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Combining passive samplers and biomonitors to evaluate endocrine disrupting compounds in a wastewater treatment plant by LC/MS/MS and bioassay analyses

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Passive sampling and biomonitoring were used to determine the amounts of endocrine disrupting compounds in wastewaters.

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ABSTRACT

Two types of integrative sampling approaches (passive samplers and biomonitors) were tested for their sampling characteristics of selected endocrine disrupting compounds (EDCs). Chemical analyses (LC/MS/MS) were used to determine the amounts of five EDCs (nonylphenol, bisphenol A, estrone, 17β-estradiol and 17α-ethinylestradiol) in polar organic chemical integrative samplers (POCIS) and freshwater mussels (*Unio pictorum*); both had been deployed in the influent and effluent of a municipal wastewater treatment plant (WWTP) in Genoa, Italy. Estrogenicity of the POCIS samples was assessed using the yeast estrogen screen (YES). Estradiol equivalent values derived from the bioassay showed a positive correlation with estradiol equivalents calculated from chemical analyses data. As expected, the amount of estrogens and EEQ values in the effluent were lower than those in the influent. Passive sampling proved to be the preferred method for assessing the presence of these compounds since employing mussels had several disadvantages both in sampling efficiency and sample analyses.

1. Introduction

Over the last two decades the presence in the environment of compounds with estrogenic and androgenic properties that can alter the normal functioning of the endocrine system in wildlife and humans has emerged as a major environmental issue. It has generated a considerable amount of attention among the worldwide scientific community, and the potential adverse effects of these endocrine disrupting compounds (EDCs) have been well documented in the literature (Hill et al., 2002; Madsen et al., 2004). Furthermore, scientific results are continuously feeding into regulatory measures, some compounds have been banned, and appropriate tools are needed to study the presence of these compounds in discharges to the aquatic environment.

Natural estrogens (such as estrone, E1, and 17β-estradiol, E2) and synthetic estrogens (e.g. 17α-ethinylestradiol, EE2, the active component of oral contraceptives) are very powerful EDCs. They derive mainly from excreta of humans and livestock (Williams et al., 2003). However, man-made chemicals such as nonylphenol (NP) and bisphenol A (BPA) are also known to influence the hormonal system of aquatic organisms (Fromme et al., 2002; Madsen et al., 2004). Alkylphenols and BPA are used to manufacture flame retardants, household surfactants, pesticide formulations, industrial products and polycarbonate and epoxy resins.

EDCs from domestic, agricultural or industrial sources are released directly or indirectly into the aquatic environment (Birkett and Lester, 2003). Wastewater treatment plants (WWTPs) are important sources of pollution, since EDCs are not fully removed by the treatment processes. The occurrence of these EDCs in WWTPs has been well documented in numerous studies worldwide (Nakada et al., 2006; Vogelsang et al., 2006), including the Mediterranean area (Gomez et al., 2007; Gonzalez et al., 2007).

Monitoring for these compounds can be challenging due to the rapid dilution of the effluents and thus requires sensitive analytical techniques, intensive sampling programs and large sample volumes. Analytical methods for the determination of EDCs in aqueous matrices, such as wastewater and river water, have often been described in the literature and are mostly based on spot sampling followed by laboratory-based extraction and analysis (Céspedes et al., 2005; Quednow and Püttmann, 2008). This approach, however, provides only an instantaneous measurement of pollutant levels and suffers from the uncertainty of short- and long-term concentration variations.

In order to obtain a time-integrated evaluation of the bioavailability of different types of chemicals, bivalve molluscs have been used as bioindicators. Bivalves are filter-feeding organisms and are

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thus exposed to large amounts of chemical pollutants, even if they are present in fairly low concentrations, and are capable of bioconcentrating environmental contaminants to many thousands times background levels. Among bivalve molluscs, the most commonly used is the marine mussel *Mytilus edulis* (UNEP, 1992) or its freshwater counterpart *Dreissena polymorpha* (Neumann and Jenner, 1992), but also representatives of the Unionacea have been used (Manly and George, 1977; Hickey et al., 1995). The unionids *Unio pictorum* are mobile, burrowing suspension feeders which have often been used for the monitoring of trace elements (Ravera et al., 2003).

An alternative approach to the use of biomonitoring is passive sampling. In the last few years there has been a rapid development in the use of passive sampling devices, which allow for continuous and integrative monitoring of aqueous pollutants. Passive samplers can integratively sample a wide variety of environmental contaminants over their exposure period.

Several studies have applied the polar organic chemical integrative sampler (POCIS) to study the presence of EDCs in rivers or effluents (Alvarez et al., 2005; Vermeirssen et al., 2005a). The POCIS contains a sorbent phase sandwiched between two microporous polyethersulphone membranes. Chemicals diffuse from the water over the membrane and adsorb in to the sorbent phase (e.g. OASIS HLB) and are extracted from the sampler after deployment.

The aim of our study was to compare the passive sampling technique, using the POCIS, with bioaccumulation, using freshwater mussels. Concentrations of selected EDCs were analyzed using liquid chromatography–tandem mass spectrometry (LC/MS/MS). In addition we assessed total estrogenicity using the yeast estrogen screen (YES). Samples originated from two sequential treatment lanes at a wastewater treatment plant in Genoa, Italy. The two different sampling techniques were compared to evaluate their suitability in terms of cost-effectiveness and the relative ability to reliably monitor the analytes of interest. To this end, different tissues of the mussels were considered in order to identify the main target in the process of bioaccumulation. Combining chemical and biological assay data allowed us to estimate the magnitude of the contribution of the selected EDCs to total estrogenic activity in wastewater.

2. Experimental

2.1. Materials and chemicals

Standards of nonylphenol, bisphenol A, 17β-estradiol, estrone and 17α-ethinylestradiol were from Sigma–Aldrich (Buchs, Switzerland). Bisphenol A-d_{16} was purchased from Sigma–Aldrich, estradiol-2,4-d_2 and estrone-2,4,16,16-d_4 from CDN Isotopes (Ausburg, Germany), 17α-ethinylestradiol-2,4,16,16-d_4 and *n*-nonylphenol ring-^{13}C from LGC Promochem GmbH (Wesel, Germany). Estradiol used in the YES was purchased from Fluka (Buchs, Switzerland). Individual stock solutions of the studied compounds were prepared in methanol and stored at −20 °C. All solvents were p.a. or HPLC grade. Acetone and hexane were purchased from Fluka, methanol and acetonitrile from Multisolvent (Scharlau, Switzerland) and the 25% ammonia solution was obtained from Merck (Darmstadt, Germany). Water was purified by a Milli-Q system (Millipore, Watford, Hertfordshire, UK). Lichrolut RP18 (500 mg, 3 mL, 40–63 μm) cartridges were purchased from VWR (Dietikon, Switzerland) and Lichrolut Silica SPE (500 mg, 3 mL, 40–63 μm) from Merck. POCISs (Pest-POCIS) were supplied by EST (St. Joseph, USA; Alvarez et al., 2004).

The yeast cells used for the yeast estrogen screen were kindly provided by J.P. Sumpter from Brunel University, Uxbridge, UK.

2.2. Sampling sites

The study was focused on a WWTP in Genoa (Liguria, Italy) that receives mainly domestic wastewater. This WWTP utilizes a primary mechanical treatment for solids and sludge removal (gridding, degritting, settling) and a secondary aerobic treatment (oxidation ditches) for reduction of BOD; the final effluent is discharged into the sea. Influent before degritting and final effluent were chosen as the sampling sites.

2.3. Field deployment of passive samplers

A continuous flow of the influent was pumped into a 100 L tank in which duplicate passive samplers were deployed for two subsequent 5-day periods (from 2nd to 12th February).

Similarly, the effluent was pumped into a 100 L tank in which two POCISs were placed for 14 days (from 2nd to 16th February); in the same tank an additional set of duplicate samplers was deployed for the first 7 days (i.e. from 2nd to 9th) and another set for the second period (from 9th to 16th February).

One passive sampler was deployed in a tank filled with artificial freshwater for 14 days and served as a quality control blank.

The flow of wastewater around the samplers was identical for both influent and effluent, about 15 000 L/day with a linear velocity of 7–10 cm/s at the surface; no water exchange was provided for the control tank.

Upon retrieval, the samplers were rinsed with Milli-Q water, wrapped in aluminum foil, transported to the laboratory and stored frozen at −20 °C until further processing.

2.4. Processing of biomonitors

Freshwater mussels (*Unio pictorum*) were collected from Lago Maggiore (Ispra, Italy). Live organisms were transported to the laboratory where they were held for a few days in aerated artificial freshwater to settle down and eliminate possible contamination. Some individuals were then used as the control sample and some others were placed in stainless steel cages and deployed together with the POCISs at two sites of the monitored wastewater treatment plant for the whole exposure period (10 days in the influent and 14 days in the effluent). Part of exposed mussels, both in influent and effluent, was collected after 5 and 7 days of exposure respectively. Control organisms were kept in the same tank as the control POCIS for 14 days.

Ten individuals from each group of control and exposed mussels were dissected to separate the gills, the mantle and the rest; the tissues obtained were then pooled, freeze-dried for 48 h, ground and stored at −80 °C before LC/MS/MS analysis. Each of the organ pools was mixed and treated as a single sample.

2.5. Extraction of EDCs

2.5.1. Passive samplers

Prior to processing, the POCISs were allowed to reach room temperature; any remaining debris was rinsed away with distilled water. Each POCIS was carefully dismantled and methanol was used to transfer the sorbent into a 1 cm i.d. glass syringe cartridge fitted with a Teflon frit and glass wool. The solid phase was eluted with 50 mL of dichloromethane/methanol/toluene (8:1:1 v/v/v) according to the manufacturer's instructions. The eluate was collected in a flask and reduced in a rotavapory evaporator to about 1 mL. The extract was transferred to a glass vial and the flask was washed with 1 mL of methanol. Subsequently, the combined eluate and methanol wash was completely dried down under a nitrogen stream and the sample was redissolved in 2 mL of dichloromethane/methanol/toluene (8:1:1 v/v/v). A 500 μL aliquot of the sample was dried down and taken up in 500 μL of ethanol prior to YES analysis. A second aliquot of 500 μL was evaporated to dryness and then redissolved in 200 μL of methanol prior to LC/MS/MS analysis.

2.5.2. Freshwater mussels

Freeze-dried mussel tissue (0.5 g) was introduced into a 50 mL glass tube and then spiked with 100 μL of a 1000 ng/mL methanolic solution of labelled bisphenol A/*n*-nonylphenol and with 30 μL of a 1000 ng/mL methanolic solution of labelled estrogens.

Rehumidification of the tissues was carried out by adding 1 mL of methanol and sonicating for 10 min. Tubes were stored at −20 °C overnight. The resulting slurry was extracted three times with 10 mL of methanol in an ultrasonic bath for 20 min, and then the suspension was centrifuged at 3500 rpm for 10 min. The supernatant was collected, evaporated to dryness and reconstituted with 2 mL of methanol/Milli-Q water (1:1). The extract was transferred to a glass vial before clean-up. Preliminary experiments showed that high matrix loads in the samples led to a remarkable ion suppression during LC/MS/MS analysis. Therefore, a more elaborate clean-up procedure – using two solid-phase extractions – was necessary. The first step was carried out using C_{18} SPE cartridges, which were conditioned with 6 mL of hexane, 2 mL of acetone, 6 mL of methanol and then 10 mL of Milli-Q water. After loading the sample into the column, the stationary phase was washed with 8 mL of 70% methanol /30% Milli-Q water and then with 6 mL of 30% acetonitrile/70% Milli-Q water. After drying of the solid phase with nitrogen, analytes were eluted with 4 mL of acetone, concentrated to 0.1 mL under a nitrogen stream and finally taken up with 2 × 200 μL of hexane/acetone (3:2). The second clean-up step was carried out using silica SPE cartridges, which were first conditioned with 4 mL of hexane/acetone (3:2) and then 4 mL of hexane. The sample was loaded onto the column and then eluted with 7 mL hexane/acetone (3:2). The eluate was completely dried down using a stream of nitrogen and redissolved in 200 μL of methanol before LC/MS/MS analysis.

2.6. Analytical determination

Analyses were performed using an HP Series 1100 high-performance liquid chromatograph (Hewlett–Packard, Waldbronn, Germany), equipped with an online vacuum degasser (DG4, Henggeler Analytic Instruments, Riehen, Switzerland), a binary high-pressure gradient pump, an autosampler and a heated column compartment kept at 23 °C. Separation was achieved with a 100 × 2.1 mm Waters X Terra, MS C18, 3.5 μm column (Waters, Rupperswil, Switzerland). A 10 μL aliquot of sample extract was injected and eluted off the column with 0.25 mL/min of 10% acetonitrile in Milli-Q water for 2 min, followed by a linear increase to 90% acetonitrile at 19 min and 3 min hold at 90% acetonitrile. A triple quadrupole mass spectrometer (API 4000, Applied Biosystems, Rotkreuz, Switzerland) was used in negative ion mode electrospray. Ionization efficiency was increased by postcolumn addition of a 2.5% ammonia solution at 10 μL/min using a micro-HPLC syringe pump (Phoenix 20 CU, Carlo Erba Instruments, Milan, Italy). Nitrogen was used as the drying and nebulizer gas. Quantitative analysis was performed in multiple reaction monitoring (MRM) in order to maximize sensitivity. The concentrations were calculated relative to the internal standards. Retention times, log K_{ow}, relative potencies and monitored ions of considered compounds and relative internal standard are shown in Table 1.

2.7. In vitro analysis (yeast estrogen screen (YES))

Estrogenic activity was assessed using the YES, an in vitro yeast reporter gene assay (Routledge and Sumpter, 1996). Four dilutions of POCIS extracts in ethanol (20 μL) were tested in triplicate. The standard in the assay is 17β-estradiol, consequently, the estrogenicity of a sample is expressed as 17β-estradiol equivalents (EEQ, ng/POCIS). Duplicate POCIS extracts for each sampling point were split between two plates (I and II). The EEQ of an extract was interpolated from the average of the standard curves on both plates.

EEQ values can be dependent on the amount of sample used in the assay, as the dilution curves of the sample and standard are not always parallel (Vermeirssen et al., 2005b). Our POCIS samples showed good parallelism; EEQ values were calculated for the highest sample dilution.

3. Results and discussion

3.1. Passive sampling

3.1.1. HPLC/MS/MS analyses

Data acquired during the exposure experiments are summarized in Table 2. Values are expressed as nanograms of analyte per POCIS; instrumental standard deviation was about 6% for each compound. All EDCs were present in the samples, except for ethinylestradiol which was always below the detection limit and therefore it is not reported in the table. A general trend showing a decrease of all the analyte concentrations after the wastewater treatment can be observed, especially considering that the samplers were deployed in the influent for 5 days instead of 7, due to a rather fast fouling.

The industrial chemicals, nonylphenol and bisphenol A, were present in higher amounts (ranging from 102 to 218 and from 310 to 2464 ng/POCIS, respectively) compared to the natural hormones (9–70 for E1 and 6–55 ng/POCIS for E2) in both influent and effluent. Bisphenol A levels are one order of magnitude higher than nonylphenol in almost all samples. Nonylphenol mainly originates from the degradation of nonylphenol polyethoxylates, which are used as nonionic surfactants in many household products. Bisphenol A is released through its use in polycarbonate plastics and epoxy resins, which are utilized, for example, in food storage containers. Since most of the influent to the WWTP is from domestic sources, high amounts of alkylphenols and bisphenol A are not unusual.

In the effluent, two POCISs were deployed for the whole experimental period of 2 weeks, showing higher concentrations than those obtained in samplers deployed for half the time, as expected from an integrative sampler. For both E1 and E2, the sum of the two "1-week" sampling slots matches the data from the "2-week" sampling quite well. For bisphenol A and nonylphenol this comparison is less good.

Although the sampling windows for effluent and influent did not match exactly, it can be interesting to evaluate the reduction of EDC amount due to the WWTP; to this aim, data reported in Table 2 were normalized to obtain the amount of analyte sampled per day per sampler.

Estradiol levels were reduced by about 87% by the treatment process (from 8 ng/POCIS per day in the influent to 1 ng/POCIS per day in the effluent) while for estrone and bisphenol A the reduction was 70% (from 7.5 to 2.2 and from 295 to 91 ng/POCIS per day, respectively). For nonylphenol the reduction was only 55% but we have to consider its relatively low levels for an ubiquitous chemical (from 35.5 to 16 ng/POCIS per day).

Estradiol was also found in the control tank (6 ng/POCIS). The source of this compound is uncertain; a possible explanation could be the release by the freshwater mussels deployed together with the passive samplers. The presence of estradiol has already been reported by Halm et al. (2002) in control tanks from in vivo studies on fish exposed to different amounts of estradiol. The concentration of E2 in the control tanks was highest at the beginning of the experiment and decreased afterwards, suggesting a possible link with stress to the fish. There are no similar data for mussels but some excretion of estradiol into the water cannot be discarded. A contributing factor to the E2 found in the control POCIS may be the absence of water renewal in the control tank.

3.1.2. In vitro bioassay (YES)

EEQ values of the POCIS samples ranged between 5 and 78 ng/POCIS. The highest values were always found in the influent, while the effluent showed a consistent lower amount than the total estrogenicity of the sample, with a removal rate of 80%. As for E1 and E2, the sum of the EEQs of the two 1-week POCIS exposures matched the amount of EEQ in the POCIS that had been exposed for 2 weeks.

We observed suppression of the YES signal at high doses of POCIS sample influent due to toxicity (i.e. cell death). However, at

Table 1
Characteristics of various EDCs analyzed by LC/MS/MS and bioassay (YES).

Compound	Abbreviation	log K_{ow} [a]	Relative potencies[b]	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	LOD (ng/ml)
Nonylphenol	NP	5.8	2.5×10^{-5}	21.82	219	133	1.0
Nonylphenol ring-^{13}C (internal standard)		5.8		21.82	225	112	
Bisphenol A	BPA	3.3	1.1×10^{-4}	16.06	227	212	1.0
Bisphenol A-d_{16} (internal standard)		3.3		16.06	242	143	
Estrone	E1	3.1	0.4	17.07	269	145	0.5
Estrone-2,4,16,16-d_4 (internal standard)		3.1		17.07	273	147	
Estradiol	E2	4.0	1	16.55	271	145	1.0
Estradiol-2,4-d_2 (internal standard)		4.0		16.55	273	147	
Ethinylestradiol	EE2	4.15	1.19	16.84	295	145	2.0
Ethinylestradiol-2,4,16,16-d_4 (internal standard)		4.15		16.84	299	273	

[a] Data from Arditsoglou and Voutsa (2008).
[b] Data from Rutishauser et al. (2004).

Table 2
Amounts of EDCs and estrogenic activity in passive samplers expressed as ng/POCIS. In the sample code, IN refers to the influent, OUT to the effluent, p1 and p2 to the first and the second period of deployment, respectively, pTot to the whole period, a and b indicate the individual POCIS duplicates.

Sample code	Deployment duration (day)	NP	BPA	E1	E2	bioEEQ[a]	calEEQ[b]
IN-p1a	5	175	1270	70	53	47	81
IN-p1b	5	218	2464	27	55	78	66
IN-p2a	5	123	967	35	30	38	44
IN-p2b	5	190	1207	20	23	40	31
OUT-p1a	7	[c]	956	21	6	22	14
OUT-p1b	7	102	810	22	10	20	19
OUT-p2a	7	122	473	12	9	8	14
OUT-p2b	7	113	310	9	6	5	10
OUT-pTota	14	152	2919	41	20	24	36
OUT-pTotb	14	172	3180	38	26	33	41
Blank POCIS	14	72	83	<LOD	6	4	6

[a] Estradiol equivalents (bioEEQ) obtained from yeast estrogen screen (YES).
[b] Calculated estradiol equivalents (calEEQ) based on EDC concentrations obtained from LC/MS/MS analysis.
[c] Value not available for the loss of the internal standard.

higher dilutions, all the samples were parallel or very close to parallel with the 17β-estradiol standard in the YES (data not shown; see also Vermeirssen et al., 2005b).

3.1.3. Comparison of calculated estrogenicity versus estrogenicity measured with the YES

The expected estrogenic activity in a sample can be calculated from chemical analysis data, based on the concept of concentration addition (Kortenkamp and Altenburger, 1998). Consequently, concentrations of each analyte were multiplied by the relative potency of each compound in the YES. Subsequently, the values for the five compounds were added together. The relative potencies determined with the yeast estrogen screen cover a range of five orders of magnitude, with the natural and synthetic steroid hormones being the most potent (see Table 1). Data from the five exposure periods (Fig. 1) were used to analyze the correlation between measured estrogenicity (bioEEQ) and calculated estradiol equivalents (calEEQ). A good correlation between bioEEQ and calEEQ ($y = 0.85x$; $R^2 = 0.67$; $n = 10$) can be observed although the data set is small. This result confirms the general robustness of both chemical and biological analyses. Furthermore, these data indicate that the natural and synthetic steroid hormones represent the major contributors to the measured estrogenicity. It has to be stressed though, that the YES results are based on the binding of compounds to the estrogen receptor. Effects on organisms can be more subtle than mere receptor binding. Hence, the contribution of bisphenol A and nonylphenol to the endocrine disrupting potential of the investigated effluent cannot be excluded.

3.2. Freshwater mussels exposure experiment

Passive sampling was developed as a time-integrated method to sample the bioavailable fraction of different types of chemicals, as an alternative to sampling organisms. Bivalve molluscs have been largely employed as pollution indicators since they are filter-feeders exposed to large volumes of water (UNEP, 1992). The freshwater mussels *Unio pictorum* were deployed in both influent and effluent of the wastewater treatment plant to evaluate the bioaccumulation of EDCs in the main organs involved in this uptake process; EDC levels were determined in the "gills", "mantle" and remaining tissue ("rest") by LC/MS/MS analysis.

There was no indication of a strong bioconcentration of nonylphenol and bisphenol A in any sample. Replicate analyses were quite variable (see error bars in Fig. 2), especially for the "rest" samples (whole tissue except for gills and mantle). This was probably due to the different nature of the various tissues that make up the "rest". Despite a careful homogenization of the sample, the whole tissue maintained a lower homogeneity compared with the single organs.

Estradiol levels in mussels from the control tank and from effluent were in the range of the levels naturally occurring in mussels along their reproductive cycle (0.6–1.1 ng/g), while mussels exposed in the influent for 5 days showed very high levels of estradiol exclusively in the mantle, within which the gonads are located. However, mussels exposed in the influent for 10 days did not present the same bioaccumulation and estradiol levels were comparable to those found in the other samples (Fig. 2). Estrone was detected at very low concentration in all tissue pools and ethinylestradiol levels were lower than the limit of detection in all samples.

Normally, free estradiol levels in mussel tissues are usually rather stable in studies of exposure; a significant increase was noted just for very high exposure doses (2000 ng/L; Janer et al., 2005).

The substantial decrease of estradiol concentration in the mantle after 10 days of exposure could be due to metabolism. Conjugation of the excess of estradiol by esterification might act as

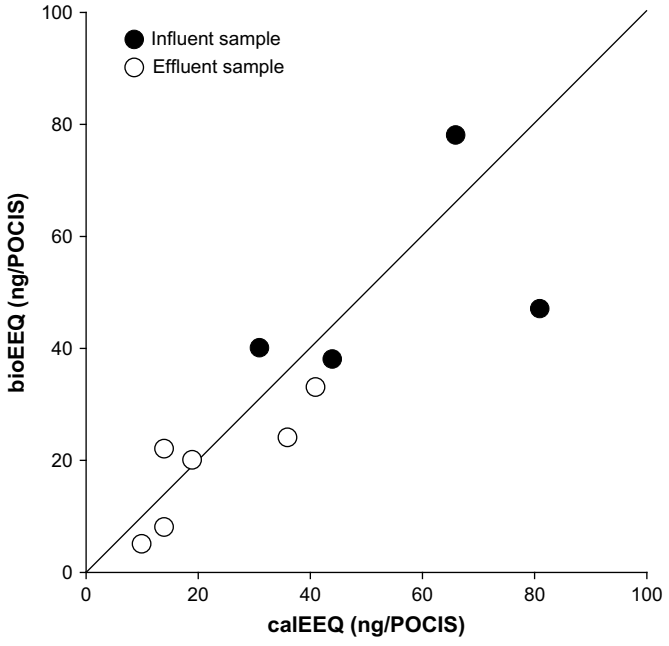

Fig. 1. Correlation between estrogenicity data obtained with yeast estrogen screen (YES; 17β-estradiol equivalents, bioEEQ) and calculated estrogenicity (calEEQ), based on chemical analysis of EDCs in passive samplers (the line illustrates a 1 to 1 relationship).

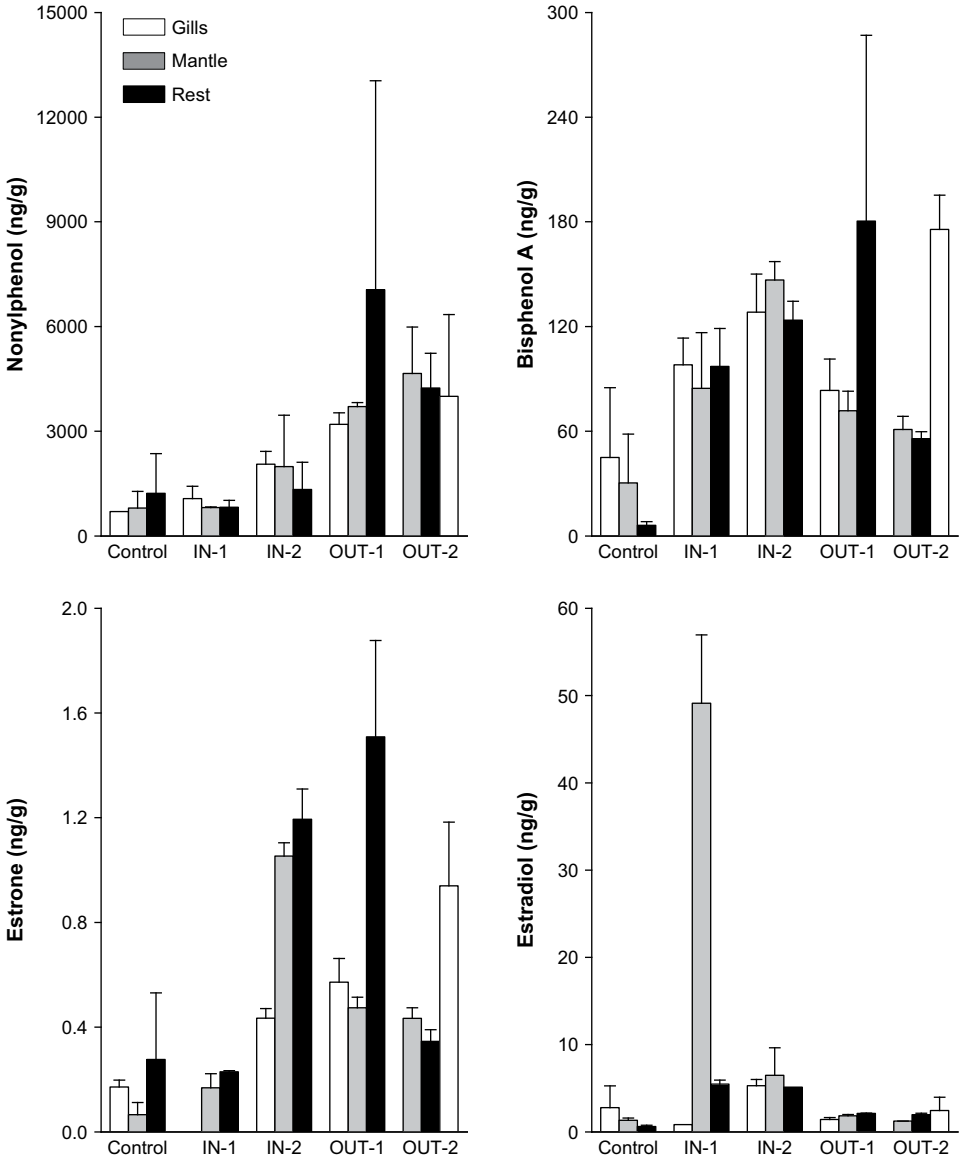

Fig. 2. Concentration of selected EDCs in freshwater mussel tissues (*Unio pictorum*). In the sample code, IN refers to the influent, OUT to the effluent, 1 to the first half period (5 days for influent and 7 days for effluent) and 2 to the whole period (10 days for influent and 14 days for effluent) of deployment, respectively. The error bars illustrate the methodological variation over two separate analyses of the same tissue pool.

a homeostatic mechanism to maintain endogenous levels of free estradiol stable. Data regarding conjugation of steroids in molluscs are rather limited; some studies have revealed that sex steroids are not predominantly metabolized via classical vertebrate routes (glucuronic and sulfate conjugates) but are often esterified to form lipophilic conjugates of long chain fatty acids (Janer et al., 2005; Labadie et al., 2007). Fatty acid esterification turns steroids into an apolar form, which is retained in the lipoidal matrices of the body, and reduces their activity, bioavailability and susceptibility to elimination (Borg et al., 1995).

Although E1 concentrations found in POCIS samples were higher than E2, no E1 bioaccumulation was found in mussels. This trend is similar to that observed in *M. edulis* sampled nearby a sewage treatment plant in Le Havre in France, where only E2 (and no E1) was detected, while chemical analysis of the effluent was shown to contain both E2 and E1 (Labadie et al., 2007). The absence of E1 in mussel tissue could be due to the mussel potent C17β-hydroxysteroid dehydrogenase (C17-HSD) activity, so that any E1 present in mussel tissues is rapidly metabolized to E2. C17β-HSD enzymes are ubiquitous in the animal kingdom and are reported to be highly active in gonad tissues (De Longcamp et al., 1974).

3.3. Comparison of passive sampling and biomonitoring for selected EDCs

The main objective of the study was to compare the two sampling methods in terms of accumulation capacity and complexity of the analytical methods. Although biomonitoring is obviously highly relevant, and potentially allows for studies on effects on organisms, we found that employing mussels held several disadvantages for assessing the presence of endocrine disruptors. As the more potent endocrine disruptors are natural estrogens, knowledge gaps in the endocrine system of molluscs impede a sound interpretation of the data. For example, it is uncertain to what extent accumulated estrogens are being metabolized. Also, estradiol is naturally present in the mussels, confounding the interpretation of elevated E2 levels in effluent exposed mussels. Besides these issues, the sample processing of the organisms is much more elaborate than the processing of the

passive samplers. Even after a complex preparation, ion suppression was still noticeable in the biological samples (about 70%).

Using mussels as biomonitors for the EDCs considered in this study may be difficult; the interpretation of data is obviously easier for contaminants such as pesticides and heavy metals, which are not responsible for metabolism and endogenous processes. However, they remain a valuable tool in determining endocrine disrupting effects of effluents.

The data from the POCIS confirm observations from earlier studies that highlight their usefulness as passive samplers for EDCs (Vermeirssen et al., 2005a; Arditsoglou and Voutsa, 2008). They hold the additional advantage that, after a rigorous calibration of the device, it is possible to calculate time weighted average concentrations.

4. Conclusions

In the present study five estrogens or estrogen mimics were considered; four of them were detected in wastewaters with both the passive sampling and the biomonitor approach.

In the effluent of a wastewater treatment plant the amount of estrogens, measured by means of LC/MS/MS, was lower than that in the influent.

EEQ values obtained with YES showed a good correlation with the chemical analyses.

Passive sampling turned out to be a good method for assessing the presence of EDCs; furthermore, it can be combined with toxicity assessments for a more complete evaluation of the estrogenic potency of wastewaters.

Mussels showed low bioaccumulation efficiency and several drawbacks both in sample preparation and analysis. On the other hand, the use of organisms (e.g. mussels or fish) is crucial when studying the possible effects of EDCs in the aquatic environment.

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