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Estrogenic Compounds: Chemical Characteristics, Detection Methods, Biological and Environmental Effects

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Abstract Several chemical compounds are being studied for their capacities to cause imbalances in several biological systems. Some of those are able to affect the endocrine system and are known as endocrine disruptors. Many negative effects can be induced in the organisms by the action of these chemicals, highlighting the capacity to cause a decrease in the fertility rate, sex inversion, and problems in embryonic development and even cancer in humans. Those contaminants can be found in different environmental conditions, in groundwater, sediments, residual waters, sludges, and even in drinking water. The purpose of this review is to provide a general overview of the main estrogenic endocrine disruptors and their effects on living organisms, showing the most frequently used tools to detect these contaminants in environmental matrices. According to the data found, there is a need to develop more studies and improve the techniques, in order to effectively determine the mechanism of action of these contaminants and, thus, establish appropriate strategies for their removal from the environment and reduce their actions on living beings.

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Abbreviations

17αEE2	17α-Ethinylestradiol
17βE2	17β-Estradiol
4-CP	4-Cumylphenol
APEO	Alkylphenol ethoxylates
APS	Alkylphenols
BBzP	Butylbenzyl phthalate
BCFs	Bioconcentration Factors
BPA	Bisphenol A
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DES	Diethylstilbestrol
DiBP	Diisobutyl phthalate
DiDP	Diisodecyl phthalate
DiNP	Diisononyl phthalate
DMP	Dimethyl phthalate
DnBP	Di-n-butyl phthalate
DOP	Di-n-octhyl phthalate
E1	Estrone
E3	Estriol
ED	Endocrine disruptor
EDsA	Androgenic compounds
EDsE	Estrogenic endocrine disruptors
EDsT	Thyroidal compounds
EEQ	Estradiol equivalent
EFSA	European Food Safety Authority
ER	Estrogen receptor
ERBA	Estrogen receptor binding assay

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GSI	Gonadal somatic index
GSI	Somatic index
HIS	Hepatosomatic index
LOAEL	Lowest observed adverse effect level
NMDRC	Nonmonotonic dose-response curve
NP	Nonylphenols
NP1EO	Nonylphenol-mono-ethoxylate
NP2EO	Ethoxylate
OP	Octylphenols
PAHs	Polycyclic aromatic hydrocarbons
PBB	Polybrominated biphenyls
PCB	Polychlorinated biphenyls
PVC	Polyvinyl chloride
SS	Sewage sludge
STP	Sewage treatment plant
TCDD	2,3,7,8-Tetrachloridibenzo-p-dioxin
TDI	Tolerable daily intake
VTG	Vitellogenin
WTPs	Water treatment plants
WWTP	Wastewater treatment plants
YES	Yeast estrogen screen

1 Introduction

The great variety of chemical compounds found in the environment may lead to many detrimental disorders in living organisms, due to the possibility of the interaction of these compounds with cellular components. Some of them can also induce hormonal responses, similar to those produced naturally by organisms.

Several natural and synthetic compounds, such as pharmaceutical drugs, pesticides, phytoestrogens, complex mixtures derived from effluents, some industrial wastes and several metals, can bind to estrogen receptors (Hilscherova et al. 2000). When compounds interact with estrogen receptors, they can interfere on the synthesis, secretion, transport, binding, action, or elimination of natural hormones, damaging the organic responses: blocking the effects of the hormones and promoting or inhibiting the endocrine system. The compounds that have these characteristics are called endocrine disruptors (EDs) (Rasier et al. 2006; USEPA 2011).

Campbell et al. (2006) cite many natural and synthetic compounds that act as EDs, among them are agrochemicals (atrazine, dieldrin, and toxaphene), surfactants (alkylphenol ethoxylates (APEOs)), natural

hormones (17β-estradiol (17βE2), estrone (E1), and estriol (E3)), synthetic hormones (17α-ethinylestradiol (17αEE2), diethylstilbestrol (DES)), and phytoestrogens (isoflavones and coumestrol). Moreover, other industrial compounds such as the bisphenol A (BPA), dioxin (2,3,7,8-TCDD), nonylphenols (NP), octylphenols (OP), phthalates, styrenes, polybrominated biphenyls (PBB), and polychlorinated biphenyls (PCB) (Diamanti-Kandarakis et al. 2010), and metals such as cadmium, lithium, barium, chromium, arsenic, and antimony are also inducers of alterations in the endocrine systems (Choe et al. 2003; Diamanti-Kandarakis et al. 2010).

Trudeau and Tyler (2007) separated the EDs in three different classes: androgenic compounds (EDsA), which mimic or block natural testosterone; thyroidal compounds (EDsT), which cause direct and indirect effects on the thyroid gland; and estrogenic compounds (EDsE), which mimic or block natural estrogen.

Estrogenic compounds are those that possess similar properties to the hormone 17βE2 (main natural estrogen produced by the ovaries), the effects of which are induced by interactions with the estrogen receptor and cell systems (Schug et al. 2011). In addition, according to Kiyama and Wada-Kiyama (2015), EDsE can act on two main pathways: the genomic path, linked to target gene transcription, and the nongenomic path, linked to signaling of specific membrane receptors. These contaminants are routinely present in the environment and can induce estrogenic effects, even at very low concentrations, causing reproductive and developmental disorders, both in humans and wild animals (Jugan et al. 2009). Due to the relevance, diversity, and complexity of adverse effects and chemical compounds related to estrogenic activity, only the EDsE will be discussed in this review.

Thus, the present review intends to carry out a concise approach regarding the main EDsE studied, describing their known effects and mechanism of action. Additionally, the most frequently used bioassays to detect estrogenic activities in different environmental samples will be also highlighted.

2 Major Contributors to Estrogenic Activity in Environmental Samples

Currently, many different chemicals have been recognized as potentially estrogenic; however, the EDsE may

not be grouped by their chemical similarity since they present a high variety of chemical structures. According to Brzozowski et al. (1997), the presence of an aromatic ring in the structure is the only requirement to act as an effective ligand and bind to the estrogenic receptor.

Classical examples of EDsE that are frequently found in the environment, include natural and synthetic hormones and chemicals as BPA, phthalates, alkylphenols (APs), polycyclic aromatic hydrocarbons (PAHs), and agrochemicals.

BPA belongs to groups derived from diphenylmethanol and isophenol (Yang et al. 2015). In acid pH and high temperature, BPA results in a monomer that is used in the production of plastic and polycarbonates, which serve to fabricate plastic bottles, CDs, and DVDs (Chen et al. 2010; Kawahata et al. 2004; Staples et al. 1998; Sun et al. 2013; Vogel 2009). Several studies have established that BPA has the capacity to reduce the synthesis of steroid hormones (steroidogenesis) by the cortex of adrenal gland (Mandich et al. 2007). According to Bergeron et al. (1999), Letcher et al. (2005), and Levy et al. (2004), BPA mainly acts by interacting with estrogen receptors, by analogously operating and blocking the physiological activity of estrogen receptors or acting as antagonists of testosterone in the cell (Vagi et al. 2014).

Phthalates (diesters of phthalic acid) are synthetic chemical compounds widely used in medicine and industry (Department of Health and Human Services 2012) that may interact with organisms through ingestion, inhalation, or absorption (Chen et al. 2014; Meeker et al. 2009). Some phthalates, such as diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), and diisobutyl phthalate (DiBP), are found in products for self-care, solvents, adhesives, and pharmaceutical drugs (Kelley et al. 2012; Sun et al. 2013; USEPA 2012). Others such as butylbenzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP), and diisodecyl phthalate (DiDP) are frequently used as plasticizers for the production of polymeric materials such as polyvinyl chloride (PVC) (Schecter et al. 2013; Stringer et al. 2000; USEPA 2012). Among the phthalates, DEHP is one of the most commonly found in the environment (especially in water) and in food. According Hsieh et al. (2012b), several studies have shown that phthalates are involved in the development of prostate and breast cancer, which is a consequence of the induction of abnormalities on endocrine functions and on steroid receptors. Some phthalates, as DnBP,

present estrogenic activities that result in several alterations in the spermatogonia, spermatocytes, and spermatids, as well as inducing vitellogenin production in fishes (Bhatia et al. 2014).

APs are synthetic compounds constituted of antioxidants, modified polystyrene, surfactants, and PVC. Generally, they are present in various effluents and are constituted by different sized alkyl groups. The estrogenicity of those substances is related to the reduction in the alkyl chain size; in other words, the smaller the chain, the greater the estrogenicity (Oliveira et al. 2007; Servos 1999; USEPA 2006, 2010; White et al. 1994). The APs and their products, such as APEO, can affect the reproduction of organisms that are exposed to then because they mimic the estrogenic hormones that are responsible for development and sexual behavior. The NP, for example, mimic the natural hormone 17βEE2 and compete with the endogenous hormones by binding to the ER (estrogen receptor) (Soares et al. 2008). According to Barber et al. (2015), NP is the AP with the major estrogenic effect.

PAHs are compounds generated from anthropic activities resulting from the incomplete combustion of fuels and certain industrial activities or even from natural sources, such as the formation of sedimentary rocks (Finkelman et al. 1999; Kavouras et al. 2001; Pleil et al. 2004). These compounds, including its metabolites, are considered highly toxic, mainly due to their physical-chemical characteristics (Tsay et al. 2013; Yamamoto et al. 2003). Van de Wiele et al. (2005) reported that PAHs can be bioactivated into estrogenic metabolites and show EE2-like activities. In general, the estrogenic activities occur through transcription factor activation (ER), which interact with response genes that are hormone-sensitive. However, only a few PAHs and their hydroxyl metabolites that have similar structures to estrogen are known to interact with ER (Fontenele et al. 2010; Nakata et al. 2014; Nesaretnam and Darbre 1997; Sievers et al. 2013). Therefore, there is discussion about how PAHs act as estrogenic substances: (1) with an estrogen-like action, duplicating the physiological effect; (2) by competing with hormones, inhibiting their effects; and (3) by indirect actions, changing the estrogen availability of target tissues (Santodonato 1997).

Several agrochemicals have a proven estrogenic activity (Pickford et al. 2015). This chemical group embraces a whole variety of chemical compounds; for this reason, the mechanism of action of endocrine interference is very diverse. According the review by Mnif et al.

(2011), some agrochemicals may interact with hormone receptors and affect the cellular signaling pathway, whereas others may inhibit the activity of enzymes which are responsible for the synthesis of steroid hormones or even induce the enzymes responsible for this process, resulting in an increase or decrease in hormone levels (Kiyama and Wada-Kiyama 2015). Those interactions may also result in the formation of endocrine-disrupting metabolic products which are even more powerful than the original compounds (Andersen et al. 1999; Andersen et al. 2002; Astiz et al. 2014).

When evaluating the estrogenic activity of 200 pesticides belonging to different chemical groups, Kojima et al. (2004) observed that 51 were able to induce ER-mediated transcriptional responses in Chinese hamster ovary cells. According to these authors, among the tested compounds, o,p'-DDT, β-BHC, δ-BHC, methoxychlor, α-endosulfan, dicofol, CNP-amino, prothiofos, cyanofenphos, and EPN showed the highest estrogenic activity. Kiyama and Wada-Kiyama (2015) also presented a long list of pesticides that are potentially recognized as EDsE, including aldrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, glyphosate, permethirndiazinon, prothiofos pyriproxyfen, thiabendazole, and tolclofos-methyl.

Natural and synthetic estrogenic hormones also can be frequently found in the environment and, depending on the concentration, they can cause harmful effects to the exposed organisms. Hormones are defined as chemical substances synthesized by the endocrine glands, which when released in the blood stream, maintains a balance in the functioning of the whole organism (Bila and Dezotti 2007; Díaz-Cruz et al. 2003; Ghiselli and Jardim 2007; Shimada et al. 2001; Srivastava 2015).

Among the natural estrogens, three categories stand out: (1) steroid hormones like 17β-E2, the primary female sex hormone, besides the E1 and E3, which are major female endogenous estrogens that exert estrogenic hormonal activity; (2) phytoestrogens, such as isoflavones, genistein, and daidzein, which are polyphenols with estrogen-like structure and function, found naturally in plants and, consequently, in many foods (Lezcano et al. 2017; Morissette et al. 2018), whose presence in the environment occurs predominantly due to agricultural sources; (3) mycotoxins, such as zearalenone and zearalenol, that are nonsteroidal estrogenic compounds produced by fungi belonging to the genus *Fusarium* and *Gibberella* (Ghiselli and Jardim 2007;

Lintelmann et al. 2003; Shimada et al. 2001; Shore et al. 1993; Yang et al. 2007).

Synthetic estrogen hormones, as for example, mestranol, DES, and 17αEE2 (Wenzel et al. 1998), are artificial compounds used as pharmaceuticals in contraceptive and in hormone replacement therapy formulations that due their high use are widely found in the environment (Darbre 2015).

Among the harmful effects caused by exposure to the natural and synthetic hormones are alterations in reproduction such as infertility, menstrual irregularities, miscarriages, and breast cancer. In ecosystems, exposure can cause sex reversal in fishes and other animals (Bhatt 2000; Sweeney et al. 2015). Both the natural and synthetic hormones undergo biotransformation before being excreted by the organism. It is known that 60% of these drugs are excreted in the urine as conjugates and several polar metabolites. The most common dissemination paths of these hormones are domestic effluents discharged into rivers and seas, deficiency in the sanitation infrastructure or by the inefficient treatment of these effluents, resulting in major environmental contamination (Erickson 2002; Gilman et al. 2003; Sweeney et al. 2015).

Nevertheless, in the environment, EDsE and natural and synthetic hormones are generally found in a complex mixture of different chemical nature. These agents may interact with each other, often forming compounds whose negative effect on the exposed organisms is greater than that observed for each individual compound in the mixture. According to Payne et al. (2000, 2001), some studies have shown that even if some components of a mixture are in concentrations that do not cause individual effects, a synergistic effect may be observed for the mixture due to the combination of the components. In some studies, authors have reported different effects resulting from the interaction between EDs, such as additives, synergists or antagonists (Barouki 2017).

Although 95% of the studies performed with these compounds are still done with isolated substances, the study of the interactions between EDsE becomes more relevant because the exposure to them usually occurs in complex mixtures, generally derived from effluents discharges (Kortenkamp 2007). However, Sun et al. (2009) and Zhou et al. (2017) highlight the absence of effective techniques to evaluate the interaction between EDs, mainly due to the differences between the chemicals belonging to this group.

Studies performed by Charles et al. (2002) showed that mixtures contained 17βE2, 17αEE2, and DES present additive effects when each compound is present at levels in the linear range of their individual dose-response curves. However, in higher concentrations, the effect is lower than expected. According to the authors, such effect is due to a saturation event.

Given the paucity of data on the effects of the combination of EDs, studies intended at evaluating the impact induced by the mixture of these compounds must be encouraged, since this kind of exposure points us to the real situation of exposure to EDs, which in most cases are not found isolated in the environment.

Moreover, due to their easy release and high prevalence in the environment, added to their difficult removal by common treatments and bioaccumulation at different trophic levels, there is an urgent need to reduce or optimize the use of compounds that can act as endocrine disruptors, and mainly generate public policies aimed at reducing their use and to improve the techniques of treatments that may prevent these compounds from reaching the environment.

3 Effects Induced by Estrogenic Agents

Compounds with estrogenic properties are used in excess in several areas, meaning that substances can be found in all types of environments (Niemuth and Klaper 2015). As a result of the persistence and bioactive characteristics of these substances on soil and hydric resources (Halling-Sorensen et al. 1998; Meyer et al. 1999), the compounds with estrogenic activities can be considered environmental pollutants that may contaminate the entire food chain. Besides environmental contamination, humans can be exposed to these compounds by occupational pathways, during production, use or waste (Baccarelli et al. 2000; Ying 2012).

3.1 General Effects

Estrogenic agents present in the aquatic ecosystem can result in a major population imbalance of several animal species. The continuous exposure in the long term to these substances, even if at minimal concentrations, may compromise the existence of endemic species at contaminated locations, because those compounds are able to induce male feminization and infertility in exposed organisms (Cavallin et al. 2016; Gorga et al. 2015; Vajda

et al. 2008). Furthermore, when those substances come into contact with females, effects can be even worse, because of the possible transmission to eggs, the placenta and breast milk, therefore exposing all descendants to this, and ultimately interfering with the development of the offspring (Bila and Dezotti 2007).

The effects of estrogens present in the water on organisms depend on the exposure time. Fishes, for example, are more significantly affected by hormones than humans, because they are constantly in contact with water (Snyder et al. 2003). However, Baldigo et al. (2015) stated that exposure to low levels of EDsE can affect organisms at the top of food chain, as a result of the bioaccumulative character of these substances. In female fishes, the presence of estrogen in the water can be identified by means of early vitellogenesis (Jones et al. 2000) or by plasma level alterations of vitellogenin (precursor protein in calves, where liver production is stimulated by estrogen). Both male and female fishes have estrogen receptors, but only females are normally exposed to natural estrogens (Baldigo et al. 2015). Consequently, when nonvitellogenic male and female fishes are found which produce vitellogenin, there are signs of estrogen present in the environment (Kime et al. 1999). The induction of vitellogenin in male fishes is related to a decrease of plasmatic testosterone (Hashimoto et al. 2000; Mills et al. 2001), renal and gonadal pathology, and ovary follicles on testicles (Simpson et al. 2000). Anderson et al. (2003) and Okoumassoun et al. (2002) confirmed that vitellogenin presence in male fishes can lead to reproductive problems, such as reduced fertility, which may promote alterations in the population density of river fishes.

Another parameter which indicates the presence of EDsE in environment is the existence of intersex animals (Liney et al. 2006; Niemuth and Klaper 2015); in other words, dioic individuals that presented sexual characteristics of males and females simultaneously (Kelly et al. 2004). The studies of Jobling et al. (2002) and Osman et al. (2015) showed that intersex decreased the fertility of *Rutilus rutilus* males, resulting in gametes with low motility and, furthermore, low ability for fertilization and decreased viable descendant production.

Pimentel et al. (2016) characterized the morphofunctional parameters of the tropical fish *Sphoeroides testudineus* and investigated the eventual occurrence and disruptive endocrine effects on Pacoti River (Ceará, Brazil). Collected fishes were weighed, measured and dissected for the evaluation of gender

(male, female, indeterminate) and histological and vitellogenin analyses. The gonadal weight of indeterminate fishes was significantly lower than that of males and females. Although differences in gonadal weight were observed, the gonadal somatic index (GSI) showed different standards only between males and the other two groups analyzed (female and indeterminate). The authors also reported that the expression of vitellogenin (VTG), which was identified in several mature males and indeterminate fishes, indicated endocrine dysfunction. The EDsE were identified and quantifies (E1, 17αE2, 17βE2, 17αEE2, DES, and E3) in sediment from river spots. Those results were considered indicative of river involvements by contamination with estrogenic compounds. Specimens of *Mullus barbatus* fish were exposed to water of the northwest Mediterranean region, which suffered the impact of sewage treatment plant (STP) effluents; those fishes presented high concentrations of NP (average of 0.34 to 28.31 μg/g) and OP (0.03 to 0.25 μg/g) in bile. The individuals also presented gonadal alterations, such as hypertrophy of connective tissue, fibrosis, necrosis, intersex, and delay in the maturation process of gametes (Martin-Skilton et al. 2006). Several authors have correlated the presence of higher levels of NP in water with intersex and vitellogenin in male fish (Jobling et al. 1998; Lavado et al. 2004). However, some gonadal alterations observed by Martin-Skilton et al. (2006) did not arise from the presence of polyphenols, but may come from a mixture of urban and industrial contaminants, including PCB.

Several species of fish, including *Cyprinus carpio*, *Micropterus* spp., and *Ictalurus punctatus*, collected at different points of the Colorado River (EUA), showed reproductive disorders like intersex (males with testicular oocytes and females with sperm in the ovaries), changes on the plasmatic vitellogenin in males and females, as well as underdeveloped gonads in males. At regions of the Colorado River, the percent of intersex males reached 70% (Hinck et al. 2007). In their article, the authors mention various works which report the presence of high concentrations of metals and organochlorine pesticides, such as DDT and its metabolites, in water, sediment, and river-associated biota. The estrogenicity of organochlorine pesticides, such as lindane, dieldrin and heptachlorine, was confirmed by Okoumassoun et al. (2002), who verified the production of vitellogenin in male fish from the species *Sarotherodon melanotheron* living in Ouémé River, Republic of Benin, Africa, which is contaminated with these pollutants.

In a study performed by Moresco et al. (2014), the authors recorded malformations in the gonads of frogs *Physalaemus cuvieri* found in the south of Brazil. They collected and removed the testicles from 20 males. By histological analysis, they observed the presence of both sperm and oocytes in 4 of the 20 analyzed specimens. The chemical analysis of water samples of the place confirmed the presence of organochlorines, organophosphates, and carbonates. Among the pesticides identified, only Dieldrin was found at values above those permitted by Brazilian law. The authors assumed that this high presence of this organic compound can be due to its cumulative potential.

The transference of EDs to humans occurs mainly by the consumption of aquatic animals which may have bioaccumulated EDs, as well as by the ingestion of drinking water arising from rivers that receive chemical residues, which mimic the effects of estrogen (Maurício et al. 2006).

Although man sits at the top of the food chain and is able to ingest high amounts of estrogenic compounds, which bioaccumulate in other organisms, the estrogenic effects that humans are subject depend on the toxicological properties of the contaminants and their synergistic or antagonistic in vivo effects, on their concentration and on the rate of consumption of directly exposed organisms, like fish (Pinto et al. 2008).

In human beings, the main effects of estrogenic compounds include the reduction in sperm quantity, and an increase in the occurrence of breast, testicular and prostate cancer and endometriosis (Bila and Dezotti 2007). According to Mueller (2004), estrogen receptors in the human body can be expressed in the reproductive system, mammary glands, kidneys, liver, bones, adipose tissue, immune and cardiovascular system, brain, and lungs.

Although human exposure to synthetic estrogen usually occurs at low levels, these compounds tend to accumulate in the adipose tissue of the organism as a result of its lipophilic character and its nonimmediate metabolism. In this way, breast cells, which are localized in a fatty region, become targets for cancer development (Darbre 2001). Knower et al. (2014) state that there is a strong relationship between the exposure to EDsE and breast cancer development. The authors reported that different types

of EDsE, like BPAs, synthetic estrogen, DES, PAHs, and the pesticides 2,3,7,8-tetrachloridibenzo-p-dioxin (TCDD), DDT, and vinclozolin, for example, are involved in the development of breast cancer in direct and indirectly exposed women and other female animals, as mice and *Rhesus* monkeys.

In vertebrate animals, the action of EDsE can be mediated by receptors. When this receptor interacts with strength, a signal is sent to the nucleus of the cell, which stimulates the synthesis of specific proteins (Harvey and Johnson 2002; Shaw and McCully 2002). These sites can be occupied by xenobiotics, which have similar chemical features to estrogens, acting by analogy or blockage of the natural hormones, binding to specific sites and causing undue responses, such as an excess or lack of certain proteins (Jacobs 2001; Shaw and McCully 2002). Another mechanism of action of EDsE is the interference of the enzymatic sequence, which leads to the premature elimination of hormones, the deactivation of breakdown enzymes or incapacitation (by destruction or inactivation) of the execution of hormonal activities (Harvey and Johnson 2002; Janošek et al. 2006; Shaw and McCully 2002).

3.2 Epigenetic Effects

During the process of growth and development of an organism, some EDs may activate or deactivate some genes in specific locations of the genome by means of epigenetic mechanisms (Shahidehnia 2016). Epigenetic mechanisms can be defined as inheritable changes in gene expression, without alteration in the DNA sequence. Thus, phenotypic alterations but not genotypic can be observed (Buckland 2015). Several studies have correlated the epigenetic effects of ED exposure with an increase in the incidence of breast cancer, reproductive disorders, abnormal growth patterns, defects in neurological development in children, and modifications in immune system (Shahidehnia 2016).

Epigenetics plays an important role in cell differentiation, growth, metabolism, and regulation of gene expression through the silencing and activation of specific genes (Bernstein et al. 2007; Esteller 2007; Jirtle and Skinner 2007). The major epigenetic modifications include DNA methylation, posttranslational modifications of histone proteins, and alterations associated with non-coding RNAs (ncRNAs) (Tammen et al. 2013).

There is a great concern about exposure to EDsE during development since epigenetic modification will be transferred to subsequent generations by germline in a process termed transgenerational epigenetic (Zama and Uzumcu 2010).

A very representative example of inheritance of the effects induced by the ED exposure is the case of DES. This synthetic nonsteroidal estrogen was widely used by millions of pregnant women to prevent miscarriages in the 1970s. Among the adverse effects induced in men and women born from exposed mothers to DES are breast cancer, reproductive tract cancer, prostate cancer, infertility, and many others reproductive disorders (Shahidehnia 2016).

Hsu et al. (2009) evaluated the effects of the DES in in vitro breast epithelial cells and observed a downregulation of miR-9-3 (microRNA associated with breast cancer) and repressive chromatin marks as H3K27me3 and H3K9me2. These modifications were accompanied by recruitment of DNMT1 that caused an increase in DNA methylation of the promoter CpG island. According the authors, the hypermethylation of this microRNA may be a mark for early breast cancer development.

Some pesticides may also cause harmful effects by epigenetic mechanisms. Milesi et al. (2017) exposed newborn female rats at 6 or 600 μg/kg/day of endosulfan every 48 h on postnatal days to 1 from 7. When sexually mature, the rats became pregnant and on the fifth day of gestation, uterine samples were collected. The results showed that neonatal exposure to endosulfan increases the expression of ERα during the pre-implantation period. This finding was correlated with a predominant hypomethylation of CpG islands of the ERα promoters observed in the uterus. Thus, the authors suggest that epigenetic changes mediated by methylation are one of the possible mechanisms by which endosulfan causes subfertility. In previous studies, Milesi et al. (2015) have demonstrated that low doses of endolsulfan induces subfertility in female rats, characterized by a drop in the pregnancy rate and in number of implanted embryos. In addition to these effects observed by Milesi et al. (2015, 2017), endosulfan may also cause uterine changes (Varayoud et al. 2008) and affect male and female postnatal reproductive development (Lafuente and Pereiro 2013; Milesi et al. 2012).

The plasticizer butyl benzyl phthalate (BBP) is an ED whose effects are correlated with high risks of endometriosis (Upson et al. 2013), several reproductive disorders in male (Foster 2006) and breast and prostate cancer (Hsieh et al. 2012a). Kang and Lee (2005)

evaluated the mechanism of action of BBP in human breast cancer MCF7 cells and found an increase in ERα mRNA expression levels and a demethylation of ERα promoter-associated CpG islands. From these findings, the authors suggest that the modifications in ER mRNA expression by BBP might be related to abnormal DNA methylation in the promoter region of ERα.

BPA is another ED that has demonstrated to induce epigenetic effects. According Manikkam et al. (2013), exposure to BPA may induce early puberty, prostate disease and cancer, mammary abnormalities including pre-neoplastic lesions, disorders in uterus and ovaries. Liu et al. (2017) evaluated the effects of BPA on ovary and eggs from female mice and found that this estrogenic compound acts mainly via modifications in histone acetylation, and it can affect the oocyte maturation, fertilization rate, and early embryonic development. The authors observed an upregulation of HDAC1 mRNA levels and a downregulation of HDAC5 and HDAC7 mRNA levels in egg and ovarian tissues. Among the class II HDACs, HDAC7 was the most sensitive target of BPA and it was considered an important element in epigenetic maintaining during the process of egg development.

Benzopyrene exposure is associated with pregnancy loss, alterations in fetal development (Zhang et al. 2016), alterations in weights of the reproductive organs, damage in ovarian follicles, and infertility and abnormal fetal development in laboratory animal models (Borman et al. 2000; Csaba et al. 1993; MacKenzie and Angevine 1981; Rigdon and Rennels 1964). Analysis of epigenetic effects of benzopyrene was studied in four breast cancer cell lines (MCF-7, T47-D, MDA MB 231, and HCC-1806) by Sadikovic and Rodenhiser (2006). Analysis of DNA methylation indicated altered DNA methylation patterns suggesting that benzopyrene may lead to dynamic, sequence-specific patterns of hypo- and hypermethylation in all four cell lines analyzed and reinforced the relation between benzopyrene exposure, DNA methylation and breast cancer.

The isoflavonoid genistein is an ED whose uterine or neonatal exposure is correlated to induction of several effects in female mice as reproductive disorders (Drummond and Fuller 2010; Patisaul 2009), increase in ano-genital distance (masculinization), accelerated puberty and irregular estrous cycles (Jefferson et al. 2005). Preconception exposure to genistein also affects gestation time, parturition time, litter size, pup weight, and pup mortality in mice (Patel et al. 2017). According

to Whirledge et al. (2015), genistein can modulate the ER activity, leading to the development of various uterine pathologies in humans. Yu et al. (2016) found that the exposure of human uterine leiomyoma cells to genistein leads to an upregulation of genes involved in cell proliferation and tumorigenesis. Genistein exposure induced epigenetic regulation of transcription factor promoter regions by a mitogen-activated protein kinase (MAPK $_{p44/42}$) through mitogen- and stress-activated protein kinase (MSK1) and histone H3 phosphorylation at serine 10 site. These results demonstrate that ginestein is a compound that may regulate the transcription of certain genes correlated to cancer induction through epigenetic mechanisms by histone phosphorylation.

As can be seen from the examples above, exposure to EDsE may alter the epigenetic programming of an individual and cause important modifications in the expression of genes associated with various diseases, including cancer and problems of reproductive order. Furthermore, exposure to some EDs during gestation and the early stages of development may lead to epigenetic modifications that can be transmitted to future generations and interfere with the normal development of the offspring.

Since that epigenetic mechanisms are reversible events, knowing the mechanism of action of the EDs upon the cells and more specifically upon the genetic material of organisms, is an important step, if not fundamental, in the improve and development of new techniques to combat and/or prevent diseases such as cancer as well diseases affecting female and male reproductive systems that may compromise the next generations.

Currently, studies on epigenetic drugs are being conducted and many of them are already in the test phase. With this, not only the individual directly exposed to EDs could be benefited, but also their offspring.

3.3 Nonmonotonic Dose-Response Curves for Endocrine Disrupting Chemicals

Recently, many studies have been pointing out that the EDs do not exhibit a typical dose-response relationship, where an increase in their concentration leads to a proportional increase in the observed response until reaching a maximum limit, resulting in a sigmoid curve. In contrast, for the majority of the EDs a nonmonotonic dose-response curve (NMDRC) has been frequently observed (Gioiosa et al. 2015; Lagarde et al. 2015; Vandenberg 2014a). By definition, an NMDRC or a

biphasic dose-response curve represents a dose-response relationship characterized by modifications in the slope directions over the interval of tested concentrations (Beausoleil et al. 2013). In this relationship, the most prominent results are observed in intermediate concentrations range, resulting in an inverted-U shaped curve; or when the lowest and highest tested doses induce the highest effects, producing a U-shaped curve.

Lagarde et al. (2015) reported in an extended review that more than 170 EDs showed an NMDR profile when evaluated in studies in vitro and in vivo. Among the substances, BPA, followed by the 17βE2, were the most studied ones, inducing several physiological effects in different organs (mammary and pituitary glands, liver) and systems (reproductive, cardiovascular, immune). According to these same authors, two main mechanisms were often cited to justify the NMDR relationship observed in the studies. The first one is related to the affinity of a same ED to bind to different molecular targets (e.g., different receptors), which exhibit oppositive effects when activated. In this case, in low concentrations, only one receptor would be activated (probably the one with more affinity with the ED), but, in higher concentrations, more types receptors can be activated and then, to inhibit the effect observed for the low tested concentrations. Thus, the absence of an increased response in higher concentrations leads to the formation of an NMDRC. The second mechanism proposed is the occurrence of negative feedback system to regulate the concentrations levels of hormones, avoiding an increased response in high concentrations of EDs. Vandenberg (2014a) also mentioned the overlap of effects caused by an ED to explain the occurrence of an NMDRC profile, such as the induction of cytotoxicity or cell proliferation inhibition associated with estrogenic activity response. For these specific cases, higher concentrations would lead to an absence of estrogenicity due to the cytotoxic effect or inhibition of proliferation.

3.4 The Low-Dose Effects Concept for EDs

Another important concept strictly related to the EDs is called "low-dose effects." This principle is based on the fact that endocrine hormones circulate and operate at very low doses, in nanomolar and, even, picomolar levels. Thus, it is expected that the EDs may exhibit biological response at similar doses (Vandenberg 2014b). Additionally, specifically for the EDs, the lack of observed effects at high tested concentrations cannot be representative of the expected results at low tested doses, as in the classical toxicology (Nadal et al. 2018).

According to Melnick et al. (2002), a low-dose effect can be defined as any biological alteration induced by EDs in low concentrations. Moreover, the concentration ranges must represent a high environmental relevance, causing disorders even in concentrations below the lowest observed adverse effect level (LOAEL), which was determined in the previous studies (Vandenberg et al. 2012).

Many studies corroborate the low-dose effect for the EDs, and it seems to be the new trend in this field of research. Vandenberg et al. (2012) showed in a review that almost 30 EDs, belonging to different chemical groups (pesticides, synthetic hormones, phytoestrogen, detergents, plasticizers, UV blocker, flame retardant, and others involved in several industrial processes) presented significant results for endocrine activity when tested in low doses, providing pieces of evidence for the low-dose hypothesis. However, although some advances were obtained in this sense, the great majority of data is related to few substances, such as BPA. A review conducted by vom Saal and Welshons (2006) about the effects of BPA in low concentrations reported the presence of more than 100 studies showing significant effects for this chemical when tested in concentrations below the LOAEL. In a recent study, Lejonklou et al. (2017) observed alterations in the lipid metabolism in rats caused by developmental exposure to a BPA concentration eight times lower than the tolerable daily intake (TDI) established by the European Food Safety Authority (EFSA). Other studies also observed a low-dose effect for BPA inducing neurological disorders in zebrafish embryos (Kinch et al. 2015) and physiological impairments in mice (Gioiosa et al. 2015).

Although the low dose effects concept and the NMDRC can be interrelated, they cannot be confused, since they present distinct definitions and mechanisms involved. However, both must be taken into consideration when conducting toxicological studies with ED compounds. Furthermore, the NMDR relationship is not restricted to the low tested doses (Vandenberg 2014b).

Thus, from what it has been reported here, it is evident that the EDs represent a challenging field of research and still require more study. Many safety exposure limits of EDs, previously established by regulatory agencies, must surely be revised, especially because the observed effects can differ along the concentrations range and low concentrations can exert relevant effects

to the organisms even when the higher concentrations do not cause any effect. Additionally, specific data regarding the presence or absence of NMDR pattern and the low-dose effect are missing for a major number of EDs. Moreover, long-term exposure studies and the effects of interactions among EDs in complex mixtures must be considered in future researches due to their expressive environmental relevance. Finally, these facts must demand new efforts to perform an adequate risk assessment for the EDs.

4 Studies for the Detection of Estrogenic Activity in Environmental Samples

Rivers and their sediments are the major reservoirs of the EDsE, being responsible for many alterations in aquatic ecosystems because they act by destabilizing the normal functions of the exposed organisms. The estrogenicity verified in water and sediments of the aquatic systems is mostly related to the dumping of effluents from domestic, industrial (Dias et al. 2015; Li et al. 2008), agricultural, and livestock (Verderame et al. 2016) activities. For example, the domestic effluents are composed by a great diversity of chemical substances present in cleaning products and drugs, as well as natural hormones that are discharged or excreted directly into the environment (Dias et al. 2015). In the USA, many pharmaceutical drugs, including the hormones present in oral contraceptives, have been found in almost every analyzed river (Kolpin et al. 2002), with concentrations that are sufficient (normally in ng/L) to cause estrogenic responses in exposed animals (Gorga et al. 2015; Petrovic et al. 2001). Moreover, factors such as temperature and microbial activity can alter the estrogenic activity of an environment (Hemming et al. 2004), as compounds including estrogen and NP can be degraded by microorganisms in rivers, sediments and soil (Hseu 2006; Yin et al. 2002; Ying et al. 2002).

Many researches have been performed to identify the presence of EDsE under the most varied environmental matrices. In this regard, there has been a great effort in developing assays that can determine the presence of EDsE in environmental samples and, also, to detect which concentrations of these substances can have harmful effects on living beings. Thus, based on reports from the scientific literature, this section describes some bioassays that have been used satisfactorily in the evaluation of estrogenic activity in different environmental samples, particularly in surface water, sediments, effluents, and sludge.

4.1 Sewage and Wastewater Treatment Plant (WWTP)

Residual waters derived from both water and sewage treating constitute a great concern with regard to the introduction of estrogenic contaminants into the environment, mostly because traditional methods conventionally used by wastewater treatment plants (WWTPs), such as dredging (removal of floating particles and solids of bigger dimensions), adsorption and decantation (removal of the resulting primary sludge), and aerobic and/or anaerobic degradation (Fontenele et al. 2010; Lester and Edge 2001; Rosa 2008), are inefficient and need to be reevaluated (Rosa 2008). Besides, with WWTPs, the transformation of substances present in the effluents, many times, results in higher levels of reactive compounds than at the initial sewage and, when they are discharged into the aquatic environment, they may induce estrogenic responses in many animals (Folmar et al. 2002). Nonionic surfactants, such as alkyphenol polythoxylates, when degraded by metabolic processes in WWTPs transform into NP or OP (Arukwe and Goksøyr 2003; Bennie et al. 1997), which induce the production of vitellogenin in male organisms of various fish species (Jobling et al. 1998; Schwaiger et al. 2000). Additionally, EDsE can also be found in sewage sludge since, according to Urase and Kikuta (2005), a large part of these compounds is transferred from the liquid effluent to the sludge during sewage treatment.

Gou et al. (2016) combined chemical measurements and bioassays to evaluate the presence and risks of EDsE in seven WWTPs located in north, south, and central Taiwan, from 2011 to 2012. In this study, the E-screen, which quantifies MCF-7 breast cancer cell proliferation induced by EDsE and T47D-Kbluc, a cell-based reporter gene assay with T47D human breast adenocarcinoma transfected with estrogen-responsive luciferase reporter gene, were performed to detect total estrogenic activity. The authors observed, in all studied samples, a higher detection rate of DEHP (82.1%) than other compounds. The bioassays performed in the first collection showed that, of the 56 studied samples, 23% presented significant amounts of estradiol equivalent (EEQ), being the E-screen the most sensitive assay. Thus, the authors concluded that the combination of E-screen and chemical analysis can grant a good strategy for EDsE detection in environmental samples.

The influents and effluents of four different domestic WWTPs and the effluent of a bleaching pulp industrial site were evaluated by Fernandez et al. (2007) in terms of chemical compositions and estrogenic activity. Among the found substances, 19-noretindrone was the most abundant synthetic hormone in the influents, while 17αEE2 was the most frequent synthetic hormone in the studied effluents. The synthetic nonsteroids NP and DEHP were present at high levels in both wastewaters and treated waters (effluents) and nontreated waters (influent). Domestic effluents showed superior amounts of steroids estrogens when compared to industrial effluents, which mainly contain the plant steroid stigma sterol. The variation in EDsE found in the samples is related to the different substances being used in each treatment. In the case of domestic WWTPs, 19-noretindrone was present because it is the most frequently used synthetic hormone for birth control in Canada, while, in the case of the effluent from the bleaching pulp site, plant steroids were found due to their release during wood processing. By using the in vitro assay with recombinant yeast carrying the human estrogen hormone receptor (ER-RYA) to detect receptor binding affinity in the samples, similar values of EEQ to domestic and industrial effluents and higher values than influents were observed. The authors concluded that, although the estrogenic activity is mainly related to domestic effluents due to the presence of steroid estrogens, the high levels of phthalate esters and natural steroids also can contribute to this effect.

The analysis of an industry effluent in Taiwan that processes products derived from fossil fuels (like plastic) using the MVLN assay confirmed the presence of compounds with estrogenic activity. This assay employs the MVLN cells (a cell line derived from MCF-7 cell, with estrogen-specific transcription and a reporter gene that produces luciferase in the presence of estrogen) to identify estrogenic activity related to all pollutants by the detection of luciferase activity verified with the aid of a luminometer. The estrogenicity values varied between 0.54 and 47.86 ng EEQ/L, and the highest values were probably related to the presence of phenols (Chen et al. 2004). According to the authors, the relative quantity of estrogenicity found in analyzed industrial effluents may signify the environmental risk in the case of these effluents being discharged without previous efficient treatment.

Cargouët et al. (2004) performed the estrogenic activity evaluation of influent and treated effluent samples from WWTPs around Paris, France, by in vitro bioassays with MELN cells (an estrogen-responsive human cell line). These cells present a luciferase reporter gene placed under the control of estrogen-responsive elements to enable to assess the induction of the estrogen hormone receptor α (Erα)-mediated gene expression. In this study, the authors evaluated two different classes of pollutants (polar and nonpolar substances). They observed the estrogenic activity only in samples with the presence of nonpolar substances, indicating that most of the contaminants with estrogenic activity are nonpolar.

Jugan et al. (2009) also used the MELN cell line in order to verify the estrogenicity induced by influents and effluents of two STPs and by surface waters from the Seine River (Paris, France). Efficiency superior to 90% was observed for both WWTPs with relation to the decreased estrogenic activity. However, an increase in estrogenicity of the river waters was detected after effluent disposal (2.5 ng/L of EEQ for STP-A and 2 ng/L for STP-B). These observed values constitute a risk for ecosystems, in particular aquatic ones, because at these concentrations, or even lower ones, they can compromise the endocrine functions of exposed organisms.

The analysis of effluent samples from the Apulia region, Italy, before their treatment in STPs, revealed the presence of E1, 17βE2, 17αEE2, BPA, and OP. Among these compounds, BPA (103.4 ng/L) and OP (35.2 ng/L) showed the highest concentrations on average, followed by E1 (32 ng/L), 17βE2 (23.6 ng/L), and 17αEE2 (17.6 ng/L). In this study, Balest et al. (2008) stated that, although the estrogenic activity of industrial activity derived compounds is lower than that of natural steroids, they can induce comparable effects to natural estrogens, due to the high concentrations that they are found at in the environment (usually ng/L for estrogens and at least μg/L for industrial compounds).

In natural wastewater arising from urban and industrial activities in Zagreb (Croatia) were studied in terms of their estrogenic potential, using vitellogenin induction in rainbow trout hepatocyte cultures (*Oncorhynchus mykiss*) (Grung et al. 2007). Among the 30 tested fractions obtained by solid phase extraction, 9 induced an increase in the amount of vitellogenin. The authors attributed the observed effect to the presence of E2 and E3, as well as to different APs, benzophenones and methylparabens.

Salste et al. (2007) investigated the presence of estrogens and the estrogenic activity of a domestic effluent of Turku/Åbo (Finland), by chemical analyses and by

the bioluminescent yeast assay (containing estrogen receptor α (Erα) and the luciferase constitutive gene (Ffluc)). The data indicated estrogenicity values from 4 to 7 ng/L of EEQ. This effect was mainly attributed to the presence of E1, since this was the most abundant estrogen found in the studied samples.

Sludge derived from water treatment plants (WTPs) and WWTPs, as well as the resulting biosolids from stabilization processes of these sludges, can constitute a source of EDsE in the environment. As the sludge and the biosolid are compounds which are rich in organic matter, their use as agricultural fertilizers has been suggested. However, Yamamoto et al. (2003) warned that the use of these materials in agricultural practices can contaminate the soil and groundwater with estrogenic substances, because of hydrophobicity coefficients and the moderately high organic carbon partition of estrogenic compounds, which means that a large part of these substances stay bonded to solid phase during the treatment process of WTPs and WWTPs.

In this sense, Mazzeo et al. (2016) evaluated the organic and aqueous extracts obtained from naturally attenuated sewage sludge (SS) as to its estrogenic potential by means of yeast-based bioassays (ER-RYA). However, the results did not show substantially estrogenic activity in samples in the initial period, which significantly decreased after attenuation. The authors state that the data found in this study support the efficiency of natural attenuation and potential of the use of SS for agricultural purposes.

Giudice and Young (2011) studied, on a pilot scale, the estrogenicity of leachate and surface runoff water of soils fertilized with biosolids, for six different EDsE. In this study, 17αEE2 and BPA were not detected in any of the samples. N-NP and triclosan were only present in runoff water. Despite the substances triclocarban and OP being present both in runoff water and leachate, the high registered concentrations were found in runoff water. The author still observed, by the chemical-activated luciferase gene expression ER-CALUX bioassay, a higher estrogenic activity to samples corresponding to runoff water (43 to 55% nM E2), in relation to the referent leachates (15 to 42% nM E2).

4.2 Aquatic Environment

Environmental pollution by estrogenic compounds is not only due to the resulting effluents from treatment processes, but also from direct discharges with estrogenic action along the riverbank, mainly because of domestic activities (Ma et al. 2007). In these cases, the levels of EDsE are, in general, much higher, and can contribute to a drastic increase in the estrogenic potential of waters. On the other hand, when there is no other effluent discharge along the river, these compounds tend to be diluted during their path, consequently decreasing their absorption availability by aquatic organisms (Maurício et al. 2006) and their estrogenic effects (Hemming et al. 2004). Due to the constant presence of EDs in aquatic environments, as reported by the following studies, much attention must be given since they can represent a potential risk to exposed organisms.

In a monitoring study performed between 2010 and 2012 by Selvaraj et al. (2015), the authors evaluated the presence and the ecotoxicological risks of ester phthalates found in surface water and sediment samples collected from the Kaveri River (India). During the study period, the presence of diethyl phthalate (DEP) and dimethyl phthalate (DMP) was observed in all samples; however, the results showed that the percentage of phthalates found in the river did not present risks to human health, based on drinking water consumption. Further, according to the chronic values of ECOSAR software, the water quality presented little threat to the sensitive organism regarding the presence of DEHP and di-n-octyl phthalate (DOP). For the sediments, the evaluation indicated that DEHP was the residue which presented the greater concern to the environment.

Zhao et al. (2011) reported multiple hormonal activities, among them the estrogenic activity in surface water and sediments of the Pearl River basin (Liuxi, Shijing, and Zhujiang Rivers) south of China. By applying the estrogen receptor reporter gene assay YES (yeast estrogen screen), the authors found estrogenic activity levels that varied from 0.23 to 324 ng/L EEQ in surface waters and from 0 to 101 ng/g EEQ in sediments. Shijing River presented higher levels of hormonal activities than Zhujiang and Liuxu rivers, indicating that it receives a greater influence of chemical products regarding the action of EDs. The EEQ concentrations observed in this study were higher than those considered in the literature as being responsible for adverse effects on aquatic organisms, confirming the commitment of the studied water bodies regarding their high estrogenic potential.

In Lisbon, Portugal, Rocha et al. (2015) collected water samples from nine sites in Tagus River during the four seasons of 2011. By using gas chromatography,

the authors evaluated 17 EDsE, including natural, pharmaceutical, industrial and domestic pollutants, phytoestrogens and phytosterols. The estrogens derived from industrial/domestic activities were found in two of the nine sampled sites (both in Lisbon region). The toxicological values obtained from E-screen assays (expressed as ethinylestradiol equivalent (ng/L)) showed an estrogenic index of 13 ng/L for estrogens, 2.3 ng/L for domestic/industrial pollutants, and 43 ng/L for phytoestrogens. From the obtained results, the authors assigned the toxicological significance, especially regarding the ecological importance of the studied sites. These data was supported by the physical chemical analysis, enabling the authors to conclude that the presence of EDs in these samples was due to anthropogenic action, resulting from domestic and industrial discharges and the presence of drugs.

Praveena et al. (2016) investigated the occurrence of estrogenic activity in surface waters and in sediment from Langat River (Selangor, Malaysia). The presence of steroid estrogens, such as E1, 17β-E2, and E3 was verified, along with the synthetic estrogen 17αEE2. The results indicated the continuous disposal of estrogens from sewage and agricultural activity residues. However, the concentrations showed a low risk to aquatic organisms. Even so, the authors suggested that it would be interesting to verify the presence of other pollutants with endocrine disruptor potential such as the presence of alkyl phenolic components.

Wang et al. (2016) assessed the incidence, sources of phenolic contaminants and the endocrine disruptor bioaccumulation potential in Panlong River, China. The phenolic EDsE (ethoxylate (NP2EO), nonylphenol-mono-ethoxylate (NP1EO), 4NP, BPA, 4-cumylphenol (4-CP), and 4-t-OP) are strongly present in this river with greatly increased residual levels in water and sediment from certain areas. The concentrations of 4-NP, BAP, 4CP, 4-t-OP, and the total phenolic EDsE levels in three prevailing fish species (*Carassius auratus*, *Cyprinus carpio*, and *Anabarilius alburnops*) were 63, 114, 12, 14, and 201 ng/g, respectively. The characteristics of the distribution of phenolic EDsE in water were similar to those found in the sediment; however, in fish, the distribution was different. The incidence, bioaccumulation, and sources of phenolic EDsE in fish were directly related to the distribution characteristics of industrial, residential, and agricultural areas on the Panlong River basin. The concentration of phenolic EDsE in urban areas was especially high compared to suburban areas, so the authors concluded that an imminent risk of estrogenicity to aquatic organisms and even to humans already exists in urban areas of the river.

Wu et al. (2016) performed a study to determine the concentrations of BPA, NP, OP, BP, and ND in bile extract of fish collected from Xin'an River as well as those obtained from Shanghai market, China. The concentrations of EDsE on the commercially acquired fish were higher, with high detection rates compared to fish collected from the river. The average concentrations of BPA, NP, OP, BP, and ND in country fish were 30.1, 203, 4.69, 7.84, and 0.514 μg/L, respectively, and in commercial fish were 240, 528, 76.5, 12.8, and 5.26 μg/L, respectively. The total average estrogenic activity of the river water was 3.32 ng/L estradiol equivalent. Moreover, the authors analyzed the BCFs (bioconcentration factors) in fish bile after 30 days of exposure to environmentally relevant concentrations of BPA in a research lab. The general data suggest that the analysis of fish bile can be an efficient method for monitoring the conditions of aquatic biota. According to Krishnan et al. (1993), the affinity of BPA to bind to estrogen receptors is weaker than the affinity of natural estrogen, but still has this binding ability (Kwon et al. 2000). Besides the presence of estrogenic compounds, some lakes from subtropical regions in China also present APs in sediments (Wu et al. 2006).

Compounds like NP, OP, E1, E2, and BPA were also found in Tama River, Tokyo. This river receives effluents from WWTPs which predominantly treat compounds from domestic origins. The detected concentrations of NP (51.6 to 147 ng/L) and OP (6.9 to 81.9 ng/L) were insufficient to induce estrogenicity in the MVLN cells, but the E1 (6.4 to 85.6 ng/L), E2 (0.5 to 12.3 ng/L), and BPA (4.8 to 76.3 ng/L) concentrations were sufficient to induce estrogenic responses (Furuichi et al. 2004). However, Fenet et al. (2003) reported that the low AP concentrations in water samples do not exclude its estrogenic potential, as these compounds can bioaccumulate in aquatic organisms, even when in low concentrations in the environment.

Wang et al. (2012) analyzed, by YES assay, the estrogenicity of surface waters and sediments from 15 different sites of Yellow River, China. In general, the estrogenic compounds present in water and sediments from Yellow River showed low estrogenicity levels, which others authors characterized from medium to minimum estrogenic risks for most of the sites, with

the exception of the site relating to the east of Lanshou, which presented high estrogenic levels.

Vega-López et al. (2007) evaluated, by in vitro tests performed with MCF-7 cells, water samples collected from Texcoco and Zumpango Mexican lakes, as these lakes received effluents from WWTPs and are the only place where the fish *Girardinichthys viviparous* is found. The authors noticed the estrogenicity in the water of these lakes and, by in vitro tests, the induction of vitellogenin in both sexes of *G. viviparous* fish descendants, when they reproduced in laboratory. The vitellogenin in studied males reached higher levels than the one produced by females.

The plasmatic vitellogenin increase in male fish is considered one of the most common biomarkers for the detection of estrogenic compounds in aquatic environments (Snyder et al. 2003). Strict related locals with the presence of species under threat of extinction deserve special attention with relation to the dumping of contaminants that may complicate the reproduction of them and, consequently, contribute to their extinction.

Song and Wang (2016) performed a study to investigate the estrogenic activities of four regions from Yellow River, by assays developed with the MVLN cell line. Besides establishing the EEQs of the samples, the VTG induction, GSI, and the hepatosomatic index (HSI) in adult males of the rice fishes (*Oryzias latipes*) exposed to the water collect from the river were verified. In another bioassay, a number of parameters were set: incubation time, hatchability, progeny survival, and VTG induction during a complete life cycle of the fish. The analyses confirmed the presence of endocrine disruptor chemicals in Yellow River. The authors warned that the fish collected from the Yiluohe and Qinhe sites are under reproductive risk and show compromised development. However, in the Xinmanghe site, no endocrine disruption risk was observed; this region may have other chemicals that are affecting the hatchability and survival of the fish.

Gagné et al. (2012) evaluated the estrogenicity of water extracts of a river and a lake under the influence of bituminous sands from Athabasca River, in primary culture of rainbow trout hepatocytes. The authors investigated the alterations caused on the estradiol receptors β2 (ER) and VTG gene expressions. They observed that exposure to the water samples from the river and lake caused the highest expression of both genes, with VTG showing higher expression.

Garcia-Reyero et al. (2005) evaluated, using the RYA, the estrogenic activity of the extracts of sediment samples of 83 lakes from European mountains. Among the evaluated samples, 26 did not show any estrogenic potential (levels under 1 pg/g EEQ), while 13 showed high estrogenicity levels, reaching 350 ng/g EEQ. The chemical analysis performed by the authors revealed a strong correlation between estrogenic activity and the presence of contaminants of anthropogenic origin.

The analyses of sediments from Tokyo basin, next to locations which receive effluent dumping of WWTPs, revealed the presence of high levels of NP in samples (142 to 20,700 ng/g dry weight). Besides showing estrogenic activity, as determined by the bioassay with the double-hybrid system in yeast (*Saccharomyces cerevisiae* Y190 with human estrogen receptor (Erα) and a coactivator (TIF2)), the NP was also the main factor responsible for more than 90% of the induction of plasmatic vitellogenin in male fish of the species *Fundulua heteroclitus* (Kurihara et al. 2007).

Bicchi et al. (2009) evaluated the influence of effluent discard in a river north of Italy, regarding the possible estrogenic activities of this receptor body. The authors associated chemical with biological analyses to monitor the estrogenicity of both urban WWTP effluent samples and surface water at the receptor river, before and after disposal. The main substances found in three different studied samples were BPA, DEP, DBP, DEHP, E1, and 4-t-BP. Regarding estrogenic activity, evaluated by E-screen assay, the values found were about 57 to 60% lower up- and downstream of the river, respectively, than the effluent samples. These findings suggest that the effluent did not contribute to a significant increase in estrogenicity of the hydric resource, perhaps due to the capacity of self-depuration and/or for the dilution of the effluent along the river course. However, the authors warned that, even with the low contribution of the effluent to the increasing estrogenicity in river waters, the estrogenic activity of these waters already indicates that the depuration process is insufficient to completely remove the EDsE, which can compromise its endemic biota.

The estrogenicity of effluents from two municipal WWTPs, as well as surface waters from the Rhine River in Worms City (Germany), collected after the disposal of these effluents, was evaluated by Pawlowski et al. (2004) in a study that used the YES assay. The estrogenic activity of the solid-phase extracts was expressed in EEQ, and was about

twice as high (65.96 ± 10.4 ng/L) as the effluent from STP 1 than the effluent from STP 2 (34.1 ± 7.18 ng/L) and about three to five times higher than the river water (11.97 ± 0.7 ng/L in the left riverside and 19.42 ± 2.8 ng/L in the right riverside). As a result, the authors stated that, due to the high amount of steroid estrogens (especially E2 and EE2), as well as the high biological activity detected by the YES, the studied effluents can maximize the EDsE concentration in the water of this river, inducing estrogenic effects in the exposed aquatic organisms.

Schilirò et al. (2009) quantified the estrogenic activity of a WWTP effluent and water samples collected from September 2006 to May 2007, from a river in the northeast region in Italy which receives this effluent. The evaluation was performed with extracts of pre-concentrated samples and their respective dilutions, submitted to the E-screen assay. The cellular proliferation was dose-dependent and the 1:10 and 1:100 dilutions stimulated maximum proliferations. The test that was used was shown to be appropriate for determining the estrogenic activity of an environmental extract.

Raw waters with various degrees of pollution and after being treated were collected from four sites distributed in rivers in Sao Paulo state, Brazil. These waters were evaluated in terms of estrogenic activity by Bergamasco et al. (2011), using the *Saccharomyces cerevisiae* in vitro test BLYES (bioluminescent yeast estrogen assay). The highest rates of estrogenic activity were found at sites with higher pollution. Treated waters did not show estrogenic potentials or estrogenic compounds. The BLYES test showed high sensibility and good detection limits proving that this was an adequate tool to evaluate and monitor water samples.

4.3 Techniques to Remove the EDs from Aquatic Environments

New additional techniques, such as the oxidative and ozonation processes, have shown promising results for the removal of EDsE from water (Esplugas et al. 2007; Guedes Maniero et al. 2008). Reungoat et al. (2012) demonstrated, using the E-screen assay, that the use of ozonation by biological filtration with activated carbon in a WWTP in Australia enabled the removal of more than 95% of the estrogenic effluent. This study concluded that this type of treatment was sufficient to diminish the estrogens and

xenoestrogens in the effluent and should be incorporated into the traditional treatment systems to ensure the preservation of water resources related to this type of effect. In addition, the authors stated that the incorporation of biological assays could complement the information provided by the chemical analyses, allowing a more satisfying evaluation of the efficiency of the treatment.

Dias et al. (2015) evaluated the estrogenicity in collected waters from Parnaiba do Sul and Guandu Brazilian Rivers by the YES assay. The samples were collected from the river close to WWTP disposal. In the water samples collected from the Guandu River, the efficiency of the removal of estrogenic activity was measured by ozonation and chlorination processes, after conventional water treatment (clarification and sand filtration), by investigating the estrogens and BPA peaks. The results indicated an alarming incidence of estrogenic activities at levels higher than 1 ng/L for some sites of the river. Another concern was the number of WWTP samples that exceeded 1 ng/L of EDsE. However, the oxidation methods (ozonation and chlorination) were efficient for the removal of estrogenic activities and the combination of both methods carried good results, using fewer oxidants.

Recently, Hamilton et al. (2016) explored the efficacy of a WWTP in Australia to remove the EDsE during the normal flow and effluent disposal peaks, using analyses of chemical traces for selected EDsE and in vitro bioassays to estrogenicity. The used bioassays were the estrogen receptor binding assay ERBA (determines estrogenic activity by measuring the amount of radiolabeled 17βE2 bound to the ER), E-screen and yeast two-hybrid assay. During summer, the effluent volume increased to 68% and, consequently, also increased the nutrient concentration to at least 27%. The hydraulic retention period was reduced by 40% compared to base flow data. Despite this pressure on the treatment system, the concentrations and the load of E1, 17β-E2, E3, BPA, OP, and NP were not significantly higher during the flow peak when compared to the base flow conditions. The chemical analyses and in vitro bioassays showed that the EDs removal efficacy of the WWTP was not affected by the different flow discharges, confirming that large flow variations are not capable of reducing the efficacy of EDsE removal from the STP.

These studies discussed in this section are compiled in Table 1.

Table 1 Summary of environmental studies conducted to assess estrogenicity in various ecosystems

Environmental sample	Main contaminant	Concentration or EEQ ranges	Test system	Technique	Reference
Wastewater	DEHP; DBP	0.150–2.05 μg/L	Human cell lines (MCF-7 and T47D-KBluc)	E-screen; T47D-KBluc assay; GC-MS	Gou et al. (2016)
	17αEE2; 19-noretindrone; DEHP; NP	9–106 ng E2/L	Recombinant yeast	ER-RYA; GC-HRMS	Fernandez et al. (2007)
	Phenols	0.54–47.86 ng E2/L	Human cell line (modified MCF-7)	MVLN estrogen-specific transcription assay	Chen et al. (2004)
	E1; 17βE2; E3; 17αEE2; nonpolar compounds	1.0–17.6 ng/L	Human cell line (modified MCF-7)	MELN assay; GC-MS	Cargouët et al. (2004)
	E1; 17βE2; 17αEE2; BPA; OP	Hormones, 17.6–32 ng/L; BPA, 103.4 ng/L; OP, 35.2 ng/L [a]	–	HPLC-MS	Balest et al. (2008)
	17βE2; E3; APs; benzophenones; methylparabens	2–15 ng E2/L	Fish cell line (rainbow trout hepatocytes)	Vitellogenin induction; HPLC-MS; GC-MS	Grung et al. (2007)
	Antibiotics; medicines; pesticides	–	Human cell line (MCF-7)	E-screen; HPLC-MS	Reungoat et al. (2012)
Wastewater and surface water	E1; 17βE2; E3; BPA; OP; NP	Hormones, 7.5–297 ng/L; others, <16.4–574.6 ng/L	Recombinant yeast; Human cell line (MCF-7)	ERBA; E-screen; yeast two-hybrid assay; GC-MS	Hamilton et al. (2016)
	E1; 17βE2; 17αEE2; NP	15–94 ng E2/L	Human cell line (modified MCF-7)	MELN assay; HPLC-MS/MS; HPLC-UV	Jugan et al. (2009)
	E1	4–7 ng/L	Bioluminescent yeast	Bioluminescence yeast assay (FFluc); HPLC-MS/MS	Salste et al. (2007)
	E1; 17βE2; 17αEE2	3.8–65.96 ng/L	Recombinant yeast	YES; GC-ion trap-MS/MS	Pawlowski et al. (2004)
	BPA	0–16 ng E2/L	Yeast	YES	Dias et al. (2015)
	E1; BPA; DEP; DBP; DEHP; 4-t-BP	4.4–11.1 ng E2/L	Human cell line (MCF-7)	E-screen; GC-MS	Bicchi et al. (2009)
	Trihalomethanes	5–23.3 ng E2/L	Human cell line (MCF-7 BUS)	E-screen; GC-ECD	Schilirò et al. (2009)
Sludge/biosolid	–	3.15–5.3 ng E2/g	Recombinant yeast	ER-RYA	Mazzeo et al. (2016)
	OP; TCS; triclocarban	<4.9–203 ng/L; <51–309 ng/L; 2.3–17.3 ng/L	Cell line	ER-CALUX; HPLC-MS	Giudice and Young (2011)
Surface water and sediment	DEP; DMP; DEHP; DOP	313–1640 ng/L (water); 2–1438 ng/g (sediment)	–	GC-MS; ECOSAR Software	Selvaraj et al. (2015)
	–	0.23–324 ng E2/L (water); 0–101 ng E2/g (sediment)	Recombinant yeast	YES	Zhao et al. (2011)
	E1; 17βE2; NP; OP; BPA; TCS	1.3; ND; 577.9; 4.7; 46.7; 6.8 ng/L (water); ND; ND; 0.5; 35.7; 1.7; 0.7 ng/g (sediment)	Recombinant yeast	YES; GC-MS	Wang et al. (2012)
	E1; 17βE2; E3; 17αEE2	<ND – 5.3[e] – 5 μg/g	–	HPLC-MS/MS	Praveena et al. (2016)
Surface water	E1; 17βE2; 17αEE2; NP; OP; OPEO; NPEO; BPA; phytoestrogens	2.3–43 ng E2/L	Human cell line (MCF-7)	E-Screen; GC-MS	Rocha et al. (2015)

Table 1 (continued)

Environmental sample	Main contaminant	Concentration or EEQ ranges	Test system	Technique	Reference
	NP; OP, BPA; 4CP	86–429 ng/L [a]	Fishes (Carassiusauratus, Cyprinus carpio and Anabarilius alburnops)	GC-MS	Wang et al. (2016)
	NP; OP, BPA; BP; ND	3.32 ng E2/L [a]	Fishes Hypophthalmichthys nobilis, Parabramis pekinensis, Carassius auratus, Lateolabrax japonicus (bile extract)	HPLC–MS/MS	Wu et al. (2016)
	E1; 17βE2; NP; OP, BPA	0.49–0.97 (E1); 0.29–1.12 (E2) ng E2/L [d]	Human cell line (modified MCF-7)	MVLN assay: HPLC–MS/MS and HPLC–MS	Furuichi et al. (2004)
	PCBs	9.4 ng/L	Cell line (MCF-7); Fish (Girardinichthys viviparus)	E-screen; vitellogenin induction; GC	Vega-López et al. (2007)
	—	0.72–1.19 ng E2/L	Human cell line (modified MCF-7); fish (Oryzias latipes)	MVLN assay; vitellogenin induction; endocrine indexes	Song and Wang (2016)
	Heavy PAHS	–	Fish cell line (rainbow trout hepatocytes)	Estradiol and vitellongenin gene expressions	Gagné et al. (2012)
	E1; 17βE2; E3; BPA	≤8.7 ng E2/L	Recombinant yeast	BLYES; HPLC–MS/MS	Bergamasco et al. (2011)
Sediments	PAHs; PCBs	2.4–1000 ng/g; 170–9700 pg/g [b]	Recombinant yeast	ER-RYA; GC-MS	Garcia-Reyero et al. (2005)
	NP	20,700 ng/g [c]	Yeast (Saccharomyces cerevisiae Y190); fish (Fundulua heteroclitus)	Double-hybrid system in yeast; vitellogenin induction; GC-MS	Kurihara et al. (2007)

[a] Average concentration

[b] Values for individual compounds

[c] High concentration found

[d] Compounds that showed estrogenic activity

ND not detected, *EEQ* estradiol equivalent, *DEHP* di(2-ethylhexyl) phthalate, *DBP* dibutylphthalate, *17αEE2* 17α-ethinylestradiol, *NP* nonylphenols, *E1* estrone, *17βE2* 17β-estradiol, *E3* estriol, *BPA* bisphenol A, *OP* octylphenols, *APs* alkylphenols, *DEP* diethyl phthalate, *4-t-BP* 4-t-butylphenol, *TCS* triclosan, *DOP* di-n-octyl phthalate, *DMP* dimethyl phthalate, *OPEO* octylphenol-ethoxylate, *NPEO* nonylphenol-mono-ethoxylate, *4CP* 4-cumylphenol, *PCBs* polychlorinated biphenyls, *PAHS* polycyclic aromatic hydrocarbons, *GC* gas chromatography, *HPLC* high-performance liquid chromatography, *MS* mass spectrometry, *HRMS* high-resolution mass spectrometry, *ECD* electron capture detector, *YES* yeast estrogen screen, BLYES bioluminescent yeast estrogen assay

5 Final Considerations

Like many other substances that persist in the environment, the EDsE accumulate in soils, hydric resources, river sediments and along the food chain, representing, along with other pollutants, a serious risk to the health of organisms that are exposed to them. The EDsE cover a wide class of substances with distinct structures, including synthetic and natural hormones, natural substances and a great number of synthetic substances, with both organic and inorganic features that are also derived from residues or subproducts of many compounds used in industries, homes, and agriculture. Moreover, some current human activities can contribute to the dispersion of these contaminants, since many EDsE are present in plastic packages, in self-care products (such as soaps, shampoos, sunscreens and other), in contraceptives, in various medical/dental treatments, and in household cleaner products.

As discussed previously, the EDsE can react with the biological environment and negatively interfere with the endocrine system of organisms, in their most varied stages of development (from embryonic to adult). Thus, these emergent pollutants present a harmful potential over the exposed biota, including humans.

For this reason, many studies have been monitoring the presence of these substances in the environment. Due to the great variety of substances belong to the EDsE group and the wide variation in their concentrations in environmental samples, a combination of chemical and biological assays seems to be an interesting approach for the detection of these contaminants. As the EDsE are, in general, present in concentrations in the order of ng, high-performance chromatography is required to precisely quantify them. Among the bioassays, as highlighted by this review, methods using the human culture cell MCF-7 and recombinant yeast carrying the vertebrate estrogenic receptor are the most routinely employed. Additionally, studies that evaluate alterations in vitellogenin levels in fish, as an endpoint of estrogenicity, have also been frequently performed. However, despite all of the studies that have already been carried out to identify the presence of EDs in the most varied environmental matrices, the data are insufficient to elucidate the real action of these compounds on living beings (Bila and Dezotti 2007), especially due to the lack of knowledge regarding the existence of many compounds with estrogenic action.

Another important and critical issue to be considered is the need for data regarding the behavior of EDsE in the most diverse environmental matrices in relation to their persistence, accumulation, degradation, and mineralization. Furthermore, new technologies must be developed to improve the efficiency of wastewater treatments, since treated and untreated effluents represent the main source of introduction of EDsE to the environment. Therefore, due to their environmental relevance, this review reinforces the importance and necessity of studies related to the EDsE.

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