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Analytical tools monitoring endocrine disrupting chemicals

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ABSTRACT

Endocrine disrupting chemicals (EDCs) are harmful, xenobiotic compounds requiring a multi-tiered analytical approach for a reliable management. Although worth efforts worldwide, comprehensive EDCs monitoring and risk-assessment still require improvements. This article covers possible risks for public health due to EDCs exposure, and revises the maturity reached in different analytical detection fields, with a special focus on biosensor technology. Among validated laboratory-techniques, hyphenated mass-spectrometry-based chromatography provides high selectivity and multi-analyte detection, while *in vitro* bioassays enable reliable toxicological testing. However, none of these methods is suitable for fast *in field*, continuous or semi-continuous operations. Due to advances in material science and synthetic biology, now biosensor technology holds the promise to close this gap and, although not included yet in routinely screening programs, fulfill the necessary requirements to sustain a coherent and global strategy to assess the state of environmental pollution.

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Contents

Abbreviations: AChE, Acetylcholinesterase; BPA, Bisphenol A; BChE, Butyrylcholinesterase; *CALUX*, Chemical Activated LUciferase *gene* eXpression; DNAzyme, Deoxyribozyme; EEC, European Economic Community; EDCs, Endocrine Disrupting Chemicals; ELISA, Enzyme-Linked Immunosorbent Assay; ELRAs, Enzyme-Linked Receptor Assays; EU, European Union; EE2, 17-α-Ethynylestradiol; HGH, Human Growth Hormone; LOD, Limits of Detection; MEMS, Micro-Electro Mechanical System; MWCNT, Multi-Walled Carbon Nanotube; NPs, Nonylphenols; OPs, Octylphenols; OPH, Organophosphate Hydrolase; OPP, Organophosphorus; PCBs, Polychlorinated Biphenyls; PCDDs, Polychlorinated Dibenzo-p-Dioxins; PCDFs, Polychlorinated Dibenzofurans; POPs, Persistent Organic Pollutants; SPEs, Screen Printed Electrodes; TCDD, 2,3,7,8-Tetrachlorodibenzo Para Dioxin; TBT, Tributyltin; USEPA, United States Environmental Protection Agency; YAS, Yeast Androgen Screen; YES, Yeast Estrogen Screen.

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1. Introduction

Anthropogenic activities and life-style of modern society often lead to intentional or unintentional discharge in the environment of thousands of chemicals, part of them interfering with hormone function. These compounds have been described as endocrine-disrupting chemicals (EDCs) that may produce a wide spectrum of adverse effects on both human health and wildlife.

Back in 1996, the enactment of Food Quality Protection Act mandated the US Environmental Protection Agency (USEPA) to develop a screening program to determine the substances with potential negative effects on endocrine human systems. This resulted in the first definition of an EDC as "an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle". Later on, USEPA, European Union (EU) and World Health Organization (WHO) implemented the EDC definition on the basis of experimental results deriving by clinical research, basic science, epidemiology, and clinical practice [1,2].

EDCs are a heterogeneous class of xenobiotic, persistent, environmental contaminants, which interfere with the endocrine system by mimicking or antagonizing natural hormones, or hindering their metabolic pathways. Nowadays, EDCs include more than 800 different chemical structures (Table 1) that have been found in air, land, drinking water, and foodstuffs of plant and animal origin, consumer goods and personal care products, fuels, pharmaceuticals, and synthetic hormones [3]. Among them, plasticisers (bisphenol A, BPA; phthalates), surfactants (alkylphenols), preservatives in cosmetic and pharmaceutical products (parabens), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, brominated flame-retardants, insecticides such as DDT and its metabolites, dioxins and perfluorinated compounds shape an extensive list of potent EDCs that is rapidly getting longer. Similarly, several classes of pesticides, including organophosphorous (OPP) and carbamic insecticides, and triazinic, diazinic and ureic herbicides have also been recognized as potential EDCs [4]. Besides, xenohormones, widely used in veterinary medicine as growth stimulants or treatment for estrogen-deficiency disorders [5], are also EDCs. An increasing amount of evidences documents endocrine system malfunctioning initiated by heavy metals, including cadmium, arsenic, mercury, nickel, lead, zinc [6], and organometallic compounds (tributyltin, TBT) [7]. Although the presence of EDCs in the environment and associated effects on humans and wildlife are of main concern, their monitoring and evaluation still require efforts. Suspected EDCs could be widely detected in the environment, but their quantification is extremely hard without extensive pre-concentration procedures as well as elaborated analytical methodologies, due to their occurrence at very low concentration (ng/L or below), often in complex mixtures containing tens to hundreds of different compounds. Finally, some EDCs are still unknown and their occurrence/identification could be possible only following the assessment of their activity. Presence of EDCs in air is also a serious task to be addressed, as their inhalation could seriously affect human health. A recent study, in fact, revealed high concentrations of specific EDCs in outdoor and indoor air, and highlighted the necessity to properly design monitoring studies useful to investigate temporal and spatial distributions suitable to identify the source, the transport and the fate of EDCs in the atmospheric compartment [8]. For these reasons, recent international directives, public opinion concerns, and the attention of diagnostic research community have been focused on EDCs identification and their regulation (Table 2). Environmental Quality Standards have been set by EU (Directive 2008/105/EC, recently amended by Directive 2013/39/EU) for surface waters, on the base of a list of 45 priority substances, most of them being known or suspected EDCs. Several strategies have been employed for revealing EDCs in air, water, and soil, without providing a complete picture of the involved ecological risks and observed impacts. USEPA has mandated several programs over the years to screen, test, and identify potential EDCs, and determine their effects on human and environmental health. Nevertheless, several challenges still need to be addressed in this sense requiring global resolutions.

To underline the relevance and the complexity of EDCs management, the next section summaries main endocrine disorders associated to exposure to EDCs and their mechanisms of action. Following, a deep overview of both already available analytical methods and new challenge-driven technologies for EDCs monitoring is presented. A special focus is devoted to the description of biosensor technology to point out the maturity reached in this field. Finally, strengths and limitations of the presented methods were critically addressed in order to emphasize the need to adopt an integrated analytical approach for EDCs managing.

2. Main endocrine disorders associated to EDCs exposure

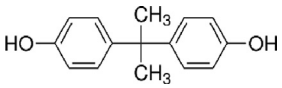
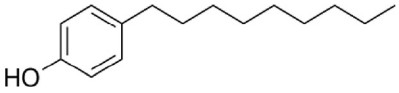
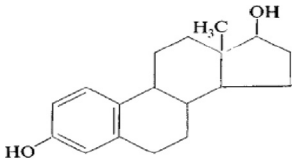
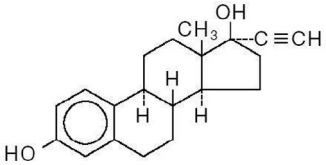
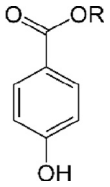
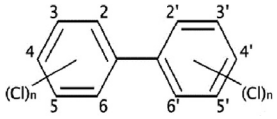
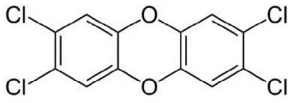
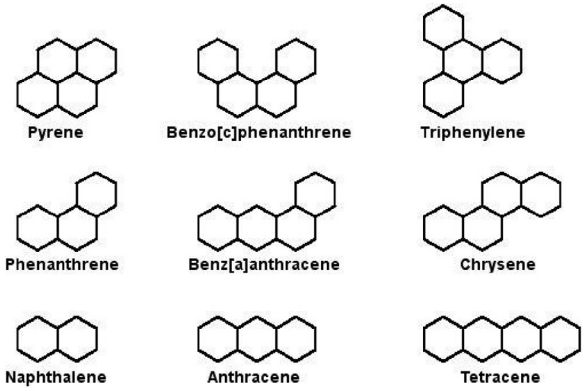
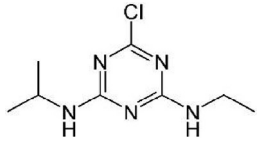
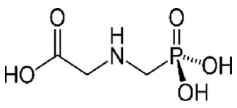
A bulk of research studies correlates EDCs exposure to the occurrence of many diseases and disorders that are mainly associated to estrogens, androgens and thyroid hormones. Due to their structural similarity to endogenous ligands EDCs could i) interact with nuclear or hormonal receptors agonizing or antagonizing their functions; ii) activate hormone-transporter proteins; iii) stimulate/inhibit enzymes involved in hormone metabolism unbalancing their homeostasis; iv) reduce/increase the number of receptors per cell affecting the extent of response to natural or artificial hormones; v) modify the production of natural hormones by interfering with signal transduction in the thyroid, immune and nervous systems [3].

Human organisms may absorb EDCs by inhalation, skin contact or ingestion. Afterward, these compounds freely pass through cell membranes and are subsequently released into the bloodstream.

An extensive literature, including *in vitro* and *in vivo* studies as well as observational epidemiology, widely reported relevant health damages associated with exposure to EDCs, which may cause short-term acute effects or long-term (chronic) adverse effects occurring even months or years after exposure. Acute health effects have been evidenced in people occupationally exposed to pesticides manifesting stinging eyes, rashes, blisters, blindness, nausea, asthma, and diarrhea [3]. Numerous studies provided evidence of herbicide-induced endocrine disruption in humans and amphibians [9]. To the other side, dramatic chronic effects includes cancer, birth defects, reproductive harm, neurological and developmental toxicity, and immunotoxicity [10]. EDCs-induced short-term effects at neurological level may provoke dizziness, lightheaded, confusion, reduced coordination and ability to think, while long-term exposure can result in reduced intelligence quotient, learning disabilities and permanent brain damages, especially in children living in areas with high concentrations of pesticides in water and food. Obesity and diabetes have also been linked to EDCs exposure. Considering the newly identified endocrine function of adipocytes, it became fundamental to investigate whether exposure to EDCs during the critical period of cell differentiation is correlated to obesity or any of its associated pathologies. By modifying the endocrine function of organs involved in carbohydrates and lipids metabolism EDCs may, in fact, induce insulin resistance possibly leading to diabetes and obesity, increasing the risk of cardiovascular diseases [10,11].

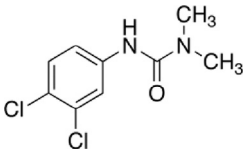
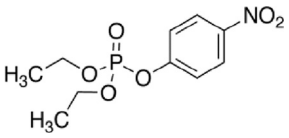
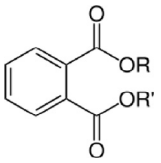
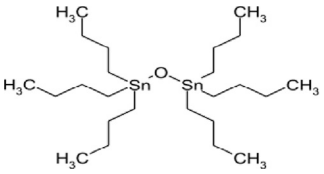
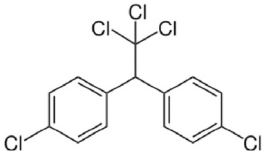
Furthermore, the complexity of endocrine-malfunction related disorders is based on the high probability for contemporary multi-EDCs exposures in presence of various environmental factors that an individual may experience during his lifespan. This threat could be particularly harmful for children, and particular care should be taken to prevent their contact with EDCs mixtures.

Table 1
List of the main pollutants classified as EDCs

EDCs	Structure	Source
Bisphenol A		Used in plastics and epoxy resins
Nonylphenol		Metabolite of the nonionic surfactants NPEOs used in industrial, domestic and commercial applications
17-β-estradiol		Natural steroidal hormone
17-α-ethinylestradiol		Synthetic steroidal hormone used in contraceptive pills
Parabens		Used as preservatives by cosmetic and pharmaceutical industries
Polychlorinated biphenyl (PCBs)		Used as dielectric and coolant fluids
Dioxin		Used in industrial processes (bleaching paper pulp, chemical and pesticide manufacture)
Polycyclic aromatic hydrocarbons		Atmospheric pollutants formed by natural or anthropogenic process of thermal decomposition
Atrazine		Runoff from triazinic herbicides used on crops
Glyphosate		Runoff from herbicide use

(*continued on next page*)

Table 1
(*continued*)

EDCs	Structure	Source
Diuron		Runoff from ureic herbicides used on crops
Paraoxon		Insecticide widely used on field crops
Phtalates		Used as plasticizers, including food and beverage packaging, excipients in drug formulations, cosmetics, insecticides and paints
Tributyltin		Used as biocides in many fields
Dichloro diphenyl trichloroethane (DDT)		Organochlorine insecticide used in several forms

The exposure to EDCs during critical developmental time windows may form the basis for adult diseases. EDCs not only affect the person exposed, but also the person's offspring through epigenetic modifications. EDCs have been considered also potential genotoxic chemicals: experimental data revealed that various EDCs exhibited mutagenic properties inducing gene mutations, chromosomal aberration or DNA damage. Nowadays, evidences correlating endocrine-related diseases and EDCs exposures experienced during foetal development and puberty and translated into adulthood, have significantly increased [12].

The concern of EU Commission on the incidence and risk-assessment of EDCs came from the fact that it is difficult to evaluate clearly the associated toxicity, since endocrine disruption cannot be considered as a measurable end-point, the noxious action of these pollutants being the result of several mechanisms and metabolic pathways [4]. A schematic representation of the main endocrine disorders associated to EDCs exposure is depicted in Fig. 1.

3. Chemical and bioanalytical tools

The need of a multi-tiered analytical strategy for EDCs monitoring emerges as a priority considering the complex relationships existing among EDCs release in the environment, human and wildlife exposure, and onset of endocrine disorders. Specifically, monitoring of EDCs should include not only identification and quantification of chemical compounds with endocrine disruption potential, but also evaluation of the effects on living beings through the elucidation of dose-response mechanisms induced by specific classes or mixtures of EDCs. Furthermore, direct *in field* and continuous assessment of EDCs is becoming a new urgency to timely screen contaminated areas, and map new sites of potential contamination. Unpicking these critical points could guide policy makers to promulgate adequate regulations for a better management of EDCs. The following sub-sections provide: *i)* a detailed description of biosensor devices specifically developed for different classes of EDCs to evaluate their potential to be included in accredited programs for *in field* screening (Table 3); and *ii)* a global description of chromatographic techniques and *in vitro* assays to showcase trusted methods commonly employed by certified laboratories and accepted by regulatory agencies.

3.1. Biosensor technology

Biosensing technology started to be addressed as an emergent industry with high-potential growth mainly due to its wide range of applications and the continuously increase of assays feasibility. Furthermore, new developments in material sciences and genetic engineering laid a foundation to solve significant custom-tailored detection issues, as those raised by EDCs assessment, by improving response specificity and methods sensitivity of biosensors. In the following subsections several examples on biosensor technology applications for determination of different EDCs are presented.

3.1.1. Pesticides

Pesticides belong to the substances with recognised endocrine disrupting effects already regulated and/or addressed by the currently in-force legislation. Specific pesticide compounds are listed in Table 1. Electrochemical biosensors based on the inhibition of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) are among the most implemented methods for the determination of different

Table 2
EU directives to identify and establish the maximum residues levels of toxic compounds in water, food and feedstuff (http://eur-lex.europa.eu/en/index.htm)

Legislation	Application	Details	Main amending
79/117/EEC	*Prohibition the placing on market and use of plant protection products containing certain active substances*	The Directive aims at the prohibition or severe restriction of manufacture and use of intentionally produced POPs; introduces restrictions on the export and import of intentionally produced POPs; formulates measures for the safe handling of stockpiles and environmentally sound disposal of waste containing POPs; and presents proposals for the reduction of emissions of unintentionally produced POPs.	***850/2004 2004/259/EC 1195/2006 172/2007 323/2007 219/2009 304/2009 756/2010 757/2010 519/2012***
91/321/EC	*Infant formulae and follow-on formulae*	This document or decree is a specific Directive within the meaning of Art. 4 of Directive ***89/398/EEC*** and lays down compositional and labeling requirements for infant formulae and follow-on formulae intended to provide infants with safe nutrition for healthy development from day one. Amending Directive was subsequently made in order to prohibited the existence even of pesticides traces in infant formulae and follow-on formulae.	***96/4/EC 1999/50/EC 2003/14/EC 2006/82/EC***
91/414/EC	*Introduction of plant protection products on the market*	The Regulation establishes the maximum admissible quantity of pesticide residues in food products of animal or vegetable origin intended for human or animal consumption. The maximum pesticide residue level in foodstuffs is debase up to 0.01 mg/kg.	***94/37/EC 2001/21/EC 94/43/EC 2001/28/EC 94/79/EC 2001/36/EC 95/36/EC 2001/87/EC 96/12/EC 2003/82/EC 96/46/EC 2004/66/EC 96/68/EC 396/2005 97/57/EC 2008/149/EC 98/47/EC 1107/2009 2000/80/EC***
96/5/EC	*Processed cereal-based foods and baby foods for infants and young children*	This Directive covers foodstuffs for particular nutritional use fulfilling the specific requirements of infants and young children in good health in the Community and are intended for use by infants while they are being weaned and by young children as a supplement to their diet and/or for their progressive adaptation to ordinary food.	***98/36/EC 1999/39/EC 2003/13/EC 2006/125/EC***
98/83/EC	*The quality of water intended for human consumption*	The Directive established as allowable limit for all pesticide 0.1 μg/L and no more than 0.5 μg/L for total pesticides content.	***1882/2003 596/200***
2000/60/EC	*A framework for Community action in the field of water policy*	The purpose of this Directive is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater.	***2455/2001/EC 2008/32/EC 2008/105/EC 2009/31/EC 2013/39/EU***

classes of pesticides [13–16]. In this context, several research activities have been focused on the inhibitory effect of OPP and organothiophosphorous pesticides on AChE and butyrylcholinesterase (BChE; EC 3.1.1.8), also known as *plasma cholinesterase*, found primarily in the liver [17,18]. The principle of AChE electrochemical biosensor involves measurement of the enzymatic activity before and after exposure to the pollutant; a decrease in enzymatic response is attributed to the presence of OPP and carbamate pesticides in the sample. Numerous amperometric biosensors based on AChE have been developed allowing the determination of I_{50} (50% of inhibition) values for aldicarb (50 ppb), carbaryl (85 ppb), paraoxon (4 ppb), and chlorpyrifos-methyl oxon (1 ppb) [17,18]. Although reliable, the first native and recombinant AChE-based biosensors were not enough selective being inhibited by several classes of pesticides and heavy metals, or sensitive. Recently, an ultra-sensitive biosensors based on *Drosophyla* engineered-AChE immobilized in poly (vinyl alcohol) and modified with Fe-Ni nanocomposite was developed reaching limit of detection (LOD) of approximately 0.1 nM

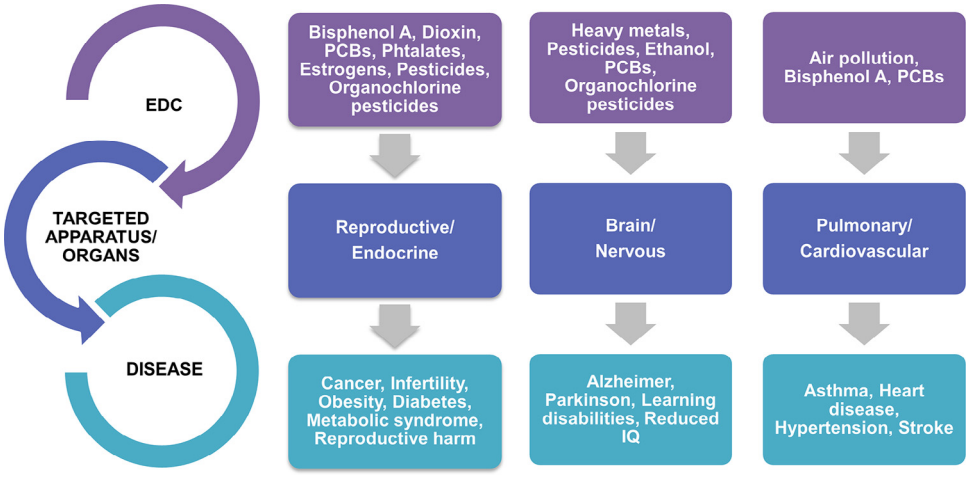


Fig. 1. List of the main pollutants classified as endocrine disruptors and related effects on human health.

Table 3
List of the main classes of EDCs and relative sensing/biosensing analytical detection methodologies

EDCS	Analytical method	LOD	Reference
Aldicarb	Amperometric biosensor based on AChE and BChE	50 ppb	[13]
Carbaryl		85 ppb	
Paraoxon		4 ppb	
Chlorpyrifos-methyl oxon		1 ppb	
Chlorpyrifos	Optical biosensor based on AChE	7.5 μM	[17]
Paraoxon	Cyclic voltammetry (CV) and UV–vis methods on the MWCNT–(PEI/DNA)2/OPH/AChE biosensor	0.5 μM	[20]
Carbaryl		1.0 μM	
Diuron	AChE immobilized on the gold nanoparticles modified-screen printed carbon electrode	50 nmol/L	[22]
Paraoxon	Automatable flow system based on BChE and Prussian Blue nanoparticles	1 ppb	[23]
Paraoxon, ethyl parathion, methyl parathion, diazinon	Colorimetric assay based on organophosphorous hydrolase	μM range	[24]
Atrazine	Optical biosensor based on PSII	10^{-9} M	[28]
Atrazine	Optical biosensor based on PSII	6.8×10^{-10} M	[30]
Prometryn		6.5×10^{-10} M	
Terbuthylazine		2.5×10^{-10} M	
Diuron		0.8×10^{-11} M	
Linuron		0.9×10^{-11} M	
Atrazine	Optical biosensor based on PSII	1.60×10^{-9} M	[14]
Prometryn		1.00×10^{-9} M	
Diuron		0.65×10^{-9} M	
Catechol, BPA	Electro-optical biosensor based on tyrosinase and laccase	25 μM	[17]
		10 μM	
Thiolated-c	ER-α assembled on the Au nanoparticle surface	2.4–2.9 nM	[39]
4-nonylphenols	Biacore biosensor immunoassay	10 ng g^{-1}	[41]
Nonylphenol	Thermal immune biosensor	1 μg/L	[42]
AFB1	AChE electrochemical assay	2 ppb	[15]
di(2-ethylhexyl) phthalate (DEHP)	Electrochemical impedance spectroscopy technique incorporating a novel interdigital capacitive thin film gold micro-electrodes	ppb	[50]
dibutyl phthalate (DBP)	Electrochemical sensor based on biomimetic layers	12.5–35.2 pM	[51]
di(2-ethylhexyl) phthalate (DEHP)			
dicyclohexyl phthalate (DCHP)			
diethyl phthalate (DEP) benzyl butyl phthalate (BBP)			
Estrone	Surface plasmon resonance assay based on human recombinant ERα	4.29×10^{-9} M	[54]
Estradiol		4.04×10^{-10} M	
Estriol		8.35×10^{-10} M	
Tamoxifen		2.16×10^{-8} M	
Diethylstilbestrol		1.46×10^{-10} M	
BPA		1.35×10^{-6} M	
4-nonylphenol		7.49×10^{-6} M	
Human growth hormone	Surface plasmon resonance immunobiosensor	2.47 nM	[55]
17β-estradiol	Electrochemical impedance spectroscopy based biosensor using receptor molecules	0.1 pM	[56]
Diethylstilbestrol	Electrochemical immunosensor based on MSN-GNPs-MWCNTs nanocomposites and HRP-Ab-GNPs-PB-MWCNTs bioconjugates	1.2×10^{-7} mg/mL	[57]
Hg(II)	Fluorescence assay based on a poly(aryleneethynylene) carrying leucine, glycine and methionine	50–100 ppb	[16]

The sensitivity (when available) of each methodological approach is also presented as LOD.
Legend: MWCNT–(PEI/DNA)2/OPH/AChE: multi-walled carbon nanotubes- polyethyleneimine/DNA/ organophosphate hydrolase/acetylcholinesterase; PSII: photosystem II; ERα: Estrogen receptor; MSN-GNPs-MWCNTs: mesoporous silica-gold nanoparticles-multiwall carbon nanotubes; HRP-Ab-GNPs-PB-MWCNTs: horseradish peroxidase-antibody-Prussian blue-multiwall carbon nanotubes.

for phosmet (an OPP pesticide) after a rapid extraction from olive oil. As reported by the authors, the use of Fe–Ni nanomaterial allowed to improve selectivity by working a lower potential, and to amplify the response current compared to non-modified biosensor [19].

To improve selectivity of biosensors, an effective solution could rely in the fabrication of multi-enzyme bio-hybrids. The work by Zhang and co-workers reported for the first time the construction of a novel bi-enzyme biosensor successfully discriminating OPP and non-OPP pesticides among other compounds. The system incorporated multi-walled carbon nanotube (MWCNT)–organophosphate hydrolase and MWCNT–AChE along with a set of cushioning bilayers consisting of MWCNT–polyethyleneimine and MWCNT–DNA on glassy carbon electrode. Electrochemical and optical methods were used to characterise the performance of the biosensor in real samples (apple juice) revealing good stability and reproducibility. The LOD was ~0.5 μM for OPP pesticide paraoxon, and 1 μM for non-OPP pesticide carbaryl [20].

Recently, mimetic molecules were used to develop a disposable biochemical sensor to detect carbamate pesticides. Sgobbi and colleagues propose the synthesis, characterization and application of hydrazones as mimetic molecules, including 4-[(1E)ethanehydrozanoyl]benzoic acid, cyclopropyl methyl ketone hydrazone, 3-methyl-2-butanone hydrazone and hydrazine. These synthetic molecules exhibited the same catalytic properties and a lower synthetic cost compared to AChE providing an effective route for the construction of commercial disposable device [21].

In addition, in order to provide *on-line* sample analysis, several biosensors have been coupled with flow systems, and the analyte detection was carried out during the flow of the samples through

the detector. This configuration would be a crucial improvement in the automating of the analysis, if compared with stationary measurements. In this context, an interesting example is provided by the study of Janegitz and co-workers, which developed a newly automated and portable microcontrolled pumping flow system suitable to detect diuron pesticides in water samples. The system was coupled to an amperometric biosensor made by disposable carbon screen printed electrodes (SPEs) modified with gold nanoparticles and AChE. In this study a linear dose/response calibration curve for diuron in the range from 80 to 1400 nmol/L was reported corresponding to LOD of 50 nmol/L [22].

Recently, a novel amperometric biosensor for paraoxon determination was developed by adhering BChE on SPEs modified with Prussian Blue nanoparticles, and embedded in a flow system. This set up showed the possibility to detect paraoxon at ppb level using an automatable and cost-effective bioanalytical approach suitable for *in field* analyses [23].

Alternatively, by exploiting the catalytic properties of organophosphate hydrolase (OPH) is possible to detect a number of OPP pesticides including paraoxon, ethyl parathion, methyl parathion and diazinon, generating p-nitrophenol, which is both an electroactive and chromophore compound, hence equally useful to set up both amperometric and optical biosensors [24].

The exploitation of photosynthetic proteins, tissues or whole microorganisms as indicators of herbicides occurrence, such as phenylurea, triazine, diazines, and phenolic herbicides has been widely explored [25–27]. The proof of concept of photosynthesis-based biosensors relies on the capability of several herbicides to bind to photosynthetic active reaction centre proteins (such as the photosystem II D1 protein in plant, algae and cyanobacteria, or the L reaction centre protein in bacteria) competing with plasto/ubi-quinones, the natural ligands involved in the light-induced photosynthetic electrons transport chain. Herbicide binding leads to inhibition of the electron flow and generation of physico-chemical modifications detectable by electrochemical-optical transducers [28]. Also in this context, molecular biology and bioinformatic tools played an important role for the design and creation of new biological recognition elements able to detect a broad variety of chemicals in a wide range of concentrations [26,28–31].

3.1.2. Phenols

Phenolic compounds are important contaminants due to their toxicity and accumulation in the environment being, as pesticides, substances already regulated with proved evidence of endocrine disrupting effect. The detection of phenolic compounds is efficiently performed by electrochemical biosensors exploiting oxidase enzymes such as tyrosinase (EC 1.14.18.1) and laccase (EC 1.10.3.2) [32,33]. These enzymes are usually immobilised on the surface of conductive materials (e.g. SPEs) and catalyse the oxidation of phenols to quinone compounds. The resulted quinone species are electrochemically reduced on the electrode surface and, consequently, the phenolic compound is detected in a concentration-dependent manner. Several electrochemical biosensors based on the use of oxidase enzymes were recently developed, aiming to increase the number of chemical species that can be monitored. Particular attention was paid to phenols with high endocrine disrupting activity, such as catechol and BPA. As an example, a biosensing system using tyrosinase from *Agaricus bisporus* and laccase from *Trametes versicolor* was recently realized for the detection of catechol and BPA [17]. The limits of detection reached in this study (10 μM for catechol and 50 μM for BPA) were consistent in regards to the maximum residue level of these contaminants imposed by EC [34].

Similar electrochemical biosensors have been set-up, where improvement of sensitivity and stability of the sensing element was achieved by immobilizing the enzymes on different surface materials, such as graphene oxide electrodes [35], quantum-dot/chitosan composites electrode [36], boron-doped diamond electrodes [37], or magnetic nickel nanoparticles [38]. Recently, it has been also reported the construction of a simple ready-to-use tyrosinase-based biosensor for *in field* screening of phenolic compounds, using a paper-strip absorption method. Tyrosinase and a chromogenic compound were immobilised onto a solid surface. The rapid conversion of the coloured compound in the presence of oxidized phenols was used to evaluate the presence and quantify different type of phenols. Although the sensitivity of the newly developed biosensor was lower compared to previously developed electrochemical tyrosinase biosensors, it holds substantial advantages because it does not requires additional instrumentation, has low fabrication costs, and enables *on-site* qualitative monitoring of phenols [39].

Finally, a new improved piezoelectric biosensor for detection of BPA was developed exploiting a competitive binding assay which incorporate a specific probe, designed for a thiolated-BPA, on a ceramic resonator surface. This configuration provided high selectivity, and LOD in the nanomolar range (2.4–2.9 nM) and allowed to use a very small sample volume (1.5 μL) [40].

The different approaches discussed above have proven their potential as suitable diagnostic tools for phenols monitoring and control, demonstrating LOD values similar to the residues levels enforced by the EU Legislation.

3.1.3. Alkylphenols

Nonylphenols (NPs) and octylphenols (OPs) have raised interest in the last decades being able to interfere with reproductive systems of aquatic organisms by eliciting estrogenic activity.

A Biacore chip immunosensor was developed by synthesizing and immobilising mono- and polyclonal antibodies with high cross-reactivity towards NPs in shellfish samples. The sensor was less sensitive compared to cognate enzyme-linked immunosorbent assay (ELISA), but proved to be useful for fast-response preliminary screening methods [41]. A thermal immune biosensor for specific determination of NPs in the environment was also developed and its performance was compared to surface plasmon resonance (SPR) biosensors [42]. The proof-of-principle of this thermal biosensor was based on measuring the heat released as a result of the interaction between hapten and specific antibodies, providing direct detection of NPs at about 1 μg/L, in an overall time of analysis of about 20–30 min. The study showed that in spite of a lower sensitivity of the thermal biosensor, it was less sensitive to admixtures in real samples and simpler in use than the SPR biosensor, hence more suitable for *in field* analysis.

3.1.4. Dioxins and dioxin-like substances

Dioxins and dioxin-like compounds are persistent pollutants mainly found in food and fatty tissue of animals. 2,3,7,8-tetrachlorodibenzo para dioxin (TCDD) is one of the most hazardous compound, however, “dioxins” also include the chemically related polychlorinated dibenzo para dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), as well as the dioxin-like PCBs [43]. As a whole, these compounds share similar toxic properties, based on their capability to interact with the intracellular aryl hydrocarbon receptor involved, among other roles, in the regulation of endogenous hormones genes expression [44]. The exploitation of nanomaterials allowed to enhance the performance of biosensors for dioxin and PCBs detection. As an example, Centi and colleagues constructed immunosensors for detecting PCBs in milk samples by combining graphite screen-printed low-density arrays and magnetic beads to a solid-phase extraction of the sample which allowed the simultaneous measurements of differently processed samples [45]. Two different antibodies were tested that demonstrated their capability for broad-spectrum screening. In particular,

this set-up greatly improved the kinetics assay. The electrochemical magneto-immunosensor was used to detect PCBs mixtures as Aroclor 1242 and Aroclor 1248, being able to detect both mixtures at concentration levels of μg/mL. The calculated LOD for Aroclor 1242 and Aroclor 1248 were 60 and 70 ng/mL, respectively [45]. Immunosensors based on quartz crystal microbalance as transducer were also successfully developed for TCDD detection in ash samples with LOD value of 1 part per trillion [46].

A biomimetic approach has been also exploited to produce oligopeptides mimicking the aryl hydrocarbon receptor binding site to reveal dioxins in food products (chicken, eggs, and milk). These synthetic peptides were immobilized on a gold surface, reaching range of detection of 1–5 ppb, 1–10 ppb, and 1–20 ppb, for TCDD, a dioxin mixture, and PCBs, respectively [47].

Other biosensors for detection of dioxins and dioxin-like compounds exploiting different transductions, such as surface-enhanced Raman scattering spectroscopy, fluorescence quenching, surface photo voltage and porous anodic alumina based capacitive sensors have been also successfully developed as reviewed in the literature [43].

3.1.5. Phthalates

Esters of phthalic acid, also known as phthalates, are commonly used as additives or plasticizers in a wide range of industrial applications (Table 1). These compounds, leaking from plastic lattice, contaminate foodstuffs and environment. As a consequence, several health and environment monitoring agencies worldwide (e.g. USEPA) issued regulations and recommended guidelines for permitted daily exposure limits for specific phthalates, indicating safe maximum concentrations at micromolar levels [48].

During the last years, several innovative electroanalytical methods were successfully exploited for large scale monitoring of phtalates due to their high sensitivity, tunable selectivity and easy miniaturization [49]. Advances in biotechnology and material science, recently supported the fabrication of electrochemical sensors. As an example, the group of Zia and co-workers developed a real time detection technique, using planar interdigital sensors fabricated on the basis of thin film micro-electromechanical system (MEMS) semiconductor device, exploiting molecularly imprinted polymer. This method permitted the detection of di(2-ethylhexyl) phthalate in 1–50 ppb range in spiked water samples [50]. Noh and co-workers developed a microfluidic channel device interfaced to a surface-modified electrode (sensing probe) made of conducting phospholipid layers and an organic cationic molecule, to provide a hydrophobic and positively charged biomimetic surface able to interact with negatively charged target molecules. This biosensor was tested on real samples, plastic and mammalian cell cultures revealing LOD between ~12.5 and ~35.2 pM. As stated by the authors, this device is potentially applicable to different research fields and to monitor various EDCs in biological and environmental samples [51].

3.1.6. Hormones and xenohormones

Xenohormones represent a group of harmful, synthetic compounds whose concentration has recently increased due to the growing population and intensive farming. Council Directive 96/22/EC of 29 April 1996 prohibited the use of certain substances having a hormonal or thyrostatic action (e.g. testosterone, progesterone, zeranol) for animal farming, and identified strict practices for their exploitation. Among human steroid hormones, 17-α-ethynylestradiol (EE2), an oral contraceptive component, is an EDC of great concern, due to its harmful effect also at very low concentrations (<ng/L) [52]. This has alerted scientists to set up analytical techniques able to monitor hormones similar to EE2 such as 17-β-estradiol, estrone. Several electrochemical biosensors were developed for hormones analyses that exploited the specific binding ability of natural estrogen receptors to xenohormones [53].

In particular, a SPR BIACORE-based biosensor was developed as a high throughput screening method of endocrine receptors binding capacity without the use of radio- or fluorescence-labelled compounds. This method allowed to determine the dissociation constant estrone (4.29×10^{-9} M), 17-β-estradiol (4.04×10^{-10} M), estriol (8.35×10^{-10} M), tamoxifen (2.16×10^{-8} M), diethylstilbestrol (1.46×10^{-10} M), BPA (1.35×10^{-6} M) and NPs (7.49×10^{-6} M) [54]. In addition, a SPR technique was used to develop a label-free detection method using antibodies against the human growth hormone (anti-HGH). The specific binding of monoclonal anti-HGH antibody on the HGH-modified surface was examined in concentration ranging from 0.25 nM to 10 μM, with LOD for anti-HGH of 2.47 nM [55]. Habauzit and co-workers developed a new electrochemical biosensor based on estrogen receptor α for label-free detection of 17-β-estradiol. This biosensor is based on a receptor molecule (i.e. a specific aptamer, an antibody or an estrogen receptor) able to irreversibly interact with 17-β-estradiol and, consequently, to influence the electron charge transfer with a redox probe present in the measuring medium. Based on this principle and using electrochemical impedance spectroscopy, the achieved detection limits was in the order of picomolar concentrations (0.1 pM) [56].

A nanobiosensor for the determination of EE2 using 3-mercaptopropionic acid capped gallium selenide (Ga_2Se_3) nanoparticles in conjunction with cytochrome P450 iso-enzyme modified gold electrode has been developed [57]. It was characterised by very low detection potential of -220 mV. This makes it appropriate for the fabrication of energy serving and battery-operated sensor devices for field application. Moreover, the sensor exhibits very low detection limits for EE2 in aqueous medium.

Finally, following the strong endorsement about nanomaterials, an ultrasensitive electrochemical immunosensors based on mesoporous silica-gold nanoparticles-MWCNT nanocomposites and horseradish peroxidase-antibody-Prussian blue- MWCNT bioconjugates were developed for the detection of diethylstilbestrol, a synthetic hormone once used as an agricultural growth promoter, banned in EU in 1981 [58]. Under the optimized conditions, a calibration plot for diethylstilbestrol was obtained with a linear range between 3.3×10^{-7} mg/mL and 4.5×10^{-3} mg/mL ($r = 0.996$), with a LOD of 1.2×10^{-7} mg/mL (signal to noise ratio = 3).

3.1.7. Metals and organometals

Nowadays, toxic metals, commonly indicated as heavy metals are all systemic toxicants known to induce multiple organ damage, even at low levels of exposure.

TBT compounds are a sub-group of the trialkyl organotyn family of compounds. They have been extensively used as biocides in many fields such as wood treatment and preservation, antifouling of boats (in marine paints) antifungal action in textile and industrial water systems, wood pulp and paper mill systems. A biosensor based on genetically modified bacteria, with a specific TBT-sensitive chromosome fused with luciferase genes, was developed and found suitable for *on-line* and *in situ* TBTs measurements in water [59].

More recently, several optical and electrochemical biosensors were described as sensitive systems for multiplexing detection of heavy metal ions, including lead, mercury, zinc and copper ions by exploiting as biosensing element DNAzyme (Deoxyribozyme), or bacteria, or graphene/DNA complexes. These hybrid systems were designed and realised combining novel synthetic bio-materials with engineered biological components to obtain integrated biosensing devices with unique features such as very low detection limits and high selectivity in complex matrices [60–63].

4. *In vitro* bioassays and combined arrays

In vitro bioassays can complement chemical analysis for screening purposes enabling measurements of biological endpoints in a sample. This achievement allows to reveal the presence of active compounds not detectable by a compositional analysis, and to identify new contaminated sites.

In this context, a series of *in vitro* bioassays has been developed, and some of them, also standardized and commercialized, which are suitable to measure synergistic effects of different chemicals in a mixture [64]. Usually, these assays are mechanism-based and exploit as template either whole cells, proteins, or other active cell subcomponents to quantify EDCs or give global information on the estrogenic potency of the sample. In receptor-binding assays, the displacement of radio-labelled high affinity ligands is examined against known active compounds, providing analytical chemical information. This information is suitable for screening purposes, but does not allow to classify agonist or antagonist compounds, as they only determine binding parameters. On the contrary, reporter gene-assays enable monitoring of receptor activation through gene expression analyses. Usually, these tests are produced mainly by transfecting mammalian or yeast cell lines with specific receptors, and include a trans-activated signalling protein (reporter gene), e.g. Luciferase. These assays include the well-known and widely used cell-proliferation (E-SCREEN), chemical activated luciferase gene expression (CALUX) and yeast estrogen/androgen screen (YES/YAS) assays that provide information on proliferating activity, estrogenic, androgenic and thyroidal activities [65–67].

It is worth to mention that also ELISA has been widely used for EDCs monitoring, and more recently also used to quantify the expression of vitellogenin, a biomarker protein of estrogen-induced activity [68,69]. For example, a strategy based on protein-ligand interaction was adopted to detect estrogens and xenoestrogens with very high sensitivity. In particular, an enzyme-linked receptor assays (ELRAs) was developed for 17-β-estradiol analysis, yielding a detection limit of 0.1 μg/L [70]. A similar method for the recognition of an array of analytes with low detection limits was afterwards elaborated, exploiting the renowned versatility and specificity of this method [71].

As previously discussed, EDCs may produce genotoxicity, that is a primary risk factor for long-term effects such as carcinogenic and reproductive toxicology. Indeed, the genotoxicological monitoring may be a useful tool to estimate the genetic risk deriving by exposure to complex mixtures of chemicals. To this purpose, a plethora of bioanalytical *in vitro* assays has been already optimised and successfully used to quantify and characterize the mode of action of some EDCs, as demonstrated by a bulk of review literature [72,73]. In this context, it should be mentioned that a set of well-established (and some even standardized) bio-assays are based on bacteria and micro-organisms. For example, acute lethality tests with *Daphnia magna* have been standardized, based on the exposition of the organisms to target contaminants or real samples under controlled conditions, by counting living organisms after the required incubation time. These tests have been adopted by several organisations, including the International Organisation for Standardization (ISO) [74], or the USEPA [75].

Luminescent microorganisms have been also used in the production of several toxicity tests; the most used microorganism is the marine bioluminescent bacterium, *Vibrio fischeri*. The bioluminescence inhibition of the *V. fischeri* test has been standardized and it is commercially available in different versions [76].

Once properly set-up and standardized, similar bioassays are useful laboratory-diagnostic tools as alarm systems, capable to indicate a “contaminated state” in a controlled environmental site that, if necessary, could be further analysed by hyphenated mass spectrometry based chromatography for quantitative determination of unknown compounds.

Recently, the explosive development of material sciences, sensor and genomics technology led to the development of sensor arrays as complementary tool to bio-assays. According to the sample spot dimensions, these could be either macroarrays or microarrays. Microarray construction requires advanced technologies and robotics, mainly to ensure the appropriate immobilization of biological elements (DNA and RNA, or antibodies) mostly on semiconductor arrays as silicon oxides or indium thin oxides [77]. Based on their capability of gene expression profiling, microarrays give information on reaction mechanisms, allowing to differentiate compounds that directly interact with DNA from those that produce genotoxicity as a result of secondary reactions/mechanisms [78]. As an example, Jeong and co-worker, by combining microarray experiments and reproduction assays on *D. Magna*, highlighted a reduction in the reproductive activity in response to BPA exposure, and identified novel biomarkers for BPA toxicity, providing relevant insights into the genes and biological processes affected by this endocrine disrupting compound [79]. Furthermore, a combined approach of DNA-microarray and mechanism-specific toxicity assay exploiting *Zebrafish* embryos were performed to investigate the pollution of river sediments. The study proved the usefulness of the combined techniques to efficiently monitor aquatic toxicology. However, conclusions relating specific gene regulation patterns of biological effect are not consistently formulated, more studies being required [80].

5. Chromatographic techniques

The most widely used methods for the determination of various EDCs are high- performance liquid chromatography (HPLC), liquid chromatography coupled with electrochemical detection (LC-ED), liquid chromatography coupled with mass spectrometry (LC-MS), capillary electrophoresis (CE), gas chromatography (GC), and gas chromatography coupled with mass spectrometry (GC–MS). Other methods such as HPLC with fluorescence or diode array detection are less frequently used in the analysis of EDCs.

The improvements achieved during the last few years in terms of sensitivity are mostly due to the development of hyphenated chromatography-mass spectrometry techniques, which nowadays are the methods of choice for the determination of trace of organic analytes in environmental and biological samples. It can be stated that liquid chromatography combined with tandem mass spectrometer (LC-MS/MS) and GC-MS are the most often used techniques in determining EDCs [81,82], allowing the ion fragmentation needed for accurate and precise determination of analytes, and providing information on the molecular structure of the compounds. The use of MS/MS increases the selectivity and sensitivity of the method. Atmospheric pressure chemical and electrospray ionization are modes of ionization interfaces that are the most widely used with LC-MS/MS. The former is more suitable for determination of low or medium polar compounds, while the latter is conducted for polar analytes. Low or medium polar compounds are determined by atmospheric pressure chemical, and the analysis of polar analytes is conducted using electrospray ionization. One of the biggest difficulties with LC-MS/MS is interference due to the matrix, that causes the strengthening or suppression of the analyte signal, producing erroneous results. As a consequence, an efficient clean-up of complex environmental samples is required. In GC-MS analysis, the matrix effect occurs less frequently compared to LC-MS/MS, even if it could be more time-consuming when a derivatization step is necessary.

The huge interest in the application of LC-MS techniques has significantly stimulated developments and improvements in mass-analyser technology. When highly complex matrices are investigated, triple quadrupole MS is used for an unequivocal identification of the target compounds [83]. Recently, more advanced MS

technologies, such as time-of-flight or linear ion trap, have been introduced and represent powerful new identification tools. New hybrid quadrupole-time-of-flight mass spectrometry allows the acquisition of full-scan product-ion spectra, which provide the accurate mass of the product ion. Based on the product-ion spectra, the structural elucidation of unknown compounds as well as the identification of target compounds can be achieved with a much greater degree of certainty.

At the same time, the demand for shortening of the analytical run times (mainly called by high-throughput analysis) is growing. Three main modern approaches in HPLC methods enable the reduction of analytical time without compromising resolution and separation efficiency: the use of monolith columns, liquid chromatography conducted at high temperatures, and liquid chromatography at ultra-high pressures (UHPLC) [84,85].

A proper chromatographic multi-residue analysis is usually accompanied by a selective extraction step, the most frequently used being solid-phase extraction. In this field, the most recent tendencies encompass automation (by coupling sample preparation units with detection systems), evolution of advanced sorbents, and adoption of "green" reduced-solvent techniques. Miniaturisation has been a key factor to achieve these objectives. Microextraction techniques for liquid samples, including solid-phase microextraction, stir-bar sorptive extraction, and liquid-phase microextraction, allow high-enrichment factors and minimise solvent consumption [81]. For solid samples, the commonly used Soxhlet extraction procedure is going to be replaced by ultrasonic extraction, microwave-assisted extraction, and more recently by pressurised liquid extraction and supercritical fluid extraction [82]. These techniques offer short extraction time, decreased solvent consumption and sample handling.

As in the case of biosensors and bioassays, nanomaterials offer several advantages also in chromatography due to their high surface-to-volume ratio which can increase separation efficiency. Recently, these nanomaterials have been applied as stationary phases both in sample pre-concentration and chromatographic columns, e.g. monolith columns [84].

The main advantages associated to chromatographic determination of EDCs in environmental matrices are generally related to high specificity, sensitivity and multi-residue analysis. Multi-residue methods have the ability to test for a broad range of chemicals in a single sample and therefore are useful for laboratory screening purposes. Specifically, high resolution separation, nanomolar sensitivity, and dynamic range of response are distinguishable features of these analytical methods. Moreover, chromatographic methods are validated and have a wide application in standardized analytical protocols. Several examples of chromatographic methods applied to routine analysis and complying with sensitivity requests imposed by in force regulations are given below. For instance, this allegation is supported by the USEPA Method 1613B that consists in high-resolution gas chromatography mass spectrometry (HRGCMS) applied to dioxins assessment at ultra-low detection limits in various matrices: feed, food, environmental samples etc. Furthermore, triple quadrupole GC-MS enables the detection of more than 91% of EU regulated pesticides in a routine analysis with LOD ~ 5 ng/g (which is lower than the current EU maximum residue levels). The challenging issue of hormones detection presented in EPA 539 method is based on LC-MS/MS used in both negative and positive mode, thus providing LOD of 0.1 part per trillion for few hormones. The routines application of this method has been driven by current advances in the development of chromatographic technologies based on the exploitation of new materials for column fabrication. Despite all achievements of the new technologies applied in development of chromatographic techniques some intrinsic disadvantages limit their exploitation for *in field* screening methods and as alarm analytical tools, including high costs, highly skilled staff, sample specific pre-treatment and impossibility to perform real-time analyses. Furthermore, these techniques did not provide information on the biological activity of EDCs that are mandatory for evaluating health risks.

6. Discussion and future perspectives

Strategies adopted by research institutes worldwide should exploit validated methods for screening and testing chemicals to identify/quantify potential EDCs, determine adverse effects, dose-response, short- and long-term effects, evaluate risk, and finally manage risk under current laws. The efforts should be focused on the adoption of a universal line-of-attack to foster the development of detailed protocols to determine EDCs through alternative analytical techniques, define a priority list of EDCs that are relevant for environmental control, and provide a predictive model on the behaviour and fate of EDCs in environment. Such assessments call for a comprehensive and reliable strategy able to characterize the extent of contamination at relevant spatial scales and in terms of its eco-effects, and to provide inclusive information with the aim to obtain a more representative picture of the quality of the environment.

The best choice for laboratory screening purposes is hyphenated mass-spectrometry-based chromatography that provides excellent sensitivity and selectivity for qualitative and quantitative determination, displaying also a wide range of advantages in terms of commercial availability, reuse, specificity, and standardization. The main challenge to be faced when using chromatographic techniques is generally related to multi-residue/multi-analytes analysis since the analytical protocols have to take into account both the analyte physical-chemical specificity and the required sensitivity. Nevertheless, the major drawbacks of chromatography are associated to high-costs, requirement of skilled-operators, sample pre-treatment, and extensive use of solvents with negative impact on the environment. In this context, the exploitation of nanomaterials allowed to mitigate consumption of chemicals, but further improvements are still required. Furthermore, chromatographic methods are not suitable for *in field* studies, continuous monitoring, and biological evaluation of EDC's effects on living organisms. Indeed, a proper risk assessment requires information not only on chemical composition of a sample, but also knowledge on the magnitude and mechanisms of endocrine homeostasis, and how it is impacted by xenobiotics. These tasks can be realized only by using a well-chosen bioassays battery. In recent years, there has been a significant increase in the importance of the biological methodologies in environmental research because of their numerous advantages. *In vitro* assays allow to evaluate the hazard and risk assessment of EDCs, which should follow a specific toxicology package taking into account intrinsically additive effects on endogenous hormones activity, and their inherently low threshold activity. They provide high specific responses for a wide range of different EDCs, achieve limits of detection in the micromolar range and enable high throughput screening, even if they are poorly selective [65]. Furthermore, from a biological point of view, bioassays are highly efficient in determining molecular mechanisms and signal networks underlying hormonal end-point [66]. In addition, many bioassays inspired the development of affinity bio-sensors for EDCs identification and quantification. As an example, biomimetic aptamer-based sensors represent an innovative and sensitive approach that revealed useful to detect estrogens or BPA [85].

Technologies like biosensors are demonstrating their suitability for *in-situ* and spatiotemporal data acquisition on EDCs, enabling to quantify concentrations of single or classes of contaminants, identify known and also unknown chemicals, and determine their biological effects (toxicity, cytotoxicity, genotoxicity or endocrine disrupting effects) [86]. By coupling micro/nanofluidics and

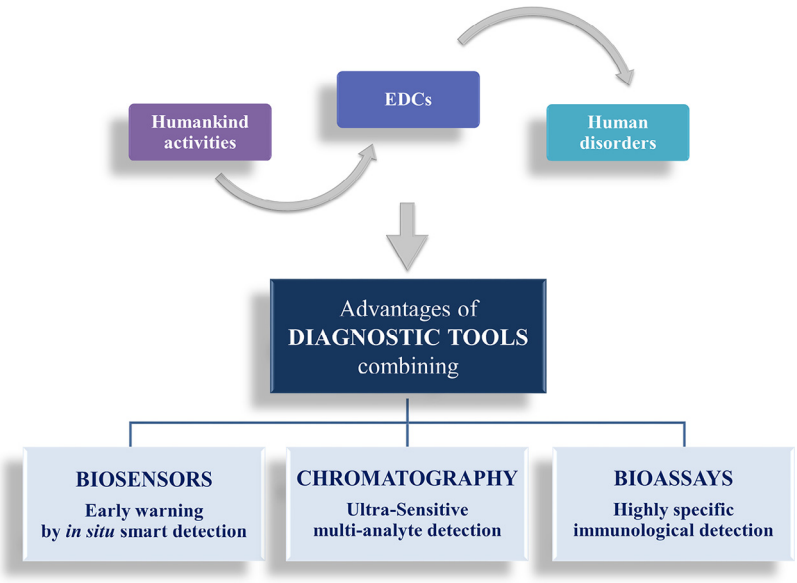


Fig. 2. Schematic sketch of a multidisciplinary integrated measuring chain for EDCs monitoring.

lab-on-chip technologies to biosensor technology it would be possible to perform *in-situ*, continuous or semi-continuous analyses, reducing possible errors deriving by collection and transportation of samples, and enhancing timely intervention strategy [87]. In addition, progress in nanotechnology, material science, and protein engineering provided the opportunity to design new materials with novel properties and functions for a wide range of applications. These novel bio-inspired materials offer numerous possible improvements in terms of response tuning, signal processing, and direct interface with electronic devices, enhancing biosensor potential in terms of sensitivity, costs, and *in field* use [88–91]. Finally, remote sensing technologies and communication networks are permitting more effective monitoring, resource management, and mitigation of environmental risks, thus reducing costs and increasing access to information by scientists as well as governmental agencies [92].

In this contest, biosensors seem a valuable complementary technology either to standard chemical methods or *in vitro* tests in terms of miniaturization, portability, handy use, fast response, real-time detection, and assessment of biological effects. On the other hand, the sensitivity of this method is not comparable yet to the very low detection limits of chromatographic methods [93], even if recent efforts in computational biology, protein engineering and synthetic biology enable the development of new molecules with more high specificity, sensitivity and robustness [94]. Similarly, the exploitation of newly characterised nanomaterials including nanoparticles and quantum dots are improving selectivity and LOD, while nanotubes, nanowires, and carbon-based materials, such as graphite, graphene allow to optimise immobilization protocols and enhance electrochemical transduction [94]. Nanomaterial-engineered biosensors could be seriously taken into consideration as reliable methods to be validated for *in field* screening purposes, even if, for a routinely use improvements of regenerative property and long-term usage are mandatory. Last, but not least, biosensors represent a "green" option compared to the even most advanced chemical methods, requiring less amount of solvents, and reducing waste.

7. Conclusions

It seems clear that a multidisciplinary approach can represent the right way to face the most important challenges in the management of EDCs. We hence encourage the adoption of an integrated measuring system combining standard chemical methods, biosensors and bioassays (Fig. 2) with emerging technologies including microfluidics, molecular engineering, nanotechnology, and information and communication technology. This multi-tiered approach could surely help to obtain more reliable and comprehensive information to guide the accountable agencies to promulgate adequate regulations and legislations for prevention/monitoring policy.

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References

[1] J.M. Vogel, Perils of paradigm: complexity, policy design, and the endocrine disruptor screening program environmental health: a global access science source, Environ. Health 4 (2) (2005) 1–11.
[2] R.T. Zoeller, T.R. Brown, L.L. Doan, A.C. Gore, N.E. Skakkebaek, A.M. Soto, et al., Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society, Endocrinology 153 (9) (2012) 4097–4110.
[3] S. De Coster, N. van Larebeke, Endocrine-Disrupting Chemicals: associated disorders and mechanisms of action, J. Environ. Public Health 713696 (2012) (2012) 1–52.
[4] Document SEC, 1635, COMMISSION STAFF WORKING DOCUMENT on the implementation of the "Community Strategy for Endocrine Disrupters" – a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706), (COM (2001) 262) and (SEC (2004) 1372). 2007.
[5] L.N. Vandenberg, T. Colborn, T.B. Hayes, J.J. Heindel, D.R. Jacobs, D.-H. Lee Jr., et al., Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses, Endocr. Rev. 33 (3) (2012) 378–455.
[6] B. Georgescu, C. Georgescu, S. Dărăban, A. Bouaru, S. Paşcalău, Heavy metals acting as endocrine disrupters, SPASB 44 (2011) 89–93.
[7] C.L. McGinnis, J.F. Crivello, Elucidating the mechanism of action of tributyltin (TBT) in zebrafish, Aquat. Toxicol. 103 (1–2) (2011) 25–31.
[8] N. Salgueiro-González, M.J. López de Alda, S. Muniategui-Lorenzoa, D. Prada-Rodríguez, D. Barceló, Analysis and occurrence of endocrine-disrupting chemicals in airborne particles, Trends Analyt. Chem. 66 (2015) 45–52.
[9] T.B. Hayes, L.L. Anderson, V.R. Beasley, S.R. de Solla, T. Iguchi, H. Ingraham, et al., Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes, J. Steroid Biochem. Mol. Biol. 127 (1–2) (2011) 64–73.
[10] G. Nalbone, A. Cicolella, S. Laot-Cabon, Endocrine disruptors and metabolic diseases: a major public health challenge, Sante Publique 25 (1) (2012) 45–49.
[11] R.R. Newbold, E. Padilla-Banks, R.J. Snyder, T.M. Phillips, W.N. Jefferson, Developmental exposure to endocrine disruptors and the obesity epidemic, Reprod. Toxicol. 23 (3) (2007) 290–296.

[12] J.D. Meeker, Exposure to environmental endocrine disruptors and child development, Arch. Pediatr. Adolesc. Med. 166 (10) (2012) 952–958.
[13] F. Arduini, F. Ricci, C.S. Tuta, D. Moscone, A. Amine, G. Palleschi, Detection of carbamic and organophosphorous pesticides in water samples using a cholinesterase biosensor based on Prussian Blue-modified screen-printed electrode, Anal. Chim. Acta 580 (2) (2006) 155–162.
[14] V. Scognamiglio, D. Raffi, M. Lambreva, G. Rea, A. Tibuzzi, G. Pezzotti, et al., *Chlamydomonas reinhardtii* genetic variants as probes for fluorescence sensing system in detection of pollutants, Anal. Bioanal. Chem. 394 (4) (2009) 1081–1087.
[15] B.I. Rejeb, F. Arduini, A. Arvinte, A. Amine, M. Gargouri, L. Micheli, et al., Development of a bio-electrochemical assay for AFB1 detection in olive oil, Biosens. Bioelectron. 24 (7) (2009) 1962–1968.
[16] D. Compagnone, A. Ricci, M. Del Carlo, M. Chiarini, A. Pepe, C.L. Sterzo, New poly(aryleneethynylene)s as optical active platforms in biosensing. Selective fluorescent detection of Hg(II) obtained by the use of aminoacidic groups anchored on conjugated backbones, Microchim Acta. 170 (2010) 313–319.
[17] V. Scognamiglio, I. Pezzotti, G. Pezzotti, J. Cano, I. Manfredonia, K. Buonasera, et al., Towards an integrated biosensor array for simultaneous and rapid multi-analysis of endocrine disrupting chemicals, Anal. Chim. Acta 751 (2012) 161–170.
[18] F. Arduini, A. Amine, D. Moscone, G. Palleschi, Biosensors based on cholinesterase inhibition for insecticides, nerve agents and aflatoxin B1 detection (review), Microchim Acta. 170 (3–4) (2010) 193–214.
[19] A.Y. El-Moghazy, E.A. Soliman, H.Z. Ibrahim, T. Noguer, J.L. Marty, G. Istamboulie, Ultra-sensitive biosensor based on genetically engineered acetylcholinesterase immobilized in poly (vinyl alcohol)/Fe–Ni alloy nanocomposite for phosmet detection in olive oil, Food Chem. 203 (2016) 73–78.
[20] Y. Zhang, M.A. Arugula, M. Wales, J. Wild, A.L. Simonian, A novel layer-by-layer assembled multi-enzyme/CNT biosensor for discriminative detection between organophosphorus and non-organophosphrus pesticides, Biosens. Bioelectron. 67 (2015) 287–295.
[21] L.F. Sgobbi, V.D. Pinho, M.F. Cabral, A.C.B. Burtoloso, S.A.S. Machado, Hydrazone molecules as mimics for acetylcholinesterase. A new route towards disposable biosensors for pesticides?, Sens. Actuators B Chem. 182 (2013) 211–216.
[22] B.C. Janegitz, F. Campanhã Vicentini, V.B. dos Santos, T.B. Guerreiro, O. Fatibello-Filho, A portable microcontrolled pumping flow system developed to in loco determination of diuron at nanomolar levels employing a disposable biosensor, J. Anal. Bioanal. Tech. 6 (3) (2015) 1–8.
[23] F. Arduini, D. Neagu, V. Scognamiglio, S. Patarino, D. Moscone, G. Palleschi, Automatable flow system for paraoxon detection with an embedded screen-printed electrode tailored with butyrylcholinesterase and prussian blue nanoparticles, Chemosensors 3 (2015) 129–145.
[24] A. Mulchandani, W. Chen, P. Mulchandani, J. Wang, K.R. Rogers, Biosensors for direct determination of organophosphate pesticides, Biosens. Bioelectron. 16 (2001) 225–230.
[25] D.J. Swainsbury, V.M. Friebe, R.N. Frese, M.R. Jones, Evaluation of a biohybrid photoelectrochemical cell employing the purple bacterial reaction centre as a biosensor for herbicides, Biosens. Bioelectron. (2014) 172–178.
[26] M.D. Lambreva, M.T. Giardi, I. Rambaldi, A. Antonacci, S. Pastorelli, I. Bertalan, et al., A powerful molecular engineering tool provided efficient Chlamydomonas mutants as bio-sensing elements for herbicides detection, PLoS ONE 8 (4) (2013) e61851.
[27] C.J. Choi, J.A. Berges, E.B. Young, Rapid effects of diverse toxic water pollutants on chlorophyll a fluorescence: variable responses among freshwater microalgae, Water Res. 46 (8) (2012) 2615–2626.
[28] G. Rea, F. Polticelli, A. Antonacci, V. Scognamiglio, P. Katiyar, S.A. Kulkarni, et al., Structure-based design of novel *Chlamydomonas reinhardtii* D1-D2 photosynthetic proteins for herbicide monitoring, Prot. Sci. 18 (10) (2009) 2139–2151.
[29] V. Scognamiglio, P. Stano, F. Polticelli, A. Antonacci, M.D. Lambreva, G. Pochetti, et al., Design and biophysical characterization of atrazine-sensing peptides mimicking the Chlamydomonas reinhardtii plastoquinone binding niche, Phys. Chem. Chem. Phys. 15 (31) (2013) 13108–13115.
[30] M.T. Giardi, V. Scognamiglio, G. Rea, G. Rodio, A. Antonacci, M.D. Lambreva, et al., Optical biosensors for environmental monitoring based on computational and biotechnological tools for engineering the photosynthetic D1 protein of *Chlamydomonas reinhardtii*, Biosens. Bioelectron. 25 (2) (2009) 294–300.
[31] E. Peña-Vázqueza, E. Maneiro, C. Pérez-Conde, M.C. Cruz Moreno-Bondi, E. Costas, Microalgae fiber optic biosensors for herbicide monitoring using sol–gel technology, Biosens. Bioelectron. 24 (2009) 3538–3543.
[32] M.M. Rodríguez-Delgado, G.S. Alemán-Nava, G.M. Rodríguez-Delgado, G. Dieck-Assad, S.O. Martínez-Chapa, D. Barceló, et al., Laccase-based biosensors for detection of phenolic compounds, Trends Analyt. Chem. 74 (2015) 21–45.
[33] N. Zehani, P. Fortgang, M.S. Lachgar, A. Baraket, M. Arab, S.V. Dzyadevych, et al., Highly sensitive electrochemical biosensor for bisphenol A detection based on a diazonium-functionalized boron-doped diamond electrode modified with a multi-walled carbon nanotube-tyrosinase hybrid film, Biosens. Bioelectron. 74 (2015) 830–835.
[34] European Commission, Community strategy for endocrine disrupters in December 1999 (COM (1999)706). 1999.
[35] K.K. Reza, M.A. Ali, S. Srivastava, V.V. Agrawal, A.M. Biradar, Tyrosinase conjugated reduced graphene oxide based biointerface for bisphenol A sensor, Biosens. Bioelectron. 74 (2015) 644–651.
[36] E. Han, Y. Yang, Z. He, J. Cai, X. Zhang, X. Dong, Development of tyrosinase biosensor based on quantum dots/chitosan nanocomposite for detection of phenolic compounds, Anal. Biochem. 486 (2015) 102–106.
[37] M. Lv, M. Wei, F. Rong, C. Terashima, A. Fujishima, Z.-Z. Gua, Electrochemical detection of catechol based on as-grown and nanograss array boron-doped diamond electrodes, Electroanalysis 22 (2) (2010) 199–203.
[38] R.S. Alkasir, M. Ganesana, Y.H. Won, L. Stanciu, S. Andreescu, Enzyme functionalized nanoparticles for electrochemical biosensors: a comparative study with applications for the detection of bisphenol A, Biosens. Bioelectron. 26 (1) (2010) 43–49.
[39] Ö. Şenyurt, F. Eyidoğan, R. Yılmaz, M.T. Öz, V.C. Özalp, Y. Arıca, et al., Development of a paper-type tyrosinase biosensor for detection of phenolic compounds, Biotechnol. Appl. Biochem. 62 (1) (2015) 132–136.
[40] L.-S. Hua, C.-C. Fonga, L. Zoua, W.-L. Wongc, K.-Y. Wongc, R.S.S. Wud, et al., Label-free detection of endocrine disrupting chemicals by integrating a competitive binding assay with a piezoelectric ceramic resonator, Biosens. Bioelectron. 53 (2014) 406–413.
[41] J.V. Samsonova, N.A. Uskova, A.N. Andresyuk, M. Franek, C.T. Elliott, Biacore biosensor immunoassay for 4-nonylphenols: assay optimization and applicability for shellfish analysis, Chemosphere 57 (8) (2004) 975–985.
[42] A.V. Demchenko, V.G. Melnik, N.F. Starodub, Thermal biosensor for detecting nonylphenol in the environment, Ukr. Biokhim. Zh. 79 (5) (2007) 212–215.
[43] J. Chobtang, I.J. De Boer, R.L. Hoogenboom, W. Haasnoot, A. Kijlstra, B.G. Meerburg, The need and potential of biosensors to detect dioxins and dioxin-like polychlorinated biphenyls along the milk, eggs and meat food chain, Sensors. 11 (12) (2011) 11692–11716.
[44] K. Eichbaum, M. Brinkmann, L. Nuesser, S. Buchinger, G. Reifferscheid, G. Codling, et al., Bioanalytical and instrumental screening of the uptake of sediment-borne, dioxin-like compounds in roach (Rutilus rutilus), Environ. Sci. Pollut. Res. Int. (2016) 1–15.
[45] S. Centi, E. Silva, S. Laschi, I. Palchetti, M. Mascini, Polychlorinated biphenyls (PCBs) detection in milk samples by an electrochemical magneto-immunosensor (EMI) coupled to solid-phase extraction (SPE) and disposable low-density arrays, Anal. Chim. Acta 594 (2007) 9–16.
[46] S. Kurosawa, H. Aizawa, J.W. Park, Quartz crystal microbalance immunosensor for highly sensitive 2,3,7,8-tetrachlorodibenzo-p-dioxin detection in fly ash from municipal solid waste incinerators, Analyst 130 (2005) 1495–1501.
[47] M. Mascini, A. Macagnano, D. Monti, M. del Carlo, R. Paolesse, B. Chen, et al., Piezoelectric sensors for dioxins: A biomimetic approach, Biosens. Bioelectron. 20 (2004) 1203–1210.
[48] S. Net, R. Sempéré, A. Delmont, A. Paluselli, B. Ouddane, Occurrence, fate, behaviour and ecotoxicological state of phthalates in different environmental matrices, Environ. Sci. Technol. 49 (7) (2015) 4019–4035.
[49] M.S. Qureshi, A.R.B.M. Yusoff, M.D.H. Wirzal, J. Barek, H.I. Afridi, Z. Üstündag, Methods for the determination of endocrine disrupting phthalate esters, Crit. Rev. Anal. Chem. 46 (2) (2016) 146–159.
[50] A.I. Zia, S.C. Mukhopadhyay, P.L. Yu, I.H. Al-Bahadly, C.P. Gooneratne, J. Kosel, Rapid and molecular selective electrochemical sensing of phthalates in aqueous solution, Biosens. Bioelectron. 67 (2015) 342–349.
[51] H.B. Noh, N.G. Gurudatt, M.S. Won, Y.B. Shim, Analysis of phthalate esters in mammalian cell culture using a microfluidic channel coupled with an electrochemical sensor, Anal. Chem. 87 (14) (2015) 7069–7077.
[52] M. Muller, F. Rabenoellina, P. Balaguer, D. Patureau, K. Lemenach, K. Budzinski, et al., Chemical and biological analysis of endocrine-disruptors hormones and estrogenic activity in an advanced sewage treatment plant, Env Toxicol and Chem 27 (8) (2008) 1649–1658.
[53] E.B. Bahadır, M.K. Sezgintürk, Electrochemical biosensors for hormone analyses, Biosens. Bioelectron. 68 (2015) 62–71.
[54] M. Usami, K. Mitsunaga, Y. Ohno, Estrogen receptor binding assay of chemicals with a surface plasmon resonance biosensor, J. Steroid Biochem. Mol. Biol. 81 (2002) 47–55.
[55] A. Kausaite-Minkstimiene, A. Ramanaviciene, A. Ramanavicius, Surface plasmon resonance biosensor for direct detection of antibodies against human growth hormone, Analyst 134 (2009) 2051–2057.
[56] D. Habauzit, S. Bayle, D. Benimelis, J. Chopineau, B. Roig, Impact of biochemical design on estrogen receptor/estrogen response element interaction by surface plasmon resonance technology, Arch. Biochem. Biophys. 541 (2014) 61–66.
[57] E. Nxusani, P.M. Ndangili, R.A. Olowu, A.N. Jijana, T. Waryo, N.R.F. Jahed, et al., 3-Mercaptopropionic acid capped Ga2Se3nanocrystal-CYP3A4 biosensor for the determination of 17-alpha-ethinyl estradiol in water, Nano Hybrids 1 (2012) 1–22.
[58] S. Liu, Q. Lin, X. Zhang, X. He, X. Xing, W. Lian, et al., Electrochemical immunosensor based on mesoporous nanocomposites and HRP-functionalized nanoparticles bioconjugates for sensitivity enhanced detection of diethylstilbestrol, Sens. Actuators B 166 (2012) 562–568.
[59] G. Thouand, H. Horry, M.J. Durand, P. Picart, L. Bendriaa, P. Daniel, et al., Development of a biosensor for on-line detection of tributyltin with a recombinant bioluminescent *Escherichia coli* strain, Appl. Microbiol. Biotechnol. 62 (2003) 218–225.
[60] C.S. Wu, M.K. Khaing, X. Oo, X. Fan, Highly sensitive multiplexed heavy metal detection using quantum-dot-labeled DNAzymes, ACS Nano 4 (10) (2010) 5897–5904.
[61] S. Ravikumar, I. Ganesh, I.K. Yoo, S.H. Hong, Construction of a bacterial biosensor for zinc and copper and its application to the development of multifunctional heavy metal adsorption bacteria, Process Biochem. 47 (2012) 758–765.

[62] Y. Zhang, H. Zhao, Z. Wu, Y. Xue, X. Zhang, Y. He, et al., A novel graphene-DNA biosensor for selective detection of mercury ions, Biosens. Bioelectron. 48 (2013) 180–187.
[63] I. Gammoudi, H. Tarbague, A. Othmane, D. Moynet, D. Rebière, R. Kalfat, et al., Love-wave bacteria-based sensor for the detection of heavy metal toxicity in liquid medium, Biosens. Bioelectron. 26 (4) (2010) 1723–1726.
[64] V. Kokkali, W. van Delft, Overview of commercially available bioassays for assessing chemical toxicity in aqueous samples, Trends Analyt. Chem. 61 (2014) 133–155.
[65] C. Arnold, New tool for EDC research: in vivo assay screens for estrogenic effects, Environ. Health Perspect. 123 (4) (2015) A95.
[66] G. Kerdivel, D. Habauzit, F. Pakdel, Assessment and molecular actions of endocrine-disrupting chemicals that interfere with estrogen receptor pathways, Int. J. Endocrinol. 2013 (2013) 501851.
[67] M.N. Jacobs, S.C. Laws, K. Willett, P. Schmieder, J. Odum, T.F. Bovee, In vitro metabolism and bioavailability tests for endocrine active substances: what is needed next for regulatory purposes?, ALTEX 30 (3) (2013) 331–351.
[68] N.D. Denslow, M.C. Chow, K.J. Kroll, L. Green, Vitellogenin as a biomarker of exposure for estrogen and estrogen mimics, Ecotoxicol 8 (1999) 385–398.
[69] B.M. Nilsen, K. Berg, J.K. Eidem, S.I. Kristiansen, F. Brion, J.M. Porcher, et al., Development of quantitative vitellogenin-ELISAs for fish test species used in endocrine disruptor screening, Anal. Bioanal. Chem. 378 (2004) 621–633.
[70] M. Seifert, S. Haindl, B. Hock, Development of an enzyme linked receptor assay (ELRA) for estrogens and xenoestrogens, Anal. Chim. Acta 386 (3) (1999) 191–199.
[71] Y. Xu, Z. He, Q. He, Y. Qiu, B. Chen, J. Chen, et al., The use of clonable peptide-MBP fusion protein as mimetic coating antigens in the standardized immunoassay for mycotoxin ochratoxin A, J. Agric. Food Chem. 62 (35) (2014) 8830–8836.
[72] M. Grimaldi, A. Boulahtouf, V. Delfosse, E. Thouennon, W. Bourguet, P. Balaguer, Reporter cell lines for the characterization of the interactions between human nuclear receptors and endocrine disruptors, Front Endocrinol 6 (2015).
[73] A.J. Murk, E. Rijntjes, B.J. Blaauboer, R. Clewell, K.M. Crofton, M.M. Dingemans, et al., Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals, Toxicol. In Vitro 27 (4) (2013) 1320–1346.
[74] ISO, Water Quality: Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera, Crustacea) e Acute Toxicity Test ISO 6341, Geneva, Switzerland. 1996.
[75] ISO, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, fifth ed. U.S. Environmental Protection Agency Office of Water (4303T), 1200 Pennsylvania Avenue, NW Washington, DC 20460. EPA-821-R-02-012. 2002.
[76] ISO, Water Quality Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio fischeri (Luminescent Bacteria Test) Part 2: Method Using Liquid-Dried Bacteria. ISO 11348-2, Geneva, Switzerland. 1998.
[77] S.B. Nimse, K. Song, M.D. Sonawane, D.R. Sayye, T. Kim, Immobilization techniques for microarray: challenges and applications, Sensors (Basel) 14 (12) (2014) 22208–22229.
[78] R.K. Newton, M. Aardema, J. Aubrecht, The utility of DNA microarrays for characterizing genotoxicity, Environ. Health Perspect. 112 (4) (2004) 420–422.
[79] S.W. Jeong, S.M. Lee, S.S. Yum, T. Iguchi, Y.R. Seo, Genomic expression responses toward bisphenol-A toxicity in Daphnia magna in terms of reproductive activity, Mol Cell Toxicol 9 (2) (2013) 149–158.
[80] T. Kosmehl, J.C. Otte, L. Yang, J. Legradi, K. Bluhm, C. Zinsmeister, et al., A combined DNA-microarray and mechanism-specific toxicity approach with zebrafish embryos to investigate the pollution of river sediments, Reprod. Toxicol. 33 (2) (2012) 245–253.
[81] Z. Sosa-Ferrera, C. Mahugo-Santana, J.J. Santana-Rodríguez, Analytical methodologies for the determination of endocrine disrupting compounds in biological and environmental samples, Biomed Res. Int. (2013) 1–23.
[82] B. Singh, A. Kumar, A.K. Malik, Recent advances in sample preparation methods for analysis of endocrine disruptors from various matrices, Crit. Rev. Anal. Chem. 44 (3) (2014) 255–269.
[83] K. Wille, H.F. De Brabander, E. De Wulf, P. Van Caeter, C.R. Janssen, L. Vanhaecke, Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment, Trends Analyt. Chem. 35 (2012) 87–108.
[84] W.C. Tseng, K.C. Hsu, C.S. Shiea, Y.L. Huang, Recent trends in nanomaterial-based microanalytical systems for the speciation of trace elements: a critical review, Anal. Chim. Acta 884 (2015) 1–18.
[85] G. Contreras Jiménez, S. Eissa, A. Ng, H. Alhadrami, M. Zourob, M. Siaj, Aptamer-based label-free impedimetric biosensor for detection of progesterone, Anal. Chem. 87 (2) (2015) 1075–1082.
[86] M. Gavrilescu, K. Demnerová, J. Aamand, S. Agathos, F. Fava, Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation, N biotechnol. 32 (1) (2015) 147–156.
[87] J.C. Jokerst, J.M. Emory, C.S. Henry, Advances in microfluidics for environmental analysis, Analyst 137 (2012) 24–34.
[88] Y. Shao, W. Jun, W. Hong, L. Jun, I.A. Aksay, L. Yuehe, Graphene based electrochemical sensors and biosensors:a review, Electroanalysis 22 (10) (2010) 1027–1036.
[89] T. Sun, G. Qing, Biomimetic smart interface materials for biological applications, Adv. Mater. 23 (12) (2011) H57–H77.
[90] J. Liu, Y. Liu, X. Yang, K. Wang, Q. Wang, H. Shi, et al., Exciton energy transfer-based fluorescent sensing through aptamer-programmed self-assembly of quantum dots, Anal. Chem. 85 (22) (2013) 11121–11128.
[91] V. Scognamiglio, A. Antonacci, M.D. Lambreva, S.C. Litescu, G. Rea, Synthetic biology and biomimetic chemistry as converging technologies fostering a new generation of smart biosensors, Biosens. Bioelectron. 74 (2015) 1076–1086.
[92] L.M. Hilty, B. Page, Information technology and renewable energy–modelling, simulation, decision support and environmental assessment, Environ. Impact Assess. Rev. 52 (2015) 1–68.
[93] S. Rodriguez-Mozaz, M.J.L. de Alda, D. Barceló, Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography–mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water, J. Chromatogr. A 1152 (1) (2007) 97–115.
[94] W. Zhang, A.M. Asiri, D. Liu, D. Du, Y. Lin, Nanomaterial-based biosensors for environmental and biological monitoring of organophosphorus pesticides and nerve agents, Trends Analyt. Chem. 54 (2014) 1–10.