliquout (5 g) of suspended matter was spiked with 25 ng EQ and E1-D4. This was loaded in the extraction cells with cellulose filter disks (Dionex, Sunyvale, CA, USA) and acetone: methanol (1:1) was used as extraction solvent (2 cycles) with an oven temperature and pressure of, respectively, 100 °C and 2000 psi. The oven heat up time and static time were both 5 min. Purge time was 60 s and flush volume was 60% of the extraction cell volume.

2.5. Sample clean-up

Water samples were cleaned-up using a combination of Si and NH_2 cartridges (100 mg, 1 mL, Sopachem nv, The Netherlands) as described previously by Noppe et al. (2005).

ASE extracts were evaporated to dryness under a gentle stream of nitrogen (Turbovap® LV evaporator, Zymark Co., Hoptkinton, MA, USA), reconstituted in 120 μL ethanol and used for HPLC fractionation. In total, 100 μL was injected on column (Beckman ODS Ultrasphere High Performance Column, 10 mm × 25 cm, USA) and collected in 4 fractions (L-5200 Fraction Collector, Merck Hitachi, VWR, Darmstadt, Germany) using a water:methanol (MeOH) gradient program (initial 25:75 Water: MeOH, after 1.1 min to 100% MeOH, after 2.2 min back to 25:75 water:MeOH, after 24 min to 100% MeOH and finally, after 27.1 min back to 25:75 water:MeOH) and a Lachrom Merck Hitachi L-6200 HPLC apparatus and a Hitachi UV-detector (VWR, Darmstadt, Germany) (Smets et al., 1997). After HPLC fractionation, a drying step and derivatization using the MSTFA mixture (See *Standards and reagents*) (1 h at 60 °C), the samples were analyzed by GC–electron impact (EI)–MS–MS as described by Noppe et al. (2005).

2.6. Chromatographic analysis

2.6.1. GC–MS–MS apparatus

All chromatographic and spectrometric analyses were performed using a Trace Gas Chromatograph 2000 fitted with a Polaris ion trap mass spectrometer (Thermo Finnigan, Austin, TX, USA) with a Carlo Erba autosampler AS2000 (Thermo Finnigan, Austin, TX, USA). Helium (99.99% purity, Air Liquide, France) was used as carrier gas at a flow rate of 1 mL min^{-1}. FC43 (Perfluorotributylamine) (Ultra Scientific, North Kingstown, USA) was used as calibration gas. A volume of 1 μL was injected (spit flow 20 mL/min, splitless time 1 min).

2.6.2. GC–MS–MS conditions

Separation of the target analytes was performed on a BPX-5 (SGE Inc., Austin, TX, USA) (25 m × 0.22 mm I.D.) fused silica capillary column with 5% phenyl liquid phase (film thickness 0.25 μm). Injector, ion source and transfer line temperature were, respectively, 250, 200 and 275 °C. Temperature program: initial 100 °C; ramp at 17 °C min^{-1} to 250 °C; ramp at 2 °C min^{-1} to 268 °C and final ramp at 30 °C min^{-1} to 300 °C (hold 1.30 min).

Multiple MS acquisition method parameters: 1 microscan, several scan segments with scan events, mass range depending on the selected precursor ion, activating potential between 0.85 and 1.30 V. The spectra were obtained in EI mode at 70 eV. The target compounds were identified based on relative retention time and ion ratio. Calibration curves in ultrapure water were used for the quantification of the target estrogens in water (see Noppe et al., 2005), spikes of blanc suspended matter samples were used for quantification of estrogens in suspended matter.

2.7. Quality assurance

Prior to the sample analysis, a dilution series (0.1–1 ng) of standard mixture of the target estrogens was injected to check the operation conditions of the GC–EI–MS–MS apparatus. For the quantitative analysis of water samples a range (0.25–5 ng L^{-1}) of calibration standards was spiked in ultrapure water. The limit of quantification (LOQ) for analysis of water samples was set at the lowest calibration point, namely 0.25 ng L^{-1}. Analyte recoveries were determined by adding known concentrations of the working standard mixture solutions to blank samples and ultrapure water. Recoveries of 105 ± 20% (EQ) were obtained for all estrogens considered (Table 1, more details are previously described by Noppe et al., 2005).

Quantification of the estrogens in suspended matter was performed using a series (0.2–2 ng g^{-1}) of spiked blanc samples. The method LOQ for the target estrogens in suspended matter was 0.2 ng g^{-1}. Recoveries of estrogens from fortified suspended matter samples over the assumed range of concentrations were satisfactory, nl 112 ± 32% (Table 1).

Prior to extraction, the procedure internal standards, EQ and deuterated estrone (E1-D4) were added to every sample at a concentration of 5 ng L^{-1} for water or 5 ng g^{-1} for suspended matter. After extraction, ethinyltestosterone (ET) was added to check the performance of the chemical analysis method. Prior to derivatization, androsterone (And) was added to every sample to check the derivatization efficiency.

Identification of the target estrogens was based on retention time and the ion ratio of the 3 most abundant ions in the spectrum as described earlier (Noppe et al., 2005, EC/2002/657).

Quality control of the method was performed by regular analyses of blank, spiked estuarine and/or ultrapure water samples (Table 1, Noppe et al., 2005). Because no certified reference material was available, matrix interference was evaluated by spikes of the target compounds to blanc samples. The recovery of EQ as internal standard (calculated using And) was 138% for water and 74% for suspended matter (all non-compliant samples considered).

3. Results

Of the estrogens targeted in this study, estrone (E1), estradiol (E2), estriol (E3) and ethinylestradiol (EE2), E1 was detected most frequently and at the highest concentrations in the water samples (Table 2). Concentrations ranged from the LOQ up to 10 ng L^{-1}. Except for samples taken in the June 2003 campaign, a clear correlation between contaminant levels and sampling locations was observed: E1 was detected most frequently at the most upstream sampling stations, Antwerp and Temse (Fig. 1 and Table 2) whereas E2 was detected only once and only as α-isomer (June 2003 campaign) at the Vlissingen and Terneuzen stations, i.e. the two most downstream and marine sampling points. αE2 concentrations were in the very low ng L^{-1} range, 0.25 and 0.27 ng L^{-1}, respectively. Contrary to studies reporting EE2, E3 and βE2 in sewage treatment effluents and freshwater systems (Belfroid et al., 1999; Baronti et al., 2000; Johnson et al., 2000; Aerni et al., 2004) concentrations of these estrogens in the Scheldt estuary were all below the LOQ; i.e. 0.25 ng L^{-1} for all estrogens considered in this study.

Table 2
Detected concentrations of E1 (ng L^{-1}) in water and environmental parameters of the campaigns: sample depth (m), temperature of the water (°C), Salinity range (psu), Turbidity (ftu) and dissolved oxygen range (mL L^{-1}) (Boarding reports http://www.vliz.be/projects/endis)

Sampling point	Campaign	Concentration E1 (ng L^{-1})	Stdev (ng L^{-1})	Depth (m)	Salinity (PSU)	Temperature (°C)	Turbidity (FTU)	Diss O2 (mg L^{-1})
S12	Dec/02	1.7	—	6.62	3.28	9.81	187.50	5.54
S22	Dec/02	8.0	—	4.76	0.46	8.7	71.8	79.2
S22	Mar/03	2.0	—	—	0.73	8.71	49.87	3.19
S01	Jun/03	0.37	0.17	3.75	30.99	19.16	5.57	5.55
S07	Jun/03	0.74	0.19	4.27	19.43	20.08	22.47	5.43
S12	Jun/03	2.6	0.62	4.01	12.32	21.12	38.19	4.48
S15	Jun/03	1.0	0.57	3.93	11.79	21.24	37.9	9.85
S22	Feb/04	7.5	0.59	10.89	0.45	7.27	79.25	6.38
S22	May/04	6.3	6.2	4.92	1.19	15.57	44.06	6.82
Temse	Sep/04	10	2.5	3.22	0.77	20.9	56.9	6.2
Temse	Dec/04	2.5	0.91	3.25	0.61	7.67	39.87	8.31

All other detected concentrations were below the LOQ (= 0.25 ng L^{-1} for all targeted compounds).

Given the relative low polarity of the targeted estrogens in this study (log K_{ow} between 2 and 4, The Merck Index, 2001; Lai et al., 2002; Beausse, 2004; Kuster et al., 2004; Young et al., 2004), sorption onto suspended matter and tendency to accumulate in sediments was expected (Petrović et al., 2001). E1 and βE2 were indeed detected in suspended matter samples (see Table 3 and Fig. 2) at concentrations up to 0.84 and 0.25 ng g^{-1} dw, respectively. In 16% of the samples ($n = 37$) E1 was detected whereas βE2 was detected only in 5% of the samples. In the downstream locations Vlissingen (S01), Terneuzen (S04) and Hansweert (S07) detected levels were below the LOQ. αE2 and E3 were not detected in any of the suspended matter samples and also EE2, which was expected to be presented in suspended matter fraction due to its higher log K_{ow} ($pK_{ow} = -\log K_{ow}$), was not found to be present.

Table 3
Detection concentrations of estrone (E1) and β-estradiol (βE2) in suspended matter in ng g^{-1} DW

Sampling point	Campaign	Concentration E1	Concentration βE2
S09	Dec/02	0.84	—
S12	Mar/03	0.23	0.21
S22	Mar/03	0.70	0.25
S12	Sep/04	0.39	—
S15	Sep/04	0.31	—
S22	Sep/04	0.49	—

All other detected concentrations were below the LOQ (= 0.2 ng g^{-1} DW for all targeted compounds).

4. Discussion

The spatial pattern of the detected estrogens in the water samples observed in this study can be explained by the fact that the Scheldt and the Scheldt estuary receive major inputs of industrial and domestic waste water (both treated and non-treated). In the vicinity of the river Scheldt and the Scheldt estuary approximately 50 WWTPs are located,

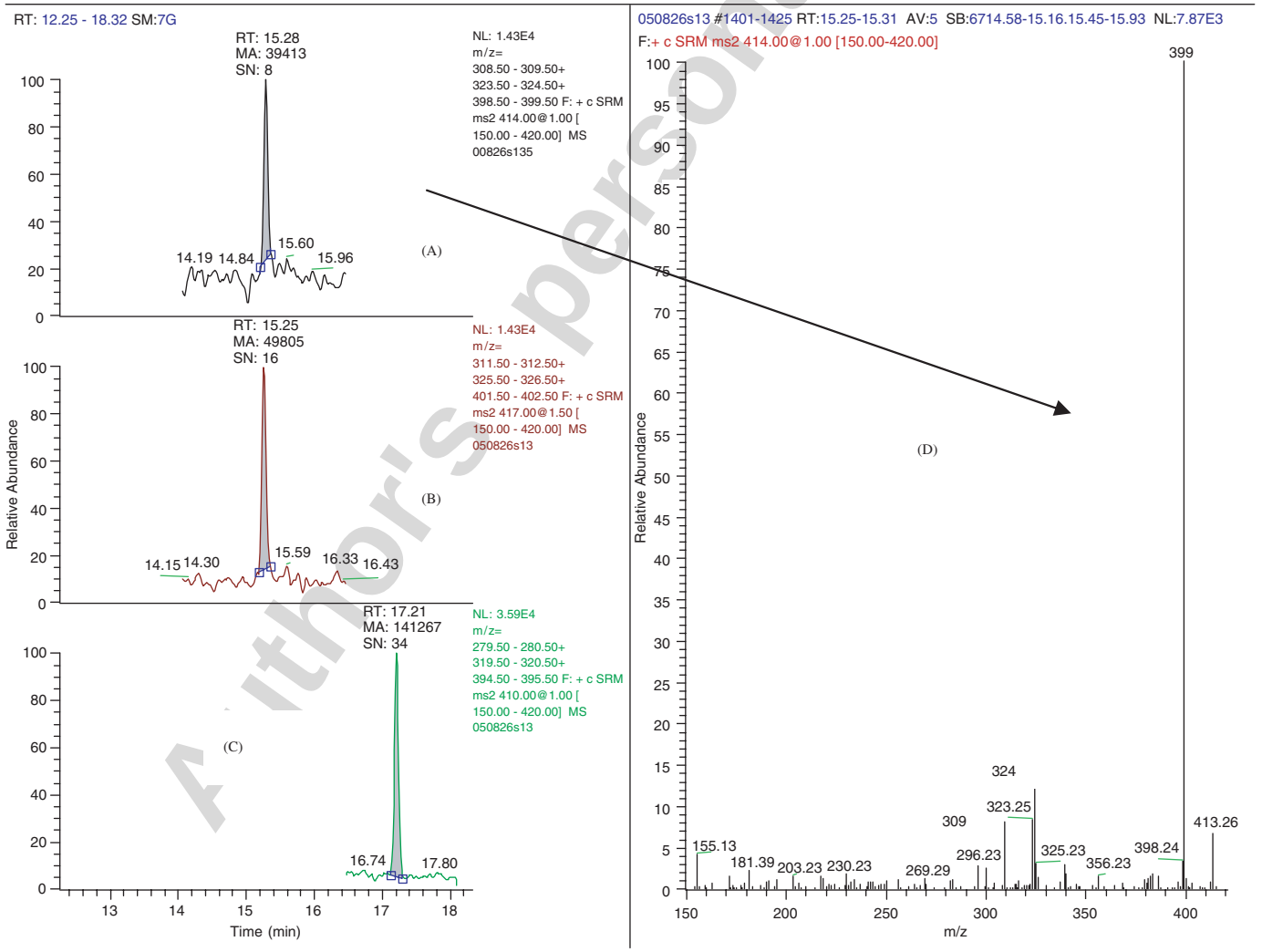

Fig. 2. Chromatograms (shaded zones = peak area) and spectrum of a fortified suspended matter sample (0.2 ng g^{-1} DW): (A) Chromatogram of estrone (E1), (B) chromatogram of Deuterated estrone (E1-D4 = internal standard), (C) chromatogram of equilinine (EQ = second internal standard) and (D) spectrum of estrone (E1).

with Deurne, Ghent and Antwerp facilities having the largest capacity in IE (respectively, 153,897,000, 122,037,676, and 94,200,388 IE for 2004) (IE = inhabitant equivalent, which is the daily amount of waste water produced by 1 inhabitant). Effluents of the latter two plants are discharged directly in the Scheldt near Antwerp (Aquafin nv., Data Management, Operations, Van Gestel N., Personal Communication).

The occurrence of αE2 in the water samples may be due to the agricultural use of sewage and/or manure or by sewage treatment discharge in the vicinity of these sampling stations. Earlier studies in the Dutch part of the Scheldt estuary at the Terneuzen location reported E1 at concentrations of up to 7 ng L^{-1} (Belfroid et al., 1999; Vethaak et al., 2002, 2005), which corroborate our findings. These authors also reported that in Doel and Vlissingen, E1 concentrations were below the detection limit. Other studies examining estrogens in waste water effluents (Belfroid et al., 1999; Baronti et al., 2000; Johnson et al., 2000, 2004; Vethaak et al., 2002; Aerni et al., 2004; Carballa et al., 2004) have reported E1 concentrations in the 5–20 ng L^{-1} range whereas E2 concentrations varied from 1 to 10 ng L^{-1}. Generally, reported concentrations of EE2 in these types of waste waters vary but are mostly <1 ng L^{-1} (Belfroid et al., 1999; Ternes et al., 1999; Baronti et al., 2000; Vethaak et al., 2002). Freshwater studies in Germany, the US, the UK and the Netherlands, corroborate our findings and conclude that E1 is the most frequently detected estrogen at concentrations up to 12 ng L^{-1} but mostly <5 ng L^{-1} (Belfroid et al., 1999; Williams et al., 2001; Soto et al., 2004). It can therefore be concluded that in (freshwater) surface waters and waste water treatment effluents, E1 concentrations are consistently higher than those of E2 and are detected more frequently. Our study demonstrates that the same pattern is observed in the estuarine part of the river Scheldt. In this context, it has to be recognized that non-detection of estrogens—priority substances and listed on the OSPAR (treaty of Oslo and Paris) list of substances of possible concern—is as valuable as detection in field monitoring, as these observations contribute to establishing a correct picture on the occurrence or absence of these chemicals in the environment.

There is an increasing amount of research on the fate and behavior of estrogens (and other lipophilic compounds) in freshwater environments which indicate that hydrodynamics, suspended sediment transport, biodegradation and hydrophobic sorption are the main processes that determine their environmental fate (Jürgens et al., 1999). However, in marine and in estuarine systems the distribution and transport of natural and synthetic estrogens is poorly understood. In this context it is important to note that because the residence time increases quickly with increasing vertical mixing due to dilution of the freshwater in a large amount of sea water, the freshwater residence time in the Scheldt estuary is high (2–3 months) (Baeyens et al., 1998). This could also explain the spatial distribution pattern observed in this study. Also, previous studies in the Scheldt estuary have found high concentrations of PCBs, PAHs, PBDEs (flame retardants), organotins, nonylphenol ethoxylates (NPE) and pesticides, compounds that have been shown to possess endocrine disrupting activity, in the zone of high turbidity at the head of salt water intrusion (around Antwerp) with little transport to the North Sea (Vuksanovic et al., 1996; Steen et al., 2001; Vethaak et al., 2002; Voorspoels et al., 2004; Jonkers et al., 2005; Verslycke et al., 2005).

It has been established that E2 concentrations decrease rapidly over time and E1 is formed (Ying et al., 2002). Compared to freshwater systems it has been suggested that E2 degradation in estuarine waters is considerably longer: i.e. 6–10 days vs. 14 up to 49 days, respectively (Jürgens et al., 1999). Similar to the situation in freshwater systems (Williams et al., 2001), faster microbial degradation of E2 can be expected with increasing water temperature. Indeed, when relating the observed E1 concentrations to the water temperatures, higher concentrations of E1 were detected at higher temperatures (except for June 2003) (Table 1). Degradation of steroid molecules may also occur through photolysis; however, the importance of this phenomenon in the field is difficult to establish (Williams et al., 2001).

As stated by Bowman et al. (2002), salinity is another important parameter that could affect the partitioning and fate of organic pollutants when—like in the Scheldt estuary—a clear salinity gradient is present. They stated that the partition coefficient between sediment and water (K_p) for E1 increases with increasing salinity. This is due to the decrease in its aqueous solubility resulting from the presence of salts. In this study, estrogens concentrations in the water phase were generally higher at sampling points with a salinity <1 psu, and lower at sampling points with a salinity >10 psu (Table 1). Hence, it can be suggested that, due to both dilution and degradation, there is little transport of estrogens from the Scheldt estuary to the North Sea.

Finally, it needs to be stressed that as the suspended matter content is highly variable due to variations in (freshwater) river inputs, rainfall, dredging, shipping, mixing of freshwater and seawater and sedimentation processes (Table 2, Bowman et al., 2002), objective interpretation of the spatial and temporal trends in the suspended solid estrogen concentrations we observed in the Scheldt estuary is currently not possible.

5. Conclusion

This study aimed at contributing to the evaluation and assessment of the presence of natural and synthetic hormones in marine systems. Similar to what has been demonstrated in fresh water habitats, we demonstrated that estuarine waters and the associated suspended matter are contaminated with the same estrogenic compounds which are found at similar concentrations as those reported in freshwater environments and waste waters.

This underlines the importance to study potential hormone-disruptive effects in estuarine and marine environments, particularly in the case of the Scheldt estuary which is highly contaminated with other known endocrine disruptors. Ongoing studies within the ENDIS-RISKS project are measuring natural and synthetic hormones, as well as a wide range of putative hormone disruptors (organotins, pesticides, phthalates, phenols, flame retardants and other polyaromatic compounds), in water, suspended solids, sediment and biota (e.g. mysids, shrimp, fish).

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