Chapter 7

Estrogenic Compounds in Estuarine and Coastal Water Environments of the Iberian Western Atlantic Coast and Selected Locations Worldwide — Relevancy, Trends and Challenges in View of the EU Water Framework Directive

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Additional information is available at the end of the chapter

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1. Introduction

Water is vital to life and thus its availability and quality has increasingly been the object of intense concern and disputes by multiple agents that include from people directly relying on ecological services, or simply living in impacted zones, to influential non-governmental environmental organizations and regulatory governmental authorities. The scientific community has increasingly intervened, particularly through the production of sound diagnostic and mechanistic data and also prognostic models, which fundamentally rely on both chemical and biological environmental monitoring. Despite the global increasing of pollution and its impacts, it appears that there is an uncontrollable expansion of anthropogenic activities, mainly in countries where there are no strict environmental policies. Therefore, pollution continues to negatively affect the quality of water and, in consequence, the vast ecosystems associated with it. Presently, it is estimated that hundreds of new chemicals with harmful potential are recorded daily in the CAS® (Chemical Abstracts Service, http://www.cas.org/cashome). Thus, the subject "Water Quality" has been the target of many reflections, particularly in Europe, where in general, all state members of the European Union (EU) have shown their concern about the near future possibility of water shortages, for all, both in quantity and quality [1]. Related to this aspect, it is also recognized as of major importance the need to protect biodiversity and natural ecosystems; an example of such recognition is depicted in the 2012 European Parliament resolution on the "EU Biodiversity Strategy 2020" [2].

In line with the referred intentions, at the beginning of the millennium the EU adopted legislation — the EU "Water Framework Directive" (WFD) [3] — that was considered very innovative at the time. The WFD called for a comprehensive and integrated approach of water protection and management, having as its ultimate goal that all European waters (continental surface waters, transitional waters, coastal waters and groundwaters) reached good chemical and ecological status within a 15 years period, since the date of publication of that directive. To achieve these requirements, temporal incremental goals were established amongst all EU states to ensure the success of this program. Thus, it was decided *inter alia* to: (*i*) apply all necessary measures to avoid the damage of superficial water bodies; (*ii*) impose fines on violators responsible for deteriorating the status of surface water bodies; (*iii*) achieve good ecological and chemical status for all artificial or heavily modified water bodies; (*iv*) progressively reduce the pollution of priority compounds, some of these focused in this Chapter, by limiting their emissions, their discharge and/or runoff into the environment.

However, as the water situation in each EU state was different and unique, since 2000 and up to the present, it has been necessary to make adjustments to overcome this aspect. Indeed, just one year after the WFD 2000/60/EC publication, this directive was updated [4]. In the renovated document (2455/2001/EC), among other measures, it was published a list containing thirty-three harmful compounds which presence in surface waters should be limited or at least/reduced under limit values [4]. Among these compounds stands out the persistent organic pollutants (POPs), such as, pesticides, polycyclic aromatic hydrocarbons and alkylphenols. Later, the number of harmful compounds were expanded up to forty five and additional environmental quality standards were included in (2008/105/EC) [5]. Nonetheless, because further toxicity details each pollutant are becoming known, the number of compounds in the WFD list tends to increase. In this vein, the current directive 2013/39/EU integrates new substances in its watch lists [6]. Among those are EDCs such as the extremely potent 17β-oestradiol (E_2) and 17α-ethinylestradiol (EE_2), as discussed in this Chapter.

2. Estrogenic endocrine disrupting compounds in surface waters

Accordingly to the National Institute of Environmental Health Sciences (USA), endocrine disrupting compounds are natural or man-made compounds that may mimic or interfere with the function of hormones in the body, producing a variety of adverse effects over the reproductive, the neurological and the immune systems of humans and both domestic and wild animals [7]. In the case of EDCs with oestrogenic activity, these substances can act: (*i*) on the hypothalamus, inhibiting the release of gonadotropin releasing hormones [8]; (*ii*) on the pituitary, inhibiting the release of gonadotropins [8]; (*iii*) on the gonads, interfering with the production of steroid hormones, namely E_2 [9]; (*iv*) on the circulation of endogenous hormones, as these compounds have the ability to bind to the same plasmatic carriers [8]; (*v*) on the same cellular receptors used by the endogenous hormones causing important structural changes [10]. In males of various fish species, situations of *ovotestis*, *i.e.*, presence of oocytes in testes, were reported in polluted systems that include some in Portugal [11].

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Here, it is exposed the environmental concentrations of sixteen oestrogenic EDCs, which were chosen taking in account the following features: (*i*) *in vivo* and *in vitro* potency, such as the natural oestrogens and EE_2 [12]; (*ii*) abundance in terms of incidence and concentration), such as bisphenol A and the alkylphenols (and their ethoxylates) [13, 14], and (*iii*) the ubiquitous, but paradoxically less studied, phytoestrogens [14, 15].

2.1. Oestrogens

2.1.1. Main characteristics and environmental origins

Both natural and synthetic oestrogens, including oestrone (E_1), E_2 and EE_2 (Figure 1) may induce, even in low (ng/L) concentrations, from mild to extremely harmful effects over the endocrine system [16]. In particular, these compounds have been associated with the occurrence of endocrine disorders such as those over the reproductive system of a wide range of species, including molluscs, crustaceans, fish, birds and mammals [17-22]. Another disruption effect observed in animals, collected from waters containing high levels of oestrogenic contamination, is the decrease of their immune system responses disorders [23]; this phenomenon also seems to occur when humans are exposed to the same type of EDCs [24, 25]. These observations, together with the abovementioned facts, justified the recent incorporation of these compounds in the WFD watching lists [6]. In this sense, and in order to be aware about the concentrations of E_1, E_2 and EE_2 in surface waters, studies were initiated to monitor their presence and appraise their temporal evolution. It is known that the primary sources of these three EDCs are their excretion by urine and faeces [26]. So, these compounds reach the rivers, estuaries and coastlines either through the discharge of effluents coming from sewage treatment plants (STPs) — or directly from sewages (hence untreated) that deliver their content into waterways.

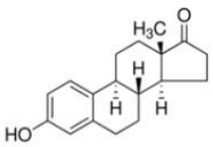
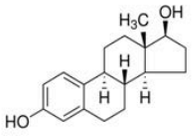
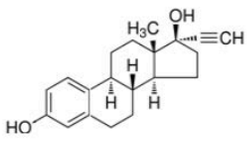
Class	Example	Chemical structure	Origin
Natural oestrogens	Oestrone (E_1)		Urine and faeces excretion [26].
	17β-Oestradiol (E_2)		
Synthetic oestrogens	17α-Ethinylestradiol (EE_2)		Urine and faeces of women consuming birth control pills [26].

Figure 1. Natural and synthetic oestrogens included (E_2 and EE_2) or to be included (E_1) in the watch list of compounds under surveillance by the WFD [6].

Although the concentrations of the referred EDCs are typically in the order of few ng/L (Table 1), it has been experimentally proven that such amounts are potentially harmful [12]. For example, just 4 ng/L of E_2 resulted in the formation of *ovotestis* in male Japanese medaka (*Oryzias latipes*) [27]. Also the male mummichog (*Fundulus heteroclitus*) exposed to 100 ng/L of EE_2 showed a large increase in the gonadosomatic index, decrease in testosterone production and liver synthesis of vitellogenin [28].

2.1.2. Oestrogens in surface waters

The highest concentrations of E_1 in surface waters were reported in Japan (Tama River) [29], Taiwan (Shui-Dan River) [30] and, China (Pearl Rivers) [31, 32] where its levels ranged from78.7 ng/L to 85.6 ng/L. The highest amounts of E_2 were measured in Italy (Venice Lagoon), Japan (Tama River) and Taiwan (Shui-Dan River) where its levels ranged from12 ng/L to175 ng/L. Finally the highest levels of EE_2 were found in Spain (Ebre River) [33], China (Pearl Rivers) [31, 32] and Italy (Venice Lagoon) [34] where its levels ranged from 34 to 130 ng/L. From the *in vivo* and *in vitro* experiments referred in section 3.1.1., among many other, it is concluded that those levels are environmental conditions for the occurrence of endocrine disrupting events. In north/central Europe, the surface water concentrations of these compounds are typically lower than those described in Asia and Brazil (Table 1). Even so, significant biological impacts associated with pollution were detected in Europe. For example, roach (*Rutilus rutilus*) and gudgeon (*Gobio gobio*) males caught in rivers and estuaries of the United Kingdom showed high occurrence of *ovotestis* [35-38]. In Portugal, in the early 2000s, *ovotestis* was observed in flounder (*Platichthys flesus*) and mullets (*Mugil cephalus*) caught from Douro estuary [11]. However, as it can be seen from Table 1, at that time no information existed about the degree of oestrogenic contamination in Iberian western Atlantic coast surface waters - this led to our integrated studies shown in this Chapter, which first data were published in 2009 (Table 2).

EDC	Sampling area	Concentration (ng/L)	References
	Estuarine and coastal waters, The Netherlands	0.1 - 3.4	[26]
	Scheldt Estuary, Belgium – The Netherlands	ND - 10.0	[39]
	The Netherlands (surface water)	<0.30 - 7.2	[40]
	Rivers in Germany	0.10 - 4.1	[41]
	Coastal area of Baltic Sea, Germany	0.08 - 0.54	[42]
	Seine River, France	1.0 - 3.2	[43]
E_1	Tiber River, Italy	5.0 - 12	[44]
	Venice Lagoon, Italy	<1.20 - 10	[34]
	Ebro River, Spain	ND - 4.9	[56]
	Llobregat River, Spain	<LOD - 22	[45]
	Llobregat River, Spain	0.82 - 5.81	[46]
	Thermaikos Gulf, Nothern Aegean Sea, Greece	<LOD	[47]
	Buyukcekme watershed, Istambul, Turkey	1.40 - 5.74	[48]

EDC	Sampling area	Concentration (ng/L)	References
	Acushnet Estuary, USA	0.73 - 1.20	[49]
	South Florida, USA	0.88 - 5.20	[50]
	Brazilian surface water	ND - 39	[51]
	Tama River, Japan	6.4 - 85.6	[29]
	Dan-Shui River, Taiwan	22.4 - 66.2	[30]
	Yellow River, China	ND - 15.6	[52]
	Pearl rivers, South China	ND - 78.7	[31, 32]
	Yangtze River estuary, China	ND - 1.43	[53]
	Songhua River, Northern China	ND - 3.05	[54]
	Tamagawa River, Japan	3.4 - 6.6	[55]
E_2	Estuarine and coastal waters, The Netherlands	0.3 - 5.5	[26]
	Scheldt Estuary, Belgium - The Netherlands	ND	[39]
	The Netherlands (surface water)	<0.8 - 1.0	[40]
	Rivers in Germany	0.15 - 2.0	[41]
	Coastal area of Baltic Sea, Germany	ND	[42]
	Seine River, France	1.0 - 3.2	[43]
	Tiber River, Italy	2.0 - 6.0	[44]
	Ebro River, Spain	ND - 1.9	[56]
	Llobregat River, Spain	ND	[46]
	Thermaikos Gulf, Nothern Aegean Sea, Greece	ND	[47]
	Buyukcekme watershed, Istambul, Turkey	1.10 - 5.39	[48]
	Acushnet Estuary, USA	0.56 - 0.83	[49]
	South Florida, USA	ND - 1.80	[50]
	Brazilian surface water	ND - 7.3	[51]
	Tama River, Japan	0.5 - 12	[29]
	Dan-Shui River, Taiwan	1.4 - 33.9	[30]
	Yellow River, China	ND - 2.3	[52]
	Pearl rivers, South China	ND - 7.72	[32]
	Yangtze River estuary, China	ND - 1.4	[53]
	Songhua River, Northern China	ND - 1.16	[54]
	Tamagawa River, Japan	0.6 - 1.0	[55]
EE_2	Estuarine and coastal waters, The Netherlands	0.1 - 4.3	[26]
	Scheldt Estuary, Belgium – The Netherlands	ND	[39]
	The Netherlands (surface water)	<0.3 - 0.4	[40]
	Rivers in Germany	0.1 - 5.1	[41]
	Coastal area of Baltic Sea, Germany	ND - 17.9	[42]
	Seine River, France	1.0 - 4.0	[43]

EDC	Sampling area	Concentration (ng/L)	References
	Ebro River, Spain	30 - 130	[33]
	Ebro River, Spain	ND	[56]
	Llobregat River, Spain	ND	[46]
	Tiber River, Italy	ND - 1.0	[44]
	Venice Lagoon, Italy	<1.0 34	[34]
	Thermaikos Gulf, Nothern Aegean Sea, Greece	ND	[47]
	Buyukcekme watershed, Istambul, Turkey	11.7 - 14.0	[48]
	Acushnet Estuary, USA	3.01 - 4.67	[49]
	South Florida, USA	NA	[50]
	Brazilian surface water	ND - 25	[51]
	Tama River, Japan	< 0.20	[29]
	Dan-Shui River, Taiwan	7.53 - 27.4	[30]
	Yellow River, China	NA	[52]
	Pearl rivers, South China	ND - 53.4	[31, 32]
	Songhua River, Northern China	ND	[54]
	Yangtze River estuary, China	ND - 0.11	[53]

NA: Not Available. ND: Not Detected. LOD: Limit of Detection.

Table 1. Concentrations of oestrogens in surface waters (minimum-maximum) measured in surface waters worldwide.

2.1.3. Oestrogens in surface waters from the west Iberian coast (Portugal)

Being Portugal one of the EU members that signed the commitment with the European Commission (EC) to accomplish the directives referred in the WFD document, systematic efforts have been made by our research group to develop and validate methods adequate to the measurement of EDCs in complex environmental matrices (seawater, estuarine and river waters). Also, a complementary effort has been dedicated to both gather all monitoring data and address their potential risk. In this sense, the evaluations done in Portuguese surface waters warned about risks of environmental impacts of oestrogens and can assist the competent authorities to take measures to prevent and clean up these habitats from having EDCs, either by eliminating or at least put them at concentrations below those know to be able to promote biological adverse effects.

The results revealed that the average amounts of oestrogens in Portuguese superficial waters were ≈ 6 ng/L for E_1 and ≈ 10 ng/L for E_2 and EE_2. These concentrations, accordingly with the *in vivo* studies referred previously (section 3.1.1.) are not only able of causing disruptive effects in aquatic animals, but also even induce negative impacts in human health [25, 57]. The findings become additionally relevant in view of the fact that these habitats are commonly used by residents and/or tourists, both for recreational and fishing purposes.

Analyzing the values of Table 2, and concerning the concentrations of all evaluated oestrogens, it concluded that habitats we studied so far have chemical quality deficiencies. Table 2 also

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points that each analysed geographical zone had quite diverse minimum-maximal amounts. This fact corresponds to spatial differences among sampling sites, in line with the presence/absence of STPs effluents and domestic discharges in the sampled areas. The last inference is directly correlated with the data obtained in the latest national *census*, which revealed a high number of houses (over 17,000) without any sort of connection to sewers [58]. Beyond this, there is also an additional factor that is the huge number of tourists that seasonally arrive to several studied zones located in both west and south of the Iberian Peninsula [59, 60]. Due to drastic increases of the number of inhabitants, that may rise up to 50%, mainly in summer the concentration of oestrogens in surface waters raised significantly, causing seasonal damages in local biota. So, it becomes clear that the physicochemical parameters typically used to assess the quality of surface waters (i.e., temperature, pH, dissolved O_2, nitrites, nitrates and phosphates) are not sufficient to guarantee the protection of both environmental and human health.

Comparing the values compiled in Tables 1 and 2 it is concluded that, in general, the levels of oestrogens tend to be higher in the west Iberian Peninsula than in the rest of central/north Europe. In fact, in average, these substances in the current study area are almost similar to those measured in many Asian countries. Despite this, it is exalted the positive efforts of remediation conducted in some Portuguese environments, such as those produced in the Ave River, considered in the past as one of the most polluted of Europe [61]. There are also commendable the important efforts occurred in the Douro River estuary [62]. In fact, since our first monitoring studies in the last estuary it was possible to observe that the surface waters collected in 2005 [63] contained significantly higher amounts of oestrogens than those collected in 2009, a fact that demonstrates an important improvement of water quality [62]. Unfortunately, because there are no other data prior to 2005, with regard to the levels of E_1, E_2 and EE_2 in other Portuguese aquatic systems it is not possible yet to establish with certainty, trend lines relating to a progressive decrease in the concentrations of these EDCs in Portuguese surface waters. In spite of this, other important data provided by our studies demonstrate that: (*i*) several areas commonly seen as "pristine" (e.g., the Mira River) contain high levels of those oestrogens (*unpublished data*); (*ii*) there is an underestimation of the efficacy of the STPs, which at times are not adequately dimensioned, namely for coping with seasonal/touristic influxes; (*iii*) currents from both Atlantic Ocean and/or estuaries can channel pollutants, such as these oestrogens, towards protected areas (e.g., the natural reserve of the Sado River estuary) [59]. From Table 1 it is observed that also in Spain, particularly in the Ebro River for EE_2 [33, 56], there are efforts that seem to have been effective in reducing the environmental amounts of these EDCs.

The data repertoire summarized in Table 2 for E_1, E_2, and EE_2 is one of more systematic ones available in the international literature about the oestrogenic status of a particular European country. This information, which can be viewed as a benchmark for the concentrations of oestrogens in Portuguese waters, makes it possible to everybody to monitor the effectiveness of the implementation of measures that may lead to the reduction of environmental levels of those EDCs along the time.

EDC	Sampling area	Concentration (ng/L)	References
	Lima River estuary and Atlantic coast of Viana-Castelo	4.6 - 36.3	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	0.5 - 7.2	[61]
	Leça River estuary and Atlantic coast of Porto	4.9 - 10.4	[65]
	Douro River estuary	<15 - 113	[63]
	Douro River estuary and Atlantic coast of Porto	1.5 - 4.6	[62]
E_1	Mondego River estuary	<5.0	[66]
	Mondego River and its estuary	1.0 - 14.6	[67]
	Tagus River and its estuary	≈ 2 - ≈ 6	*Unpublished*
	Sado River and its estuary	1.0 - 9.8	[59]
	Mira River and its estuary	≈ 3 - ≈ 12	*Unpublished*
	Ria Formosa	1.0 - 2.0	[60]
	Lima River estuary and Atlantic coast of Viana-Castelo	2.4 - 24.4	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	1.6 - 9.4	[61]
	Leça River estuary and Atlantic coast of Porto	3.3 - 5.9	[65]
	Douro River estuary	<7.0	[63]
	Douro River estuary and Atlantic coast of Porto	5.4 - 8.5	[62]
E_2	Mondego River estuary	<3.0	[66]
	Mondego River and its estuary	1.5 - 18.4	[67]
	Tagus River and its estuary	≈ 3 - ≈ 20	*Unpublished*
	Sado River and its estuary	1.2 - 10.8	[59]
	Mira River and its estuary	≈ 4 - ≈ 62	*Unpublished*
	Ria Formosa	1.3 - 10.1	[60]
	Lima River estuary and Atlantic coast of Viana-Castelo	0.3 - 19.4	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	0.3 - 20.4	[61]
	Leça River estuary and Atlantic coast of Porto	2.1 - 4.4	[65]
	Douro River estuary	18 - 102	[63]
	Douro River estuary and Atlantic coast of Porto	<1.3 - 4.5	[62]
EE_2	Mondego River estuary	<12	[66]
	Mondego River and its estuary	0.3 - 11.3	[67]
	Tagus River and its estuary	≈ 4 - ≈ 20	*Unpublished*
	Sado River and its estuary	1.1 - 3.2	[59]
	Mira River and its estuary	≈ 4 - ≈ 67	*Unpublished*
	Ria Formosa	12.1 - 25.0	[60]

Table 2. Concentrations (minimum–maximum) of natural and pharmaceutical oestrogens in Portuguese surface waters.

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2.2. Industrial and household products

2.2.1. Main characteristics and environmental origins

There are industrial and household compounds prone to promote oestrogenic effects in wildlife and humans [68-70]. Some of these EDCs are compounds such as phenols (bisphenol A, BPA) and alkylphenols (APs) — *viz*. octylphenols (OPs) and nonylphenols (NPs) — and their ethoxylates (APEOs) (Figure 2).

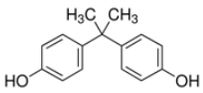
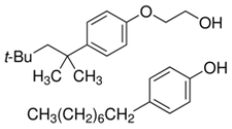
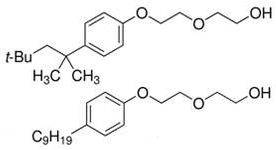
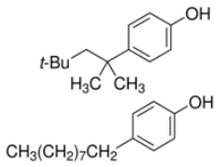
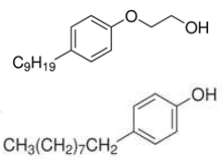
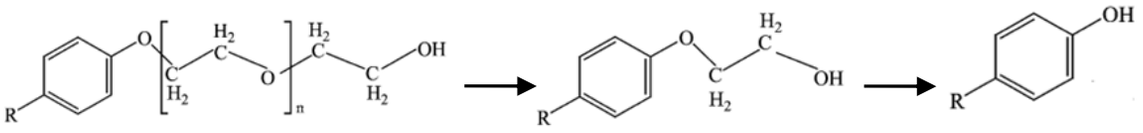
Class	Example	Chemical structure	Origin
Phenols	**Bisfenol A (BPA)**		Industrial and domestic usages [71].
Alkylphenol ethoxylates (APEOs)	**Octylphenol and nonylphenol ethoxylates**		Industrial, domestic and as pesticide additives [14, 73].
	Octylphenol and nonylphenol diethoxylates		
Alkylphenols (APs)	**Octylphenols (OPs)**		Environmental Degradation of APEOs [73].
	Nonylphenols (NPs)		

Figure 2. Industrial and household products included in the list of compounds under surveillance by the WFD (European 2013).

Although the disruptive activity of these compounds is much lower than that of natural and synthetic oestrogens, as they may reach levels in the order of tens to hundreds of μg/L they can became harmful for aquatic fauna. Because of this, and despite great controversy between diverse agents, these compounds become subjected to strict laws that included both APs and APEOs, in the group of "priority substances in the field of water policy 2455/2001/EC" [4]. Presently, the WFD established that the concentration of NPs in surface waters should not exceed 2 μg/L [6].

BPA origin in the environment comes from the fact that it is the monomer of the polycarbonate (plastic) used in an huge variety of domestic stuffs and as an intermediate in the synthesis of epoxy resins, flame retardants and many other products [71]. In spite of its vast usage, controversial opinions do exist among those calling for its banning and those that devaluate the BPA toxic effects in realistic scenarios. The February 2007 study "Toxic Baby Bottle's", by the Environment California Research and Policy Center [72], showed that even small amounts of BPA may be one cause of diseases, including breast cancer, prostatic hyperplasia, diabetes, obesity, and hyperactivity disorders involving the immune system. Infertility and early puberty are also among the possible effects caused by BPA, and all of them are associated with the compound's ability to deregulate the endocrine system.

The APEOs sources in the environment are due to their commonly usage as non-ionic surfactant compounds and dispersants [14, 73]. Due to these properties APEOs are being used in detergents and additives of pesticide formulations. Currently, these applications are prohibited in the EU [4] as these compounds are known to readily degrade, in both aerobic and anaerobic conditions, into APs that are more toxic compounds than APEOs (Figure 3). In spite of the APs toxicity, it is known that their lifetime is not long, since they usually degrade in about 10 to 15 hours after sunlight exposure [74]. Thus, it is concluded that the ubiquitous presence of APs in surface waters implies the continuous entrance of APEOs in the environment [60].



Alkylphenol Ethoxylate (AP_nEO *n*= 0-20) | **Alkylphenol Monoethoxylate** (AP_1EO) | **Alkylphenol** (AP)

Figure 3. Summarized degradation mechanism of APEOs in the environment adapted from Warhurst [75].

The endocrine disrupting activity of APEOs and (especially) that of APs, whether they are octylphenols (4-OP and 4-t-OP) or nonylphenols (4-NP and 4-n-NP) is derived from the existence of similarities between the structure of these compounds and that of E_2 (Figure 4) [76].

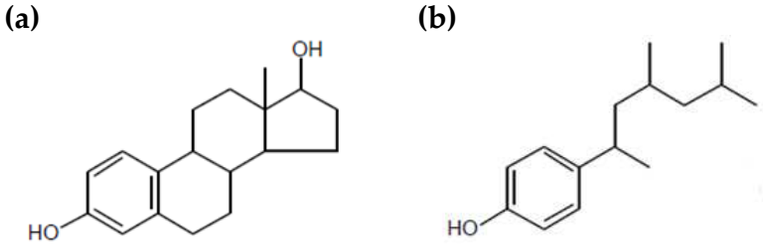


Figure 4. Comparison between the chemical structures of E_2 (**a**) and 4-NP (**b**).

As such, APs may induce oestrogenic effects using the same paths described in Section 3. These effects were proved, for example, after *in vivo* exposures of male rainbow trout (*Oncorhynchus mykiss*) to 30 μg/L of 4-OP, and of male Japanese medaka (*O. latipes*) to 30 μg/L of 4-NP, eliciting, respectively, an inhibition of the testicular growth and hermaphroditism [27, 77, 78]. Alas, as it is possible to purchase in EU countries imported products containing APEOs — see an interesting record in "APEOs Investigation Report" [79] — these EDCs continue to exist in European surface waters.

Currently, we theorize that the most likely source of APEOs in the aquatic systems is the discharge of wastewater effluents from STPs, nevertheless not neglecting the possible leaching caused by the runoff of these EDCs from landfills and agricultural areas (as APEOs are emulsifiers in some pesticide formulations).

2.2.2. BPA, APEOs and APs in surface waters

Table 3 shows an overview of BPA, APEOs and APs in estuaries and rivers worldwide. In line with the broad use of these EDCs it is observed that, at least up to 2005, their concentrations in surface waters were in the order of μg/L. For instance, ten years ago BPA reached concentrations of 5.0 μg/L in several German rivers [80] and the alkylphenols, 4-OP and 4-NP, levels attained 26 to 37 μg/L in several Spanish rivers and estuaries [81]. In line with these observations, it could be hypothesized that if there was not the pressure exerted by the program set out by the WFD, together with the directive 76/769/EEC [82] that established restrictions on the marketing and use of NPs and NPEOs, the current environmental content of these EDCs in European surface waters would probably be worse than that found nowadays. It is important to stress that the last directive advocates the banishment of NP and NPEOs in formulations when their levels are equal or higher than 0.1% by mass. This measure leaded to a decrease in the use of NPEOs or even their deliberate discontinuity in Europe (case of Germany) and, consequently, the number of studies that accompanied the temporal evolution of these compounds began decreasing. This aspect increases the difficulty to assess the actual amounts of APEOs and APs in European surface waters, being assumed that their usage was deprecated; a presumption that is not entirely correct, as we have been witnessing.

As shown in Table 3, the levels of APs in several EU countries continue to show potential toxicity (<0.5-37,300 ng/L). This poses obstacles to the compliance defined by WFD, since it seems impossible to achieve concentrations of ≤100 ng/L for the OPs and, ≤300 ng/L for NPs, in all EU states up to 2015. However, it is possible to observe that recent studies revealed that there is positive effort towards the reduction of the global amounts of both APs and BPA in all European countries. So, presently, and also accordingly to the compilation in Table 3, it is observed that in general the levels of APs and BPA are generally higher in Asia and South America than those measured in the EU.

In opposition, and with the exception of the two works [83, 84], before 2005 there were virtually no data about the levels of these EDCs in the west Iberian Peninsula, inc. Portuguese surface waters. These observations leaded to the creation of regular monitoring programs for these compounds, in order to make a general assessment of the situation in various locations and, when possible, monitor their evolution in time. Such temporal results are central to define if

the implementation of WFD policies are being successful in the field, alone or together and correlated with biologic outputs (e.g., biomarkers data). The efforts done in the last decade for evaluating BPA, APEOs and APs pollution in Portuguese surface waters from estuaries, rivers and coastal waters are compiled in Table 4.

EDC	Sampling area	Concentration (ng/L)	References
BPA	Gulf of Gdansk, Poland	26.9 - 48.1	[85]
	Estuaries and rivers, The Netherlands	<8.8 - 1,000	[40]
	German Rivers	0.5 - 14	[86]
	Elbe River and its tributaries, Germany	3.8 - 92.0	[80]
	Coastal area of Baltic Sea, Germany	ND - 5.7	[42]
	Baden-Württemberg Rivers, Germany	<50 - 272	[87]
	Glatt River, Switzerland	9.0 - 76	[88]
	Sussex River, England	<5.3 - 10	[89]
	Ebro River, Spain	10 - 20	[33]
	Ebro River, Spain	ND - 61	[56]
	Llobregat, Cardener, Anoia, Riera de Rubi River, Spain	<90 - 2,970	[81]
	Venice Lagoon, Italy	<1.0 - 145	[34]
	Thermaikos Gulf, Nothern Aegean Sea, Greece	10.6 - 52.3	[47]
	New Orleans Waters,USA	0.9 - 158	[90]
	South Florida, USA	4.8 - 32	[50]
	Brazilian surface water	25 - 84	[51]
	Coastal waters of Shenzhen, China	11.2 - 776.6	[91]
	Dianchi Lake, China	35 - 1,081	[92]
	Liao River, China	12.3 - 116.5	[52]
	Songhua River, Northern China	8.24 - 263	[54]
	Pearl rivers, South China	4.35 - 1,390	[31, 32]
	Yangtze River estuary, China	0.98 - 43.8	[53]
	River waters of South Korea and seven Asian countries	3.0 - 100	[93]
	Tama River, Japan	4.8 - 76.3	[29]
	Coastal waters, Singapore	ND - 2,470	[94]
OPs	Gulf of Gdansk, Poland	<5.0 - 65.9	[85]
	Estuaries and rivers, The Netherlands	<50 - 6,300	[40]
	German Rivers	0.8 - 54	[86]
	Baden-Württemberg River, Germany	<20 - 189	[87]

EDC	Sampling area	Concentration (ng/L)	References
	Elbe River and its tributaries, Germany	<0.5 - 5.0	[80]
	Ombrone River, Italy	33 - 85	[95]
	Glatt River, Switzerland	6.0 - 22	[88]
	Sussex River, England	2.6 - 25	[89]
	Ebro River, Spain	20 - 70	[33]
	Ebro River, Spain	2.9 - 5.3	[56]
	Llobregat, Cardener, Anoia, Riera de Rubi Spain	<90 - 21,900	[81]
	Thermaikos Gulf, Nothern Aegean Sea, Greece	1.7 - 18.2	[47]
	Danube River, Hungary	1.6 - 178	[96]
	Back River, Chesapeake Bay, USA	<0.3	[97]
	Brazilian surface water	ND	[51]
	Dianchi Lake, China	2.0 - 73	[92]
	Liao River, China	2.3 - 13.2	[52]
	Songhua River, Northern China	1.54 - 45.8	[54]
	Yellow River, China	14.66 - 17.72	[98]
	Tama River, Japan	6.9 - 81.9	[29]
NPs	Gulf of Gdansk, Poland	12.9 - 132.9	[85]
	The Netherlands (surface water)	<110 - 4,100	[40]
	Baden-Württemberg Rivers, Germany	ND - 458	[87]
	Elbe River and its tributaries, Germany	0.06 - 2,970	[80]
	Rivers in Germany	6.7 - 134	[41]
	Glatt River, Switzerland	68 - 326	[88]
	Ombrone River, Italy	<2	[95]
	Venice Lagoon, Italy	<0.05 - 211	[34]
	Ebro River, Spain	ND	[33]
	Ebro River, Spain	ND- 15	[56]
	Llobregat, Cardener, Anoia, Riera de Rubi, Spain	<150- 37,300	[81]
	Thermaikos Gulf, Nothern Aegean Sea, Greece	22- 201	[47]
	Danube River, Hungary	8.0 - 428	[96]
	Brazilian surface water	ND	[51]
	Back River, Chesapeake Bay, USA	140 - 200	[97]
	Buenos Aires rivers, Argentina	100 - 7,000	[99]
	Dianchi Lake, China	5 - 57	[92]

EDC	Sampling area	Concentration (ng/L)	References
	Liao River, China	112 - 900.7	[52]
	Songhua River, Northern China (4-t-NP)	106 - 344	[54]
	Songhua River, Northern China (4-n-NP)	0.35 - 3.77	[54]
	Area of Chongqing, China	100 - 7,300	[100]
	Pearl River, China	36 - 33,000	[31]
	Tama River, Japan	51.6 - 147.0	[29]
	River waters of South Korea and 7 Asian Countries	<LOD - 2,097	[93]
OPEOs	The Netherlands (surface water)	<160 - 1,700	[40]
	Elbe River and its tributaries, Germany	0.6 - 9.6	[80]
	Seine River, France	55 - 63	[101]
	Ombrone River, Italy (4-t-OP_1EO)	<94	[95]
	Ombrone River, Italy (4-t-OP_2EO)	6 - 34	[95]
	Ebro River, Spain (OP_2EO)	1.7 - 8.3	[56]
	Thermaikos Gulf, Greece (OP_1EO)	<4 - 9.5	[47]
	Thermaikos Gulf, Greece (OP_2EO)	<4 - 11.7	[47]
	Estuaries and rivers, USA	7 - 400	[102]
NPEOs	Back River, Chesapeake Bay, USA (OP_1EO)	<0.2	[97]
	Back River, Chesapeake Bay, USA (OP_2EO)	<0.02	[97]
	The Netherlands (surface water)	<180 - 8,700	[40]
	Elbe River and its tributaries, Germany	<0.5 - 124	[80]
	Seine River, France (NP_1EO)	9 - 11	[101]
	Ombrone River, Italy (4-t-NP_1EO)	<122	[95]
	Ombrone River, Italy (4-t-NP_2EO)	9 - 35	[95]
	Ebro River, Spain (NP_2EO)	9.4 - 275	[56]
	Thermaikos Gulf, Greece (NP_1EO)	15.2 - 270	[47]
	Thermaikos Gulf, Greece (NP_2EO)	14.6 - 346	[47]
	Estuaries and rivers, USA	220 - 1,050	[102]
	Back River, Chesapeake Bay, USA (NP_1EO)	<0.2 - 67	[97]
	Back River, Chesapeake Bay, USA (NP_2EO)	12 - 57	[97]
	Buenos Aires, Argentina (NP_1EO)	100 - 9,200	[99]
	Buenos Aires, Argentina (NP_2EO)	100 - 5,400	[99]
	Songhua River, Northeastern China (NP_1EO)	8.9 - 385	[54]
	Songhua River, Northeastern China (NP_2EO)	19.6 - 321	[54]

EDC	Sampling area	Concentration (ng/L)	References
	Dianchi Lake, China (NP_1EO)	54 - 1,942	[92]
	Dianchi Lake, China (NP_2EO)	98 - 2,074	[92]

ND: Not Detected. LOD: Limit of Detection.

Table 3. Concentrations (minimum–maximum) of industrial and household products in surface waters worldwide.

2.2.3. BPA, APEOs and APs in west Iberian Peninsula surface waters

Recent studies revealed that surface waters taken from Portuguese aquatic environments show average concentrations of 650 ng/L for BPA, 1,000 ng/L for APEOs and 360 ng/L for APs (Table 4). Comparing such data with those reported in other countries (Table 3) it is observed that in Portugal the global amounts of these compounds are still quite high. Nevertheless, it is important to note that the values measured before/during 2005 [63, 66, 83, 84], namely for BPA and APs, are significantly higher than those measured in 2010-2011. Thus, and assuming that these results represent the wider reality of the west Iberian Peninsula, it seems that good efforts are being done to reduce the levels of industrial and household pollution (Table 4). In spite of this, Table 4 shows that APEOs exist in amounts that are still approximately one hundred fold higher than those recommended by the European legislation. These observations may be due to the presence of several textile industries and also large agricultural fields located near the sampling areas (Table 4); it should be noticed that several pesticide formulations use APEOs as dispersants. However, since similar amounts of APEOs were found in the Spanish and Greek waters [68, 69] it is possible that these EDCs are still being used, and therefore constitute a global problem of coastal areas.

EDC	Sampling area	Concentration (ng/L)	References
BPA	Lima River estuary and Atlantic coast of Viana-Castelo	1.9 - 35.7	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	7.9 - 521.8	[61]
	Leça River estuary and Atlantic coast of Porto	30.6 - 62.4	[65]
	Douro River estuary	<80 - 10,700	[63]
	Douro River estuary and Atlantic coast of Porto	20.4 - 314.0	[62]
	Mondego River estuary	<6.6 - 880	[66]
	Mondego River and its estuary	8.5 - 184.6	[67]
	Tagus River estuary	≈ 13 - ≈ 320	*Unpublished*
	Sado River estuary	7.3 - 28	[103]
	Sado River estuary	12.2 - 28.9	[59]
	Mira River and its estuary	≈ 7 - ≈ 360	*Unpublished*
	Ria Formosa	6.5 - 71.7	[60]
	Portuguese rivers and estuaries	200 - 4,000	[83]
	Portuguese rivers and estuaries	0.2 - 5,000	[84]
4-OP	Lima River estuary and Atlantic coast of Viana-Castelo	6.2 - 86.5	[64]

EDC	Sampling area	Concentration (ng/L)	References
	Ave River estuary and Atlantic coast of Vila-Conde	0.6 - 8.3	[61]
	Leça River estuary and Atlantic coast of Porto	27.0 - 68.3	[65]
	Douro River estuary and Atlantic coast of Porto	< 3.5	[62]
	Mondego River and its estuary	0.7 - 1,279	[67]
	Tagus River and its estuary	≈ 6 - ≈ 150	*Unpublished*
	Sado River estuary	2.8 - 27.8	[59]
	Mira River and its estuary	≈ 6 - ≈ 28	*Unpublished*
	Ria Formosa	3.5 - 8.5	[60]
	Portuguese rivers and estuaries	0.1 - 30,000	[84]
4-t-OP	Lima River estuary and Atlantic coast of Viana-Castelo	5.7 - 105.0	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	1.3 - 25.4	[61]
	Leça River estuary and Atlantic coast of Porto	7.9 - 60.0	[65]
	Douro River estuary and Atlantic coast of Porto	5.1 - 30.6	[62]
	Mondego River and its estuary	30 - 27,502	[67]
	Tagus River and its estuary	≈ 2 - ≈ 71	*Unpublished*
	Sado River and its estuary	11.4 - 22.0	[59]
	Mira River and its estuary	≈ 4 - ≈ 27	*Unpublished*
	Ria Formosa	4.3 - 40.9	[60]
4-n-NP	Lima River estuary and Atlantic coast of V. Castelo	3.0 - 35.4	[64]
	Ave River estuary and Atlantic coast of V. Conde	0.3 - 16.8	[61]
	Leça River estuary and Atlantic coast of Porto (north)	28.8 - 63.5	[65]
	Douro River estuary and Atlantic coast of Porto	3.3 - 116.0	[62]
	Mondego River and its estuary	20.8 - 2,770	[67]
	Tagus River and its estuary	≈ 1 - ≈ 41	*Unpublished*
	Sado River estuary	2.6 - 27.3	[59]
	Mira River and its estuary	≈ 2 - ≈ 33	*Unpublished*
	Ria Formosa	3.4 - 14.6	[60]
4-NP	Lima River estuary and Atlantic coast of Viana-Castelo	3.9 - 649.8	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	43.3 - 154.9	[61]
	Douro River estuary and Atlantic coast of Porto	88.2 - 170.0	[62]
	Mondego River and its estuary	80.6 - 1,003	[67]
	Tagus River and its estuary	≈ 200 - ≈ 1,600	*Unpublished*
	Sado River estuary	129.2 - 239.9	[59]
	Mira River and its estuary	≈ 52 - ≈ 289	*Unpublished*
	Ria Formosa	12.2 - 546.6	[60]
	Portuguese rivers and estuaries	200 - 30,000	[83]
	Portuguese rivers and estuaries	0.3 - 25,000	[84]

EDC	Sampling area	Concentration (ng/L)	References
	Lima River estuary and Atlantic coast of Viana-Castelo	8.8 - 125.0	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	2.3 - 31.7	[61]
	Leça River estuary and Atlantic coast of Porto	20.8 - 72.9	[65]
	Douro River estuary and Atlantic coast of Porto	33.0 - 60.0	[62]
OP_1EO	Mondego River and its estuary	10.7 - 2,337	[67]
	Tagus River and its estuary	≈ 6 - ≈ 142	*Unpublished*
	Sado River estuary	13.0 - 109.4	[59]
	Mira River and its estuary	≈ 9 - ≈ 63	*Unpublished*
	Ria Formosa	6.9 - 35.6	[60]
	Lima River estuary and Atlantic coast of Viana-Castelo	21.5 - 374.0	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	44.1 - 208.7	[61]
	Leça River estuary and Atlantic coast of Porto	29.7 - 213.2	[65]
	Douro River estuary and Atlantic coast of Porto	100.0 - 424.0	[62]
OP_2EO	Mondego River and its estuary	21.5 - 2,330	[67]
	Tagus River and its estuary	≈ 4 - ≈ 67	*Unpublished*
	Sado River and its estuary	60.0 - 384.2	[59]
	Mira River and its estuary	≈ 4 - ≈ 43	*Unpublished*
	Ria Formosa	46.5 - 182.1	[60]
	Lima River estuary and Atlantic coast of Viana-Castelo	44.9 - 259.1	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	29.9 - 227.8	[61]
	Leça River estuary and Atlantic coast of Porto	115.6 - 923.3	[65]
	Douro River estuary and Atlantic coast of Porto	101.0 - 354.0	[62]
NP_1EO	Mondego River and its estuary	95.4 - 7,794	[67]
	Tagus River and its estuary	≈ 15 - ≈ 340	*Unpublished*
	Sado River and its estuary	60.0 - 311.4	[59]
	Mira River and its estuary	≈ 14 - ≈ 816	*Unpublished*
	Ria Formosa	41.4 - 278.9	[60]
	Lima River estuary and Atlantic coast of Viana-Castelo	47.3 - 467.0	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	142.6 - 750.6	[61]
	Leça River estuary and Atlantic coast of Porto	723.1 - 2,132	[65]
	Douro River estuary and Atlantic coast of Porto	212.0 - 1,148	[62]
NP_2EO	Mondego River and its estuary	118.5 - 18,327	[67]
	Tagus River and its estuary	≈ 40 - ≈ 300	*Unpublished*
	Sado River and its estuary	167.0 - 1,096	[59]
	Mira River and its estuary	≈ 111 - ≈ 3,600	*Unpublished*
	Ria Formosa	49.1 - 779.7	[60]

Table 4. Concentrations (minimum–maximum) of industrial and household products in Portuguese surface waters.

2.3. Naturally occurring compounds present in plants

2.3.1. Phytoestrogens, characteristics and their environmental origin

Accordingly to the U.S. Food Standards Agency [104], phytoestrogens are "any compound of vegetable origin, or their(s) metabolite(s) with structural similarities to E_2, which results in their ability to mimic or block the action of the endogenous sexual hormone in Man". Phytoestrogens fall into two classes: (*i*) flavonoids; and (*ii*) non-flavonoids. The flavonoids are subdivided into the three subclasses: (*i*) isoflavones (Figure 5); (*ii*) coumestans; and (*iii*) prenylflavonoids. The non-flavonoids include the lignans [104].

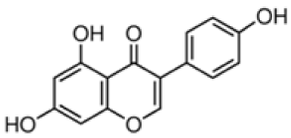
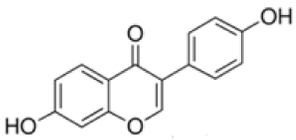
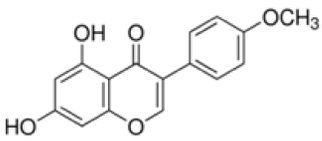
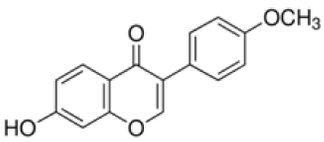
Isoflavone	Chemical structure	Origin
Daizein (DAID)		Natural: Leachates from the decomposition of vascular plants (either living in the margins or in the waters of rivers, estuaries or coastal areas) [14, 15]. Industrial: Debris from industrial processes [105].
Genistein (GEN)		
Biochanin A (BIO-A)		
Formononetin (FORM)		

Figure 5. Phytoestrogens with potential oestrogenic activity.

In this Chapter it is focused the group of isoflavones, which include compounds such as daidzein (DAID), genistein (GEN), biochanin A (BIO-A) and formononetin (FORM), due to their structural resemblance with E_2. Although not steroids, isoflavones exhibit high affinity for oestrogen receptors. In fact, both *in vivo* and *in vitro* studies have demonstrated their ability to induce abnormal liver synthesis of vitellogenin in male goldfish (*Carassius auratus*) and in rainbow trout (*O. mykiss*) [106-108], and the triggering of hermaphroditism in fish, such as the Japanese medaka (*O. latipes*) exposed to 1,000 μg/L of GEN [15]. Thus, although these compounds have not been included yet in the list of substances under the supervision by the WFD, but because their global levels in superficial waters can easily reach levels 1,000 times higher than those of E_2, i.e., in the order of μg/L or even mg/L, it is plausible to think that phytoestrogens may induce effects equivalent to those described for E_2 in spite of having a much lower potency [109]. Despite this, there are not many studies dedicated to the evaluation of these EDCs in surface waters. Also, little information exists about their origins and persistence. Presently, the main source of phytoestrogens in the aquatic environment is attributed either

to the presence of the seagrass *Zostera noltii*, and/or to the leaching from the margins where plants rich in these compounds may exist, e.g., *Typha* spp., *Phragmites communis*, *Juncus acutus*, *Fuirena pubescens*, *Carex riparia* and *Carex hispida*, *Cladium mariscus*, *Callitriche stagnalis* and *Potamogeton* spp., *Trifolium* spp. and *Papilionaceae* [110-112].

Other equally important source of phytoestrogens in surface waters is their disposal as a result of industrial processes — especially in food processing industries and paper mills [105]. So, as the water levels of these compounds can quickly mount up to mg/L, it is possible that they exert in the wilderness the disruptive biological effects attributed to them [112-114]. Nonetheless, if the levels of these EDCs do not surpass ng/L and, in the absence of other potentially stressful compounds, it is most likely that phytoestrogens are harmless [115].

2.3.2. Phytoestrogens in surface waters

Comparatively to the other EDCs, the evaluation of phytoestrogens in aquatic environments, particularly in surface waters of estuaries or rivers, in less studied than the other EDCs referred in this Chapter. This is an important gap in the current knowledge since one the possible origins of these compounds is the natural aquatic flora, which generally is very developed in areas where the organic load is high and prone for eutrophication [116], which is a banal occurrence worldwide. In Table 5 it is shown a collection of studies done in several continents, and the data reveal that the highest concentrations of DAID (42,900 ng/L) and GEN (143,000 n/L) were found in Asia, in the Japanese Kanzaki River. In contrast, the highest amounts of FORM (157 ng/L) and BIO-A (59.4 ng/L) were measured in Europe, at various locations in Switzerland.

EDC	Sampling area	Concentration (ng/L)	References
DAID	Tiber River, Italy	2.0 - 3.0	[44]
	Glatt, Töss, Swiss Midlands, Switzerland	ND - 31.5	[117]
	Surface waters, Switzerland	*Detected*	[118]
	Drainage waters, Switzerland	5.0 - 30	[118]
	Rhine River, Germany	< 10	[119]
	Several rivers, Iowa, USA	10.5 - 41	[120]
	Lake Vadnais and Metro Plant effluent channel, USA	1.6 - 1.8	[115]
	Straight Lake, USA	ND	[115]
	Several Rivers, Brazil	36.2 - 276	[121]
	Waters from Mullet Creek, Australia	3.0 - 7.0	[122]
	Macquarie Rivulet River, Australia	14 - 33	[122]
	Mullet Creek water, Australia	2.0 - 12	[122]
	Toolijooa surface dam (water), Australia	ND - 120	[122]
	Kanzaki River, Japan	LOD - 42,900	[123]
	Zhangcun River, China	ND - 1,490	[124]
GEN	Tiber River, Italy	4.0 - 7.0	[44]
	Glatt, Töss, Swiss Midlands, Switzerland	ND - 24.2	[117]
	Surface waters, Switzerland	ND	[118]
	Drainage waters, Switzerland	*Detected* - 14	[118]
	Several rivers, Iowa, USA	ND - 8.0	[120]

EDC	Sampling area	Concentration (ng/L)	References
	Waters from Upper Midwest (USA)	1.4 - 1.6	[115]
	Straight Lake, USA	ND	[115]
	Several Rivers, Brazil	3.96 - 336	[122]
	Waters from Mullet Creek, Australia	ND - 1.0	[122]
	Macquarie Rivulet River, Australia	1.0 - 8.0	[122]
	Toolijooa surface dam (water), Australia	1.0 - 20	[122]
	Yeongsan and Seomjin Rivers, Korea	ND - 0.7	[93]
	Salut, Malaysia	ND	[93]
	Khong River, Thailand	ND	[93]
	Long Xuyen city, Vietnam	1.5 - 2.4	[93]
	Siem Reap, Cambodia	4.4	[93]
	Fenhe, China	3.6 - 5.0	[93]
	Zhangcun River, China	ND - 2,650	[124]
	Kanzaki River (Japan)	LOD - 143,000	[123]
FORM	Tiber River, Italy	ND	[44]
	Glatt, Töss, Swiss Midlands, Switzerland	ND - 217	[117]
	Surface waters, Switzerland	*Detected* - 21	[118]
	Drainage waters, Switzerland	44 - 157	[118]
	Several rivers, Iowa, USA	5.3 - 13.5	[120]
	Straight Lake, USA	ND	[115]
	Lake Vadnais, USA	0.9 - 1.1	[115]
	Macquarie Rivulet River, Australia	ND - 2.0	[122]
	Waters from Mullet Creek, Australia	ND - 1.0	[122]
	Toolijooa surface dam (water), Australia	ND - 35	[122]
BIO-A	Tiber River, Italy	1.0 - 3.0	[44]
	Glatt, Töss, Swiss Midlands, Switzerland	ND - 59.4	[117]
	Surface waters, Switzerland	*Detected* - 12	[118]
	Drainage waters, Switzerland	7 - 22	[118]
	Several rivers, Iowa, USA	1.7 - 5.6	[120]
	Lake Vadnais and Metro Plant effluent channel, USA	ND - 1.1	[115]
	Straight Lake, USA	ND	[115]
	Waters from Mullet Creek, Australia	ND - 0.1	[122]
	Macquarie Rivulet River, Australia	ND - 1.0	[122]
	Toolijooa surface dam (water), Australia	ND - 4.0	[122]

ND: Not Detected. LOD: Limit of Detection.

Table 5. Concentrations (minimum–maximum) of phytoestrogens in surface waters worldwide.

2.3.3. Phytoestrogens in Portuguese surface waters

Concerning the Iberian peninsula west Atlantic coast, it was found that surface waters from the Rivers Douro (ca., 19 μg/L BIO-A), Mondego (ca., 5.5 μg/L of FORM and 12 μg/L DAID)

and Tagus (ca., from 10 μ/L) were those holding the higher amounts of phytoestrogens (Table 6). As in these habitats there were occasions when the concentrations of the isoflavones were more than 1,000 times higher than those measured for oestrogens (mainly in spring and summer), it is supposed that those compounds may contribute significantly to endocrine disrupting phenomena occurring in those ecosystems. So, although the phytoestrogens are much less active than oestrogens (E_2) their very high concentrations make them worth studying and relevant in monitoring programs.

EDC	Sampling area	Concentration (ng/L)	References
DAID	Lima River estuary and Atlantic coast of Viana-Castelo	2.9 - 78.5	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	7.7 - 74.3	[61]
	Douro River estuary	<10 - 888	[62]
	Douro River estuary and Atlantic coast of Porto	6.7 - 24.2	[63]
	Mondego River estuary	<3.0 - 526	[66]
	Mondego River and its estuary	52.9 - 11,945	[67]
	Tagus River estuary	≈ 4 - ≈ 20	*Unpublished*
	Sado River estuary	8.4 - 160	[103]
	Sado River and its estuary	3.4 - 32.3	[59]
	Mira River and its estuary	≈ 5 - ≈ 40	*Unpublished*
	Ria Formosa	4.6 - 14.0	[60]
GEN	Lima River estuary and Atlantic coast of Viana- Castelo	18.5 - 120.3	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	36.6 - 682.3	[61]
	Douro River estuary	<3.2 - 197	[62]
	Douro River estuary and Atlantic coast of Porto	16.6 - 137.8	[63]
	Mondego River estuary	<2.6 - 507	[66]
	Mondego River and its estuary	127.9 - 5,093	[67]
	Tagus River and its estuary	≈ 5 - ≈ 4,500	*Unpublished*
	Sado River and its estuary	8.6 - 100	[103]
	Sado River and its estuary	24.5 - 113.4	[59]
	Mira River and its estuary	≈ 3 - ≈ 47	*Unpublished*
	Ria Formosa	404.8 - 1,158	[60]
FORM	Lima River estuary and Atlantic coast of Viana-Castelo	90.0 - 801.0	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	83.0 - 362.3	[61]
	Douro River estuary and Atlantic coast of Porto	68 - 341	[63]
	Mondego River and its estuary	25.8 - 5,495	[67]
	Tagus River and its estuary	≈ 3 - ≈ 8	*Unpublished*
	Sado River and its estuary	423.4 - 2,605	[59]
	Mira River and its estuary	≈ 3 - ≈ 91	*Unpublished*
	Ria Formosa	186.3 - 1,041	[60]
BIO-A	Lima River estuary and Atlantic coast of Viana-Castelo	23.5 - 350.0	[64]
	Ave River estuary and Atlantic coast of Vila-Conde	99.0 - 398.1	[61]
	Douro River estuary	<12 - 191	[62]
	Douro River estuary and Atlantic coast of Porto	728.4 - 19,091	[63]

EDC	Sampling area	Concentration (ng/L)	References
	Mondego River estuary	<8.4 - 60	[66]
	Mondego River and its estuary	50.1 - 590.0	[67]
	Tagus River and its estuary	≈ 6 - ≈ 85	*Unpublished*
	Sado River and its estuary	10 - 30	[103]
BIO-A	Sado River and its estuary	130.8 - 844.5	[59]
	Mira River and its estuary	≈ 5 - ≈ 460	*Unpublished*
	Ria Formosa	91.2 - 261.4	[60]

Table 6. Concentrations (minimum–maximum) of phytoestrogens measured in Portuguese surface waters.

3. Individual compounds versus total estrogenic load and its endocrine disruption potential

To better understand and predict the effect of the measured concentrations of all EDCs compiled in this Chapter, in terms of action strength and consequent endocrine disrupting effects, the oestrogenic potency of each compound was estimated relative to that of the standard reference oestrogen, the EE_2, the most potent environmental oestrogen at this date. Thus, the average levels of each analysed EDC at every studied area in the west of the Iberian Peninsula were all converted in EE_2 equivalents (EE_{2eq}). The use of these units facilitates the interpretation of the data. The EE_{2eq} estimates of the estrogenic potential of the sixteen EDCs referred in this Chapter followed the next formula [125]:

$$EE_{2eq} = C \; x \; F$$

Here, ***C*** concerns to the measured concentration of a given EDC and ***F*** refers to the EE_2 equivalency factor, as determined from *in vitro* assays [125]. Although this type of interpretation is very useful, the ***C*** may vary with the assay and it does not exempt the *in vivo* testing.

Interpreting the results presented here, in light of that normalization, and joining the EDCs by groups (i.e., oestrogens, APs + BPA, APEOs and phytoestrogens) it can be deduced that before 2005 the Portuguese surface waters taken from the rivers Douro, Mondego and Sado exhibited values of EE_{2eq} that "hovered" between 24 and 198 ng/L, being the Douro River estuary the habitat with the highest oestrogen load (Table 7). After 2005, possibly due to the application of some of the regulations proposed by WFD, it was observed a significant decrease of the EE_{2eq} in the Douro River estuary surface waters, which displayed values that stand in 12 ng/L, even considering a larger spectrum of analysed EDCs. For the Mondego and Sado Rivers it is also observed that even analysing almost the twice number of EDCs, the data obtained from surface waters in 2005 [66, 103] had similar EE_{2eq} (24 ng/L) than those observed in waters from the same sampling areas in 2010-2011. Besides, from the analysis of Table 7 it is also possible to observe which group of compounds contribute the most to the final values of $EE_{2eq.}$ Thus, it is concluded that by order of importance the compounds that contribute the most for

the EE_{2eq} values in the Portuguese surface waters were: (*i*) oestrogens; (*ii*) phytoestrogens; (*iii*) APs + BPA; and (*iv*) APEOs. So, both oestrogens and phytoestrogens are important "key points" to consider when the purpose of achieving good water quality by 2015 is the main goal of the European Environment Agency (EEA). Overall, it is proposed herein that an improvement of the sewerage system could surely promote reduction of the concentration of oestrogens and eutrophication. In addition, it is also suggested that the authorities should equate ways to reduce impacts caused by the use of products containing APEOs, e.g., by regulating their imports.

Sampling areas		EE_{2eq} (ng/L)				Total (ng/L)
		Oestrogens	APs+BPA	APEOs	Phytoestrogens	
Lima River	[64]	18	0.5	0.000	13	32
Ave River	[61]	9.0	0.4	0.001	12	22
Leça River	[65]	10	0.01	0.000	*NA*	10
Douro River	[63]	192	0.25	NA	5.1*	198
Douro River	[62]	9.0	0.45	0.002	2.9	12
Mondego River	[66]	16	0.04	NA	9.0*	25
Mondego River	[67]	12	2.0	0.004	58	72
Tagus River	*Unpublished*	≈ 11	≈ 1	≈ 0	≈ 43	55
Sado River	[103]	16	0.005	*NA*	8.7*	25
Sado River	[59]	9.2	0.6	0.001	12	22
Mira River	*Unpublished*	≈ 52	≈ 1	≈ 0	≈ 1	54
Ria Formosa	[60]	24	0.8	0.001	28	52

Data not available (NA) or (*) summations containing different number of analysed EDCs.

Table 7. Estimation of the estrogenic potential of several Portuguese surface waters.

4. Status of waters in the European Union and in Portugal

Since the beginning of the implementation of the WFD, the EC was aware that it would not be an easy task to attain the proposed quality goals stated in all EU member states within a limited period of time — 15 years [3]. Therefore, although strict targets ought to be accomplished in all states, it was considered some temporal flexibility to completely achieve the main goals, as it was considered that each nation has its own environmental (and social) characteristics. In this vein, a 2007 report from the EC revealed that nineteen EU states still showed significant weaknesses in the implementation of the WFD and, called attention to the risk of the purposes set for 2015 may not be met. Therefore, in order to coerce the accomplishment of the WFD requirements, the EC appointed the EEA as a periodic gauge which role has been the evaluation of the water quality in each country that assumed to apply the WFD. In this context, during the last evaluation by the EEA, Portugal was identified as having not yet implemented a plan for the management of all national watersheds (Judgment from 21 June 2012 in Case C-223/11,

Portugal) [126]. As this task was considered essential for the implementation of various articles defined in the WFD, including the Article 8 that aims the implementation of standards for water monitoring, Portugal together with others (Spain, Greece and Luxembourg) were condemned by the Court of Justice of the European Community. Besides this occurrence, in 2013 the WFD published a list of other, most common, defaults recorded in many states [127]: (*i*) existence of severe gaps in the levels of chemical pollutants from anthropogenic origins in surface waters; (*ii*) 60% of groundwater resources in cities were over-exploitation; (*iii*) 25% of the groundwater was polluted; (*iv*) 47% of the surface waters showed bad ecological status; and (*v*) 50% of the wetlands showed extinction risks of indigenous species. Considering the first item of this list, it is demonstrated the need of implement chemical monitoring programs for all states involved in the implementation of the WFD. As a corollary, we do emphasize herein the relevance of the regular chemical monitoring and the implementation of strategies for reducing the levels of the EDCs referred in this Chapter.

5. Impact of natural and xenoestrogenic compounds in human health

Estimating with certainty the contribution of aquatic environmental pollution — namely by the above mentioned EDCs — to the burden of disease in humans is extremely difficult and consequently quite polemic [10, 128]. This fact comes from the difficulty to measure and link exposures with the health disorders that may occur in humans, as these are not in regular contact with "oestrogenic waters" as aquatic animals. However, the consumption of contaminated drinking water and/or seafood together with some other sporadic contact between humans and contaminated waters, e.g., during recreational activities either in sea or fluvial beaches [60, 64], may change some preconception about this issue. In fact, and despite the confounding variables, recent studies have linked the presence of environmental natural oestrogens, phytoestrogens and xenoestrogens with the development of a range of disorders that go from immune deficiencies, birth defects, chronic endocrine diseases to cancer (Table 8).

During the last decade researchers devoted to both environmental health and human oncology have shown an increasing interest in the environmental impacts of EDCs over human health as the chemical structures of some of these chemicals, namely those refereed in this work, resemble that of E_2 which is a molecule that evolution maintained conserved amongst different species [10]. This means that, alike fishes and other aquatic animals, the distribution of oestrogen receptors in mammalian/human tissues is so wide that the presence of these EDCs are able to interfere with the orchestration of an important number of pathways, some of which, are close related with the development of cancer [129-132]. Besides, in both fish and mammals high levels of oestrogens induce the production of reactive oxygen species causing hypomethylation and microsatellite instability [133, 134]; these phenomena, which is an early step in the process of carcinogenesis, cause DNA adducts and other genetic damages, seen, e.g., by the emergence of micronuclei, a fact that was observed by our group in fish caught in areas described here as having high estrogenic loads [135].

EDCs	Human health disorders	References
E_1, E_2 and EE_2	Immune deficiencies	[23, 24]
	Ovarian Cancer	[136]
	Cancer in children and adolescent	[137]
	Abnormal prostate development	[138]
BPA and APs	Breast Cancer	[10, 139]
	Human reproduction	[140]
	Reproductive development	[141]
	Premature puberty and endometriosis development	[142]
	Fetal development	[143]
	Defects in human male germ cells	[144]
Phytoestrogens	Breast cancer	[145]
	Hypospadias	[146]
	Puberty disorders	[147]
	Masculine infertility	[148]
	Endocrine modulation	[149, 150]

Table 8. Examples of some disorders promoted by the EDCs focused in this Chapter.

Some epidemiologic studies also support the correlation between oestrogenic EDCs and cancer as, it was found that in Europe childhood cancer incidence is having an annual increase of 1% [137]. This worrying result it is also associated with a rising trend of other cancer types such as the soft tissue sarcoma, brain tumours, germ-cell tumours, lymphomas, renal cancers, leukaemia, breast cancer and lung cancer in women [10, 151, 152]. These occurrences are much preoccupant and alert all society to the possible risk that these compounds can pose for public health.

6. Conclusion and perspectives

As demonstrated in this Chapter, with regard to the xenobiotics that can act as oestrogens there is still much to do in both Portugal and other countries in order to reduce this type of chemical pollution in the surface waters from rivers, estuaries and coastal areas. Thus it would be very useful to conceive national monitoring plans, coordinated in time and space (location of the areas under evaluation), using not only chemical methods but also biological tools (e.g., via the usage of biomarkers). This type of plans, involving and networking public and private agents, would make it possible to assess risks and if measures of prevention and remediation that are being promoted on the ground produce, the desired effects, namely as required by the implementation of the WFD. With regard to research activities done in the west Iberian Peninsula, we seek to continue developing projects that allow the diagnosis of the aquatic systems, not only focusing the attention in the type of EDCs discussed here but also in others judged relevant, such as emerging pharmaceutical compounds, PAHs, PCBs and pesticides — some data are already published concerning these compounds [153-159]. Introduction of

passive sampling methods should be pursued too, to get time-integrated characterizations. In parallel, we also view as utterly important to contribute with knowledge about the mechanisms of action that underlie the disruptive effects in aquatic organisms, as illustrated in works we co-authored [160-169]. At last, it is very relevant to reinforce the efforts to investigate cause-effect associations related with potential long term risks of drinking (inc. tape) waters contaminated with estrogenic compounds, both by monitoring the types and quantities of compounds [170] and by epidemiological approaches [171].

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