Environmental Research 143 (2015) 56–64

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe

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ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form
30 June 2015
Accepted 14 September 2015
Available online 26 September 2015

Keywords:
Pharmaceutical
Endocrine disrupting compounds
Fish
Bivalves
Algae
Prioritisation

ABSTRACT

The ocurrence and levels of PhACs, Endocrine Disrupting and related Compounds (EDCs) in seafood from potential contaminated areas in Europe has been studied. Macroalgae (*Saccharina latissima* and *Laminaria digitata*), bivalves (*Mytilus galloprovincialis*, *Mytilus spp.*, *Chamalea gallina* and *Crassostrea gigas)* and fish (*Liza aurata* and *Platichthys flesus*) from Portugal, Spain, Italy, Netherlands, and Norway were analysed following 4 different analytical protocols depending on the organism and target group of contaminants. The results revealed the presence of 4 pharmaceutical compounds in macroalgae samples, 16 in bivalves and 10 in fish. To the best of our knowledge, this is the first time that PhACs have been detected in marine fish and in macroalgae. Besides, this is also the first time that dimetridazole, hydrochlorothiazide and tamsulosin have been detected in biota samples. The highest levels of PhACs corresponded to the psychiatric drug velanfaxine (up to 36.1 ng/g dry weight (dw)) and the antibiotic azithromycin (up to 13.3 ng/g dw) in bivalves from the Po delta (Italy). EDCs were not detected in macroalgae samples, however, the analysis revealed the presence of 10 EDCs in bivalves and 8 in fish. The highest levels corresponded to the organophosphorus flame retardant tris(2-butoxyethyl)phosphate (TBEP) reaching up to 98.4 ng/g dw in mullet fish from the Tagus estuary. Bivalves, in particular mussels, have shown to be good bioindicator organisms for PhACs and fish for EDCs. Taking into consideration the concentrations and frequencies of detection of PhACs and EDCs in the seafood samples analysed, a list of candidates' compounds for priorization in future studies has been proposed.

1. Introduction

Pharmaceutically Active Compounds (PhACs) and Endocrine Disrupting Compounds (EDCs) are considered Contaminants of Emerging Concern (CEC). They are not currently included in routine monitoring programmes and may be candidates for future regulation depending on their (eco)toxicity, potential health effects, public perception, and frequency of occurrence in environmental media (Workgroup, 2008). Recently, the European Union has included three of these chemicals (diclofenac, ethinylestradiol (EE2), and β-estradiol) in the European monitoring list, the so-called watch list (Union, 2013), but there is a huge set of compounds that might be potential candidates for future regulations and more research is needed in order to prioritise those.

PhACs are designed to invoke specific biological effects in humans or animals at low doses. They include a wide variety of therapeutic families such as: antibiotics, psychiatric drugs, analgesics/anti-inflammatories, tranquilisers, β-blockers and diuretics, and each of them is composed of different chemicals. Contaminants classified as EDCs are substances that alter functions of the endocrine system and consequently adversely affect the health of exposed organisms (Programme, W.H.O.U.N.E., 2013). EDCs include, among others, plasticizers, pesticides, fungicides, surfactants, flame retardants, and PhACs like hormones. The main sources of PhACs and EDCs in marine environments are sewage

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http://dx.doi.org/10.1016/j.envres.2015.09.018
0013-9351/

effluents, waste disposal, aquaculture, animal husbandry and horticulture (Gaw et al., 2014). They provide an incessant loading of these compounds into the aquatic system and as a result they have been detected at ng-μg/L range in marine waters (Nodler et al., 2014; Bayen et al., 2013; Arditsoglou and Voutsa, 2012; Lolic et al., 2015; Moreno-González et al., In preparation). Due to the continuous exposure of aquatic organisms to these compounds one of the main concerns is their transference between the surrounding media and the aquatic biota, and consequently their potential bioaccumulation. The associated risks of PhACs and EDCs' exposure like the well-known feminisation of male fish due to estrogens exposure (Sumpter, 1995), or the antimicrobial resistance (Cabello, 2006; Sapkota et al., 2008), need to be taken into consideration not only at the species level but also at the consumer level through the possible ingestion of contaminated seafood. In order to protect public health The European Community has set in 2009 maximum residue limits (MRLs) for a variety of these chemicals in foodstuffs of animal origin including seafood species (Commission regulation (EU), 2010). This list needs to be regularly updated and for this purpose information regarding the occurrence of PhACs and EDCs in seafood, together with their potential risk (a hazard-based approach), is extremely helpful for policy makers.

EDCs, in particular, have attracted a lot of attention in the last decade and the number of published studies, describing EDCs levels in marine organisms, is considerably high. The concentrations depend on the type of contaminant and the origin of the samples ranging from few ng/g up to a thousand ng/g when they are expressed as a summatory of homologues. As examples, mean concentrations of parabens ranging from 0.005 ng/g to 1.45 ng/g wet weight (ww) were found in fish and seafood from China (Liao et al., 2013), between 2 and 59 ng/g ww of bisphenol A were detected in canned fish from different locations (Podlipna and Cichna-Markl, 2007), up to 38 ng/g dw of 17α-ethinylestradiol in mussels from a Lagoon in Venice (Italy) (Pojana et al., 2007), and up to a maximum of 1255 ng/g ww of total akylphenolic compounds (APEs) were measured in shrimps from Fiumicino (Italy) (Ferrara et al., 2008). However, the number of papers published reporting the occurrence and levels of PhACs in marine organisms is considerably lower. They have been performed mainly in mollusks (Wille et al., 2011; McEneff et al., 2014; Klosterhaus et al., 2013; Martinez Bueno et al., 2013, 2014; Li et al., 2012; Dodder et al., 2014) and freshwater fish (Gelsleichter and Szabo, 2013; Maruya et al., 2012; Azzouz et al., 2011). The levels found are usually in the low ng/g (i.e. sertraline 1.4 ng/g dw (Dodder et al., 2014), venlanfaxine 2.7 ng/g dw (Martinez Bueno et al., 2014), azithromycin 3.0 ng/g dw (Alvarez-Muñoz et al., 2015), carbamazepine 11 ng/g dw (Wille et al., 2011), but depending on the compound they can reach up to a few hundreds of ng/g (i.e. ciprofloxacin 208 ng/g dw (Li et al., 2012), paracetamol 115 ng/g dw and salicylic acid 490 ng/g dw (Wille et al., 2011). Carbamazepine has been the compound most frequently reported in marine organisms, a total of 4 studies has been published (Wille et al., 2011; McEneff et al., 2014; Klosterhaus et al., 2013; Martinez Bueno et al., 2013), followed by sertraline (Klosterhaus et al., 2013; Dodder et al., 2014; Gelsleichter and Szabo, 2013), venlafaxine (Martinez Bueno et al., 2014; Gelsleichter and Szabo, 2013) and diazepam (Klosterhaus et al., 2013; Maruya et al., 2012), all psychiatric drugs. This reveals a lack of knowledge regarding other pharmaceutical compounds and their detection frequency in marine biota, especially in edible species.

The aims of the present research were: (1) to monitor the occurrence and levels of PhACs and EDCs in ecologically relevant species of seafood from potential contaminated areas in Europe, and (2) to propose a preliminary list of compounds that can be prioritised based on their concentration, occurence and frequency of detection and (3) to compare total contamination due to PhACs and EDCs in seafood from the hotspots areas. For this purpose different species of macroalgae, bivalves, and fish were selected. Fish and bivalves are world wide recognised seafood products, whereas macroalgaes or seaweeds are widely consumed in East-Asia they have a limited tradition as fresh food in Europe, being mostly used as a gelling agent from kelps (called alginate) in several food, toothpaste and cosmetics. The selection of PhACs and EDCs for the screening was based on two criteria. First, the compounds were known or suspected to bioaccumulate in biota from previous surveys, and second, the availability of robust analytical multi-residue methods capable of processing the large set of samples.

2. Material and methods

2.1. Standards and reagents

All PhACs and EDCs standards were of high purity grade (> 90%) and they were purchased from Sigma-Aldrich except n-acetyl-sulfamethoxazole, o-demethyl-venlafaxine, 10,11-epoxycarbamazepine and 2-hydroxycarbamazepine that were obtained from Toronto Research Chemicals (TRC), propyphenazone, sertraline, venlafaxine, paroxetine, loratadine, and diltiazem from European Pharmacopeia (EP), metroprolol from US Pharmacopeia (USP), and codeine from Fluka. Isotopically labelled compounds, used as internal standards, atenolol-d_7, venlafaxine-d_6, carbamazepine-d_{10}, citalopram-d_4, diazepam-d_5, hydrochlorothiazide-d_2, estrone-d_4, 17β-estradiol-d_2, 17α-ethimylestradiol-d_4, bisphenolA-d_4, methylparaben-d_4, triclosan-d_3, 1H-benzotriazole-d_4, and caffeine-d_3 were purchased from CDN isotopes, ronidazole-d_3, ibuprofen-d_3, diazepam-d_5, fluoxetine-d_5, erythromycin-N,N$^{13}C_2$, azaperone-d_4 and trisphenylphosphate-d_{15} from Sigma-Aldrich, sulfamethoxazole- d_4 and azithromycin-d_3 from TRC, and meloxicam-d_3 from Fluka, and progesterone-d8 from Cambridge Isotope Laboratories. Individual stock standards and isotopically labelled internal standards were prepared in methanol at a concentration of 1000 mg/L. Stock solutions and 20 mg/L mixtures in methanol were stored at −20 °C. Working standard solutions, containing either PhACs or EDCs (1 mg/L), and a mixture of isotopically labelled internal standards were prepared in methanol/water (10:90, v/v) and (1:1, v/v) for PhACs and EDCs, respectively, before each analytical run.

2.2. Sampling species

The target species were selected according to different criteria such as their use for human consumption, ability to accumulate contaminants, ecological relevance, a wide geographic distribution, and abundance. *Saccharina latissima* (sugarkelp) and *Laminaria digitata* (oarweed) were chosen as macroalgae species, *Mytilus galloprovincialis*, *Mytilus spp.* (Mediterranean or Atlantic mussel), *Chamalea gallina* (striped venus clam), and *Crassostrea gigas* (pacific oyster) were the selected bivalves, and *Liza aurata* (mullet) and *Platichthys flesus* (flounder) were the chosen fish species. All specimens from each species were of similar size and they satisfied the legal requirements of harvestable size or weight for human consumption. The total number of individual organisms collected from the sampling sites was 20 for macroalgae, 25 for fish, and 50 for bivalve species. A pool was prepared with the edible content of the specimens corresponding to the same species. In the case of macroalgae the frond was used, for bivalves the shell was discarded and all edible tissue together with intervalvar liquid was added to the pool, and for fish the skin was removed and only the muscle was used. Each species' pool was grinded, homogenised, freeze-dried and kept at −20 °C until its analysis.