

# Pharmaceuticals in the Aquatic Environment: A Short Summary of Current Knowledge and the Potential Impacts on Aquatic Biota and Humans

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**EPA Research Programme 2014-2020**

# **Pharmaceuticals in the Aquatic Environment: A Short Summary of Current Knowledge and the Potential Impacts on Aquatic Biota and Humans**

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The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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# Executive Summary

Reports concerning the quantitative analysis of pharmaceuticals in marine ecosystems are somewhat limited. With an increasing human population in coastal regions, elevated levels of anthropogenic pollution can be anticipated. The detection of novel contaminants, such as human pharmaceuticals in marine and coastal waters, is a new challenge. The knowledge of the potential toxicological effects of these novel contaminants in non-target organisms remains incomplete. It is necessary to determine pharmaceutical fate and assess any potential risk of exposure for aquatic species and, ultimately, for seafood consumers. However, in Ireland, very little research has been carried out to determine the presence of pharmaceutical residues in the aquatic environment.

This report aims to collate the knowledge base by documenting what is currently known and understood about pharmaceuticals in the aquatic environment and their potential impacts on aquatic biota and ultimately humans. This

summary document provides the background for a larger and more detailed report entitled 'The assessment and potential human impact of exposure to environmental contaminants on marine and freshwater bivalves' derived from a five-year, EPA-funded Developing Environmental Research Potential (DERP) study (2007-DRP-3), available for download at <http://erc.epa.ie/safer/reports>. The project summary report is published as EPA Report No. 143.

The objectives of this report are to provide information on:

- the occurrence of pharmaceuticals in the aquatic environment
- the occurrence and toxicological effects of pharmaceuticals in aquatic biota
- the potential risk of pharmaceuticals to human health via dietary intake
- the methods utilised for the sampling and analysis of pharmaceuticals in water and biota.



## 1.1 Pharmaceuticals as Environmental Pollutants

Over the last three decades, public awareness of environmental health issues has soared in Ireland, due to the increase in funding for environmental research from national and international policy makers, such as the Irish Environmental Protection Agency (EPA) and the European Union (EU) respectively (Colgan and Donlon, 2010, EUR-OP, 2012). In particular, the assessment and monitoring of environment exposure was found to be a leading research topic, accounting for 20.8% of all environmental publications released between the years 1995 and 2005 (Tarkowski, 2007). Initially, the emphasis of environmental exposure research was on the so-called 'priority pollutants', such as pesticides and industrial intermediates. As environmental interest grew and research progressed, so too did the technologies employed, resulting in the development of more advanced techniques and the discovery of a new group of emerging contaminants, collectively referred to as 'chemicals of emerging concern (CECs)' (Bhandari, 2009). These compounds include pharmaceuticals and personal care products (PPCPs), endocrine disrupting compounds (EDCs), perfluorinated compounds (PFCs), surfactants, gasoline additives, disinfection by-products, algal and cyanobacterial toxins, organometallic compounds, brominated and organophosphate flame retardants, plasticisers and nanoparticles (Gros et al., 2006b).

For the purpose of this report, the array of compounds encompassed in the scope of PPCPs will be defined to include over-the-counter/prescription pharmaceuticals for human and animal use, excluding hormones. Pharmaceuticals have a specific structure, mode of action and biological effect, which determines their function and therapeutic class.

Pharmaceuticals can be classified into numerous therapeutic classes, including anti-inflammatories, antibiotics, antipsychotics, antihypertensives, antidiabetics, antihistamines, lipid regulators, anticonvulsant,  $\beta$ -blockers, stimulants and statins. The amount of pharmaceutical production, consumption and, ultimately, discharge into the aquatic environment is steadily increasing (EEA, 2010, Santos et al., 2010). In Ireland, the Irish Medicines Board (IBM) has licensed > 6,000 medicines for human use and > 1,000 for veterinary use (Barron et al., 2008). Ireland is a leading location for the pharmaceutical industry, housing nine of the 10 largest pharmaceutical companies in the world. The Irish market for pharmaceutical products in 2010 was valued at just under €2.3 billion by the Irish Pharmaceutical Healthcare Association (IPHA), indicating a high level of pharmaceutical consumption in Ireland (IPHA, 2012).

Pharmaceuticals were first introduced as environmental contaminants by Richardson and Bowron (1985), but their negative environmental effect was only later acknowledged in the late nineties when they were described as 'agents of subtle change' (Daughton and Ternes, 1999). As previously mentioned, concern about pharmaceutical products in the environment results from the rapid technological advances in recent years, which have enabled the improvement of analytical performance, in terms of resolution and sensitivity and the detection of these now-termed CECs. Lacey et al. (2008) previously reported the presence of pharmaceuticals in Irish wastewater effluent at low  $\mu\text{g.L}^{-1}$  concentrations. The release of pharmaceuticals in this concentration range has been shown to impact on the quality of the surrounding aquatic environment in other

European countries and America (Corcoran et al., 2010; Huerta et al., 2012). Although hormones are not within the scope of this thesis, it is worth mentioning that the release of the hormone medroxyprogesterone acetate in Irish industrial waste consequently resulted in the infertility of pigs in Holland. This hormone was detected in the pig feed and traced back to contaminated waste, sourced from a large U.S. pharmaceutical company based in Ireland, which was accidentally mixed with other waste exported to Holland and Belgium to be recycled and processed in pig feed (Van Leengoed et al., 2002). The best-known example confirming the negative effects of pharmaceuticals on wildlife concerned the decline of three species of vultures in Asia over the last 20 years. The anti-inflammatory, diclofenac, was administered to livestock and reported to have caused acute renal failure in vultures preying on their carcasses (Oaks et al., 2004).

In Europe, a number of policies and directives have been enforced in order to protect the environment from exposure to harmful chemicals, including the Integrated Pollution Prevention and Control (IPPC), Urban Wastewater Treatment, Habitats, Nitrates and Water Framework Directive (WFD). Annex x of the WFD (2000/60/EC) lists 33 priority pollutants and eight other pollutants, which must be regulated and monitored in all European wastewaters (EU WFD Bose and Bhattacharya 2000, EU WFD 2008/105/EC). Pharmaceuticals have remained outside the scope for regulation and monitoring under this directive, but in the past year, the EU Commission has revised the

list of priority pollutants and following recent findings, has included the regulation of 12 additional substances and the monitoring of three pharmaceutical compounds, including the anti-inflammatory, diclofenac, and the hormones, 17 $\alpha$ -ethinylestradiol and 17 $\beta$ -estradiol (EU WFD 2013/39/EU). These compounds have been added to a 'watch list', which are not subject to EU standards, but are instead closely monitored in EU surface waters for possible future addition to the priority list. The recently implemented Marine Strategy Framework Directive (MSFD) extends EU water legislation to the marine environment. Thereby, it follows the approach of the WFD to achieve and ensure a 'Good Environmental Status' by the year 2020. Both directives (WFD and MSFD) are linked to one another and will provide protection and management of Europe's freshwater, coastal and marine waters. These regulatory directives provide the framework for environmental toxicity testing and for this current research.

The Oslo-Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) is the only regulatory body to have considered pharmaceuticals as a threat to the environment as early as 2002. This convention monitors environmental conditions and issues standards on the release of hazardous and radioactive materials, eutrophication and marine biodiversity. Under this legislation, clotrimazole, an antifungal agent, is listed for priority action, together with a number of other pharmaceuticals listed as chemicals of possible concern (Table 1.1).

**Table 1.1. OSPAR list of pharmaceuticals of possible concern to the marine environment**

Pharmaceutical compound	Class	CAS no.
Chloroquine	Antimalarial	54-05-7
Chloroquine diphosphate	Antimalarial	50-63-5
Chlorpromazine	Antipsychotic	50-53-3
Mitotane	Antineoplastic	53-19-0
Prochlorperazine	Antipsychotic/Antiemetic	58-38-8
Fluphenazine	Antipsychotic	69-23-8
Fluphenazine dihydrochloride	Antipsychotic	146-56-5
Trifluoperazine dihydrochloride	Antipsychotic	440-17-5
Trifluoperidol	Antipsychotic	749-13-3
Prochlorperazine edisylate	Antipsychotic/Antiemetic	1257-78-9
Pimozide	Antipsychotic	2062-78-4
Dimetacrine tartrate	Antidepressant	3759-07-7
Niflumic acid	Anti-inflammatory	4394-00-7
Dimetacrine	Antidepressant	4757-55-5
Niclofolan	Anthelmintic	10331-57-4
Miconazole nitrate	Antifungal	22832-87-7
Timiperone	Antipsychotic	57648-21-2
Midazolam	Anxiolytic	59467-70-8
Diammonium N-ethylheptadecafluoro-N-[2-(phosphonatoxy)ethyl] octanesulfonamidate	Chemical Auxiliary Agent	67969-69-1
Penfluridol	Antipsychotic	26864-56-2
Terofenamate	Anti-inflammatory	29098-15-5
Flunarizine	Antihypertensive	52468-60-7

The current list of pharmaceutical compounds deemed harmful to the aquatic environment is compiled based on ecotoxicology test results showing acute toxicity in the exposed organism.

Pharmaceuticals exerting biological effects on organisms over time, i.e. chronic exposure, are not currently regulated, but should also be considered a threat to the aquatic environment.

## **1.2 Sources of Pharmaceuticals in the Aquatic Environment**

Human actions, termed as 'involuntarily' and 'purposefully', are primarily responsible for the release of pharmaceuticals into the environment (Daughton, 2007). Involuntary actions include pharmaceutical excretion through the body or washing of topical medicines down the drain. Human pharmaceuticals are excreted into the sewage system as a mixture of the parent compound and metabolites, comprising mostly of transformation products and conjugated glucuronides (Heberer, 2002). Conjugated compounds have previously been shown to be easily cleaved during wastewater treatment, releasing the parent compound into the treated wastewater, and subsequently into the environment (Ternes, 1998, Jelic et al., 2011). In contrast, purposeful actions include the disposal of unused or out-of-date medicines down the drain or into refuse waste. Medicines disposed of inappropriately into refuse waste enter landfill sites, where the unchanged bioactive compounds can leach into the soil. Agricultural medicines administered to farmed animals are also a source of pharmaceutical pollution.

Excreted manure, containing the metabolite/unchanged pharmaceutical mix, is often used as a fertiliser, resulting in further exposure of these compounds to soil. Sludge from wastewater treatment plants (WWTPs) is also used as a soil fertiliser and may also be a source of pharmaceutical contamination in the environment. Soil leaching and groundwater recharge, caused by heavy precipitation, are the main modes of transportation for pharmaceuticals through the soil and into the aquatic environment. Other sources of pharmaceutical pollution of the aquatic environment include industrial spills and aquaculture. The origins and pathways of pharmaceuticals into the aquatic and terrestrial environment are depicted in Figure 1.1 (Boxall, 2004).

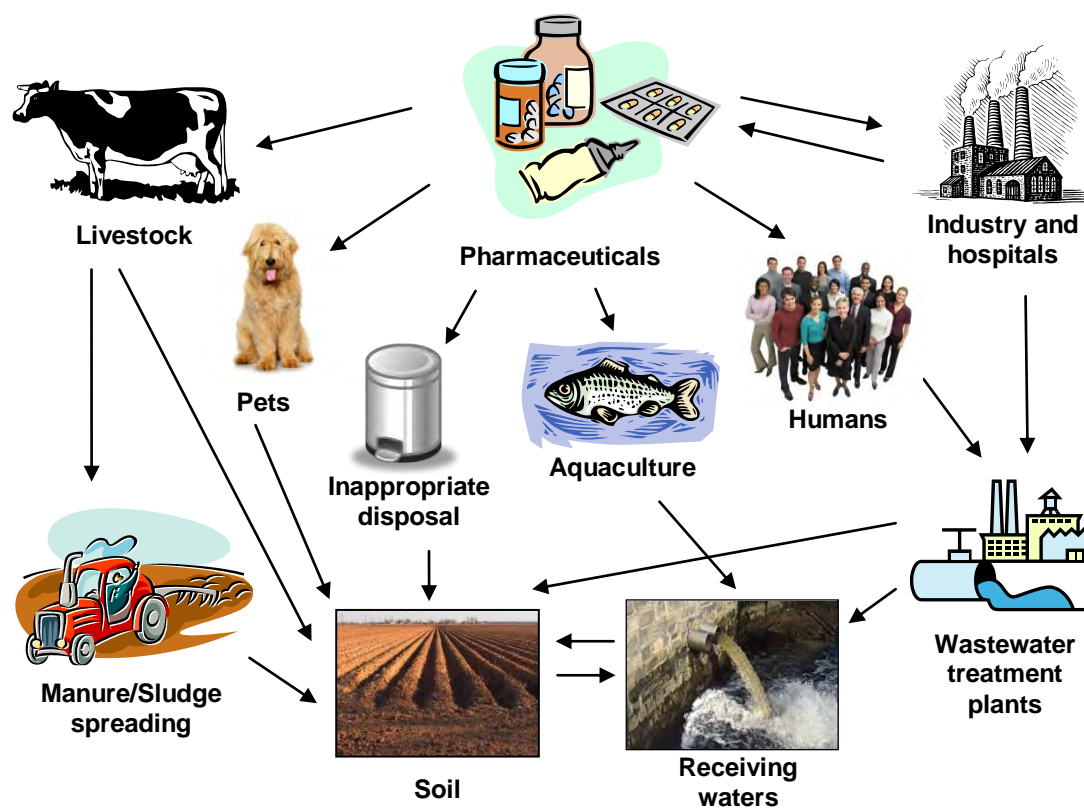


Figure 1.1. Sources of pharmaceuticals in the environment (Boxall, 2004)

## 1.3 Drug Metabolism

The fate of pharmaceuticals in the aquatic environment depends on numerous factors, such as the degree of transformation of the parent drug, the structure of the newly-formed metabolites and the quantity of parent drug and metabolites excreted. Pharmacokinetics is the study of the processes by which a drug is absorbed, distributed, modified and excreted by the body (Rosenbaum, 2011). Currently, pharmaceuticals are designed in such a way that the active ingredient can be released at a targeted site to give the required pharmacological effect. In order to reach specific sites, pharmaceuticals must possess lipophilic properties to pass through the cell membranes of the body. Metabolism is an enzymatic process necessary for the transformation of lipophilic compounds to more polar metabolites suitable for elimination (Gumbleton, 2005). It is often considered as a deactivating process for drugs, but for some compounds, known as 'prodrugs', metabolism is required to release the active parent compound and produce a pharmacological effect (Rautio et al., 2008). Drug metabolism mainly takes place in the liver but other organs, such as the intestine, lungs and kidneys, also have the ability to metabolise drugs (Gumbleton, 2005).

There are two reaction processes involved in the metabolism of compounds within the body; Phase I reactions involve the addition or exposure of a reactive functional group on the parent molecule and Phase II reactions conjugate the parent compound and/or the Phase I metabolite to a highly polar moiety. Phase I reactions include oxidation, reduction, hydrolysis, hydration, dethioacetylation and isomerisation (Gibson and Skett, 2001).

Oxidation is the most common Phase I reaction, as it performs numerous different types of functionalisation reactions. It is mostly controlled by cytochrome P450 enzymes, present in the endoplasmic reticulum of liver tissue. Cytochrome P450-catalysed mixed function oxidase (MFO) reactions occur in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and an oxidisable drug substrate. Phase II reactions include glucuronidation, glycosidation, sulfation, methylation, acetylation, condensation and amino acid, glutathione and fatty acid conjugation (Gibson and Skett, 2001). The large and diverse group of enzymes involved in Phase II reactions can only act on drug substrates in the presence of specific cofactors. For example, glucuronidation is the most common Phase II reaction, due to the constant presence of the uridine-diphosphate (UDP)-glucuronosyltransferase (UGT) enzyme, and its individual cofactor, UDP-glucuronic acid (UDPGA).

The result of Phase I and Phase II reactions is the production of highly hydrophilic drug conjugates, which are soluble in urine and easily eliminated from the kidneys via urine. There are various metabolic pathways for drugs, some more dominant than others and some equally as important. Phase II conjugation can take place directly for compounds containing reactive hydroxyl, carboxyl, amino and sulfhydryl groups (Faed, 1984). The lipid regulator, gemfibrozil, is a carboxylic acid containing compound and is primarily metabolised by Phase II glucuronidation to form the acyl glucuronide, gemfibrozil 1-O- $\beta$ -glucuronide, shown in Figure 1.2 (Ogilvie et al., 2006). Acyl glucuronides are highly reactive electrophiles, derived from carboxylic acids (Hornig et al., 2013). Further oxidation of

gemfibrozil 1-O- $\beta$ -glucuronide by hydroxyl groups has been reported at positions R<sub>1</sub>, R<sub>2</sub> or R<sub>3</sub>, also

shown in Figure 1.2 (Ogilvie *et al.*, 2006).

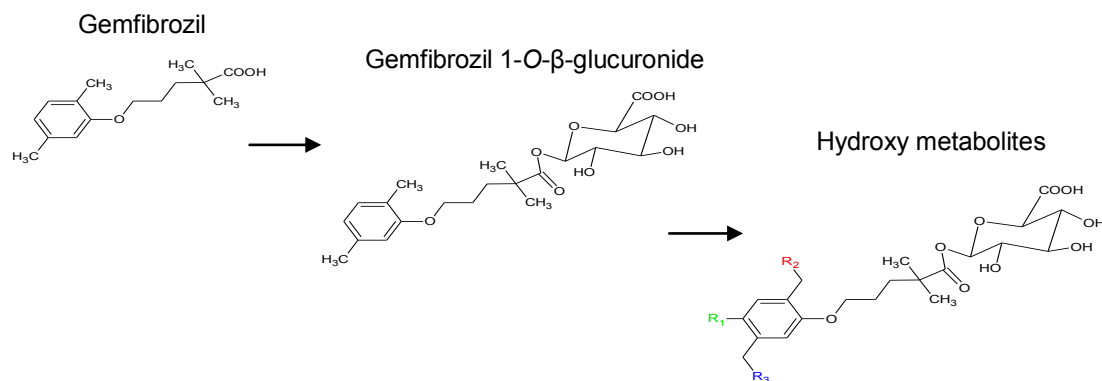


Figure 1.2. The primary metabolic pathway of gemfibrozil (Ogilvie *et al.*, 2006)

Metabolites of pharmaceuticals cannot be rendered unreactive or harmless as in the cases of paracetamol and amitriptyline, which are partly metabolised to highly reactive compounds (Rudorfer and Potter, 1997, Graham *et al.*, 2013). Under experimental conditions, water-exposed rainbow trout were found to metabolise

pharmaceuticals readily with higher concentrations of metabolites detected in fish bile and plasma than the parent drug (Lahti *et al.*, 2011). Besides the toxicological concerns, the possibility of uptake and metabolism of pharmaceutical compounds in exposed aquatic organisms still warrants further investigation.

## **1.4 The Wastewater Treatment Process**

Municipal wastewater consists of the discharge from primarily domestic sources, with additional input from road run-off and industrial sources, and is comprised of approximately 99.9% water and 0.1% dissolved and suspended solid material (Gray, 2004). The concentration of solid wastewater components can vary greatly and is influenced by the surface run-off volume, the level of treatment in the plant and potential input of industrial effluent in the catchment area. The main constituents include micro-organisms, biodegradable organic material and non-biodegradable organic material (oil, solvents and persistent organic pollutants (POPs)), nutrients, metals and inorganic materials (Henze et al., 2002). The collection, treatment and discharge of wastewater is legally controlled in Ireland under national legislation transposing the EU directive 91/271/EEC (EPA, 1997). The main objective of WWTPs is to prevent or minimise the risk of ecological impact on effluent receiving waters and its surrounding environments, by reducing suspended, organic and inorganic matter. The functions of individual WWTPs vary and are very much dependent on the nature of the receiving waters (Forster, 1985).

The treatment of municipal wastewater is a sequential process of mechanical, biological and chemical processes. A typical layout of a wastewater treatment plant is shown in Figure 1.3 (Mjalli et al., 2007). Physical and mechanical processes are carried out during preliminary and primary treatment. Preliminary treatment aims to prevent plant operational problems by screening

large objects, debris, wood, grit and oil and by facilitating storm overflow (EPA, 1997). Primary treatment involves the sedimentation of suspended solids using large clarifier tanks for sufficient lengths of time. After settling, suspended solids and floating organic matter are physically removed from the tank and the wastewater is ready for secondary treatment. Biological processes are carried out during secondary treatment to remove biodegradable and trace organic constituents, colloidal solids and nutrients present in municipal wastewaters (Tchobanoglous et al., 2003). Activated sludge treatment is the most common type of secondary treatment. This type of treatment consists of two phases, aeration and sludge settlement, and aims to reduce the biological oxygen demand (BOD) of the wastewater. In suspended growth processes, such as activated sludge treatment, this process exposes wastewater to a diversified range of micro-organisms, i.e. bacteria, protozoa, rotifiers and nematodes, each with distinct trophic levels, forming a complete ecosystem (Gray, 2004). These various micro-organisms form 'flocs' or active microbial biomasses, which feed on organic matter and absorb colloidal and suspended materials (Horan, 1996). Oxygen diffusers or mechanical aerators, such as paddles, encourage high rates of microbial growth and respiration by providing aeration and help to keep flocs in suspension, enabling maximum contact with organic matter in the wastewater (Gray, 2004).

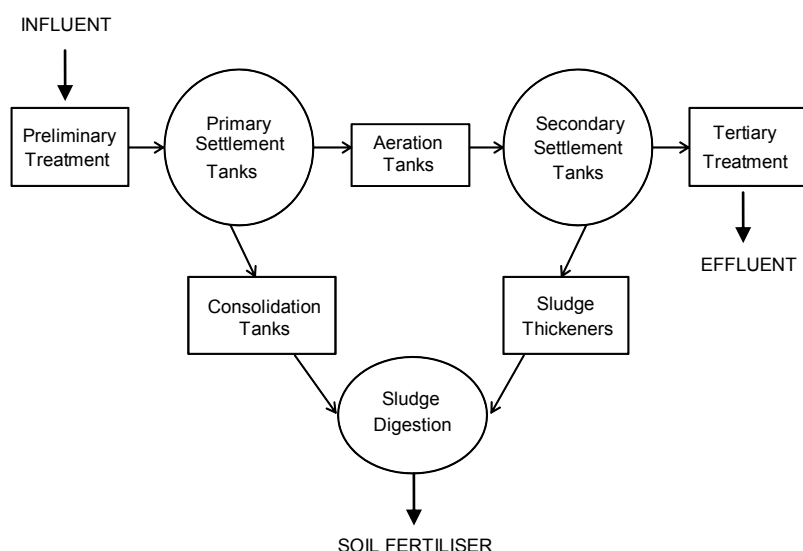


Figure 1.3. Schematic diagram of the basic overview of an activated sludge wastewater treatment plant (Mjalli *et al.*, 2007)

The final stage of secondary treatment is clarification of the wastewater by settlement. The formation of flocs is also useful in yielding a clarified effluent, as the well-formed flocs settle rapidly into a sludge form, which is recycled back into the aeration tanks for further BOD removal (Horan, 1996). The remainder of solids in the settlement tank exits the WWTP and can be used as soil fertiliser or be disposed of by incineration. At this stage, the treated water is released from the WWTP as effluent or undergoes tertiary treatment (EPA, 1997).

Where discharges are made to more sensitive waters, such as bathing waters or shellfish growing areas, more stringent treatments are necessary (EPA, 1997). Tertiary treatments are mainly physical in action and are utilised for the removal of pathogens, residual suspended solids, dissolved organic matter (DOM) and eutrophication-causing nutrients, such as nitrates and phosphates. Nitrogen, primarily removed during the activated sludge treatment due to

nitrification and denitrification processes, can be removed by the air stripping of ammonia. Chemical removal of phosphorous is a low-cost technique, which requires the addition of metal salts/lime and the subsequent removal of precipitates from the water. Disinfection via ultra-violet (UV) treatment is usually the most common final step of the wastewater treatment process and is important for the removal of harmful pathogens (Tchobanoglous *et al.*, 2003). The removal of DOM has become an important issue in recent years, due to the exposure of their negative biological effects on aquatic biota (Farre *et al.*, 2008b). Advanced wastewater treatments are carried out for the removal of dissolved organic and inorganic pollutants, using both physical and chemical methods. Techniques for their removal include activated carbon, membrane filtration and advanced oxidation processes (AOPs), such as ozonation (Tchobanoglous *et al.*, 2003).

## 1.5 Pharmaceuticals in the Wastewater Treatment Process

There are three possible outcomes for pharmaceuticals travelling through a WWTP process; persistence throughout the process and release into receiving waters, transformation to more hydrophobic compounds and sorption to solids/activated sludge, and removal after settlement or degradation to less harmful compounds. The degree of hydrophobicity of a compound is important when assessing their fate in the WWTP, as more non-polar compounds adsorb onto activated sludge and more polar compounds remain in the water phase, passing through the system unchanged and travelling into receiving waters.

Previous studies have reported low removal efficiencies of pharmaceuticals in the conventional activated sludge process, by

comparing pharmaceutical concentrations in the influent to those released in the effluent (Ternes, 1998, Jelic et al., 2011). A list of commonly-used pharmaceuticals and their removal efficiencies in the biological wastewater treatment process has been compiled in Table 1.2. From the selected literature, it is clear that removal efficiencies can vary as a function of the type of compound being removed. Removal efficiencies for antibiotics ranged from 40 to 86%, anti-inflammatories between 66 and 95%, with the exception of diclofenac,  $\beta$ -blockers between 30 and 95% and lipid regulators generally between 50 and 83%, but for one measurement of gemfibrozil, removal efficiencies of <10 % were recorded. Amitriptyline was removed efficiently during secondary treatment (>95% efficiency)

**Table 1.2. Removal efficiencies of selected pharmaceuticals in the conventional activated sludge process**

Pharmaceutical class	Compound	% removal	Reference
<i>Antibiotic</i>	Trimethoprim	40-50, 60-70	(Kasprzyk-Hordern et al., 2009, Jelic et al., 2011)
	Ciprofloxacin	86	(Vieno et al., 2007)
<i>Antidepressant</i>	Amitriptyline	>95	(Kasprzyk-Hordern et al., 2009)
<i>Antiepileptic</i>	Carbamazepine	7, -44	(Ternes, 1998, Vieno et al., 2007)
<i>Anti-inflammatory</i>	Diclofenac	-34-40	(Lishman et al., 2006, Kasprzyk-Hordern et al., 2009)
	Ibuprofen	90, 95	(Ternes, 1998, Lishman et al., 2006)
	Naproxen	66, 85, 93	(Ternes, 1998, Lishman et al., 2006, Kasprzyk-Hordern et al., 2009)
<i><math>\beta</math>-blocker</i>	Atenolol	80-95, 63	(Vieno et al., 2007, Kasprzyk-Hordern et al., 2009)
	Propranolol	96, 30-75	(Ternes, 1998, Kasprzyk-Hordern et al., 2009)
<i>Lipid regulator</i>	Gemfibrozil	69, <10, 66	(Ternes, 1998, Lishman et al., 2006, Jelic et al., 2011)
	Bezafibrate	83, 50	(Ternes, 1998, Stumpf et al., 1999)

unlike carbamazepine and diclofenac, which increased in concentration, suggesting the cleavage of glucuronised metabolites and conversion back to their original form. The reconversion of glucuronised compounds was previously demonstrated by Ternes et al. (1999), while studying the behaviour of estrogens during the activated sludge process.

Removal efficiencies of WWTPs are also dependent on the treatment technology in place, the wastewater retention time in each phase of treatment, the solid retention time (SRT) and weather conditions such as rainfall (Vieno et al., 2007). Of all of these parameters, SRT is the most critical in the wastewater treatment process, as it has been proved that longer SRTs greatly improve the removal efficiencies of pharmaceuticals (Clara et al., 2005, Lishman et al., 2006), possibly by adsorption, potentially increasing the contamination of sewage sludge. A longer period of activated sludge treatment allows for the growth of diverse bacteria capable of further reducing the concentration of persistent pharmaceuticals (Clara et al., 2005).

Carballa et al. (2004) focused upon sampling wastewater at each stage of the treatment process, in an attempt to ascertain which treatment step provided the highest rate of removal for selected pharmaceuticals. Preliminary and primary treatments revealed no reduction in concentrations for the anti-inflammatories, ibuprofen and naproxen, and the antibiotic, sulfamethoxazole, however, removal efficiencies of between 40-70% were achieved following biological treatment. Joss et al. (2006) carried out a comprehensive study investigating the biodegradation of PPCPs after biological treatment and found that only four out of 35 compounds were degraded by >90%, while 17 compounds were removed by <50%. The main removal mechanism of some persistent

pharmaceuticals in biological WWTPs is sorption on activated sludge, rather than biodegradation (Kim et al., 2008). Previous studies have reported the sorption of pharmaceuticals, such as triclosan, sertraline and citalopram, on digested sludge collected from several WWTPs worldwide at up to  $\mu\text{g.g}^{-1}$  concentrations (Barron et al., 2009, Jelic et al., 2011). Other types of biological treatments in operation have not shown removal efficiencies for pharmaceuticals significantly greater than those produced after activated sludge treatment. Membrane bioreactor (MBR) systems use a suspended growth bioreactor, similar to the activated sludge process, but with micro-/ultrafiltration as an alternative to gravity sedimentation (Harper Jr. et al., 2008). With longer SRTs and higher mixed liquor suspended solids concentrations, recent reports have only shown slightly higher efficiencies, if any, in comparison to similarly-operated conventional systems (Clara et al., 2004). A trickling filter did not perform as well at removing pharmaceuticals, showing efficiencies approximately 12-66% lower than those yielded by activated sludge treatment (Stumpf et al., 1999).

A comprehensive study, carried out by Snyder et al. (2008), evaluated advanced WWTP processes individually and in combination for their removal efficiency of selected pharmaceuticals. Studies revealed that UV treatment alone was not enough to give sufficient removal rates for pharmaceuticals, but when in combination with AOPs, such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), reductions of >90% were reported for the majority of the selected pharmaceuticals. Ozonation was another treatment found to be highly effective for the removal of most target pharmaceuticals, with average removal efficiencies of >90%.

## **1.6 Pharmaceutical Fate in the Environment**

Designed to be robust and stable, most pharmaceuticals have a polar and non-volatile nature, making it difficult for their degradation in the aquatic environment. Pharmaceuticals are predominately released into the aquatic environment with treated wastewater effluent, but can also be leached through the soil from landfills and from contaminated sewage sludge and manure used as fertiliser (Boxall, 2004). Knowledge concerning the fate of pharmaceuticals is essential when attempting to assess the potential risk posed by these micro-pollutants. The regular use and continuous introduction of pharmaceuticals into the aquatic environment confers some degree of pseudo-persistence (Hernando et al., 2006). To date, over 80 pharmaceuticals have been detected at substantial concentrations in various environmental matrices, including surface waters (Ternes, 1998, Ollers et al., 2001, Roberts and Thomas, 2006, Wille et al., 2010), groundwaters (Lapworth et al., 2012) and soils (Barron et al., 2008). Several processes can occur on compounds in the aquatic environment, including photochemical degradation, sorption onto solid matrices and, of particular importance in this work, dilution and transportation within the aquatic environment with potential uptake in biological species, which will be covered in more detail in the next section.

Pharmaceuticals are mainly administered orally and are therefore considered resistant to hydrolysis, with previous studies confirming this assumption (Buser et al., 1998b, Lam et al., 2004). Photodegradation is the primary pathway for the abiotic transformation of pharmaceuticals in the aquatic environment. Two types of photolytic reactions can occur; direct absorption of light by the compound, known as direct photolysis, or interaction with a reactive

intermediate of another species produced by its absorption of light, known as indirect photolysis (Andreozzi et al., 2003). Both reactions cause instability within the compound, causing it to degrade into numerous photoproducts. With only a small number of published papers on this subject to date, research on the photochemical fate of pharmaceutical chemicals is limited. Buser et al. (1998b) investigated the photochemical fate of the non-steroidal anti-inflammatory drug (NSAID), diclofenac, in fortified natural lake water and found it was rapidly degraded to <1% of the initial concentration, after four days of exposure to natural sunlight. High rates of photodegradation were observed in similar experiments, where diclofenac was exposed to natural sunlight (Poiger et al., 2001, Schmitt-Jansen et al., 2007). Other pharmaceuticals which readily underwent phototransformation include naproxen, sulfamethoxazole, ofloxacin, propranolol and fluoxetine (Andreozzi et al., 2003, Packer et al., 2003, Lam et al., 2005). In comparison, carbamazepine and clofibric acid were observed to have almost negligible rates of phototransformation upon irradiation (Andreozzi et al., 2003). The antibiotics, oxolinic acid and ciprofloxacin, were also found to degrade at slow rates, with oxolinic acid degrading much slower than ciprofloxacin (Turiel et al., 2005). This highlights the persistence of antibiotics in the aquatic environment and the risk of microbial resistance posed by quinolone antibiotics, in particular. Photoproducts derived from pharmaceuticals, such as ciprofloxacin, naproxen and furosemide, have been found to contain the active group of the pharmaceutical and may have the potential to be more toxic than the parent molecules. Both the parent compound and their degradation products should be considered when

carrying out environmental risk assessments of pharmaceuticals (Isidori et al., 2005, Turiel et al., 2005, Isidori et al., 2006).

As previously mentioned, sludge from WWTPs are often applied as fertiliser over farmed land, introducing both human and veterinary pharmaceuticals into the environment. Barron et al. (2009) investigated the occurrence of PPCPs in sludge and sludge-enriched agricultural soils in Ireland. Significant levels of triclosan and carbamazepine were identified in dried sludge samples, while lower, but still relevant, concentrations of triclosan and nimesulide were found in the fertilised soil. Pharmaceuticals enter and persist within the soil depending on their capacity for sorption, resistance to photodegradation and affinity for water, which, if high, will cause leaching from the solid into water systems (Diaz-Cruz et al., 2003). The distribution of pharmaceuticals between the aqueous phase and the solid phase is an equilibrium process, represented by the solid-water distribution coefficient ( $K_D$ ) whereby;

$$K_D = C_{\text{solid}} / C_{\text{aqueous}} \quad \text{Eqn. 1.1}$$

$C_{\text{solid}}$  and  $C_{\text{aqueous}}$  are the pharmaceutical concentrations in the solid and water phases respectively.  $K_D$  values predict whether a substance will show appreciable adsorption or if they will remain in the aqueous phase. Compounds with higher  $K_D$  values are considered more of a possible threat to the soil

environment. A previous study on the sorption and fate of 49 pharmaceuticals in biosolid-enriched soil revealed over two-thirds of all selected pharmaceuticals showed low affinity to the soil matrix with  $K_D$  values  $\leq 50 \text{ L.kg}^{-1}$  (Barron et al., 2009). Compound properties such as  $K_D$ , octanol-water partition coefficients ( $\log K_{ow}$ ) and normalised organic carbon sorption coefficients ( $K_{oc}$ ) have previously been used to interpret sorption behaviours of pharmaceuticals, but can only be used as an approximate guide, due to the complexity of solid matrices and the nature of pharmaceuticals (Carballa et al., 2008). A more recent solids column study reported the leaching of salbutamol, sulfamethoxazole, sulfamethazine, ketoprofen and bezafibrate from sludge and spiked soil. Carbamazepine was mostly retained in the soil, with low levels leached into the aqueous phase. The majority of the other pharmaceuticals, including  $\beta$ -blockers and trimethoprim, were also highly retained in the soil, but in the case of indomethacin, residues were mostly retained in the sludge. Interestingly, the majority of pharmaceuticals retained in the sludge or soil were detected at overall concentrations approximately 75% less than those originally spiked, suggesting high levels of transformation within the solid matrices (Barron et al., 2010). Oppel et al. (2004) also reported the high retention of carbamazepine in soil, along with diazepam and ibuprofen, while clofibric acid was found to seep out with the leachate.

## **1.7 Pharmaceutical Occurrence in the Aquatic Environment**

### **1.7.1 Surface water**

In recent years, monitoring studies have focused on the presence of pharmaceuticals in freshwater environments with comparatively little knowledge regarding the occurrence, distribution and fate of pharmaceuticals in marine or estuarine environments. This may be due to the difficulty of working with a more complex matrix or the assumption that pharmaceutical residues are diluted to negligible concentrations in the marine environment. Considering the vast number of studies on pharmaceutical occurrence in freshwater environments, this report will specifically focus on the monitoring of pharmaceuticals in marine surface waters only.

In a study carried out by Thomas and Hilton (2004), 14 pharmaceuticals were monitored in British estuaries of the Thames, the Tyne, the Mersey, the Tees and Belfast Lough. The selected pharmaceuticals were chosen based on priority lists of the UK Environmental Agency and the OSPAR Commission (Hilton et al., 2003). From the targeted list, nine pharmaceuticals were detected in the estuarine water samples collected. Ibuprofen and trimethoprim were detected at the highest concentrations, measuring at 928 ng.L<sup>-1</sup> and 569 ng.L<sup>-1</sup> respectively, and clotrimazole was the most frequently detected pharmaceutical, with a median concentration of 7 ng.L<sup>-1</sup>. A number of other studies have analysed marine water samples collected from the North Sea, its estuaries and harbours. In two separate studies, clofibric acid was the only pharmaceutical detected in the North Sea at concentrations between 1-2 ng.L<sup>-1</sup>, but further into the River Elbe estuary, clofibric acid was detected at concentrations of 18 ng.L<sup>-1</sup>, along with diclofenac

and ibuprofen measuring at 6.2 ng.L<sup>-1</sup> and 0.6 ng.L<sup>-1</sup> respectively (Buser et al., 1998a, Weigel et al., 2002). A more recent study, carried out by Wille et al. (2010), frequently detected residues of salicylic acid, carbamazepine and propranolol in marine surface water collected from several Belgian harbours and estuaries, with lower and less frequent residues of the antibiotics, trimethoprim and sulfamethoxazole, also detected. Further offshore, salicylic acid and carbamazepine were detected up to concentrations of 237 ng.L<sup>-1</sup> and 12 ng.L<sup>-1</sup> respectively, in the Belgian coastal area of the North Sea. Residues of ibuprofen and its metabolites were detected in effluent-receiving marine waters in Norway at concentrations up to 7.7 ng.L<sup>-1</sup> (Weigel et al., 2004). Langford and Thomas (2011) also reported the presence of the active metabolites, carbamazepine-10, 11-epoxide and simvastatin hydroxy carboxylic acid, in marine coastal waters from Oslofjord, at concentrations greater than their parent compounds, highlighting the need for environmental risk assessments of active metabolites.

Monitoring studies in the Mediterranean Sea revealed the presence of pharmaceuticals in the low ng.L<sup>-1</sup> range. Eight pharmaceuticals, including verapamil, atenolol and metolol, were detected in marine surface water collected from several bays around the island of Mallorca (Rodriguez-Navas et al., 2013). Munaron et al. (2012) deployed passive samplers in French Mediterranean coastal waters for a period of 14-28 days and detected the presence of carbamazepine, theophylline and terbutaline.

Outside of Europe, Comeau et al. (2008) investigated pharmaceutical residues in

Canadian marine surface water collected downstream from a WWTP. Although low residues of naproxen, gemfibrozil, ibuprofen and salicylic acid were detected in the effluent-receiving waters, none of these acidic compounds were detected in the marine surface waters sampled at an estuary further downstream. However more recently, a list of 108 PPCPs and alkylphenols were targeted in a monitoring study carried out in San Francisco Bay, California. From this list, 10 pharmaceuticals were detected in the marine waters at all five sampling locations, with carbamazepine, gemfibrozil, sulfamethoxazole, and valsartan among the highest concentrations, measuring between 38-92 ng.L<sup>-1</sup> (Klosterhaus et al., 2013). The detection of pharmaceuticals and their metabolites in marine surface waters suggests a high level of persistence of these compounds in the aquatic environment. This was proven to be the case in Ireland where, as part of this DERP study, pharmaceutical residues were measured in Irish marine surface waters at concentrations up to 1.41 µg.L<sup>-1</sup> for carbamazepine and with the other selected compounds (trimethoprim, diclofenac, mefenamic acid and gemfibrozil) quantified in the mid to high ng.L<sup>-1</sup> concentration range (McEneff et al., 2014).

### 1.7.2 Aquatic biota

One of the main concerns surrounding pharmaceutical release into surface waters is their potential to bioaccumulate in aquatic organisms. Bioconcentration is the concentration of a compound in or on an organism through water exposure alone, whereas bioaccumulation is the uptake of a chemical by an organism through a combination of water, food, sediment and air, as occurs in the natural aquatic environment (Arnot and Gobas, 2006). There are several elements that can influence the bioaccumulation of a pharmaceutical, including

its physicochemical nature, its bioavailability in the aquatic system, biotic factors relating to the exposed aquatic organism and the temperature, pH, flow and quality of its residing waters (Bremle et al., 1995, Nakamura et al., 2008, Rendal et al., 2011).  $K_{ow}$  values determine the partition of a compound between octanol and aqueous phases, however, due to the ionisation of pharmaceuticals, the octanol-water distribution coefficient ( $D_{ow}$ ) is a more reliable measure for bioaccumulation potential, as both the neutral and ionised fractions of the compound are considered at a given pH (Cunningham, 2008).

Several studies have investigated the occurrence of pharmaceutical residues in wild aquatic species, focusing mainly on accumulation in wild fish species. The first of such work was carried out by Brooks et al. (2005), whereby numerous antidepressants were screened for in the tissues of wild fish residing in two separate effluent-dominated streams in the US. Two antidepressants, fluoxetine and sertraline, and their two respective metabolites, norfluoxetine and desmethylsertraline, were detected at levels greater than 0.1 ng.g<sup>-1</sup> wet weight (WW) in all tissues, with highest concentrations measured in the liver and brain. Additional selective serotonin reuptake inhibitors (SSRIs) and their metabolites have also been detected in similar studies carried out more recently in the US, again at low ng.g<sup>-1</sup> concentrations (Chu and Metcalfe, 2007, Schultz et al., 2010). In 2009, Ramirez et al. (2009) led a national pilot study, to investigate the occurrence of PPCPs in fish fillet and liver from effluent-dominated rivers throughout the United States. All fish tissues tested positive for norfluoxetine, sertraline, diphenhydramine, diltiazem and carbamazepine, with gemfibrozil and fluoxetine additionally detected in the liver. At one particular exposure site, sertraline was measured at concentrations up to 545 ng.g<sup>-1</sup> wet weight (WW) in the liver of the wild white sucker

fish species. In Sweden, studies on juvenile rainbow trout sampled from several sewage effluent outfall sites revealed the presence of ibuprofen, diclofenac, naproxen, ketoprofen and gemfibrozil in the fish plasma, with gemfibrozil detected at the highest concentrations (Brown et al., 2007). More recently, Huerta et al. (2013) collected 11 wild fish species from four Mediterranean rivers in Spain for pharmaceutical and metabolite analysis. From the 20 selected compounds, nine pharmaceuticals were detected in fish homogenates and liver tissues. Diclofenac was the most recurrent compound, detected in 9% of all samples collected, but carbamazepine measured highest at a concentration of  $18 \text{ ng.g}^{-1}$  in fish liver. Bivalves, such as mussels, are natural filter feeders, which have been widely utilised in POP monitoring programmes because of their high bioaccumulation capacities, fixed habitat and high populations in marine waters (Monirith et al., 2003, Hunt and Slone, 2010). The uptake of pharmaceuticals, such as antibiotics, has been previously observed in mussel species collected from the Bohai Sea in China over a period of four years (Li et al., 2012). Most recently, wild ribbed horse mussels, sampled from five near-shore sites in San Francisco Bay, were found to contain low residues of carbamazepine and sertraline at concentrations up to  $5.3 \text{ ng.g}^{-1}$  and  $1.4 \text{ ng.g}^{-1}$  WW respectively (Klosterhaus et al., 2013).

The use of caged sample studies involves the caging of uncontaminated species at effluent outfall sites for a number of days. Fick et al. (2010) exposed cages of rainbow trout at three different effluent exposure sites for a period of 14 days. Out of the 25 pharmaceuticals present in the effluent, 16 were detected in the fish plasma. In a similar study, two antidepressants were detected at high  $\text{ng.L}^{-1}$  levels in the bile of rainbow trout exposed downstream to a Canadian WWTP for 14 days (Togunde et al., 2012). With regard to pharmaceutical exposure studies using caged mussels, a recent study deployed five cages of blue mussels off the Belgian coast. Over a six-month period, five pharmaceuticals were detected in mussel tissues, including salicylic acid residues that measured up to  $490 \text{ ng.g}^{-1}$  dry weight (DW) (Wille et al., 2011). In a separate study in North Carolina, the antidepressant, fluoxetine, was detected in caged mussel tissues up to  $79 \text{ ng.g}^{-1}$  WW, after 14 days exposure in a wastewater effluent channel (Bringolf et al., 2010). In Ireland, the uptake of the antibiotic, trimethoprim, was reported in wild and caged *Mytilus* spp. at concentrations of  $6.68 \text{ ng.g}^{-1}$  and  $9.22 \text{ ng.g}^{-1}$  respectively, after exposure to one of the most contaminated marine surface water sites on the Irish coast. Carbamazepine and mefenamic acid were also detected in caged marine mussel tissues from this site (McEneff et al., 2014).

## 1.8 Environmental Risk Assessment and Ecotoxicity of Pharmaceuticals

The European Medicines Evaluation Agency (EMA) is responsible for the licensing and registration of medicinal products in the EU. Under the EU Directive 93/39/EEC, an environmental risk assessment is required prior to drug licensing, to determine any significant toxicological risks associated with a drug (Straub, 2002). The EMA risk assessment procedure is carried out over three sections, with the first involving the calculation of a predicted environmental concentration (PEC) value. PEC values are calculated by taking into account the defined daily dose in mg per person per day, population size treated, volume of wastewater produced per person per day, and dilution factor in receiving waters (Huschek et al., 2004). Calculated PEC values must be  $<0.01 \mu\text{g.L}^{-1}$  to complete the risk assessment, otherwise the investigation proceeds to the next section, where the predicted no effect concentration (PNEC) value is calculated. This value is based on acute toxicity data generated from standardised testing on algae, *daphnia* or fish. The assessment is complete if the PEC/PNEC ratio is  $<1$ , but if it exceeds this value, completion of the third section is required, which may include further testing and safety measures, such as chronic toxicity testing, modelling environmental fate, field studies, specific product labelling and restricted use (Straub, 2002).

Pharmaceuticals are designed to target specific receptors in humans and animals, and thus are extremely potent, even at low concentrations. As previously mentioned, the main concern surrounding pharmaceutical release into surface waters is the potential for pharmaceuticals to target similar receptors in non-target aquatic species, causing similar effects or side-effects (Fent et al., 2006). Under the current regulatory guidelines, environmental toxicity testing of pharmaceuticals only requires that standard acute toxicity tests are carried out, unless the PEC/PNEC ratio is  $>1$ , in which case further testing is required. Standardised toxicity tests on aquatic organisms are generally limited to algae, *daphnia* and/or fish from freshwater environments. This approach may not reduce the uncertainty of the environmental impact of chemicals on other freshwater and marine aquatic species, and even on the tested aquatic species, due to intra species variations, such as age and gender, resulting in different sensitivities (Jones et al., 2007). Toxic effects observed upon exposure of aquatic organisms to environmentally-relevant pharmaceutical concentrations are listed in Table 1.3. The main effects observed in exposed aquatic biota were increased oxidative stress conditions and alterations in fish organs, mainly the kidneys.

**Table 1.3. Effects of pharmaceuticals at environmentally-relevant concentrations on non-target species following chronic exposure studies**

Compound	Organism	Exposed concentration	Exposure Period	Toxicity	Reference
Antidepressants	Fish	WWTP effluent	3 mth	Inhibited activity of the ATP-dependent Na/K-ATPase	(Lajeunesse et al., 2011)
Carbamazepine	Mollusc	1-1000 nM	7 d	Increased oxidative stress	(Contardo-Jara et al., 2011)
	Cnidarian	0.1-5 $\mu$ M	48 h	Increased oxidative stress >30 nM	(Quinn et al., 2004)
Diclofenac	Fish	1-100 $\mu$ g.L <sup>-1</sup>	28 d	Alterations to kidney >1 $\mu$ g.L <sup>-1</sup>	(Triebkorn et al., 2007)
	Fish	1-500 $\mu$ g.L <sup>-1</sup>	28 d	Kidney lesions and alterations in gills >5 $\mu$ g.L <sup>-1</sup>	(Schwaiger et al., 2004)
	Fish	1-500 $\mu$ g.L <sup>-1</sup>	28 d	Alterations to liver, kidney, gills & intestine >1 $\mu$ g.L <sup>-1</sup>	(Triebkorn et al., 2004)
	Fish	0.5-25 $\mu$ g.L <sup>-1</sup>	21 d	Kidney & intestinal lesions >1 $\mu$ g.L <sup>-1</sup>	(Mehinto et al., 2010)
	Fish	1-100 $\mu$ g.L <sup>-1</sup>	14 d	Effects on hepatic gene expression	(Cuklev et al., 2011)
	Mollusc	1 $\mu$ g.L <sup>-1</sup>	96 h	Increased oxidative stress	(Quinn et al., 2011)
	Mollusc	1 $\mu$ g.L <sup>-1</sup>	14 d	Increased oxidative stress	(Schmidt et al., 2014)
Gemfibrozil	Mollusc	1 $\mu$ g.L <sup>-1</sup>	96 h	Increased oxidative stress	(Quinn et al., 2011) (Schmidt et al., 2011)
	Mollusc	1 $\mu$ g.L <sup>-1</sup>	14 d	Increased oxidative stress	(Schmidt et al., 2014)
Ibuprofen	Mollusc	1-1000 nM	7 d	Increased oxidative stress	(Contardo-Jara et al., 2011)
	Amphipod Crustacean	1-1x10 <sup>6</sup> ng.L <sup>-1</sup>	2 h	Behavioural activity changes >10 ng.L <sup>-1</sup>	(De Lange et al., 2006)
Metoprolol	Fish	1-100 $\mu$ g.L <sup>-1</sup>	28 d	Alterations to liver >1 $\mu$ g.L <sup>-1</sup>	(Triebkorn et al., 2007)

Over recent decades, in order to investigate the sub-lethal chronic effects of environmental pollutants, biomarkers have been used as early warning tools in the assessment of environmental quality of coastal waters. Biomarkers can indicate xenobiotically induced changes in cellular or biochemical components, processes or structures in organisms (Lam, 2009). These enzymes are, in general, present throughout the phylogenetic scale and, hence, form a good indicator of xenobiotic metabolism. Various biomarkers, with different degrees of specificity, have been developed over recent years (Picado *et al.*, 2007, Lam, 2009). Their successful usage in laboratory studies, as well as in environmental monitoring in the field, has increased over previous years (Bolognesi *et al.*, 1999, Cajaraville *et al.*, 2000, Amiard *et al.*, 2006, Canesi *et al.*, 2008, Hagger *et al.*, 2008, Martínez-Díaz *et al.*, 2009, Garmendia *et al.*, 2011).

Biomarkers are classified according to their function and interaction (Gagné and Blaise, 2005). Those indicating stress, such as the detoxification enzyme glutathione S-transferase (GST) or metallothionein (MT), provide information of changes in the antioxidant defences mechanisms and may indicate the onset of an early biological effect. GST is the catalyst for the conjugation of tripeptide glutathione (GSH) with endogenous and xenobiotic compounds. With this conjugation, the solubility and therefore excretion of the compound is increased (Livingstone, 2001). Metallothionein (MT) plays a vital role in the detoxification of metals and is therefore widely recognised as an indicator for metal pollution (Amiard *et al.*, 2006). In addition to its preference to bind to metals, due to its high cysteine content, it can be induced by other environmental stressors that generate oxidative stress (Amiard *et al.*, 2006). MT has the ability to scavenge

reactive oxygen species (ROS), like hydroxyl (OH) and superoxide ( $O_2^-$ ) in order to maintain the homeostasis of the antioxidant system (Amiard *et al.*, 2006). ROS are naturally produced in biological systems, but in the presence of chemical contaminants, their production is increased. In response, a number of antioxidant enzymes are induced, which have the potential to cause damage to biological tissue, resulting in biochemical lesions. Oxidative stress is caused by an imbalance in favour of the production of ROS instead of their neutralisation by antioxidant defence mechanisms (Romeo and Giamberini, 2013). Due to the relationship between environmental pollutants and molecular responses of oxidative stress, the use of antioxidant enzymes, such as glutathione S-transferase and glutathione, as biomarkers of exposure, has been reported to successfully highlight oxidative stress in exposed organisms (Hellou *et al.*, 2012). However, the changes in these biomarkers are not necessarily translated into adverse toxic effects. Within the class of biomarkers of damage, a direct measurement of toxic damage at a cellular level can be assessed (Gagné and Blaise, 2005). Examples of biomarkers of damage are the evaluation of lipid peroxidation (LPO) or DNA damage. Elevated formation of ROS can lead to oxidative damage, such as peroxidation of membrane phospholipids, measured as LPO (Wills, 1987). Such effects could influence the health and survival of an organism in the long term (Gagné and Blaise, 2005). Some pollutants found in the aquatic environment have the potential to imitate functions of the endocrine system. The decrease of vitellogenesis in females or an increase of vitellogenin levels in males can be used as biomarkers of reproduction, indicating an endocrine-disrupting effect. Such effects could have a direct impact on the reproduction and,

hence, the population viability (Gagné and Blaise, 2005).

In general, it is recommended to use a suite of different biomarkers together, when using this method for monitoring of biological effects of exposure to xenobiotic compounds. The successful use of biomarkers has been established in a variety of environmental biomonitoring programmes (Lam and Gray, 2003). Biomarkers are a more sensitive endpoint than standard toxicity tests, however their response can be influenced by multiple confounding abiotic (e.g. temperature, salinity, etc.) and biotic factors (reproduction and age) (Monsinjon and Knigge, 2007). Seasonal fluctuations, for example on food availability, have an impact on the physiology of organisms. Consequently, this and other relationships with co-factors have to be taken into account in environmental monitoring studies (Lemly, 1997, Sheehan and Power, 1999). These issues were addressed in the DERP project (2007-D RP-3), and summarised in the EPA Report No. 143, by investigating the effects of season (Schmidt et al., 2013) and shore location (Schmidt et al., 2012) on biomarker expression, providing valuable baseline data to help overcome these uncertainties. Within these limitations, more recent work has focused on the application of OMICS methodologies (proteomics, genomics and metabolomics) in the assessment of environmental stress (Campos et al., 2012, Schmidt et al., 2014). The global observation of gene and protein expression enables the examination of the potentially unforeseen responses (Sanchez et al., 2011). The first application of a proteomic approach in aquatic toxicology was undertaken by Shepard et al. (2000), where blue mussels were exposed to copper, polychlorinated biphenyls and salinity stress.

Through the identification of a Protein Expression Signature (PES) for a particular contaminant, potential candidates for novel, specific biomarkers can be found. In addition, PES helps in the understanding in the toxicity mechanisms of a pollutant and may also allow the identification of proteins leading to functional linkages to higher-order effects (Shepard et al., 2000). Since this first application of environmental proteomics, further studies have generated protein patterns that react to contaminants (Campos et al., 2012). An example of the successful application in the field is the study by Apraiz et al. (2009), which investigated oil pollution and its effects after the accident of the Prestige in northwest Spain. Furthermore, several laboratory and field studies have used proteomics to investigate the protein response after the exposure to a wide range of pollutants (e.g. metals, polyaromatic hydrocarbons, diallylphtalate, polybrominated diphenyl ethers) (Rodríguez-Ortega et al., 2003, Knigge et al., 2004, Olsson et al., 2004, Manduzio et al., 2005, Apraiz et al., 2006, Dowling et al., 2006, Romero-Ruiz et al., 2006, Chora et al., 2009, Leung et al., 2011, Riva et al., 2011, Thompson et al., 2012). With respect to pharmaceuticals, both biomarker expression (Quinn et al., 2011, Schmidt et al., 2011) and PES (Schmidt et al., 2014) were investigated during the process of this DERP study to investigate chronic, sub-lethal effects in non-target organisms, with effects being observed at environmentally relevant ( $\mu\text{g.L}^{-1}$ ) concentrations.

Computerised models using quantitative structural activity relationships (QSARs) have also been employed to assess the potential risk posed by pharmaceuticals. QSARs compare pharmaceuticals to similar, previously-tested compounds, based on their structure and composition, to predict toxicity data (Perkins et al., 2003). Due to the lack of ecotoxicological

data, QSAR models have proved useful by providing reasonable environmental risk data on the chronic toxicity of pharmaceuticals in surface waters (Sanderson et al., 2003). A previous study ranked 2,986 different pharmaceuticals relative to the risk they pose to algae, daphnids and fish, using a QSAR model. In this study,

cardiovascular pharmaceuticals ranked the most hazardous therapeutic class of pharmaceuticals. Daphnids and algae were found to be the most and least susceptible species (respectively) impacted following pharmaceutical exposure (Sanderson et al., 2004).

## 1.9 Pharmaceutical Exposure to Humans

Unbeknownst to us, human health can also be exposed to pharmaceuticals through food and drinking water. The widespread use of veterinary medicines in aquaculture and livestock has generated great concern of developing antibiotic resistance. This may be the reason as to why antibiotics are the most investigated compounds when it comes to assessing the potential risk of pharmaceutical exposure to humans via trophic level transfer. In Canada, a 'Total Diet Study' was carried out to test the presence of veterinary drugs in fresh and canned fish and shrimp produced for human consumption. Results confirmed the exposure of humans to low  $\text{ng.g}^{-1}$  concentrations of some banned and unapproved veterinary drug residues, via consumption of these farmed seafoods (Tittlemier et al., 2007). The European Food Safety Authority (EFSA) conducts an annual report on the monitoring of veterinary pharmaceutical residues in live animals and animal products from 27 member states (EFSA, 2013). The most recent report detailed the results from the year 2011 with tested animals and animal produce, including bovines, pigs, sheep and goats, horses, poultry, rabbit, farmed game, wild game, aquaculture, milk, eggs and honey. For antibiotics, 0.2% of just over 400,000 tested samples were deemed non-compliant, with the highest reoccurrence in honey. Other drugs such as NSAIDs and steroids were also detected in less than 0.7% and 0.1% of tested samples respectively. The potential for human health risks, due to exposure to contaminated seafood and animal produce, are not confined geographically to one location, due to global trade, making this a worldwide issue (Sapkota et al., 2008). The potential human health risk was investigated during this DERP

project, with the study showing that domestic cooking by steaming resulted in an overall increase in pharmaceutical residues in the contaminated mussel tissue and cooking water (McEneff et al., 2013). Diclofenac, gemfibrozil and mefenamic acid residues in the mussel tissue increased by more than a factor of 20, and in the case of mefenamic acid, concentrations increased from  $1.6 \mu\text{g.g}^{-1}$  in the raw mussel to  $89.6 \mu\text{g.g}^{-1}$ , after cooking. Acidic pharmaceuticals undergo high rates of biotransformation in the mussel, due to large increases in glutathione S-transferase enzyme. These glucuronides are generally stable but it is hypothesised that their reconversion to the parent compound occurred following thermal treatment, resulting in the increases found compared to the uncooked mussels (McEneff et al., 2013).

Pharmaceutical residues found in drinking water are mostly polar compounds with a weak binding affinity to soil particles, which enable them to pass through the soil to the groundwater. The presence of pharmaceuticals in groundwater is a cause for concern, as groundwater is often reused and recycled as potable water. As previously mentioned, pharmaceuticals have been detected in drinking water supplies at low  $\text{ng.L}^{-1}$  concentrations, providing direct entry for pharmaceuticals into the human body (Stan and Heberer, 1997, Heberer et al., 2000, Focazio et al., 2008, Benotti et al., 2009). The extent of exposure to humans has been deemed as negligible in most cases, citing 'no appreciable risk to humans exist', but the risk of chronic exposure to pharmaceuticals needs to be further assessed (Schwab et al., 2005, Cunningham et al., 2009).

## **1.10 Pharmaceutical Analysis of Environmental Samples**

Since the initial detection of pharmaceuticals in the environment, instrumentation has advanced considerably, enabling the quantification of pharmaceuticals at part per trillion levels in very complex matrices. Within this review, the objective of this section is to highlight the most recent advances and current approaches in the methods carried out for the analysis of pharmaceuticals in the natural aquatic environment, covering both water and aquatic biota samples.

Most recently, the need for more efficient sample preparation and instrumental methods of analysis has resulted in the development of numerous multi-class detection methods for pharmaceuticals (Gros et al., 2006a, Petrovic et al., 2006, US, 2007, Ferrer et al., 2010, Gracia-Lor et al., 2011). These methods vary in procedure and technique, but the basic sample preparation steps, depicted in Figure 1.4, are fundamental to all methods of analysis for pharmaceuticals in solid and aqueous samples (Reemtsma and Quintana, 2006, Comerton et al., 2009).

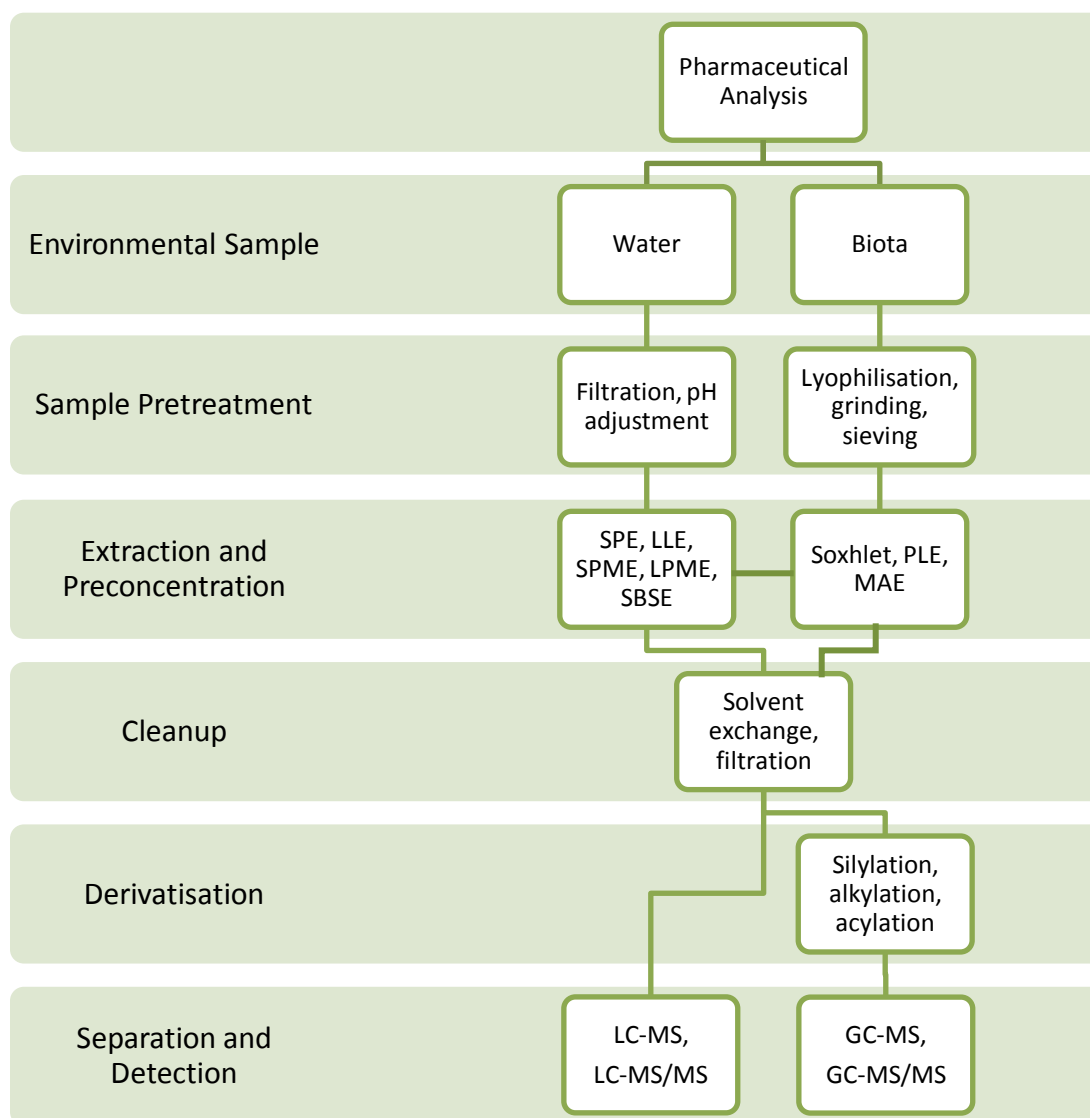


Figure 1.4. Sample preparation and analysis of pharmaceuticals in aqueous and biotic matrices (Reemtsma and Quintana, 2006)

Water samples include wastewater influent and effluent, marine water, freshwater groundwater and drinking water. Solid samples include biological tissue, soil and sludge, but within this review, we will discuss the techniques carried out to date on aquatic biological tissues only.

### 1.10.1. Water analysis

In order to minimise any initial losses of analytes, certain approaches are taken for sample collection and pre-treatment. Water samples are

filtered soon after collection, typically with glass fibre filters (0.2-1.2 µm), to remove suspended particulate matter (SPM) present in the sample and reduce the clogging of extraction sorbents (Jjemba, 2008). Adjustment of sample pH is carried out to determine analyte speciation and promote interactions with the solid phase extraction (SPE) sorbent, enhancing analyte recovery (Comerton et al., 2009).

For complex environmental samples, extraction is required to isolate and pre-concentrate target

analytes, reduce or remove unwanted matrix components and overall, increase method sensitivity. A wide range of multi-class pharmaceuticals have been found to be reasonably extracted from aqueous samples and pre-concentrated using a technique known as solid phase extraction (SPE) (Li *et al.*, 2006, Gros *et al.*, 2006b). Currently, the most common sample preparation technique in environmental analysis, SPE, is less time-consuming, less wasteful and more sensitive than the traditionally used liquid-liquid extraction (LLE) (Pichon, 2000, Wu *et al.*, 2010). Two basic approaches to SPE are off-line, i.e. extraction is separate to the analyte separation step and the SPE sorbent is usually packed in disposable cartridges, and on-line, i.e. extraction is coupled with the analyte separation step by means of packing the sorbent into a pre-column in the injection loop of a high performance liquid chromatography (HPLC) system (Pichon, 2000). Off-line SPE can be time-consuming and laborious, but in comparison to on-line SPE, the risk of contamination from sorbent reuse is eliminated and costs are somewhat reduced. Both off-line and on-line SPE techniques yield comparable values for precision and sensitivity (Trenholm *et al.*, 2009), but for the vast majority of methods, off-line SPE is the method of choice (Gros *et al.*, 2009, Petrovic *et al.*, 2010).

A large variety of off-line SPE sorbents are commercially available, with selection based on the nature of the analyte and the sample matrix type. Mixed-mode SPE sorbents have emerged onto the market with the advantage of displaying both hydrophobic and ion exchange properties and allowing for multi-class analysis of pharmaceuticals in aqueous samples. The most popular of these new polymeric sorbents are Oasis HLB, a copolymer of divinylbenzene and vinylpyrrolidone, manufactured by Waters, and Strata-X, a polydivinylbenzene resin containing

piperidone groups, manufactured by Phenomenex. These sorbents have shown the highest extraction efficiencies for numerous pharmaceuticals of varied classes (Cahill *et al.*, 2004, Roberts and Bersuder, 2006, Gomez *et al.*, 2007, Lacey *et al.*, 2008, McEneff *et al.*, 2013, McEneff *et al.*, 2014). Other mixed-mode cartridges used for pharmaceutical extraction include Oasis MXC (mixed-mode cation exchange) (Castiglioni *et al.*, 2005, Batt *et al.*, 2008, Kasprzyk-Hordern *et al.*, 2008) and Oasis MAX (mixed-mode anion exchange) (Laven *et al.*, 2009, Sousa *et al.*, 2011), both manufactured by Waters. Following extraction, analytes retained on SPE sorbents will remain stable until further analysis, once stored at  $-20^{\circ}\text{C}$  (Baker and Kasprzyk-Hordern, 2011).

More recently, molecularly imprinted polymers (MIPs) were developed to overcome the problem of poor recovery of polar analytes. This highly selective extraction technique only recognises molecules matching the shape and functional group position of the template in the polymer (Buszewski and Szultka, 2012). Although there have been several studies which have utilised MIPs for pharmaceutical extraction from environmental water samples (Beltran *et al.*, 2007, Gros *et al.*, 2008, Sun *et al.*, 2008, Zorita *et al.*, 2008), this is not a practical method for the analysis of multi-class pharmaceuticals. Sorptive extraction methods have also been used for the extraction of low-level pharmaceutical residues from complex water samples and include solid phase micro-extraction (SPME) (Rodriguez *et al.*, 2004, Wen *et al.*, 2006, Araujo *et al.*, 2008) and stir-bar sorptive extraction (SBSE) (Quintana *et al.*, 2007, Van Hoeck *et al.*, 2009, Luo *et al.*, 2011). Low detection limits were also determined for NSAIDs using liquid phase micro-extraction (LPME) in conjunction with liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass

spectrometry (GC-MS) analysis (Quintana et al., 2004, Es'haghi, 2009). Although alternative techniques to SPE may be speedier, more cost efficient and less wasteful, higher sensitivity and precision values are achieved when using SPE for the extraction of multi-class pharmaceuticals (Fatta et al., 2007).

Highly sensitive methods are required to determine the low levels of contaminants present in complex environmental samples. Hyphenated techniques, such as GC-MS and LC-MS, combine chromatographic separation with spectrometric detection and produce a highly sensitive analytical technique with high specificity. The traditional GC-MS approach is only suitable for the analysis of thermally stable and volatile analytes, hence, a lengthy derivatisation procedure is required prior to pharmaceutical analysis. Such an approach is unfavourable, as not only is it time-consuming, but it also increases the level of variability of the method (Fatta et al., 2007). For these reasons, LC-MS has been extensively employed for the identification and quantification of a wide range of multi-class pharmaceuticals in environmental samples (Wu et al., 2010, Buchberger, 2011, McEneff et al., 2013, McEneff et al., 2014).

Liquid Chromatography (LC) is most commonly used in reverse phase, with the mobile phase consisting of an aqueous phase (water) and an organic phase (usually acetonitrile or methanol). The aqueous phase often has the addition of additives, such as ammonium acetate or formic acid, to enhance ionisation efficiencies of basic and acidic pharmaceuticals respectively. Ultra performance liquid chromatography (UPLC) is a new technique, which exploits sub 2  $\mu\text{m}$  particle-packed columns to produce faster and more resolved separations of pharmaceuticals (Wille et al., 2012). Numerous analytical methods have been developed using UPLC and separations of up to 70 pharmaceutical residues in less than

seven minutes have been reported (Petrovic et al., 2006, Kasprzyk-Hordern et al., 2007, Batt et al., 2008, Farre et al., 2008a, Kasprzyk-Hordern et al., 2008, Gracia-Lor et al., 2011). Limits of detection (LODs) were in the low  $\text{ng.L}^{-1}$  range when combined with an MS (Mass Spectrometry) detector, similar to conventional HPLC analysis.

Mass spectrometry has been the detection technique of choice for the analysis of pharmaceutical residues in aqueous samples for many years. Over the past two decades, LC-MS technologies have greatly advanced in reliability, sensitivity and selectivity, with detection limits in the  $\text{ng.L}^{-1}$  range and lower. Electrospray ionisation (ESI) is the most commonly used atmospheric pressure ionisation interface for the coupling of LC with mass spectrometry, rather than atmospheric pressure chemical ionisation (APCI) or atmospheric pressure photoionisation (APPI) (Krauss et al., 2010). A drawback associated with LC is the co-elution of unwanted matrix constituents, with analytes of interest resulting in a decrease in ionisation efficiencies. The use of internal standards has somewhat overcome this problem, but depending on costs, availability and variability of the matrix, standard addition has proved to be just as effective (Van De Steene et al., 2006, Botitsi et al., 2007).

Quadrupole mass analysers set up in tandem are most frequently used for routine target analysis of complex environmental samples, due to their relatively low cost and ability to fragment ions necessary for the accurate identification of analytes (Comerton et al., 2009). The most common types of quadrupole mass analysers used are the triple quadrupole (QqQ) mass analyser and the ion trap, with typical limits of detection for pharmaceuticals in contaminated waters measuring in the low  $\text{ng.L}^{-1}$  concentration range (Petrovic et al., 2005). These analysers offer high sensitivity and selectivity in selected reaction monitoring (SRM) mode for target

analysis (Zwiener and Frimmel, 2004). For the accurate mass screening of both known and unknown compounds, the time-of-flight (TOF) and orbitrap mass analysers are applied due to their high resolving power, high mass accuracy and high sensitivity, down to the femtogram range (Krauss *et al.*, 2010). However, these techniques are much more expensive to run and have not been carried out as often in environmental analysis (Radjenović *et al.*, 2007, Nurmi and Pellinen, 2011). Recently, a new type of hybrid instrument, combining the detection and identification capabilities of two different mass spectrometers, has emerged.

Triple quadrupole linear ion trap (QqLIT) (Bueno *et al.*, 2007, Jelic *et al.*, 2009, Huerta-Fontela *et al.*, 2010, Gros *et al.*, 2012), quadrupole time-of-flight (QTOF) (Petrovic and Barcelo, 2006, Farre *et al.*, 2008a, Ibanez *et al.*, 2009, Magner *et al.*, 2010) and linear ion trap orbitrap (Hogenboom *et al.*, 2009, Cahill *et al.*, 2012) have the abilities to unequivocally identify pharmaceuticals in complex environmental matrices, due to their full-scan, product-ion spectrum and high-resolution, exact mass measurement of both precursor and product ions. In order to avoid the reporting of false positives of pharmaceutical residues in complex environmental matrices, the European Union Commission Decision 2002/657/EC requires the detection of at least four identification points for LC-MS/MS analysis. This can be achieved with the detection of one precursor ion and two daughter ions or two precursor ions each with one daughter ion (EU Decision 2002/657/EC, 2002).

LC, in tandem with spectrophotometric detection, such as UV detection, diode array detection (DAD) and fluorescence are less expensive techniques, which have been also previously utilised in the analysis of pharmaceuticals in environmental waters (Benito-Pena *et al.*, 2006, Seifrtova *et al.*, 2008, Garcia *et al.*, 2009, Kim *et*

*al.*, 2013). However, the need for higher sensitivities in more complex environmental matrices is required, and the wider availability of mass spectrometers has resulted in a decrease in studies utilising these techniques.

A combination of both LC-MS and GC-MS techniques is recommended for the reliable confirmation and measurement of a wider range of compound properties (Comerton *et al.*, 2009). GC-MS is generally carried out using electron impact (EI) ionisation, with previous studies reporting LODs for pharmaceuticals in the low ng.L<sup>-1</sup> concentration range in environmental waters (Reddersen and Heberer, 2003, Togola and Budzinski, 2008)

### 1.10.2 Biota analysis

Prior to clean-up and extraction, biotic samples are de-shelled/deboned, if required, and dissected. Tissues are usually separated and pooled before homogenisation. Depending on the method, extraction can then be carried out or frozen samples can be freeze-dried, ground down to a powder and sieved prior to extraction. In comparison to studies on water, sediment and food, relatively fewer studies have investigated the presence of pharmaceutical residues in aquatic species. In most cases, the methods developed for sediment and food matrices can be easily adapted for the analysis of aquatic biota. In solid samples, soxhlet extraction has been replaced by alternative methods, which use significantly less volumes of organic solvent. The most common extraction techniques carried out on aquatic biota include LLE (Huang *et al.*, 1997, Brooks *et al.*, 2005, Konwick *et al.*, 2006, Dussault *et al.*, 2009, Nallani *et al.*, 2011, Klosterhaus *et al.*, 2013) followed by less solvent consuming techniques, such as pressurised liquid extraction (PLE) (Chu and Metcalfe, 2007, Ramirez *et al.*, 2007, Schultz *et al.*, 2010, Wille *et al.*, 2011) and SPME (Zhou *et al.*, 2008, Togunde

et al., 2012). Microwave-assisted extraction (MAE) with micellar media is another extraction technique recently developed and previously employed for the quantification of six pharmaceuticals in mussel tissue (Cueva-Mestanza et al., 2008). Following extraction of pharmaceuticals from solid samples, further clean-up of the aqueous extract may be required using extraction methods, such as SPE.

A recent review, carried out by Huerta et al. (2012), compiled a list of previously applied analytical techniques for pharmaceutical analysis in biological tissues. From this list, LC-MS/MS was the most widely applied technique, with the QqQ mass analyser utilised for the majority of studies, with the QqLIT also utilised in two of the reported studies for the analysis of pharmaceuticals in both fish and molluscs (Schultz et al., 2010, Contardo-Jara et al., 2011, McEneff et al., 2013, McEneff et al., 2014). Most of the LODs reported using LC-MS/MS techniques were at concentrations  $<1 \text{ ng.g}^{-1}$ . More recent studies, not included in the review, have analysed wild marine mussels for up to 104 PPCPs by LC-QqQ analysis (Klosterhaus et al., 2013); combined UPLC with a QqQ mass analyser for the quantification of 11

pharmaceuticals in caged blue mussels (Wille et al., 2011); detected a range of multi-class pharmaceuticals in the tissues of wild fish analysed by LC-QqLIT (Togunde et al., 2012, Huerta et al., 2013) and quantified five pharmaceuticals in wild marine mussels from the Mediterranean Sea using an orbitrap mass analyser (Bueno et al., 2013). Besides MS detection, other techniques previously used in tandem with LC for the detection of pharmaceuticals in biota include UV, DAD and fluorescence detection (Schroder and Machetzki, 2007, Cueva-Mestanza et al., 2008, Uno et al., 2010, Fernandez-Torres et al., 2011). Although these methods of analysis had LODs at low  $\text{ng.g}^{-1}$  concentrations, these LODs were still slightly higher than those produced by LC-MS/MS analysis. GC-MS has also been utilised in the detection of diclofenac and antidepressants in fish tissues (Schwaiger et al., 2004, Brooks et al., 2005, Nakamura et al., 2008). While there are a number of recently developed analytical techniques for the detection of pharmaceuticals in aquatic biota, there is still a need for more sensitive, reproducible and transferable methods which can be applied to a greater range of aquatic species.

## Conclusions

The current literature on the presence of pharmaceuticals in the aquatic environment focuses mainly on their occurrence in wastewater and freshwater, however, knowledge on their occurrence, fate and effects in the marine environment is still lacking. Pharmaceuticals have previously been reported to exert specific biological effects on aquatic organisms exposed *in vivo*, but their potential to bioaccumulate and biomagnify in the natural aquatic environment is relatively unknown. The need for data on marine species is particularly important in order to meet requirements set out by the Water Framework Directive for coastal and transitional waters, and by the Marine Strategy Framework Directive for marine waters. Additionally, the potential for trophic level transfer of pharmaceuticals through the food chain and human exposure, via ingestion of contaminated seafood, has also not been previously addressed. The measurement of pharmaceuticals in aquatic species important in terms of human consumption, such as mussels, may be very useful in estimating human

exposure and dietary intake of these pharmaceuticals.

Chemical analysis in this area still proves to be very challenging, primarily due to the complexity of the environmental sample matrices involved. LC-MS/MS remains the technique of choice for trace analysis of pharmaceuticals in environmental matrices, however, matrix effects and laborious sample preparation processes continue to limit its applicability to all samples. In Ireland, limited chemical analysis and biological assessment of pharmaceuticals has been undertaken. This gap in knowledge has been addressed by the EPA DERP project (2007-DRP-3), and summarised in the EPA Report No. 143, providing data on both chemical analysis and biological effects of pharmaceuticals in the Irish aquatic environment. Integration of the biomarker approach with standardised toxicity tests may increase the accuracy of toxicity assessment of pharmaceuticals. The requirement for a detailed risk assessment of pharmaceuticals is a priority, to ensure no major risks to the environment and human health exist.

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## Acronyms

AOP	Advanced Oxidation Process
APCI	Atmospheric Pressure Chemical Ionisation
APPI	Atmospheric-Pressure Photoionisation
BOD	Biological Oxygen Demand
CECs	Chemicals of Emerging Concern
DAD	Diode Array Detection
DERP	Developing Environmental Research Potential
DNA	Deoxyribonucleic Acid
DOM	Dissolved Organic Matter
DW	Dry Weight
EDCs	Endocrine Disrupting Compounds
EFSA	European Food Safety Authority
EI	Electron Impact
EMA	European Medicines Evaluation Agency
EPA	Environmental Protection Agency
ESI	Electrospray Ionisation
EU	European Union
GC-MS	Gas Chromatography-Mass Spectrometry
GSH	Glutathione
GST	Glutathione S-Transferase
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
HPLC	High Performance Liquid Chromatography
IMB	Irish Medicines Board
IPHA	Irish Pharmaceutical Healthcare Association
IPPC	Integrated Pollution Prevention and Control
LC	Liquid Chromatography
LC-MS-MS	Liquid Chromatography–Tandem Mass Spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limits of Detection
LPME	Liquid Phase Micro-Extraction
LPO	Lipid Peroxidation
MAE	Microwave-Assisted Extraction
MAX	Mixed-mode Anion Exchange
MBR	Membrane Bioreactor
MFO	Mixed Function Oxidase
MIP	Molecularly Imprinted Polymers
MS	Mass Spectrometry
MSFD	Marine Strategy Framework Directive
MT	Metallothionein
MXC	Mixed-mode Cation Exchange
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NSAID	Non-Steroidal Anti-Inflammatory Drug
O <sub>2</sub> <sup>-</sup>	Superoxide
OH	Hydroxyl
OSPAR	Oslo- Paris Convention for the Protection of the Marine Environment of the North-East Atlantic
PEC	Predicted Environmental Concentration
PES	Protein Expression Signature
PFC	Perfluorinated Compounds
PLE	Pressurised Liquid Extraction
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutants
PPCPs	Pharmaceuticals and Personal Care Products
QqQ	Triple Quadrupole
QqLIT	Triple Quadrupole Linear Ion Trap
QSAR	Quantitative Structural Activity Relationship
QTOF	Quadrupole Time-of-Flight
ROS	Reactive Oxygen Species

SBSE	Stir-Bar Sorptive Extraction
SPE	Solid Phase Extraction
SPM	Suspended Particulate Matter
SPME	Solid Phase Micro-Extraction
SRM	Selected Reaction Monitoring
SRT	Solid Retention Time
SSRIs	Selective Serotonin Reuptake Inhibitors
TOF	Time-of-Flight
UDP	Uridine Diphosphate
UDPGA	Uridine Diphosphate Glucuronic Acid
UGT	Uridine Glucuronosyltransferase
UPLC	Ultra Performance Liquid Chromatography
US	United States of America
UV	Ultra-Violet
WFD	Water Framework Directive
WW	Wet Weight
WWTP	Wastewater Treatment Plant

# EPA Research Report 142

## Pharmaceuticals in the Aquatic Environment: A Short Summary of Current Knowledge and the Potential Impacts on Aquatic Biota and Humans



Authors: Gillian McEneff, Wiebke Schmidt, Brian Quinn

Current literature on the presence of pharmaceuticals in the aquatic environment is primarily focused on residues detected in wastewater and freshwater. In comparison, knowledge on the occurrence of pharmaceuticals in the marine environment is still lacking. This report provides a summary of the current knowledge on pharmaceuticals in the aquatic environment with a focus on monitoring studies previously conducted in marine surface water and marine biota and recent trends in analytical techniques. Following recent concern, pharmaceutical ecotoxicity and the potential for human exposure via contaminated drinking water and seafood are also discussed and evaluated.

### Identifying Pressures

Ireland has a high level of pharmaceutical consumption with many of these drugs being insufficiently removed during the treatment of municipal effluents. As the majority of waste water treatment plants are located on the coast, it is necessary to quantify the levels of these contaminants entering the marine environment, to investigate their toxic potential and the potential for human exposure via contaminated seafood. This literature review report aims to collate the knowledge base by documenting what is currently known and understood about pharmaceuticals in the aquatic environment and their potential impacts on aquatic biota and ultimately humans.

### Informing Policy

For the first time pharmaceutical compounds have recently been added to the EU Water Framework Directive (WFD) watch list and are currently monitored in EU surface waters. Both the WFD and the Marine Strategy Framework Directive (MSFD) aim to achieve 'Good Environmental Status' in European waters. This literature review report aims to collate the knowledge base by documenting what is currently known and understood about pharmaceuticals in the aquatic environment and can inform our implementation of these Directives.

### Developing Solutions

Marine ecosystems provide ecological, economic and societal services that are under increasing pressure from the threat of pollution. The analytical chemistry techniques and biological assessment using both established and developing new methods, not only enhances Ireland's capacity towards the integrated monitoring of contaminants but also adds valuable baseline data necessary for effective monitoring. During the course of this project, which is published as EPA Report No. 143, new techniques and protocols were developed to aid in the monitoring of contaminants in the Irish marine environment.