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Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain

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HIGHLIGHTS

- Our work analyzes a total of 30 EDCs and others emergent or suspected EDCs.
- Flame retardants, alkylphenolics and anticorrosives are the most abundant compounds.
- NP and OP were detected at higher concentrations than the annual average EQS.
- Alkylphenolics are the major estrogenicity contributors in all samples.
- None of the samples exceeded the EEQ 1 ng/L with estrogenic effects on organisms.

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ABSTRACT

Endocrine-disrupting compounds (EDCs) are chemical compounds with the ability to alter the hormonal systems of organisms. Such compounds are used in several industrial and domestic activities and reach the aquatic environment via wastewater discharge. The aim of this study is to assess the occurrence of 30 EDCs and related compounds in the surface waters of central Spain and to determine the overall estrogenic activity of environmental samples. This study analyzed a large number of EDCs and other emergent or suspected compounds with endocrine-disrupting activity. The results have shown the presence of 19 EDCs at concentrations ranging from 2 to 5928 ng L^{-1}. Organophosphorus-based flame retardants, alkylphenolic compounds and anticorrosives were found at the highest concentrations. Furthermore, although insufficient data are available to calculate an average over time, these preliminary results show the need to monitor the waters in both rivers studied. Alkylphenolic compounds, particularly nonylphenol, were the main contributors to overall estrogenicity. A higher concentration of the compounds studied was detected in the river Jarama, although the estrogenicity expressed as estradiol equivalents (EEQs) was higher in the river Manzanares due to a higher concentration of nonylphenol. However, the total estrogenicity did not exceed 1 ng L^{-1} (EEQ), which is the level that may cause estrogenic effects in aquatic organisms, in any of the samples. In conclusion, the potential estrogenic risk in both rivers is low, although organophosphorus-based flame retardants may increase this risk as they were found at high levels in all samples. Unfortunately, these compounds could not be taken into account when calculating the estrogenic activity due to the lack of activity data for them. For future investigations, it will be important to assess the estrogenicity provided by these flame retardants. Due to the significant concentrations of EDCs detected in both rivers, further studies in this region are required.

1. Introduction

The presence of chemical compounds that can alter the hormonal systems of organisms, known as endocrine-disrupting compounds (EDCs), is arousing great interest among the scientific community due to their industrial and domestic applications and potential adverse effects (Deblonde et al., 2011). In recent years, a relationship between exposure to EDCs and changes in the metabolism, development, growth and reproduction of organisms has been demonstrated (Jackson and Sutton, 2008). The effects observed include reduced

Abbreviations: AA-EQS, Annual Average Environmental Quality Standard; EC_{50}, Effective Concentration; E2 equiv, Relative Estrogenicity Factor; STP, Sewage Treatment Plant; EEQ, Estradiol Equivalents; EQS, Environmental Quality Standard; EDC, Endocrine-Disrupting Compound; ISOPA, Isocyanate Producers Association; LOD, Limit of Detection; LOQ, Limit of Quantification; MAC-EQS, Maximum Allowed Concentration Environmental Quality Standard; MR, Madrid Region; NE, Northeast; NW, Northwest; RSP, River Sampling Point; SW, Southwest; SRMSelected, Reaction Monitoring.

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fertility, feminization, reproductive organ anomalies and changes in the sexual behavior of various aquatic organisms (fish, algae, frogs, benthic organisms, etc) (Pal et al., 2010). Due to their impact on multiple hormonal activities, recent studies in humans have focused on the potential effects of EDCs on the reproductive system as well as on other physiological systems, such as the immune, cardiovascular and neuronal systems. Specifically, it has been shown that some organophosphorus compounds with hormonal activity may be involved in an increased incidence of certain metabolic disorders, such as obesity and type 2 diabetes (Lyche et al., 2011). A key issue in this respect is the point in the life cycle at which exposure to EDCs occurs, with some studies observing higher severity when exposure occurs during embryonic development. Exposure to EDCs during pregnancy or childhood can alter the development of organs and regulatory circuits and lead to permanent toxic effects, which may manifest later in life (Lyche et al., 2011).

The presence of chemicals that are currently considered to be EDCs in water is still poorly regulated. In this respect, the European Union has evaluated 553 compounds from the final original list of candidate substances in relation to their disrupting activity and has classified 194 substances as category 1 (Clear evidence of endocrine disrupting effects in an intact organism) and 125 substances as category 2 (potential for endocrine disruption in intact organisms) (ENC.D.4/ETU/2005/0028r). The occurrence of synthetic EDCs, and their concentrations, in a natural ecosystem is an excellent marker of human impact on the environment and a good indicator of water quality.

Alkylphenolic compounds are one of the most important groups of EDCs. Indeed, nonylphenol (NP) and octylphenol (OP) belong to category 1 of the endocrine disrupter priority list and present clear endocrine disrupting effects in wildlife and humans. In addition, both compounds are among the 33 priority substances in the European Water Framework Directive 2000/60/EC, as amended by Directive 2008/105/EC on environmental quality standards in the field of water policy, and are classified as priority hazardous substances. The metabolites nonylphenol diethoxylate (NP_2EO) and nonylphenol monocarboxylate (NP_1EC) belong to categories 1 and 2, respectively, of the endocrine disrupter priority list for wildlife and human health. Alkylphenolic compounds interfere in the endocrine system and are bioaccumulative in aquatic organisms due to their pronounced lipophilicity. As a result, long-term toxic effects can occur at concentrations of 3.3 mg L^{-1} (ECB, 2002). In particular, it has been shown that NP induces the production of vitellogenin (an egg precursor protein) in male rainbow trout and reduces egg production in female zebrafish (Zoller, 2006).

Bisphenol A (BPA) belongs to category 1 of the endocrine disrupter priority list for wildlife and human health and was recently included in the list of substances under review for identification as a priority or hazardous substance (Directive 2008/105/EC). To date, this review has concluded that there is insufficient evidence of a significant risk to the aquatic environment. Nevertheless, the European Commission periodically re-evaluates information the available and makes the proposed listing where appropriate. The ecotoxicological effects of BPA include reduced fertility and sperm quality, inhibition of ovulation in several species of fishes, alteration of the development of tadpoles' tails in frog species and anomalies in the development of zebrafish otoliths during early embryogenesis (Gibert et al., 2011). In addition, several recent studies (An et al., 2013; Teng et al., 2013) have analyzed the possible estrogenic activity of BPA (e.g. interference with uterine contractility in rats and effects on androgen receptor function).

Since the last proposal from the European Commission (COM(2011) 876 final), the estrogenic compounds 17-α-ethinylestradiol (EE2) and 17-β-estradiol (E2) are currently under review for identification as priority substances due to their high estrogenic potency. The estrogenicity of these substances may affect aquatic organisms by altering their normal hormone functions, as seen in fish exposed to extremely low levels of E2 and EE2 (0.1 to 10 ng L^{-1}) (Céspedes et al., 2004).

The preservatives ethylparaben (EtP) and propylparaben (PrP) belong to category 1 of the endocrine disrupter priority list for wildlife and human health. As such, in recent years, attention has focused on the potential long-term effects of these compounds on aquatic organisms and in humans. Furthermore, parabens are easily absorbed by the skin and have been found in breast milk, serum and urine (Blanco et al., 2009). Several studies (Terasaki et al., 2009) have suggested that although parabens produce an estrogenic response, its potency is less than that of natural estradiol. However, the *in vitro* estrogenic activity of parabens in human breast cancer cells is higher (Okubo et al., 2001; Dagher et al., 2012).

Although other compounds such as antimicrobials, anticorrosives and organophosphorus-based flame retardants are not included in the endocrine disrupter priority list, many are considered to be emergent or suspected EDCs. For example, Triclosan (TCS) is bioaccumulative and produces acute and chronic toxicity in aquatic species (algae, fishes, amphibians, etc.). Furthermore, as a result of the development of cross-resistance, TCS can produce bacteria strains that are resistant to antimicrobials and antibiotics. The potential environmental impact of antimicrobial resistance in aquatic ecosystems is low, although it may have major implications for human health (Brausch and Rand, 2011). Information about the potential toxicological impacts of organophosphorus-based flame retardants (TBEP, TCPP and TCEP) is still very scarce. Of these, TCEP and TCPP are the most important due to their toxicity, persistence and mobility. Some studies have shown that TCEP is carcinogenic and neurotoxic for organisms (ECB, 2008), and others (Liu et al., 2012) have demonstrated the possible estrogenic activity of these compounds as a result of their ability to alter sex hormone balance via several mechanisms, including alterations to steroidogenesis or estrogen metabolism. Finally, benzotriazole (BT) and tolyltriazole (TT) show limited biodegradability and BT is classified as toxic to aquatic organisms (Kasprzyk-Hordern et al., 2008).

Some of the limited research in this field has shown the occurrence of EDCs in wastewaters (Jackson and Sutton, 2008; Zhou et al., 2010), natural waters (fluvial, groundwater and marine waters; Comeau et al., 2008; Jonkers et al., 2010; Wang L et al., 2012; Wang G et al., 2012) and drinking waters (Amiridou and Voutsa, 2011; Fram and Belitz, 2011; Li et al., 2010) from around the world. These compounds are released as a result of different manufacturing processes and are not fully eliminated by sewage treatment systems. As the discharge of these compounds is continuous, they do not need to be persistent to be found in the environment.

There are few published studies concerning the detection of EDCs, and their estrogenic activity, in Spanish river basins. Most such studies have focused on northeast (NE) and northwest (NW) river basins. Thus, in the Galicia region (NW Spain), Carballa et al. (2008a, 2008b) detected estrogen EE2 in wastewaters and Rodil et al. (2012) demonstrated the occurrence of organophosphorus-based flame retardants and the antimicrobial triclosan (TCS) in surface and drinking water. In the Llobregat river basin (NE Spain), studies conducted by Muller et al. (2008), Kuster et al. (2010) and López-Roldán et al. (2010) detected the estrogens E2, EE2, estrone (E1), estrone 3-sulfate (E1-3S), and estriol (E3) in wastewaters and drinking water. Moreover, in the same river basin, Blanco et al. (2009), Montes et al. (2009), Brix et al. (2010) and Pelayo et al. (2011) found other EDCs such as parabens, triclosan (TCS) and alkylphenolic compounds. In the Ter river basin (NE Spain), the work of Céspedes et al. (2006) showed the presence of alkylphenolic compounds and BPA. Other studies in SW Spain, such as that conducted by Camacho-Muñoz et al. (2010) in the Doñana National Park, detected EE2 and E2 in samples taken during the summer months. Most of these studies focused on a limited number of compounds, mainly natural and synthetic estrogens, bisphenol A and alkylphenolic compounds. In contrast, our work analyzes a total of 30 compounds with endocrine disrupting activity (natural and synthetic estrogens, bisphenol A, alkylphenolic compounds and preservatives) and others emergent or suspected EDCs (antimicrobials, anticorrosives and organophosphorus-