
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 17 β -oestradiol (E₂) and 17 α -ethinylestradiol (EE₂), 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 E₂ [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].

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 EE₂ [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 (E₁), E₂ and EE₂ (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 E₁, E₂ and EE₂ 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.

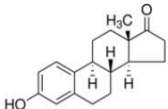
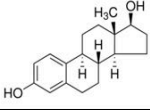
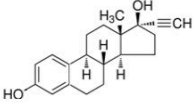
Class	Example	Chemical structure	Origin
Natural oestrogens	Oestrone (E ₁)		Urine and faeces excretion [26].
	17β-Oestradiol (E ₂)		
Synthetic oestrogens	17α-Ethinylestradiol (EE ₂)		Urine and faeces of women consuming birth control pills [26].

Figure 1. Natural and synthetic oestrogens included (E₂ and EE₂) or to be included (E₁) 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 E₂ 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 EE₂ 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 E₁ 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 from 78.7 ng/L to 85.6 ng/L. The highest amounts of E₂ were measured in Italy (Venice Lagoon), Japan (Tama River) and Taiwan (Shui-Dan River) where its levels ranged from 12 ng/L to 175 ng/L. Finally the highest levels of EE₂ 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
E ₁	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]
	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, Northern Aegean Sea, Greece	<LOD	[47]
	Buyukcekme watershed, Istanbul, 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]
	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, Northern Aegean Sea, Greece	ND	[47]
E ₂	Buyukcekme watershed, Istanbul, 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]
	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]
EE ₂			

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, Northern Aegean Sea, Greece	ND	[47]
	Buyukcekme watershed, Istanbul, 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 known 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

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 O₂, 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 E₁, E₂ and EE₂ 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 EE₂ [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 E₁, E₂, and EE₂ 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
E ₁	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]
	Mondego River estuary	<5.0	[66]
	Mondego River and its estuary	1.0 - 14.6	[67]
	Tagus River and its estuary	≈ 2 - ≈ 6	<i>Unpublished</i>
	Sado River and its estuary	1.0 - 9.8	[59]
	Mira River and its estuary	≈ 3 - ≈ 12	<i>Unpublished</i>
Ria Formosa	1.0 - 2.0	[60]	
E ₂	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]
	Mondego River estuary	<3.0	[66]
	Mondego River and its estuary	1.5 - 18.4	[67]
	Tagus River and its estuary	≈ 3 - ≈ 20	<i>Unpublished</i>
	Sado River and its estuary	1.2 - 10.8	[59]
	Mira River and its estuary	≈ 4 - ≈ 62	<i>Unpublished</i>
Ria Formosa	1.3 - 10.1	[60]	
EE ₂	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]
	Mondego River estuary	<12	[66]
	Mondego River and its estuary	0.3 - 11.3	[67]
	Tagus River and its estuary	≈ 4 - ≈ 20	<i>Unpublished</i>
	Sado River and its estuary	1.1 - 3.2	[59]
	Mira River and its estuary	≈ 4 - ≈ 67	<i>Unpublished</i>
Ria Formosa	12.1 - 25.0	[60]	

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

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).

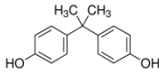
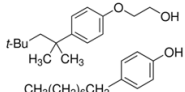
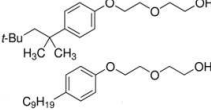
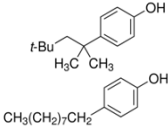
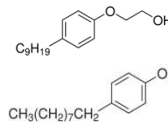
Class	Example	Chemical structure	Origin
Phenols	Bisfenol A (BPA)		Industrial and domestic usages [71].
Alkylphenol ethoxylates (APEOs)	Octylphenol and nonylphenol ethoxylates		Industrial, domestic and as pesticide additives [14, 73].
	Octylphenol and nonylphenol diethoxylates		
Alkylphenols (APs)	Octylphenols (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 $\mu\text{g/L}$ they can become 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 $2 \mu\text{g/L}$ [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 devalue 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].

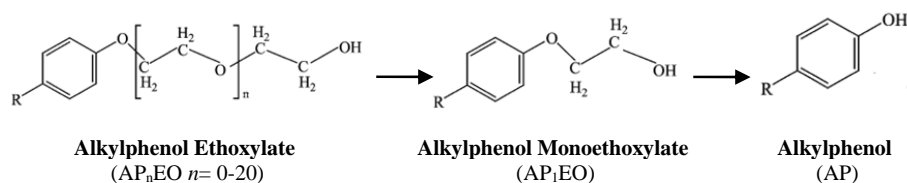


Figure 3. Summarized degradation mechanism of APEOs in the environment adapted from Warhurst [75].

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 E₂ (Figure 4) [76].

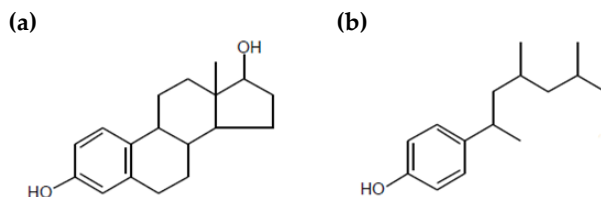


Figure 4. Comparison between the chemical structures of E₂ (a) and 4-NP (b).

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 led 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 led 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
	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]
BPA	Thermaikos Gulf, Northern 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]
	Gulf of Gdansk, Poland	<5.0 - 65.9	[85]
	Estuaries and rivers, The Netherlands	<50 - 6,300	[40]
OPs	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]
	Ombro 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, Northern 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]
	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]
	Ombro River, Italy	<2	[95]
	Venice Lagoon, Italy	<0.05 - 211	[34]
NPs	Ebro River, Spain	ND	[33]
	Ebro River, Spain	ND- 15	[56]
	Llobregat, Cardener, Anoia, Riera de Rubi, Spain	<150- 37,300	[81]
	Thermaikos Gulf, Northern 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]
	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]
	Ombro River, Italy (4-t-OP ₁ EO)	<94	[95]
OPEOs	Ombro River, Italy (4-t-OP ₂ EO)	6 - 34	[95]
	Ebro River, Spain (OP ₂ EO)	1.7 - 8.3	[56]
	Thermaikos Gulf, Greece (OP ₁ EO)	<4 - 9.5	[47]
	Thermaikos Gulf, Greece (OP ₂ EO)	<4 - 11.7	[47]
	Estuaries and rivers, USA	7 - 400	[102]
	Back River, Chesapeake Bay, USA (OP ₁ EO)	<0.2	[97]
	Back River, Chesapeake Bay, USA (OP ₂ EO)	<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 ₁ EO)	9 - 11	[101]
	Ombro River, Italy (4-t-NP ₁ EO)	<122	[95]
	Ombro River, Italy (4-t-NP ₂ EO)	9 - 35	[95]
	Ebro River, Spain (NP ₂ EO)	9.4 - 275	[56]
NPEOs	Thermaikos Gulf, Greece (NP ₁ EO)	15.2 - 270	[47]
	Thermaikos Gulf, Greece (NP ₂ EO)	14.6 - 346	[47]
	Estuaries and rivers, USA	220 - 1,050	[102]
	Back River, Chesapeake Bay, USA (NP ₁ EO)	<0.2 - 67	[97]
	Back River, Chesapeake Bay, USA (NP ₂ EO)	12 - 57	[97]
	Buenos Aires, Argentina (NP ₁ EO)	100 - 9,200	[99]
	Buenos Aires, Argentina (NP ₂ EO)	100 - 5,400	[99]
	Songhua River, Northeastern China (NP ₁ EO)	8.9 - 385	[54]
	Songhua River, Northeastern China (NP ₂ EO)	19.6 - 321	[54]

EDC	Sampling area	Concentration (ng/L)	References
	Dianchi Lake, China (NP ₁ EO)	54 - 1,942	[92]
	Dianchi Lake, China (NP ₂ EO)	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	<i>Unpublished</i>
	Sado River estuary	7.3 - 28	[103]
	Sado River estuary	12.2 - 28.9	[59]
	Mira River and its estuary	≈ 7 - ≈ 360	<i>Unpublished</i>
	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	<i>Unpublished</i>
	Sado River estuary	2.8 - 27.8	[59]
	Mira River and its estuary	≈ 6 - ≈ 28	<i>Unpublished</i>
	Ria Formosa	3.5 - 8.5	[60]
	Portuguese rivers and estuaries	0.1 - 30,000	[84]
	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]
4-t-OP	Mondego River and its estuary	30 - 27,502	[67]
	Tagus River and its estuary	≈ 2 - ≈ 71	<i>Unpublished</i>
	Sado River and its estuary	11.4 - 22.0	[59]
	Mira River and its estuary	≈ 4 - ≈ 27	<i>Unpublished</i>
	Ria Formosa	4.3 - 40.9	[60]
	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]
4-n-NP	Mondego River and its estuary	20.8 - 2,770	[67]
	Tagus River and its estuary	≈ 1 - ≈ 41	<i>Unpublished</i>
	Sado River estuary	2.6 - 27.3	[59]
	Mira River and its estuary	≈ 2 - ≈ 33	<i>Unpublished</i>
	Ria Formosa	3.4 - 14.6	[60]
	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]
4-NP	Tagus River and its estuary	≈ 200 - ≈ 1,600	<i>Unpublished</i>
	Sado River estuary	129.2 - 239.9	[59]
	Mira River and its estuary	≈ 52 - ≈ 289	<i>Unpublished</i>
	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
OP ₁ EO	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]
	Mondego River and its estuary	10.7 - 2,337	[67]
	Tagus River and its estuary	≈ 6 - ≈ 142	<i>Unpublished</i>
	Sado River estuary	13.0 - 109.4	[59]
	Mira River and its estuary	≈ 9 - ≈ 63	<i>Unpublished</i>
	Ria Formosa	6.9 - 35.6	[60]
OP ₂ EO	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]
	Mondego River and its estuary	21.5 - 2,330	[67]
	Tagus River and its estuary	≈ 4 - ≈ 67	<i>Unpublished</i>
	Sado River and its estuary	60.0 - 384.2	[59]
	Mira River and its estuary	≈ 4 - ≈ 43	<i>Unpublished</i>
	Ria Formosa	46.5 - 182.1	[60]
NP ₁ EO	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]
	Mondego River and its estuary	95.4 - 7,794	[67]
	Tagus River and its estuary	≈ 15 - ≈ 340	<i>Unpublished</i>
	Sado River and its estuary	60.0 - 311.4	[59]
	Mira River and its estuary	≈ 14 - ≈ 816	<i>Unpublished</i>
	Ria Formosa	41.4 - 278.9	[60]
NP ₂ EO	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]
	Mondego River and its estuary	118.5 - 18,327	[67]
	Tagus River and its estuary	≈ 40 - ≈ 300	<i>Unpublished</i>
	Sado River and its estuary	167.0 - 1,096	[59]
	Mira River and its estuary	≈ 111 - ≈ 3,600	<i>Unpublished</i>
	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].

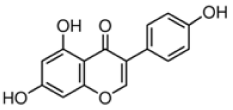
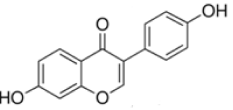
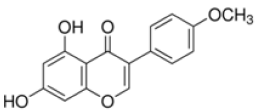
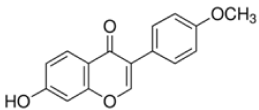
Isoflavone	Chemical structure	Origin
Daizein (DAID)		<p>Natural: Leachates from the decomposition of vascular plants (either living in the margins or in the waters of rivers, estuaries or coastal areas) [14, 15].</p> <p>Industrial: Debris from industrial processes [105].</p>
Genistein (GEN)		
Biochanin 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 $\mu\text{g/L}$ 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 $\mu\text{g/L}$ 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, is less studied than the other EDCs referred in this Chapter. This is an important gap in the current knowledge since one of 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
Glatt, Töss, Swiss Midlands, Switzerland		ND - 217	[117]
Surface waters, Switzerland		<i>Detected</i> - 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	<i>Detected</i> - 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 (E₂) 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	<i>Unpublished</i>
	Sado River estuary	8.4 - 160	[103]
	Sado River and its estuary	3.4 - 32.3	[59]
	Mira River and its estuary	≈ 5 - ≈ 40	<i>Unpublished</i>
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	<i>Unpublished</i>
	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	<i>Unpublished</i>
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	<i>Unpublished</i>
	Sado River and its estuary	423.4 - 2,605	[59]
	Mira River and its estuary	≈ 3 - ≈ 91	<i>Unpublished</i>
	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	<i>Unpublished</i>
	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	<i>Unpublished</i>
	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]:

$$EE_{2eq} = C \times F$$

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	<i>Unpublished</i>	~ 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	<i>Unpublished</i>	~ 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
E₁, E₂ and EE₂	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. tap) waters contaminated with estrogenic compounds, both by monitoring the types and quantities of compounds [170] and by epidemiological approaches [171].

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References

- [1] Barnett TP, Adam JC, Lettenmaier DP. Potential Impacts of a Warming Climate on Water Availability in Snow-Dominated Regions. *Nature* 2005; 438(7066) 303-309.
- [2] Commission E. The Eu Biodiversity Strategy to 2020. Publications Office of the European Union 2011.
- [3] WFD. Directive 2000/60/Ec of the European Parliament and of the Council, in, Official Journal of the European Communities, 2000.

- [4] WFD. Decision No 2455/2001/Ec of the European Parliament and of the Council of 20 November 2001 Establishing the List of Priority Substances in the Field of Water Policy and Amending Directive 2000/60/Ec, in, Official Journal of the European Communities, 2001.
- [5] WFD. Directive 2008/105/Ec of the European Parliament and of the Council of 16 December 2008 on Environmental Quality Standards in the Field of Water Policy, Amending and Subsequently Repealing Council Directives 82/176/Eec, 83/513/Eec, 84/156/Eec, 84/491/Eec, 86/280/Eec and Amending Directive 2000/60/Ec of the European Parliament and of the Council in, Official Journal of the European Union, 2008.
- [6] WFD. Directive 2013/39/Eu of the European Parliament and of the Council of 12 August 2013 Amending Directives 2000/60/Ec and 2008/105/Ec as Regards Priority Substances in the Field of Water Policy, in, Official Journal of the European Union, 2013.
- [7] NIH. Endocrine Disruptors, in, National Institutes of Health U.S. Department of Health and Human Services, 2010.
- [8] Kordon C, Gaillard RC. Hormones and the Brain. Research and Perspectives in Endocrine Interactions. In: Springer-Verlag Berlin Heidelberg New York; 2006. p
- [9] Tierney KB, Farrell AP, Brauner CJ, (Eds.). Fish Physiology: Organic Chemical Toxicology of Fishes, Academic Press; 2013.
- [10] Fucic A, Gamulin M, Ferencic Z, Katic J, Kraye von Krauss M, Bartonova A, Merlo D. Environmental Exposure to Xenoestrogens and Oestrogen Related Cancers: Reproductive System, Breast, Lung, Kidney, Pancreas, and Brain. Environmental Health 2012; 11(Suppl 1) S8.
- [11] Ferreira M, Antunes P, Gil O, Vale C, Reis-Henriques MA. Organochlorine Contaminants in Flounder (*Platichthys flesus*) and Mullet (*Mugil cephalus*) from Douro Estuary, and Their Use as Sentinel Species for Environmental Monitoring. Aquatic Toxicology 2004; 69(4) 347-357.
- [12] Mills LJ, Chichester C. Review of Evidence: Are Endocrine-Disrupting Chemicals in the Aquatic Environment Impacting Fish Populations? Science of the Total Environment 2005; 343(1-3) 1-34.
- [13] Ying GG, Kookana RS, Chen ZL. On-Line Solid-Phase Extraction and Fluorescence Detection of Selected Endocrine Disrupting Chemicals in Water by High-Performance Liquid Chromatography. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 2002; 37(3) 225-234.
- [14] Lintelmann J, Katayama A, Kurihara N, Shore L, Wenzel A. Endocrine Disruptors in the Environment - (Iupac Technical Report). Pure and Applied Chemistry 2003; 75(5) 631-681.

- [15] Kiparissis Y, Hughes R, Metcalfe C, Ternes T. Identification of the Isoflavonoid Genistein in Bleached Kraft Mill Effluent. *Environmental Science and Technology* 2001; 35(12) 2423-2427.
- [16] Gutendorf B, Westendorf J. Comparison of an Array of *in Vitro* Assays for the Assessment of the Estrogenic Potential of Natural and Synthetic Estrogens, Phytoestrogens and Xenoestrogens. *Toxicology* 2001; 166(1-2) 79-89.
- [17] Sumpter JP. Endocrine Disrupters in the Aquatic Environment: An Overview. *Acta Hydrochimica et Hydrobiologica* 2005; 33(1) 9-16.
- [18] McLachlan JA, Simpson E, Martin M. Endocrine Disrupters and Female Reproductive Health. *Best Practice & Research Clinical Endocrinology & Metabolism* 2006; 20(1) 63-75.
- [19] Milnes MR, Bermudez DS, Bryan TA, Edwards TM, Gunderson MP, Larkin ILV, Moore BC, Guillette LJ. Contaminant-Induced Feminization and Demasculinization of Nonmammalian Vertebrate Males in Aquatic Environments. *Environmental Research* 2006; 100(1) 3-17.
- [20] Scott AP, Sanders M, Stentiford GD, Reese RA, Katsiadaki I. Evidence for Estrogenic Endocrine Disruption in an Offshore Flatfish, the Dab (*Limanda limanda* L.). *Marine Environmental Research* 2007; 64(2) 128-148.
- [21] Hotchkiss AK, Lambright CS, Ostby JS, Parks-Saldutti L, Vandenberg JG, Gray LE, Jr. Prenatal Testosterone Exposure Permanently Masculinizes Anogenital Distance, Nipple Development, and Reproductive Tract Morphology in Female Sprague-Dawley Rats. *Toxicological Sciences* 2007; 96(2) 335-345.
- [22] Matthiessen P. An Assessment of Endocrine Disruption in Mollusks and the Potential for Developing Internationally Standardized Mollusk Life Cycle Test Guidelines. *Integrated environmental assessment and management* 2008; 4(3) 274-284.
- [23] Ansar Ahmed S. The Immune System as a Potential Target for Environmental Estrogens (Endocrine Disrupters): A New Emerging Field. *Toxicology* 2000; 150(1-3) 191-206.
- [24] Chalubinski M, Kowalski ML. Endocrine Disrupters - Potential Modulators of the Immune System and Allergic Response. *Allergy* 2006; 61(11) 1326-1335.
- [25] Waring RH, Harris RM. Endocrine Disrupters: A Human Risk? *Molecular and Cellular Endocrinology* 2005; 244(1-2) 2-9.
- [26] Belfroid AC, Van der Horst A, Vethaak AD, Schafer AJ, Rijs GBJ, Wegener J, Cofino WP. Analysis and Occurrence of Estrogenic Hormones and Their Glucuronides in Surface Water and Waste Water in the Netherlands. *Science of the Total Environment* 1999; 225(1-2) 101-108.
- [27] Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE, Potter T. Estrogenic Potency of Chemicals Detected in Sewage Treatment

- Plant Effluents as Determined by *in Vivo* Assays with Japanese Medaka (*Oryzias latipes*). *Environmental Toxicology and Chemistry* 2001; 20(2) 297-308.
- [28] Peters REM, Courtenay SC, Cagampan S, Hewitt ML, MacLatchy DL. Effects on Reproductive Potential and Endocrine Status in the Mummichog (*Fundulus heteroclitus*) after Exposure to 17 Alpha-Ethynylestradiol in a Short-Term Reproductive Bioassay. *Aquatic Toxicology* 2007; 85(2) 154-166.
- [29] Furuichi T, Kannan K, Glesy JP, Masunaga S. Contribution of Known Endocrine Disrupting Substances to the Estrogenic Activity in Tama River Water Samples from Japan Using Instrumental Analysis and *in Vitro* Reporter Gene Assay. *Water Research* 2004; 38(20) 4491-4501.
- [30] Chen CY, Wen TY, Wang GS, Cheng HW, Lin YH, Lien GW. Determining Estrogenic Steroids in Taipei Waters and Removal in Drinking Water Treatment Using High-Flow Solid-Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry. *Science of the Total Environment* 2007; 378(3) 352-365.
- [31] Peng X, Yu Y, Tang C, Tan J, Huang Q, Wang Z. Occurrence of Steroid Estrogens, Endocrine-Disrupting Phenols, and Acid Pharmaceutical Residues in Urban Riverine Water of the Pearl River Delta, South China. *Science of the Total Environment* 2008; 397(1-3) 158-166.
- [32] Zhao JL, Ying GG, Chen F, Liu YS, Wang L, Yang B, Liu S, Tao R. Estrogenic Activity Profiles and Risks in Surface Waters and Sediments of the Pearl River System in South China Assessed by Chemical Analysis and *in Vitro* Bioassay. *Journal of Environmental Monitoring* 2011; 13(4) 813-821.
- [33] Brossa L, Marcé RM, Borrull F, Pocurull E. Occurrence of Twenty-Six Endocrine-Disrupting Compounds in Environmental Water Samples from Catalonia, Spain. *Environmental Toxicology and Chemistry* 2005; 24(2) 261-267.
- [34] Pojana G, Gomiero A, Jonkers N, Marcomini A. Natural and Synthetic Endocrine Disrupting Compounds (Edcs) in Water, Sediment and Biota of a Coastal Lagoon. *Environment International* 2007; 33(7) 929-936.
- [35] Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty GC, Sumpter JP, Tyler CR. Altered Sexual Maturation and Gamete Production in Wild Roach (*Rutilus rutilus*) Living in Rivers That Receive Treated Sewage Effluents. *Biology of Reproduction* 2002; 66(2) 272-281.
- [36] Lange A, Paull GC, Hamilton PB, Iguchi T, Tyler CR. Implications of Persistent Exposure to Treated Wastewater Effluent for Breeding in Wild Roach (*Rutilus rutilus*) Populations. *Environmental Science & Technology* 2011; 45(4) 1673-1679.
- [37] Tyler CR, Lange A, Paull GC, Katsu Y, Iguchi T. The Roach (*Rutilus rutilus*) as a Sentinel for Assessing Endocrine Disruption. *Environmental Sciences* 2007; 14(5) 235-253.

- [38] van Aerle R, Nolan M, Jobling S, Christiansen LB, Sumpter JP, Tyler CR. Sexual Disruption in a Second Species of Wild Cyprinid Fish (the Gudgeon, *Gobio gobio*) in United Kingdom Freshwaters. *Environmental Toxicology and Chemistry* 2001; 20(12) 2841-2847.
- [39] Noppe H, Verslycke T, De Wulf E, Verheyden K, Monteyne E, Van Caeter P, Janssen CR, De brabander HF. Occurrence of Estrogens in the Scheldt Estuary: A 2-Year Survey. *Ecotoxicology and Environmental Safety* 2007; 66(1) 1-8.
- [40] Vethaak AD, Lahr J, Schrap SM, Belfroid AC, Rijs GBJ, Gerritsen A, de Boer J, Bulder AS, Grinwis GCM, Kuiper RV, Legler J, Murk TAJ, Peijnenburg W, Verhaar HJM, de Voogt P. An Integrated Assessment of Estrogenic Contamination and Biological Effects in the Aquatic Environment of the Netherlands. *Chemosphere* 2005; 59(4) 511-524.
- [41] Kuch HM, Ballschmiter K. Determination of Endocrine-Disrupting Phenolic Compounds and Estrogens in Surface and Drinking Water by Hrgc-(Nci)-Ms in the Pico-gram Per Liter Range. *Environmental Science & Technology* 2001; 35(15) 3201-3206.
- [42] Beck IC, Bruhn R, Gandrass J, Ruck W. Liquid Chromatography-Tandem Mass Spectrometry Analysis of Estrogenic Compounds in Coastal Surface Water of the Baltic Sea. *Journal of Chromatography A* 2005; 1090(1-2) 98-106.
- [43] Cargouet M, Perdiz D, Mouatassim-Souali A, Tamisier-Karolak S, Levi Y. Assessment of River Contamination by Estrogenic Compounds in Paris Area (France). *Science of the Total Environment* 2004; 324(1-3) 55-66.
- [44] Lagana A, Bacaloni A, De Leva I, Faberi A, Fago G, Marino A. Analytical Methodologies for Determining the Occurrence of Endocrine Disrupting Chemicals in Sewage Treatment Plants and Natural Waters. *Analytica Chimica Acta* 2004; 501(1) 79-88.
- [45] Rodriguez-Mozaz S, López de Alda MJ, Barceló D. Monitoring of Estrogens, Pesticides and Bisphenol a in Natural Waters and Drinking Water Treatment Plants by Solid-Phase Extraction-Liquid Chromatography-Mass Spectrometry. *Journal of Chromatography A* 2004; 1045(1-2) 85-92.
- [46] López-Roldán R, de Alda ML, Gros M, Petrovic M, Martín-Alonso J, Barceló D. Advanced Monitoring of Pharmaceuticals and Estrogens in the Llobregat River Basin (Spain) by Liquid Chromatography-Triple Quadrupole-Tandem Mass Spectrometry in Combination with Ultra Performance Liquid Chromatography-Time of Flight-Mass Spectrometry. *Chemosphere* 2010; 80(11) 1337-1344.
- [47] Arditoglou A, Voutsas D. Occurrence and Partitioning of Endocrine-Disrupting Compounds in the Marine Environment of Thermaikos Gulf, Northern Aegean Sea, Greece. *Marine Pollution Bulletin* 2012; 64(11) 2443-2452.

- [48] Aydin E, Talinli I. Analysis, Occurrence and Fate of Commonly Used Pharmaceuticals and Hormones in the Buyukcekmece Watershed, Turkey. *Chemosphere* 2013; 90(6) 2004-2012.
- [49] Zuo Y, Zhang K, Deng Y. Occurrence and Photochemical Degradation of 17 α -Ethinylestradiol in Acushnet River Estuary. *Chemosphere* 2006; 63(9) 1583-1590.
- [50] Singh S, Azua A, Chaudhary A, Khan S, Willett K, Gardinali P. Occurrence and Distribution of Steroids, Hormones and Selected Pharmaceuticals in South Florida Coastal Environments. *Ecotoxicology* 2010; 19(2) 338-350.
- [51] Sodré FF, Pescara IC, Montagner CC, Jardim WF. Assessing Selected Estrogens and Xenoestrogens in Brazilian Surface Waters by Liquid Chromatography-Tandem Mass Spectrometry. *Microchemical Journal* 2010; 96(1) 92-98.
- [52] Wang L, Ying GG, Zhao JL, Liu S, Yang B, Zhou LJ, Tao R, Su HC. Assessing Estrogenic Activity in Surface Water and Sediment of the Liao River System in Northeast China Using Combined Chemical and Biological Tools. *Environmental Pollution* 2011; 159(1) 148-156.
- [53] Shi J, Liu X, Chen Q, Zhang H. Spatial and Seasonal Distributions of Estrogens and Bisphenol a in the Yangtze River Estuary and the Adjacent East China Sea. *Chemosphere* 2014; 111(0) 336-343.
- [54] Zhang Z, Ren N, Kannan K, Nan J, Liu L, Ma W, Qi H, Li Y. Occurrence of Endocrine-Disrupting Phenols and Estrogens in Water and Sediment of the Songhua River, Northeastern China. *Archives of Environmental Contamination and Toxicology* 2014; 66(3) 361-369.
- [55] Isobe T, Shiraishi H, Yasuda M, Shinoda A, Suzuki H, Morita M. Determination of Estrogens and Their Conjugates in Water Using Solid-Phase Extraction Followed by Liquid Chromatography-Tandem Mass Spectrometry. *Journal of Chromatography A* 2003; 984(2) 195-202.
- [56] Gorga M, Petrovic M, Barceló D. Multi-Residue Analytical Method for the Determination of Endocrine Disruptors and Related Compounds in River and Waste Water Using Dual Column Liquid Chromatography Switching System Coupled to Mass Spectrometry. *Journal of Chromatography A* 2013; 1295(0) 57-66.
- [57] Safe SH. Endocrine Disruptors and Human Health - Is There a Problem? An Update. *Environmental Health Perspectives* 2000; 108(6) 487-493.
- [58] Census. Resultados Definitivos - Portugal, in: I.N.d. Estatística, (Ed.), Lisboa, 2011.
- [59] Rocha MJ, Cruzeiro C, Reis M, Rocha E, Pardal MA. Determination of 17 Endocrine Disruptor Compounds and Their Spatial and Seasonal Distribution in the Sado River Estuary (Portugal). *Toxicological & Environmental Chemistry* 2013; 95(2) 237-253.
- [60] Rocha M, Cruzeiro C, Reis M, Rocha E, Pardal M. Determination of Seventeen Endocrine Disruptor Compounds and Their Spatial and Seasonal Distribution in Ria For-

- mosa Lagoon (Portugal). *Environmental Monitoring and Assessment* 2013; 185(10) 8215-8226.
- [61] Rocha MJ, Cruzeiro C, Rocha E. Quantification of 17 Endocrine Disruptor Compounds and Their Spatial and Seasonal Distribution in the Iberian Ave River and Its Coastline. *Toxicological & Environmental Chemistry* 2013; 95(3) 386-399.
- [62] Rocha MJ, Cruzeiro C, Rocha E. Development and Validation of a Gc-MS Method for the Evaluation of 17 Endocrine Disruptor Compounds, Including Phytoestrogens and Sitosterol, in Coastal Waters - Their Spatial and Seasonal Levels in Porto Costal Region (Portugal). *Journal of Water & Health* 2013; 11(2) 281-296.
- [63] Ribeiro C, Tiritan M, Rocha E, Rocha M. Seasonal and Spatial Distribution of Several Endocrine-Disrupting Compounds in the Douro River Estuary, Portugal. *Archives of Environmental Contamination and Toxicology* 2009; 56(1) 1-11.
- [64] Rocha M, Cruzeiro C, Peixoto C, Rocha E. Annual Fluctuations of Endocrine-Disrupting Compounds at the Lower End of the Lima River, Portugal, and in Adjacent Coastal Waters. *Archives of Environmental Contamination and Toxicology* 2014; 67(3) 389-401.
- [65] Rocha MJ, Ribeiro M, Ribeiro C, Couto C, Cruzeiro C, Rocha E. Endocrine Disruptors in the Leça River and Nearby Porto Coast (Nw Portugal): Presence of Estrogenic Compounds and Hypoxic Conditions. *Toxicological & Environmental Chemistry* 2012; 94(2) 262-274.
- [66] Ribeiro C, Pardal M, Martinho F, Margalho R, Tiritan M, Rocha E, Rocha M. Distribution of Endocrine Disruptors in the Mondego River Estuary, Portugal. *Environmental Monitoring and Assessment* 2009; 149(1-4) 183-193.
- [67] Rocha M, Cruzeiro C, Reis M, Pardal M, Rocha E. Spatial and Seasonal Distribution of 17 Endocrine Disruptor Compounds in an Urban Estuary (Mondego River, Portugal): Evaluation of the Estrogenic Load of the Area. *Environmental Monitoring and Assessment* 2014; 186(6) 3337-3350.
- [68] Arditsoglou A, Voutsas D. Passive Sampling of Selected Endocrine Disrupting Compounds Using Polar Organic Chemical Integrative Samplers. *Environmental Pollution* 2008; 156(2) 316-324.
- [69] David A, Fenet H, Gomez E. Alkylphenols in Marine Environments: Distribution Monitoring Strategies and Detection Considerations. *Marine Pollution Bulletin* 2009; 58(7) 953-960.
- [70] Knez J. Endocrine-Disrupting Chemicals and Male Reproductive Health. *Reproductive BioMedicine Online* 2013; 26(5) 440-448.
- [71] Rochester JR. Bisphenol a and Human Health: A Review of the Literature. *Reproductive Toxicology* 2013; 42(0) 132-155.

- [72] Gibson RL. Toxic Baby Bottles. Scientific Finds Leaching Chemicals in Clear Plastic Baby Bottles, in, Environment California Research and Policy Center, 2007, pp. 1-36.
- [73] Ying G-G, Williams B, Kookana R. Environmental Fate of Alkylphenols and Alkylphenol Ethoxylates—a Review. *Environment International* 2002; 28(3) 215-226.
- [74] Ahel M, Schaffner C, Giger W. Behaviour of Alkylphenol Polyethoxylates Surfactants in the Aquatic Environment - III. Occurrence and Elimination of Their Persistent Metabolites During Infiltration of River Water to Groundwater. *Water Research* 1996; 30(1) 37-46.
- [75] Warhurst AM. An Environmental Assessment of Alkylphenol Ethoxylates and Alkylphenols, in, 1995.
- [76] Wang SL, Chang YC, Chao HR, Li CM, Li LA, Lin LY, Papke O. Body Burdens of Polychlorinated Dibenzo-P-Dioxins, Dibenzofurans, and Biphenyls and Their Relations to Estrogen Metabolism in Pregnant Women. *Environmental Health Perspectives* 2006; 114(5) 740-745.
- [77] Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. Inhibition of Testicular Growth in Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Estrogenic Alkylphenolic Chemicals. *Environmental Toxicology and Chemistry* 1996; 15(2) 194-202.
- [78] Balch G, Metcalfe C. Developmental Effects in Japanese Medaka (*Oryzias latipes*) Exposed to Nonylphenol Ethoxylates and Their Degradation Products. *Chemosphere* 2006; 62 (8) 1214-1223.
- [79] Group B. Apeos Investigation Report as of July 2013, in, 2013.
- [80] Stachel B, Ehrhorn U, Heemken O-P, Lepom P, Reincke H, Sawal G, Theobald N. Xenoestrogens in the River Elbe and Its Tributaries. *Environmental Pollution* 2003; 124(3) 497-507.
- [81] Céspedes R, Lacorte S, Raldúa D, Ginebreda A, Barceló D, Piña B. Distribution of Endocrine Disruptors in the Llobregat River Basin (Catalonia, NE Spain). *Chemosphere* 2005; 61(11) 1710-1719.
- [82] EEC. Council Directive of 27 July 1976 on the Approximation of the Laws, Regulations and Administrative Provisions of the Member States Relating to Restrictions on the Marketing and Use of Certain Dangerous Substances and Preparations (76/769/Eec), in, 1976.
- [83] Azevedo DA, Lacorte S, Viana P, Barceló D. Occurrence of Nonylphenol and Bisphenol-a in Surface Waters from Portugal. *Journal of the Brazilian Chemical Society* 2001; 12(4) 532-537.
- [84] Quirós L, Céspedes R, Lacorte S, Viana P, Raldúa D, Barceló D, Piña B. Detection and Evaluation of Endocrine-Disruption Activity in Water Samples from Portuguese Rivers. *Environmental Toxicology and Chemistry* 2005; 24(2) 389-395.

- [85] Staniszewska M, Falkowska L, Grabowski P, Kwasniak J, Mudrak-Cegiolka S, Reindl AR, Sokolowski A, Szumilo E, Zgrundo A. Bisphenol A, 4-Tert-Octylphenol, and 4-Nonylphenol in the Gulf of Gdansk (Southern Baltic). *Archives of Environmental Contamination and Toxicology* 2014; 67(3) 335-347.
- [86] Kuch HM, Ballschmiter K. Determination of Endocrine-Disrupting Phenolic Compounds and Estrogens in Surface and Drinking Water by Hrgc-(Nci)-Ms in the Pico-gram Per Liter Range. *Environmental Science & Technology* 2001; 35(15) 3201-3206.
- [87] Bolz U, Hagenmaier H, Körner W. Phenolic Xenoestrogens in Surface Water, Sediments, and Sewage Sludge from Baden-Württemberg, South-West Germany. *Environmental Pollution* 2001; 115(2) 291-301.
- [88] Voutsas D, Hartmann P, Schaffner C, Giger W. Benzotriazoles, Alkylphenols and Bisphenol A in Municipal Wastewaters and in the Glatt River, Switzerland. *Environmental Science and Pollution Research* 2006; 13(5) 333-341.
- [89] Liu Y, Zhou JL, Wilding A. Simultaneous Determination of Endocrine Disrupting Phenolic Compounds and Steroids in Water by Solid-Phase Extraction-Gas Chromatography-Mass Spectrometry. *Journal of Chromatography A* 2004; 1022(1-2) 179-189.
- [90] Boyd GR, Palmeri JM, Zhang SY, Grimm DA. Pharmaceuticals and Personal Care Products (Ppcps) and Endocrine Disrupting Chemicals (Edcs) in Stormwater Canals and Bayou St. John in New Orleans, Louisiana, USA. *Science of the Total Environment* 2004; 333(1-3) 137-148.
- [91] Liu Y, Guan Y, Tam N, Mizuno T, Tsuno H, Zhu W. Influence of Rainfall and Basic Water Quality Parameters on the Distribution of Endocrine-Disrupting Chemicals in Coastal Area. *Water, Air, & Soil Pollution* 2010; 209(1-4) 333-343.
- [92] Wang B, Huang B, Jin W, Zhao S, Li F, Hu P, Pan X. Occurrence, Distribution, and Sources of Six Phenolic Endocrine Disrupting Chemicals in the 22 River Estuaries around Dianchi Lake in China. *Environmental Science and Pollution Research* 2013; 20(5) 3185-3194.
- [93] Duong CN, Ra JS, Cho J, Kim SD, Choi HK, Park J-H, Kim KW, Inam E, Kim SD. Estrogenic Chemicals and Estrogenicity in River Waters of South Korea and Seven Asian Countries. *Chemosphere* 2010; 78(3) 286-293.
- [94] Basheer C, Lee HK, Tan KS. Endocrine Disrupting Alkylphenols and Bisphenol-a in Coastal Waters and Supermarket Seafood from Singapore. *Marine Pollution Bulletin* 2004; 48(11-12) 1161-1167.
- [95] Ciofi L, Ancillotti C, Chiuminatto U, Fibbi D, Checchini L, Orlandini S, Del Bubba M. Liquid Chromatographic-Tandem Mass Spectrometric Method for the Simultaneous Determination of Alkylphenols Polyethoxylates, Alkylphenoxy Carboxylates and Alkylphenols in Wastewater and Surface-Water. *Journal of Chromatography A* 2014; 1362(0) 75-88.

- [96] Nagy P, Fekete J, Sharma VK. Octylphenol and Nonylphenol in Surface Water of Ráckevei-Soroksári Danube Branch, Hungary. *Journal of Environmental Science and Health, Part A* 2005; 40(9) 1679-1688.
- [97] Loyo-Rosales JE, Schmitz-Afonso I, Rice CP, Torrents A. Analysis of Octyl- and Nonylphenol and Their Ethoxylates in Water and Sediments by Liquid Chromatography/Tandem Mass Spectrometry. *Analytical Chemistry* 2003; 75(18) 4811-4817.
- [98] Yang X, Liu M, Wang Z, Li Q, Zhang Z. Determination of 4-Tert-Octylphenol in Surface Water Samples of Jinan in China by Solid Phase Extraction Coupled with GC-MS. *Journal of Environmental Sciences* 2013; 25(8) 1712-1717.
- [99] Babay PA, Itria RF, Ale EER, Becquart ET, Gautier EA. Ubiquity of Endocrine Disruptors Nonylphenol and Its Mono- and Di-Ethoxylates in Freshwater, Sediments, and Biosolids Associated with High and Low Density Populations of Buenos Aires, Argentina. *Clean-Soil Air Water* 2014; 42(6) 731-737.
- [100] Shao B, Hu J, Yang M, An W, Tao S. Nonylphenol and Nonylphenol Ethoxylates in River Water, Drinking Water, and Fish Tissues in the Area of Chongqing, China. *Archives of Environmental Contamination and Toxicology* 2005; 48(4) 467-473.
- [101] Cladière M, Bonhomme C, Vilmin L, Gasperi J, Flipo N, Tassin B. Modelling the Fate of Nonylphenolic Compounds in the Seine River – Part 1: Determination of in-Situ Attenuation Rate Constants. *Science of the Total Environment* 2014; 468-469(0) 1050-1058.
- [102] Ferguson PL, Iden CR, Brownawell BJ. Distribution and Fate of Neutral Alkylphenol Ethoxylate Metabolites in a Sewage-Impacted Urban Estuary. *Environmental Science & Technology* 2001; 35(12) 2428-2435.
- [103] Ribeiro C, Pardal M, Tiritan M, Rocha E, Margalho R, Rocha M. Spatial Distribution and Quantification of Endocrine-Disrupting Chemicals in Sado River Estuary, Portugal. *Environmental Monitoring and Assessment* 2009; 159(1-4) 415-427.
- [104] FSA. Food Standards Agency, Working Group on Phytoestrogens and Health of the Committee of Toxicology of Chemicals in Food, Consumer Products and the Environment: Phytoestrogens and Health, in: C. Copyright, (Ed.), COT Report 2003.
- [105] Kelly MM, Fleischhacker NT, Rearick DC, Arnold WA, Schoenfuss HL, Novak PJ. Phytoestrogens in the Environment, Ii: Microbiological Degradation of Phytoestrogens and the Response of Fathead Minnows to Degradate Exposure. *Environmental Toxicology and Chemistry* 2014; 33(3) 560-566.
- [106] Ishibashi H, Kobayashi M, Koshiishi T, Moriwaki T, Tachibana K, Tsuchimoto M, Soyano K, Iguchi T, Mori C, Arizono K. Induction of Plasma Vitellogenin Synthesis by the Commercial Fish Diets in Male Goldfish (*Carassius auratus*) and Dietary Phytoestrogens. *Journal of Health Science* 2002; 48(5) 427-434.
- [107] Latonnel K, Fostier A, Le Menn F, Bennetau-Pelissero C. Binding Affinities of Hepatic Nuclear Estrogen Receptors for Phytoestrogens in Rainbow Trout (*Oncorhynchus*

- mykiss*) and Siberian Sturgeon (*Acipenser baeri*). General and Comparative Endocrinology 2002; 129(2) 69-79.
- [108] Latonnelle K, Le Menn F, Kaushik SJ, Bennetau-Pelissero C. Effects of Dietary Phytoestrogens *in Vivo* and *in Vitro* in Rainbow Trout and Siberian Sturgeon: Interests and Limits of the *in Vitro* Studies of Interspecies Differences. General and Comparative Endocrinology 2002; 126(1) 39-51.
- [109] Hoerger CC, Wettstein FE, Hungerbühler K, Bucheli TD. Occurrence and Origin of Estrogenic Isoflavones in Swiss River Waters. Environmental Science & Technology 2009; 43(16) 6151-6157.
- [110] Mackova Z, Koblovská R, Lapčík O. Distribution of Isoflavonoids in Non-Leguminous Taxa – an Update. Phytochemistry 2006; 67(9) 849-855.
- [111] Booth NL, Overk CR, Yao P, Totura S, Deng Y, Hedayat AS, Bolton JL, Pauli GF, Farnsworth NR. Seasonal Variation of Red Clover (*Trifolium Pratense* L., Fabaceae) Isoflavones and Estrogenic Activity. Journal of Agricultural and Food Chemistry 2006; 54(4) 1277-1282.
- [112] Benassayag C, Perrot-Appianat M, Ferre F. Phytoestrogens as Modulators of Steroid Action in Target Cells. Journal of Chromatography B 2002; 777(1-2) 233-248.
- [113] Clotfelter ED, Rodriguez AC. Behavioral Changes in Fish Exposed to Phytoestrogens. Environmental Pollution 2006; 144(3) 833-839.
- [114] Cheshenko K, Pakdel F, Segner H, Kah O, Eggen RIL. Interference of Endocrine Disrupting Chemicals with Aromatase Cyp19 Expression or Activity, and Consequences for Reproduction of Teleost Fish. General and Comparative Endocrinology 2008; 155(1) 31-62.
- [115] Rearick DC, Fleischhacker NT, Kelly MM, Arnold WA, Novak PJ, Schoenfuss HL. Phytoestrogens in the Environment, I: Occurrence and Exposure Effects on Fathead Minnows. Environmental Toxicology and Chemistry. 2014; 3(33) 553-559.
- [116] Dolbeth M, Cardoso P, Pardal M. Impact of Eutrophication on the Seagrass Assemblages of the Mondego Estuary (Portugal). In: A.A. Ansari, S. Singh Gill, G.R. Lanza, W. Rast (eds.) Eutrophication: Causes, Consequences and Control. Springer Netherlands; 2011. p225-246.
- [117] Hoerger CC, Wettstein FE, Hungerbuehler K, Bucheli TD. Occurrence and Origin of Estrogenic Isoflavones in Swiss River Waters. Environmental Science & Technology 2009; 43(16) 6151-6157.
- [118] Erbs M, Hoerger CC, Hartmann N, Bucheli TD. Quantification of Six Phytoestrogens at the Nanogram Per Liter Level in Aqueous Environmental Samples Using ¹³C₃-Labeled Internal Standards. Journal of Agricultural and Food Chemistry 2007; 55(21) 8339-8345.

- [119] Pawlowski S, Ternes T, Bonerz M, Kluczka T, van der Burg B, Nau H, Erdinger L, Braunbeck T. Combined *In Situ* and *In Vitro* Assessment of the Estrogenic Activity of Sewage and Surface Water Samples. *Toxicological Sciences* 2003; 75(1) 57-65.
- [120] Kolpin DW, Hoerger CC, Meyer MT, Wettstein FE, Hubbard LE, Bucheli TD. Phytoestrogens and Mycotoxins in Iowa Streams: An Examination of Underinvestigated Compounds in Agricultural Basins. *Journal of Environmental Quality* 2010; 39(6) 2089-2099.
- [121] Kuster M, Azevedo DA, de Alda MJL, Neto FRA, Barcelo D. Analysis of Phytoestrogens, Progestogens and Estrogens in Environmental Waters from Rio de Janeiro (Brazil). *Environment International* 2009; 35(7) 997-1003.
- [122] Kang J, Price WE, Hick LA. Simultaneous Determination of Isoflavones and Lignans at Trace Levels in Natural Waters and Wastewater Samples Using Liquid Chromatography/Electrospray Ionization Ion Trap Mass Spectrometry. *Rapid Communications in Mass Spectrometry* 2006; 20(16) 2411-2418.
- [123] Kawanishi M, Takamura-Enya T, Ermawati R, Shimohara C, Sakamoto M, Matsukawa K, Matsuda T, Murahashi T, Matsui S, Wakabayashi K, Watanabe T, Tashiro Y, Yagi T. Detection of Genistein as an Estrogenic Contaminant of River Water in Osaka. *Environmental Science & Technology* 2004; 38(23) 6424-6429.
- [124] Wang C, Wang L, Zhao QS, Chen JH, Zheng L, Zheng MG, Zhang RT, Wang ZJ. Cloud Point Extraction Coupled with Hplc-Dad for the Determination of Genistein and Daidzein in River Water. *Analytical Methods* 2013; 5(15) 3688-3692.
- [125] Beck I-C, Bruhn R, Gandrass J. Analysis of Estrogenic Activity in Coastal Surface Waters of the Baltic Sea Using the Yeast Estrogen Screen. *Chemosphere* 2006; 63(11) 1870-1878.
- [126] EUR-Lex. Case C-223/11: Judgment of the Court (Fifth Chamber) of 21 June 2012 — European Commission V Portuguese Republic (Failure of a Member State to Fulfil Obligations — Environment — Directive 2000/60/Ec — European Union Water Policy — River Basin District Management Plans — Publication and Notification to the Commission — None — Information and Consultation of the Public on the Envisaged Management Plans — None), in, 2012.
- [127] EC. European Commission - a Water Blueprint for Europe, in, *Blueprint*, 2013.
- [128] Briggs D. Environmental Pollution and the Global Burden of Disease. *British Medical Bulletin* 2003; 68(1-24).
- [129] Chen G, Zeng Q, Tse G. Estrogen and Its Receptors in Cancer. *Medicinal Research Reviews* 2008; 28(6) 954-974.
- [130] Gonzales R, Ansar S, Duckles S, Krause D. Androgenic/Estrogenic Balance in the Male Rat Cerebral Circulation: Metabolic Enzymes and Sex Steroid Receptors. *J Cereb Blood Flow Metab* 2007; 27(11) 1841-1852.

- [131] Lemmen J, Arends R, van Boxtel A, van der Saag P, van der Burg B. Tissue and Time Dependent Estrogen Receptor Activation in Estrogen Reporter Mice. *Journal of Molecular Endocrinology* 2004; 32(3) 689-701.
- [132] Roy D, Liehr J. Estrogen DNA Damage and Mutations. *Mutation Research* 1999; 424((1-2) 107-115.
- [133] Cavalieri E, Stack D, Devanesan P. Molecular Origin of Cancer: Catechol Estrogen-3,4-Quinones as Endogenous Tumor Initiators. *Proc Nat Acad Sci USA* 1997; 94(20) 10937 - 10942.
- [134] Liehr J. Genotoxicity of the Steroidal Oestrogens Oestrone and Oestradiol: Possible Mechanism of Uterine and Mammary Cancer Development. *Human Reproduction Update* 2001; 7(3) 273-281.
- [135] Carrola J, Santos N, Rocha M, Fontainhas-Fernandes A, Pardal M, Monteiro RF, Rocha E. Frequency of Micronuclei and of Other Nuclear Abnormalities in Erythrocytes of the Grey Mullet from the Mondego, Douro and Ave Estuaries—Portugal. *Environmental Science and Pollution Research* 2014; 21(9) 6057-6068.
- [136] Ganmaa D, Sato A. The Possible Role of Female Sex Hormones in Milk from Pregnant Cows in the Development of Breast, Ovarian and Corpus Uteri Cancers. *Medical Hypotheses* 2005; 65(6) 1028-1037.
- [137] Pritchard-Jones K, Kaatsch P, Steliarova-Foucher E, Stiller C, Coebergh J. Cancer in Children and Adolescence in Europe: Developments over 20 Years and Future Challenges. *European Journal of Cancer* 2006; 42(13) 2183-2190.
- [138] Taylor JA, Richter CA, Ruhlen RL, Saal FSV. Estrogenic Environmental Chemicals and Drugs: Mechanisms for Effects on the Developing Male Urogenital System. *Journal of Steroid Biochemistry and Molecular Biology* 2011; 127(1-2) 83-95.
- [139] Kim EJ, Lee D, Chung BC, Pyo H, Lee J. Association between Urinary Levels of Bisphenol-A and Estrogen Metabolism in Korean Adults. *Science of the Total Environment* 2014; 470 1401-1407.
- [140] Sugiura-Ogasawara M, Ozaki Y, Sonta SI, Makino T, Suzumori K. Exposure to Bisphenol A Is Associated with Recurrent Miscarriage. *Human Reproduction* 2005; 20(8) 2325-2329.
- [141] Caserta D, Di Segni N, Mallozzi M, Giovanale V, Mantovani A, Marci R, Moscarini M. Bisphenol A and the Female Reproductive Tract: An Overview of Recent Laboratory Evidence and Epidemiological Studies. *Reproductive Biology and Endocrinology* 2014; 12 37
- [142] Dhaini HR, Nassif RM. Exposure Assessment of Endocrine Disruptors in Bottled Drinking Water of Lebanon. *Environmental Monitoring and Assessment* 2014; 186(9) 5655-5662.

- [143] Lee BE, Park H, Hong YC, Ha M, Kim Y, Chang N, Kim BN, Kim YJ, Yu SD, Ha EH. Prenatal Bisphenol a and Birth Outcomes: Moceh (Mothers and Children's Environmental Health) Study. *International Journal of Hygiene and Environmental Health* 2014; 217(2-3) 328-334.
- [144] Rouiller-Fabre V, Habert R, Livera G. Effects of Endocrine Disruptors on the Human Fetal Testis. *Annales d'Endocrinologie* 2014; 75(2) 54-57.
- [145] Albini A, Rosano C, Angelini G, Amaro A, Esposito AI, Maramotti S, Noonan DM, Pfeffer U. Exogenous Hormonal Regulation in Breast Cancer Cells by Phytoestrogens and Endocrine Disruptors. *Current Medicinal Chemistry* 2014; 21(9) 1129-1145.
- [146] Carmichael SL, Cogswell ME, Ma C, Gonzalez-Feliciano A, Olney RS, Correa A, Shaw GM, Natl Birth Defects P. Hypospadias and Maternal Intake of Phytoestrogens. *American Journal of Epidemiology* 2013; 178(3) 434-440.
- [147] Patisaul HB. Effects of Environmental Endocrine Disruptors and Phytoestrogens on the Kisspeptin System. In: A.S. Kauffman, J.T. Smith (eds.) *Kisspeptin Signaling in Reproductive Biology*. New York: Springer; 2013. p455-479.
- [148] Eustache F, Mondon F, Canivenc-Lavier MC, Lesaffre C, Fulla Y, Berges R, Cravedi JP, Vaiman D, Auger J. Chronic Dietary Exposure to a Low-Dose Mixture of Genistein and Vinclozolin Modifies the Reproductive Axis, Testis Transcriptome, and Fertility. *Environmental Health Perspectives* 2009; 117(8) 1272-1279.
- [149] Vollmer G, Starcke S, Wober J, Zierau O. Endocrine Modulation and the Fragile Balance of Homeostasis - an Overview. *Neuroendocrinology Letters* 2002; 23(2) 37-42.
- [150] Patisaul HB, Jefferson W. The Pros and Cons of Phytoestrogens. *Frontiers in neuroendocrinology* 2010; 31(4) 400-419.
- [151] Ferlay J, Autier P, Boniol M, Heaune M, Colombet M, Boyle P. Estimates of the Incidence and Mortality in Europe in 2006. *Annals of Oncology* 2007; 18(3) 581-592.
- [152] Giannandrea F, Paoli D, Figà-Talamanca I, Lombardo F, Lenzi A, Gandini L. Effect of Endogenous and Exogenous Hormones on Testicular Cancer: The Epidemiological Evidence. *The International Journal Of Developmental Biology* 2013; 57(2-4) 255-263.
- [153] Madureira TV, Barreiro JC, Rocha MJ, Cass QB, Tiritan ME. Pharmaceutical Trace Analysis in Aqueous Environmental Matrices by Liquid Chromatography-Ion Trap Tandem Mass Spectrometry. *Journal of Chromatography A* 2009; 1216(42) 7033-7042.
- [154] Madureira TV, Rocha MJ, Cass QB, Tiritan ME. Development and Optimization of a Hplc-Dad Method for the Determination of Diverse Pharmaceuticals in Estuarine Surface Waters. *Journal of Chromatographic Science* 2010; 48(3) 176-182.
- [155] Madureira TV, Barreiro JC, Rocha MJ, Rocha E, Cass QB, Tiritan ME. Spatiotemporal Distribution of Pharmaceuticals in the Douro River Estuary (Portugal). *Science of the Total Environment* 2010; 408(22) 5513-5520.

- [156] Rocha MJ, Ferreira PC, Reis PA, Cruzeiro C, Rocha E. Determination of Polycyclic Aromatic Hydrocarbons in Coastal Sediments from the Porto Region (Portugal) by Microwave-Assisted Extraction, Followed by Spme and Gc-Ms. *Journal of Chromatographic Science* 2011; 49(9) 695-701.
- [157] Rocha MJ, Ribeiro MFT, Cruzeiro C, Figueiredo F, Rocha E. Development and Validation of a Gc-Ms Method for Determination of 39 Common Pesticides in Estuarine Water - Targeting Hazardous Amounts in the Douro River Estuary. *International Journal of Environmental Analytical Chemistry* 2012; 92(14) 1587-1608.
- [158] Madureira TV, Rocha MJ, Cruzeiro C, Rodrigues I, Monteiro RAF, Rocha E. The Toxicity Potential of Pharmaceuticals Found in the Douro River Estuary (Portugal): Evaluation of Impacts on Fish Liver, by Histopathology, Stereology, Vitellogenin and Cyp1a Immunohistochemistry, after Sub-Acute Exposures of the Zebrafish Model. *Environmental Toxicology and Pharmacology* 2012; 34(1) 34-45.
- [159] Madureira TV, Velhote S, Santos C, Cruzeiro C, Rocha E, Rocha MJ. A Step Forward Using Quechers (Quick, Easy, Cheap, Effective, Rugged, and Safe) Based Extraction and Gas Chromatography-Tandem Mass Spectrometry-Levels of Priority Polycyclic Aromatic Hydrocarbons in Wild and Commercial Mussels. *Environmental Science and Pollution Research* 2014; 21(9) 6089-6098.
- [160] Rocha MJ, ReisHenriques MA. Plasma and Urine Levels of C₋₁₈, C₋₁₉ and C₋₂₁ Steroids in an Asynchronous Fish, the Tilapia *Oreochromis mossambicus* (Teleostei, Cichlidae). *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* 1996; 115(3) 257-264.
- [161] Rocha MJ, Reis-Henriques MA. Plasma Levels of C₋₁₈, C₋₁₉- and C₋₂₁-Steroids in Captive and Feral Female Sea Bass. *Journal of Fish Biology* 1999; 55(1) 26-34.
- [162] Rocha MJ, Reis-Henriques MA. Steroid Metabolism by Ovarian Follicles of the Sea Bass *Dicentrarchus labrax*. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* 2000; 125(1) 85-91.
- [163] Rocha MJ, Reis-Henriques MA. Steroid Metabolism by Ovarian Follicles of the Tilapia *Oreochromis mossambicus* (Teleostei, Cichlidae). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 1998; 121(1) 85-90.
- [164] Castro LFC, Rocha MJ, Lobo-da-Cunha A, Batista-Pinto C, Machado A, Rocha E. The 17 Beta-Hydroxysteroid Dehydrogenase 4: Gender-Specific and Seasonal Gene Expression in the Liver of Brown Trout (*Salmo trutta* f. *fario*). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 2009; 153(2) 157-164.
- [165] Ribeiro C, Urbatzka R, Castro LFC, Carrola J, Fontainhas-Fernandes A, Monteiro RAF, Rocha E, Rocha MJ. *In Vitro* Exposure of Nile Tilapia (*Oreochromis niloticus*) Testis to Estrogenic Endocrine Disrupting Chemicals: mRNA Expression of Genes Encoding Steroidogenic Enzymes. *Toxicology Mechanisms and Methods* 2012; 22(1) 47-53.

- [166] Urbatzka R, Rocha E, Reis B, Cruzeiro C, Monteiro RAF, Rocha MJ. Effects of Ethinylestradiol and of an Environmentally Relevant Mixture of Xenoestrogens on Steroidogenic Gene Expression and Specific Transcription Factors in Zebrafish. *Environmental Pollution* 2012; 164 28-35.
- [167] Castro LFC, Lobo-da-Cunha A, Rocha MJ, Urbatzka R, Rocha E. Pex11 Alpha in Brown Trout (*Salmo trutta* f. *fario*): Expression Dynamics During the Reproductive Cycle Reveals Sex-Specific Seasonal Patterns. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* 2013; 164(1) 207-214.
- [168] Silva P, Rocha MJ, Cruzeiro C, Malhao F, Reis B, Urbatzka R, Monteiro RAF, Rocha E. Testing the Effects of Ethinylestradiol and of an Environmentally Relevant Mixture of Xenoestrogens as Found in the Douro River (Portugal) on the Maturation of Fish Gonads - A Stereological Study Using the Zebrafish (*Danio rerio*) as Model. *Aquatic Toxicology* 2012; 124 1-10.
- [169] Sousa ML, Silva A, Malhao F, Rocha MJ, Rocha E, Urbatzka R. Viability Analysis of Oocyte-Follicle Complexes and Gonadal Fragments of Zebrafish as Baseline for Toxicity Testing. *Toxicology Mechanisms and Methods* 2014; 24(1) 42-49.
- [170] Esteban S, Gorga M, González-Alonso S, Petrovic M, Barceló D, Valcárcel Y. Monitoring endocrine disrupting compounds and estrogenic activity in tap water from Central Spain. *Environmental Science and Pollution Research* 2014; 21(15) 9297-9310.
- [171] Brody JG, Aschengrau A, McKelvey W, Swartz CH, Kennedy T, Rudel RA. Breast cancer risk and drinking water contaminated by wastewater: a case control study. *Environmental Health* 2006; 5:28.

