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A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves

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HIGHLIGHTS

- Pharmaceuticals detected in wastewater effluent at concentrations up to 3.16 $\mu g \cdot g^{-1}$
- Pharmaceuticals detected in Irish marine waters at concentrations up to 1.41 $\mu g \cdot g^{-1}$
- Three pharmaceuticals detected in marine mussels after *in situ* exposure
- Trimethoprim measured in mussels at concentrations up to 9.22 $ng \cdot g^{-1}$ dry weight
- Low risk of pharmaceutical exposure to humans via ingestion of exposed mussels

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ABSTRACT

Reports concerning the quantitative analysis of pharmaceuticals in marine ecosystems are somewhat limited. It is necessary to determine pharmaceutical fate and assess any potential risk of exposure to aquatic species and ultimately, seafood consumers. In the work presented herein, analytical methods were optimised and validated for the quantification of pharmaceutical residues in wastewater effluent, receiving marine waters and marine mussels (*Mytilus* spp.). Selected pharmaceuticals included two non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac and mefenamic acid), an antibiotic (trimethoprim), an antiepileptic (carbamazepine) and a lipid regulator (gemfibrozil). This paper also presents the results of an in situ study in which caged *Mytilus* spp. were deployed at three sites on the Irish coastline over a 1-year period. In water samples, pharmaceutical residues were determined using solid phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). The extraction of pharmaceuticals from mussel tissues used an additional pressurised liquid extraction (PLE) step prior to SPE and LC–MS/MS. Limits of quantification between 15 and 225 $ng \cdot L^{-1}$ were achieved in wastewater effluent, between 3 and 38 $ng \cdot L^{-1}$ in marine surface water and between 4 and 29 $ng \cdot g^{-1}$ dry weight in marine mussels. Method linearity was achieved for pharmaceuticals in each matrix with correlation coefficients of $R^2 \geq 0.976$. All five selected pharmaceuticals were quantified in wastewater effluent and marine surface waters. This work has demonstrated the susceptibility of the *Mytilus* spp. to pharmaceutical exposure following the detection of pharmaceutical residues in the tissues of this mussel species at measurable concentrations.

1. Introduction

Current knowledge on the distribution pathways and fate of pharmaceuticals in the aquatic environment is somewhat limited and has emerged as an environmental issue. Unlike other environmental contaminants, pharmaceuticals have many physicochemical and biological properties which must be taken into account when predicting or assessing their fate in the environment. Human pharmaceuticals are excreted into the sewage system as a mixture of the parent compound and metabolites, comprising mostly of either transformation products or conjugated glucuronides (Heberer, 2002). These conjugates are easily cleaved during wastewater treatment, releasing the parent compound

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into the treated wastewater, and subsequently into the environment (Jelic et al., 2011; Ternes, 1998). Veterinary medicines also enter the environment, mainly via medicated fish feed and agricultural soil leaching (Boxall, 2004; Heberer, 2002). Although susceptible to degradation or transformation, their continuous introduction into the aquatic environment in reality confers some degree of pseudo-persistence (Hernando et al., 2006). The seemingly ubiquitous presence of pharmaceuticals in the aquatic environment has been reported over the past decade or so, with over 80 pharmaceuticals and their metabolites detected at low $\mu g \cdot L^{-1}$ concentrations in municipal sewage effluent, surface water and groundwater worldwide (Fatta-Kassinos et al., 2011; Heberer et al., 2000; Lapworth et al., 2012; Roberts and Thomas, 2006; Stan and Heberer, 1997; Ternes, 1998). In order to investigate their fate, the quantitative determination of pharmaceuticals in aquatic ecosystems is necessary.

One of the main concerns surrounding pharmaceutical release into surface waters is their potential for bioaccumulation in biota. The polar nature of most pharmaceuticals makes them directly bioavailable to filter feeding organisms such as bivalves. Bivalves, such as mussels, are natural filter feeders which have been previously utilised in persistent organic pollutant (POP) monitoring programmes because of their high bioaccumulation capacities, fixed location and high populations in marine waters (Hunt and Slone, 2010; Monirith et al., 2003). The uptake of pharmaceuticals has been previously observed in wild mussel species collected from the Mediterranean Sea, San Francisco Bay and the Bohai Sea in China (Bueno et al., 2013; Klosterhaus et al., 2013; Li et al., 2012). The use of caged sample studies allows for the measurement of exposure levels as a function of time, making it easier to assess and compare the extent of pollution between contaminated sites. With regard to pharmaceutical exposure studies using caged mussels, recent studies carried out by Bringolf et al. (2010) and Wille et al. (2011) have involved the exposure of caged *Mytilus* spp. to effluent contaminated surface waters and the detection of salicylic acid, carbamazepine and fluoxetine in exposed mussel tissues.

There is comparatively larger knowledge of the fate of pharmaceuticals in the human body and during wastewater treatment processes (Debska et al., 2004; Fent et al., 2006), but little research has been performed regarding pharmaceutical fate studies after effluent release into surface waters, particularly in the marine environment. Pharmaceuticals in the environment need to be quantified by means of in situ studies in order to assess the pharmaceutical residues present in 'real' environmental matrices. Pharmaceuticals are mostly polar compounds and are designed to be biologically active at low concentrations. Numerous effects on the reproduction and growth of non-target aquatic species have been observed following toxicity studies of pharmaceuticals at environmentally relevant concentrations (Boxall, 2004; Huerta et al., 2012; Quinn et al., 2011; Schmidt et al., 2011). Besides toxicity to aquatic species, trace pharmaceutical concentrations have been previously detected in drinking water in Greece and the US (Benotti et al., 2009; Heberer et al., 2002) and in cooked seafood (McEneff et al., 2013; Uno, 2002; Uno et al., 2006a; Uno et al., 2006b; Uno et al., 2010). The presence of pharmaceuticals in water and seafood may potentially act as a risk to the consumer either through direct effect or indirectly through potential antimicrobial resistance (Cabello, 2006). In order to study the possible environmental and human health risks posed by these contaminants at environmentally relevant concentrations, information regarding their occurrence in the aquatic environment, particularly in aquatic species, is urgently required.

The aim of this study was to measure the occurrence and relative distribution of five pharmaceuticals in samples of wastewater effluent, marine surface water and marine mussels collected from three sites around the Irish coastline. Based on sales data for Ireland (Irish Health Service Executive, 2010), the UK (The National Health Scheme Information Centre of England, 2010) and previous reports of pharmaceuticals detected in Irish effluent (Lacey et al., 2008), five pharmaceuticals were chosen from four different therapeutic classes: an antiepileptic (carbamazepine); two NSAIDs (diclofenac and mefenamic acid); a lipid regulator (gemfibrozil); and an antibiotic (trimethoprim). The chemical structures of these compounds and their physicochemical properties are given in Table 1. To the author's knowledge, this study was the first to quantify a range of pharmaceuticals in marine waters and marine mussels across a 1-year period.

2. Materials and methods

2.1. Reagents, chemicals and consumables

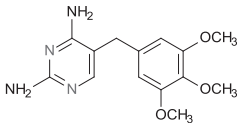
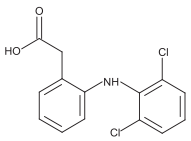
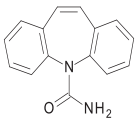
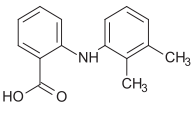
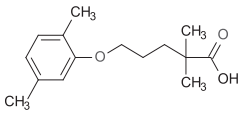
Spectranal grade acetonitrile and water and analytical grade acetone, acetonitrile, ethyl acetate and methanol were purchased from Fisher Scientific (Cheshire, UK). Dichloromethane, dichlorodimethylsilane, ammonium hydroxide solution, acetic acid and sulphuric acid were purchased from Aldrich (Gillingham, UK). Analytical grade carbamazepine (≥98%), diclofenac sodium salt (≥98%), gemfibrozil (≥99%) and mefenamic acid (≥99%) were obtained from Sigma-Aldrich (Steinheim, Germany) and trimethoprim (≥98%) was ordered from Fluka (Buch, Switzerland). Ultra-pure water was obtained from a Millipore Milli-Q water purification system (Bedford, MA, USA). Ottawa sand (20–30 mesh) was ordered from Fisher Scientific (Cheshire, UK) and activated, neutral aluminium oxide was ordered from Sigma-Aldrich (Steinheim, Germany).

Stock solutions (1000 $mg \cdot L^{-1}$) of individual analytes were prepared in methanol and stored in a freezer at −20 °C and in the dark, for optimum stability. Working mixed standards were prepared weekly in either methanol or, where required, in 80:20 13 mM ammonium acetate in water:acetonitrile (v:v).

2.2. Sampling and experimental design

Blue mussels (*Mytilus* spp. which includes both *Mytilus edulis* and *Mytilus galloprovincialis*) were sourced in the west of Ireland (Lettermullen, Co. Galway), from a Class A bivalve mollusc production area, designated by the Sea-Fisheries Protection Authority of Ireland under EC Regulation 854/2004. Animals chosen for this study were of the same size class (4–6 cm) and were collected in

Table 1
Chemical structure, class and physicochemical properties (Bones et al., 2006; Brown et al., 2007) of selected pharmaceutical compounds.

Trimethoprim	Diclofenac	Carbamazepine	Mefenamic acid	Gemfibrozil
				
Antibiotic	Anti-inflammatory	Antiepileptic	Anti-inflammatory	Lipid regulator
CAS No. 738-70-5	CAS No. 15307-79-6	CAS No. 298-46-4	CAS No. 61-68-7	CAS No. 25812-30-0
M_r 290.32	M_r 296.15	M_r 236.27	M_r 250.34	M_r 241.28
pK_a 6.60	pK_a 4.15	pK_a 13.90	pK_a 4.20	pK_a 4.80
LogK_{ow} 0.65	LogK_{ow} 3.91	LogK_{ow} 2.30	LogK_{ow} 4.16	LogK_{ow} 3.56