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REVIEW

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Endocrine disrupting compounds and other emerging contaminants in the environment: A survey on new monitoring strategies and occurrence data

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Abstract An overview of Toxicity Identification and Evaluation (TIE) procedures, used for the effect-based analysis of endocrine disrupting compounds (EDCs) in environmental samples, is presented. Future trends in advanced chemical analysis of EDCs and some emerging contaminants are outlined. The review also gives an overview of concentration levels found in environmental samples and discusses the correlation of calculated estrogenicity (based on chemical analysis of target EDCs) with that measured by various bioassays.

Keywords Toxicity Identification and Evaluation (TIE) procedures · Endocrine disrupting compounds · Emerging contaminants · Environmental analysis

Introduction

Chemical analysis of endocrine disrupting compounds (EDC) is generally insufficient to assess contaminants present in the environment and to estimate their endocrine potential. Beside recognized EDCs, numerous new chemicals are synthesized each year and released into the environment with unforeseen consequences, and evidence for their endocrine potential is constantly emerging. However, chemical identification of all compounds, responsible for the observed endocrine disrupting effect is an impossible task. Therefore, the assesment of endocrine disrupting activity in complex environmental mixtures requires application of integrative procedures combining chemical analysis and specific bioassays. This approach, focused on health and environmentally relevant compounds, is based on the Toxicity Identification and Evaluation (TIE) procedure that was developed by the US-EPA at the beginning of the 1990s and was originally aimed at investigating wastewater [1, 2]. However, during the last decade the TIE approach has become an established and powerful tool for determining the causes of the effects (such as acute toxicity, (geno)toxicity, endocrine disrupting potential) in aqueous and solid environmental samples.

This paper surveys the current state of new monitoring strategies involving the application of integrated chemical-biological procedures for the determination of EDC, including fractionation procedures and potency balance calculations. Current trends and future perspectives in chemical analysis for several groups of EDCs that are of priority within European Union and US research activities: steroid sex hormones, alkylphenols, polybrominated diphenyl ethers (PBDEs), and phthalates, are outlined.

Additionally, several selected classes of so-called "new" or "emerging contaminants", such as surfactants, human and veterinary drugs, fragrances and antiseptics are included in this review. Emerging contaminants are unregulated contaminants, which may be candidates for future regulation depending on research on their potential health effects and monitoring data regarding their occurrence. This group is mainly composed of products used in everyday life and for most of them ecotoxicological data are not yet available, and therefore it is difficult to predict what health effects they may have on humans and aquatic organisms. However, recent studies showed that some emerging contaminants are weak endocrine disruptors. For example, by means of the E-screen assay, a number of musk fragrances such as *p*-amino musk xylene were shown in vitro to possess estrogenic activity [3], while changes in fin length of Japanese medaka fry (*Oryzias latipes*) and non-significant trends in sex ratio suggest that triclosan, an antibacterial agent commonly used in households and industry, is potentially weakly androgenic [4]. Finally, levels of selected EDCs and emerging contaminants found in environmental samples are reviewed.

TIE procedures for EDCs

The general scheme of TIE for the effect-based analysis of environmental samples is shown in Fig. 1. This strategy

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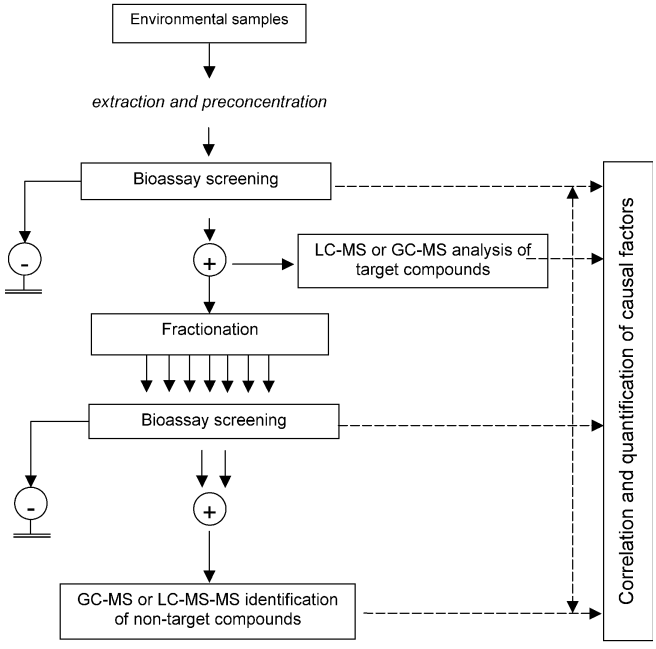

Fig. 1 Toxicity Identification Evaluation (TIE) scheme used for the effect-based analysis of environmental samples

enables determination of the causative agents, and the results obtained by applying bioassays in the first stage are used to direct attention to detailed chemical analysis of fractions until the correlation is achieved. The whole process can be repeated, applying an additional fractionation procedure until the chemical complexity of the fractions is reduced sufficiently. Thus non-target chemical analysis enables detection or identification of unknown compounds responsible for the observed effect.

Different TIE procedures that have been developed for the assessment of endocrine disrupting potential of environmental samples, integrating chemical analysis of target EDCs and a variety of biological screening assays, are summarized in Table 1.

Fractionation schemes

TIE procedures usually involve the stepwise fractionation and simplification of a sample in order to isolate compounds responsible for the observed effects. Fractionation is usually directed by a bioassay and is performed either using sequential SPE or (semi)preparative HPLC. Several authors reported the use of multiple-step SPE procedures in order to achieve a versatile and broad extraction, since a single extraction under neutral or acidic conditions is limited to hydrophobic or only weakly polar compounds. Prior to toxicity determination, Reemtsma et al [5] applied the four step SPE procedure, using a combination of C18 and polymeric cartridges and different pH conditions for separating the extract into hydrophobic neutral, polar neutral, acidic and strongly acidic fractions. A similar approach was used by Castillo et al [6, 7] for the determination of more polar toxic wastewater constituents.

Another approach employed sequential elution of compounds bound to the C18 cartridge using a gradient of polarity: water-methanol mixture from 0% to 100% methanol, followed with final elution with hexane [8] or methanol/water followed by solvents of low polarity to non-polar solvents (diethylether, 50/50 diethylether/hexane and finally hexane) [9].

However, the most often applied fractionation scheme involves the reversed phase (RP) HPLC fractionation, used directly to fractionate bulk extracts or for fine fractionation of SPE fractions. Linear gradients of methanol and water [9, 10, 11, 12] or acetonitrile and water [13], respectively were preferably used to split the components in relation to their Kow range.

In vitro bioassays

A variety of in vitro biological assays have been developed for the screening of EDCs in environmental samples. They are designed to facilitate sensitive, rapid and relatively inexpensive prediction of endocrine disrupting potential of complex mixtures, and their development has clearly sped up the process in identifying potential estrogenic and anti-estrogenic compounds in the environment. The most widely used bioassays are: receptor binding assays that measure binding to estrogen, androgen or progesterone receptors; cell proliferation assays, usually using estrogen-dependent human breast cancer cell lines such as MCF-7 (E-screen) or T47D; and reporter gene assays measuring the ability of a substance to activate transcription of a hormone (for instance estrogen)-sensitive promoter in, usually, mammalian or yeast cells. These in vitro assays enable assessment of total biological activity of a sample integrating possible interactions among chemicals that act through the same mode of action and therefore offer the possibility of providing an early screen prior to targeted chemical analysis. Several review papers give summary of in vitro assays used for the assessment of the estrogenic potential of natural and synthetic estrogens and xenoestrogens, including details on mechanisms, performances, applications, advantages and limitations [14, 15, 16, 17]. In most cases these in vitro assays were used to assess the estrogenic/antiestrogenic potential of pure compounds and less often for the analysis of real environmental samples. TIE procedures applying bioassays such as Yeast based recombinant estrogen receptor-reporter assay (YES), MCF-7 cell proliferation (E-screen), and Estrogen receptor-mediated chemical activated luciferase gene expression assay (ER-CALUX), respectively were successfully used to characterize estrogenic activity of surface waters, sediments, suspended particles, wastewaters and biological samples (see Table 1). One of the issues to be raised when combining bioassays and chemical analysis is the importance of specific sample preparation needed for bioassay due to different requirements of two methods in terms of compatibility of solvents. Therefore, it is necessary to develop and optimize clean-up procedures that permit us to achieve a versatile and broad extraction with high and reproducible recoveries of analytes.

Table 1 Survey of TIE procedures for testing EDCs in environmental samples

Sample	Extraction method	Fractionation	Chemical analysis	Target compounds	Bioassay	Reference
STP effluent	SPE C18	1. SPE sequential elution	GC-MS	17β-estradiol	Yeast estrogen screen (YES)	[9]
		2. RP HPLC		17α-ethylestradiol		
		3. RP HPLC (shallow gradient)		estrone		
Estuary water, sediment	C8-polystyrene based polymer-nonporous carbon	RP HPLC	GC-MS	17β-estradiol, DEHP, Nonylphenol, androsterone	Yeast estrogen screen (YES)	[10]
River water, STP effluents	SPE C18	–	LC-MS	Nonylphenol, NPEOs, steroids (estrogens, progestogens)	Yeast estrogen screen (YES)	[66]
STP influent and effluent	Not given	–	GC-MS-MS	Steroids	Yeast estrogen screen (YES)	[69]
River water and sediment	SPE C18	SPE sequential elution	GC-MS	Alkylphenols	Recombinant receptor-reporter gene assay (MELN)	[8]
STP influent and effluent	SPE polystyrene copolymer resin and C18	–	GC-MS	Alkylphenols, Bisphenol A	MCF-7 cell proliferation (E-screen)	[67]
River sediment	Soxhlet	Florisil – sequential elution	GC-ECD	OC pesticides	MCF-7 cell proliferation (E-screen)	[68]
			GC-MS	PCDDs/DFs, PCBs, PAHs		
			HPLC-FL	Alkylphenols		
			GC-MS	Phthalates, Nonylphenol		
			LC-MS	NPEO		
Sediment, suspended particles, biota	Soxhlet	–	GC-MS	Alkylphenols, APEOs, phthalates	Estrogen receptor-mediated chemical activated luciferase gene expression (ER-CALUX)	[65, 70]

Currently, the main limitation is the lack of testing guidelines, and recent activities have been oriented toward defining strategies for short-term and long-term testing of EDCs and validating of existing tests. Most of in vitro bioassays used nowadays are not validated and their comparability is rarely investigated. A comparison study showed that the relative estrogenic potency of complex environmental mixtures determined with different assays having a different end point might vary greatly [15], not predicting reliably the outcome of in vivo testing due to differences in metabolic capabilities of the test systems. Therefore, a battery of tests, including both in vitro and in vivo assays that assess both receptor and non-receptor mediated mechanisms of action, seems the most appropriate way to assess the potential of EDCs in complex environmental samples [17].

Quantification of estrogenic activity – relative potency calculations

There is an ongoing debate on predictivity and comparability of different bioassays used in the assessment of EDCs. Contrary to in vivo tests, in vitro assays provide insights on mechanisms of action of a specific substance, but their capacity to mimic whole animal uptake, distribution and availability of contaminants in organisms is restricted. Additionally, because of the different end points used in different assays and the diverse range of mechanisms by which EDCs may act, the comparison of in vitro assays and correlation of the results obtained is not always good. Analog to the calculation of toxicity equivalents (TEQ-values) for dioxins and dioxin-like compounds, the estrogenic potential of individual EDCs may be expressed relative to 17β-estradiol (estradiol equivalent factors - EEF), and that of complex mixtures can be calculated in estradiol equivalents (EEQ).

Table 2 Relative estrogenic potency as determined by different bioassays (expressed as EEF – the molar based 17β-estradiol equivalency factor)

Compounds	YES [65, 71, 72, 73]	MCF7 assay (E-screen) [15, 65, 67, 71, 74]	ER-CALUX [65]
Steroid sex hormones			
17β-estradiol	1	1	1
Estriol	3.7×10^{-1}		
Ethinylestradiol	1.9×10^{-1}–1.2	1.25–1.9	1.2
Diethylstilbestrol	4.5×10^{-2}–1.1	2.5	
Estrone	1.9×10^{-2}–1.0×10^{-1}	1.0×10^{-2}	5.6×10^{-2}
Alkylphenolic compounds			
Nonylphenol	7.2×10^{-7}–4.1×10^{-4}	1.3×10^{-5}–1.0×10^{-4}	2.3×10^{-5}
Octylphenol	1.0×10^{-5}–4.9×10^{-4}	1.0×10^{-4}–2.5×10^{-4}	1.4×10^{-6}
Nonylphenol monoethoxylate	4.0×10^{-6}–1.3×10^{-5}		3.8×10^{-6}
Bisphenolic compounds			
Bisphenol A	1.0×10^{-5}–8.1×10^{-5}	2.5×10^{-5}–6.0×10^{-5}	7.8×10^{-6}
Phthalates			
Di(ethylhexyl)phthalate	2.5×10^{-5}		
Dibutylphthalate	1.0×10^{-7}–1.0×10^{-5}		1.8×10^{-8}
Benzylbutylphthalate	1.0×1^{-6}–8.9×10^{-6}		1.4×10^{-6}
Dimethylphthalate	1.0×10^{-6}–7.0×10^{-6}		1.1×10^{-5}
Diethylphthalate	5.0×10^{-7}–4.7×10^{-6}		3.2×10^{-8}
Fragrances			
Musk xylol		3.3×10^{-5}	
Musk ketone		7.0×10^{-5}	

Table 2 summarizes relative potencies of selected EDCs determined using different in vitro assays. EEF were calculated from the EC_{50} values determined from the dose response curves, which for some xenoestrogens are biphasic with a decrease in response at greater dose due to citotoxicity. Generally, data show major discrepancies between the relative estrogenic potencies of the compounds tested, requiring a careful interpretation of the results when such bioassays are used to estimate the estrogenic potential of complex mixtures. Partly, the differences are due to the specificity of the assays and different experimental conditions, which means that EEF should be determined experimentally for each bioassay and specific conditions. Possible false-negative and false-positive results, as discussed by Hoogenboom et al [14] may be reduced by the use of specific assay conditions and the application of more selective clean-up procedures, whereas competitive antiestrogenic effects and possible citotoxicity of mixtures should be evaluated in bioassays with the same cells that were used in receptor mediated measurements [16].

Chemical analysis of endocrine disruptors and emerging contaminants

Target analysis

A number of analytical methodologies have been reported for the analysis of specific classes of EDC and other emerging contaminants in aqueous and solid environmental samples, and several comprehensive reviews were recently published on this issue (Table 3). Thus, this review does not include a detailed discussion on the methodology for a specific group of compounds and only outlines the major tendencies in advanced instrumental analysis of trace EDCs.

Due to the chemical diversity of endocrine disrupting compounds the range of instrumental techniques applicable to their analysis is also very wide. Within modern analytical techniques applicable to trace analysis of endocrine disrupting compounds, GC and LC combined with MS and tandem MS, respectively, play a pivotal role providing sufficient selectivity and inherent sensitivity in the analysis of complex environmental matrices. Most of the analytical procedures developed for environmental determination of EDCs and emerging contaminants have been designed for analysis of single particular classes of compounds, and the key issue is to develop multi-residue methods in which different compound classes can be determined in a single analysis.

Additionally, because of the generally very low environmental levels of EDCs and emerging contaminants and the complexity of some matrices, very efficient extraction/purification methods, in addition to selective and sensitive analytical techniques, are required. In this respect, it is worth noting the application of (i) immunosorbents for the purification of extracts prior to the analysis, (ii) dual column switching LC-MS for the integrated purification and analysis, and (iii) a fully automated methodology for the on-line SPE and analysis [18].

Regarding the instrumental analysis, driven by the estrogenic potency of some compounds (for example steroid sex hormones) and low environmental concentrations, the detection limits required for the monitoring of endocrine

Table 3 Reviews of analytical methodology for the analysis of selected classes of EDC and other emerging contaminants in environmental samples

Compound classes	Remark	Reference
Emerging contaminants	Review of mass spectrometric methods for analysis of emerging contaminants (including several classes of EDCs)	[75]
Alkylphenolic compounds	LC-MS and GC-MS methods for the analysis of aqueous and solid environmental samples	[76]
	Review of sample preparation techniques (sediment, sludge biota)	[77]
Pharmaceuticals	Analytical methods for the determination of pharmaceuticals in aqueous environmental samples	[78]
	Analysis and sample preparation of soil, sediment and sludge samples	[79]
Steroid sex hormones	LC-MS and GC-MS methods for the analysis of aqueous and solid environmental samples	[76]
	Analysis of hormones in wastewater samples	[80]
	Solid-phase extraction for sample preparation in the determination of estrogens and progestogens in sediment and water	[81]
Plasticizers (Phthalates, BPA)	LC-MS and GC-MS methods for the analysis of aqueous and solid environmental samples	[76]
Musks	Analysis of polycyclic musk	[52]
	Analysis of musk xylene and musk ketone amino metabolites	[53]
Brominated flame retardants	Review of chromatographic techniques for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons	[82]
	Determination of brominated flame retardants in environmental samples	[83]
	Method for the analysis of polybrominated diphenylethers in sediments and biota	[84]

disruptors are being pushed from the µg/L to ng/L range and even to below the ng/L range. For example, the sensitivity of the techniques for the determination of steroid hormones in complex environmental matrices improved in the order LC-MS ($LOD_{absolute}$ 200 pg/µl)< GC-MS-MS ($LOQ_{absolute}$ 20 pg/µl)< LC-MS-MS ($LOQ_{absolute}$ 5 pg/µl) [19]. For alkylphenolic compounds LODs achieved are in the low ng/L range [20] (for the preconcentration of 500 ml of water samples).

The overall trend in chromatographic analysis of environmental samples involves employment of fast-LC and fast-GC methods using short, narrow bore columns, high mobile phase flow-rates and ultra-high pressures. Shortening the analytical run times is an important step towards high sample throughput often required in laboratories conducting monitoring studies. Run times of several tens of minutes are not tolerable for truly high-throughput analyses and emphasis is directed towards achievement of maximum chromatographic resolution in a drastically reduced time.

The use of advanced separation systems and narrow-bore columns that produce extremely narrow chromatographic peaks must be accompanied by compatible instrumentation for trace level analysis. Technologies that have significantly advanced in the last recent years include tandem (MS-MS) systems, time-of-flight MS (ToF-MS), and quadrupole-time-of-flight (Q-ToF-MS). The added power of MS-MS, applying a variety of scan functions and modes (in other words product ion scan, precursor ion scan, neutral loss, multiple reaction monitoring-MRM), improved analytical performances (reliability and sensitivity) and allowed a gradual shift from the detection of parent compounds to the analysis of metabolites and transformation products. LC-MS-MS analysis of steroid sex hormones, as reported by Lagana et al [21], is about two hours faster, less affected by error, and significantly more sensitive than a conventional GC-MS method. However, an advantage of GC-MS, compared with LC-MS, is the availability of extensive libraries of mass spectra useful for identification of unknown peaks in estrogenically active fractions.

Identification of unknown compounds

Comprehensive screening protocols, such as GC-MS or LC-MS of fractionated samples or two dimensional GC (GC×GC), are able to identify several hundreds of individual components in complex environmental samples, and combined with the potential of tandem MS technique might be used to identify unknown compounds. However, such general screening for unknown compounds is time consuming and expensive, and is often related to problems such as lack of authentic standards and of MS spec-

tra libraries. Moreover, all compounds detected or identified represent only a small part (usually only several %) of the dissolved organic carbon (DOC) content.

Although the sensitivity, selectivity and efficiency of the MRM approach are all excellent, qualitative information needed to support the structural elucidation of compounds other than the target analytes is lost. In full scan mode, this information can be obtained; however, the lack of compound databases and mass spectra libraries often represent an obstacle for efficient structural elucidation of unknown compounds.

Coupling of API technology and ToF-MS combines high accuracy and excellent sensitivity due to the high-frequency sampling of all ions simultaneously recorded across a full mass range. An orthogonal-acceleration time-of-flight MS instrument (oaToF-MS) coupled to LC proved to be a powerful tool for identification of micro-constituents in complex mixtures and/or confirmation of their presence. Such an instrument provides mass determinations with an accuracy of 5–10 ppm, which is an impressive improvement over the conventional nominal-mass information of a quadrupole instrument. Hogenboom et al [22, 23] demonstrated the capability of oaTOF MS for multiresidue screening in water analysis where accurate mass determinations were used for confirmation and identification of organic micro-contaminants in surface water.

Another variation of tandem hybrid instruments that has gained popularity in the last few years is a combination of a quadrupole instrument and an orthogonal acceleration ToF-MS (Q-ToF). Such an instrument enables accurate mass measurement with accuracies of <5 ppm, which allows removal of interpretation ambiguities and easy differentiation of charge states even in weak collisionally-activated decomposition tandem mass spectra [24]. Although environmental applications are still scarce, Q-ToF mass spectrometers are often used in bioanalysis for the identification of small molecules (<1000 Da) due to the advantages of the ion separation and detection principle. The possibilities of a Q-ToF-MS in a LC-MS/MS screening and identification of organic micro-pollutants in surface and sea water [25, 26] are being explored and further development and more environmental applications are expected. A recent book [27] on LC-MS-MS and ToF-MS analysis of emerging contaminants focuses on wide range of compounds from pesticides, pharmaceuticals, surfactants, plasticizers, steroids and hormones, to disinfection by-products, and brings together many applications of these two techniques in the field of environmental analysis.

Environmental data

Concentration levels

Numerous field studies, designed to provide basic scientific information related to the occurrence and potential transport of contaminants in the environment, are being continuously conducted with the aim of identifying which contaminants enter the environment, at what concentrations, and in which combinations. A large body of literature exists on occurrence of specific groups of organic contaminants in the environment. However, for a long time research priorities have been focused on priority pollutants, such as POPs, pesticides, toxic metals, radionuclides, and only recently has the attention of the scientific community started to shift to endocrine disruptors and other emerging contaminants.

Alkylphenol etoxylates and their metabolites

The globally increased interest toward endocrine disruptors resulted in various research and monitoring programs conducted by official environmental organizations and scientific groups, with the objective to assess the occurrence, distribution and impact of alkylphenol ethoxylates (APEOs) and their metabolites in natural systems. Table 4 summarizes the concentrations reported in the last 10 years [28]. Generally, the highest concentrations of alkylphenolic compounds were found in industrial areas, so the elevated concentrations are likely to be attributed to discharge of industrial wastewaters. However, alkylphenolic compounds were also detected in areas without significant industrial activity, but with an intensive agricultural practice, where one of the possible sources of alkylpenolic compounds is the use of sewage sludge as fertilizer [29]. Concentrations of nonylphenol (NP) in surface waters receiving STP effluents ranged from low ng/L up to several tens of µg/L, showing a clear increase in concentrations at sites downstream of STP. Levels detected in some rivers exceed, or are close to the NOEC (no-observed effect concentration) for the induction of vitellogenesis in caged trout (5–20 µg/L) and suggest that long-term exposure may exert an estrogenic effect on fish populations [30].

Nonylphenol and short chain nonylphenol ethoxylates (NPEOs) are lipophilic compounds with a log K_{ow} of 4.48 (NP) and around 4.2 (NPE_1O, NPE_2O and NPE_3O) [31] so they partition preferentially to the organic fraction of sediments and show considerable potential to bioaccumulate in aquatic organisms. Therefore, in the majority of sediments and biological samples analyzed NP was a predominant alkylphenolic compound. In rivers and lakes, concentrations found span two to three orders of magnitude, mainly depending on the vicinity of local industrial and urban sources. In river sediments levels of alkylphenolic compounds found upstream of point sources of pollution were generally lower than 200 ng/g. Significantly higher concentrations (up to hundred µg/g) were observed at locations situated near STPs, or downstream of discharges of industrial wastewaters.

As a result of restrictions on industrial cleaning applications of APEOs the general trend of decline in APEO concentrations was observed, especially in the Scandinavian countries, Denmark, Netherlands, Switzerland, Germany and UK. For example, the down core sediment profiles of NP concentrations in Swiss rivers and lakes indicate that this toxic and estrogenic surfactant metabolite occurred at

Table 4 Reported concentrations of alkylphenol ethoxylates and their metabolites in environmental and wastewater samples

Matrix/location	Compound	Concentration (water) µg/L	Concentration (sediment/ soil/sludge) mg/kg	Reference
Freshwater aquatic systems				
Spain (Catalonia), water and sediment	NPEO, NPEC, NP	<0.1–31 (NPEO), <0.1–15 (NP), <0.1–35 (NPEC)	0.010–0.820 (NPEC), 0.022–0.645 (NP)	[29]
Germany (Baden-Württenberg)	NP, OP	<0.01–0.49 (NP), <0.01–0.19 (OP)	<0.05–0.26 (NP)	[85]
USA and Canada (Great Lakes)	NP, OP	<0.01–0.92 (NP), <0.005–0.084 (OP), <0.02–7.8 (NPE_1O), <0.02–10 (NPE_2O)	0.17–72 (NP), <0.01–1.08 (OP), <0.015–38 (NPE_1O), <0.015–6.0 (NPE_1O)	[86, 87]
USA streams (30 rivers)	NP, NPEO	<0.11–0.64 (NP), <0.06–0.60 (NPE_1O), <0.07–1.2 (NPE_2O), <1.6–14.9 ($NPE_{3-17}O$)	<0.003–2.96 (NP), <0.003–0.17 (NPE_1O)	[88]
Japan	NPEO, NP, OP	0.04–0.42 (NPE_1O), 0.04–0.52 (NPE_2O), <0.02–0.3 (NP), <0.02–0.09 (OP)	–	[89]
Soil				
Sludge-amended soil	NPEO, NP	–	0.11–1.1 (NPE_1O), 0.095–0.012 (NPE_2O)	[90, 91]
Sludge-amended soil	NPEO, NP, OP	–	0.07–1.21 (NPE_1O), 0.08–0.39 (NPE_2O), 2.35–4.61 (NP), 0.05–0.18 (OP)	[92]
Marine and estuarine environment				
Spanish coastal area	NPEO, NPEC, NP	<0.2–11 (NPEO), <0.1 (NPEC), <0.15–4.1 (NP)	0.01–0.62 (NPEO), <0.01–1.05 (NP)	[93]
Venice lagoon (Italy) – Estuarine water	NPEO, NPEC	1.1–38.5 (NPEO), 0.6–102 (NPEC)	–	
Krka estuary (Croatia)	NPEO, NP	<0.02–0.44 (NP_1EO), <0.02–1.3 (NP_2EO), 0.1–0.7 (NP3–18EO), <0.02–1.2 (NP)	–	[94, 95]
English estuaries	NPEO, NP, OP	<0.6–76 (NP1+2EO), <0.08–5.8 (NP), <0.1–13 (OP)	0.16–3.97 (NP1EO), 0.03–9.05 (NP), 0.002–0.34 (OP)	[96, 97, 98]
Netherlands (Scheldt Estuary)	NPEO, NP, NPEC	0.04–2.7 (NPEO), 0.04–2.0 (NP), 0.09–12 (NPEC)	0.04–0.25 (NPEO)	[99]
USA (Jamaica Bay)	NPEO, NP, OPEO, OP, halogenated NP	0.16–0.94 (NP1–3EO), 0.077–0.42 (NP)	0.05–30 (NPEO), <0.001–0.027 (BrNP)	[100, 101]
Israel coast (sea water)	NPEO	<1.0–25	–	[102]

Abbreviations: NPEO – nonylphenol ethoxylates, NP – nonylphenol, NPE – nonylphenol carboxylates, OPEO – octylphenol ethoxylates, OP – octylphenol, BrNP – brominated nonylphenol

much higher concentrations in the early 1980s (maximum concentration 1.4 mg/kg) than at present (<0.1 mg/kg) [32].

Hormones and contraceptives

There is little information in the literature on the fate and persistence of synthetic ovulation-inhibiting hormones in the aquatic environment. Natural and synthetic estrogens and progestogens entering wastewater treatment plants from urban and industrial discharges are subject to a variety of treatment processes of varying efficiency and in some cases they are finally released into surface waters [33, 34, 35, 36].

Moreover, it is been reported that the less active conjugated forms (glucuronides, sulfates), in which estrogens are excreted, can be deconjugated during wastewater treatment and in the environment to generate the more potent parent compound [9, 37, 38]. In the very few studies that have attempted the determination of conjugated estro-

Table 5 Reported concentrations of pharmaceuticals and sex hormones in environmental samples

Matrix (Location)	Compounds	Concentration (ng/L or ng/g)	Reference
Drinking water			
(Germany)	Phenazone drugs	<5–900	[103]
(Germany)	Natural and synthetic estrogens	<0.1–2.1	[104]
(Germany)	Clofibric acid	<5–170	[105]
(U.K.)	Synthetic estrogens/progestogens	<1–10	[106]
Groundwater			
(Germany)	60 pharmaceuticals	1.8–1100	[107]
(Germany)	18 antibiotics	<20–470	[108]
(Germany)	13 pharmaceuticals	n.d.–7300	[105]
River water			
(Germany)	18 antibiotics	<20–6000	[108]
(Germany)	32 drugs	<10–4100	[109]
(Germany)	Natural and synthetic estrogens	<0.1–5.1	[105]
(U.K.)	Natural and synthetic estrogens	<0.2–17	[110]
(U.K)	Synthetic estrogens/progestogens	2–17	[106]
(Spain)	Estrogens and progestogens	0.2–71.1	[29]
(USA)	Antibiotics	14–100	[47]
(USA)	Prescrition drugs	7–260	[47]
(USA)	Nonprescrition drugs	9–80	[47]
(USA)	Steroids and hormones	5–2000	[47]
(Canada)	Steroids	2–67	[111]
Marine/estuarine water			
(North Sea)	Neutral/acidic pharmaceuticals	<0.002–18.6	[112]
Solid samples			
River sediment (Germany)	Natural and synthetic estrogens	<0.2–1.5	[36]
River sediment (Spain)	Estrogens and progestogens	0.05–22.8	[45]
Marine sediment (Washington, USA)	Antibacterial drugs	<0.2–1.7 µg/g	[113]
Fertilized soil (Germany)	Antibiotics (TCs and tylosin)	0.1–4 µg/g	[114]
Activated and digested sludge (Germany)	Natural and synthetic estrogens	<2–49	[36]
Activated sludge (Israel)	Estrogen	19–64	[33]
Biota			
Rainbow trout bile (Sweden)	Natural and synthetic estrogens	<0.1–2.5 µg/g	[46]
Red rock crab meat (Washington, USA)	Antibacterial drugs	<0.1–3.8 µg/g	[113]
Mussel (Canada)	Coprostanol	32252	[111]

gens in STP effluents and surface waters, concentrations below the limit of determination have been most often found [38, 39].

The presence of both natural and synthetic estrogens and progestogens in the various types of water has, in most instances, been reported to occur at the low ng/L range up to the tens of ng/L range (see Table 5), and only on a few occasions have concentrations been higher, reaching µg/L levels [33, 40].

Of the various estrogens most frequently monitored in environment programs, the natural hormone estradiol and the synthetic estrogen ethynyl estradiol are the most relevant because of their high estrogenic potency. However, these two compounds are often the least frequently detected in environmental waters, and estriol and estrone, the main metabolites of estradiol, the most ubiquitous [29, 38]

Downstream of STPs, the steroid concentrations in the river are normally considerably lower than those of the corresponding effluent, decreasing with distance from the STP and, very often, the compounds present in the effluent are not further detected in the river [39, 41].

On the other hand, given the relatively low polarity of these compounds, which present octanol-water partition coefficients mostly between 10^3 and 10^6 [42], sorption to bed-sediments appears as a quite likely process. Under the anaerobic, dark conditions normally present in the sub-surface layers of river sediments, these compounds are expected to undergo low photodecomposition and biodegradation. River sediments can therefore act as sinks where estrogens and progestogens may persist for long periods of time, be transported to other areas, and be eventually released back to the water column [43].

According to Jurgens et al [44], between 13% and 92% of the estrogens entering a river system would end up in the bed-sediment compartment with the majority of sorption occurring within the first 24 h of contact. Furthermore, the synthetic estrogens (mestranol, ethynyl estra-

diol) have been shown to partition to the sediment to a greater extent than the natural estrogens [43].

To our knowledge, the presence of estrogens and progestogens in marine sediments has never investigated, and there are just a few works reporting their occurrence in river sediments. According to these works, estrogens and progestogens are present in river sediments at the pg/g or ng/g level and tend to accumulate in sediments [36, 45].

On the other hand, in a study conducted by Larsson et al [46], it was observed that the bile of fish caged downstream of STPs contained estrogenic substances at concentrations 10^4–10^6 times higher than water levels, which indicates that exposure to environmental estrogens results in bio-accumulation of prominent amounts of these substances.

Human and veterinary drugs, and personal care products

The study of pharmaceuticals and their bioactive metabolites in the environment has become an issue of much interest worldwide in the last few years, as the high volume of papers dealing with it shows. The increasing observance of bacterial resistance caused by the extensive use of antibiotics in animal and fish farming and the extended practice of addition of manure and sewage sludge to agricultural fields is of particular concern.

Most of the studies carried out to assess the environmental occurrence of drugs have focused on aquatic media, especially in connection to drinking water. Soils, sludge and sediments have been scarcely investigated compared to water media. Table 5 summarizes the concentrations of pharmaceuticals reported in the last years. The most comprehensive reconnaissance of the occurrence of pharmaceuticals, hormones, and other organic wastewater contaminants so far was conducted in the USA from 1999 to 2000 by the U.S. Geological Survey [47]. Concentrations of 95 organic water contaminants, including many of emerging environmental concern, were measured in samples from 139 streams in 30 states, chosen according to their susceptibility to contamination (in other words they were taken downstream of intense urbanization and livestock production). Compounds from a wide range of industrial, residential and agricultural origins, such as veterinary and human antibiotics, prescription drugs, nonprescription drugs, and other wastewater related compounds, were detected in this study with a median of seven, but with as many as 38 compounds found in a given water sample.

The amounts of pharmaceuticals and their bioactive metabolites being introduced into the environment are likely to be low. However, their continuous environmental input may lead to a high long-term concentration and promote continual but unnoticed adverse effects on aquatic and terrestrial organisms.

From all potential sources of environmental exposure to drugs – manufacturing processes, the disposal of unused or expired products, and excreta – animal excreta is the most important. Most of the drugs used in veterinary medicine end up in manure. When this manure is dispersed on agricultural fields, the non-metabolized drugs present in the manure (or their biologically active metabolites) may threaten the ground water (depending on their mobility in the soil system) and affect terrestrial and aquatic organisms due to leaching from fields.

The same situation is found when the sludge originated in sewage treatment plants – which may contain drugs that are non-biodegradable by the activated sludge, such as sulphonamides [48, 49] – is used to fertilise soils. Various authors have underlined the incomplete removal of several drugs in wastewater treatment plants, which represents a clear risk for humans [40]. As a result, diverse pharmaceutical compounds are currently being considered as possible candidates to be monitored in the near future as drinking water contaminants.

Other disposal options for the sewage sludge are landfilling, incineration, and dumping at sea. Among them, the most popular for solid waste disposal today is landfilling. However, many of the disposal sites are open dumps without protective barriers or leachate collection systems, which involve a risk for the quality of the groundwaters nearby. The investigation of the presence of pharmaceutical compounds in landfill leachates and groundwater at different positions with respect to the disposal site has been the focus of some recent works. In a study conducted by Ahel and Jelicic [50] on the occurrence of phenazone analgesics in leachate samples, concentrations of prophyphenazone in the range of 3.7–60 µg/L and much lower concentrations of aminopyrine and antipiryne were reported. The occurrence and distribution of several pharmaceutical compounds in the groundwater downgradient of a landfill in Denmark has also been investigated [16]. In this work, a rapid decrease in concentration of the identified compounds with distance from the landfill was reported by Holm et al, who attributed the observed large attenuation of pharmaceuticals to the strongly anaerobic conditions existing in certain parts of the leachate plume.

The persistence of a drug in sediment or soil depends mostly on its photo stability, its binding and adsorption capability, its degradation rate, and the leaching in the water. Also, the rate of sedimentation highly conditions the half-life of the chemical [18]. Strongly sorbing pharmaceuticals tend to accumulate in the soil or sediment. On the other hand, highly mobile pharmaceuticals tend to leach to the groundwater and be transported with the groundwater, drainage water, and surface run-off to surface waters.

Pharmaceuticals, especially those presenting the greatest consumption and persistence, have been found in environmental matrices at considerably higher concentrations (in the ng or µg per liter or per gram range), than those reported for estrogens and progestogens (in the pg or ng per liter or per gram range). Their occurrence in the various aquatic compartments has been investigated in different countries, including Italy, Spain, Sweden, Switzerland, Netherlands, UK, Israel, USA, and Canada, but, the largest amount of data has been produced by Germany.

Several reviews reported levels of various personal care products in the aquatic environment. Daughton and Ternes [51] prepared a comprehensive review on occurrence of over 50 individual pharmaceutical and personal care products (PPCPs), or metabolites from more than 10 broad classes of therapeutic agents, or personal care products in environmental samples, mainly in sewage, surface, and ground waters and much less frequently in drinking waters.

Rimkus reviewed data on the occurrence of polycyclic musk [52] and musk xylene and musk ketone amino metabolites [53] in water, sediment and suspended particulate matter, sewage sludge and biota. The highest concentrations of polycyclic musks (HHCB and AHTN) were found in water (max. concentration 6 μg/L of HHCB and 4.4 µg/L of AHTN) and sludge (max. concentrations 63 and 34 mg/kg for HHCB and AHTN, respectively) from sewage plants. In other samples from different aquatic ecosystems these chemicals were also detected at varying concentrations (ranging from low ng/L to μg/L level) dependent on the distance to STP.

Occurrence of iodine containing diagnostic agents in aqueous matrices was reported by Hirsch et al [54]. Levels from 10 to 70 ng/L were reported for tap water, 40 to 400 ng/L for surface water and from 0.09 to 3.070 µg/L for STP effluents. Iodinated contrast media exhibit high polarity and are very persistent against metabolism by the organism and environmental degradation. Degradation studies [55] revealed their poor elimination during wastewater treatment. For example, diatrizoate was released almost quantitatively in non-metabolized form, while approximately 85% of iopromide was transformed into two highly hydrophilic metabolites.

Brominated flame retardants

PBDEs were first discovered in the environment in 1981, when they were found in pike from western Sweden. Subsequent reports, based on analyses of sediments, document the ubiquitous distribution of PBDEs in the environment. Considerable data is available on the levels and composition of PBDEs in the environment and biota. On the basis of the available data, the environmental behaviour and fate could be summarized as follows. Because of their high lipophilicity (log K_{OW}>6) and resistance to degradative processes, PBDEs are expected to bio-accumulate easily. The higher brominated compounds are less mobile in the environment, possibly due to their low volatility, low water solubility and strong adsorption to sediments. Therefore, the higher brominated compounds tend to end up in sediments at high residue levels near their emission sources but not so in marine organisms. On the other hand, the lower brominated compounds are predicted to be more volatile, water soluble, and bio-accumulative than the higher brominated compounds. The environmental behavior and fate of lower brominated compounds are therefore thought to be similar to chlorinated pollutants. Although deca-BDE accounts for most PBDE consumption, it is the lower congeners, tetra-BDE and penta-BDE, and in particular BDE-47 and BDE-99, that are most commonly found in the environment. To explain this discrepancy, some scientists theorize that higher congeners break down in the environment.

Considerable data exists on the occurrence of PBDEs in fish and mammals, although PBDE levels in biota varied widely depending on the species and the collection sites (Table 6). Until recently, PBDE concentrations detected in wildlife have been below concentrations of organochlorine compounds, such as PCBs. However, for certain species, contamination levels are in the same range as for the PCB congeners. BDE-47 is dominant in biota, followed by BDE-99, BDE-100, BDE-153 and BDE-154. On the other hand, BDE-209 is rarely found in biota. There may be several reasons for the absence of BDE-209 in wildlife samples. It may disappear (and possibly degrade to lower brominated congeners) because of chemical, or microbial degradation, or it may be taken up at reduced rates because it is difficult for their relatively large molecule to cross cell membranes. Levels of BDE-47, BDE-99 and BDE-100 are low in mammals and birds at low trophic levels in the terrestrial ecosystem. Higher concentrations of these PBDE congeners have been found in biota samples (fish, birds, mammals) in aquatic and marine ecosystems. In a Swedish study on a wide variety of animal species (various fish, rabbit, starling, moose, birds, bird eggs) the levels of PBDEs in terrestrial species were low compared with the levels in aquatic species [56]. A spatial trend is apparent with highest levels in biota from the Netherlands coast followed by the Baltic Sea, with lower levels in the North Sea and lowest levels in northern Sweden and the Arctic. The spatial trend is very similar to that found for PCB and DDT. A similar spatial trend seems to be indicated along the Pacific coast of North America compared to the Canadian Arctic in marine mammals [57].

The levels of BDE-47, BDE-99 and BDE-100 in fish were high compared with levels in sediment (4.6 to 36-fold) indicating the bioavailability of the congeners. Moreover, concentrations have been found to be much lower in edible muscle tissue of fish than in fish liver, indicating that they were transported to and stored in the most lipid-rich tissues. The PBDE levels have been found to increase with age of the fish, indicating bioaccumulation. Different studies also showed a clear evidence of bio-magnification of PBDEs [58]. Burreau et al [59] calculated the bio-magnification potential for several PBDE congeners and found that these were all positive, meaning that all the studied congeners bio-magnify. However, there were differences, with tetra- and penta-BDEs biomagnifying to a similar degree, the tri-BDEs biomagnifying somewhat less and the hexa-BDEs biomagnifying considerably less.

Several studies show an increasing trend in the concentrations of PBDEs in the environment since 1970s. Some differences are seen between Europe and North America. In North America, temporal trends in lake trout from Lake Ontario, ringed seal, and beluga from the Canadian Arctic all indicate steady and continuing increases in PBDE concentrations, with no indications of leveling off. The

Table 6 Reported concentrations of PBDEs in environmental samples

Matrix (Location)	Compounds	Concentration	Reference
Sediment			
River sediments (Japan)	Tetra- + Penta-BDEs	21–59 ng/g	[115]
Sediments downstream of plastic industry (Sweden)	BDE-47	490 ng/g	[116]
	BDE-99	750 ng/g	
	BDE-100	170 ng/g	
Sediments from a river with textile industries (Sweden)	BDE-47+99+100	nd–9.6 ng/g	[117]
	BDE-209	nd–360 ng/g	
Sediment (Baltic Sea)	Sum PBDE	nd–1.1 ng/g	[118]
River mouth sediments (Europe)	BDE-47	<0.17–6.2 ng/g	[119]
	BDE-99	<0.19–7.0 ng/g	
Sewage Sludge			
Digested sludge (Gothenburg, Sweden)	Sum PBDE	20–30 ng/g	[120]
Sewage sludge (Germany)	Sum PBDE	0.4–15 ng/g	[121]
Digested sludge (Stockholm, Sweden)	BDE-47	39–91 ng/g	[122]
	BDE-99	48–120 ng/g	
	BDE-100	11–28 ng/g	
	BDE-209	140–350 ng/g	
Biota			
Fish from background areas (Sweden)	Sum PBDE	26–1200 ng/g fat	[56, 123]
Fish (Sweden)	BDE-47+99+100	19–4600 ng/g fat	[124]
Osprey found dead (Sweden)	Sum PBDE	2100 ng/g fat	[119, 123]
Marine fish and shellfish (Japan)	Tetra- + Penta-BDEs	0.1–17 ng/g fat	[125]
Freshwater fish (North-Rhine Westphalia)	Sum PBDE	18–983 ng/g fat	[126]
Salmon (Baltic sea)	BDE-47	167–190 ng/g fat	[58]
	BDE-99	52 ng/g fat	
Cow's milk (Germany)	Sum PBDE	2.5–4.5 ng/g fat	[126]
Reindeer and moose (Sweden)	Sum PBDE	0.47–1.7 ng/g fat	[127]
Chickens (US)	Sum PBDE	3.6–35.1 ng/g fat	[128]

trends in Baltic guillemot indicate that levels of BDE-47, BDE-99 and BDE-100 have begun to decline in the Baltic Sea since voluntary withdrawal of use in a number of countries [60]. The trends in pike from Lake Bolmen, Sweden, indicate a leveling off of PBDE levels in the 1990s. The continuously increasing trends in North America may be more reflective of the fact that PBDE technical products are still being used to a larger extent than in Europe.

On the other hand to the organochlorine compounds, the concentrations of PBDEs have increased during the period 1972–1997, indicating a doubling of the levels every 5 years [61]. Since this report on drastic increase of PBDE concentrations in human milk from Sweden, monitoring for temporal trends of PBDE were carried out in other countries, such as Canada [62] and United States [63]. These studies confirmed that human exposure to PBDEs is increasing. The data showed that PBDEs are global contaminants that have a tendency to increase in concentration in the environment and human samples in some parts of the world, in particular in North America. The accumulation and ongoing increase in the levels of PBDEs calls for immediate measures to stop the environmental pollution and human exposure to PBDEs.

Plasticizers

In all reported studies di(ethylhexyl)phthalate (DEHP) was found to be predominant phthalate ester (PAE), due to its high production (nearly 90% of European plasticizer use) and its physico-chemical properties (low solubility and relatively high K_{ow}). It was estimated that 15–17% of the low molecular weight phthalates (dimethyl phthalate-DMP to benzylbutyl phthalate-BBP) was particulate bound while for DEHP and di(n-octyl)phthalate (DnOP) 53 to 74% of the total concentration was sorbed to suspended particulate matter.

The most systematic study on the occurrence of PAEs in the aquatic environment was conducted by Fromme et al [64]. The levels of DEHP and dibutyl phthalate (DBP) were reported for 116 surface water-samples, 35 sediments from rivers, lakes and channels, 39 sewage effluents and 38 sewage sludges collected in Germany. The phthalate burden was mainly from DEHP, whilst DBP was found in minor concentrations and BBP at concentrations near the detection limit. The concentrations found ranged from 0.3–98 µg/L (surface water), 1.7–182 µg/L (sewage effluent), 28–154 mg/kg dw (sewage sludge) and 0.2–8.4 mg/kg (sediment). The highest concentrations found were closely related to the input of industrial wastewaters from plastic production and were limited to a few kilometers downstream of the source of contamination.

In the same study high concentration of bisphenol A (BPA) was confirmed in waste dump water and compost water samples as well as in the liquid manure samples (from 61 to 1112 µg/L). In surface waters concentrations ranged from 0.5 to 410 ng/L, in sewage effluents 18–702 ng/L, up to 0.19 mg/kg in sediments and up to 1.4 mg/kg in sewage sludge.

Estrogenicity of environmental samples

Correlation of calculated estrogenicity (EEQ based on chemical analysis of target EDCs) and measured estrogenicity (based on bioassays) is often bad, since the calculated EEQs include the contribution of only a limited number of EDCs (target compounds) and do not include interaction and antagonism between compounds. Generally, the picture emerging from bioassays is somewhat different from the one coming from the chemical analysis of the same samples. The most prominent compounds found in most samples were NP and its derivatives, whereas very potent steroid hormones were found in low concentrations, often just above the LOD of the analytical method used, or were not chemically detected. Therefore, a measured level is relatively uncertain and because of the high EEF values of these compounds the calculated EEQ results in high uncertainty. Additionally, chemical analysis generally includes only several target compounds, underestimating the presence of other known and unknown EDCs.

The percentage of estrogenic potency explained by chemical analysis is generally low. Analysing steroid sex hormones in STP influents Murk et al [65] explained 70% of the ER-CALUX activity. However, in the effluent samples the explained fraction decreased to 20%, which coincides with a lower contribution of hormones and possible formation of bioactive metabolites.

The natural steroid 17β-estradiol, originating from domestically derived STP effluents, was the major component (84–90%) causing activity in the YES assay in the surface waters of UK estuaries, while androsterone, NP, DEHP and other unknown agents contributed to a lesser extent [10]. In the study of Desbrow et al [9] the most active fraction (>80% total activity in domestic effluent) was found to contain low levels of natural and synthetic steroidal estrogens.

Garcia-Reyero et al [66] found that the YES is rather insensitive to NP, and possible inhibitory effects might easily mask some compounds with high detection limits. For example, in their study YES was apparently unable to detect NP in some STP effluents although its concentration surpassed the calculated LOD. They found that the key problem of the use of YES to test environmental samples is the presence of inhibitory compounds, whose nature is still elusive, suggesting that the use of an appropriate panel of different yeast systems (perhaps including the androgen and the glucocorticoid receptors, among others) will be useful in the characterization of both estrogenic and antiestrogenic compounds.

Körner et al [67] showed that the levels of estrogenic compounds chemically detected in STP influents and effluents accounted only for 0.7 to 4.3% of the total estogenic activity detected by E-screen (expressed as EEQ value). Among the detected target compounds (phenolic xenoestrogens) the contribution of NP was the largest, however natural and synthetic estrogens were not analyzed.

Hilscherova et al [68] determined that, based on mass balance calculations, certain PAHs and their metabolites were the most likely compounds contributing to the estrogenicity, while the contribution of some other compounds such as PCNs and PAH derivatives was very low. In their study, the contribution of alkylphenols to the calculated EEQ for riverine sediments was only 2%.

Conclusions

TIE is recommended as the most appropriate approach for the identification of estrogenic compounds in environmental samples. Bioassays, combined with toxicity-based fractionation, can account for interactions within complex mixtures that are not possible to consider in conventional chemical residue analysis. However, at present, many different assays are being used and one of the problems to be solved is the standardization and inter-comparison of the different effects measured. Additionally, due to different modes of action and end-points one single bioassay is not sufficient and a battery of tests, including both in vitro and in vivo assays that assess both receptor and non-receptor mediated mechanisms of action, seems to be the most appropriate way to assess the potential of EDCs in complex environmental samples.

Further improvements in analytical protocols will allow additional research, which is needed to evaluate environmental presence and impact of unknown EDCs. Due to the diversity of chemical compounds that are responsible for endocrine disruption, tailor-made specific analytical protocols are required for their determination. As EDCs are chemically extremely heterogeneous and range from very polar and very soluble to very hydrophobic, effect-based analysis still requires further optimization and validation of extraction procedures in order to reveal the identity of compounds causing the effects observed. Within modern analytical techniques, only GC and LC combined with MS and tandem MS, respectively, provide sufficient selectivity and inherent sensitivity in analyzing EDCs in complex samples. However, further improvements are needed to lower the LODs for some EDCs, particularly for ethynylestradiol and other compounds from the same group. Since these compounds are affecting aquatic organisms at levels as low as 1 ng/L, the chemical analysis of such analytes should be able to quantify these molecules at 0.1–1 ng/L, which is quite difficult at present.

In any case, the complex issue of EDCs in the environment needs further research, especially in order to gain more insight into the factors that determine their bioavailability and release. Combined exposure to mixtures of EDCs (even at concentration levels lower than the no-observed effect level) that may produce additive effects still has to be assessed, as well as the fate and behavior of numerous, still undiscovered EDCs.

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