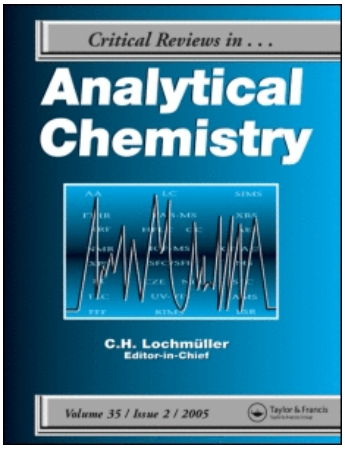
This article was downloaded by:
On: *17 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Determination of Estrogenic Endocrine Disruptors in Environmental Samples—A Review of Chromatographic Methods

Katarzyna Kozlowska-Tylingo[a]; Jacek Namieśnik[b]; Tadeusz Górecki[c]
[a] Faculty of Chemistry, Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Gdańsk, Poland [b] Faculty of Chemistry, Department of Analytical Chemistry, Gdańsk University of Technology, Gdańsk, Poland [c] Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada

Online publication date: 02 August 2010

To cite this Article Kozlowska-Tylingo, Katarzyna , Namieśnik, Jacek and Górecki, Tadeusz(2010) 'Determination of Estrogenic Endocrine Disruptors in Environmental Samples—A Review of Chromatographic Methods', Critical Reviews in Analytical Chemistry, 40: 3, 194 — 201
To link to this Article: DOI: 10.1080/10408347.2010.490488
URL: http://dx.doi.org/10.1080/10408347.2010.490488

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Critical Reviews in Analytical Chemistry, 40:194–201, 2010
Copyright © Taylor and Francis Group, LLC
ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408347.2010.490488

Determination of Estrogenic Endocrine Disruptors in Environmental Samples—A Review of Chromatographic Methods

Katarzyna Kozłowska-Tylingo,[1] Jacek Namieśnik,[2] and Tadeusz Górecki[3]

[1]*Faculty of Chemistry, Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Gdańsk, Poland*

[2]*Faculty of Chemistry, Department of Analytical Chemistry, Gdańsk University of Technology, Gdańsk, Poland*

[3]*Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada*

Natural and synthetic estrogens are characterized by the largest endocrine disrupting potential, as confirmed by both *in vitro* and *in vivo* studies. Estrogens have been detected in a large fraction of samples (50–95%) of purified wastewaters introduced to natural water bodies. Their presence in drinking water has also been reported. Thus, there is an urgent need to introduce or modify the legislation regulating the production of meat with the use of feed containing hormonal supplements, the use of compounds with proven endocrine disrupting activity in the industry, the circulation and purification of wastewaters, as well as monitoring of EDCs in the environment. The latter requires that proper methodologies are developed and validated. Determination of Endocrine Disrupting Compounds (EDCs) in biological samples (blood, urine) is often based on bioanalytical techniques (YES, ELISA, E-Screen). Speciation analysis, both qualitative and quantitative, usually employs chromatographic techniques at the stage of the final determination, especially for samples with aqueous matrices.

Keywords EDC's, estrogens, hormons, HPLC, drinking water, wastewater

Downloaded At: 11:55 17 January 2011

INTRODUCTION

Among the plethora of environmental pollutants, endocrine disrupting compounds (EDCs) garner increasing attention. United States Environmental Protection Agency (US EPA) defines an endocrine disruptor as an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes. EDCs are responsible for the feminization, infertility and hermaphroditism in aquatic organisms. In humans, particularly men, they might lead to enlargement of breasts, infertility and increased rates of testicular cancer (1,2).

Investigations into the fate and action of EDCs in the environment are advancing rapidly, with some of the mechanisms of their detrimental action well characterized and described. Among the EDCs, natural and synthetic estrogens have been recognized as having the greatest endocrine-disrupting potential. Emissions of estrogens into the environment originate mainly from pharmaceutical and veterinary industries (manufacturing of hormonal preparations including contraceptives), sewage (excretion of drugs and/or their metabolites by humans and animals), disposal of expired drugs from households (small scale) and hospitals (much larger scale), as well as wastewater treatment plants (inefficient purification methods). The large endocrine disrupting potential of estrogens has been confirmed by both *in vitro* and *in vivo* studies (3–10).

Metabolites of oral contraceptives, which enter surface waters with sewage, are the main source of estrogens in the environment. While the amounts of ethinylestradiol (E_2) and progesterone in modern contraceptives are very small, older generations of these drugs contained significantly higher amounts of the hormones. The residues of these older generation drugs are still present in the environment and exert endocrine disrupting effects. Literature provides abundant information on the levels of EDCs (including estrogens) in municipal sewage and animal farms wastewaters, as well as on their effect on

Address Correspondence to Katarzyna Kozłowska-Tylingo, Department of Pharmaceutical Technology and Biochemistry, Chemical Faculty, Gdańsk University of Technology, 11/12 G. Narutowicza Street, Gdańsk 80-952, Poland; E-mail: ka.ko@wp.pl

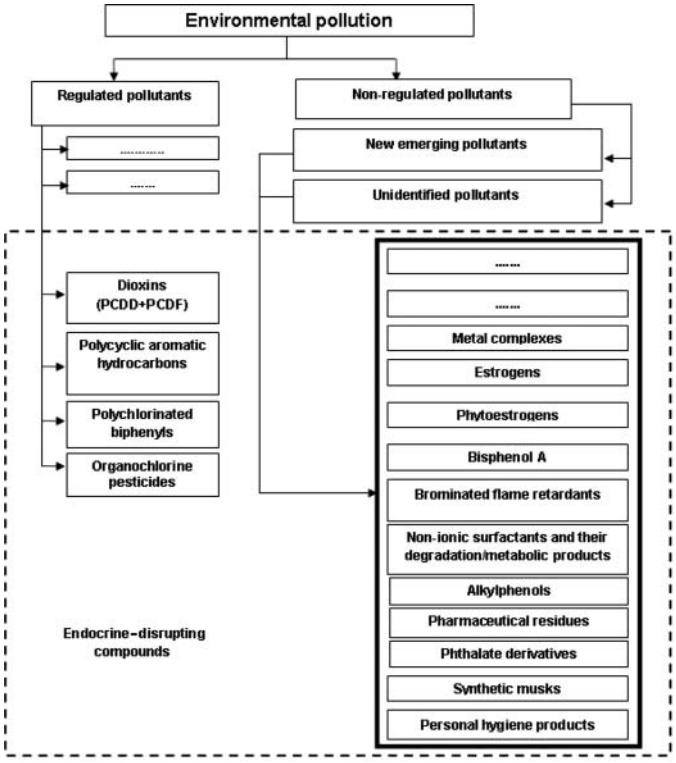


FIG. 1. Classification of environmental pollutants.

biocenosis and aquatic organisms (5–9). Estrogens have been detected in a large fraction of samples (50–95%) of purified wastewaters introduced to natural water bodies in countries including the United Kingdom, USA, Italy, Germany, Spain, The Netherlands, Sweden, Israel and Canada. Their presence in drinking water has also been reported. Many environmental pollutants act as endocrine disruptors, yet the maximum allowable levels in the environment or food have been established only for selected few of them (including dioxins, PAHs, PCBs and some pesticides) (3–5, 10–15). The remaining EDCs are not regulated in any way (see Fig. 1). Taking into account the potential dangers related to these compounds, determining the actual effects exerted by them on the environment and biota is urgently called for.

No standards or regulations regarding the treatment of sewage containing hormonal residues, their maximum allowable levels and/or determination methods have been established thus far in Poland. The process is the most advanced in the USA, with Europe generally lagging behind the US in this area. Poland is still at the early stages with regard to recognizing problems related to the increasing use of various drugs and the resulting increase in the amounts of drug residues in the environment (16–20). Before any standards can be introduced, though, methods for EDC determination in environmental samples should be established first. When developing analytical methods, the following characteristics of EDCs should be taken into account:

1. Their levels are generally very low (ng/L), and the matrix composition of the samples is typically very complex; hence, analyte isolation and preconcentration methods should aim at maximizing the analyte recovery.
2. EDC concentrations might fluctuate both in time and in space.
3. Standards (e.g., deuterated ones) and reference materials are not easily available.

METHODS FOR THE DETERMINATION OF ESTROGENS IN ENVIRONMENTAL SAMPLES (WATER, SEWAGE)

Selection of the proper methods is dictated by the type of analyte, its concentration, source and type of information produced by the method. Table 1 lists the limits of detection of various methods used in the analysis of EDCs. A significant number of those methods, especially ones used for biological samples, are based on bioanalytical techniques. Rather than providing the analyte concentration, such methods generally determine its endocrine disrupting potential. The determination is based on coupling of the analyte with specific reagents (e.g., antibodies,

TABLE 1
Limits of detection of various methods used for the determination of EDCs (mainly estrogens) (21–27).

Techniques	Acronym	Limits of detection (ng/L)
Bioanalytical	E-Screen	0.27
	ER-CALUX[a]	0.14
	YES[b]	0.3–30
	ELISA[c]	20–40
	LC-MS/MS[d]	0.08–33
	GC-MS[e]	0.2–2
	GC-MS/MS[f]	0.05–2.4
	SPME-HPLC[g]	0.064–1.2
	MISPE –HPLC/DAD[h]	0.00047
	HPLC/ESI –MS/MS[i]	0.2–1
Chromatographic	MEKC[j]	44–89

[a]ER-CALUX: estrogen responsive chemically activated luciferase expression
[b]YES: yeast estrogen screen
[c]ELISA: enzyme-linked immunosorbent assay
[d]LC-MS/MS: liquid chromatography-tandem mass spectrometry
[e]GC-MS: gas chromatography-mass spectrometry
[f]GC-MS/MS: gas chromatography-tandem mass spectrometry
[g]SPME-HPLC: solid-phase microextraction-high performance liquid chromatography
[h]MISPE-HPLC/DAD: molecularly imprinted solid-phase extraction - high performance liquid chromatography/diode array detector.
[i]HPLC/ESi-MS/MS: high-performance liquid chromatography with positive electrospray ionization and tandem mass spectrometry;
[j]MEKC: micellar electrokinetic chromatography

Downloaded At: 11:55 17 January 2011

TABLE 2
Comparison of the chromatographic methods used for the determination of natural and synthetic sex hormones (estrogens, androgens) in wastewater samples.

Analyte	Sample volume (L)	Analyte isolation/ preconcentration method	LOD (ng/L)	Recovery (%)	Concentration range found (ng/L)	Reference	No.
EE[d]				GC–MS			
	20	SPE[a] (C18)	0.2	Du[c]	<LOD[n] –76	Desbrow et al. (1998)	28
E1[e]		HPLC fract. LLE					
E2[f]	15	SPE (ENV+) LLE	du	du	1.1–5.8	Larsson et al. (1999)	8
E3[g]		GPC Hydrolysis					
17□–E2[h]	1	LLE	58	35–95	<LOD	Barber et al. (2000)	29
PROG[i]	20	SPE (EN/C18) Silica gel 60	du	du	1–13	Kuch et al. (2000)	30
MES[j]		Derivatization					
	2	SPE (C18)	0.5–1.0	du	1.7–220	Rodgers–Gray et al. (2000)	31
NOR[k]		HPLC fract.					
LEV[l]	7	SPE (C18 discs)	74	du	<LOD	Siegener et al. (2000)	32
DES[m]		SPE (ENVI/SDB–XC discs) SPE (C18/EN)	0.5	78–83 75	du	Mödler et al. (2007)	33
				GC–MS–MS			
	1	SPE (SDB–XC discs) Hydrolysis SPE (C18 lub NH_2) HPLC fract.	0.1–2.4	88–98	<LOD–9	Belfroid et al. (1999)	34
	1	SPE (C18 or EN) Silica gel Derivatization	1	41–90	<LOQ[o]	Ternes et al. (1999)	35
	1	SPE (SDB–XC discs) SPE (C18/ NH_2) HPLC fract.	0.1–1.8	88–98	<0.4–140	Johnson et al. (2000)	36
	2.5	SPE (C18 discs)	1	92–100	<1–55	Kelly (2000)	37
	2	SPE (C18 discs) Hydrolysis HPLC fract.	0.2–0.4	du	du	Huang et al. (2001)	23
	1–2	SPE (HLB) derivatization	1–3	79–108	du	Quintana et al. (2004)	38
				HPLC–DAD			
	0.2	SPE on line (PLRP–s)	10–200	96–111	du	Lopez de Alda et al. (2001)	39
	0.03	SBSE–LD[b]	300–1000	15–100	du	Almeida et al. (2006)	40
				HPLC-DAD–MS			
	0.5	SPE (C18)	2–500	57–112	du	Lopez de Alda et al. (2000)	41
	0.2–1	SPE (off line, on line)	0.2–5	44–112	<LOD– 22.8	Lopez de Alda et al. (2001)	42

(Continued on next page)

Downloaded At: 11:55 17 January 2011

TABLE 2
Comparison of the chromatographic methods used for the determination of natural and synthetic sex hormones (estrogens, androgens) in wastewater samples. *(Continued)*

Analyte	Sample volume (L)	Analyte isolation/ preconcentration method	LOD (ng/L)	Recovery (%)	Concentration range found (ng/L)	Reference	No.
	0.5	SPE (C18)	2–15	90–119	4–22	Rodrigez–Mozaz (2004) et al.	43
	0.7	SPE (C18)	0.9–4.3	du	1.3–196	Vulliet (2007) et al.	3
			HPLC–MS—MS				
	1	SPE (Envi–Carb)	0.5–1.0	87–94	du	Lagana et al. (2000)	44
	0.15–0.4	SPE (Carbograf–4)	du	86–91	0.3–188	Baronti et al. (2000)	45
	0.5–1	SPE (Carbograf–4)	0.2–0.5	88–97	<0.5–120	Johnson et al. (2000)	37
	1	LLE	1–2	88–95	1–10	Ingrand et al.	46
	1	SPE(C18 Speedisk)	0.65	85–120	19–26	Chen et al. (2007)	47
	2	SPE (C18) Derivatization	0.26–6.3	du	du	Lin et al. (2007)	48
			HPLC–FLD				
	5	SPE (SDB–XC discs)	4	72–78	<LOD-3.7	Snyder et al. (1999)	49
GLC							
	20–80	LLE Hydrolysis TLC	10	du	ng–μg/L	Tabak et al. (1981)	50

[a]SPE—solid-phase extraction
[b]SBSE-LD—stir bar sorptive extraction and liquid desorption
[c]du—data unavailable
[d]EE—17β–ethinylestradiol
[e]E1—estrone
[f]E2—estradiol
[g]E3—estriol
[h]17α—ethinylestradiol
[i]PROG—progesterone
[j]MES—mestranol
[k]NOR—norethindrone
[l]LEV—levonogestrel
[m]DES—diethylstilbestrol
[n]LOD—limit of detection
[o]LOQ—limit of quantitation

Downloaded At: 11:55 17 January 2011

luciferase). The product yields new properties (e.g., luminescence or fluorescence), which can be used for the determination of its estrogenic activity with proper detection methods. Techniques based on biologically active compounds are analyte specific, or selective at the least, which makes detection of the analyte's derivatives (e.g., its conjugates or metabolites) practically impossible. They are mainly geared towards the analysis of biological samples (blood, urine, tissue). To identify the individual estrogenic compounds in the environmental samples, biological assay results must be combined with the analysis by gas or liquid chromatography coupled with mass spectrometry. Such a combination, however, is both impractical and costly; consequently, EDC identification and quantitation are typically carried out by chromatography alone (especially in aqueous matrices).

Chromatographic Techniques

A number of methods for the qualitative and quantitative determination of estrogenic compounds in surface waters, groundwater, wastewater and drinking water have been published in the recent years. Each of them addresses the basic steps of the analytical procedure, including sample collection, preservation and storage, filtration, analyte isolation and pre-concentration (e.g., by SPE) and/or derivatization (mainly when GC-MS is used), followed by purification of the extract and final determination. Table 2 summarizes the information on the analytical methods used for the determination of estrogens and androgens in drinking water and waste water using chromatography for the final determination of the analytes. The individual stages of the analytical procedure are characterized below.

Downloaded At: 11:55 17 January 2011

Sample Collection, Preservation and Storage

Wastewater samples are typically collected into amber flasks pre-cleaned by solvent rinse and drying. Analyte stability tests in the absence of chemical preservatives demonstrated that estrogens decompose within 24 to 48 hrs. Consequently, such samples have to be stored at low temperatures (4 to −20 °C) prior to the analysis. Further improvement of storage stability can be accomplished by the use of chemical preservatives, including formaldehyde, methanol and mercuric chloride. These compounds reduce microbial activity in the sample. Sample integrity can also be preserved by sorption of the analytes onto solid sorbents used for analyte isolation and pre-concentration (e.g., C18-modified silica gel or graphitic sorbents). Sample volume is determined by the sensitivity of the method used for the final determination. The typical volume is ∼2 L when the analysis is performed by HPLC with diode array or fluorescent detection; it can be lower by a factor of ten when MS/MS is used.

Filtration

Filtration is necessary when wastewater samples are analyzed, as such samples typically contain significant amounts of suspended solids that tend to plug the pores of the sorbents used for analyte isolation and pre-concentration (typically silica gel). This, in turn, might lead to analyte breakthrough. Filtration is usually not required when liquid-liquid extraction is used for analyte isolation from the aqueous matrix (used mainly with gas chromatography).

Analyte Isolation and Concentration

Analytes present in complex matrices, including wastewater, need to be isolated and/or concentrated prior to qualitative and quantitative analysis. Solid phase extraction (SPE) is the most popular and reliable extraction method when HPLC is used for the final determination of the analytes. Typical sorbents used in the analysis of EDCs include C18-modified silica gel (packed in columns or trapped in extraction disks) and graphitic carbon (usually packed in columns) (39, 42, 50) . Extraction disks provide a larger contact area between the sorbent and the matrix, which reduces plugging and speeds up the extraction process. In addition, the risk of sample contamination with the components of the extraction disk itself (e.g., plasticizers) is reduced because of the short extraction time. On the other hand, SPE columns usually have larger breakthrough volumes and the extraction process is easier to automate.

Derivatization

Derivatization is carried out to improve the stability of the analytes, as well as the precision and sensitivity of GC analysis. Derivatization is typically performed for compounds which are thermolabile, polar, and/or characterized by low volatility, including estrogens. This additional sample preparation step might lead to analyte losses. Derivatization is rarely used with HPLC-MS and HPLC-MS/MS to improve the sensitivity and/or selectivity of the analysis. Table 3 lists common derivatization agents used in the analysis of estrogens.

TABLE 3
Derivatization agents used for estrogen analysis (48,52–57).

Derivatization agent	Final determination
Pentafluorobenzyl bromide (PFBBr)	GC-(NCI)-MS
Pentafluorobenzyl-trimethylsilyl	GC-(NCI-CI)-MS
N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)	GC-MS
p-Nitrobenzoyl chloride	HPLC-FLD
Dansyl chloride	HPLC-(ESI)-MS/MS

Extract Purification and Removal of Excess Solvent

Wastewater extracts usually require purification by liquid chromatography before the final determination. This is usually performed with the help of solid adsorbents, e.g., using columns packed with C18- or NH_2-modified silica gel. Graphitic sorbents can also be used for this purpose with good results. The process itself is akin to SPE, except that the sample extract is passed through the sorbent rather than the aqueous sample itself.

Removal of excess solvent is the last stage of sample preparation prior to chromatographic analysis. Its goal is to concentrate the analyte and thus improve the sensitivity of the method. The choice of the method for excess solvent removal (e.g., using a rotary evaporator or performing the evaporation under the stream of inert gas) is determined by the volume of the extract. The conditions (e.g., temperature) have to be carefully controlled and optimized, as solvent removal might lead to significant analyte losses.

Final Determination

Determination of steroid sex hormones in environmental samples is usually carried out by GC. However, the use of HPLC for this purpose has been increasing in the recent years. In contrast to GC-MS techniques, HPLC-MS is not limited to volatile compounds of low molecular weight. This allows the determination of the various forms of occurrence of the estrogens (e.g., conjugates) without the need for prior derivatization, as is the case with GC-MS separations. HPLC separations of the estrogens are usually carried out using columns packed with C^{18}-modified silica gel, and the typical column dimensions are 250 mm × 4.6 mm. Acetonitrile-water mixtures are usually used as the mobile phase. Gradient elution has to be applied due to the different polarities of the analytes. The gradient typically starts at 20–50% of the organic phase, and ends at 100%. Detection sensitivity can be improved by post-column modification of the mobile phase. Triethylamine is often used as a modifier enhancing the deprotonation of acidic estrogen derivatives

TABLE 4
Comparison of different analytical methods used for the determination of sex hormones.

	Techniques		
	Bioanalytical	Chromatographic	
Requirements	*E-Screen, ER-Calux, YES, ELISA*	*Gas*	*Liquid*
Application/outcome	Determination of the estrogenic potential/activity	Qualitative and quantitative analysis	Qualitative and quantitative analysis
Sample type	Mainly biological materials (blood, urine, sperm)	Water, wastewater	Water, wastewater
Sample volume (mL)	1-20	200-20000	150-5000
Sample preparation	Specific antigens	SPE, LLE, HPLC fractionation; derivatization required	SPE
Detection method	Colorimetry fluorescence, luminescence	MS, MS-MS	DAD FLD MS-MS
Limits of detection (ng/L)	From 0.14	From 0.05	From 0.06
Ability to determine analyte derivatives (metabolites, conjugates)	No	No	Yes
Suitability for routine application in wastewater analysis	No	Yes	Yes

Downloaded At: 11:55 17 January 2011

and increasing the response of mass spectrometers when using electrospray ionization in negative ion mode. Mass spectrometry provides improved qualitative and quantitative information (lower limits of detection) compared to other detection techniques, but it requires a significant up-front investment. There are some reports in the literature on the use of diode array (DAD) or fluorescent detection (FLD) for the determination of estrogens. This is possible because both natural and synthetic estrogens contain chromophores absorbing light at a wavelength range of ∼197–200 nm. However, to the best of our knowledge, no studies have been published on the comparison of the various detection methods (DAD, FLD and MS) in the determination of sex hormones.

Table 4 presents a comparison of the different (bio)analytical methods used for the determination of sex hormones. In general, chromatographic techniques are the most suited for the determination of these compounds in environmental samples (water, wastewater). Even though GC is more popular than HPLC as the final determination method, the latter technique offers more flexibility. For example, detection methods other than MS can be used (DAD, FLD), and sample preparation is much simpler as analyte derivatization prior to the analysis is not necessary. In addition, HPLC allows the determination of estrogen derivatives, which is not possible with GC or the bioanalytical techniques.

ACKNOWLEDGMENTS

This work has been financially supported in the formwork of grant attributed by Polish Ministry of Science and High Education.

REFERENCES

1. A. M. Soto, J. M. Calabro, N. Prechtl, A. Yau, E. Orlando, A. Daxenberger, A. L. Kolok, J. Guillette, B. L. Bizec, I. Lange, and C. Sonnenschein, Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in Eastern Nebraska. *Environ. Health Perspec* 112 (2004):346–352.
2. J. Brian, C. Harris, M. Scholze, T. Backhaus, P. Booy, M. Lamoree, G. Pojana, N. Jonkers, T. Runnalls, A. Bonfa, A. Marcomini, and J. Sumpter, Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. *Environ. Health Perspec.* 113 (2005):721–728.
3. M. P. Fernandez, M. G. Ikonomou, and I. Buchanan, An assessment of estrogenic organic contaminants in Canadian wastewaters. *Sci. Total Environ.* 373 (2007):250–269.
4. E. Vulliet, J. B Baugros, M. M. Flament-Waton, and M. F. Grenier-Loustalot, Analytical methods for the determination of selected steroid sex hormones and corticosteriods in wastewater. *Anal. Bioanal. Chem* 387 (2007):2143–2149.
5. W. Korner, P. Speengler, U. Bolz, W. Schuller, V. Hanf, and J. Metzger, Substances with estrogenic activity in effluents of sewage

treatment plants in southwestern Germany. 2. Biological analysis. *Environ. Toxicol. Chem* 20 (2001):2142–2152.
6. B. Gutendorf, and J. Wastendorf, Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. *Toxicology* 166 (2001):79–87.
7. J. P. Sumpter, and S. Jobling, Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environ. Health Perspect.* 103 (1995):173–180.
8. D. G. J. Larsson, M. Adolfsson-Erici, J. Parkkonen, M. Patterson, A. H. Berg, P. E. Olsson, and L. Förlin, Ethinyloestradiol—An undesired fish contraceptive? *Aquat. Toxicol.* 45 (1999):91–97.
9. E. P. Ko˙odziej, T. Harter, and D. L. Sedlak, Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. *Environ. Sci. Technol.* 38 (2004):6377–6383.
10. D. M. Fry, and C. K. Toone, DDT–induced feminization of gull embryos. Science 213 (1981):922–924.
11. K. C. Fertuck, S. Kumar, H. C. Sikka, J. B. Matthews, and T. R. Zacharewski, Interaction of PAH-related compounds with the α and β isoforms of the estrogen receptor. *Toxicol. Let.* 121 (2001):167–177.
12. C. A. Harris, P. Henttu, M. G. Parker, and J. P. Sumpter, The estrogenic activity of phthalate esters in vitro. *Environ. Health. Perspect.* 105 (1997):802–809.
13. H. S. Kim, T. S. KIM, J. H. Shin, H. J. Moon, I. H. Kang, I. Y. Kim, J. Y. Oh, and S. Y Han, Neonatal exposure to di(n-butyl) phthalate (dbp) alters male reproductive-tract development. *J. Toxicol. Environ. Health Part B* 67 (2004):2045–2060.
14. J. D McKinney and C. L Waller, Molecular determinants of hormone mimicry: Halogenated aromatic hydrocarbon environmental agents. *J. Toxicol. Environ. Heath Part B. Crit Rev.* 1 (1998):27–58.
15. F. Yadetie and F. Male, Effects of 4-nonylphenol on gene expression of pituitary hormones in juvenile Atlantic salmon (*Salmo salar*). *Aquat. Toxicol.* 58 (2002):113–129.
16. U.S. EPA, Special report on environmental endocrine disruption: an effect assessment and analysis, Office of Research and Development. EPA/630/R–96/012, 2006.
17. Environmental Agency, "Endocrine—disrupting substances in environment; what should be done?" Environmental Issue series, Consultative Report, 1998.
18. European Commission, "European workshop on the impact of endocrine disrupters on human health and wildlife." Report of the Proceeding, 2–4 December 1996, Weybridge, UK, Report EUR 17549, 1996.
19. Waste Water Cluster, European Commission-DG XII-DI, *Water Technologies*, WWC Newsletter No. 2 (1999).
20. The Identification and Assessment of Estrogenic Substances in Sewage Treatment Effluents, R&D Publication, Nov. 1996, The Environment Agency, Bristol, 1996.
21. C. G. Campbell, S. E. Borglin, F. B. Green, A. Grayson, E. Wozei, and W. Stringfellow, Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. *Chemosphere* 65 (2006):1265–1280.
22. Y. Fan, M. Zhang, S.-L. Da, and Y.-Q. Feng, Determination of endocrine disruptors in environmental waters using poly(acrylamide-vinylpyridine) monolithic capillary for in-tube solid-phase microextraction coupled to high-performance liquid chromatography with fluorescence detection. *Analyst* 130 (2005):1065–1069.
23. CH. Huang and D. L. Sedlak, Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. *Environ. Toxicol. Chem.* 20 (2001):133–139.
24. I. Histerkamp, J. Ganrass, and W. Ruck, Bioassay-directed chemical analysis utilizing LC–MS: A tool for identifying estrogenic compounds in water samples? *Anal. Bioanal. Chem.* 378 (2004):709–715.
25. F. Zhang, M.J. Bartels, R. Kookana, J. C. Brodeur, E. L. McClymont, and K. B. Woodburn, Quantitation of 17-ethinylestradiol in aquatic samples using liquid-liquid phase extraction, dansyl derivatization, and liquid chromatography/positive electrospray tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 18 (2004):2739–2742.
26. M. J. López de Alda, S. Díaz-Cruz, M. Petrowic, and D. Barceló, Liquid chromatography–(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment. *J. Chromatogr. A* 1000 (2003):503–526.
27. R. Gadza˙a-Kopciuch, J. Ričanyová, and B. Buszewski, Isolation and detection of steroids from human urine by molecularly imprinted solid–phase extraction and liquid chromatography. *J. Chromatogr. B* 877 (2009):1177–1184.
28. N. Voulvoulis, Methods for the determination of endocrine disrupters, in *Endocrine Disrupters in Wastewater and Sludge Treatment Processes* (CRC Press LLC, New York, 2003), Ch. 3, 59–101.
29. C. Desbrow, E. J. Routledge, G. C. Brighty, J. P. Sumpter, and M. Waldock, Identification of estrogenic chemicals in STW effluent. 1. chemical fractionation and in vitro biological screening. *Environ. Sci. Tech.* 32 (1998):1549–1558.
30. L. B. Barber, G. K. Brown, and S. D. Zaugg, Potential endocrine disrupting organic chemicals in treated municipal wastewater and river water. *ACS Symposium Series, Analysis of Environmental Endocrine Disruptors*, 747 (2000):97–123.
31. H. M. Kuch, and K. Ballschmiter, Determination of endogenous and exogenous estrogens in effluents from sewage treatment plants at ng/l-level. *J. Anal. Chem.* 366 (2000):392–395.
32. T. P. Rogers-Gray, S. Jobling, S. Morris, C. Kelly, S. Kirby, A. Janbakhsh, J. E. Harries, M. J. Waldock, J. P. Stumper, and C. R. Tyler, Long-term temporal changes in the estrogenic composition of treated sewage effluent and its biological effects on fish. *Environ. Sci. Technol.* 34 (2000):1521–1528.
33. R. Siegener and R. F. Chen, Detection of pharmaceuticals entering Boston Harbor. *Anal. Environ. Endoc. Disrupt ACS Symp. Ser.* 747 (2000):125–132.
34. M. Möder, P. Braun, F. Lange, S. Schrader, and W. Lorenz, Determination of endocrine disrupting compounds and acidic drugs in water by coupling of derivatization, gas chromatography and negative-chemical ionization mass spectrometry. *Clean.* 35 (2007):444–451.
35. A. C. Belfroid, A. Van der Horst, A. D. Vethaak, A. J. Schäfer, G. B. J. Rijs, J. Wegener, and W. P. Cofino, Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. *Sci. Tot. Environ.* 225 (1999):101–108.
36. T. A. Ternes, M. Stumpf, J. Mueller, K. Haberer, D. Wilken, and M. Servos, Behavior and occurrence of estrogens in municipal

Downloaded At: 11:55 17 January 2011

sewage treatment plants—I. Investigations in Germany, Canada and Brazil. *Sci. Total Environ.* 225 (1999):81–88

37. A. C. Johnson, A. Belfroid, and A. Di Corcia, Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. *Sci. Total Environ.* 256 (2000):163–171.
38. C. Kelly, Analysis of steroids in environmental water samples using solid-phase extraction and ion-trap gas chromatography–mass spectrometry and gas chromatography–tandem mass spectrometry. *J. Chromatogr. A* 872 (2000):309–314.
39. J. B. Quintana, J. Carpinterio, I. Rodríguez, R. A. Lorenzo, A. M. Carro, and R. Cela, Determination of natural and synthetic estrogens in water by gas chromatography with mass spectrometric detection. *J. Chromatogr. A* 1024 (2004):177–185.
40. M. J. López de Alda and D. Barceló, Determination of steroid sex hormones and related synthetic compounds considered as endocrine disrupters in water by fully automated on-line solid-phase extraction–liquid chromatography–diode array detection. *J. Chromatogr. A* 911 (2001):203–210.
41. C. Almeida and J. M. F. Nogueira, Determination of steroid sex hormones in water and urine matrices by stir bar sorptive extraction and liquid chromatography with diode array detection. *Pharm. Biomed. Analysis* 41 (2006):303–311.
42. M. J. López de Alda and D. Barceló, Determination of steroid sex hormones and related synthetic compounds considered as endocrine disrupters in water by liquid chromatography–diode array detection–mass spectrometry. *J. Chromatogr. A* 892 (2000):391–406.
43. M. J. López de Alda and D. Barceló, Review of analytical methods for the determination of estrogens and progestogens in waste waters. *Fresenius J. Anal. Chem.* 371 (2001): 437–447.
44. S. Rodriguez-Mozaz, M. J. M. J. López de Alda, and D. Barceló, Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid–phase extraction–liquid chromatography–mass spectrometry. *J. Chromatogr. A* 1045 (2004):85–92.
45. A. Laganá, A. Bacaloni, G. Fago, and A. Marino, Trace analysis of estrogenic chemicals in sewage effluent using liquid chromatography combined with tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 14 (2000):401–407.
46. C. Baronti, R. Curini, G. D'Ascenzo, A. Di Corcia, A. Centili, and R. Samperi, Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environ. Sci. Technol.* 34 (2000):5059–5066.
47. V. Ingrand, G. Herry, J. Beausse, and M-R. de Roubin, Analysis of steroid hormones in effluents of wastewater treatment plants by liquid chromatoraphy–tandem mass spectrometry. *J. Chromatogr. A* 1020 (2003):99–104.
48. C.-Y. Cen, T.-Y. Wen, G.-S. Wang, H.-W. Cheng, Y.-H. Lin, and G.-W. Lin, Determining estrogenic steroids in Taipei waters and removal in drinking water treatment using high-flow solid-phase extraction and liquid chromatography/tandem mass spectrometry. *Sci. Total Environ.* 378 (2007):352–359.
49. Y.-H. Lin, C.-Y. Chen, and G.-S. Wand, Analysis of steroid estrogens in water using liquid chromatography/tandem mass spectrometry with chemical derivatizations. *Rapid Commun. Mass Spectrom.* 21 (2007):1973–1983.
50. S. A. Snyder, T. L. Keith, D. A. Verbrugge, E.M. Synder, T.S. Gross, K. Kannan, and J. P. Giesy, Analytical methods for detection of selected estrogenic compounds in aqueous mixtures. *Environ. Sci. Technol*, 33 (1999):2814–2820.
51. H. H. Tabak, R. N. Bloomhuff, and R. L. Bunch, Steroid hormones as water pollutants. II. Studies on the persistence and stability of natural urinary and synthetic ovulation-inhibiting hormones in untreated and treated wastewaters. *Dev. Ind. Microbiol.* 22 (1981):497–519.
52. X. Y. Xiao, D. V. McCalley, and J. McEvoy, Analysis of estrogens in river water and effluents using solid-phase extraction and gas chromatography–negative chemical ionisation mass spectrometry of the pentafluorobenzoyl derivatives. *J. Chromatogr. A* 923 (2001):195–204.
53. F. Courant, J.-P. Antignac, D. Maume, F. Monteau, A.-M. Andersson, N. Skakkebaek, F. Andre, and B. Le Bizec, Exposure assessment of prepubertal children to steroid endocrine disrupters: 1. Analytical strategy for estrogens measurement in plasma at ultra-trace level. *Anal. Chim. Acta* 586 (2007):105–114.
54. D. D. Fine, G. P. Breidenbach, T. L. Price, and S. R. Hutchins, Quantitation of estrogens in ground water and swine lagoon samples using solid-phase extraction, pentafluorobenzyl/trimethylsilyl derivatizations and gas chromatography–negative ion chemical ionization tandem mass spectrometry. *J. Chromatogr. A* 1017 (2003):167–185.
55. W. Z. Shou, X. Y. Jiang, and N. D. Weng, Development and validation of a high-sensitivity liquid chromatography/tandem mass spectrometry (LC/MS/MS) method with chemical derivatization for the determination of ethinyl estradiol in human plasma. *Biomed. Chromatogr.* 18 (2004):414–421.
56. F. Zhang, M. J. Bartels, J. C. Brodeur, E. L. McClymont, and K. B. Woodburn, Quantitation of 17α-ethinylestradiol in aquatic samples using liquid-liquid phase extraction, dansyl derivatization, and liquid chromatography/positive electrospray tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 18 (2004):2739–2742.
57. M. R. Anari, R. W. Burton, S. Gopaul, and F. S. Abbott, Metabolic profiling of valproic acid by cDNA-expressed human cytochrome P450 enzymes using negative–ion chemical ionization gas chromatography–mass spectrometry. *J. Chromatogr. B* 742 (2000):217–227.

Downloaded At: 11:55 17 January 2011