Occurrence and effects of pharmaceuticals in estuaries

Sarah Letsinger

Submitted in accordance with the requirements for the degree of Doctor of Philosophy

School of Geography

University of Leeds

April 2019

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 3

Letsinger S, Kay P (2019) Comparison of prioritisation schemes for human pharmaceuticals in the aquatic environment. Environ Sci Pollut R 26(4): 3479-3491 doi: 10.1007/s11356-018-3834-9

SL (the candidate) conducted the analysis, prepared all figures and wrote the manuscript. PK provided guidance, supervision and gave critical feedback on the draft of the manuscript.

Chapter 4

Letsinger S, Kay P, Rodríguez-Mozaz S, Villagrassa M, Barceló D, Rotchell JM (2019) Spatial and temporal occurrence of pharmaceuticals in UK estuaries. Sci Tot Environ 678: 74-84 doi: 10.1016/j.scitotenv.2019.04.182

SL planned and conducted field work, carried out sample preparation, conducted data analysis, prepared the figures and wrote the manuscript. SR, MV and DB performed LC-MS analysis of surface water samples. All authors commented on a draft of the manuscript, with more extensive feedback from PK and JMR (PhD supervisors). PK and JMR also provided guidance and supervision.

Chapter 5

Letsinger S, Kay P, Rotchell JM (*In preparation*) Effects of diclofenac and metformin on ragworms, *Hediste diversicolor*

SL carried out all laboratory work including exposure experiment, characterisation and isolation of target genes from *H. diversicolor*, optimisation of qPCR assays and qPCR. JRM helped with preparation of samples from exposure experiments. SL conducted data analysis, interpreted results, prepared figures and wrote the manuscript. JRM and PK provides guidance throughout and gave critical feedback on the draft of the manuscript.

Thesis by Alternative Format Rationale

This thesis is submitted as an alternative style of doctoral thesis including published material. This format is appropriate for the thesis because two (Chapters 3 and 4) out of the three data chapter have been accepted for publication in peer-reviewed journals, and the third manuscript (Chapter 5) is developed and close to being ready for submission. The three manuscripts are preceded by an introduction (Chapter 1) and a method development section (Chapter 2). The introduction provides rationale for the project and an outline to the main research questions. Chapter 2 provides context and further rationale for the pharmaceuticals and methods selected for the following data chapters. Chapter 6 is a synthesis of the research and draws together the findings from the preceding chapters and discusses the wider implications of the findings. It also contains the limitations of the study and directions for future work.

Acknowledgements

This research would not have been possible without the help and support of numerous people. I am so grateful for the help of my supervision team. First and foremost, I would like to thank Paul Kay and Jeanette Rotchell, who have been generous with their time offering continued support and encouragement over the past three and a half years. In addition to my main supervisors, I would also like to say thank Lee Brown and Kevin Welham for their guidance and feedback throughout this project.

I am so grateful to Emma Chapman for her help, guidance and patience in the lab. Thank you for taking the time to teach me techniques, help me run exposure experiments and making me feel so welcome in Hull.

Field work has been a large component of this project and it would not have been possible without so many people. I am hugely appreciative of Eleanor Pearson (who even sacrificed her wellies to get samples), Sonia Jennings, Vic Swetez and Rose Wilcox who visited field sites with me, provided valuable knowledge and came out with me over a year to collect samples from the Humber Estuary. The sampling of other estuaries would not have been possible without additional funding from Water@Leeds. A huge thank you to Jo Clarke, Anwen Bowers, Georgina Hunt and Stu Phillips for spending long hours in the car with me, tolerating the (often) rainy weather and getting very muddy in the name of science.

I have been fortunate to work with so many amazing people over the course of my PhD and it's almost too difficult to name everyone. Special thanks to Sarah Fell, Selina Keeton, Alice Noble, Annie Connolly, Alice Butler, James Bell, Jess Baker, Sam Eze, Zora, Alex Austin and Tom for providing me with so many opportunities, helping me to get to grips with R and reminding me that there is a life outside of the office.

Finally, I would like to give a huge thank you to my parents, Katherine, Keith and Oliver, and my brothers John and Luke for their endless and unconditional support throughout my studies. I would never have gotten here without them. Thank you to my mom for coming out with me to collect samples and her continued enthusiasm for being involved with my work. Special thanks to Daniel, Julia, Maya and Nova for their support and encouragement.

Abstract

Pharmaceuticals have been identified as emerging contaminants of concern due to their widespread occurrence in the aquatic environment and potential to be biologically active, yet the implications of their presence in the environment is not fully known. There is a plethora of pharmaceuticals commercially available making it unfeasible to carry out detailed investigations on all of these compounds, and prioritisation schemes can provide a useful tool to determine how best to direct resources. Different prioritisation schemes were carried out on the fifty most prescribed drugs in the UK, and their results were compared in order to assess the efficacy of these schemes. Many failed to accurately identify these risks, but a holistic approach using more than one method to generate a priority list of compounds, may provide better protection for the environment. To date, most monitoring and ecotoxicological studies have focused on pharmaceuticals in freshwater, and there is less understanding of their occurrence and effects in estuaries. In order to gain insight into their spatio-temporal patterns, five pharmaceuticals were monitored in the Humber Estuary every other month for twelve months. Patterns in their spatial and temporal occurrence were related to source points, consumption patterns and environmental conditions. Eleven further estuaries were monitored to give an overall picture of pharmaceutical pollution in the UK. The Humber Estuary contained highest levels of pharmaceuticals and concentrations of ibuprofen were the highest measured globally. Finally, ragworms *(Hediste diversicolor*) were exposed to diclofenac and metformin in a controlled experimental exposure, and the expression of selected target genes, ATP synthase and c-amp activated protein kinase was measured. Highest levels of metformin (1 µg l^{-1}) were found to significantly increase expression of ATP synthase, indicating that this drug induces environmental stress in *H. diversicolor*. Overall, this body of research has further contributed to the knowledge of pharmaceuticals as emerging contaminants in estuaries, and information on the occurrence, current levels and biological effects of the drugs studied may be of interest to regulators in their management decisions for such environments.

Contents

List of Tables

List of Figures

List of Equations

List of Appendices

List of Abbreviations

AChE - Acetylcholinesterase
ALP - Alkaline Phosphatase
AMPK - c-AMP Activated Protein Kinase
AOP - Adverse Outcome Pathway
ATP - Adenosine Triphosphate
ATPS - ATP Synthase
BCF - Bioconcentration Factor
CAT - Catalase
CEC - Critical Environmental Concentration
COX - Cyclooxygenase
CYP - Cytochrome P450
CSO - Combined Sewer Overflow
DBF - Dubenzylfluroscein Dealkylase
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure Activity Relationship
EF1 - Elongation Factor 1
EMEA - European Medicines Agency
ER - Effective Ratio
ERA - Environmental Risk Assessment
EPA - Environment Protection Agency
EROD - Ethoxyresorufin-O-deethylase
FPM - Fish Plasma Model
$F_{SS}PC$ - Fish Steady State Plasma Concentration
GPx - Glutathione Peroxidase
GR - Glutathione Reductase
GST - Glutathione S-Transferase
HSP - Heat Shock Protein
H_TPC - Human Therapeutic Plasma Concentration
K_D - Sorption Coefficient
LC - Liquid Chromatography
LC-MS/MS - Liquid Chromatography with Mass Spectrometry
LDH - Lactate Dehydrogenase
LPO - Lipid Peroxidase
LO - Lysosomal Activity
Log_{KOW} - Octanol-water Partition Coefficient

LMS	-	Lysosomal Membrane Stability
MDL	-	Method Detection Limit
MEC	-	Measured Environmental Concentration
MAO	-	Monoamine Oxidase
MDA	-	Malondiadelhyde
MET	-	Mitochondrial Electron Transport
MF	-	Methyl Farnesoate
MoA	-	Mode of Action
MQL	-	Method Quantification Limit
MRM	-	Multiple Reaction Monitoring
NO	-	Nitric Oxide
NOEC	-	No-Observed Effect Concentration
NSAID	-	Nonsteroidal Anti-inflammatory
OTC	-	Over The Counter
PBT	-	Persistence, Bioaccumulation and Toxicity
PCOS	-	Polycystic Ovarian Syndrome
PCR	-	Polymerase Chain Reaction
PE	-	Population Equivalent
PEC	-	Predicted Environmental Concentration
pKa	-	Disassociation Constant
PNEC	-	Predicted No Effect Concentration
qPCR	-	Quantitative Real-Time Polymerase Chain Reaction
QSAR	-	Quantitative Structure-Activity Relationship
REACH	-	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROS	-	Reactive Oxygen Species
RQ	-	Risk Quotient
SD	-	Standard Deviation
SOD	-	Superoxide Dismutase
SPE	-	Solid Phase Extraction
SRM	-	Selective Reaction Monitoring
SSRI	-	Selective Serotonin Reuptake Inhibitor
TBA	-	Thiobarbituric Acid
TFA	-	Trifluoroacetic Acid
UGT	-	Diphosphate-glucuronosyltransferase
WFD	-	Water Framework Directive

WWTP - Wastewater Treatment Plant

Chapter 1: Introduction

This introductory chapter seeks to place the thesis into context by providing background information and reviewing previous research conducted on pharmaceuticals as emerging contaminants. Pharmaceuticals are consumed in large quantities, with annual production for the most widely consumed pharmaceuticals in the kiloton range (Beretta et al. 2014). The average global per capita consumption is 15 g of drugs per day with developed countries consuming 3 - 10 times more pharmaceuticals than less economically developed ones (Pal et al. 2010). In addition to compounds used in human medicine, pharmaceuticals are also available for veterinary use (Capleton et al. 2006). Pharmaceuticals are unique contaminants, as they are designed to be biologically active, and are therefore likely to have an effect in non-target organisms (Küster and Adler 2014). This review aims to bring together research on the occurrence and ecotoxicology of pharmaceuticals in the marine and estuarine environments and identify potential knowledge gaps.

Pharmaceuticals have the potential to enter the aquatic environment as a mixture of parent compounds, metabolites and transformation products (Backhaus 2014). After consumption, a proportion of the drug is used by the body, and then is excreted into the sewage system via urine and faeces (Figure 1.1; Hutchinson et al. 2014). Topical pharmaceuticals may also enter sewage systems after being washed off or directly into the aquatic environment (Ruhoy and Daughton 2008). It has been estimated that approximately 30% of topical ointments applied to the skin will be washed off and not absorbed into the body, however, these products only make up a small proportion of pharmaceuticals available on the market (Bound and Voulvoulis 2006). Some pharmaceuticals, such as sertraline, are excreted as less than 1% of the parent compound, whereas other such as gabapentin are excreted largely unchanged (Drugbank, 2018). Drugs may also enter sewage through improper disposal of unused or out of date pharmaceuticals, however, data is insufficient to determine if this is a significant route of entry (Ruhoy and Daughton 2008). Bound and Voulvoulis (2005) found 64% of surveyed people in the US had disposed of medicines through household waste, and the amount of incorrectly disposed pharmaceuticals is estimated to be as high as 2.3% of those sold in the US (Ruhoy and Daughton 2008). Wastewater treatment plants (WWTPs) may further remove some pharmaceuticals through bacterial degradation, UV degradation or absorption to sludge (Boreen et al. 2003; Cuong et al. 2011). Pharmaceuticals have different sorption properties and those with a low sorption coefficient (K_d) are more likely to enter the environment as they will not bind to suspended solids as easily (Liu et al.

2013). Biodegradation is the most prominent form of removal in WWTPs, with sludge retention time and compound structure, the most important factors in determining the efficiency of this (Sipma et al. 2010). Even the most advanced WWTPs will be unable to completely remove all pharmaceuticals, which can lead to the continuous input of low levels into the aquatic environment (Fabbri 2015). 41% of the global population lives in coastal areas, and as a result, a high amount of sewage is being released into coastal waters or estuaries (Gaw et al. 2014). Sewage may also be discharged from ships and cruise liners, therefore there is the potential for drugs to be found in marine waters further from the coast, however these concentrations are likely to be small (Backhaus 2014).

Agriculture and aquaculture provide another route of entry into the aquatic environment (Figure 1.1; Pal et al. 2010). Many of the pharmaceuticals used in these industries, particularly antibiotics are also registered for human use (Kim et al. 2016). Veterinary pharmaceuticals also have the potential to enter the aquatic environment and pose a threat. However, the scope of this review will only focus on those which are registered for human use. In offshore aquaculture, up to 75% of medicines administered can be lost to surrounding waters and in some areas of Asia, fish in aquaculture are fed with treated sewage sludge, which will contain low levels of pharmaceuticals (Gaw et al. 2014). The spreading of manure contaminated with pharmaceuticals, and runoff from agriculture can also contribute to their entrance into the aquatic environment.

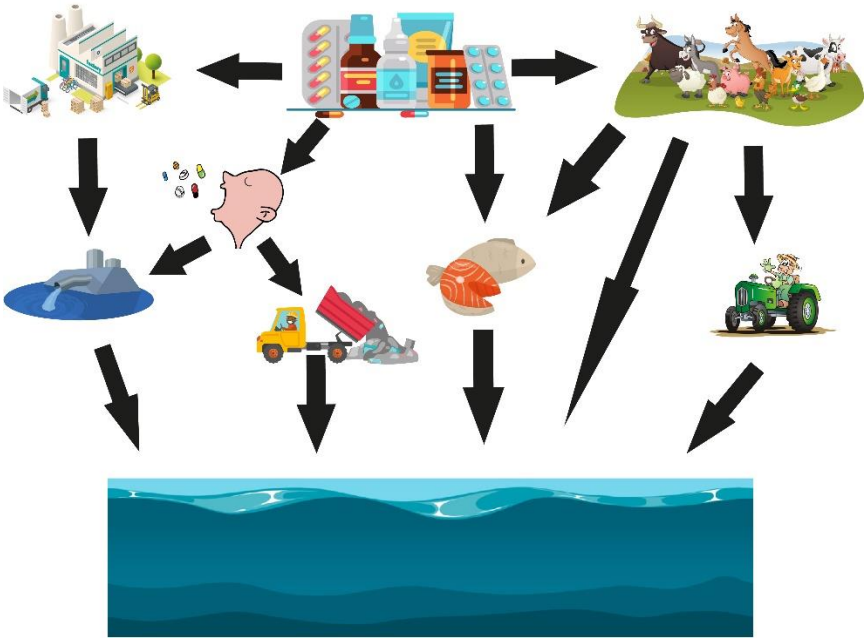


Figure 1.1: Diagram outlining the sources of pharmaceuticals in the aquatic environment. Pharmaceuticals can enter the aquatic environment through the manufacturing process, human consumption, improper disposal, aquaculture, run off from agriculture and the spreading of manure.

Once in the aquatic environment, pharmaceuticals may absorb to sediment or suspended particles, enter biological organisms or be further degraded or transformed (Yang et al. 2011; Liu et al. 2013). In surface waters, photodegradation is the most efficient form of removal, as it is likely that many of the pharmaceuticals present have already experienced biodegradation in WWTPs and will therefore be resistant to this (Boreen et al. 2003; Cuong et al. 2011). The efficiency of photodegradation depends on the chemical structure of the compound and light intensity, and therefore is likely to be more efficient in some seasons and geographical areas than others (Cuong et al. 2011). Sorption to sediment is the other main method of pharmaceutical removal from surface waters, however, there is limited data on the fate of pharmaceuticals once they reach aquatic sediment (Maskaoui and Zhou 2010; Liu et al. 2013). They are likely to become bioavailable to different organisms, but depending on the biogeochemistry may become buried or resuspended (Beretta et al. 2014).

There are many parameters which can affect the partitioning of pharmaceuticals between water and sediment (Oh et al. 2016).Pharmaceuticals with a high molecular weight and high octanol-water partition coefficient (Log_{KOW}) are less soluble and more easily sorbed to sediments. However, this is not the only predictor of sorption to sediment, and pharmaceuticals with a low log_{KOW}, such as trimethoprim (log_{KOW} <1), have been detected in sediments (Lara-Martín et al. 2014). This partitioning of pharmaceuticals between sediment and water is not only determined by chemical properties, but also environmental factors, and sediment properties. Pharmaceuticals often have one or more ionisable groups and the ionisation of these compounds is often pH dependent (Martínez-Hernández et al. 2014). As a result, sorption to sediment can also be influenced by water and sediment pH. When the pH of a compound is less than its dissociation constant (pKa) then it will be protonated, and more likely to absorb to sediment (Yamamoto et al. 2009). As a result acidic (pKa < 7) pharmaceuticals such as ibuprofen and diclofenac have showed lower affinity to bind to suspended solids in the environment in comparison to compounds with basic characteristics such as antidepressants (Zenker et al. 2014; Oh et al. 2016). For instance, ibuprofen has a pKa of 4.5 and in experimental studies, has been shown to have a higher sorption tendency to sediment with a pH below this, and almost no sorption to sediment at pH 7 due to increased solubility and decreased log_{KOW} (Oh et al. 2016). A linear relationship between the organic content of sediment and the K_D of a compound has been observed. Al-Khazrajy and Boxall (2016) assessed the sorption behaviour of amitriptyline, atenolol, cimetidine, diltiazem and mefenamic acid to ten types of sediment and found that there was a positive relationship between sorption of cimetidine (pKa 6.8) to the organic and clay content of sediment, as the result of a greater presence

of the neutral form fraction. These characteristics will also influence the uptake of pharmaceuticals by aquatic organisms. Log_{KOW} is often used as a predictor of pharmaceutical bioaccumulation, however, due to the ionisation of these compounds, is often found to be inaccurate, and the potential for bioaccumulation of pharmaceuticals is dependent on pH (Schreiber et al. 2011). Whilst lipophilicity of pharmaceuticals plays a role in the uptake of pharmaceuticals, this can differ between tissue type and organisms (Moreno-González et al. 2016; Ojemaye and Petrik 2019).

Current research on the fate of pharmaceuticals in the aquatic environment has focused on freshwater. However, this may not be transferable to the marine and estuarine environments due to different physical-chemical properties (Gaw et al. 2014). Changes in pH and salt within an estuary will have an influence on the ionisation of many compounds which can lead to changes in solubility and sorption (Fabbri and Franzellitti 2016). Typically, seawater has a pH of 8, which may increase the lipophilicity of compounds, leading to enhanced affinity to be absorbed to sediment or taken up by organisms. Additionally, the increased salt content will decrease the solubility of neutral compounds as the result of the salting-out effect (Turner 2003). Tides and currents are key process in these environments and are likely to play a role in the transport of pharmaceuticals, changes in pH, and interaction of pharmaceuticals with suspended sediment (Zhao et al. 2015).

1.1 Prioritisation of pharmaceuticals

In 2004 the first pieces of legislation (2004/27/EC and 2004/28/EC) to require an environmental risk assessment (ERA) for pharmaceutical compounds came into effect, requiring an ERA assessment to be completed for all new marketing authorisation applications under regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; Adler et al. 2008). Under REACH, an ERA must include an assessment on the risk and hazards of the given compound in aquatic and terrestrial compartments (Tarazona et al. 2007). A risk-benefit analysis for veterinary drugs was introduced where compounds could be banned from use if the environmental risks outweighed the potential benefits, however, the benefit to human medicine is always seen to outweigh the potential environmental risks (Küster and Adler 2014). Under this legislation, ERAs must include an assessment of the amount of the compound in different compartments (e.g. freshwater, terrestrial and marine environments) and if a trigger level is reached an assessment on the risk to biota in these compartments must be undertaken (Tarazona et al. 2007). In the aquatic environment this usually comprises of predicted no effect concentrations (PNEC) or acute toxicity tests with *Daphnia magna*, green algae and

zebrafish (*Danio rerio*; Tarazona et al. 2007). Prior to this, compounds were released into the environment unregulated with little to no knowledge of their potential hazards (Roos et al. 2012). Currently, human pharmaceuticals must be disposed of through hazardous waste, but there are not any regulations surrounding their usage and environmental consequences. Since this legislation came into effect, approximately 10% of pharmaceuticals were found to pose an environmental risk (Kuster and Adler, 2014). Diclofenac and ethinylestradiol are examples of compounds which pose a risk to the aquatic environment (Adler et al. 2008). They were added to priority watch lists under the water framework directive in 2013, recognising for the first time that pharmaceuticals have the potential to be a serious environment risk (Mavragani et al. 2016).

There is still a lot of uncertainty surrounding the occurrence of pharmaceuticals in the aquatic environment, and their environmental risk. There is evidence that they are occurring in the environment (Hughes et al. 2013; Fabbri and Franzellitti 2016), however, the implications of this is not yet fully understood (Taylor and Senac 2014). It is only within recent years that analytical methods have been able to detect these compounds, and prior to this, little research was conducted on pharmaceuticals as environmental contaminants. Despite the widespread and ubiquitous usage of pharmaceuticals, we know relatively little about their environmental impacts. Unlike other pollutants, there is already extensive knowledge surrounding the pathways of pharmaceuticals in vertebrates, but there is some uncertainty over the potential effects on non-target organisms (Fabbri 2015). With so many pharmaceuticals commercially available, it would use a great deal of resources to monitor their occurrence in the environment and determine the effects in non-target organisms. Prioritisation schemes are frequently used in the literature to identify a smaller subset of compounds which are likely to be found in the environment and pose a risk (Mansour et al. 2016). This can help direct resources and determine where scientific research should be invested. Further research into the environmental effect of pharmaceuticals can impact legislation, by further determining those which need to be regulated.

1.1.1 Exposure assessment

Many prioritisation schemes use predicted environmental concentrations (PECs) as a basis to these assessments, assuming that if the compound is not found in the environment, or is found at low concentrations then there is no risk. Most prioritisation schemes include the assessment of pharmaceutical concentrations in surface water, but not other compartments (Besse and Garric 2008). The EU technical guidance advises that PECs are calculated by modelling discharge and fate processes or that measured environmental concentrations (MECs) are used where available (Ehrlich et al. 2011). Guidelines are also given on assessing concentrations in other compartments such as

sediment. The use of MECs is often difficult for pharmaceuticals as there aren't many monitoring schemes in place and fate of pharmaceuticals in the aquatic environment is not fully understood (Fabbri and Franzellitti 2016). PECs used in the prioritisation literature are often simplified versions of those found in ERAs. Most PECs are calculated from usage data on the volume of drugs produced per year or number of prescriptions filled and then are refined based on metabolism, removal in WWTPs and dilution (Ashton et al. 2004; Besse et al. 2008). Dilution is a key process affecting the fate of pharmaceuticals in the aquatic environment, and some studies have included localised data into these equations (Ferrari et al. 2004; Burns et al. 2017). Other schemes have estimated that 5 to 15% of oral pharmaceuticals and 30% of topical pharmaceuticals will never be consumed (and therefore not enter the environment) and have been included into PEC calculations (Kostich and Lazorchak 2008). Prescription data and usage of over the counter (OTC) medicines are not available in many regions, making it difficult to predict environmental concentrations. The European medicines agency (EMEA) guidelines advise use of a PEC calculation which does not require prescription data, as it involves predicting environmental concentrations from the maximum dosage per person and market penetration (EMEA 2006).

Few prioritisation schemes include the assessment of pharmaceuticals in sediment. This is reflected in the literature, with most environmental monitoring having been carried out in effluent and surface waters (Fabbri and Franzellitti 2016). The K_D of pharmaceuticals is often used to determine the likelihood of their presence in sediment, however, this value is heavily influenced by temperature and pH, which will differ between regions (Al-Khazrajy and Boxall 2016). The EMEA requires a risk assessment on the fate of pharmaceuticals in sediment, however, experimental data does not currently exist for many compounds (EMEA, 2006).

1.1.2 Predicting toxicity

Many prioritisation schemes assess the risk of pharmaceuticals using traditional ERAs. Risk quotients using a ratio of PEC:PNEC are calculated and if the result is greater than 1, then it is deemed to pose a threat (Hoyett et al. 2016). PNECs are usually calculated by selecting the most sensitive LC_{50} and applying an assessment factor (Thomas Backhaus and Faust 2012). Such experimental data is often unavailable in the literature and is time consuming to generate for prioritisation schemes. Many authors have used quantitative structure-activity relationships (QSARs), which are allowed under REACH and US environmental protection agency (EPA) guidelines to model the potential toxicity of these compounds (Sanderson et al. 2004; Ortiz de García et al. 2013). These models predict the physico-chemical properties of an unknown chemical by comparing them to other known

chemicals based on their structure (Guillén et al. 2012). There are many different software packages which can be used in these assessments, of which, ECOSAR is the most widely used in the prioritisation literature (Guillén et al. 2012). The use of QSARs to model toxicity, has been widely debated, and has been found to be a poor predictor of toxicity for many compounds (de Roode et al. 2006). Ashton et al. (2004) estimated PNECs using a different method, taking the maximum therapeutic dose in humans and applying an assessment factor of 1000. The rationale of which is that there are many conserved drug targets between humans and non-target organisms, and those which are more biologically active in humans, may be so in other organisms (Gunnarsson et al. 2008).

Persistence, bioaccumulation and toxicity (PBT) assessments are alternatives to risk quotients for ERAs under REACH (Ehrlich et al. 2011). In prioritisation schemes, PBT assessments are often used alongside PECs. Most commonly, this is assessed through the half-life of compounds in the environment (persistence), bioconcentration factor (BCF; bioaccumulation) and no-observed effect concentrations (NOECs) or PNECs (toxicity), however where data is lacking it allows flexibility, and different approaches have been used within the prioritisation literature (Ortiz de García et al. 2013). For example, Sangion and Gramatica (2016) used modelled PBT data using QSARs, whilst Daouk et al. (2015) used removal in wastewater to determine the persistence of compounds in effluent.

Due to difficulty in obtaining experimental data on PBT of compounds and the limitations of QSARs in modelling toxicity, several studies have suggested modelling the effects of pharmaceuticals on aquatic species by utilising information on pathways of these pharmaceuticals in mammals. Due to difficulty in obtaining experimental data on PBT of compounds and the limitations of QSARs in modelling toxicity, several studies have suggested modelling the effects of pharmaceuticals on aquatic species by utilising information on pathways of these pharmaceuticals in mammals. The under-pinning assumption in these models, is that drug-targets in mammals are conserved across other species and function in the same way, however, novel functions may arise as the result of evolution of such targets and it may not always be possible to translate the effects seen in vertebrates to non-target organisms (Thornton 2000; Ankley et al. 2010). There is conflicting evidence as to the conserved function of these targets across species, and the ability to extrapolate this information has been debated (Adler et al. 2008). Gunnarsson et al. (2008) looked at 1318 drug targets across 16 species and determined that 86% were conserved in zebrafish, 61% in daphnia and 35% in green algae, suggesting that the pathways of pharmaceuticals could be predicted in a variety of species. They also found that whilst enzymes are well conserved across species, the function of receptors are not. Whilst many of these receptors are present in other species, there is often a poor

mechanistic understanding, and when differences in their function arise, it can be difficult to translate effects to other organisms (Rand-Weaver et al. 2013). For example, an estrogen receptor (ER) ortholog has been described in some molluscs, but is not activated by estrogen (Bannister et al. 2000; Thornton et al. 2003). Furthermore, steroid hormones have been characterised in molluscs, however, there function is poorly understood, and there is not a consensus in the scientific literature as to their role in reproduction (Scott et al. 2013). Despite this, ethinylestradiol has caused reproductive changes in molluscs, such as increased vitellogenin and increased egg laying (Jobling et al. 2004; Ciocan et al. 2010; Benstead et al. 2011). This suggests that ethinylestradiol could mediate its effect through a non-ortholog receptor or through conserved pathways that have yet to be characterised. Regardless, this highlights the limitations of methods which are underpinned by assuming the conservation of drug-targets.

The fish plasma model (FPM), which was originally developed by Huggett et al. (2003), is one method which utilises information on the activity of pharmaceuticals in mammals. It estimates the plasma concentration in fish based upon the human therapeutic plasma concentration of a pharmaceutical. This is compared to environmental concentrations and is often used as an alternative to RQs in prioritisation schemes (Fick et al. 2010, Schrieber et al. 2011, Roos et al. 2012). There are two main assumptions with this model: that drug targets are conserved across human and fish species, and that the therapeutic concentration at which an effect is exerted is the same (Schreiber et al. 2011). Brown et al. (2014) determined the conservation of 459 drug targets across 14 fish species and found that between 65 and 86% were conserved, which suggests the difficulty in translating the effects of pharmaceuticals between fish species.

Many authors have also suggested the use of adverse outcome pathways (AOPs) for prioritising pharmaceuticals (Ankley et al. 2010, Caldwell et al. 2014). AOPs look at the effect of a chemical at a molecular, cellular, individual and population level, linking an effect with a molecular initiating event (Figure 1.2). For example, a molecular initiating event may be an estrogen receptor (ER) antagonist, which will lead to a decline in vitellogenin synthesis and concentrations which ultimately leads to decreased spawning and fecundity in females, and ultimately a declining population (Ankley et al. 2010, Figure 1.2). This allows for better cross-species prediction, which is particularly useful for pharmaceutical assessments as pathways of pharmaceuticals in humans are relatively well understood (Caldwell et al. 2014, LaLone et al. 2014). However, there is often complexity in linking molecular initiating events with a population effect, which is further complicated by uncertainty surrounding the conservation of drug targets across species. A number of pharmaceuticals, such as tamoxifen and ethinylestradiol are known ER antagonists and

have the potential to have an effect on fish populations. However, even with a well-defined AOP, experimental exposures often lack information on plasma concentrations, which can make it difficult to support models such as FPM (Rand-Weaver et al. 2013). As evidenced with the example of estrogen receptors in molluscs, such pathways may not be as well defined or understood in other species.

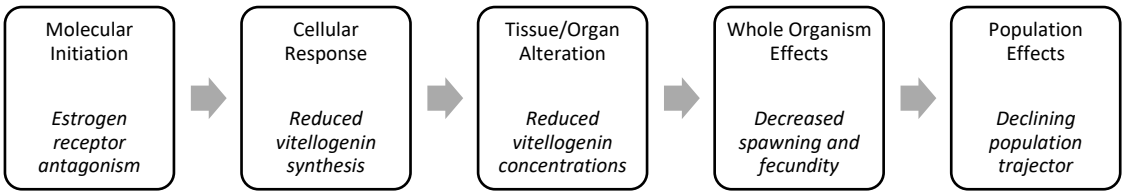


Figure 1.2 Process of adverse outcome pathways, which link a molecular initiating event to effect at a population level. Estrogen receptor antagonism in female fish as an example of how an AOP can be used to link a molecular initiating event to a population effect.

1.2 Occurrence of pharmaceuticals in estuaries

1.2.1 Surface Water

Reviews have previously summarised the occurrence of pharmaceuticals in fresh (Hughes et al. 2013) and marine waters (Fabbri and Franzellitti 2016); 155 published studies have been conducted in 41 countries and 46 published studies have been carried out in 22 countries, respectively. In contrast, 29 studies across 9 countries (China, USA, Portugal, Australia, UK, Germany, Belgium, France and Spain) have been carried out in the estuarine environment, with sulfamethoxazole being the most monitored compound (Table 1.1). It has only been within recent years, that the occurrence of pharmaceuticals in estuaries has gained more attention, as only 5 of these studies were conducted prior to 2011 (Thomas and Hilton 2004, Wiegel et al. 2004, Benotti and Brownawell 2007, Noppe et al. 2007, Tamtam et al. 2008). In total 126 of 181 target pharmaceuticals have been detected in estuarine surface waters, with median concentrations generally less than 100 ng l^{-1} (Appendix 1.1). Only five compounds (oxytetracycline, tetracycline, trimethoprim, salbutamol and phenytoin) have been found in the µg l^{-1} range (Benotti and Brownawell 2009; Hui Chen et al. 2015; Mijangos et al. 2018). Of these, the highest concentration was the antibiotic oxytetracycline, which was detected in China (Table 1.1). Antibiotics were the most studied compound type, comprising of approximately 30% of those monitored in estuaries (Table 1.2), most of which were carried out in China. China is the largest consumer and producer of antibiotics globally, and as a result some of the highest

concentrations have been seen here (Bu et al. 2013). Approximately 200,000 tons of antibiotics are produced annually in China, compared to approximately 9,000 tons in USA (Daghrir and Drogui 2013). Many of these antibiotics are also used in veterinary medicine and as growth promoters in agriculture, which can account for their high occurrence and detection frequencies (Guo et al. 2019). Antidepressants, antihypertensives, nonsteroidal anti-inflammatories (NSAIDs) and pain killers made of a further 50% of the compounds studied, with the remaining 20% made up of 19 different classes (Table 1.2). Although the literature has covered a wide range of compounds, there is often little overlap between studies, with some pharmaceuticals having only been measured in a few areas (Appendix 1.1). As a result, it is difficult to establish trends in their occurrence.

The concentrations of pharmaceuticals varied between these estuaries, with the main sources differing between geographical areas. Most of the studies in the USA and Europe attributed the input of pharmaceuticals to mostly be the result of the discharge of domestic, industrial and hospital wastewater (Beretta et al. 2014). In China on the other hand, discharge of untreated sewage and presence of agriculture and fish farming were found to be a greater source of pharmaceuticals (Cui et al. 2019; Guo et al. 2019). The elevated concentrations of some pharmaceuticals were the result of proximity to these sources.

In terms of spatial distribution, most pharmaceuticals have a negative correlation with salinity, declining in concentration from source to mouth of an estuary (Liang et al. 2013; Sun et al. 2014). These concentrations have been observed to vary as the result of flow rate, tides and currents, and as a result, dilution has been named as the biggest factor influencing the fate of these pharmaceuticals in estuaries (Cantwell et al. 2017). Additionally, concentrations are generally highest at low and ebb tide, when salinity is lowest (Lara-Martín et al. 2014), however, Munro et al. (2019) observed the opposite in the Thames Estuary, as high tide coincided with untreated effluent discharge from combined sewer overflows (CSOs), causing transport of the compounds further upstream the estuary. The variations in concentrations of pharmaceuticals between estuaries is also likely the result of flushing time, as those with a higher flushing rate are less likely to retain pharmaceuticals (Cantwell et al. 2017).

Concentrations of pharmaceuticals were also found to vary temporally as the result of changes in environmental conditions and fluctuations in input. In wastewater effluent dominated estuaries, temporal fluctuations may have been the result of seasonal differences in population or consumption patterns (Mijangos et al. 2018). Golovko et al. (2014a, 2014b) looked at seasonal variations in pharmaceutical concentrations in WWTPs. Antibiotics were found to be seasonal with concentrations highest in winter, likely

due to the increase in colds and infections (Golovko et al. 2014b). Antidepressants and lipid lowering agents were also highest during this time (Golovko et al. 2014a). However, due to the usage of these drugs to treat chronic conditions, the seasonal differences in their occurrence are more likely the result of low temperatures which leads to lower degradation and reduced input (Gonzalez-Rey et al. 2015). In general, overall concentrations were higher in estuaries during winter as the result of reduced degradation due to low temperatures and low irradiance (Hedgespeth et al. 2012). This pattern did not apply to all regions, and some areas exhibited higher concentration in the summer as the result of decreased flow (Benotti and Brownawell 2007).

Table 1.1: Top 15 pharmaceuticas measured in estuarine surface water by maximum concentrations (see Table A1.1 for full occurrence data). Detection frequency is the number of positive detections/number of samples analysed accross all studies.

Compound	Class	Median ($ng\ l^{-1}$)	Maximum ($ng\ l^{-1}$)	Detection Frequency (%)	Number of Estuaries Sampled	Reference
Oxytetracycline	Antibiotic	0	15 163	24.8	5	Yan et al. (2013), Chen et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018), Guo et al. (2019)
Tetracycline	Antibiotic	0	2 305	18.6	6	Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018), Guo et al. (2019)
Trimethoprim	Antibiotic	4.12	2 046	66.3	18	Thomas and Hilton (2004), Benotti and Brownawell (2007), Tamtam et al. (2008), Zhang et al. (2012, 2013), Klosterhaus et al. (2013), Chen et al. (2015), Cantwell et al. (2016, 2017, 2018), Mijangos et al. (2018), Reis-Santos et al. (2018), Munro et al. (2019)
Salbutamol	Bronchodilator	0	1 440	32.5	3	Benotti and Brownawell (2007), Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015)
Phenytoin	Anticonvulsant	9	1 401	18.7	3	Mijangos et al. (2018)
Indomethacin	NSAID	0	979	53.8	3	Chen et al. (2011), Sun et al. (2016), Cui et al. (2019)
Telmisartan	Antihypertensive	9	969	71.7	3	Mijangos et al. (2018)
Ibuprofen	NSAID	3.8	928	47.3	10	Thomas and Hilton (2004), Hedgespeth et al. (2012), Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018)
Nifedipine	Antihypertensive	2.5	899	35.6	2	Benotti and Brownawell (2007), Munro et al. (2019)
Metformin	Antidiabetic	234.25	832	61.3	2	Benotti and Brownawell (2007), Meador et al. (2016)
Sulfamethoxazole	Antibiotic	5.15	765	59.4	26	Thomas and Hilton (2004), Benotti and Brownawell (2007), Tamtam et al. (2008), Yang et al. (2011), Zheng et al. (2011), Zhang et al. (2012, 2013), Klosterhaus et al. (2013), Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Zhao et al. (2015), Cantwell et al. (2016), Meador et al. (2016) Sun et al. (2016), Cantwell et al. (2017, 2018), Mijangos et al. (2018), Reis-Santos et al. (2018), Guo et al. (2019)
Meclofenamic Acid	NSAID	217.5	679	66.7	1	Yang et al. (2011)
Carbamazepine	Anticonvulsant	4.35	675	67.9	14	Benotti and Brownawell (2007), Yang et al. (2011), Klosterhaus et al. (2013), Birch et al. (2015), Gonzalez-Rey et al. (2015), Zhao et al. (2015), Sun et al. (2016), Cantwell et al. (2016, 2017, 2018), Reis-Santos et al. (2018), Mijangos et al. (2018), Cui et al. (2019), Munro et al. (2019)
Diclofenac	NSAID	3.1	650	48.2	14	Thomas and Hilton (2004), Wiegel et al. (2004), Yang et al. (2011), Gonzalez-Rey et al. (2015), Sun et al. (2016), Mijangos et al. (2018), Reis-Santos et al. (2018), Cui et al. (2019)
Fluoxetine	Antidepressant	0	596	36.1	6	Benotti and Brownawell (2007), Birch et al. (2015), Gonzalez-Rey et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018), Munro et al. (2019)

Table 1.2: Summary of monitoring studies that have been conducted in estuaries, showing the number of compounds monitored, how many were detected in at least one estuary and the number of studies which monitored each compound class. Full details can be found in appendix A1.1 and A1.2

	Surface Water			Sediment		
Compound Class	**No. of Compounds**	**No. Detected**	**No. of Studies**	**No. of Compounds**	**No. Detected**	**No. of Studies**
Antibiotic	62	39	20	32	28	10
Anticonvulsant	5	5	14	1	1	5
Antidepressant	10	7	10	4	3	3
Antihypertensive	20	17	17	10	8	6
Anxiolytic	6	5	4	2	1	2
Bronchodilator	6	4	5	1	1	2
Hormone	7	5	10	1	1	1
Lipid Lowering Agent	5	4	14	3	3	3
Metabolite	10	7	5	7	4	3
Mucosal Protectant	4	4	7	3	3	3
NSAID	11	9	13	5	5	4
Pain Killer	9	8	14	3	2	2
Other	27	13	12	8	8	5

1.2.2 Sediment

Few studies have determined the occurrence of pharmaceuticals in estuarine sediments in comparison to surface water. A total of 11 studies have been carried out in four countries (Brazil, USA, China, New Zealand), comprising of 79 pharmaceutical compounds (Table 2; Appendix 1.2). Similar to studies conducted on surface water, antibiotics were the most studied compounds class (Table 1.2). Concentration of pharmaceuticals were often lower in sediment than those found in surface, with only ofloxacin, chlortetracycline and oxytetracycline, detected at concentrations above 100 ng l^{-1}, and only ten pharmaceuticals were detected above 25 ng l^{-1} (Table 1.3). Of the antibiotics measured, sulfanomides, such as sulfamethoxazole showed low sorption capacity, and were mostly absent from sediment, which could account for their high presence in surface water (Shi et al. 2014).

Few studies have looked at the spatial and temporal patterns of pharmaceuticals in sediments. Many of the compounds measured, exhibited trends similar to those observed in surface water, with the presence of pharmaceuticals related to consumption patterns and highest concentrations occurring in regions with higher populations and at sites in closer proximity to sources (Beretta et al. 2014). The presence of pharmaceuticals in sediment is dependent on their K_D, however these values are highly dependent on pH and temperature, suggesting that removal of pharmaceuticals to sediment could differ seasonally (Al-Khazrajy and Boxall 2016). The sorption capacity of estrone was found to increase with increasing salinity in the Scheldt Estuary, and as a result, concentrations in

the dissolved phase were lower further downstream the estuary (Noppe et al. 2007). The sorption capacity of pharmaceuticals has also been observed to differ with sediment type, with a positive correlation between sorption and the percentage of clay in the sediment (Beretta et al. 2014).

Table 1.3: Top 15 pharmaceuticals measured in estuarine sediment by maximum concentrations (see Table A1.2 for full occurrence data). Detection frequency is the number of positive detections/number of samples analysed across all studies.

Compound	Class	Median ($ng\ g^{-1}$)	Maximum ($ng\ g^{-1}$)	Detection Frequency (%)	Number of Estuaries Sampled	Reference
Ofloxacin	Antibiotic	2.59	458.2	54.5	4	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
Chloratetracycline	Antibiotic	0.41	184	52.1	2	Shi et al. (2014), Chen et al. (2015)
Oxytetracycline	Antibiotic	0.84	176	37.8	4	Long et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
Norfloxacin	Antibiotic	3.36	69.3	64.7	3	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015)
Erythromycin-H_2O	Metabolite	1.13	65.33	57.6	2	Klosterhaus et al. (2013), Chen et al. (2015)
Erythromycin	Antibiotic	0.24	51.5	41.9	3	Beretta et al. (2014), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
Anydrochloratetracycline	Metabolite	0	46.9	2.5	1	Long et al. (2013)
Metoprolol	Antihypertensive	2.11	44	54.7	4	Klosterhaus et al. (2013), Stewart et al. (2014), Lara-Martín et al. (2015), Cantwell et al. (2017)
Ciprofloxacin	Antibiotic	3.1	42.9	46.7	3	Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
Ibuprofen	NSAID	1.1	21.7	50.6	4	Klosterhaus et al. (2013), Beretta et al. (2014), Stewart et al. (2014), Lara-Martín et al. (2015)
Paroxetine	Antidepressant	0	21.5	11.1	1	Klosterhaus et al. (2013)
Doxycycline	Antibiotic	0	18.6	100	1	Shi et al. (2014), Chen et al. (2015)
Trimethoprim	Antibiotic	0	18.2	26.7	5	Klosterhaus et al. (2013), Stewart et al. (2014), Chen et al. (2015), Lara-Martín et al. (2015), Cantwell et al. (2017)
Tetracycline	Antibiotic	0.81	14.6	37.7	4	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
Galaxolide	Antidiabetic	9.17	14.54	100	1	Beretta et al. (2014)

1.3 Biological Effects

The ecotoxicological effects of pharmaceuticals have been extensively studied in freshwater organisms (Crane et al. 2006; Fent et al. 2006; Fabbri 2015). Studies in marine organisms are sparser, and have been summarised in Table 1.4. Despite this, there are similarities in biological systems of organisms found in both of these systems. However, differences may occur in physiology between marine and fresh water organisms, for example, to be able to cope with saline conditions, and organisms present in estuaries are often living at the edge of their tolerance zones, which can make them more sensitive to contaminants (Scaps 2002).

The effects of 38 different compounds have been assessed on marine species, with carbamazepine and fluoxetine dominating these studies, and bivalves were the most commonly studied taxa in ecotoxicity studies (Table 1.4). Bivalves are commonly used in ecotoxicology experiments as they are long-living, sessile, and filter high volumes of water, and as a result can be particularly susceptible to contaminants (Gagné et al. 2010). They are also abundantly available, of commercial importance and easy to maintain in a laboratory setting. Despite the numerous studies which have spanned a broad range of taxa and pharmaceuticals, there are still many questions about the effects and pathways of these chemicals. Many experiments use concentrations much higher than environmental ones and simple endpoints such as mortality and growth. Although these are important to know, it is essential to have a deeper understanding of pathways of pharmaceuticals in order to determine toxicity that has the potential to effect populations, and therefore is of more interest to regulators (Ankley et al. 2010). Almost all types of pharmaceuticals have been found to cause oxidative stress where reactive oxygen species (ROS) are produced as the result of pharmaceutical metabolism (Diniz et al. 2015). ROS can cause oxidation of proteins and lipids, alter gene expression, and damage cells (Diniz et al. 2015). Many organisms have developed mechanisms to minimise the damage by producing anti-oxidants such as catalase (CAT), superoxide dismutase (SOD), and Glutathione. This demonstrates that these pharmaceuticals have the potential to harm, but in many cases, the exact mechanisms of toxicity are poorly understood.

Table 1.4: Summary of the ecotoxicology literature of pharmaceuticals to marine organisms. Where species do not have a common name, class was stated

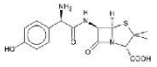
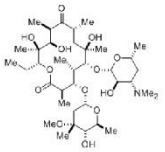
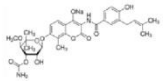
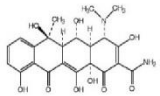
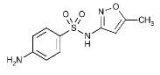
Antibiotic	Amoxicillin 	*Mytilus galloprovincialis*	Mediterranean Mussel	100 – 400 µg l^{-1}	7 Days	(i)Haemocyte Count (ii)Lactate Dehydrogenase	Matozzo et al. (2016)
		Ruditapes philippinarum	Manila Clam	100 – 400 µg l^{-1}	7 Days	(i)Haemocyte Count (ii)Lactate Dehydrogenase	Matozzo et al. (2016)
	Erythromycin 	*Mytilus edulis*	Blue Mussel	2 - 100000 µg l^{-1}	21 Hours	(i)Haemocyte Mortality (ii)Genotoxicity	Lacaze et al. (2015)
		Sparus aurata	Gilthead Bream	0.7 – 8.8 µg l^{-1}	28 Days	(i)EROD (ii)GST (iii)UGT (iv)CAT (v)GPx (vi)GR (vii)LPO (viii)TBARS (ix)genotoxicity (x)AChE (xi)Lactate dehydrogenase	Correia et al. (2018)
	Novobiocin 	*Carcinus maenas*	Edible Crab	0.1 – 50 µg l^{-1}	28 Days	(i)LMS	Aguirre-Martínez et al. (2013)
	Oxytetracycline 	*Brachionus rotundiformis*	Rotifer	10 – 1000 µg l^{-1}	10 Days	(i)CYP (ii)GST	Park et al. (2018)
		M. galloprovincialis	Mediterranean Mussel	1 – 100 µg l^{-1}	4 Days	(i)Temperature (ii)CAT (iii)GST (iv)HSP (v)LO	Banni et al. (2015)
		S. aurata	Gilthead Bream	4 – 8 mg g^{-1}	21 Days	(I)Peroxidase Activity (ii)Haemolytic Complement Activity (iii)Serum IgM Level (iv)Phagocytic Activity (v)Real Time PCR	Guardiola et al. (2012)
	Sulfamethoxazole 	*M. edulis*	Blue Mussel	0.002 - 100 mg l^{-1}	21 Hours	(i)Haemocyte Mortality (ii)Genotoxicity	Lacaze et al. (2015)
		Palaemonetes pugio	Daggerblade Grass Shrimp	60 mg l^{-1}	5 Days	(i)Hatching (ii)Metamorphosis (iii)Growth (iv)Mortality	Garcia et al. (2014)

Table 1.4: *Continued*

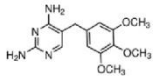
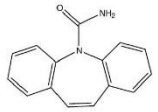
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Antibiotic	Trimethoprim 	*M. edulis*	Blue Mussel	0.002 – 100 mg l^{-1}	21 Hours	(i)Haemocyte Mortality (ii)Genotoxicity	Lacaze et al. (2015)
Anticonvulsant	Carbamazepine 	*Ampelisca brevicornis*	Malacostrata	0.05 – 500 ng g^{-1}	10 Days	(i)EROD (ii)GST (iii)GPX (iv)GR (v)AChE (vi)LPO (vii)Genotoxicity	Maranho et al. (2015a)
		C. maenas	Edible Crab	- 50 µg l^{-1}	28 Days	(i)LMS	Aguirre-Martínez et al. (2013)
		Crassostrea gigas	Oyster	10^{-7} – 10^{7} µg l^{-1}	36 Hours	(i)Larval Development (ii)Metamorphosis	Di Poi et al. (2018)
		Dunaliella tertiolecta	Chlorophycae	5 - 80 mg l^{-1}	96 Hours	(i)Cell Density	DeLorenzo and Fleming (2008)
		Echinogammarus marinus	Malacostraca	0.01 – 10 µg l^{-1}	21 Days	(i)Behaviour	Guler and Ford (2010)
		Hediste diversicolor	Ragworm	0.5 – 500 ng g^{-1}	14 Days	(i)MAO (ii)COX (iii)Total Lipids (iv)MET	Maranho et al. (2015b)
		M. edulis	Blue Mussel	0.0015 – 75 mg l^{-1}	21 Hours	(i)Haemocyte Mortality (ii)Genotoxicity	Lacaze et al. (2015)
		M. galloprovincialis	Mediterranean Mussel	0.1 – 10 µg l^{-1}	7 Days	(i)cAMP (ii)Protein Kinase A (iii)LO (iv)Lipid Content (v)GST (vi)CAT (vii)GR (vi)Genotoxicity (vii)Multixenobiotic Resistence Gene	Martin-Diaz et al. (2009)
		Perna viridis	Green Mussels	1 – 96 ng l^{-1}	7 Days	(i)AChE (ii)Phagocytosis (iii)Hemocytes (iv)EROD (v)GST (vi)LO (vii)Genotoxicity (viii)Vitellin-Like Protein (ix)CYP 1A	Juhel et al. (2017)
		S. aurata	Gilthead Bream	5 µg l^{-1}	28 Days	(i)Transciptomic Microarray	Hampel et al. (2017)

Table 1.4: *Continued*

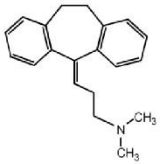
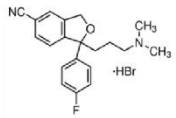
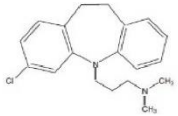
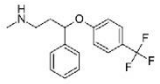
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Anticonvulsant	Carbamazepine	*Venerupis decussata*	Cross Cut Carpet Shell	0.03 – 9 µg l^{-1}	96 Hours	(i)Condition Index (ii)Clearance Rate (iii)MDA (iv)GSH (v)SOD (vi)CAT (vii)GR (viii)GR (ix)CYP3A4	Almeida et al. (2015)
		Venerupis philippinarium	Japanese Cockle	0.03 – 9 µg l^{-1}	96 Hours	(i)Condition Index (ii)Clearance Rate (iii)MDA (iv)GSH (v)SOD (vi)CAT (vii)GR (viii)GR (ix)CYP3A4	Almeida et al. (2015)
Antidepressant	Amitriptyline 	*Haliotis tuberculata*	Green Ormer	17.03 - 45.24mg l^{-1}	48 Hours	(i)MIT Assay (ii)LMS (iii)Phagocytosis (iv)ROS production (v)Esterase Activity	Minguez et al. (2014)
	Citalopram 	*H. tuberculata*	Green Ormer	7.5 - 32.94 mg l^{-1}	48 Hours	(i)MIT Assay (ii)LMS (iii)Phagocytosis (iv)ROS production (v)Esterase Activity	Minguez et al. (2014)
Antidepressant	Clomipramine 	*H. tuberculata*	Green Ormer	0.7 - 4.76mg l^{-1}	48 Hours	(i)MIT Assay (ii)LMS (iii)Phagocytosis (iv)ROS production (v)Esterase Activity	Minguez et al. (2014)
	Fluoxetine 	*A. brevicornis*	Malacostrata	0.01 –100 ng g^{-1}	10 Days	(i)EROD (ii)GST (iii)GPX (iv)GR (v)AChE (vi)LPO (vii)Genotoxicity	Maranho et al. (2015a)
		Capitella teleta	Polychaeta	0.01 - 3.3 µg g^{-1}	18 Days	(i)Body Weight (ii)Egestion Rate (iii)Sex	Méndez et al. (2013)

Table 1.4: *Continued*

Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Antidepressant	Fluoxetine	*C. maenas*	Edible Crab	0.5 – 750 µg l^{-1}	7 Days	(i)N-acetyl-β-glucosaminidase (ii)AChE (iii)GST (iv)GR (v)GPx (vi)GST (vii)LPO	Mesquita et al. (2011)
		C. gigas	Oysters	0.001 – 10 µg l^{-1}	28 Days	(i)Growth (ii)Histology (iii)GST (iv)CAT (v)LPO (vi)TBARS	Di Poi et al. (2016)
		Cyprinodon variegatus	Sheapshead Minnow	3 – 300 µg l^{-1}	96 Hours	(i)Mortality (ii)Bioaccumulation	Winder et al. (2009)
		D. tertiolecta	Chlorophycae	2.7 – 216 µg l^{-1}	96 Hours	(i)Cell Density	DeLorenzo and Fleming (2008)
		E. marinus	Malacostraca	- 1 µg l^{-1}	1 Hour – 8 Days	(i)Behaviour (ii)PCR	Bossus et al. (2014)
		E. marinus	Malacostraca	0.01 – 1 µg l^{-1}	21 Days	(i)Behaviour	Guler and Ford (2010)
		Gibbula umbilicalis	Flat Top Shell	0.001 – 1000 µg l^{-1}	4 Hours	(i)Foot Detachment (ii)Righting Time	Ford et al. (2018)
		H. diversicolor	Ragworm	0.5 – 500 ng g^{-1}	14 Days	(i)MAO (ii)COX (iii)Total Lipids (iv)MET	Maranho et al. (2015b)
				0.000001 - 0.1 ng g^{-1}	14 Days	(i) Total Protein Content (ii)EROD (iii)DBF (iv)GST (v)GPX (vi)AChE (vii)TBA (viii)Genotoxicity	Maranho et al. (2014)
		Ilyanassa obsoleta	Eastern Mudsnail	3.45 – 345 µg l^{-1}	2 Hours	(i)Foot Mediated Behaviour	Fong et al. (2017)
		Mytilus californianus	Californian Mussel	0.3 – 300 ng l^{-1}	107 Days	(i)Algal clearance rate (ii)Growth (iii)Gonadosomatic index	Peters and Granek (2016)
		M. edulis	Blue Mussel	1 – 50000 µg l^{-1}	21 Hours	(i)Haemocyte Mortality (ii)Genotoxicity	Lacaze et al. (2015)
		M.galloprovincialis	Mediterranean Mussel	0.0003 µg l^{-1}	7 Days	(i)Cyclic AMP content (ii)PKA Activity (iii)mRNA expression	Franzellitti et al. (2013)

Table 1.4: *Continued*

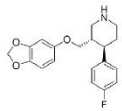
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Antidepressant	Fluoxetine	*M.galloprovincialis*	Mediterranean Mussel	0.00003 - 0.3 µg l^{-1}	7 Days	(i)Bioaccumulation (ii)Cyclic AMP Content (iii)PKA Activity (iv)mRNA expression (v)Lysosomal Membrane Activity (vi)MDA (vii)GST (viii)CAT (ix)AChE Activity	Franzellitti et al. (2014)
				0.0003 µg l^{-1}	7 Days	(i)Bioaccumulation (ii)LMS (iii)GST (iv)GSH (v)CAT (v)mRNA Expression (vi)Genotoxicity	Franzellitti et al. (2015)
				0.00075 µg l^{-1}	14 Days	(i)SOD (ii)CAT (iii)LPO (iv)AChE (v)ALP	Gonzalez-Rey and Bebianno (2013)
		Neogobius melanostomus	Round Goby	1 – 40 µg l^{-1}	28 Days	(i)Behaviour	McCallum et al. (2017)
		Osanus beta	Gulf Toadfish	25-100 µg g^{-1}	Injected Dose	(i)Serotonin (ii)Cortisol (iii)Behaviour	McDonald et al. (2011)
					24 Hours	(i)Real Time PCR (ii)Cortisol (iii)Nitrogen Excretion (iv)Survival	Morando et al. (2009)
		Sepia Officinalis	European Cuttlefish	1 – 10 µg l^{-1}	15 Days	(i)Dopamine (ii)Brain Development (iii)Behaviour (iv)LO (v)Phenoloxidase Activity	Bidel et al. (2016)
				0.0001 - 0.1 µg l^{-1}	30 Days	(i)Predatory Behaviour	Di Poi et al. (2013)
		Thalassoma bifasciatum	Bluehead Wrasse	6 µg g^{-1}	14 Days	(i)Behaviour	Semsar et al. (2004)
		V. philippinarium	Japanese Cockle	1 - 625 µg l^{-1}	7 Days	(i)Haemocyte Diameter (ii)Haemocyte Proliferation (iii)NO Uptake (iv)LO (v)AChE	Munari et al. (2014)
	Paroxetine 	*H. tuberculata*	Green Ormer	0.7 - 4.16 mg l^{-1}	48 Hours	(i)MIT Assay (ii)LO (iii)Phagocytosis (iv)ROS production (v)Esterase Activity	Minguez et al. (2014)
		I. obsoleta	Eastern Mudsnail	3.45 – 345 µg l^{-1}	2 Hours	(i)Foot Mediated Behaviour	Fong et al. (2017)

Table 1.4: *Continued*

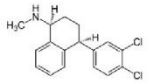
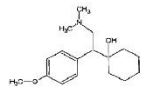
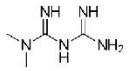
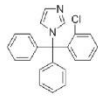
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Antidepressant	Paroxetine	*M. edulis*	Blue Mussel	0.0015 – 75 mg l^{-1}	21 Hours	(i)Haemocyte Mortality (ii)Genotoxicity	Lacaze et al. (2015)
	Sertraline 	*amphitrite*	Acorn Barnacle	0.001 – 1000 µg l^{-1}	24 - 48 Hours	(i)Mortality (ii)Immobilisation (iii)Swimming Speed	Estevez-Calvar et al. (2016)
		Brachionus plicatilis	Rotifer	0.001 – 1000 µg l^{-1}	24 - 48 Hours	(i)Mortality (ii)Immobilisation (iii)Swimming Speed	Estevez-Calvar et al. (2016)
		I. obsoleta	Eastern Mudsnail	3.45 – 345 µg l^{-1}	2 Hours	(i)Foot Mediated Behaviour	Fong et al. (2017)
		E. marinus	Malacostraca	0.001 – 1 µg l^{-1}	1 Hour – 8 Days	Sea Water Exposure	Bossus et al. (2014)
		M. galloprovincialis	Mediterranean Mussel	0.001 – 1000 µg l^{-1}	24 - 48 Hours	(i)Larval Development	Estevez-Calvar et al. (2016)
	Venlafaxine	*C. gigas*	Oyster	10^{-7} – 10^{7} µg l^{-1}	36 Hours	(i)Larval Development (ii)Metamorphosis	Di Poi et al. (2018)
		*I. obsoleta*	Eastern Mudsnail	3.45 – 345 µg l^{-1}	2 Hours	(i)Foot Mediated Behaviour	Fong et al. (2017)
		M. edulis	Blue Mussel	0.0015 - 75 mg l^{-1}	21 Hours	(i)Haemocyte Mortality (ii)Genotoxicity	Lacaze et al. (2015)
Antidiabetic	Metformin 	*M. edulis*	Blue Mussel	40 µg l^{-1}	7 Days	(i)Temperature (ii)Histology (iii)Estrogen Receptor (iv)Vitellogenin (v)Cytotoxicity	Koagouw and Ciocan (2018)
Antifungal	Clotrimazole	*Microalgal Community*		10 - 100nmol l^{-1}	4 Days	(i)SWIFT Periphyton Test	Porsbring et al. (2009)
		*Palaemon serratus*	Commn Prawn	0.25 - 10µg l^{-1}	50 Days	(i)Survival (ii)Growth Rate	González-Ortegón et al. (2013)

Table 1.4: *Continued*

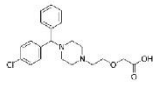
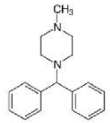
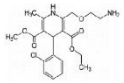
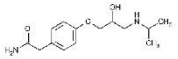
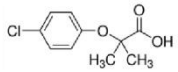
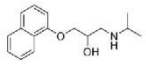
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Antihistamine	Cetrizine	*A. amphitrite*	Acorn Barnacles	1 – 10 mg l^{-1}	96 Hours	(i)Mortality (ii)Development (iii)Respiration (iv)Settlement	Al-Aidaroos et al. (2017)
		*M. galloprovincialis*	Mediterranean Mussel	0.3 – 12 µg l^{-1}	28 Days	(i)CAT (ii)SOD (iii)MET (iv)Glycogen Content (v)LPO (vi)GSH (vii)Oxidised Glutathione	Teixeira et al. (2017)
	Cyclizine	*Balanus amphrite*	Acorn Barnacle	25 µg ml^{-1}	24 Hours	(i)Mortality	Choong et al. (2006)
							
Antihypertensive	Amlodipine	*A. amphitrite*	Acorn Barnacles	1 – 5 mg l^{-1}	96 Hours	(i)Mortality (ii)Development (iii)Respiration (iv)Settlement	Al-Aidaroos et al. (2017)
							
	Atenolol	*A. amphitrite*	Acorn Barnacles	1 – 50 mg l^{-1}	96 Hours	(i)Mortality (ii)Development (iii)Respiration (iv)Settlement	Al-Aidaroos et al. (2017)
		*Sparus aurata*	Gilthead Bream	5 µg l^{-1}	28 Days	(i)Transciptomic Microarray	Hampel et al. (2017)
	Clofibric Acid	*D. tertiolecta*	Chloropycae	5 - 80mg l^{-1}	50 Days	(i)Cell Density	DeLorenzo and Fleming (2008)
		*P. serratus*	Common Prawn	26 - 1000µg l^{-1}	48 Hours	(i)Survival (ii)Growth Rate	González-Ortegón et al. (2013)
	Propranolol	*A. brevicornis*	Malacostrata	0.05 – 500 ng g^{-1}	10 Days	(i)EROD (ii)GST (iii)GPX (iv)GR (v)AChE (vi)LPO (vii)Genotoxicity	Maranho et al. (2015a)
		*Baltic Sea Community*		100 – 1000 µg l^{-1}	7 Days	(i)Community Gross Production to Respiration Ratio (ii)Respiration (iii)Mortality (iv)Ash free weight and carbon content	Oskarsson et al. (2014)
		H. diversicolor	Ragworm	0.5 - 500 ng g^{-1}	14 Days	(i) Total Protein Content (ii)EROD (iii)DBF (iv)GST (v)GPX (vi)AChE (vii)TBARS (viii)Genotoxicity	Maranho et al. (2014)

Table 1.4: *Continued*

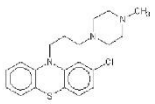
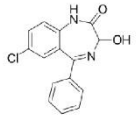
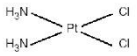
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Antihypertensive	Propranolol	*H. diversicolor*	Ragworm	0.05 – 500 ng g^{-1}	14 Days	(i)MAO (ii)COX (iii)Total Lipids (iv)MET	Maranho et al. (2015b)
		M. edulis trossulus	Bay Mussel	1 – 1000 µg l^{-1}	8-21 Days	(i)Strength and abundance of byssus threads (ii) respiration, absorption efficiency and clearance rate (iii) an energy budget (SFG) for each of the endpoints in (ii)	Ericson et al. (2010)
		M. galloprovincialis	Mediterranean Mussel	0.3 ng l^{-1}	7 Days	(i)Cyclic AMP content (ii)PKA Activity (iii)mRNA expression	Franzellitti et al. (2013)
		M. galloprovincialis	Mediterranean Mussel	0.3 ng l^{-1}	7 Days	(i)Bioaccumulation (ii)LMS (iii)GST (iv)GSH (v)CAT (v)mRNA Expression (vi)Genotoxicity	Franzellitti et al. (2015)
			Mediterranean Mussel	20 - 200 µg l^{-1}	10 Days	(i)Feeding Rate (ii)AChE Activity (iii)GST (iv)CAT (v)LPO (vi)Total Protein Content	Solé et al. (2010)
Antiemetic	Prochlorperazine 	*B. amphrite*	Acorn Barnacle	25 µg ml^{-1}	24 Hours	(i)Mortality	Choong et al. (2006)
Anxiolytic	Oxazepam 	*Rutilus rutilus*	Common Roach	1 – 300 µg l^{-1}	8 Days	(i)Behaviour	Brodin et al. (2017)
Chemotherapy Agent	Cisplatin 	*M. galloprovincialis*	Mediterranean Mussel	100 ng l^{-1}	14 Days	(i)SOD (ii)CAT (iii)GST (iv)LPO (v)AChE (vi)Genotoxicity	Trombini et al. (2016)

Table 1.4: *Continued*

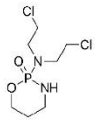
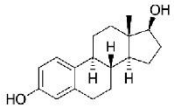
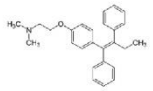
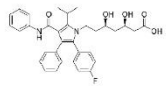
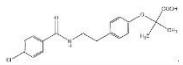
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Chemotherapy Agent	Cyclophosphamide 	*H. diversicolor*	Ragworm	10 – 1000 ng l^{-1}	14 Days	(i)CAT (ii)SOD (iii)GPx (iv)GST (v)LPO (vi)Genotoxicity	Fonseca et al. (2017)
Hormone	Estradiol 	*B. amphrite*	Acorn Barnacle	0.01 – 1 µg l^{-1}	24 Hours	(i)Total Protein	Billinghurst et al. (2000)
		Mytilus galloprovincialis	Mediterranean Mussel	25 nM	30 Minutes	(i)CAT (ii)SOD (iii)GST (iv)LPO (v)Comet Assay	Koutsogiannaki et al. (2014)
				5 – 25 pmol	24 Hours	(i)LMS (ii)Pyruvate Kinase (iii)CAT (iv)Lipid Accumulation (v)Phosphofructokinase	Canesi et al. (2007a)
	Tamoxifen 	*Pacentrotus lividus*	Purple Sea Urchin	10^{-8} – 10^{-6} M	72 Hours	(i)CAT (ii)SOD (iii)LPO	Pagano et al. (2001)
		S. aurata	Sheapshead Minnow	100 µg g^{-1}	50 Days	(i)Gene Expression (ii)ROS Production Assay (iii)IgM Levels	Rodenas et al. (2015)
		Spherechinus granularis	Purple Sea Urchin	10^{-8} – 10^{-6} M	72 Hours	(i)CAT (ii)SOD (iii)LPO	Pagano et al. (2001)
		Stronglyocentrotus purpuratus	Purple Sea Urchin	0.5 - 250 ng ml^{-1}	96 Hours	(i)Development (ii)Mortality	Roepke et al. (2005)
Lipid Lowering Agent	Atorvastatin 	*Amphibalanus amphitrite*	Acorn Barnacles	1 – 10 mg l^{-1}	96 Hours	(i)Mortality (ii)Development (iii)Respiration (iv)Settlement	Al-Aidaroos et al. (2017)
		Mytilus edulis	Blue Mussel	1.2 µg l^{-1}	11 weeks	(i)O_2 Consumption (ii)Tissue Energy Production (iii)Byssus Production (iv)P-glycoprotein activity (v)CYP (vi)GST (vii)Fatty acid metabolism	Falfushynska et al. (2019)
	Bezafibrate 	*M. galloprovincialis*	Mediterranean Mussel	2 – 20 µM	30 Minutes	(i)LMS (ii)Phagocytosis (iii)Lysosomal Enzyme Release (iv)Nitrite Production	Canesi et al. (2007b)

Table 1.4: *Continued*

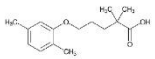
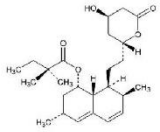
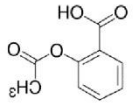
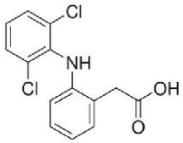
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
Lipid Lowering Agent	Gemfibrozil 	*M. galloprovincialis*	Mediterranean Mussel	2 – 20 µM	30 Minutes	(i)LMS (ii)Phagocytosis (iii)Lysosomal Enzyme Release (iv)Nitrite Production	Canesi et al. (2007b)
		S. aurata	Gilthead Bream	1.5 – 15,000 µg l^{-1}	96 Hours	(i)Swimming Behaviour (ii)GST (iii)GPx (iv)GR (v)LPO	Barreto et al. (2018)
		Mytilus spp.	Mussel Species	1 – 1000 µg l^{-1}	96 Hours	(i)Metallothionein (ii)GST (iii)LPO (iv)Genotoxicity (v)Vitellin-Like Proteins	Schmidt et al. (2011)
	Simvastatin 	*D. tertiolecta*	Chlorphycae	1.560 – 100 mg l^{-1}	96 Hours	(i)Cell Density	DeLorenzo and Fleming (2008)
		Fundulus heteroclitus	Mud Minnow	0.625 - 2.5 mg l^{-1}	96 Hours	(i)GSH (ii)LPO (iii)Ache	Key et al. (2009)
		Gammarus locusta	Malacostraca	64 – 8000 ng l^{-1}	69 Days	(i)Survival (ii)Sex Ratio (iii)Length (iv)Histology (v)Population Modelling	Neuparth et al. (2014)
		Nitocra spinipes	Harpactecoid Copepod	0.16 - 16µg l^{-1}	96 Hours	(i)Development (ii)Body Length (iii)RNA Content (iv)Growth Rate	Dahl et al. (2006)
NSAID	Acetylsalicylic Acid 	*Hediste diversicolor*	Ragworm	5 – 2000 µg l^{-1}	28 Days	(i)Histology (ii)CAT (iii)GR (iv)GPx (v)GST (vi)TBARS	Gomes et al. (2019)
	Diclofenac 	*D. tertiolecta*	Chloropycae	25 – 400 mg l^{-1}	96 Hours	(i)Cell Density	DeLorenzo and Fleming (2008)
		E. marinus	Malacostraca	0.01 – 10 µg l^{-1}	21 Days	(i)Behaviour	Guler and Ford (2010)
		M. edulis trossulus	Bay Mussel	1 - 10000 µg l^{-1}	8-21 Days	(i)Strength and abundance of byssus threads (ii) respiration, absorption efficiency and clearance rate (iii) an energy budget (SFG) for each of the endpoints in (ii)	Ericson et al. (2010)

Table 1.4: *Continued*

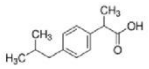
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
NSAID	Diclofenac	*M. galloprovincialis*	Mediterranean Mussel	1 – 10 µg l^{-1}	48 Hours	(i)Embryotoxicity (ii)GST (iii)SOD (iv)CAT (v)HSP (vi)Neuroendocrine Signal	Balbi et al. (2018)
				100 µg l^{-1}	7 Days	(i)Metabalomics Profile	Bonnefille et al. (2018)
				1 – 100 µg l^{-1}	72 Hours	(i)Prostaglandin Levels	Courant et al. (2018)
				2.5 µg l^{-1}	60 Days	(i)DNA Microarray (ii)CAT (iii)GST (iv)GR (v)GSH (vi)LPO (vii)AChE (viii)Acyl-CoA Oxidase	Mezzelani et al. (2018)
				250 ng l^{-1}	14 Days	(i)SOD (ii)CAT (iii)GR (iv)GST (v)LPO (vi)AChE (vii)ALP	Gonzalez-Rey and Bebianno (2014)
		Mytilus spp.	Mussel Species	1 - 1000 µg l^{-1}	96 Hours	(i)Metallothionein (ii)GST (iii)LPO (iv)Genotoxicity (v)Vitellin-Like Proteins	Schmidt et al. (2011)
		P. serratus	Common Prawn	40 - 1600 µg l^{-1}	50 Days	(i)Survival (ii)Growth Rate	González-Ortegón et al. (2013)
	Ibuprofen 	*A. brevicornis*	Malacostrata	0.05 – 500 ng g^{-1}	10 Days	(i)EROD (ii)GST (iii)GPX (iv)GR (v)AChE (vi)LPO (vii)Genotoxicity	Maranho et al. (2015a)
		C. maenas	Edible Crab	0.1 – 50 µg l^{-1}	28 Days	(i)LMS	Aguirre-Martínez et al. (2013)
		H. diversicolor	Ragworm	0.5 – 500 ng g^{-1}	14 Days	(i)MAO (ii)COX (iii)Total Lipids (iv)MET	Maranho et al. (2015b)
				0.05 – 500 ng g^{-1}	14 Days	(i) Total Protein Content (ii)EROD (iii)DBF (iv)GST (v)GPX (vi)AChE (vii)TBA (viii)Genotoxicity	Maranho et al. (2014)
		Litopenaeus spp.	Pacific White Shrmip	0.01 - 0.1 µg l^{-1}	3-5 Weeks	(i)Ovarian Maturation (ii)Spermatophore Quality	Alfaro-Montoya (2015)
		Lytechinus variegatus	Green Sea Urchin	1.5 – 1508 ng g^{-1}	24 Hours	(i)Embry-Larval Development	Pusceddu et al. (2018)
		Mytella charruana	Charru Mussel	0.02 – 1.51 ng g^{-1}	24 Hours	(i)Cytotoxicity	Pusceddu et al. (2018)

Table 1.4: *Continued*

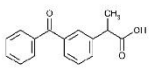
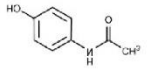
Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
NSAID	Ibuprofen	*M. edulis trossulus*	Bay Mussel	1 – 1000 µg l^{-1}	8-21 Days	(i)Strength and abundance of byssus threads (ii) respiration, absorption efficiency and clearance rate (iii) an energy budget (SFG) for each of the endpoints in (ii)	Ericson et al. (2010)
		M. galloprovincialis	Mediterranean Mussel	2.5 µg l^{-1}	60 Days	(i)DNA Microarray (ii)CAT (iii)GST (iv)GR (v)GSH (vi)LPO (vii)AChE (viii)Acyl-CoA Oxidase	Mezzelani et al. (2018)
				250 ng l^{-1}	15 Days	(i)Transcriptomic Microarray	Maria et al. (2016)
				250 ng l^{-1}	14 Days	(i)SOD (ii)CAT (iii)GR (iv)GST (v)LPO (vi)ALP	Gonzalez-Rey and Bebianno (2012)
		V. philippinarium	Japanese Cockle	100 – 1000 µg l^{-1}	1-7 Days	(i)LO (ii)AChE (iii)SOD (iv)Digestive Gland Transcriptome	Milan et al. (2013)
	Ketoprofen 	*M. galloprovincialis*	Mediterranean Mussel	2.5 µg l^{-1}	60 Days	(i)DNA Microarray (ii)CAT (iii)GST (iv)GR (v)GSH (vi)LPO (vii)AChE (viii)Acyl-CoA Oxidase	Mezzelani et al. (2018)
Pain Killer	Paracetamol 	*B. rotundiformis*	Rotifer	10 – 1000 µg l^{-1}	10 Days	(i)CYP (ii)GST	Park et al. (2018)
		C. gigas	Oyster	1 – 100 µg l^{-1}	7 Days	(i)SOD (ii)GST (iii)GR (iv)GPx (v)CYP Genes (vi)CAT (vii)Geontoxicity (viii)Glucose 6-phosphate dehydrogenase	Bebianno et al. (2017)
		M. galloprovincialis	Mediterranean Mussel	20 – 200 µg l^{-1}	10 Days	(i)Feeding Rate (ii)AChE Activity (iii)GST (iv)CAT (v)LPO (vi)Total Protein Content	Solé et al. (2010)
		R. philippinarum	Manila Clam			(i)SOD (ii)GPx (iii)GST (iv)GR (v)LPO (vi)AChE (vii)MET (viii)Glycogen Content	Nunes et al. (2017)
		S. aurata	Gilthead Bream	50 µg l^{-1}	28 Days	(i)Transciptomic Microarray	Hampel et al. (2017)
		V. decussata	Cross Cut Carpet Shell	0.05 - 0.5 mg l^{-1}	96 Hours	(i)GST, (ii)GR (iii)LPO (iv)Total Protein Content	Antunes et al. (2013)

Table 1.4: *Continued*

Class	Compound	Species	Common Name	Concentration	Duration	Endpoints	Reference
		V. philippinarium	Japanese Cockle	0.05 - 0.5 mg l^{-1}	96 Hours	(i)GST, (ii)GR (iii)LPO (iv)Total Protein Content	Antunes et al. (2013)

Acetylcholinesterase (**AChE),** Alkaline Phosphatase (**ALP**), Adenosine Triphosphate (**ATP**), Catalase (**CAT**), Cyclooxygenase (**COX**), Cytochrome P450 (**CYP**), Diphosphate-glucuronosyltransferase (**UGT**), Dubenzylfluroscein Dealkylase (**DBF**), Ethoxyresorufin-O-deethylase (**EROD**), Glutathione Reductase (**GR**), Glutathione S-Transferase (**GST**), Heat Shock Protein (**HSP**), Lipid Peroxidase (**LPO**), Lysozyme Activity (**LO**), Lysozymal Membrane Stability (**LMS**), Maltodiadelhyde (**MDA**), Mitochondrial Electron Transport (**MET**), Monoamine oxidase (**MAO**), Nitric Oxide (**NO**), Polymerase Chain Reaction (**PCR)**, Reactive Oxygen Species (**ROS**), Superoxide Dismutase (**SOD**)., Thiobarbituric acid (**TBA**)

1.3.1 Antibiotics

The greatest threat of antibiotics to aquatic ecosystems is the potential to cause antibiotic resistant bacteria and genes, which can cause the spread of antibiotic resistant infections, having implications on human and veterinary health (Cizmas et al. 2015). Antibiotics are toxic to bacteria, which can also have implications on the immunology of aquatic species (Guardiola et al. 2012). Microbes play an important role in marine ecosystems, and the presence of antibiotics could disrupt these. Antibiotics have been observed to disrupt microbial processes such as denitrification, nitrogen fixation and organic breakdown, which could have implications on water quality and aquatic health (Costanzo et al. 2005).

The literature on antibiotic toxicity has focused on antibiotic resistance, however, there is an indication that they may exert effects on aquatic organisms in different ways (Daghrir and Drogui 2013). Many bacteria have symbiotic relationships with algae, supplying them with nutrients in return for a protective environment, and inhibition of these bacteria to form biofilms as the result of antibiotic exposure could result in limited algal growth and nutrient deficiency (Guo et al. 2015). Antibiotics also cause oxidative stress in a range of species (Table 1.4). Trimethoprim and erythromycin were found to cause DNA damage in the mussels, *Mytilus edulis* and *Dreissena polymorpha* (Lacaze et al. 2015). This is in part, attributed to oxidative stress, but also to also to the ability of these drugs to interfere with DNA synthesis and replication, respectively. Oxytetracycline and amoxicillin inhibited CAT and induced GST in zebrafish at high concentrations (Oliveira et al. 2013). Oxytetracycline also caused an increase in lactate dehydrogenase (LDH) a key enzyme in energy production, and an indicator of stress (Oliveira et al. 2013).

1.3.2 Anticonvulsants

Carbamazepine is one of the most prolific pharmaceuticals in the literature. It is a psychiatric drug used to treat epilepsy, bipolar disorder, chronic nerve conditions and addiction by blocking sodium channels and reducing the firing of neurones (Jarvis et al. 2014). Due to its high consumption, low removal and long half-life, high concentrations have been found in estuaries globally (Almeida et al. 2015). It has also been found to bioaccumulate at high concentrations in bivalves, algae and crustaceans, but not cnidarian (Vernouillet et al. 2010; Almeida et al. 2015). Carbamazepine has the potential to alter behaviour leading to changes in reproduction, predator avoidance and locomotion (De Lange et al. 2006; Brandão et al. 2013; Chen et al. 2014). It reduces fecundity, breeding success, alters courtship behaviour and sperm morphology in fish (Overturf et al. 2015). In bivalves, carbamazepine caused reduction in siphoning behaviour and valve movement, which plays an important role in nutrition, defence and reproduction (Chen et al. 2014). Reduction in siphoning is a response to chemical stress which can lead to ammonia

accumulation in the tissue, reduction in oxygen and reduced feeding, and chronic exposure to carbamazepine could eventually lead to death. Low concentrations of carbamazepine also cause oxidative stress in fish and bivalves, and caused reduced LMS in the haemocytes of the crab, *Carcinus maenas* (Aguirre-Martínez et al. 2013; Brandão et al. 2013; Almeida et al. 2015). Carbamazepine caused changes of the enzymes LDH, glutamate pyruvate transamine, and glutamate oxaloacetate transamine in the gill, liver and muscle leading to tissue hypoxia and damage (Malarvizhi et al. 2012). Another anti-epileptic phenytoin caused oxidative stress in the pumpkinseed sunfish, *Lepomis gibbosus*, but did not alter behaviour (Brandão et al. 2013). Despite high concentrations of phenytoin found in estuaries (1.4 µg l^{-1}), effects on aquatic organisms is relatively unknown (Mijangos et al. 2018).

1.3.3 Antidepressants

Antidepressants, which include tricyclics, monoamine oxidise inhibitors and selective serotonin reuptake inhibitors (SSRIs), account for approximately 4% of pharmaceuticals detected in the environment (Fong and Ford 2014). The main concern surrounding antidepressants is their role as endocrine disruptors due to the alteration of serotonin and dopamine which stimulate hormone production (Fong and Ford 2014). As a result, they are often used in aquaculture to speed up growth and reproduction (Fong and Ford 2014). The chemical structure of antidepressants has several potentially mutagenic effects as DNA damage can be caused directly by the aromatic ring and/or flyorobenzene group (Lacaze et al. 2015). Serotonin and dopamine have similar metabolic pathways in aquatic invertebrates and fish to humans (Gagné et al. 2010). As a result, the side effects seen in humans such as changes in behaviour and aggression have been observed in such biota (Weinberger and Klaper 2014).

SSRIs are the most widely prescribed antidepressant (Fong and Ford 2014; Lacaze et al. 2015). They exert a therapeutic effect by inhibiting the reuptake of serotonin and therefore increasing concentrations in the body (Overturf et al. 2015). Fluoxetine has been studied most prolifically, however citalopram and venlafaxine have now surpassed fluoxetine prescriptions in the USA and Canada (Fong and Ford 2014; Lacaze et al. 2015), and citalopram is prescribed more than fluoxetine in the UK (National Health Service 2017). Serotonin plays an important role in reproduction in both vertebrates and invertebrates, and SSRIs have been found to negatively impact reproductive processes in many species (Dorelle et al. 2017). Exposure of fluoxetine (20 - 200 ng l^{-1}) to *D. polymorpha* caused decreased oocytes and spermatozoan in male and female gonads, as well as increased levels of estradiol (Lazzara et al. 2012). It also caused increased vitellogenin levels in the Mediterranean mussel, *Mytilus galloprovincialis* (Gonzalez-Rey and Bebianno 2013). In

fish, fluoxetine has caused decreased sperm production and caused aggressive behaviour (Weinberger and Klaper 2014). Fathead minnows, *Pimephales promelas*, were exposed to 1 - 100µg l^{-1} fluoxetine for four weeks, which caused changes in reproductive behaviour (Weinberger and Klaper 2014). In this species males are responsible for nest preparation and egg care; some males did not engage in reproductive behaviour, whilst others were aggressive and attacked females. Those which did mate successfully exhibited aggressive nest cleaning behaviour resulting in broken eggs. However, similar levels of the SSRI citalopram in guppies, *Poecilia reticulata*, did not induce changes in sexual behaviour (Holmberg et al. 2011).

SSRIs also cause other effects in aquatic organisms which are unrelated to the endocrine system. Serotonin controls ciliary pedal activity, pedal muscle contraction and swimming movement in gastropods (Lewis et al. 2011). Serotonin will increase these movements, which could result in altered locomotion which is vital to feeding, reproduction and predator avoidance (Estévez-Calvar et al. 2017). Exposure to SSRIs also resulted in changes in behaviour; fluoxetine also reduced learning and memory in cuttlefish, *Sepia officinalis*, at concentrations as low as 1ng l^{-1} (Di Poi et al. 2013). Fluoxetine, sertraline and venlafaxine have caused reduced predator avoidance in *P. promelas* (Painter et al. 2009). A variety of antidepressants: fluoxetine, paroxetine, amitriptyline and clomipramine cause immunotoxicity at environmentally relevant concentrations (Minguez et al. 2014). Paroxetine and fluoxetine also caused DNA strand breakage, cytotoxicity and immunotoxicity in *M. edulis* haemocytes (Lacaze et al. 2015).

The toxicity of other types of antidepressants aren't as prevalent in the literature. Tricyclics (such as amitriptyline) block serotonin and norepinephrine reuptake transporters reducing the hyperactivity of the hypothalamo-pituitary-adrenocortical axis present in depression (Yang et al. 2014). Neurotoxic side effects of tricyclics in aquatic life have been reported. Amitriptyline caused a reduction in nitric oxide (NO) production which compromised the immune system (Yang et al. 2014). The anti-inflammatory activity of amitriptyline is suggested to be associated with the inhibition of pro-inflammatory cytokines from immune cells and a decrease in NO (Yang et al. 2014).

1.3.4 Antihypertensives

There are many different anti-hypertensives including, angiotensin-converting-enzyme inhibitors, calcium channel blockers, angiotensin II receptor antagonists (sartans) and β-blockers. The most widely studied of these are β-blockers, which are the most consumed, and found in the highest concentrations in the aquatic environment (Godoy et al. 2015). A total of 34 anti-hypertensives (20 in estuaries; Appendix 1.1) have been detected globally

in aquatic ecosystems, however, ecotoxicology studies have only been conducted on 16 (4 in marine species) of these (Godoy et al. 2015). β -blockers function by binding to β-adrenergic receptors to block the binding of norepinephrine and epinephrine, resulting in decreased blood pressure (Maszkowska et al. 2014). β-blockers can either be selective, binding to a particular β-adrenergic receptor (e.g. metoprolol and atenolol) or non-selective (e.g. propranolol; Massarsky et al. 2011). β-adrenergic receptors are present in mussels and vertebrates, but not in crustaceans or echinoderms, however deleterious effects have been observed in all of these groups (Franzellitti et al. 2013).

There is debate in the literature as to the toxicity of β-blockers, with a few reaching the consensus that environmental concentrations of β-blockers do not pose a significant risk to aquatic life (Winter et al. 2008, Godoy et al. 2015). Exposure to propranolol at environmentally relevant concentrations (0.3 – 500 ng l^{-1}) caused oxidative stress and disrupted cell signalling in *M. galloprovincialis* (Solé et al. 2010, Franzellitti et al. 2013) and ragworm, *Hediste diversicolor* (Maranho et al. 2014). Sun et al. (2015) looked at the regulation of genes involved in antioxidant and detoxification responses in zebrafish to propranolol and metoprolol. Responses were not significant below 3mg l^{-1}, which is far above concentrations found in the freshwater (Hughes et al. 2013), estuaries (Appendix 1.1) or oceans (Gaw et al. 2014). β-blockers appear to have the potential to disrupt reproductive function, and affect early life stages, which could have implications at a population level. It is thought that β-adrenergic receptors may play a role in larval metamorphosis in bivalves, and as a result β-blockers could have an effect on this (Solé et al. 2010). Medaka exposed to propranolol, metoprolol and nadolol produced less viable embryos after 4 weeks, however no significant difference from the control was seen at 2 weeks (Huggett et al. 2002). In the same study, these drugs caused reproductive effects to *Daphnia magna, Hyalella azteca* and *Ceriodaphnia dubia,* however, due to the high concentrations at which these effects were observed, it is unlikely concentrations currently observed in the environment would have significant impacts on populations. Norepinephrine also plays a role in stimulating or inhibiting hormones and β-blockers have been found to decrease testosterone and luteinising hormone in fish indicating their potential as endocrine disruptors (Massarsky et al. 2011; Godoy et al. 2015).

Interestingly, propranolol is one of the few pharmaceuticals to be involved in mesocosm experiments studying inter-species dynamics in the presence of this drug. Oskarsson et al. (2014) exposed a model Baltic Sea community composed of macroalgae, mussels and amphipods to 100 and 1000µg l^{-1} propranolol. Mussels were the most sensitive, which led to a feeding shift from the algae to the mussel by the amphipod. The amphipods did not suffer negative effects and it was thought that the higher nutritious food may have

counteracted this. This shift was beneficial in turn to algae as they were no longer consumed.

Ecotoxicology studies on other types of anti-hypertensives are not as common. Calcium channel blockers block L-type calcium channels preventing the influx of calcium ions into the vascular system, reducing myocardial contractions and vascular relaxation, resulting in reduced blood pressure (Palande et al. 2015). There is evidence that the mode of action (MoA) of calcium channel blockers in fish is similar to that in humans; verapamil caused a reduced heart rate in common carp (*Cyprinus carpio*) embryos and larvae (Steinbach et al. 2013). At high levels, calcium channel blockers can cause toxicity to other organs such as kidneys due to difficulty in metabolising this drug. These drugs also block neuronal calcium channels resulting in altered behaviour. Exposure of goldfish *(Carassius auratus*) to verapamil caused loss of balance, increased ocular movement, increased swimming rate and caused capsizing (Palande et al. 2015). It has been suggested that calcium channel blockers may also impact K^+ and Na^+ channels, which would negatively affect the osmoregulatory capacity of fish (Palande et al. 2015). Verapamil caused pericardial oedemas in carp embryos, which is often indicative of osmoregulatory disruption (Steinbach et al. 2013). However, this was only seen at concentrations much higher than those found in the environment.

1.3.5 Lipid Lowering Agents

There are two types of lipid lowering medications: fibrates and statins. Fibrates are the most targeted for analytical and ecotoxicological studies (Fent et al. 2006, Overturf et al. 2012). Statins lower blood plasma lipids, whilst fibrates lower both lipids and triglycerides (Fent et al. 2006). Fibrates bind to peroxisome proliferator-activated receptors which cause them to stimulate fatty acid uptake and regulate the expression of several lipid regulatory proteins (Canesi et al. 2007b). In fish, steroid hormones are derived from cholesterol, and it's reduction caused by fibrates can disrupt steroidogenesis and spermatogenesis (Velasco-Santamaría et al. 2011). Gemfibrozil causes reduced growth and in turn lower fecundity, altered reproductive behaviour and sperm morphology leading to reduced reproductive success in fish (Overturf et al. 2015). *D. rerio* exposed to Bezafibrate altered the expression of the testis gene, suggesting it also had an effect on reproduction (Velasco-Santamaria et al. 2011). Bezafibrate had no effect on lowering cholesterol levels in *P. promelas*, however it's metabolite Clofibric acid increased the activity of fatty acetyl-coenzyme-A which plays a role in the oxidation of fatty acids (Weston et al. 2009). Clofibric acid also reduced egg production. Fibrates appear to negatively impact the immune system of some organisms. Bezafibrate and gemfibrozil injected into *M. galloprovincialis* haemocytes caused lysosomal destabilisation, NO production and decreased phagocytic

activity (Canesi et al. 2007b). Fibrates also affected the haemocyte function of freshwater bivalve *Elliptio compalnata* (Gagné et al. 2006).

Statins block mevalonic acid pathways thereby inhibiting the synthesis of cholesterol (Ellesat et al. 2010). Atorvastatin and simvastatin are prodrugs which are inactive, and are metabolised by the body into the active compound, as a result, it is their metabolites which pose the largest risk (Besse and Garric 2008). To date exposure experiments have included the parent compound, and it not known if they will be metabolised in the same way in non-target organisms. Despite this, exposure of some species to statins have resulted in deleterious effects. Atorvastatin caused upregulation of genes involved in membrane transport, oxidative stress, apoptosis and biotransformation at concentrations as low as 200ng l^{-1} in *O.mykiss* (Ellesat et al. 2012). These effects were observed in the gill, but not the liver despite this being a target organ of statins in humans. This is likely due to cholesterol levels being highest in fish gills. In humans, statin toxicity includes inhibition of membrane transport, however, Ellesat et al. (2012) did not observe any change in Na^+/K^+ -ATPase. Statins also caused impairment to reproduction. Simvastatin negatively affected reproduction of *Gammarus locusta* by disrupting the hormone methyl farnesoate (MF) and causing reduced gonadal development at concentrations as low as 320ng l^{-1} (Neuparth et al. 2014). MF is an important hormone in crustaceans and is responsible for reproductive maturation by increasing vitellogenin and stimulating gonadal growth.

1.3.6 Analgesics

1.3.6.1 NSAIDs

There are many different types of analgesics, which can be broadly split into two categories: NSAIDs and painkillers (Overturf et al. 2015). These are among the most prolifically used pharmaceuticals as they are widely prescribed and readily available OTC (Fent et al. 2006). Ibuprofen and diclofenac, are the most commonly used and studied NSAIDs (Table 1.4). NSAIDs reduce pain and inflammation by inhibiting the production of prostaglandins at the site of an injury, which are produced through the oxidation of arachidonic acid by cyclooxygenases (COX), resulting in pain and inflammation (Gan 2010). There are two isoforms of this enzyme: COX I and COX II, which are non-selectively inhibited by NSAIDs (Gravel et al. 2009). Prostaglandins are involved in other physiological processes, including thermoregulation, ovulation, sexual behaviour, homeostasis, ion transport and kidney filtration (Miller 2006). COX I is responsible for the baseline levels of prostaglandins involved in these processes, whilst COX II produces prostaglandins at the point of a stimulus such as an injury (Gan 2010). Prostaglandin function is similar in fish

to other vertebrates, but they are also found in cnidarian, bivalves and crustaceans (Ruggeri and Thoroughgood 1985; Courant et al. 2017). As both of these isoforms are inhibited by NSAIDs, there is a potential that these physiological processes could be disrupted. NSAIDs have caused toxicity in the liver and kidneys in humans, and similar toxic effects have been seen in fish (Triebskorn et al. 2004).

Diclofenac induced Acetylcholinesterase (AChE) activity in the gills of the *M. galloprovincialis* (Gonzalez-Rey and Bebianno 2014). AChE is released after cell membrane disruption, causing apoptosis and plays a role in the neuromuscular system by preventing continuous muscle contraction (Milan et al. 2013). This indicated the potential of NSAIDs to cause apoptosis. In humans, they have been studied as a candidate for cancer prevention as they have the potential to enhance cell proliferation and enhance apoptosis (Milan et al. 2013). This could be problematic in aquatic organisms and have the potential to cause neurotoxic effects. In some species, AChE activity has been linked to the disruption of estrogenic receptors, however, the endocrine disruption of NSAIDs needs to be further investigated (Gonzalez-Rey and Bebianno 2014). Experimental exposure to NSAIDs has led to altered reproduction. Ibuprofen caused increased ovary maturation in female marine shrimp (*Litopenaeus spp.*) and lowered sperm abnormalities in males (Alfaro-Montoya 2015). Whilst this could be beneficial in aquaculture, in the natural environment this could lead to poor fecundity and decreased reproductive success. Ibuprofen (<100µg l^{-1}) altered reproductive timing in medaka, *Oryzuas latipes* (Flippin et al. 2007); exposure for six weeks increased fecundity but decreased the amount of spawning events. Osmoregulatory processes are important for physiology of marine and estuarine species, and there is indication that this could be interrupted by NSAIDs. Diclofenac disrupted osmoregulation in brown trout at 1g l^{-1} and at more environmentally realistic concentrations (10ng l^{-1}) in *C. maenas* (Eades and Waring 2010). Ibuprofen and salicylic acid have also reduced osmoregulation capability in *O. mykiss* at concentrations (1 mg l^{-1}) higher than those found in the environment (Gravel et al. 2009).

1.3.6.2 Pain Killers

Paracetamol also inhibits prostaglandin synthesis, but at a central nervous system level and it also blocks pain impulses, however, doesn't have the same anti-inflammatory properties as NSAIDs (Ouellet and Percival 2001). The exact pathways of paracetamol are poorly understood. There is some evidence in vertebrates that there may be a third isoform of cyclooxygenase, COX III, which may be inhibited and account for differences in effects between paracetamol and NSAIDs (Chandrasekharan et al. 2002). However, it has been debated whether it is a target of paracetamol and it is not known if this isoform exists in aquatic species (Schwab et al. 2003; Graham et al. 2013). In humans, paracetamol is

metabolised in the liver, with high concentrations causing hepatotoxicity, and there is evidence that this may occur in fish as well (Graham et al. 2013; Guiloski et al. 2017). Paracetamol caused neurotoxicity in the freshwater shrimp, *Neocaridina denticulata* and planarian, *Dugesia japonica* (Wu and Li 2015). Paracetamol inhibited two enzymes, which are essential for normal neurological function: Cholinesterase and monoamine oxidase (MAO). It has also been found to indirectly inhibit Na^+/K^+ ATPase in the brain as the result of oxidative stress (Wu and Li 2015). Paracetamol also caused oxidative stressed in the European eel, *Anguilla anguilla*) which led to the inhibition of AChE (Nunes et al. 2015). A transcriptome analysis of gilthead bream (*Sparus aurata*) brains has shown that paracetamol may alter processes involved in gene regulation and DNA repair and may disrupt development of embryos (Hampel et al. 2017). There is also some evidence that paracetamol could impact reproductive processes in organisms; it reduced testosterone levels and inhibited spermatogenesis in male fish *(Rhamdia quelen*), at environmentally relevant concentration (>250 ng l^{-1}), however, there is currently not any further evidence in these effects in other organisms (Guiloski et al. 2017).

Opiates are another type of painkiller which have the potential to impact non-target organisms. Low levels of codeine, hydrocodone and tramadol have been found in estuaries (Benotti and Brownawell 2007; Klosterhaus et al. 2013; Birch et al. 2015; Sun et al. 2016,; Munro et al. 2019). Despite the high prescription rates and occurrence of opiates in aquatic systems, marine ecotoxicology studies are largely absent (Hughes et al. 2013; Rosi-Marshall et al. 2015). Opiates bind to opiate receptors causing the release of dopamine and reduction of serotonin which lessen pain (Gagné et al. 2010). It is likely that they would have an effect on reproduction in bivalves as dopamine plays a role oogenesis and serotonin in the maturation of gametes and spawning (Gagné et al. 2010). Morphine compromised the immune system of the freshwater mussel, *Mytilus Elliptio*, through the production of NO which downregulates immunocyte activity and inhibits phagocytosis (Gagné et al. 2006).

1.3.7 Other compound classes

The amount of pharmaceuticals with the potential to enter the marine environment is too numerous to be completely covered in this review. There are some pharmaceuticals which exist in current literature, and are relevant to this thesis, which are not included in the above sections. Cimetidine and ranitidine are H2 receptor antagonists which inhibit the action of histamine at this site (Bergheim et al. 2012). As a result, these drugs inhibit acid production and are used to treat gastric ulcers and acid reflux (Bergheim et al. 2012). In humans, H2 receptors are also present in the brain, and have side effects affecting the nervous and endocrine systems (Fent et al. 2006). Some fish, such as *C. carpio* and cod

(*Gadus spp.*) have H2 receptors, whilst others such as *D. rerio* have H3 receptors (Fent et al. 2006). Toxicity data of cimetidine and ranitidine on freshwater or marine organisms mostly focuses on acute mortality, which is low (Isidori et al. 2009). Despite this, these pharmaceuticals have been highlighted as a potential of concern due to their high excretion rates, low removal from WWTPs and chronic presence in freshwater systems (Bergheim et al. 2012). Experimental exposures of ranitidine and cimetidine have caused a decrease in testosterone in males and intersex embryos in *D. rerio* exposed to low levels (298.25 ng l^{-1}) of cimetidine (Lee et al. 2015). In humans cimetidine caused increases of luteinising hormone and follicle stimulating, but this was not seen in female *D. rerio* at such low levels (Lee et al. 2015). Ranitidine has effects on the endocrine systems of non-aquatic species; it lowered testosterone levels in rats and has the side effect of sexual dysfunction in humans (Lee et al. 2015). Histamines supress cellular immune response, and exposure of fish to cimetidine improved immune system function (Hosseinifard et al. 2013). As a result, it has been proposed that cimetidine should be used in aquaculture to prevent disease (Hosseinifard et al. 2013).

Metformin was the eleventh most prescribed drug in 2014 and in the top 20 in the preceding five years (National Health Service, 2017). Few studies have monitored this compound in the aquatic environment, however, it has been found at concentrations above 500 ng l^{-1} in freshwater (Burns et al. 2018) and estuaries (Meador et al. 2016). Metformin is an antidiabetic drug also used in the treatment polycystic ovarian syndrome (PCOS) and cancer. Metformin primarily reduces glucose output in the liver, and secondary to this, stimulate glucose uptake in peripheral tissues (Joshi 2005). It's MoA in patients with PCOS is poorly understood, but thought to be effective as insulin resistance is commonly experienced alongside PCOS (Sivalingam et al. 2014). It has the potential as an endocrine disruptor, and caused increased levels of vitellogenin in male *P. promelas*, but not testosterone (Niemuth and Klaper 2015). It is thought that this is not due to the ability of the drug to bind hormone receptors, but the indirect disruption of steroidogenesis caused by the alteration of insulin signalling (Niemuth and Klaper 2015). Further evidence that metformin could increase vitellogenin has been seen in *M. edulis*, however the cause of this is unknown, and further research into its MoA is needed (Sumpter et al. 2016, Koagouw and Ciocan 2018).

1.4 Aims and Objectives

Pharmaceuticals are occurring in the environment, and many appear to be biologically active. This review highlights the gaps in the knowledge surrounding the spatio-temporal

distributions of pharmaceuticals in estuaries. Additionally, little is known about the MoA of these drugs to non-target organisms, and their effects at environmentally relevant concentrations. As a result, it is difficult to get an overall picture of the problem of pharmaceutical pollution. The key questions which guided this thesis were:

1. Based on existing knowledge on the occurrence, fate and effects of pharmaceuticals in the aquatic environment, which compounds pose the greatest risk to the aquatic environment?
2. At what concentrations are pharmaceuticals occurring in estuaries, and do these differ spatially and temporally?
3. What are the effects of pharmaceuticals on non-target organisms?

The primary aim of this thesis was to develop a deeper understanding of the occurrence and effects of pharmaceuticals in estuaries. The specific objectives were to:

1. Explore the efficacy of prioritisation schemes used in the literature to predict the occurrence and toxicity of pharmaceuticals in the aquatic environment, through the comparison of these schemes using one dataset.
2. Create a list of priority pharmaceuticals that pose a risk to the aquatic environment.
3. Quantify the spatial and temporal occurrence of five pharmaceuticals in an estuary, and determine if they are representative of other geographical areas.
4. Examine the biological effects of pharmaceutical exposures to *H. diversicolor*, through controlled experimental exposures and use of quantitative qPCR-based assays to determine expression of targeted genes.

It is anticipated that this thesis will contribute to the sparse data on the presence of pharmaceuticals in estuaries and provide valuable insight into the patterns in their occurrence. It will also provide novel information on the effects of these contaminants to an understudied, yet ecologically important estuarine species.

1.5 Thesis Outline

A suite of methodologies were employed in order to address the aims and objectives detailed in section 1.4. The rest of this thesis contains the results of this research organised into three manuscripts, and final discussion chapter which considers the work as a whole. A summary of each chapter is described below:

- **Chapter 2**: **Method development for the analysis of pharmaceuticals in environmental samples**

This chapter outlines the rationale behind selecting the pharmaceuticals used in monitoring (chapter 4) and ecotoxicological studies (chapter 5). It also details the methodological development in the preparation of environmental samples for analysis.

- **Chapter 3: Comparison of prioritisation schemes for human pharmaceuticals in the aquatic environment**

  In this chapter, prioritisation schemes commonly used in the literature were carried out on the fifty most prescribed drugs in the UK, and their resulting rankings were compared in order to explore their efficacy. These schemes highlighted a number of priority compounds which warrant further study and may be of interest to regulators.

- **Chapter 4: Spatial and temporal occurrence of pharmaceuticals in UK estuaries**

  In this chapter, five pharmaceuticals – ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram were measured in the surface water of the Humber Estuary every other month over a twelve month period. In order to put the concentrations seen in the Humber Estuary into context, water samples from eleven further estuaries were analysed for the presence of these target compounds.

- **Chapter 5: Effects of metformin and diclofenac on the ragworm, *Hediste diversicolor***

  Two target genes ATP synthase (*ATPS*) and c-amp activated protein kinase (*AMPK)* were isolated from *H. diversicolor*, which had been experimentally exposed to metformin, diclofenac, or a control. Quantitative qPCR assays were optimised and carried out in order to determine differences in expression between these treatments.

- **Chapter 6: Discussion**

  The results obtained from the preceding chapters were considered within the context of the original research questions (section 1.4) which guided this thesis.

1.6 References

Adler NE, Koschorreck J, Rechenberg B (2008) Environmental impact assessment and control of pharmaceuticals: The role of environmental agencies. Wat Sci Technol 57: 91–97. doi: 10.2166/wst.2008.816

Aguirre-Martínez GV, Buratti S, Fabbri E, Del Valls TA, Martín-Díaz ML (2013) Stability of lysosomal membrane in Carcinus maenas acts as a biomarker of exposure to pharmaceuticals. Environ Monit and Assess 185: 3783–3793. doi: 10.1007/s10661-012-2827-2

Al-Aidaroos AM, Satheesh S, Devassy RP (2017) Effects of pharmacological compounds on the barnacle larval development, metabolism and settlement. Inter Biodeterior Biodegradation 117: 190–196. doi: 10.1016/J.IBIOD.2017.01.003

Al-Khazrajy OSA, Boxall ABA (2016) Impacts of compound properties and sediment characteristics on the sorption behaviour of pharmaceuticals in aquatic systems. J Hazard Mat. doi: 10.1016/j.jhazmat.2016.05.065

Alfaro-Montoya J (2015) The effect of Ibuprofen on female and male reproduction of the open thelyca marine shrimp, Litopenaeus. Aquacult Res 46: 105–116. doi: 10.1111/are.12163

Almeida Â, Freitas R, Calisto V, Esteves VI, Schneider RJ, Soares AMVM, Figueira E (2015) Chronic toxicity of the antiepileptic carbamazepine on the clam Ruditapes philippinarum. Comp Biochem Physiol C Toxicol Pharmacol 172–173: 26–35. doi: 10.1016/j.cbpc.2015.04.004

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL (2010) Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29: 730–741. doi: 10.1002/etc.34

Antunes SC, Freitas R, Figueira E, Gonçalves F, Nunes B (2013) Biochemical effects of acetaminophen in aquatic species: Edible clams Venerupis decussata and Venerupis philippinarum. Environ Sci Pollut R 20: 6658–6666. doi: 10.1007/s11356-013-1784-9

Ashton D, Hilton M, Thomas K V. (2004) Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Sci Total Environ 333: 167–184. doi: 10.1016/j.scitotenv.2004.04.062

Backhaus T (2014) Medicines, shaken and stirred: a critical review on the ecotoxicology

of pharmaceutical mixtures. Philos Trans R Soc Lond B Biol Sci 369(1656): 20130585–20130585. doi: 10.1098/rstb.2013.0585

Backhaus T, Faust M (2012) Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ Sci Technol 46: 2564–2573. doi: 10.1021/es2034125

Balbi T, Montagna M, Fabbri R, Carbone C, Franzellitti S, Fabbri E, Canesi L (2018) Diclofenac affects early embryo development in the marine bivalve Mytilus galloprovincialis. Sci Total Environ 642: 601–609. doi: 10.1016/J.SCITOTENV.2018.06.125

Banni M, Sforzini S, Franzellitti S, Oliveri C, Viarengo A, Fabbri E (2015) Molecular and Cellular Effects Induced in Mytilus galloprovincialis Treated with Oxytetracycline at Different Temperatures. PLoS One 10(6): e0128468. doi: 10.1371/journal.pone.0128468

Bannister R, Beresford N, Granger DW, Pounds NA, Rand-Weaver M, White R, Jobling S, Routledge EJ (2013) No substantial changes in estrogen receptor and estrogen-related receptor orthologue gene transcription in Marisa cornuarietis exposed to estrogenic chemicals. Aquat Toxicol. 140-141: 19-26 doi: 10.1016/j.aquatox.2013.05.002

Barreto A, Luis LG, Paíga P, Santos LHMLM, Delerue-Matos C, Soares AMVM, Hylland K, Loureiro S, Oliveira M (2018) A multibiomarker approach highlights effects induced by the human pharmaceutical gemfibrozil to gilthead seabream Sparus aurata. Aquat Toxicol 200: 266–274. doi: 10.1016/J.AQUATOX.2018.05.012

Bebianno MJ, Mello ACP, Serrano MAS, Flores-Nunes F, Mattos JJ, Zacchi FL, Piazza CE, Siebert MN, Piazza RS, Gomes CHAM, Melo CMR, Bainy ACD (2017) Transcriptional and cellular effects of paracetamol in the oyster Crassostrea gigas. Ecotox Environ Safe 144: 258–267 doi: 10.1016/J.ECOENV.2017.06.034

Benotti MJ, Brownawell BJ (2007) Distributions of pharmaceuticals in an urban estuary during both dry- and wet-weather conditions. Environ Sci Technol 41: 5795–5802. doi: 10.1021/es0629965

Benstead RS, Baynes A, Casey D, Routledge EJ, Jobling S (2011) 17β-Oestradiol may prolong reproduction in seasonally breeding freshwater gastropod molluscs. Aquat Toxicol. 101(2): 326-334 doi: 10.1016/J.AQUATOX.2010.11.005

Beretta M, Britto V, Tavares TM, da Silva SMT, Pletsch AL (2014) Occurrence of

pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. J Soils Sediments 14: 1278–1286. doi: 10.1007/s11368-014-0884-6

Bergheim M, Gieré R, Kümmerer K (2012) Biodegradability and ecotoxicitiy of tramadol, ranitidine, and their photoderivatives in the aquatic environment. Environ Sci Pollut R 19: 72–85. doi: 10.1007/s11356-011-0536-y

Besse JP, Garric J (2008) Human pharmaceuticals in surface waters. Implementation of a prioritization methodology and application to the French situation. Toxicol Lett 176: 104–123. doi: 10.1016/j.toxlet.2007.10.012

Bidel F, Di Poi C, Imarazene B, Koueta N, Budzinski H, Van Delft P, Bellanger C, Jozet-Alves C (2016) Pre-hatching fluoxetine-induced neurochemical, neurodevelopmental, and immunological changes in newly hatched cuttlefish. Environ Sci Pollut R 23: 5030–5045. doi: 10.1007/s11356-015-4591-7

Billinghurst Z, Clare AS, Matsumura K, Depledge MH (2000) Induction of cypris major protein in barnacle larvae by exposure to 4-n-nonylphenol and 17β-oestradiol. Aquat Toxicol 47: 203–212. doi: 10.1016/S0166-445X(99)00018-1

Birch GF, Drage DS, Thompson K, Eaglesham G, Mueller JF (2015) Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary, Australia. Mar Pollut Bull 97: 56–66. doi: 10.1016/J.MARPOLBUL.2015.06.038

Bonnefille B, Gomez E, Alali M, Rosain D, Fenet H, Courant F (2018) Metabolomics assessment of the effects of diclofenac exposure on Mytilus galloprovincialis: Potential effects on osmoregulation and reproduction. Sci Total Environ 613–614: 611–618. doi: 10.1016/J.SCITOTENV.2017.09.146

Boreen AL, Arnold WA, McNeill K (2003) Photodegradation of pharmaceuticals in the aquatic environment: A review. Aquat Sci 65: 320–341. doi: 10.1007/s00027-003-0672-7

Bossus MC, Guler YZ, Short SJ, Morrison ER, Ford AT (2014) Behavioural and transcriptional changes in the amphipod Echinogammarus marinus exposed to two antidepressants, fluoxetine and sertraline. Aquat Toxicol 151: 46–56. doi: 10.1016/j.aquatox.2013.11.025

Bound JP, Voulvoulis N (2005) Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environ Health Perspect 113: 1705–

1711. doi: 10.1289/ehp.8315

Bound JP, Voulvoulis N (2006) Predicted and measured concentrations for selected pharmaceuticals in UK rivers: Implications for risk assessment. Wat Res 40: 2885–2892. doi: 10.1016/j.watres.2006.05.036

Brandão FP, Rodrigues S, Castro BB, Gonçalves F, Antunes SC, Nunes B (2013) Short-term effects of neuroactive pharmaceutical drugs on a fish species: Biochemical and behavioural effects. Aquat Toxicol 144–145: 218–229. doi: 10.1016/j.aquatox.2013.10.005

Brodin T, Nordling J, Lagesson A, Klaminder J, Hellström G, Christensen B, Fick J (2017) Environmental relevant levels of a benzodiazepine (oxazepam) alters important behavioral traits in a common planktivorous fish, (*Rutilus rutilus*). J Toxicol Environ Health A 80: 963–970. doi: 10.1080/15287394.2017.1352214

Brown AR, Gunnarsson L, Kristiansson E, Tyler CR (2014) Assessing variation in the potential susceptibility of fish to pharmaceuticals, considering evolutionary differences in their physiology and ecology. Philos Trans R Soc Lond B Biol Sci 369 (1656): 1-11 doi: 10.1098/rstb.2013.0576

Bu Q, Wang B, Huang J, Deng S, Yu G (2013) Pharmaceuticals and personal care products in the aquatic environment in China: A review. J Hazard Mat 262: 189–211. doi: 10.1016/j.jhazmat.2013.08.040

Burns EE, Thomas-Oates J, Kolpin DW, Furlong ET, Boxall ABA (2017) Are exposure predictions, used for the prioritization of pharmaceuticals in the environment, fit for purpose? Environ Toxicol Chem 36: 2823–2832. doi: 10.1002/etc.3842

Burns EE, Carter LJ, Kolpin DW, Thomas-Oates J, Boxall ABA (2018) Temporal and spatial variation in pharmaceutical concentrations in an urban river system. Wat Res. doi: 10.1016/j.watres.2018.02.066

Caldwell DJ, Mastrocco F, Margiotta-Casaluci L, Brooks BW (2014) An integrated approach for prioritizing pharmaceuticals found in the environment for risk assessment, monitoring and advanced research. Chemosphere 115: 4–12. doi: 10.1016/j.chemosphere.2014.01.021

Canesi L, Borghi C, Fabbri R, Ciacci C, Lorusso LC, Gallo G, Vergani L (2007a) Effects of 17β-estradiol on mussel digestive gland. Gen Comp Endocrinol 153: 40–46. doi: 10.1016/J.YGCEN.2007.02.005

Canesi L, Lorusso LC, Ciacci C, Betti M, Regoli F, Poiana G, Gallo G, Marcomini A (2007b) Effects of blood lipid lowering pharmaceuticals (bezafibrate and gemfibrozil) on immune and digestive gland functions of the bivalve mollusc, Mytilus galloprovincialis. Chemosphere 69: 994–1002. doi: 10.1016/j.chemosphere.2007.04.085

Cantwell MG, Katz DR, Sullivan JC, Ho K, Burgess RM, Cashman M (2016) Selected pharmaceuticals entering an estuary: Concentrations, temporal trends, partitioning, and fluxes. Environmen Toxicol Chem 35: 2665–2673. doi: 10.1002/etc.3452

Cantwell MG, Katz DR, Sullivan JC, Ho K, Burgess RM (2017) Temporal and spatial behavior of pharmaceuticals in Narragansett Bay, Rhode Island, United States. Environmen Toxicol Chem. doi: 10.1002/etc.3710

Cantwell MG, Katz DR, Sullivan JC, Shapley D, Lipscomb J, Epstein J, Juhl AR, Knudson C, O'Mullan GD (2018) Spatial patterns of pharmaceuticals and wastewater tracers in the Hudson River Estuary. Wat Res 137: 335–343. doi: 10.1016/j.watres.2017.12.044

Capleton AC, Courage C, Rumsby P, Holmes P, Stutt E, Boxall ABA, Levy LS (2006) Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. Toxicol Lett 163: 213–223. doi: 10.1016/j.toxlet.2005.10.023

Chandrasekharan N V, Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS, Simmons DL (2002) COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci USA 99: 13926–31. doi: 10.1073/pnas.162468699

Chen Z, Wang H, Ren N, Cui M, Nie S, Hu D (2011) Simultaneous removal and evaluation of organic substrates and NH3-N by a novel combined process in treating chemical synthesis-based pharmaceutical wastewater. J Hazard Mat 197: 49–59. doi: 10.1016/j.jhazmat.2011.09.053

Chen H, Zha J, Liang X, Li J, Wang Z (2014) Effects of the human antiepileptic drug carbamazepine on the behavior, biomarkers, and heat shock proteins in the Asian clam Corbicula fluminea. Aquat Toxicol 155: 1–8. doi: 10.1016/j.aquatox.2014.06.001

Chen H, Liu S, Xu XR, Zhou GJ, Liu SS, Yue WZ, Sun KF, Ying GG (2015) Antibiotics in the coastal environment of the Hailing Bay region, South China Sea: Spatial distribution, source analysis and ecological risks. Mar Pollut Bull 95: 365–373. doi: 10.1016/j.marpolbul.2015.04.025

Choong AMF, Teo SL-M, Leow JL, Koh HL, Ho PCL (2006) A preliminary ecotoxicity study

of pharmaceuticals in the marine environment. J Toxicol Environ Health A 69: 1959–1970. doi: 10.1080/15287390600751371

Ciocan CM, Cubero-Leon E, Puinean AM, Hill EM, Minier C, Osada M, Fenlon K, Rotchell JM (2010) Effects of estrogen exposure in mussels, *Mytilus edulis*, at different stages of gametogenesis. Environ Pollut. 158: 2977-2984 doi: 10.1016/J.ENVPOL.2010.05.025

Cizmas L, Sharma VK, Gray CM, McDonald TJ (2015) Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. Environ Chem Lett 13: 381–394. doi: 10.1007/s10311-015-0524-4

Correia AT, Nunes B, Rodrigues S, Golovko O, Antunes SC, Žlábek V (2018) Assessment of toxic effects of the antibiotic erythromycin on the marine fish gilthead seabream (Sparus aurata L.) by a multi-biomarker approach. Chemosphere 216: 234–247. doi: 10.1016/j.chemosphere.2018.10.124

Costanzo SD, Murby J, Bates J (2005) Ecosystem response to antibiotics entering the aquatic environment. Mar Pollut Bull 51: 218–223. doi: 10.1016/J.MARPOLBUL.2004.10.038

Courant F, Arpin-Pont L, Bonnefille B, Vacher S, Picot-Groz M, Gomez E, Fenet H (2017) Exposure of marine mussels to diclofenac: modulation of prostaglandin biosynthesis. Environ Sci Pollut R. 1–8. doi: 10.1007/s11356-017-9228-6

Courant F, Arpin-Pont L, Bonnefille B, Vacher S, Picot-Groz M, Gomez E, Fenet H (2018) Exposure of marine mussels to diclofenac: modulation of prostaglandin biosynthesis. Environ Sci Pollut R 25: 6087–6094. doi: 10.1007/s11356-017-9228-6

Crane M, Watts C, Boucard T (2006) Chronic aquatic environmental risks from exposure to human pharmaceuticals. Sci Total Environ 367: 23–41. doi: 10.1016/j.scitotenv.2006.04.010

Cui Y, Wang Y, Pan C, Li R, Xue R, Guo J, Zhang R (2019) Spatiotemporal distributions, source apportionment and potential risks of 15 pharmaceuticals and personal care products (PPCPs) in Qinzhou Bay, South China. Mar Pollut Bull 141: 104–111. doi: 10.1016/j.marpolbul.2019.02.012

Cuong DM, Kim K-W, Toan TQ, Phu TD (2011) Review: Source, Fate, Toxicological Effect and Removal Technology of Pharmaceuticals in the Environment. Geosystem Engineering 14: 35–42. doi: 10.1080/12269328.2011.10541328

Daghrir R, Drogui P (2013) Tetracycline antibiotics in the environment: A review. Environ Chem Lett 11: 209–227. doi: 10.1007/s10311-013-0404-8

Dahl U, Gorokhova E, Breitholtz M (2006) Application of growth-related sublethal endpoints in ecotoxicological assessments using a harpacticoid copepod. Aquat Toxicol 77: 433–438. doi: 10.1016/j.aquatox.2006.01.014

Daouk S, Chèvre N, Vernaz N, Bonnabry P, Dayer P, Daali Y, Fleury-Souverain S (2015) Prioritization methodology for the monitoring of active pharmaceutical ingredients in hospital effluents. J Environ Manage 160: 324–32. doi: 10.1016/j.jenvman.2015.06.037

DeLorenzo ME, Fleming J (2008) Individual and mixture effects of selected pharmaceuticals and personal care products on the marine phytoplankton species Dunaliella tertiolecta. Arch Environ Contam Toxicol 54: 203–210. doi: 10.1007/s00244-007-9032-2

Di Poi C, Darmaillacq AS, Dickel L, Boulouard M, Bellanger C (2013) Effects of perinatal exposure to waterborne fluoxetine on memory processing in the cuttlefish Sepia officinalis. Aquat Toxicol 132–133: 84–91. doi: 10.1016/j.aquatox.2013.02.004

Di Poi C, Evariste L, Séguin A, Mottier A, Pedelucq J, Lebel J-M, Serpentini A, Budzinski H, Costil K (2016) Sub-chronic exposure to fluoxetine in juvenile oysters (Crassostrea gigas): uptake and biological effects. Environ Sci Pollut R 23: 5002–5018. doi: 10.1007/s11356-014-3702-1

Di Poi C, Costil K, Bouchart V, Halm-Lemeille M-P (2018) Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. Environmen Sci Poll Res 25: 6122–6134. doi: 10.1007/s11356-017-9306-9

Diniz MS, Salgado R, Pereira VJ, Carvalho G, Oehmen A, Reis MAM, Noronha JP (2015) Ecotoxicity of ketoprofen, diclofenac, atenolol and their photolysis byproducts in zebrafish (Danio rerio). Sci Total Environ 505: 282–289. doi: 10.1016/j.scitotenv.2014.09.103

Dorelle LS, Da Cuña RH, Rey Vázquez G, Höcht C, Shimizu A, Genovese G, Lo Nostro FL (2017) The SSRI fluoxetine exhibits mild effects on the reproductive axis in the cichlid fish Cichlasoma dimerus (Teleostei, Cichliformes). Chemosphere 171: 370–378. doi: 10.1016/j.chemosphere.2016.11.141

Drugbank (2018) Available from: http://www.drugbank.com [Accessed May 5, 2018]

Eades C, Waring CP (2010) The effects of diclofenac on the physiology of the green shore crab Carcinus maenas. Mar Environ Res 69: S46–S48. doi: 10.1016/j.marenvres.2009.11.001

Ehrlich G, Jöhncke U, Drost W, Schulte C (2011) Problems faced when evaluating the bioaccumulation potential of substances under REACH. Integ Environ Asess Manage. 7(4): 550-558 doi: 10.1002/ieam.190

Ellesat KS, Holth TF, Wojewodzic MW, Hylland K (2012) Atorvastatin up-regulate toxicologically relevant genes in rainbow trout gills. Ecotoxicology 21: 1841–1856. doi: 10.1007/s10646-012-0918-z

Ellesat KS, Tollefsen KE, Åsberg A, Thomas K V., Hylland K (2010) Cytotoxicity of atorvastatin and simvastatin on primary rainbow trout (Oncorhynchus mykiss) hepatocytes. Toxicol in Vitro 24: 1610–1618. doi: 10.1016/j.tiv.2010.06.006

EMEA (2006) EMEA Guidance for RA. Available from: http://www.emea.eu.int [Accessed January 14, 2017].

Ericson H, Thorsén G, Kumblad L (2010) Physiological effects of diclofenac, ibuprofen and propranolol on Baltic Sea blue mussels. Aquat Toxicol 99: 223–231. doi: 10.1016/j.aquatox.2010.04.017

Estevez-Calvar N, Canesia L, Montagna M, Faimali M, Piazza V, Garaventa F (2016) Adverse effects of the SSRI antidepressant sertraline on early life stages of marine invertebrates. Mar Environ Res 128: 88–97. doi: 10.1016/j.marenvres.2016.05.021

Estévez-Calvar N, Canesi L, Montagna M, Faimali M, Piazza V, Garaventa F (2017) Adverse effects of the SSRI antidepressant sertraline on early life stages of marine invertebrates. Mar Environ Res 128: 88–97. doi: 10.1016/j.marenvres.2016.05.021

Fabbri E (2015) Pharmaceuticals in the environment: Expected and unexpected effects on aquatic fauna. Ann NY Acade Sci 1340: 20–28. doi: 10.1111/nyas.12605

Fabbri E, Franzellitti S (2016) Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. Environ Toxicol Chem 35: 799–812. doi: 10.1002/etc.3131

Falfushynska H, Sokolov EP, Haider F, Oppermann C, Kragl U, Ruth W, Stock M, Glufke S, Winkel EJ, Sokolova IM (2019) Effects of a common pharmaceutical, atorvastatin, on energy metabolism and detoxification mechanisms of a marine bivalve Mytilus edulis. Aquat Toxicol 208: 47–61. doi: 10.1016/j.aquatox.2018.12.022

Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76: 122–159. doi: 10.1016/j.aquatox.2005.09.009

Ferrari B, Mons R, Vollat B, Fraysse B, Paxéus N, Lo Giudice R, Pollio A, Garric J (2004) Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environ Toxicol Chem 23: 1344. doi: 10.1897/03-246

Fick J, Lindberg RH, Tysklind M, Larsson DGJ (2010) Predicted critical environmental concentrations for 500 pharmaceuticals. Regul Toxicol Pharmacol 58: 516–523. doi: 10.1016/j.yrtph.2010.08.025

Flippin JL, Huggett D, Foran CM (2007) Changes in the timing of reproduction following chronic exposure to ibuprofen in Japanese medaka, Oryzias latipes. Aquat Toxicol 81: 73–78. doi: 10.1016/j.aquatox.2006.11.002

Fong PP, Ford AT (2014) The biological effects of antidepressants on the molluscs and crustaceans: A review. Aquat Toxicol 151: 4–13. doi: 10.1016/j.aquatox.2013.12.003

Fong PP, Bury TBS, Donovan EE, Lambert OJ, Palmucci JR, Adamczak SK (2017) Exposure to SSRI-type antidepressants increases righting time in the marine snail Ilyanassa obsoleta. Environ Sci Pollut R 24: 725–731. doi: 10.1007/s11356-016-7855-y

Fonseca TG, Morais MB, Rocha T, Abessa DMS, Aureliano M, Bebianno MJ (2017) Ecotoxicological assessment of the anticancer drug cisplatin in the polychaete Nereis diversicolor. Sci Total Environ 575: 162–172. doi: 10.1016/j.scitotenv.2016.09.185

Ford AT, Hyett B, Cassidy D, Malyon G (2018) The effects of fluoxetine on attachment and righting behaviours in marine (Gibbula unbilicalis) and freshwater (Lymnea stagnalis) gastropods. Ecotox 27: 477–484. doi: 10.1007/s10646-018-1919-3

Franzellitti S, Buratti S, Valbonesi P, Fabbri E (2013) The mode of action (MOA) approach reveals interactive effects of environmental pharmaceuticals on Mytilus galloprovincialis. Aquat Toxicol 140–141: 249–256. doi: 10.1016/j.aquatox.2013.06.005

Franzellitti S, Buratti S, Capolupo M, Du B, Haddad SP, Chambliss CK, Brooks BW, Fabbri E (2014) An exploratory investigation of various modes of action and potential adverse outcomes of fluoxetine in marine mussels. Aquat Toxicol 151: 14–26. doi: 10.1016/j.aquatox.2013.11.016

Franzellitti S, Buratti S, Du B, Haddad SP, Chambliss CK, Brooks BW, Fabbri E (2015) A multibiomarker approach to explore interactive effects of propranolol and fluoxetine in marine mussels. Environ Pollut 205: 60–69. doi: 10.1016/j.envpol.2015.05.020

Gagné F, Blaise C, Fournier M, Hansen PD (2006) Effects of selected pharmaceutical products on phagocytic activity in Elliptio complanata mussels. Comp Biochem Physiol C Toxicol Pharmacol 143: 179–186. doi: 10.1016/j.cbpc.2006.01.008

Gagné F, André C, Gélinas M (2010) Neurochemical effects of benzodiazepine and morphine on freshwater mussels. Comp Biochem Physiol C Toxicol Pharmacol 152: 207–214. doi: 10.1016/j.cbpc.2010.04.007

Gan TJ (2010) Diclofenac: an update on its mechanism of action and safety profile. Curr Med Res Opin 26: 1715–1731. doi: 10.1185/03007995.2010.486301

Garcia RN, Chung KW, DeLorenzo ME, Curran MC (2014) Individual and mixture effects of caffeine and sulfamethoxazole on the daggerblade grass shrimp *Palaemonetes pugio* following maternal exposure. Environ Toxicol Chem 33: 2120–2125. doi: 10.1002/etc.2669

Gaw S, Thomas K V, Hutchinson TH (2014) Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philosoph Trans Royal Soc Lond B Biol Sci 369. doi: 10.1098/rstb.2013.0572

Godoy AA, Kummrow F, Pamplin PAZ (2015) Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment - A review. Chemosphere 138: 281–291. doi: 10.1016/j.chemosphere.2015.06.024

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014a) Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular pharmaceuticals and UV filters in wastewater treatment plant. Environ Sci Pollut R Int 21(12): 7578-7585 doi: 10.1007/s11356-014-2654-9

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014b) Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111: 418–426. doi: 10.1016/j.chemosphere.2014.03.132

Gomes A, Correia AT, Nunes B (2019) Worms on drugs: ecotoxicological effects of acetylsalicylic acid on the Polychaeta species Hediste diversicolor in terms of biochemical and histological alterations. Environ Sci Pollut R 1–11. doi:

10.1007/s11356-019-04880-1

González-Ortegón E, Blasco J, Le Vay L, Giménez L (2013) A multiple stressor approach to study the toxicity and sub-lethal effects of pharmaceutical compounds on the larval development of a marine invertebrate. J Hazard Mat 263: 233–238. doi: 10.1016/j.jhazmat.2013.09.041

Gonzalez-Rey M, Bebianno MJ (2012) Does non-steroidal anti-inflammatory (NSAID) ibuprofen induce antioxidant stress and endocrine disruption in mussel Mytilus galloprovincialis? Environ Toxicol Pharmacol 33: 361–371. doi: 10.1016/j.etap.2011.12.017

Gonzalez-Rey M, Bebianno MJ (2013) Does selective serotonin reuptake inhibitor (SSRI) fluoxetine affects mussel Mytilus galloprovincialis? Environ Pollut 173: 200–209. doi: 10.1016/j.envpol.2012.10.018

Gonzalez-Rey M, Bebianno MJ (2014) Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel Mytilus galloprovincialis. Aquat Toxicol 148: 221–230. doi: 10.1016/J.AQUATOX.2014.01.011

Gonzalez-Rey M, Tapie N, Le Menach K, Dévier M-H, Budzinski H, Bebianno MJ (2015) Occurrence of pharmaceutical compounds and pesticides in aquatic systems. Mar Pollut Bull 96: 384–400. doi: 10.1016/J.MARPOLBUL.2015.04.029

Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF (2013) The modern pharmacology of paracetamol: Therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacology 21: 201–232. doi: 10.1007/s10787-013-0172-x

Gravel A, Wilson JM, Pedro DFN, Vijayan MM (2009) Non-steroidal anti-inflammatory drugs disturb the osmoregulatory, metabolic and cortisol responses associated with seawater exposure in rainbow trout. Comp Biochem Physiol C Toxicol Pharmacol 149: 481–490. doi: 10.1016/j.cbpc.2008.11.002

Guardiola FA, Cerezuela R, Meseguer J, Esteban MA (2012) Modulation of the immune parameters and expression of genes of gilthead seabream (Sparus aurata L.) by dietary administration of oxytetracycline. Aquaculture 334–337: 51–57. doi: 10.1016/j.aquaculture.2012.01.003

Guillén D, Ginebreda A, Farré M, Darbra RM, Petrovic M, Gros M, Barceló D (2012) Prioritization of chemicals in the aquatic environment based on risk assessment: Analytical, modeling and regulatory perspective. Sci Total Environ 440: 236–252. doi:

10.1016/j.scitotenv.2012.06.064

Guiloski IC, Ribas JLC, Piancini LDS, Dagostim AC, Cirio SM, Fávaro LF, Boschen SL, Cestari MM, da Cunha C, de Assis HCS (2017) Paracetamol causes endocrine disruption and hepatotoxicity in male fish Rhamdia quelen after subchronic exposure. Environ Toxicol Pharmacol. doi: 10.1016/j.etap.2017.05.005

Guler Y, Ford AT (2010) Anti-depressants make amphipods see the light. Aquat Toxicol 99: 397–404. doi: 10.1016/j.aquatox.2010.05.019

Gunnarsson L, Jauhiainen A, Kristiansson E, Nerman O, Larsson DGJ (2008) Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ Sci Technol 42: 5807–5813. doi: 10.1021/es8005173

Guo J, Boxall A, Selby K (2015) Do Pharmaceuticals Pose a Threat to Primary Producers? Crit Rev Environ Sci Technol 45: 2565–2610. doi: 10.1080/10643389.2015.1061873

Guo X, Feng C, Gu E, Tian C, Shen Z (2019) Spatial distribution, source apportionment and risk assessment of antibiotics in the surface water and sediments of the Yangtze Estuary. Sci Total Environ 671: 548–557. doi: 10.1016/j.scitotenv.2019.03.393

Hampel M, Blasco J, Babbucci M, Ferraresso S, Bargelloni L, Milan M (2017) Transcriptome analysis of the brain of the sea bream (Sparus aurata) after exposure to human pharmaceuticals at realistic environmental concentrations. Mar Environ Res. doi: 10.1016/j.marenvres.2017.04.012

Hedgespeth ML, Sapozhnikova Y, Pennington P, Clum A, Fairey A, Wirth E (2012) Pharmaceuticals and personal care products (PPCPs) in treated wastewater discharges into Charleston Harbor, South Carolina. Sci Total Environ 437: 1–9. doi: 10.1016/J.SCITOTENV.2012.07.076

Holmberg A, Fogel J, Albertsson E, Fick J, Brown JN, Paxéus N, Förlin L, Johnsson JI, Larsson DGJ (2011) Does waterborne citalopram affect the aggressive and sexual behaviour of rainbow trout and guppy? J Hazard Mat 187: 596–599. doi: 10.1016/J.JHAZMAT.2011.01.055

Hosseinifard SM, Ahmadpour A, Mojazi Amiri B, Razeghi Mansour M, Ebrahimpour A (2013) Immunomodulatory effect of cimetidine in common carp (Cyprinus carpio L.). Fish Physiol Biochem 39: 1505–1511. doi: 10.1007/s10695-013-9804-8

Hoyett Z, Owens MA, Clark CJ, Abazinge M (2016) A comparative evaluation of environmental risk assessment strategies for pharmaceuticals and personal care

products. Ocean Coast Manage 127: 74–80. doi: 10.1016/j.ocecoaman.2016.04.013

Huggett DB, Brooks BW, Peterson B, Foran CM, Schlenk D (2002) Toxicity of select beta adrenergic receptor-blocking pharmaceuticals (B-blockers) on aquatic organisms. Arch Environ Contam Toxicol 43: 229–235. doi: 10.1007/s00244-002-1182-7

Huggett DB, Cook JC, Ericson JF, Williams RT (2003) A Theoretical Model for Utilizing Mammalian Pharmacology and Safety Data to Prioritize Potential Impacts of Human Pharmaceuticals to Fish. Hum Ecol Risk Assess: An International Journal 9: 1789–1799. doi: 10.1080/714044797

Hughes SR, Kay P, Brown LE (2013) Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ Scu Technol 47: 661–677. doi: 10.1021/es3030148

Hutchinson TH, Madden JC, Naidoo V, Walker CH, Hutchinson TH (2014) Comparative metabolism as a key driver of wildlife species sensitivity to human and veterinary pharmaceuticals. Philosoph Trans Royal Soc Lond B Biol Sci 369: 1–9. doi: 10.1098/rstb.2013.0583

Isidori M, Parrella A, Pistillo P, Temussi F (2009) Effects of ranitidine and its photoderivatives in the aquatic environment. Environ Int 35: 821–825. doi: 10.1016/j.envint.2008.12.002

Jarvis AL, Bernot MJ, Bernot RJ (2014) The effects of the pharmaceutical carbamazepine on life history characteristics of flat-headed mayflies (Heptageniidae) and aquatic resource interactions. Ecotoxicology 23: 1701–1712. doi: 10.1007/s10646-014-1309-4

Jobling S, Casey D, Rodgers-Gray T, Oehlmann J, Schulte-Oehlmann, Pawlowski S, Baunbeck T, Turner AP, Tyler CR (2004) Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquat Toxicol. 66: 207-222 doi: 10.1016/J.AQUATOX.2004.01.002

Joshi SR (2005) Metformin: old wine in new bottle--evolving technology and therapy in diabetes. J Assoc Physicians India 53: 963–972.doi:

Juhel G, Bayen S, Goh C, Lee WK, Kelly BC (2017) Use of a suite of biomarkers to assess the effects of carbamazepine, bisphenol A, atrazine, and their mixtures on green mussels, *Perna viridis*. Environ Toxicol Chem 36: 429–441. doi: 10.1002/etc.3556

Key PB, Hoguet J, Chung KW, Venturella JJ, Pennington PL, Fulton MH (2009) Lethal and

sublethal effects of simvastatin, irgarol, and PBDE-47 on the estuarine fish, Fundulus heteroclitus. J Environ Sci Health B 44: 379–382. doi: 10.1080/03601230902801083

Kim H-Y, Lee I-S, Oh J-E (2016) Human and veterinary pharmaceuticals in the marine environment including fish farms in Korea. Sci Total Environ. doi: 10.1016/j.scitotenv.2016.10.039

Klosterhaus SL, Grace R, Hamilton MC, Yee D (2013) Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ Int 54: 92–99. doi: 10.1016/j.envint.2013.01.009

Koagouw W, Ciocan C (2018) Impact of Metformin and Increased Temperature on Blue Mussels Mytilus edulis - Evidence for Synergism. J Shellfish Res 37: 467–474. doi: 10.2983/035.037.0301

Kostich MS, Lazorchak JM (2008) Risks to aquatic organisms posed by human pharmaceutical use. Sci Total Environ 389: 329–339. doi: 10.1016/j.scitotenv.2007.09.008

Koutsogiannaki S, Franzellitti S, Fabbri E, Kaloyianni M (2014) Oxidative stress parameters induced by exposure to either cadmium or 17β-estradiol on Mytilus galloprovincialis hemocytes. The role of signaling molecules. Aquat Toxicol 146: 186–195. doi: 10.1016/J.AQUATOX.2013.11.005

Küster A, Adler N (2014) Pharmaceuticals in the environment: scientific evidence of risks and its regulation. Philosoph Trans Royal Soc Lond B Biol Sci 369: 20130587-. doi: 10.1098/rstb.2013.0587

Lacaze E, Pédelucq J, Fortier M, Brousseau P, Auffret M, Budzinski H, Fournier M (2015) Genotoxic and immunotoxic potential effects of selected psychotropic drugs and antibiotics on blue mussel (Mytilus edulis) hemocytes. Environ Pollut 202: 177–186. doi: 10.1016/j.envpol.2015.03.025

LaLone CA, Berninger JP, Villeneuve DL, Ankley GT (2014) Leveraging existing data for prioritization of the ecological risks of human and veterinary pharmaceuticals to aquatic organisms. Philosoph Trans Royal Soc Lond B Biol Sci 369: 20140022-. doi: 10.1098/rstb.2014.0022

De Lange HJ, Noordoven W, Murk AJ, Lürling M, Peeters ETHM (2006) Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquat Toxicol 78: 209–216. doi: 10.1016/j.aquatox.2006.03.002

Lara-Martín PA, González-Mazo E, Petrovic M, Barceló D, Brownawell BJ (2014) Occurrence, distribution and partitioning of nonionic surfactants and pharmaceuticals in the urbanized Long Island Sound Estuary (NY). Mar Pollut Bull 85: 710–719. doi: 10.1016/J.MARPOLBUL.2014.01.022

Lara-Martín PA, Renfro AA, Cochran JK, Brownawell BJ (2015) Geochronologies of Pharmaceuticals in a Sewage-Impacted Estuarine Urban Setting (Jamaica Bay, New York). Environl Sci Technol 49: 5948–5955. doi: 10.1021/es506009v

Lazzara R, Blázquez M, Porte C, Barata C (2012) Low environmental levels of fluoxetine induce spawning and changes in endogenous estradiol levels in the zebra mussel Dreissena polymorpha. Aquat Toxicol 106–107: 123–130. doi: 10.1016/j.aquatox.2011.11.003

Lee S, Jung D, Kho Y, Ji K, Kim P, Ahn B, Choi K (2015) Ecotoxicological assessment of cimetidine and determination of its potential for endocrine disruption using three test organisms: Daphnia magna, Moina macrocopa, and Danio rerio. Chemosphere 135: 208–216. doi: 10.1016/j.chemosphere.2015.04.033

Lewis SL, Lyons DE, Meekins TL, Newcomb JM (2011) Serotonin influences locomotion in the nudibranch mollusc Melibe leonina. Biological Bull 220: 155–60. doi: 10.1086/BBLv220n3p155

Liang X, Chen B, Nie X, Shi Z, Huang X, Li X (2013) The distribution and partitioning of common antibiotics in water and sediment of the Pearl River Estuary, South China. Chemosphere 92: 1410–1416. doi: 10.1016/j.chemosphere.2013.03.044

Liu D, Lung WS, Colosi LM (2013) Effects of sorption kinetics on the fate and transport of pharmaceuticals in estuaries. Chemosphere 92: 1001–1009. doi: 10.1016/j.chemosphere.2013.03.029

Long ER, Dutch M, Weakland S, Chandramouli B, Benskin JP (2013) Quantification of pharmaceuticals, personal care products, and perfluoroalkyl substances in the marine sediments of Puget Sound, Washington, USA. Environ Toxicol Chem 32: 1701–1710. doi: 10.1002/etc.2281

Malarvizhi A, Kavitha C, Saravanan M, Ramesh M (2012) Carbamazepine (CBZ) induced enzymatic stress in gill, liver and muscle of a common carp, Cyprinus carpio. J King Saud Uni. 24: 179–186. doi: 10.1016/j.jksus.2011.01.001

Mansour F, Al-Hindi M, Saad W, Salam D (2016) Environmental risk analysis and prioritization of pharmaceuticals in a developing world context. Sci Total Environ 557:

31–43. doi: 10.1016/j.scitotenv.2016.03.023

Maranho LA, André C, DelValls TA, Gagné F, Martín-Díaz ML (2015) Toxicological evaluation of sediment samples spiked with human pharmaceutical products: Energy status and neuroendocrine effects in marine polychaetes Hediste diversicolor. Ecotox Environ Safe. 118: 27–36. doi: 10.1016/j.ecoenv.2015.04.010

Maranho LA, Baena-Nogueras RM, Lara-Martín PA, DelValls TA, Martín-Díaz ML (2014) Bioavailability, oxidative stress, neurotoxicity and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete Hediste diversicolor as bioindicator species. Environ Res. 134: 353–365. doi: 10.1016/j.envres.2014.08.014

Maranho LA, Moreira LB, Baena-Nogueras RM, Lara-Martín PA, DelValls TA, Martín-Díaz ML (2015) A Candidate Short-Term Toxicity Test Using Ampelisca brevicornis to Assess Sublethal Responses to Pharmaceuticals Bound to Marine Sediments. Arch Environ Contam Toxicol 68: 237–258. doi: 10.1007/s00244-014-0080-0

Maria VL, Amorim MJB, Bebianno MJ, Dondero F (2016) Transcriptomic effects of the non-steroidal anti-inflammatory drug Ibuprofen in the marine bivalve Mytilus galloprovincialis Lam. Mar Environ Res 119: 31–39. doi: 10.1016/j.marenvres.2016.05.010

Martin-Diaz L, Franzellitti S, Buratti S, Valbonesi P, Capuzzo A, Fabbri E (2009) Effects of environmental concentrations of the antiepilectic drug carbamazepine on biomarkers and cAMP-mediated cell signaling in the mussel Mytilus galloprovincialis. Aquat Toxicol 94: 177–185. doi: 10.1016/J.AQUATOX.2009.06.015

Martínez-Hernández R, Meffe R, Herrera S, Arranz E, de Bustamante I (2014) Sorption/desorption of non-hydrophobic and ionisable pharmacetical and personal care products from reclaimed water onto/from a natural sediment. Sci Total Environ 472: 273-281 doi: 10.1016/J.SCITOTENV.2013.11.036

Maskaoui K, Zhou JL (2010) Colloids as a sink for certain pharmaceuticals in the aquatic environment. Environ Sci Pollut R 17: 898–907. doi: 10.1007/s11356-009-0279-1

Massarsky A, Trudeau VL, Moon TW (2011) β-blockers as endocrine disruptors: The potential effects of human β-blockers on aquatic organisms. J Exp Zool A Ecol Gen Physiol 315 A: 251–265. doi: 10.1002/jez.672

Maszkowska J, Stolte S, Kumirska J, Łukaszewicz P, Mioduszewska K, Puckowski A, Caban M, Wagil M, Stepnowski P, Białk-Bielin´ska A (2014) Beta-blockers in the environment: Part II. Ecotoxicity study. Sci Total Environ 493: 1122–1126. doi:

10.1016/j.scitotenv.2014.06.039

Matozzo V, Bertin V, Battistara M, Guidolin A, Masiero L, Marisa I, Orsetti A (2016) Does the antibiotic amoxicillin affect haemocyte parameters in non-target aquatic invertebrates? The clam Ruditapes philippinarum and the mussel Mytilus galloprovincialis as model organisms. Mar Environ Res 119: 51–58. doi: 10.1016/J.MARENVRES.2016.05.017

Mavragani A, Sypsa K, Sampri A, Tsagarakis KP (2016) Quantifying the UK online interest in substances of the EU watchlist for monitoring: diclofenac, estradiol and the macrolide antibiotic. Water. 8(11): 542-563 doi: 10.3390/w8110542

McCallum ES, Bose APH, Warriner TR, Balshine S (2017) An evaluation of behavioural endpoints: The pharmaceutical pollutant fluoxetine decreases aggression across multiple contexts in round goby (Neogobius melanostomus). Chemosphere 175: 401–410. doi: 10.1016/J.CHEMOSPHERE.2017.02.059

McDonald MD, Gonzalez A, Sloman KA (2011) Higher levels of aggression are observed in socially dominant toadfish treated with the selective serotonin reuptake inhibitor, fluoxetine. Comp Biochem Physiol C Toxicol Pharmacol 153: 107–112. doi: 10.1016/J.CBPC.2010.09.006

Meador JP, Yeh A, Young G, Gallagher EP (2016) Contaminants of emerging concern in a large temperate estuary. Environmen Poll 213: 254–267. doi: 10.1016/J.ENVPOL.2016.01.088

Méndez N, Lacorte S, Barata C (2013) Effects of the pharmaceutical fluoxetine in spiked-sediments on feeding activity and growth of the polychaete Capitella teleta. Mar Environ Res 89: 76–82. doi: 10.1016/j.marenvres.2013.05.004

Mesquita SR, Guilhermino L, Guimarães L (2011) Biochemical and locomotor responses of Carcinus maenas exposed to the serotonin reuptake inhibitor fluoxetine. Chemosphere 85: 967–976. doi: 10.1016/J.CHEMOSPHERE.2011.06.067

Mezzelani M, Gorbi S, Fattorini D, D'Errico G, Consolandi G, Milan M, Bargelloni L, Regoli F (2018) Long-term exposure of Mytilus galloprovincialis to diclofenac, Ibuprofen and Ketoprofen: Insights into bioavailability, biomarkers and transcriptomic changes. Chemosphere 198: 238–248. doi: 10.1016/j.chemosphere.2018.01.148

Mijangos L, Ziarrusta H, Ros O, Kortazar L, Fernández LA, Olivares M, Zuloaga O, Prieto A, Etxebarria N (2018) Occurrence of emerging pollutants in estuaries of the Basque Country: Analysis of sources and distribution, and assessment of the environmental

risk. Wat Res 147: 152–163. doi: 10.1016/J.WATRES.2018.09.033

Milan M, Pauletto M, Patarnello T, Bargelloni L, Marin MG, Matozzo V (2013) Gene transcription and biomarker responses in the clam Ruditapes philippinarum after exposure to ibuprofen. Aquat Toxicol 126: 17–29. doi: 10.1016/j.aquatox.2012.10.007

Miller SB (2006) Prostaglandins in Health and Disease: An Overview. Semin Arthritis Rheum 36: 37–49. doi: 10.1016/J.SEMARTHRIT.2006.03.005

Minguez L, Halm-Lemeille MP, Costil K, Bureau R, Lebel JM, Serpentini A (2014) Assessment of cytotoxic and immunomodulatory properties of four antidepressants on primary cultures of abalone hemocytes (Haliotis tuberculata). Aquat Toxicol153: 3–11. doi: 10.1016/j.aquatox.2013.10.020

Morando MB, Medeiros LR, McDonald MD (2009) Fluoxetine treatment affects nitrogen waste excretion and osmoregulation in a marine teleost fish (DOI:10.1016/j.aquatox.2009.03.011). Aquat Toxicol 95: 164–171. doi: 10.1016/j.aquatox.2009.10.015

Moreno-González R, Rodríguez-Mozaz S, Huerta B, Barceló D, León VM (2016) Do pharmaceuticals bioaccumulate in marine molluscs and fish from a coastal lagoon? Environ Res 146: 282-298 doi: 10.1016/j.envres.2016.01.001

Munari M, Marin MG, Matozzo V (2014) Effects of the antidepressant fluoxetine on the immune parameters and acetylcholinesterase activity of the clam Venerupis philippinarum. Mar Environ Res 94: 32–37. doi: 10.1016/j.marenvres.2013.11.007

Munro K, Martins CPB, Loewenthal M, Comber S, Cowan DA, Pereira L, Barron LP (2019) Evaluation of combined sewer overflow impacts on short-term pharmaceutical and illicit drug occurrence in a heavily urbanised tidal river catchment (London, UK). Sci Total Environ 657: 1099–1111. doi: 10.1016/J.SCITOTENV.2018.12.108

National Health Service (2017) Prescriptions Dispensed in the Community - Statistics for England, 2006-2016. Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescriptions-dispensed-in-the-community/prescriptions-dispensed-in-the-community-statistics-for-england-2006-2016-pas [Accessed: January 1, 2019].

Neuparth T, Martins C, Santos CB de los, Costa MH, Martins I, Costa PM, Santos MM (2014) Hypocholesterolaemic pharmaceutical simvastatin disrupts reproduction and population growth of the amphipod Gammarus locusta at the ng/L range. Aquat

Toxicol 155: 337–347. doi: 10.1016/j.aquatox.2014.07.009

Niemuth NJ, Klaper RD (2015) Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. Chemosphere 135: 38–45. doi: 10.1016/J.CHEMOSPHERE.2015.03.060

Noppe H, Verslycke T, De Wulf E, Verheyden K, Monteyne E, Van Caeter P, Janssen CR, De brabander HF (2007) Occurrence of estrogens in the Scheldt estuary: A 2-year survey. Ecotox Environ Safe 66: 1–8. doi: 10.1016/J.ECOENV.2006.04.005

Nunes B, Verde MF, Soares AMVM (2015) Biochemical effects of the pharmaceutical drug paracetamol on Anguilla anguilla. Environ Sci Pollut R 22: 11574–84. doi: 10.1007/s11356-015-4329-6

Nunes B, Nunes J, Soares AMVM, Figueira E, Freitas R (2017) Toxicological effects of paracetamol on the clam Ruditapes philippinarum: exposure vs recovery. Aquat Toxicol 192: 198–206. doi: 10.1016/J.AQUATOX.2017.09.015

Oh S, Shin WS, Kim HT (2016) Effects of pH, dissolved organic matter and salinity on ibuprofen sorption on sediment Environ Sci Pollut 23: 22882-22889 doi: 10.1007/s11356-016-7503-6

Ojemaye CY, Petrik L (2019) Occurrences, levels and risk assessment studies of emerging pollutants (pharmaceuticals, perfluoroalkyl and endocrine disrupting compounds) in fish samples from Kalk Bay harbour, South Africa. Environ Pollut 252: 562-572 doi: 10.1016/J.ENVPOL.2019.05.091

Oliveira R, McDonough S, Ladewig JCL, Soares AMVM, Nogueira AJA, Domingues I (2013) Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (Danio rerio). Environ Toxicol Pharmacol 36: 903–912. doi: 10.1016/j.etap.2013.07.019

Ortiz de García S, Pinto GP, García-Encina PA, Mata RI (2013) Ranking of concern, based on environmental indexes, for pharmaceutical and personal care products: An application to the Spanish case. J Environ Manage 129: 384–397. doi: 10.1016/j.jenvman.2013.06.035

Oskarsson H, Wiklund AKE, Thorsén G, Danielsson G, Kumblad L (2014) Community interactions modify the effects of pharmaceutical exposure: A microcosm study on responses to propranolol in Baltic Sea coastal organisms. PLoS ONE 9. doi: 10.1371/journal.pone.0093774

Ouellet M, Percival MD (2001) Mechanism of Acetaminophen Inhibition of Cyclooxygenase Isoforms. Arch Biochem Biophys 387: 273–280. doi: 10.1006/abbi.2000.2232

Overturf MD, Overturf CL, Baxter D, Hala DN, Constantine L, Venables B, Huggett DB (2012) Early life-stage toxicity of eight pharmaceuticals to the fathead minnow, pimephales promelas. Arch Environ Contam Toxicol 62: 455–464. doi: 10.1007/s00244-011-9723-6

Overturf MD, Anderson JC, Pandelides Z, Beyger L, Holdway D a (2015) Pharmaceuticals and personal care products: A critical review of the impacts on fish reproduction. Crit Rev Toxicol 8444: 1–23. doi: 10.3109/10408444.2015.1038499

Pagano G, De Biase A, Deeva IB, Degan P, Doronin YK, Iaccarino M, Oral R, Trieff NM, Warnau M, Korkina LG (2001) The role of oxidative stress in developmental and reproductive toxicity of tamoxifen. Life Sciences 68: 1735–1749. doi: 10.1016/S0024-3205(01)00969-9

Painter MM, Buerkley MA, Julius ML, Vajda AM, Norris DO, Barber LB, Furlong ET, Schultz MM, Schoenfuss HL (2009) Antidepressants at environmentally relevant oncentrations affect predator avoidance behavior of larval fathead minnows (*Pimephales promelas*). Environ Toxicol Chem 28: 2677. doi: 10.1897/08-556.1

Pal A, Gin KYH, Lin AYC, Reinhard M (2010) Impacts of emerging organic contaminants on freshwater resources: Review of recent occurrences, sources, fate and effects. Sci Total Environ 408: 6062–6069. doi: 10.1016/j.scitotenv.2010.09.026

Palande N V., Bhoyar RC, Biswas SP, Jadhao AG (2015) Short-term exposure to L-type calcium channel blocker, verapamil, alters the expression pattern of calcium-binding proteins in the brain of goldfish, Carassius auratus. Comp Biochem Physiol C Toxicol Pharmacol 176–177: 31–43. doi: 10.1016/j.cbpc.2015.07.006

Park JC, Yoon D-S, Byeon E, Seo JS, Hwang U-K, Han J, Lee J-S (2018) Adverse effects of two pharmaceuticals acetaminophen and oxytetracycline on life cycle parameters, oxidative stress, and defensome system in the marine rotifer Brachionus rotundiformis. Aquat Toxicol 204: 70–79. doi: 10.1016/j.aquatox.2018.08.018

Peters JR, Granek EF (2016) Long-term exposure to fluoxetine reduces growth and reproductive potential in the dominant rocky intertidal mussel, Mytilus californianus. Sci Total Environ 545–546: 621–628. doi: 10.1016/j.scitotenv.2015.12.118

Porsbring T, Blanck H, Tjellström H, Backhaus T (2009) Toxicity of the pharmaceutical

clotrimazole to marine microalgal communities. Aquat Toxicol 91: 203–211. doi: 10.1016/j.aquatox.2008.11.003

Pusceddu FH, Choueri RB, Pereira CDS, Cortez FS, Santos DRA, Moreno BB, Santos AR, Rogero JR, Cesar A (2018) Environmental risk assessment of triclosan and ibuprofen in marine sediments using individual and sub-individual endpoints. Environ Pollut 232: 274–283. doi: 10.1016/J.ENVPOL.2017.09.046

Reis-Santos P, Pais M, Duarte B, Caçador I, Freitas A, Vila Pouca AS, Barbosa J, Leston S, Rosa J, Ramos F, Cabral HN, Gillanders BM, Fonseca VF (2018) Screening of human and veterinary pharmaceuticals in estuarine waters: A baseline assessment for the Tejo estuary. Mar Pollut Bull 135: 1079–1084. doi: 10.1016/J.MARPOLBUL.2018.08.036

Rodenas MC, Cabas I, Abellán E, Meseguer J, Mulero V, García-Ayala A (2015) Tamoxifen persistently disrupts the humoral adaptive immune response of gilthead seabream (Sparus aurata L.). Dev Comp Immunol 53: 283–92. doi: 10.1016/j.dci.2015.06.014

Roepke TA, Snyder MJ, Cherr GN (2005) Estradiol and endocrine disrupting compounds adversely affect development of sea urchin embryos at environmentally relevant concentrations. Aquat Toxicol 71: 155–173. doi: 10.1016/j.aquatox.2004.11.003

de Roode D, Hoekzema C, de Vries-Buitenweg S, van de Waart B, van der Hoeven J (2006) QSARs in ecotoxicological risk assessment. Regul Toxicol Pharmacol 45: 24–35. doi: 10.1016/j.yrtph.2006.01.012

Roos V, Gunnarsson L, Fick J, Larsson DGJ, Rudén C (2012) Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Sci Total Environ 421–422: 102–110. doi: 10.1016/j.scitotenv.2012.01.039

Rosi-Marshall EJ, Snow D, Bartelt-Hunt SL, Paspalof A, Tank JL (2015) A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. J Hazard Mat 282: 18–25. doi: 10.1016/j.jhazmat.2014.06.062

Ruggeri B, Thoroughgood CA (1985) Prostaglandins in aquatic fauna : a comprehensive review. Tetrahedron 23: 301–306. doi: 10.3354/meps023301

Ruhoy IS, Daughton CG (2008) Beyond the medicine cabinet: An analysis of where and why medications accumulate. Environ Int 34: 1157–1169. doi: 10.1016/j.envint.2008.05.002

Sanderson H, Johnson DJ, Reitsma T, Brain RA, Wilson CJ, Solomon KR (2004) Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regul Toxicol Pharmacol 39: 158–183. doi: 10.1016/j.yrtph.2003.12.006

Sangion A, Gramatica P (2016) Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity. Environ Int 95: 131–143. doi: 10.1016/j.envint.2016.08.008

Scaps P (2002) A review of the biology, ecology and potential use of the common ragworm Hediste diversicolor (O.F. Müller) (Annelida: Polychaeta). Hydrobiologia 470: 203–218. doi: 10.1023/A:1015681605656

Schmidt W, O'Rourke K, Hernan R, Quinn B (2011) Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (Mytilus spp.) and their comparison with standardized toxicity tests. Mar Pollut Bull 62: 1389–1395. doi: 10.1016/J.MARPOLBUL.2011.04.043

Schrieber R, Gundel U, Franz S, Kuster A, Rechenberg B, Attenburger R (2011) Using the fish plasma model for comparative hazard identification for pharmaceuticals in the environment by extrapolation from human therapeutic data. Regul Toxicol Pharmacol 61: 261–275. doi: 10.1016/J.YRTPH.2011.08.006

Schwab JM, Schluesener HJ, Laufer S (2003) COX-3: just another COX or the solitary elusive target of paracetamol? Lancet (London, England) 361: 981–2. doi: 10.1016/S0140-6736(03)12841-3

Scott AP (2013) Do mollusks use vertebrate sex steroids as reproductive hormones? II. Critical review of the evidence that steroids have biologial effects. Steroids 78(2): 268-281 doi: 10.1016/j.steroids.2012.11.006

Semsar K, Perreault HAN, Godwin J (2004) Fluoxetine-treated male wrasses exhibit low AVT expression. Brain Res 1029: 141–147. doi: 10.1016/J.BRAINRES.2004.09.030

Shi H, Yang Y, Liu M, Yan C, Yue H, Zhou J (2014) Occurrence and distribution of antibiotics in the surface sediments of the Yangtze Estuary and nearby coastal areas. Mar Pollut Bull 83: 317–323. doi: 10.1016/j.marpolbul.2014.04.034

Sipma J, Osuna B, Collado N, Monclús H, Ferrero G, Comas J, Rodriguez-Roda I (2010) Comparison of removal of pharmaceuticals in MBR and activated sludge systems. Desalination 250: 653–659. doi: 10.1016/j.desal.2009.06.073

Sivalingam VN, Myers J, Nicholas S, Balen AH, Crosbie EJ (2014) Metformin in

reproductive health, pregnancy and gynaecological cancer: Established and emerging indications. Human Reprod Update 20: 853–868. doi: 10.1093/humupd/dmu037

Solé M, Shaw JP, Frickers PE, Readman JW, Hutchinson TH (2010) Effects on feeding rate and biomarker responses of marine mussels experimentally exposed to propranolol and acetaminophen. Anal Bioanal Chem 396: 649–656. doi: 10.1007/s00216-009-3182-1

Steinbach C, Fedorova G, Prokes M, Grabicova K, Machova J, Grabic R, Valentova O, Kroupova HK (2013) Toxic effects, bioconcentration and depuration of verapamil in the early life stages of common carp (Cyprinus carpio L.). Sci Total Environ 461–462: 198–206. doi: 10.1016/j.scitotenv.2013.05.002

Stewart M, Olsen G, Hickey CW, Ferreira B, Jelić A, Petrović M, Barcelo D (2014) A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand. Sci Total Environ 468–469: 202–210. doi: 10.1016/J.SCITOTENV.2013.08.039

Sumpter JP, Scott AP, Katsiadaki I (2016) Comments on Niemuth, N.J. and Klaper, R.D. 2015. Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. Chemosphere 135, 38–45. Chemosphere. doi: 10.1016/j.chemosphere.2016.08.049

Sun L, Liu F, Chen H, Wang S, Lin X, Chi J, Zhu Q, Fu Z (2015) Transcriptional Responses in Adult Zebrafish (Danio rerio) Exposed to Propranolol and Metoprolol. Ecotoxicology 24: 1352–1361. doi: 10.1007/s10646-015-1510-0

Sun L, Xin L, Peng Z, Jin R, Jin Y, Qian H, Fu Z (2014) Toxicity and enantiospecific differences of two β-blockers, propranolol and metoprolol, in the embryos and larvae of zebrafish (*Danio rerio*). Environ Toxicol 29: 1367–1378. doi: 10.1002/tox.21867

Sun Q, Li Y, Li M, Ashfaq M, Lv M, Wang H, Hu A, Yu C-P (2016) PPCPs in Jiulong River estuary (China): Spatiotemporal distributions, fate, and their use as chemical markers of wastewater. Chemosphere 150: 596–604. doi: 10.1016/J.CHEMOSPHERE.2016.02.036

Tamtam F, Mercier F, Le Bot B, Eurin J, Tuc Dinh Q, Clement M, Chevreuil M (2008) Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci Total Environ 393: 84–95. doi: 10.1016/j.scitotenv.2007.12.009

Tarazona J V., Escher B, Giltrow E, Sumpter JP, Knacker T (2007) Targeting the

environmental risk assessment of pharmaceuticals: facts and fantasies. Integr Environ Assess Manag. 6: 603-613. doi: 10.1897/IEAM_2009-052.1

Taylor D, Senac T (2014) Human pharmaceutical products in the environment – The “problem” in perspective. Chemosphere 115: 95–99. doi: 10.1016/J.CHEMOSPHERE.2014.01.011

Teixeira M, Almeida Â, Calisto V, Esteves VI, Schneider RJ, Wrona FJ, Soares AMVM, Figueira E, Freitas R (2017) Toxic effects of the antihistamine cetirizine in mussel Mytilus galloprovincialis. Wat Res 114: 316–326. doi: 10.1016/j.watres.2017.02.032

Thomas K V., Hilton MJ (2004) The occurrence of selected human pharmaceutical compounds in UK estuaries. Mar Pollut Bull 49: 436–444. doi: 10.1016/j.marpolbul.2004.02.028

Thornton JW (2001) Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. PNAS 98(10): 5671-5676 doi:10.1073/pnas.091553298

Thornton JW, Need E, Crews D (2003) Resurrecting the ancestral steroid receptor: ancient origin of estrogen signaling. Science 301(5640): 1714-1717 doi: 10.1126/science.1086185

Triebskorn R, Casper H, Heyd A, Eikemper R, Köhler H-R, Schwaiger J (2004) Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss). Aquat Toxicol 68: 151–166. doi: 10.1016/j.aquatox.2004.03.015

Trombini C, Garcia da Fonseca T, Morais M, Rocha TL, Blasco J, Bebianno MJ (2016) Toxic effects of cisplatin cytostatic drug in mussel Mytilus galloprovincialis. Mar Environ Res 119: 12–21. doi: 10.1016/j.marenvres.2016.05.004

Turner A (2003) Salting out of chemicals in estuaries: implications for contaminant partitioning and modelling. Sci Total Environ. 314-316: 599-612 doi: 10.1016/S0048-9697(03)00076-7

Vasquez MI, Lambrianides A, Schneider M, Kümmerer K, Fatta-Kassinos D (2014) Environmental side effects of pharmaceutical cocktails: What we know and what we should know. J Hazard Mat 279: 169–189. doi: 10.1016/j.jhazmat.2014.06.069

Velasco-Santamaría YM, Korsgaard B, Madsen SS, Bjerregaard P (2011) Bezafibrate, a lipid-lowering pharmaceutical, as a potential endocrine disruptor in male zebrafish

(Danio rerio). Aquat Toxicol 105: 107–118. doi: 10.1016/J.AQUATOX.2011.05.018

Vernouillet G, Eullaffroy P, Lajeunesse A, Blaise C, Gagné F, Juneau P (2010) Toxic effects and bioaccumulation of carbamazepine evaluated by biomarkers measured in organisms of different trophic levels. Chemosphere 80: 1062–1068. doi: 10.1016/j.chemosphere.2010.05.010

Weinberger J, Klaper R (2014) Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish Pimephales promelas (fathead minnow). Aquat Toxicol 151: 77–83. doi: 10.1016/j.aquatox.2013.10.012

Weston A, Caminada D, Galicia H, Fent K (2009) Effects of lipid lowering pharmaceuticals bezafibrate and clofibric acid on lipid metabolism in fathead minnow (pimephales promelas). Environ Toxicol Chem 28: 2648. doi: 10.1897/09-087.1

Wiegel S, Aulinger A, Brockmeyer R, Harms H, Löffler J, Reincke H, Schmidt R, Stachel B, Von Tümpling W, Wanke A (2004) Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57: 107–126. doi: 10.1016/j.chemosphere.2004.05.017

Winder VL, Sapozhnikova Y, Pennington PL, Wirth EF (2009) Effects of fluoxetine exposure on serotonin-related activity in the sheepshead minnow (Cyprinodon variegatus) using LC/MS/MS detection and quantitation. Comp Biochem Physiol C Toxicol Pharmacol 149: 559–565. doi: 10.1016/j.cbpc.2008.12.008

Winter MJ, Lillicrap AD, Caunter JE, Schaffner C, Alder AC, Ramil M, Ternes TA, Giltrow E, Sumpter JP, Hutchinson TH (2008) Defining the chronic impacts of atenolol on embryo-larval development and reproduction in the fathead minnow (Pimephales promelas). Aquat Toxicol 86: 361–369. doi: 10.1016/j.aquatox.2007.11.017

Wu JP, Li MH (2015) Inhibitory effects of pain relief drugs on neurological enzymes: Implications on their potential neurotoxicity to aquatic animals. Environ Toxicol Pharmacol 39: 898–905. doi: 10.1016/j.etap.2015.02.022

Yamamoto H, Nakamura Y, Moriguchi S, Nakamura Y, Honda Y, Tamura I, Hirata Y, Hayashi A, Sekizawa J (2009) Persistance and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. Wat Res 43: 351-362 doi: 10.1016/j.watres.2008.10.039

Yang M, Qiu W, Chen J, Zhan J, Pan C, Lei X, Wu M (2014) Growth inhibition and coordinated physiological regulation of zebrafish (Danio rerio) embryos upon sublethal exposure to antidepressant amitriptyline. Aquat Toxicol 151: 68–76. doi:

10.1016/j.aquatox.2013.12.029

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL (2011) Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone. J Hazard Mat 190: 588–596. doi: 10.1016/J.JHAZMAT.2011.03.092

Zenker A, Cicero MR, Prestinaci F, Bottoni P, Carere M (2014) Bioaccumulation and biomagnification of pharmaceuticals with a focus to the aquatic environment. J Environ Manage 133: 378-387 doi: 10.1016/J.JENVMAN.2013.12.017

Zhang R, Zhang G, Zheng Q, Tang J, Chen Y, Xu W, Zou Y, Chen X (2012) Occurrence and risks of antibiotics in the Laizhou Bay, China: Impacts of river discharge. Ecotox Environ Safe 80: 208–215. doi: 10.1016/J.ECOENV.2012.03.002

Zhang R, Tang J, Li J, Cheng Z, Chaemfa C, Liu D, Zheng Q, Song M, Luo C, Zhang G (2013) Occurrence and risks of antibiotics in the coastal aquatic environment of the Yellow Sea, North China. Sci Total Environ 450–451: 197–204. doi: 10.1016/J.SCITOTENV.2013.02.024

Zhao H, Zhou JL, Zhang J (2015) Tidal impact on the dynamic behavior of dissolved pharmaceuticals in the Yangtze Estuary, China. Sci Total Environ 536: 946–954. doi: 10.1016/j.scitotenv.2015.06.055

Zheng S, Qiu X, Chen B, Yu X, Liu Z, Zhong G, Li H, Chen M, Sun G, Huang H, Yu W, Freestone D (2011) Antibiotics pollution in Jiulong River estuary: Source, distribution and bacterial resistance. Chemosphere 84: 1677–1685. doi: 10.1016/j.chemosphere.2011.04.076

Chapter 2: Method development for the analysis of pharmaceuticals in environmental samples

2.1 Selection of Compounds

Due to time and financial limitations it is not feasible to carry out monitoring of all pharmaceutical compounds. There are many prioritisation schemes in the existing literature which are used to determine the likelihood of a compound to enter the environment and cause potential harm to organisms. In order to determine which pharmaceuticals to focus on in the subsequent chapters, a prioritisation exercise was carried out. Compounds which are commonly prescribed in the UK or are present in the literature were included (National Health Service, 2014). Naturally occurring compounds, such as, caffeine and nicotine were not included, leaving 80 compounds (Appendix 2.1). This method was adapted from an assessment carried out by Daouk et al. (2015) where compounds were assigned a ranking of 1-5 based on their potential to enter the environment, persist, and be taken up by organisms and cause toxicity (Table 2.1).

The potential to enter the environment was determined by calculating PECs. This is shown in Eq. 2.1, where A is the amount prescribed (kg year^{-1} calculated from National Health Service, 2014), E is the fraction of the compound excreted unchanged, V is the volume of waste water per capita per day (assumed to be 200 litres; EMEA 2006), P is the population of the UK in 2014 and D is the dilution of wastewater (assumed to be 10 times; EMEA 2006). Excretion rates were obtained from peer reviewed literature or databases such as drugbank (http://www.drugbank.com) and compendium (http://www.compendium.ch). Excretion rates were often variable, so the highest value was used, and where data was not available, excretion was assumed to be 100%. A score of 1 to 5 was given to each of the compounds based on the calculated PEC (Table 2.1).

$$\frac{A * E}{V * P * D * 365} \qquad \text{(Eq. 2.1)}$$

Pharmaceuticals were then ranked 1-5 based on removal rates during wastewater treatment (Table 2.1). Removal rates were obtained from the literature and they were assumed to be 0, when no data was available or when removal rates were negative. These rates often varied depending on technology used so the lowest rate was chosen

for this exercise. Wastewater treatment plant (WWTP) removal rates were used for persistence criteria, as there was little available experimental data on the half-life of pharmaceuticals in the environment. WWTP was used instead, as compounds which have gone through this process, are more likely to be resistant to degradation processes (Kim et al. 2014). Daouk et al. (2015) used bioconcentration factor (BCF) to determine the bioavailability of a compound, however this data is often unavailable. Instead, the $log_{KOW,}$ which is often use in prioritisation schemes as a predictor of bioconcentration was used instead, and compounds were ranked based on this information (Table 2.1).

In order to determine potential toxicity, compounds were ranked by a method adapted from Capleton et al. (2006) in order to determine how likely a compound is to exert a biological effect on aquatic organisms. Six categories of potential effects on different biological systems were chosen: reproductive health, neurotoxicity, endocrine disruption, immunotoxicity, antimicrobial and genotoxicity. Each compound was given a score 1 to 5 (Table 2.1) to determine if the compound would have an effect on the selected systems. If it was unknown whether a compound exerted a particular effect, it was decided to distinguish between whether the effects were truly unknown (score 3) or whether it was suspected to have an effect (score 2). Suspected was defined as compounds which had had a mode of action (MoA) that was likely to occur in an aquatic species, or if another compound with a similar MoA had an effect on aquatic species.

Finally, the rankings from the four categories were added together to create a final ranking. Those with the lowest score are more likely to occur in the environment and exert a biological effect, and therefore should be prioritised for study. This resulted in compounds with a ranking between 16 and 35. The top 20 ranked drugs are shown in Table 2.2, with the full scores presented in Appendix 2.1.

Table 2.1: Criteria thresholds for the ranking of pharmaceuticals

Score	Occurrence PEC ($ng\ l^{-1}$)	Persistence WWTP Removal (%)	Bioaccumulation Log_{Kow}	Toxicity
1	>1000	<20	>4	Proven
2	500-999	20-39	3-3.9	Suspected
3	200-499	40-59	2-2.9	Unknown
4	50-199	60-79	1-1.9	
5	0-49	>80	<1	No Effect

Six compounds were selected for monitoring in the Humber Estuary: citalopram, diclofenac, paracetamol, ranitidine, metformin and trimethoprim. Compounds were chosen to incorporate a variety of classes and based on their overall score (Table 2.2)

Table 2.2: Scores of priority compounds based on PECs, wastewater removal, $\log_{KOW}$ and potential for toxicity. Selected target compounds for study are shaded in grey.

Score	Compound	Class
17	Fluoxetine	Antidepressant (SSRI)
18	Paracetamol	Pain Killer
19	Bezafibrate	Lipid Lowering
	Citalopram	Antidepressant (SSRI)
	Ibuprofen	Anti-inflammatory
20	Tamoxifen	Hormone
22	Amoxicillin	Antibiotic
	Atorvastatin	Lipid Lowering
	Diclofenac	Anti-inflammatory
23	Carbamazepine	Anticonvulsant
	Metformin	Antidiabetic
24	Erythromycin	Antibiotic
	Flucloxacillin	Antibiotic
	Ketoprofen	Anti-inflammatory
	Mefenamic Acid	Anti-inflammatory
	Sodium Valproate	Anticonvulsant
25	Atenolol	Anti-hypertensive (Beta Blocker)
	Pregabalin	Anticonvulsant
	Ranitidine	Ulcer Medication
	Trimethoprim	Antibiotic

2.2 Chemicals and Reagents

Pharmaceutical standards were used to create working and stock solutions. Diclofenac sodium (≥98.5), paracetamol (≥99%), citalopram hydrobromide (≥98), ibuprofen (≥98%), metformin hydrochloride (≥98), ranitidine (>97%) and trimethoprim (≥98%) were supplied by Sigma-Aldrich Ltd. (Dorset, UK). A fresh Individual stock standard solution was prepared by weight in 100% methanol each day. Standards were prepared by appropriate dilution in 100% methanol immediately before each analytical run. Methanol, acetonitrile, hydrochloric acid, acetic acid, ammonium acetate and formic acid were

supplied by VWR chemicals (Leicestershire, UK). 0.1% Trifluoroacetic acid (TFA) in methanol was supplied by Fisher Scientific. (Loughborough, UK).

2.3 Solid Phase Extraction

There are several methods in the existing literature for the extraction of the target analytes from surface water samples, however, most of these are for determining occurrence in freshwater (Białk-Bielińska et al. 2016). A matrix effect is often seen in marine samples which can lead to poor analytical accuracy, requiring different methods than those used for freshwater samples (Vieira Madureira et al. 2009). As a result, the salinity gradient seen in estuaries can pose a challenge to the analysis of samples from this environment. In order to minimise cost and maximise efficiency, solid phase extraction (SPE) methods need to be suitable for as many of the target analytes as possible. Metformin provides an additional challenge, as it is a polar compound, and as a result requires a different analysis methods than the other compounds (Poole 2003).

2.3.1 Cartridges

The recovery of three SPE cartridges: Oasis HLB (6 cc, 150 mg; Waters Corporation, Milford, MA, USA), Oasis WXC (6 cc, 150 mg; Waters Corporation, Milford, MA, USA) and Strata-X (6 cc, 150mg; Phenomenex, Torrance, CA, USA) using five different methods was determined. Recovery was determined by dosing artificial seawater (20 ppt, Tropic Marin Synthetic Sea Salt) with 1 µg l^{-1} of citalopram, diclofenac, metformin, paracetamol, ranitidine and trimethoprim. Prior to SPE, samples were filtered through a 0.45 µm cellulose filter (Scientific Laboratory Supplies, Hessle, UK) under vacuum. The concentration for each compound was determined by comparing the peak area against a standard of the same concentration. Recovery experiments were carried out in triplicate with a blank sample (not containing pharmaceuticals). It was determined that Oasis HLB method two resulted in the best recovery for citalopram, diclofenac, trimethoprim, ranitidine and paracetamol, whereas Strata-X method two resulted in best recovery for metformin (Figure 2.1).

2.3.1.1 Oasis HLB

The first method was adapted from Petrovic et al. (2006), where HLB cartridges were used for the extraction of 27 pharmaceuticals including ranitidine, trimethoprim, and diclofenac. Cartridges were first conditioned with 5 mL 100% methanol followed 5 mL deionised water at a flow rate of 1 mL min^{-1}. 500mL samples were then loaded onto the SPE cartridge at a flow rate of 10 mL min^{-1}, during which care was taken to ensure the sorbent material did not dry out. Cartridges were then rinsed with 5 mL deionised water prior to being air dried under vacuum for 30 minutes. Elution of cartridges was then

performed with 5 mL 100% methanol twice at a flow rate of 1 mL min^{-1}. The eluent was evaporated to dryness using a rotary evaporator (40ºC, speed 7) and reconstituted with 1 mL 100% methanol. For the second method, conditioning and loading of samples was performed in the same way, but cartridges were eluted twice using 5 mL 0.1% trifluoroacetic acid (TFA) in methanol. The addition of TFA for elution improved recovery of all compounds except trimethoprim and metformin where it remained the same (Figure 2.1)

2.3.1.2 Oasis WCX

Prior to SPE, samples were acidified to pH 4 using hydrochloric acid. 500 mL samples were loaded directly onto the cartridge at a flow rate of 10 mL min^{-1} and then rinsed with 6 mL 5% ammonium hydroxide. Cartridges were left to dry under vacuum for 30 minutes, prior to elution with 6 mL 100% methanol followed by 6 mL 2% formic acid in methanol. The eluent was evaporated to dryness using a rotary evaporator (40ºC, speed 7) and reconstituted with 1 mL 100% methanol. This method resulted in moderate recovery (< 80%) for diclofenac, paracetamol and trimethoprim, however, recovery for metformin and ranitidine was poor (< 10%; Figure 2.1)

2.3.1.3 Strata-X

SPE using strata-X cartridges were carried out using two different methods. First cartridges were conditioned with 5 mL 100% methanol, followed by 5 mL deionised water. 500 mL samples were loaded onto the cartridge at 5 mL min^{-1} and then were rinsed with 5 mL 50% methanol, prior to drying under vacuum for 30 minutes. The first method consisted of elution with 5 mL 2% formic acid in methanol, twice. The second method eluted using 5 mL 2% formic acid in methanol followed by 5 mL acetonitrile. The eluent was evaporated to dryness using a rotary evaporator (40ºC, speed 7) and reconstituted with 1 mL 100% methanol. The second method, yielded improved recoveries for Metformin.

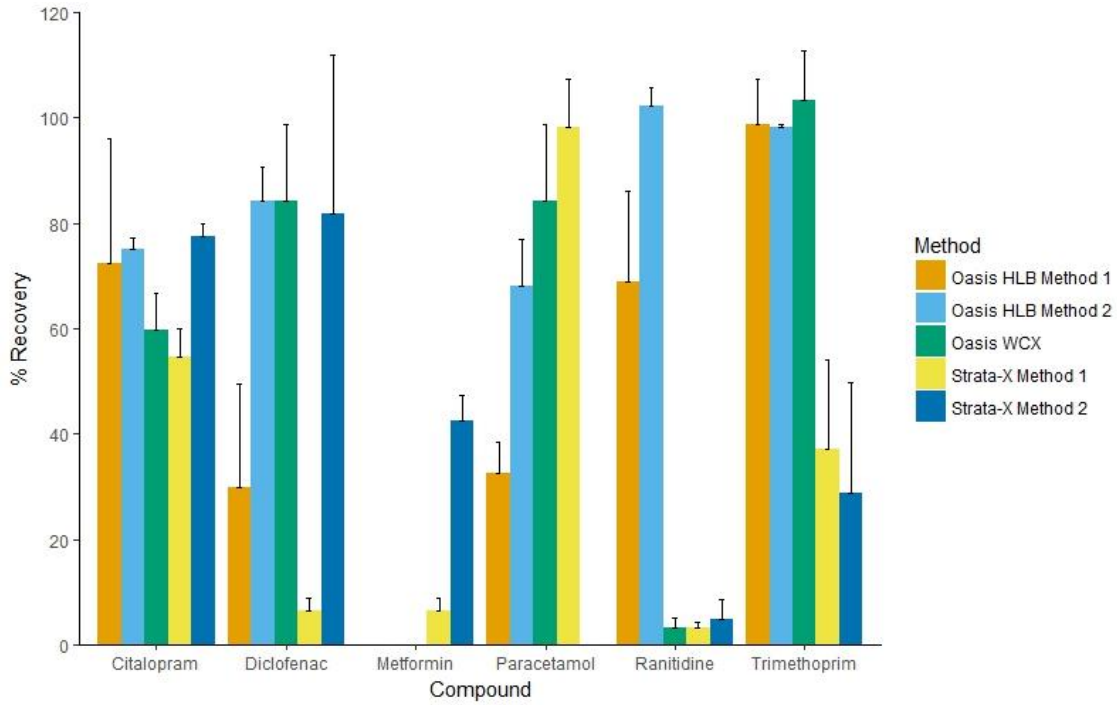


Figure 2.1 Mean recovery (±standard deviation) for target analytes: citalopram, diclofenac, metformin, paracetamol, ranitidine and trimethoprim using three SPE cartridges (n = 3).

2.3.2 Salinity

SPE recoveries can often differ between freshwater and marine samples, which can complicate analysing estuarine samples due to variable salinities (Vieira Madureira et al. 2009). In order to determine if there was a difference in recovery between salinities, artificial seawater was made to two salinities (10 and 20 ppt) using deionised water. SPE was carried out by spiking 500 mL deionised water, 10 ppt and 20 ppt samples with 500 ng of citalopram, diclofenac, paracetamol, ranitidine and trimethoprim. SPE was carried out using Oasis HLB cartridges as described in section 2.3.1.1, with 0.1% TFA in methanol as an elution solvent.

There was little difference in the recovery of samples between 10 and 20 ppt for all compounds, except ranitidine where the mean recovery differed by 33% (Figure 2.2). Use of deionised water resulted in 12 – 48% difference in recovery from saline samples. The recovery of compounds in deionised water is likely to be different from freshwater, because freshwater will have a higher ionic strength which may increase sorption of compounds to the SPE column. Additionally, an increase in salinity will have a similar effect by increasing ionic strength, decreasing solubility, and as a result can improve recovery efficiency of hydrophobic compounds such as diclofenac (Zhang and Zhou 2007). Conversely, an increase in pH as the result of salinity or other environmental differences at sites could affect the sorption of compounds and cause differences in

recovery. For example, the sorption of acidic compounds may decrease as they will not be ionised at a high pH. However, it was determined that the recovery seen with Oasis HLB cartridges would be sufficient for the analysis of estuarine samples, as most sites within the Humber had a salinity of 7-24 ppt, however variability in the recovery between the samples will be expected due to fluctuations in salinity and pH throughout the estuary.

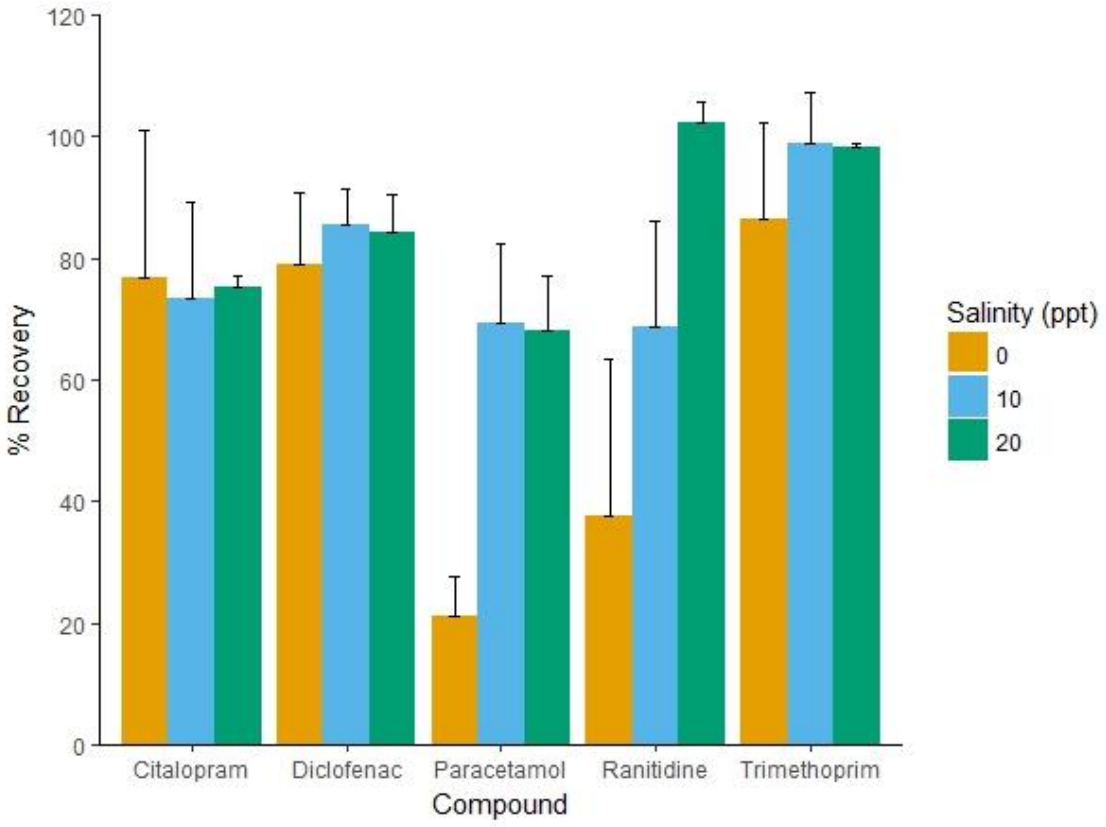


Figure 2.2 Mean recovery (±standard deviation) of target analytes: citalopram, diclofenac, metformin, paracetamol, ranitidine and trimethoprim in deionised water and artificial seawater (10 and 20 ppt, n = 3s).

2.3.3 Volume

In order to determine the optimum volume of sample to be loaded onto the cartridge SPE of citalopram, ranitidine and trimethoprim in 250 mL, 500 mL, 800 mL and 1000 litres of artificial seawater (20 ppt) was determined using Oasis HLB cartridges as outlined in section 2.3.1.1. Samples were spiked with 500 ng l^{-1} citalopram, diclofenac, paracetamol, ranitidine and trimethoprim. 800 mL and 1000 mL samples containing diclofenac and paracetamol were not analysed. Samples for 250 mL samples were also partially analysed, however, an insufficient number of samples (n = 1) were analysed as the result of technical problems with the LC-MS/MS, but those which were showed lower recovery.

Studies frequently perform SPE on 1 litre samples collected from rivers (For example Camacho-Muñoz et al. 2009; Liu et al. 2017). However, a lower recovery was seen at 800 and 1000 mL for citalopram, ranitidine and trimethoprim in comparison to 500 mL. The increased salt in the larger volumes clogged the cartridges and prevented complete filtration and sorption of the pharmaceuticals. However, at a lower volume (250 mL) recovery was lower due to lower concentrations of pharmaceuticals in these samples. As a result, 500 mL was chosen as the optimum volume for SPE.

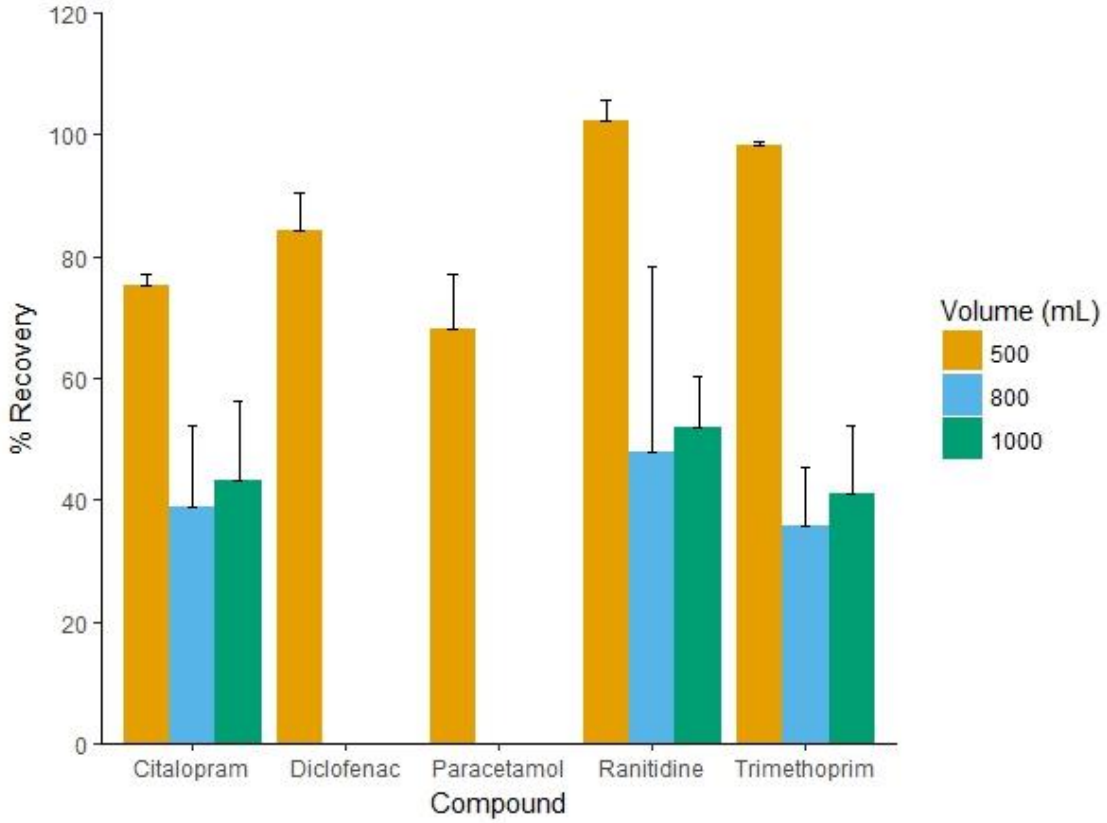


Figure 2.3 Mean recovery (±standard deviation) of target analytes citalopram, ranitidine and trimethoprim in 500 mL, 800 mL and 1000 mL artificial seawater (20 ppt) as well as diclofenac and paracetamol in 500 mL artificial seawater (20 ppt; n = 3). SPE of diclofenac and paracetamol in 800 mL or 1000 mL seawater were not analysed.

Due to technical problems with the LC-MS/MS, it was not possible to determine recovery of different volumes or salinity for metformin. As the result of these ongoing difficulties, samples were sent for external analysis by colleagues at the Catalan Institute for Water Research, and due to financial limitations, it was only possible to analyse environmental samples for the presence of ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram in the subsequent chapters.

The recovery of these compounds in spiked water samples differed between this chapter and Chapter 4. Whilst the same SPE method was used, samples were reconstituted in

10:90 (methanol: water). The use of TFA in elution of the compounds, will have further acidified compounds such as diclofenac, and use of a higher aqueous solution will have resulted in lower solubility of this compound. Additionally, optimisation of SPE was performed on samples containing artificial seawater and environmental samples will contain more complex mixtures of organic matter, salinity and pH which can also account for differences in these salinities.

2.4 Liquid Chromatography with tandem mass spectrometry (LC-MS/MS)

Liquid chromatography (LC) was performed using Agilent 1100 series and the LC eluent was directly infused into the Z-spray electrospray source of a Bruker mass spectrometer. Tandem mass spectrometry (MS/MS) was initially performed for each compound alternating between positive ion (PI) and negative ion (NI) modes. MS/MS was then performed using multiple reaction monitoring (MRM) on target compounds identifying compounds based on run time, molecular weight of the compound and the molecular weight of one or two fragments (Table 2.3). Chromatograms for the target analytes are displayed in Figure 2.4. MS/MS method was optimised by trying different temperatures, backing pressures and flow rates. Once a method was optimised, spiked methanol standards using a standard solution in dilution series were analysed in order to determine method detection limits (MDL) and method quantification limits (MQL). These were calculated using Eq. 2.2, where the standard deviation of 20 blank samples is multiplied by a factor of 3 and 10 for MDL and MQL respectively, SD is the standard deviation from 20 blank samples and b is the slope of the regression line for each of the compounds (Shrivastava and Gupta 2011)

$$MDL/MQL = \frac{F * SD}{b} \quad \text{(Eq. 2.2)}$$

Table 2.3 Precursor ion, MRM transitions and run time used for a positive identification of each of the target compounds. MDL and MQL were calculated as outlined in Eq. 2.2. Mean recovery (± standard deviation) is also provide using SPE methods outlined in section 2.3.

Compound	Precursor Ion	MRM Transition	Run Time	MDL (ng l^{-1})	MQL (ng l^{-1})	Recovery (%)
Citalopram	325	325 > 109 325 > 83	11.5	0.5	1	75.13 (2.09)
Diclofenac	296	296 > 215 296 > 214	15.5	0.4	6.5	84.12 (6.46)
Metformin	130	130 > 85	2.1	10	50	42.56 (4.70)
Paracetamol	152	152 > 110	5.2	9.9	60	67.97 (9.04)
Ranitidine	315	315 > 130 315 > 124	5.0	1	12	102.26 (3.32)
Trimethoprim	291	291 > 261 291 > 123	7.9	1	2.5	98.12 (0.56)

Citalopram, ranitidine and trimethoprim were analysed in positive ion (PI) mode whilst diclofenac and paracetamol were analysed in negative ion (NI) mode using a C18 column (Water Corporation, Milford, MA, USA). These samples were analysed with a solvent system of acetonitrile + 0.1% formic acid (Buffer A) and water + 0.1% formic acid (Buffer B) at a flow rate of 0.3 mL min^{-1} and the column held at 35ºC. After 10 µl injection the gradient was increased from 0 to 100% A over 15 minutes. This was held for four minutes followed by a decline to 0% A over 30 seconds. Reequilibriation time was 5.5 minutes. A column wash in 100% acetonitrile was carried out between each sample injection. The LC eluent was directly infused into the MS, with a backing pressure of 35psi, electrospray desolvatation temperature of 150ºC and 9 l min^{-1}. The lockspray frequency was set to scan 45ms for each ion.

Metformin was analysed in PI mode using a HILIC column according to US EPA method 1694 (EPA 2007). LC was performed using a solvent system of acetonitrile (solvent A) and 0.1% acetic acid in ammonium acetate buffer (solvent B), with a flow rate of 0.2 mL min^{-1} and the column held at 35ºC. After 10 µl injection, the gradient was kept at 98% solvent A for 5 minutes. Solvent A was then decreased to 70% and was held for 7 minutes before increasing back to 98% over 30 seconds. The column was reequilibriated for 3.5 minutes. A column wash in 100% acetonitrile was carried out between each sample injection. The LC eluent was directly infused into the MS, with a backing pressure of 35psi, electrospray desolvatation temperature of 150ºC and 9 l min^{-1}. The lockspray frequency was set to scan 45ms for each ion.

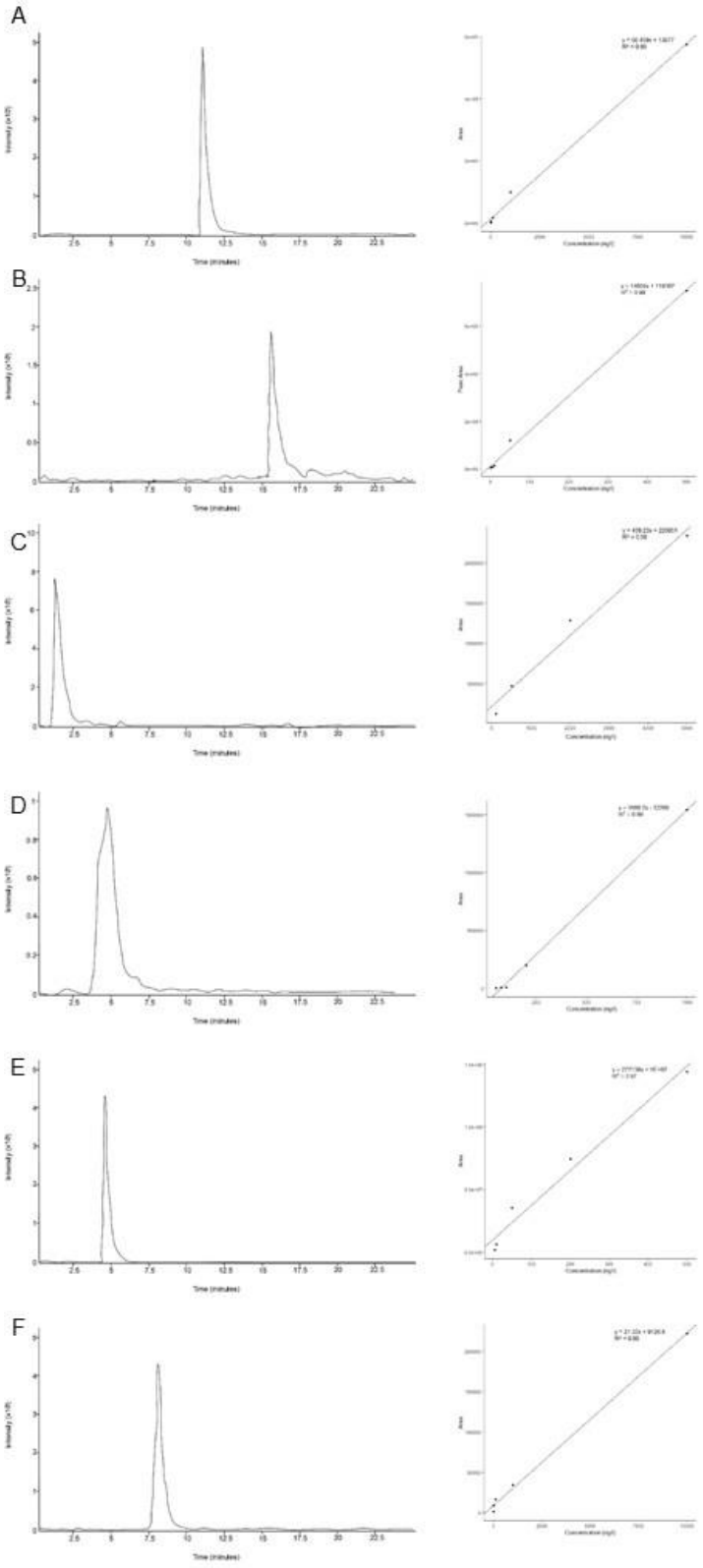


Figure 2.4: Chromatograms and calibration curves for target analyte standards (100 ng ml^{-1} in methanol) for (A)Citalopram (B)Diclofenac (C)Metformin (D)Ranitidine (E)Trimethoprim

2.5 References

Białk-Bielińska A, Kumirska J, Borecka M, Caban M, Paszkiewicz M, Pazdro K, Stepnowski P (2016) Selected analytical challenges in the determination of pharmaceuticals in drinking/marine waters and soil/sediment samples. J Pharm Biomed Anal 121: 271–296. doi: 10.1016/J.JPBA.2016.01.016

Camacho-Muñoz D, Martín J, Santos JL, Aparicio I, Alonso E (2010) Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Doñona Park (Spain) J Hazard Mat 183: 602-608 doi: 10.1016/j.jhazmat.2010.07.067

Capleton AC, Courage C, Rumsby P, Holmes P, Stutt E, Boxall ABA, Levy LS (2006) Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. Toxicol Lett 163: 213–223. doi: 10.1016/j.toxlet.2005.10.023

EMEA (2006) EMEA Guidance for RA. Available from: http://www.emea.eu.int [Accessed January 14, 2017].

Daouk S, Chèvre N, Vernaz N, Bonnabry P, Dayer P, Daali Y, Fleury-Souverain S (2015) Prioritization methodology for the monitoring of active pharmaceutical ingredients in hospital effluents. J Environ Manage 160: 324–32. doi: 10.1016/j.jenvman.2015.06.037

EPA (2007) Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. Available from: http://www.epa.gov/waterscience [Accessed February 27, 2019].

Kim M, Guerra P, Shah A, Parsa M, Alaee M, Smyth SA (2014) Removal of pharmaceuticals and personal care products in a membrane bioreactor wastewater treatment plant. Wat Sci Technol 69: 2221–2229. doi: 10.2166/wst.2014.145

Liu Y-H, Zhang S-H, Ji G-X, Wu S-M, Guo R-X, Cheng J, Yan Z-Y, Chen J-Q (2017) Occurrence, distribution and risk assessment of suspected endocrine-disrupting chemicals in surface water and suspended particulate matter of Yangtze River (Nanjing section) Ecotox Environ Safe 135: 90-97 doi: 10.1016/j.ecoenv.2016.09.035

National Health Service (2014) Prescriptions dispensed in the community, 2004-2014. Available from: http://wales.gov.uk/topics/statistics/headlines/health2013/prescriptions-dispensed-

community-2012/?lang=en. [Accessed February 10, 2019]

Petrovic M, Gros M, Barcelo D (2006) Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography–quadrupole–time-of-flight mass spectrometry. Journal Chromatogr A 1124: 68–81. doi: 10.1016/J.CHROMA.2006.05.024

Poole CF (2003) New trends in solid-phase extraction. Trends Anal Chem 22: 362–373. doi: 10.1016/S0165-9936(03)00605-8

Shrivastava A, Gupta VB (2011) Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron 2: 21–25. doi: 10.4103/2229-5186.79345

Vieira Madureira T, Barreiro JC, Rocha MJ, Cass B, Tiritan ME (2009) Pharmaceutical trace analysis in aqueous environmental matrices by liquid chromatography-ion trap tandem mass spectrometry. J Chromatogr A 1216: 7033–7042. doi: 10.1016/j.chroma.2009.08.060

Zhang Z, Zhou J (2007) Simultaneous determination of various pharmaceutical compounds in water by solid-phase extraction-liquid chromatography-tandem mass spectrometry. J Chromat A 1154(1-2): 205-213 doi: 10.1016/J.CHROMA.2007.03.105

Chapter 3: Comparison of Prioritisation Schemes for Human Pharmaceuticals in the Aquatic Environment

Published in Environmental Science and Pollution Research

Sarah Letsinger[1], Paul Kay[1]

[1]School of Geography, University of Leeds, Leeds, UK

Abstract

Only a small proportion of pharmaceuticals available for commercial use have been monitored in the aquatic environment, and even less is known about the effects on organisms. With thousands of pharmaceuticals in use, it is not feasible to monitor or assess the effects of all of these compounds. Prioritisation schemes allow the ranking of pharmaceuticals based on their potential as environmental contaminants, allowing resources to be appropriately used on those which are most likely to enter the environment and cause greatest harm. Many different types of prioritisation scheme exist in the literature and those utilising predicted environmental concentrations (PECs), the fish plasma model (FPM), critical environmental concentrations (CECs) and acute ecotoxicological data were assessed in the current study using the fifty most prescribed drugs in the United Kingdom. PECs were found to be overestimates of mean measured environmental concentrations but mainly underestimations of maximum concentrations. Acute ecological data identified different compounds of concern to the other effects assessments although the FPM and CECs methods were more conservative. These schemes highlighted antidepressants, lipid regulators, antibiotics, antihypertensive compounds and ibuprofen as priority compounds for further study and regulation.

3.1 Introduction

Concern over the presence of pharmaceuticals in the environment and the subsequent development of environmental risk assessments (ERAs) for these compounds began in the 1990s (Küster and Adler 2014). Currently, only Europe and the USA have specific ERA protocols for the assessment of pharmaceuticals, which are required to be completed in order to register them for commercial use (Adler et al. 2008). In 2006, an EU regulation on the Registration, Evaluation, Authorisation and Restriction of chemicals

(REACH) came into effect, and now all chemicals being manufactured in or imported to the EU must be assessed following ECHA guidelines, including information on potential risks and hazards to the environment (Ehrlich et al. 2011). However, prior to the implementation of such legislation, pharmaceuticals have been released into the environment unregulated for years. The number of human pharmaceuticals in use has been reported as being between 1,500 and 10,000 (Guo et al. 2016; Dong et al. 2013). Only a little over 200 of these have been monitored in freshwaters and fewer in marine waters, and even less is known about their impacts once they enter the aquatic environment (Fabbri and Franzellitti 2016; Hughes et al. 2013). This has left continuing uncertainty surrounding the environmental impacts of pharmaceuticals in the aquatic environment. The use of a prioritisation scheme can help address this by identifying a smaller set of compounds which have the potential to enter the environment and pose a biological risk. This can allow researchers and policy makers to direct resources towards further study; they can help decide which compounds need to be monitored in the environment and which require more information on their fate and biological effects (Mansour et al. 2016).

Many prioritisation schemes are based on existing ERAs, which include the calculation of predicted environmental concentrations (PECs) and an assessment of the risk to biota. PECs are usually derived from usage data on the volume of drugs produced per year, or number of prescriptions filled, which may be further refined based on processes which affect the compounds between production and entering the environment, such as metabolism, wastewater treatment, and dilution (Besse and Garric 2008). Often, where experimental data is missing or chemical properties are not known, simplified PECs, where little or no fate criteria are applied, may be calculated to facilitate quick assessment of a large number of chemicals (Ashton et al. 2004; Besse and Garric 2008; Kostich and Lazorchak 2008). As a result, the PECs calculated in such schemes give broad predictions for a country or large area and are not refined enough to give predictions at different spatial or temporal scales.

PECs are usually paired with assessments of hazards to biological organisms inhabiting the receiving environments. One such method is through the use of risk quotients, which determine if the predicted no effect concentrations (PNECs) of a compound exceed PECs. If the result is greater than 1 then the study compound is deemed to pose a threat (Hoyett et al. 2016). PNECs are usually calculated by selecting the most sensitive LC_{50} and applying an assessment factor (Backhaus and Faust 2012). Such experimental data is often unavailable in the literature, however, and it is time consuming to generate such data for a prioritisation scheme. Ecotoxicological structure-activity relationships

(ECOSAR) can be used to calculate chronic and acute LC_{50} values and are allowed under REACH guidelines (Sanderson et al. 2004, Ortiz de García et al. 2013)

Pharmaceuticals are unique contaminants as they are designed to be biologically active and, unlike many other environmental contaminants, information from the medical literature on the pathways and effects of pharmaceuticals in vertebrates is abundant. This information has been utilised to produce alternative methods of assessing the hazard of pharmaceuticals to biota. Fish are not biochemically different from vertebrates and share many of the same drug targets (Huggett et al. 2003). The fish plasma model utilises this information and compares the human therapeutic concentration to a calculated fish plasma concentration. Vertebrates are usually more sensitive to chemicals than invertebrates, due to shared targets. It is thought that this model is a scheme sufficient to predict the environmental hazard of chemicals (LaLone et al. 2014).

Despite their extensive development, the prioritisation schemes which exist in the literature are varied and often highlight different compounds of concern (Besse and Garric 2008; Donnachie et al. 2016; Roos et al. 2012). Moreover, it can be difficult to compare them as they are applied to different data sets and scenarios which can make it hard to understand which compounds really are of most concern or to select a scheme for use in research and management. The aim of this paper was, therefore, to use a range of common prioritisation schemes to assess the environmental risk of the fifty most prescribed pharmaceuticals in the UK, highlight compounds of concern, and make suggestions as to the efficacy of the different schemes.

3.2 Methods

3.2.1 Predicted Environmental Concentrations

3.2.1.1 Calculations

Information on the quantity of pharmaceuticals prescribed was obtained from data released monthly by the National Health Service England for 2014 (NHS, 2014). The 50 most prescribed compounds during this period were used for this assessment. For each compound, the monthly and annual mass of prescriptions was calculated (Appendix 3.1).

PEC_A was calculated using (Eq. 3.1), where A is the amount of pharmaceuticals dispensed (kg $year^{-1}$), E is the fraction of the compound excreted unchanged, V is the volume of waste water per capita per day (assumed to be 200 litres), P is the population of England in 2014, and D is the dilution of waste water (assumed to be 10 times; EMEA 2006). This method was derived from the approach detailed in the EU technical guidance for risk assessment of human pharmaceuticals (EU 2003). Excretion rates were obtained

from peer reviewed literature or online databases and the highest excretion rate was used in the calculation (Appendix 3.2). PEC_B further refined this equation by applying the removal rate for pharmaceuticals in wastewater treatment plants (WWTPs ;Eq. 3.2), where R is the removal rate. Removal rates were obtained from peer reviewed literature and where multiple removal rates were published for the same compound, the lowest was chosen in order to create a more conservative estimate (Appendix 3.2). If no removal rate, or a negative one, was found then it was assumed to be 0. PEC_C included further refinement; taking into account metabolism and removal in wastewater (Eq. 3.3).

$$\mathrm{PEC_A} = \frac{\mathrm{A} * \mathrm{E}}{\mathrm{V} * \mathrm{P} * \mathrm{D} * 365} \quad \text{(Eq. 3.1)}$$

$$\mathrm{PEC_B} = \frac{\mathrm{A} * (1 - \mathrm{R})}{365 * \mathrm{P} * \mathrm{V} * \mathrm{D}} \quad \text{(Eq. 3.2)}$$

$$\mathrm{PEC_C} = \frac{\mathrm{A} * \mathrm{E} * (1 - \mathrm{R})}{\mathrm{V} * \mathrm{P} * \mathrm{D} * 365} \quad \text{(Eq. 3.3)}$$

PEC_D (Eq. 3.4) is derived from the EMEA guidelines and does not require prescription data to be calculated. Instead, it includes the proportion of the population being treated with a particular drug (Fpen), where a suggested value of 1% is used (EMEA 2006). Dose is the maximum dosage per person and Cap_{stp} is the capacity of the local WWTP (assumed to be 10,000; EMEA 2006). The EMEA guidelines also suggest the inclusion of information on the fraction of the compound absorbed to suspended matter. Due to the unavailability of this data for most compounds this was not included (Besse et al. 2008).

$$\mathrm{PEC_D} = \frac{\mathrm{Elocal_{water}} * (1 - \mathrm{R})}{\mathrm{V} * \mathrm{D} * \mathrm{Cap_{stp}}} \quad \text{(Eq. 3.4)}$$

$$\mathrm{Elocal_{water}} = \mathrm{Dose} * \mathrm{E} * \mathrm{Fpen} * \mathrm{Cap_{stp}} \quad \text{(Eq. 3.5)}$$

Each compound was ranked by each of the PEC calculations (Appendix 3.3) and the mass prescribed annually in order to compare how the different schemes altered the predicted relative environmental risk.

3.2.1.2 Comparison with Environmental Concentrations

In order to compare the PECs to measured environmental concentrations (MECs) data were taken from monitoring studies carried out in the United Kingdom (Baker and Kasprzyk-Hordern 2013; Bound and Voulvoulis 2006; Burns et al. 2017; Burns et al. 2018a; Kasprzyk-Hordern et al. 2008; Kasprzyk-Hordern et al. 2009; Kay et al. 2017; Nakada et al. 2017; Roberts and Thomas 2006; Ashton et al. 2004). Only monitoring studies from surface water were included, measurements from influent and effluent were omitted. The mean MEC across all studies was calculated and compared to each of the PECs along with the maximum MEC.

3.2.2 Effect Data

3.2.2.1 Fish Plasma Model

The FPM was calculated according to Huggett et al. (2003). This model compares the human therapeutic plasma concentration (H_TPC) and the fish steady state concentration ($F_{ss}PC$) to give an effective ratio (ER), a measure of risk (Eq. 3.6). $F_{ss}PC$ was estimated for each of the PEC values calculated in 2.1.1 (Eq. 3.7) and the H_TPC was obtained by using the peak serum concentration that is reached in humans after the drug has been administered (cmax). Where multiple cmax values were found, the higher value was used in this assessment (Appendix 3.4).

$$\mathrm{ER} = \frac{H_T PC}{F_{ss} PC} \qquad \text{(Eq. 3.6)}$$

$$\mathrm{F_{ss}PC} = \mathrm{PEC\ x\ P_{Blood:Water}} \qquad \text{(Eq. 3.7)}$$

$$\mathrm{logP_{Blood:Water}} = 0.73 * \mathrm{log_{kow}} - 0.88 \qquad \text{(Eq. 3.8)}$$

The compounds were ranked from lowest to highest by ER. Huggett et al. (2003) suggested that compounds with an ER<1000 may warrant further assessment.

3.2.2.2 Critical Environmental Concentrations

Critical environmental concentrations (CECs) were proposed by Fick et al. (2010) and utilise the concept of the FPM but are independent of environmental concentrations. CECs are calculated by the ratio (Eq. 3.9) of H_TPC and $P_{Blood:Water}$ (Eq. 3.8).

$$\mathrm{CEC} = \frac{H_T PC}{P_{Blood:Water}} \quad \text{(Eq. 3.9)}$$

3.2.2.3 Risk Quotients

Information on the acute toxicity of each of the compounds was obtained from reviews containing comprehensive experimental ecotoxicological data or studies containing such data provided by pharmaceutical companies (Sanderson and Thomsen 2009, Sangion and Gramatica 2016a, Vestel et al. 2016). For compounds not included in these studies, LC_{50} values were obtained from risk assessments or scientific literature (Appendix 3.4). Values were only included if they followed standard protocols (for example, OECD, US EPA), used at least five concentrations in the exposures and at least three replicates per treatment. This data was unavailable for 12 compounds, so ECOSAR (v 1.11) was used to estimate LC_{50} values although the model was unable to estimate these for 7 of the compounds. A relative ranking, where the ranking was divided by the number of compounds in the scheme, was used in order to compare rankings across all effect schemes.

Risk quotients (RQ) were calculated by dividing the lowest LC_{50} value for fish, algae or daphnia by each of the PECs calculated in 2.1.1. An assessment factor of 1000 was applied in order to account for any uncertainties and provide a more conservative assessment. Those compounds with a RQ > 1 deemed to be hazardous to the environment.

3.3 Results

3.3.1 Exposure Criteria

3.3.1.1 Comparison of predicted environmental concentrations between schemes

Metformin, gabapentin, flucloxacillin, amoxicillin, naproxen and ibuprofen were ranked in the top 10 across all PEC schemes, whereas tamsulosin, ethinylestradiol, fluticasone, budesonide, beclomethasone, felodipine, and tiotropium were ranked in the bottom 10 (Figure 3.1). These compounds were in the top 10 and bottom 10 respectively when ranked by the amount dispensed annually. For most compounds, there was less than a 10 place difference between schemes (Appendix 3.3). Where larger differences occurred it can mostly be attributed to different results between schemes which utilised usage data (PEC_A, PEC_B and PEC_C) and PEC_D which did not. However, the PEC values for individual compounds did differ greatly depending on which scheme was used.

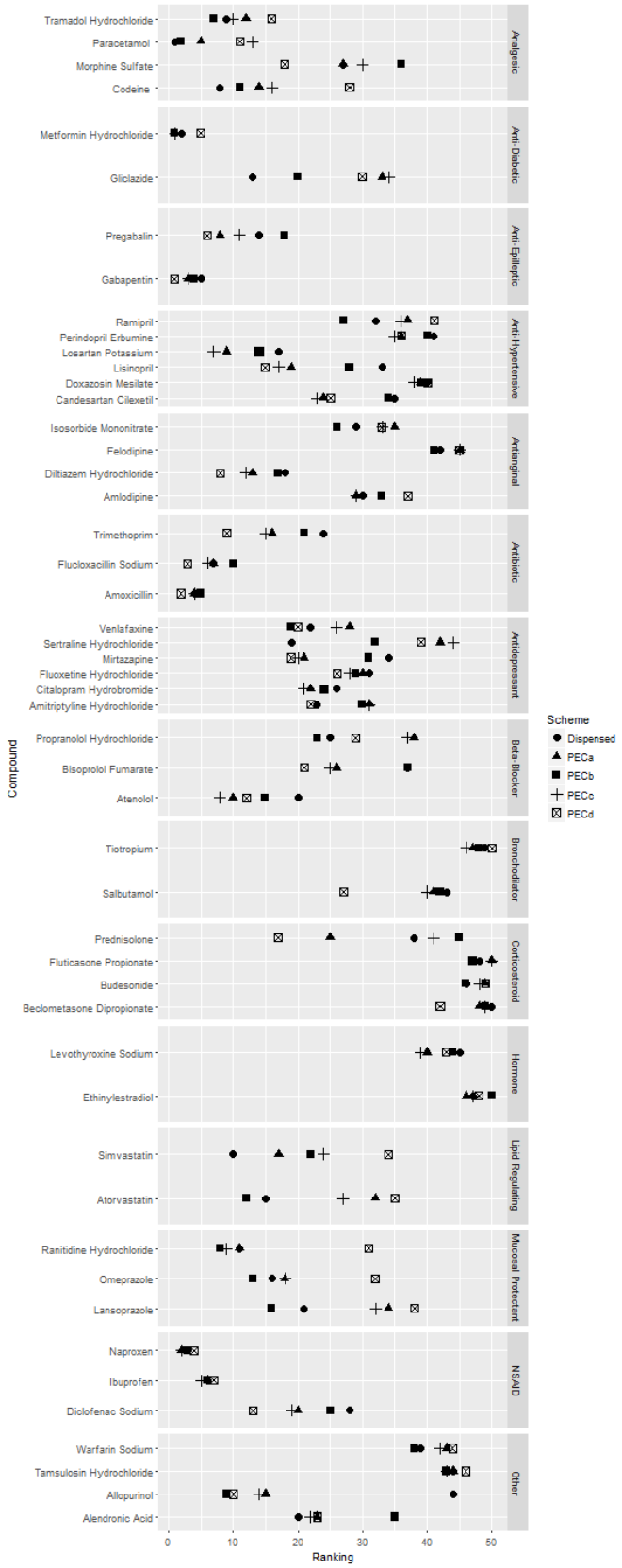


Figure 3.1 Comparison of the ranking of pharmaceuticals by compound class between predicted environmental concentration schemes

3.3.1.2 Comparison with measured environmental concentrations

MECs in the UK were available for 24 out of the 50 study compounds. Of these, warfarin sodium, sertraline prednisolone and fluticasone propionate were below the method detection limit (MDL) in all studies. All of the schemes underestimated the maximum concentrations for tramadol, salbutamol, paracetamol, ibuprofen and ethinylestradiol (Figure 3.2). Maximum MECs were overestimated for amoxicillin, diltiazem, gabapentin and naproxen by all schemes. For the other compounds, PEC_B overestimated maximum concentrations more than the other schemes.

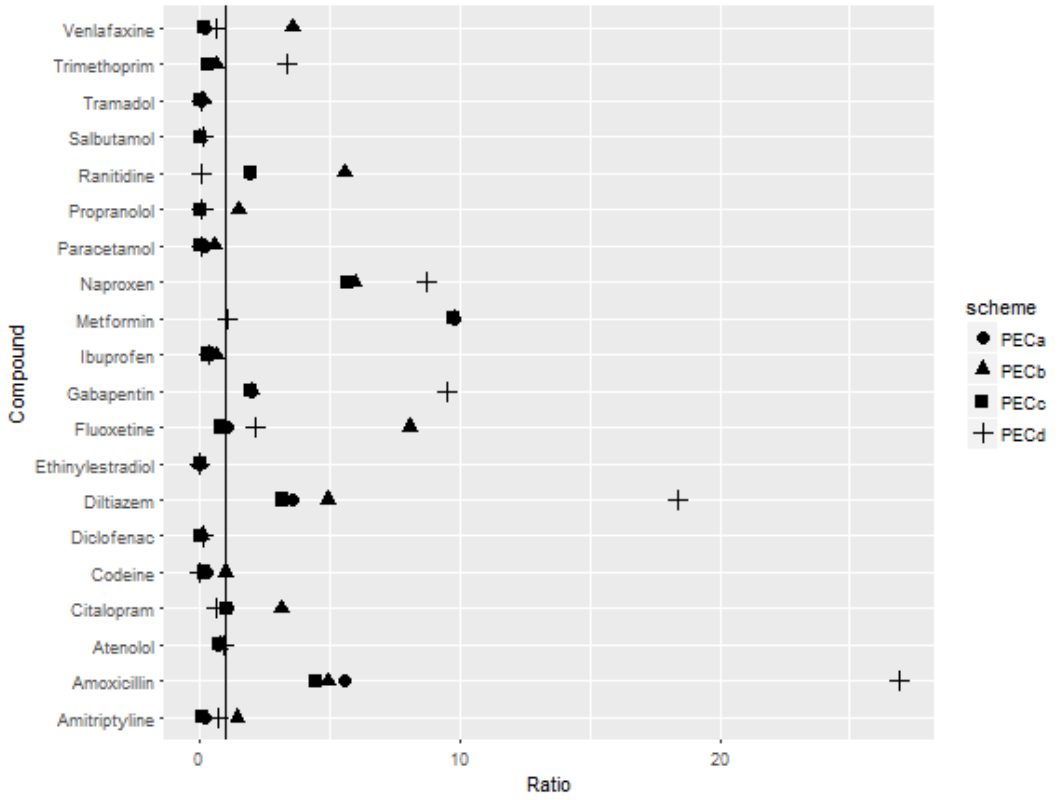


Figure 3.2 Ratio of PEC: maximum MEC for each of the schemes. The line denotes a ratio of 1.

All PECs were overestimates of mean MECs for all of the compounds, with the exception of ethinylestradiol and salbutamol (Figure 3.3). PEC_A, PEC_C and PEC_D also underestimated the MECs of propranolol and tramadol. Further to this PEC_C and PEC_D underestimated the MECs for paracetamol and codeine respectively. The ratio for mean MECs was much higher than those for maximum MECs for all compounds. PEC_D overestimated MECs to a greater degree than the other schemes, and PEC_C more accurately predicted the mean MECs than the other schemes.

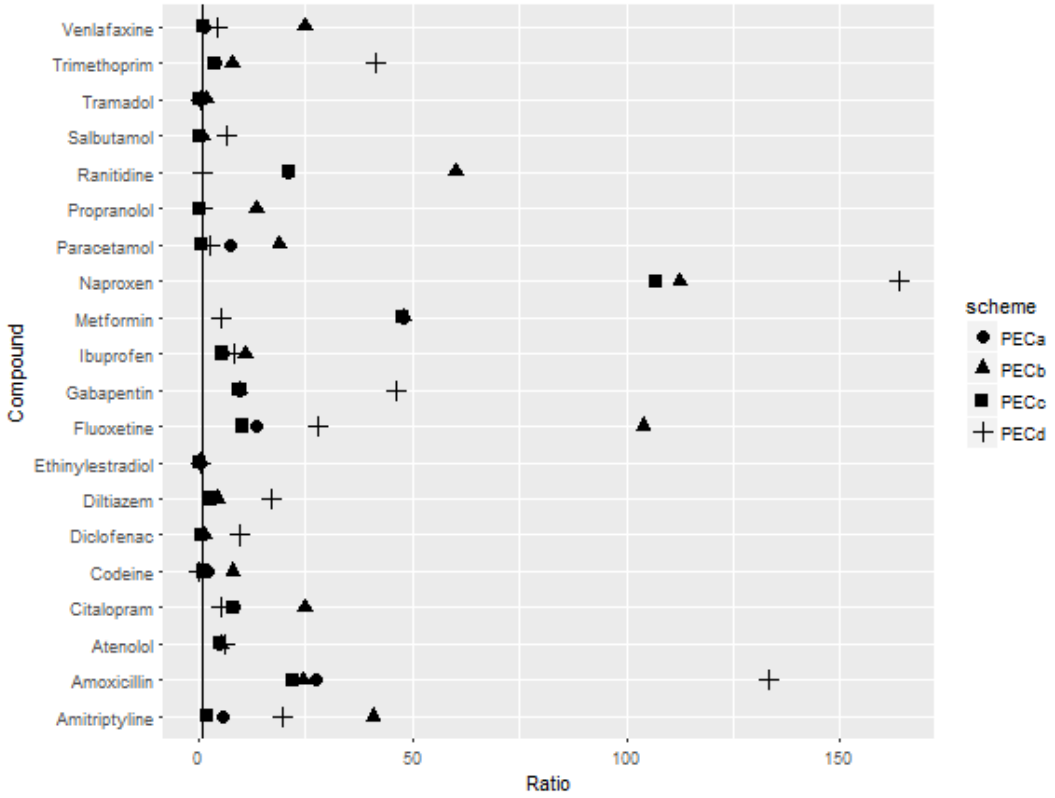


Figure 3.3 Ratios of PEC: mean MEC for each of the schemes. The line denotes a ratio of 1

3.3.2 Effect Criteria

For many of the compounds, LC_{50} values resulted in the opposite ranking to the other schemes (Appendix 3.5). The FPM, LOG_{KOW} and CEC schemes resulted in simvastatin, atorvastatin, candesartan, ibuprofen and losartan being ranked in the top 25%, however, the LC_{50} ranked these compounds as lower priority (Figure 3.4). The opposite was true for allopurinol, alendronic acid, beclomethasone and amoxicillin. Pregabalin, gabapentin, isosorbide mononitrate and tiotropium were ranked in the bottom 25% across all schemes. CECs highlighted some compounds as priority that the other schemes did not; ethinylestradiol, fluticasone propionate and beclomethasone diproprionate had a higher relative ranking before the inclusion of PEC values. As a compound class, antidepressants and antibiotics were given a high priority ranking, whereas bronchodilators and mucosal protectants were not.

All compounds had an ER ratio < 1000, with the exception of tiotropium and alendronic acid, where the ER exceeded this value with all PECs (Appendix 3.6). Isosorbide mononitrate also had an ER < 1000 for FPM_A, and FPM_C. Less compounds exceeded the RQ value of 1; all PECs resulted in an RQ > 1 for amoxicillin (Appendix 3.6). PEC_B resulted in the RQ being exceeded for the allopurinol and fluoxetine and PEC_D for allopurinol, fluoxetine and flucloxacillin.

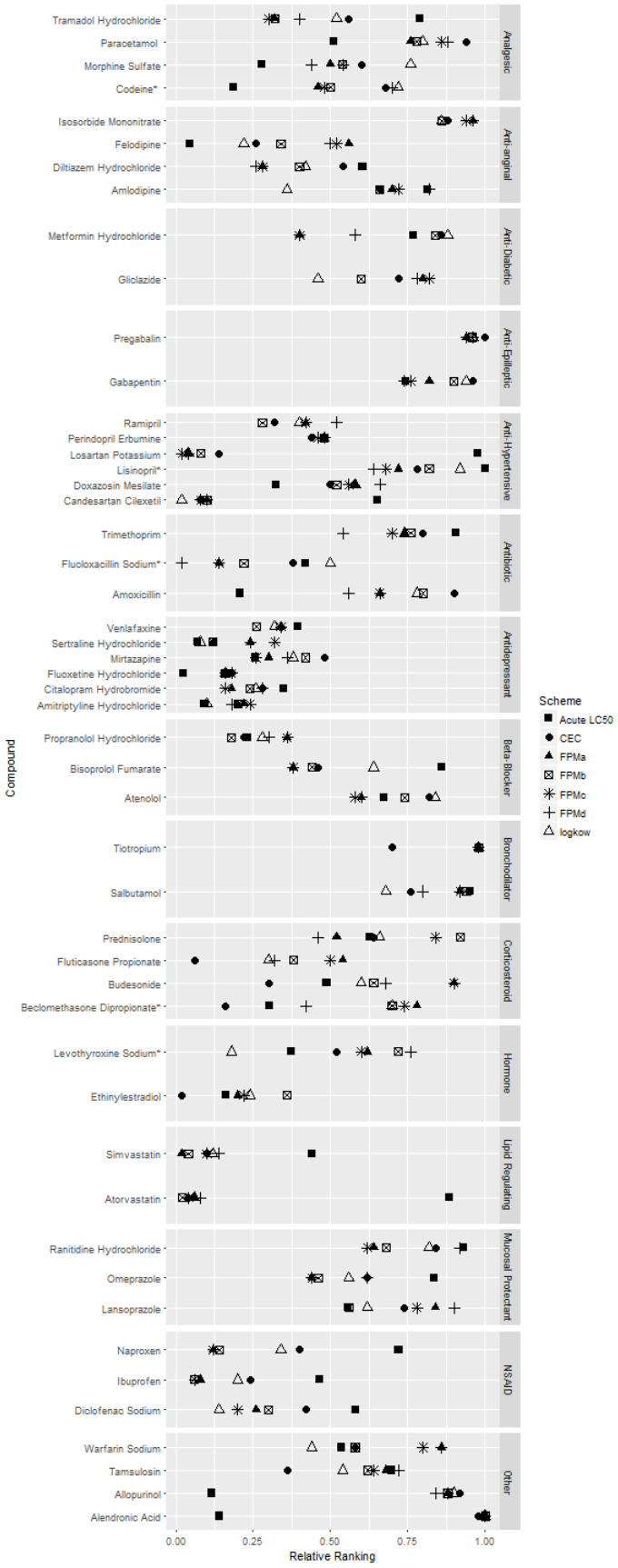


Figure 3.4 Comparison of the relative ranking of pharmaceuticals by compound class between effect schemes

3.4 Discussion

3.4.1 Comparison of schemes for predicted environmental concentrations

For many of the compounds in this assessment, the ranking within each PEC scheme was correlated with the amount dispensed, which has also been found in other prioritisation studies (Ashton et al. 2004; Roos et al. 2012). Of the compounds which were ranked in the top ten across all schemes, metformin, amoxicillin, naproxen and ibuprofen have previously occurred on many priority lists (Burns et al. 2018b). Gabapentin and flucloxacillin have only been listed of concern in one prioritisation exercise each (Helwig et al. 2013; Ortiz de Garcia et al. 2013) and, as a result, fewer monitoring studies include these compounds. PEC_D results were less closely related with the amount of compound dispensed, as this was not included in the calculation. Instead, PEC_D used the maximum dosage and assumed 1% of the population was taking the compound. It is unsurprising that compounds which have a higher dosage are also prescribed at higher masses. However, for many compounds, the usage has been found to surpass 1% (Pereira et al. 2017). As a result, the inclusion of usage data in risk assessments is very important and, where this is not available (e.g. many developing countries), its production should be seen as a high priority by governments. As over the counter (OTC) sales of some products have been attributed to up to 50% of this, it is very important that these figures are available for risk assessment purposes (Guo et al. 2016). Of the compounds assessed in the current study, paracetamol, ibuprofen, diclofenac, omeprazole and naproxen are available OTC in the United Kingdom. Even though OTC data were not available, omeprazole was ranked between 10 and 20 across all schemes and if OTC sales were also included, it could be much more important in terms of environmental impact. Furthermore, many pharmaceuticals are also used for veterinary purposes and these data are needed for more accurate PEC calculations.

Although, for the majority of compounds, ranking by the amount of pharmaceutical dispensed may be sufficient to estimate relative environmental exposure, some compounds undergo extensive metabolism or removal in WWTPs, making some refinement necessary. Amoxicillin, metformin, gabapentin, ibuprofen and naproxen are prescribed in such high numbers that the application of removal and excretion data has little impact on their relative ranking. Gliclazide, on the other hand, had a 20 place ranking difference between the amount dispensed and PEC_A due to its extensive excretion. Those which were ranked between 20 and 40 showed more variability in their ranking between schemes than those at the top and bottom end, as they were dispensed in similar amount to other compounds. Information on the metabolism of pharmaceuticals

was available in the scientific literature and pharmaceutical databases, with little variation in reported values.

Removal rates during wastewater treatment had less of an effect on the ranking of compounds than excretion rate. It is possible that this is the result of the overall lack of information of this process or variability within the data, depending on external factors such as temperature and WWTP efficiency (Golovko et al. 2014). For example, removal of metformin has been reported to be as low 0% and as high as 99% (Santos et al. 2013). Variability such as this can have a great impact on the ranking of compounds; PEC_B included the lower rate of removal of 0% which resulted in a ranking of 1, however, using the higher removal rate of 99% would have resulted in the lower ranking of 23. Furthermore, in some cases, an increase in the compound concentration has been seen in effluent as the result of conversion back to the parent compound in WWTPs and so a negative removal rate would have to be used in a PEC scheme to accurately take this occurrence into account (Paíga et al. 2016).

3.4.2 Comparison of predicted environmental concentrations with measured environmental concentrations

In the majority of cases, the PECs failed to accurately represent the MECs; mean MECs were mostly overestimated, and half of the maximum MECs were underestimated by all schemes. PEC_A, PEC_B and PEC_C were most accurate in estimating mean MECs, despite overestimations. The MECs of naproxen were the least accurately identified, with PECs overestimating maximum concentrations by a factor of 6-10, and mean concentrations by a factor of 106 to 163. Nevertheless, these afford a degree of environmental safety. When interpreting these results, the lack of available monitoring data needs to be taken into consideration and many compounds were only measured at one time point and at one or two sites. Concentrations of some pharmaceuticals have been shown to fluctuate depending on seasonal and environmental conditions, so more thorough monitoring studies are needed to further validate methods for producing PECs (Moreno-Gonzalez et al. 2015). Ferrari et al. (2004) compared PEC_B and the highest MECs for five pharmaceuticals in wastewater effluent and rivers in France and Germany. In German effluents, these concentrations were accurately predicted for carbamazepine and diclofenac, but were underestimated (although by less than a factor of 10) for propranolol, clofibric acid and sulfamethoxazole, and overestimated for oflaxin. However, in French effluents, MECs were overestimated for all compounds showing that the scenario being assessed is important when choosing a PEC model and that local factors which could affect concentrations are considered. Burns et al. (2017) also compared MECs and PECs which were calculated using local hydrological information alongside

lowest removal and highest excretion rates. MECs were accurately predicted in one river but not another, which was attributed to missing inputs. The inclusion of local hydrological information such as this may help to produce more accurate PECs.

PEC_A, PEC_B and PEC_C rely upon the accuracy of usage data to form reliable estimates. Besides the compounds available OTC, prescription data may not always be an accurate representation of the usage of compounds. It is unlikely that all pharmaceuticals prescribed will be consumed, and a survey of 400 people in South-Eastern England showed that only 53% of people finish their medication (Bound and Voulvouslis 2005), and another survey in the United States showed that more than 98% of people disposed of their unused medication in household waste or down the sink and toilets (Kupis and Krenzelok 1996). Kostich and Lazorchak (2008) tried to add estimates of unconsumed pharmaceuticals into their PEC calculations assuming that approximately 5% of drugs prescribed for long-term therapy were wasted compared to 15% prescribed for short term therapy and 33% for topical use. Whilst naproxen is a prescription only NSAID, it is often prescribed short-term or on an as needed basis for pain management, and as a result could help explain the over-estimate of its concentrations.

3.4.3 Comparison of effect based methods

FPM, Log_{KOW} and CEC schemes resulted in different rankings to acute LC_{50} and triggered different compounds for further assessment, which is concurrent with other recent studies, showing that Log_{KOW} has a strong influence on these calculations (Roos et al. 2012). Additionally, FPMs were more conservative than RQs, triggering more compounds for further assessment. Thus, simply ranking compounds by log_{KOW} could be a useful approach for determining the relative hazard pharmaceuticals pose to biota. Nevertheless, although log_{KOW} is used in FPM and CEC models, it does not necessarily indicate the compound will be toxic, but instead that it is likely to be taken up by fish at a level sufficient to have a biological effect (Schrieber et al. 2011). Instead, it is suggested that those with an ER less than 1000 warrant further assessment (Huggett et al. 2003). Log_{KOW} values have been used as predictors for bioconcentration however this measurement was originally developed for non-polar chemicals, and as a result does not work for many chemicals (Schrieber et al. 2011).

The use of acute LC_{50} and QSAR in order to assess the potential hazard of pharmaceuticals has been debated. Although LC_{50} values are derived from experimental work, they can be influenced by variables such as the number of concentrations assessed (Hoyett et al. 2016). The primary concern relating to pharmaceuticals in the environment is the potential chronic exposure to low levels, and not acute toxicity. As a

result, they may affect endpoints which are not covered by traditional risk assessments (Johnson et al. 2017). QSARs have been used to model the potential toxicity of contaminants to fish, daphnia and algae. There are several QSAR models which have been proposed for use in predicting ecotoxicity of pharmaceuticals which have found to vary in accuracy (for example, de Roode et al. 2006; Sangion and Gramatica, 2016a).

There is evidence that fish are more sensitive than algae or invertebrates as they retain many of the same drug targets as humans (Donnachie et al. 2016). The FPM was developed in order to utilise this information. A read-across approach can be used in assessing the potential risk of pharmaceuticals to invertebrates and algae. Fish share 86% of targets with humans, 61% have been found to be conserved in daphnia and 35% in algae (Gunnarsson et al. 2008). There is particular concern surrounding the toxicity of antibiotics and statins to algae, in part due to conserved pathways, but also due to the inhibition of symbiotic bacteria (Guo et al. 2015). CEC resulted in a higher ranking for statins and two of the antibiotics than LC_{50} values. Amoxicillin, on the other hand, was highlighted by its acute toxicity and not by the FPM. Only the RQ which included PEC_A exceeded 1 for amoxicillin, whereas this was exceeded by all of the FPM schemes. As a result, the FPM and CEC will add a degree of protection for organisms besides fish.

For many compounds, FPM and CECs resulted in similar rankings. The minor influence PEC has on FPM confirms what has been found in other comparisons between prioritisation schemes (Roos et al. 2012). However, ethinylestradiol, fluticasone propionate and beclomethasone were highlighted by CECs, but not by FPMs as the PEC values for these compounds were small. In this case, ethinylestradiol had a low PEC, however MECs were much higher than this. Ethinylestradiol is a compound on the EU's priority watch list due to concern over its potential effects at environmentally relevant concentrations. Johnson et al. (2017) ranked chemicals based on their measured environmental concentrations in UK Rivers and measured ecotoxicity concentrations, and found that ethinylestradiol was highlighted as posing the greatest risk. As a result it is important that PEC results are accurate if FPM is going to be used. The use of an assessment factor or ER value of 1000 allows for the most conservative estimate of risk whilst accounting for uncertainty in the PEC values.

3.4.4 Selecting a prioritisation scheme

It is important to consider the inclusion of compounds into a scheme to begin with. Metoprolol, carbamazepine, aspirin and sulfamethoxazole were four of the most cited pharmaceuticals of concern in the prioritisation literature but were not in the 50 most prescribed compounds (Donnachie et al. 2016). The high number of prescriptions does

not necessarily translate into a large mass of the compound; bronchodilators, for example, were prescribed in high numbers, but at a very low mass. As a result, certain compounds may be overlooked and it may be necessary to select compounds based on their mass as well as prescription numbers.

Of the PEC schemes used in this assessment, PEC_A is the most suitable for assessing the relative exposure risk as it requires limited data, but also conservatively estimates the likelihood of pharmaceuticals entering the environment. It can be used to select pharmaceuticals for which to further refine PECs based on local criteria before selection of compounds for monitoring in the environment. Where information on the number of prescriptions is not available, PEC_D is a better alternative as it can work within the confines of available data.

Assessment of the potential effects of pharmaceuticals should be used alongside PEC evaluations. Log_{KOW} offers a quick and easy method for assessing the relative risk, based on potential bioaccumulation. The use of CECs and FPM add an extra level of refinement, based on utilising information on mammalian effects. FPM appears to give a conservative approach to prioritising pharmaceuticals in comparison to acute RQs. As a result, those compounds which exceed the RQ threshold should be of priority. The use of CECs over FPMs allows the ranking of compounds independent of PECs. However, both exposure risk and potential effects should be included, as compounds found at small concentrations could still be enough to warrant an effect. For example ethinylestradiol was ranked as a low priority by the PEC schemes, but inclusion of effect information increased its ranking.

When prioritising pharmaceuticals, it is essential to take a holistic approach which conservatively highlights potential compounds of concern which warrant further assessment. It is important to consider why the exercise is being carried out and the question it is trying to address. There will not be a one size fits all approach, and not all schemes will be appropriate in all situations. As a result, the limitations to each of these schemes needs to be kept in mind.

3.4.5 Compounds of concern

The combination of PEC and effect criteria clearly highlight groups which should be a priority for further research. Some assessments have only added one compound from each class to the priority list, assuming that each class will have a similar mode of action and similar effect (Besse and Garric, 2008). Antidepressants were ranked high across all of the effect schemes, and moderately for PECs too. Overall ranking between compounds does not differ much, however, fluoxetine may be of most of concern due to

exceeding the RQ threshold values when none of the others did. Fluoxetine is commonly present on priority lists, however some rankings have pointed towards sertraline, citalopram and amitriptyline as representing a greater hazard (Besse and Garric 2008; Roos et al. 2012; Sangion and Gramatica 2016b). Many of these antidepressants have been found to have an effect on biota at environmentally relevant concentrations and the use of FPM also highlights this (Silva et al. 2015). To the authors' knowledge, this is the first prioritisation exercise which has highlighted mirtazapine and venlafaxine to be a potential concern.

Similarly to antidepressants, candesartan and losartan had moderate PEC rankings but high effect rankings for FPM, CEC and LC_{50}, whilst other anti-hypertensives had a low ranking across both PEC and effect schemes. These compounds are not commonly included in prioritisation exercises, however, losartan has been present on priority lists previously (Besse and Garric 2008). Candesartan had a higher ranking across schemes and as a result may be more of a concern. The lipid regulators, atorvastatin and simvastatin also had moderate to low PECs. However, their high ranking among CECs and FPM means they warrant further investigation.

Amoxicillin and flucloxacillin were two of four compounds to exceed a RQ value of 1. Both of these compounds were ranked highly as the result of PEC values. The effect rankings of flucloxacillin were much higher than those of amoxicillin. Flucloxacillin is not commonly present in monitoring or effects studies and there is still uncertainty about its occurrence and impacts so it could be seen as a priority compound.

Ibuprofen was ranked in the top 10 of all of schemes, with the exception of acute LC_{50}. Ibuprofen is the fifth most prioritised compound in the prioritisation literature (Burns et al. 2018b). The environmental impact of ibuprofen pollution has been the focus of many studies and its repeat presence on priority lists and high rankings in the current study indicate the importance in understanding its fate and effects.

Allopurinol may also warrant further assessment due to its high exposure ranking and RQ value. Whilst it had a low ranking for FPM, CEC and Log_{KOW} values it had an ER < 1000. Allopurinol has been stated to be a highly prescribed drug in other EU countries (Küster and Adler, 2014; Roos et al. 2012) although Roos et al. (2012) carried out a comparison of first-tier prioritisation schemes, including FPM, on 582 pharmaceuticals in Sweden, and did not find it to be a high priority. However, it has been highlighted on other priority lists based on exposure and effect criteria (Besse and Garric, 2008; Linert et al. 2007). Despite this, it is not present in the monitoring or ecotoxicity literature and it

has only been monitored in coastal waters in Spain, where it was not detected (Rodriguez-Navas et al. 2013).

Other compounds such as metformin and gabapentin are ranked in the top by PEC schemes, but inclusion of effect criteria decreased their ranking. However, due their high PECs, moderate effect rankings across FPM and acute LC_{50} values, they may still warrant further assessment. It is particularly important to understand their occurrence and fate. Metformin in particular may be of concern as it now a widely used drug, and its usage has increased rapidly over the last decade (Oosterhuis et al. 2013).

This assessment also clearly highlights compounds which are not of concern. Bronchodilators were ranked in the bottom of all schemes and corticosteroids were ranked at the bottom across all PEC schemes. This is concurrent with other prioritisation exercises. As a result, these compounds are not commonly featured in monitoring campaigns or experimental effects work. Although the priority ranking increased with the application of effect criteria, it was still low.

3.4.6 Future direction for the management of pharmaceuticals in the environment

There is some evidence that EU policy has not used risk assessment approaches to accurately identify compounds of concern. In the present study, ibuprofen and naproxen had a higher PEC and effect ranking than diclofenac even though the latter has been placed on the EU priority watch list. This could perhaps be attributed to the fall in diclofenac's usage over the past few years though (Mavragani et al. 2016). Ethinylestradiol is another compound included on the EU priority watch list even though it had a low PEC ranking and similar effect ranking; only CECs ranked it as a priority. Similar results were seen in comparison of first-tier risk assessments by Roos et al. (2012), where FPM did not result in a high ranking for ethinylestradiol but CEC and three other schemes did. As pharmaceuticals are designed to be biologically active, it is important that there is an understanding of these pathways in non-target organisms in order to create better risk assessments.

There has been an increasing interest in the occurrence of pharmaceuticals in environmental compartments other than effluent and water such as sediment and marine environments. Comparatively little is known about the occurrence of pharmaceuticals in these areas (Fabbri and Franzellitti 2016; Gaw et al. 2014) and use of the PEC schemes employed here may not appropriately predict presence in these compartments. Other properties, such as lipophilicity, pH and sediment type may be more relevant in predicting the presence of pharmaceuticals in sediments, and in turn the potential risks to biota which live within these systems (Al-Khazrajy and Boxall 2016). Salinity is also a defining

factor of marine waters and it is hypothesised that the physical-chemical characteristics of some compounds may change in marine waters. For example, the partition coefficient between sediment and water for estrone increases with increasing salinity, meaning concentrations will be lower (Pal et al. 2010).

All pharmaceuticals are metabolised to a different degree, yet only two prioritisation schemes have included metabolites (Besse and Garric 2008; Capleton et al. 2006). If metabolism and degradation play a significant role in the fate of pharmaceuticals then metabolites will be present in the environment. Few studies have covered the occurrence and effects of metabolites, many of which are inert, but some of which have been found to be pharmacologically active and even toxic (García-Cambero et al. 2015).

3.5 Conclusion

Prioritisation schemes should include assessments of the potential of a compound to enter the environment as well its potential toxicity. Excretion of pharmaceuticals had a large influence on the ranking of PECs for different compounds, and as a result should be included in these calculations. CECs should be used alongside PECs in order to assess potential hazard; both of these schemes result in a conservative estimate of risk, and highlight compounds which warrant further assessment. Antidepressants, statins, antibiotics candesartan, losartan and ibuprofen were highlighted as the substances of greatest environmental concern.

3.6 References

Adler NE, Koschorreck J, Rechenberg B (2008) Environmental impact assessment and control of pharmaceuticals: the role of environmental agencies. Wat Sci Technol 57. doi: 10.2166/wst.2008.816

Al-Khazrajy OSA, Boxall ABA (2016) Impacts of compound properties and sediment characteristics on the sorption behaviour of pharmaceuticals in aquatic systems. J Hazard Mater. doi: 10.1016/j.jhazmat.2016.05.065

Ashton D, Hilton M, Thomas KV (2004) Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Sci Total Environ 333: 167–184. doi: 10.1016/j.scitotenv.2004.04.062

Backhaus T, Faust M (2012) Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ Sci Technol 46: 2564–2573. doi: 10.1021/es2034125

Baker DR, Kasprzyk-Hordern B (2013) Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: New developments. Sci Total Environ 454–455: 442–456. doi: 10.1016/j.scitotenv.2013.03.043

Besse JP, Kausch-Barreto C, Garric J (2008) Exposure assessment of pharmaceuticals and their metabolites in the aquatic environment: application to the french situation and preliminary prioritization. Hum Ecol Risk Assess 14: 665–695. doi: 10.1080/10807030802235078

Bound JP, Voulvoulis N (2006) Predicted and measured concentrations for selected pharmaceuticals in UK rivers: Implications for risk assessment. Wat Res 40: 2885–2892. doi: 10.1016/j.watres.2006.05.036

Burns EE, Thomas-Oates J, Kolpin DW, Furlong ET, Boxall AB. (2017) Are exposure predictions, used for the prioritization of pharmaceuticals in the environment, fit for purpose? Environ Toxicol Chem 36: 2823–2832. doi: 10.1002/etc.3842

Burns EE, Carter LJ, Kolpin DW, Thomas-Oates J, Boxall ABA (2018a) Temporal and spatial variation in pharmaceutical concentrations in an urban river system. Wat Res 137: 72-85 doi: 10.1016/j.watres.2018.02.066

Burns EE, Carter LJ, Snape JR, Thomas-Oates J, Boxall ABA (2018b) Application of prioritization approaches to optimize environmental monitoring and testing of pharmaceuticals. J Toxicol Environ Health B Crit Rev 21(3): 115-141 doi: 10.1080/10937404.1465873

Capleton AC, Courage C, Rumsby P, Holmes P, Stutt E, Boxall ABA, Levy LS (2006) Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. Toxicol Lett 163: 213–223. doi: 10.1016/j.toxlet.2005.10.023

Daouk S, Chèvre N, Vernaz N, Bonnabry P, Dayer P, Daali Y, Fleury-Souverain S (2015) Prioritization methodology for the monitoring of active pharmaceutical ingredients in hospital effluents. J Environ Manage 160: 324-332

de Roode D, Hoekzema C, de Vries-Buitenweg S, van de Waart B, van der Hoeven J (2006) QSARs in ecotoxicological risk assessment. Regul Toxicol Pharmacol 45(1): 24-35 doi: 10.1016/j.yrtph.2006.01.012

Dong Z, Senn DB, Moran RE, Shine JP (2013) Prioritizing environmental risk of prescription pharmaceuticals. Regul Toxicol Pharmacol 65: 60–67. doi:

10.1016/j.yrtph.2012.07.003

Donnachie RL, Johnson AC, Sumpter JP (2016) A rational approach to selecting and ranking some pharmaceuticals of concern for the aquatic environment and their relative importance compared with other chemicals. Environ Toxicol Chem 35: 1021–1027. doi: 10.1002/etc.3165

EMEA (2006) Guideline on the environmental risk assessment of medicinal products for human use. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003978.pdf.

EU (2003) Environmental risk assessment for human medicinal products containing or consisting of GMOs, III/5504/94 draft 4. Available from: https://echa.europa.eu/documents/10162/16960216/tgdpart2_2ed_en.pdf

Fabbri E, Franzellitti S (2016) Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. Environ Toxicol Chem 35: 799–812. doi: 10.1002/etc.3131

Ferrari B, Mons R, Vollat B, Fraysse B, Paxéus N, Lo Giudice R, Pollio A, Garric J (2004) Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environ Toxicol Chem 23: 1344. doi: 10.1897/03-246

Fick J, Lindberg RH, Tysklind M, Larsson DGJ (2010) Predicted critical environmental concentrations for 500 pharmaceuticals. Regul Toxicol Pharmacol 58: 516–523. doi: 10.1016/j.yrtph.2010.08.025

García-Cambero J., García-Cortés H, Valcárcel Y, Catalá M (2015) Environmental concentrations of the cocaine metabolite benzoylecgonine induced sublethal toxicity in the development of plants but not in a zebrafish embryo–larval model. J Hazard Mater 300: 866–872. doi: 10.1016/j.jhazmat.2015.08.019

Gaw S, Thomas KV, Hutchinson TH (2014) Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos Trans R Soc Lon B, Biol Sci 369(1656): 1-11 doi: 10.1098/rstb.2013.0572

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014) Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111: 418–426. doi: 10.1016/j.chemosphere.2014.03.132

Guo J, Boxall ABA, Selby K (2015) Do pharmaceuticals pose a threat to primary producers? Crit Rev Environ Sci Technol 45(23): 2565-2610 doi: 10.1080/10643389.2015.1061873

Guo J, Sinclair CJ, Selby K, Boxall ABA (2016) Toxicological and ecotoxicological risk-based prioritization of pharmaceuticals in the natural environment. Environ Toxicol Chem doi: 10.1002/etc.3319

Helwig K, Hunter C, McNaughtan M, Roberts J, Pahl O (2013) Ranking prescribed pharmaceuticals in terms of environmental risk: Inclusion of hospital data and the importance of regular review. Environ Toxicol Chem 35(4): 1043-1050 doi: 10.1002/etc.3302

Hoyett Z, Owens MA, Clark CJ, Abazinge M (2016) A comparative evaluation of environmental risk assessment strategies for pharmaceuticals and personal care products. Ocean Coast Manage 127: 74–80. doi: 10.1016/j.ocecoaman.2016.04.013

Huggett DB, Cook JC, Ericson JF, Williams RT (2003) A Theoretical Model for Utilizing Mammalian Pharmacology and Safety Data to Prioritize Potential Impacts of Human Pharmaceuticals to Fish. Hum Ecol Risk Assess 9: 1789–1799. doi: 10.1080/714044797

Hughes SR, Kay P, Brown LE (2013) Global Synthesis and Critical Evaluation of Pharmaceutical Data Sets Collected from River Systems. Environ Sci Technol 47: 661–677. doi: 10.1021/es3030148

Johnson AC, Donnachie RL, Sumpter JP, Jürgens MD, Moeckel C, Pereira MG (2017) An alternative approach to risk rank chemicals on the threat they pose to the aquatic environment. Sci Total Environ 599–600: 1372–1381. doi: 10.1016/j.scitotenv.2017.05.039

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Wat Res 42: 3498–3518. doi: 10.1016/j.watres.2008.04.026

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Wat Res 43: 363–380. doi: 10.1016/j.watres.2008.10.047

Kay P, Hughes SR, Ault JR, Ashcroft AE, Brown LE (2017) Widespread, routine

occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. Environ Pollut 220: 1447-1455 doi: 10.1016/j.envpol.2016.10.087

Kostich MS, Lazorchak JM (2008) Risks to aquatic organisms posed by human pharmaceutical use. Sci Total Environ 389: 329–339. doi: 10.1016/j.scitotenv.2007.09.008

Küster A, Adler N (2014) Pharmaceuticals in the environment: scientific evidence of risks and its regulation. Philos Trans R Soc Lon B, Biol Sci 369(1656): 1-8 doi: 10.1098/rstb.2013.0587

LaLone CA, Berninger JP, Villeneuve DL, Ankley GT (2014) Leveraging existing data for prioritization of the ecological risks of human and veterinary pharmaceuticals to aquatic organisms. Philos Trans R Soc Lon B, Biol Sci 369(1656): 1-10 doi: 10.1098/rstb.2014.0022

Linert J, Güdel K, Escher BI (2007) Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. Environ Sci Technol 41(12): 4471-4478 doi: 10.1021/es0627693

Mansour F, Al-Hindi M, Saad W, Salam D (2016) Environmental risk analysis and prioritization of pharmaceuticals in a developing world context. Sci Total Environ 557: 31–43. doi: 10.1016/j.scitotenv.2016.03.023

Mavragani A, Sypsa K, Sampri A, Tsagarakis K (2016) Quantifying the UK Online Interest in Substances of the EU Watchlist for Water Monitoring: Diclofenac, Estradiol, and the Macrolide Antibiotics. Water 8(11): 1-21 doi: 10.3390/w8110542

Moreno-González R, Rodriguez-Mozaz S, Gros M, Barceló D, León VM (2015) Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). Environ Res 138: 326–344. doi: 10.1016/j.envres.2015.02.016

Nakada N, Hanamoto S, Jürgens MD, Johnson AC, Bowes MJ, Tanaka H (2017) Assessing the population equivalent and performance of wastewater treatment through the ratios of pharmaceuticals and personal care products present in a river basin: Application to the River Thames basin, UK. Sci Total Environ 575: 1100–1108. doi: 10.1016/J.SCITOTENV.2016.09.180

NHS (2014) GP practice prescribing presentation-level data. Available from: http://content.digital.nhs.uk.

Oosterhuis M, Sacher F, ter Laak TL (2013) Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. Scie Total Environ 442: 380-388 doi: 10.1016/J.scitotenv.2012.10.046

Ortiz de García S, Pinto GP, García-Encina PA, Mata RI (2013) Ranking of concern, based on environmental indexes, for pharmaceutical and personal care products: An application to the Spanish case. J Environ Manage 129: 384–397. doi: 10.1016/j.jenvman.2013.06.035

Paíga P, Santos LHMLM, Ramos S, Jorge S, Silva JG, Delerue-Matos C (2016) Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Sci Total Environ 573: 164–177. doi: 10.1016/j.scitotenv.2016.08.089

Pal A, Gin KYH, Lin AYC, Reinhard M (2010) Impacts of emerging organic contaminants on freshwater resources: Review of recent occurrences, sources, fate and effects. Sci Total Environ 408: 6062–6069. doi: 10.1016/j.scitotenv.2010.09.026

Pereira AMPT, Silva LJG, Lino CM, Meisel LM, Pena A (2017) A critical evaluation of different parameters for estimating pharmaceutical exposure seeking an improved environmental risk assessment. Sci Total Environ 603: 226–236. doi: 10.1016/j.scitotenv.2017.06.022

Roberts PH, Thomas K V. (2006) The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ 356: 143–153. doi: 10.1016/j.scitotenv.2005.04.031

Rodrígues-Navas C, Björklund E, Bak SA, Hansen M, Krogh KA, Maya F, Forteza R, Cerdà V (2013) Pollution pathways of pharmaceutical residues in the aquatic environment on the Island of Mallorca, Spain. Arch Environ Contam Toxicol 65(1) 56-66 doi: 10.1007/s00244-013-9880-x

Roos V, Gunnarsson L, Fick J, Larsson DGJ, Rudén C (2012) Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Sci Total Environ 421–422: 102–110. doi: 10.1016/j.scitotenv.2012.01.039

Sanderson H, Johnson DJ, Reitsma T, Brain RA, Wilson CJ, Solomon KR (2004) Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regul Toxicol Pharmacol 39: 158–183. doi: 10.1016/j.yrtph.2003.12.006

Sanderson H, Thomsen M (2009) Comparative analysis of pharmaceuticals versus

industrial chemicals acute aquatic toxicity classification according to the United Nations classification system for chemicals. Assessment of the (Q)SAR predictability of pharmaceuticals acute aquatic toxicit. Toxicol Lett 187: 84–93. doi: 10.1016/j.toxlet.2009.02.003

Sangion A, Gramatica P (2016a) Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity. Environ Int 95: 131-143 doi: 10.1016/j.envint.2016.08.008

Sangion A, Gramatica P (2016b) PBT assessment and prioritisation of contaminants of emerging concern: Pharmaceuticals. Environ Res 147: 297-306 doi: 10.1016/j.envres.2016.02.021

Santos LH, Gros M, Rodriguez-Mozaz S, Delerue-Matos C, Pena A, Barceló D, Montenegro MC (2013) Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. Sci Total Environ 461–462: 302–316. doi: 10.1016/J.SCITOTENV.2013.04.077

Schrieber R, Gundel U, Franz S, Kuster A, Rechenberg B, Attenburger R (2011) Using the fish plasma model for comparative hazard identification for pharmaceuticals in the environment by extrapolation from human therapeutic data. Regul Toxicol Pharmacol 61: 261–275. doi: 10.1016/J.YRTPH.2011.08.006

Silva LJG, Pereira AMPT, Meisel LM, Lino CM, Pena A (2015) Reviewing the serotonin reuptake inhibitors (SSRIs) footprint in the aquatic biota: Uptake, bioaccumulation and ecotoxicology. Environ Pollut 197: 127–143. doi: 10.1016/j.envpol.2014.12.002

Vestel J, Caldwell DJ, Constantine L, D'Aco VJ, Davidson T, Dolan DG, Millard SP, Murray-Smith R, Parke NJ, Ryan JJ, Straub JO, Wilson P (2016) Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environ Toxicol Chem 35: 1201–1212. doi: 10.1002/etc.3260

Chapter 4: Spatial and Temporal Occurrence of Pharmaceuticals in UK Estuaries

Accepted to Science of the Total Environment

Sarah Letsinger[1], Paul Kay[1], Sara Rodríguez-Mozaz[2], Marta Villagrassa[2], Damià Barceló[2] Jeanette M. Rotchell[3]

[1] School of Geography, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT

[2] Catalan Institute for Water Research (ICRA), H_2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, E-17003, Girona, Spain

[3] School of Environmental Sciences, University of Hull, Cottingham Road, Hull, HU6, 7RX

Abstract

There is a lack of data on the occurrence of pharmaceuticals in estuaries worldwide, with little understanding of their temporal and spatial variations globally. Ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram were measured in twelve estuaries in the UK. Initially, these compounds were monitored in the Humber Estuary, where samples were taken every two months over a twelve month period in order to assess their spatial and temporal variations. Ibuprofen was found at some of the highest concentrations ever measured in an estuary globally (18 – 6297ng l^{-1}), with paracetamol also measured at relatively high concentrations (4 – 917 ng l^{-1}) in comparison to the other compounds. In terms of spatial distribution, a pattern was observed, where highest concentrations were found at a site where wastewater is discharged, whilst compound concentrations were often lower upstream and downstream of this site. The downstream profile of pharmaceuticals differed temporally with concentrations highest downstream when input from wastewater effluent was highest. Eleven further estuaries were sampled around the UK in order to put the occurrence of pharmaceuticals seen in the Humber Estuary into a wider context. Pharmaceutical concentrations in the other estuaries sampled were less than 210 ng l^{-1}, but, again, ibuprofen and paracetamol were found at concentrations higher than other compounds, whereas diclofenac and citalopram were absent from many estuaries. The Humber, which is the receiving environment for the sewage effluent for approximately 20% (13.6 million people) of the population of England, was observed to have the highest overall concentration of pharmaceuticals in

contrast to the other estuaries sampled, thereby representing a worst case scenario for pharmaceutical pollution.

4.1 Introduction

Despite the extensive and long-term use of pharmaceuticals, it has only been in the past few decades that interest in pharmaceutical pollution has gained popularity and now hundreds of pharmaceuticals have been detected in the aquatic environment (Hughes et al. 2013; Gaw et al. 2014). Their presence in the aquatic environment is sustained through continuous input from wastewater treatment plants (WWTPs), as well as from improper disposal, agriculture and aquaculture (Godoy et al. 2015). Pharmaceuticals are designed to be biologically active, often at low levels, and their presence in surface water has led to concern over their potential biological effect (Santos et al. 2010). Many pharmaceuticals (e.g. diclofenac and fluoxetine) have been found to illicit a negative response on biota in laboratory exposures at concentrations similar to those found in the aquatic environment (Eades and Waring 2010; Franzellitti et al. 2013; Minguez et al. 2016).

The fate of pharmaceuticals is best understood in the freshwater environment, with input, environmental conditions, biological degradation and sediment-related processes playing a prominent role in their spatial and temporal distribution (Li 2014). Pharmaceuticals often show a decline in concentration downstream from input sources as the result of dilution, degradation and partitioning to sediment (Kunkel and Radkle 2012). However, due to the prevalence of WWTPs, this leads to the continuous input of pharmaceuticals into the environment. As a result, these processes are not enough to sufficiently remove compounds leading to their high detection in the aquatic environment and potentially, transportation into estuaries and coastal waters (Ebele et al. 2017).

Estuaries are receiving waters, often for many rivers, acting as a confluence for contaminants, therefore increasing the potential risk of pharmaceutical pollution in these environments (Ridgway and Shimmield 2002). Estuaries are ecologically important to ecosystem services, providing habitat for many species and acting as an area for recreation and transport (Ridgway and Shimmield 2002). Despite this, few studies have measured the occurrence of pharmaceuticals in estuaries, and those that do, exist typically lack the resolution to determine spatial and temporal patterns (Table 4.1). Studies which have investigated the spatial and temporal patterns of pharmaceuticals are often locally focused, monitoring only one estuary (for example Tamtam et al., 2012; Hedgespeth et al. 2012; Cantwell et al. 2017) and it is important to determine if any patterns seen are relevant at a wider scale. It is important to examine the fate of these

compounds across a wider spatial scale in order to determine whether they pose a risk to the environment.

Table 4.1: Maximum concentrations of ibuprofen, paracetamol, diclofenac and trimethoprim detected in estuaries globally (ng l^{-1}) Citalopram has not previously been monitored in any estuaries.

Region	Estuary	Ibuprofen	Paracetamol	Diclofenac	Trimethoprim	Reference
Asia	Jiulong, China	21	13	11		Sun et al. (2016)
	Hailing Bay, China				37	Chen et al. (2015)
	Qinzhou Bay, China			7		Cui et al. (2019)
	Yangtze, China			<MDL		Yang et al. (2011)
	Yangtze, China				330	Zhang et al. (2012)
	Yangtze, China		<MDL			Zhao et al. (2015)
Europe	Seine, France				45	Tamtam et al. (2008)
	Elbe, Germany	1		1		Weigel et al. (2002)
	Arade, Portugal	28	88	31		Gonzalez-Rey et al. (2015)
	Douro, Portugal				16	Madureira et al. (2010)
	Tejo, Portugal	<MDL	11	52	8	Reis-Santos et al. (2016)
	Bilbao, Spain		440	650	2046	Mijangos et al. (2018)
	Plentzia, Spain		49	22	6	Mijangos et al. (2018)
	Urdaibai, Spain		321	35	3	Mijangos et al. (2018)
	Belfast Lough, UK	376	<MDL	<MDL	32	Thomas and Hilton (2004)
	Mersey, UK	386	<MDL	195	569	Thomas and Hilton (2004)
	Tees, UK	88	<MDL	191	17	Thomas and Hilton (2004)
	Thames, UK	928	<MDL	125	<MDL	Thomas and Hilton (2004)
	Thames, UK				19	Munro et al. (2019)
	Tyne, UK	755		90	46	Thomas and Hilton (2004)
North America	Charleston Harbour, USA	8	28			Hedgespeth et al. (2012)
	Jamaica Bay, USA	38	156		125	Benotti and Brownawell (2007)
	Narragansett Bay, USA		60		18	Cantwell et al. (2017)
	New York Bay, USA		162		14	Cantwell et al. (2018)
	San Francisco, USA				4	Klosterhaus et al. (2013)
Oceania	Sydney, Australia		31			Birch et al. (2015)

This study aimed to further contribute to the overall picture of pharmaceutical contamination in estuaries. Five target compounds — ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram were chosen for the present study, based on their prevalent usage and predicted risk to the aquatic environment (National Health Service 2017; Roos et al. 2012). To the author's knowledge, citalopram has not previously been monitored in the estuarine environment (Table 4.1). Moreover,

monitoring of the aforementioned compounds is limited, with some of these measurements dating back almost 15 years. The target compounds were measured every other month over a twelve month period at various sites in the Humber Estuary to determine their spatial and temporal occurrence. In addition, eleven further estuaries, located in other parts of the UK, were selected in order to determine whether concentrations observed in the Humber were representative of other estuaries.

4.2 Methods

4.2.1 Study Area

The Humber Estuary is a macrotidal estuary located in Yorkshire, on the East Coast of England, UK (Figure 4.1). It is 303 km^2, has an average depth of 6.5 m and is the confluence for the Rivers Ouse, Trent and Hull which pass through some of the largest urban areas in the UK, thus it is the receiving water for approximately 20% of UK effluent (European Environment Agency, 2017; Table 4.2). Samples were collected from nine sites along a 65 km stretch on the North side of the estuary (Figure 4.1). Two of these were located in the River Ouse: A1 (20 km from Humber) was the furthest upstream and A2 was located less that 1km upstream from the confluence with the Humber Estuary. The furthest site upstream in the Humber Estuary (R1) was the receiving site for effluent from Melton WWTP, which serves a population equivalent (PE) of 12,255 (European Environment Agency, 2017). Three sites (R2-R4) were positioned every 2 km downstream from R1. Three final sites (A3-A5) were located 20km from R1 in the lower estuary and 15 km from the mouth. Further information on site location can be found in Appendix 4.1. The Humber Estuary is an important site for conservation and has been designated as a Special Protection Area (SPA), also containing a Special Area of Conservation (SAC). It is also a vital habitat for many species of international importance, providing habitat for 4.1% of the red knot (*Calidris canutus*) and 5.7% of the common redshank *(Tringa tetanus*) international populations, and as a result has also been designated as a RAMSAR site (Buck et al. 1997)

Samples were also collected from eleven further estuaries which encompassed a range of estuary types, tidal ranges and sizes (Table 4.2). The total PE was calculated for the WWTPs in the catchment area of each estuary (Table 4.2); further information on the proximity of WWTPs to the sampling sites in each estuary can be found in Appendix 4.2. Many of these estuaries have been designated as SACs, SPAs and RAMSAR sites as the result of the sensitive and important species resident to them.

Table 4.2: Information on the type and size of estuaries sampled (Davidson et al.1991). Information on the number of WWTPs and the population equivalent served in 2014 was calculated from an interactive wastewater treatment map (European Environment Agency 2017).

Estuary	Type	Estuary Area (km^2)	Tidal Type	Number of WWTPs in Catchment	Total PE (000s)
Cromarty	Complex	92.3	Mesotidal	3	15.6
Forth	Complex	84.0	Macrotidal	33	1 613.3
Humber	Coastal Plain	303.6	Macrotidal	304	13 674.7
Mersey	Coastal Plain	89.1	Macrotidal	30	3 689.7
Portsmouth	Ria	15.9	Macrotidal	2	383
Severn	Coastal Plain	556.8	Macrotidal	171	6 724.4
Solway	Complex	420.6	Macrotidal	20	314.9
Tay	Complex	121.3	Mesotidal	12	167.6
Tees	Coastal Plain	13.5	Macrotidal	9	844.9
Thames	Coastal Plain	46.5	Macrotidal	198	16 510.5
Tyne	Complex	7.9	Macrotidal	6	1 092.8
Ythan	Barbuilt	2.8	Mesotidal	1	11.2

4.2.2 Sampling

4.2.2.1 Seasonal monitoring

Sampling was carried out in the Humber Estuary, UK, every two months from October 2016 to August 2017 at sites R1-R4 (Figure 4.1). Samples were also collected from four additional sites (A1-A2 and A4-A5) in October, February and June, and a further site (A3) in February and June (Figure 4.1). Sampling was carried out during a high neap tide (± 3 hours) to minimise differences in diurnal concentrations as the result of tides (Lara-Martin et al. 2014). At each site, 3 x 1 L of surface seawater were collected in amber glass bottles and temperature, pH and dissolved oxygen were determined using a HACH meter and salinity (0 – 27 ppt) measured with a refractometer (Appendix 4.1). Water samples were kept on ice or in the fridge at 4 °C and extracted within 48 hours for analysis of pharmaceuticals.

4.2.2.2 UK wide monitoring

Sampling was carried out in eleven additional UK estuaries in order to provide a wider context for the concentrations of pharmaceuticals seen in the Humber Estuary (Figure 4.1). Sampling was carried out in August and September 2017 and samples were also collected during high tides (± 3 hours). Within each estuary, sites were chosen in the upper, middle and lower parts of the estuary and 1 L of water was collected at each of these in amber glass bottles. (Appendix 4.2). Temperature, pH, dissolved oxygen and salinity (0-34 ppt) were determined as above and samples were stored and extracted in the same manner (Appendix 4.2).

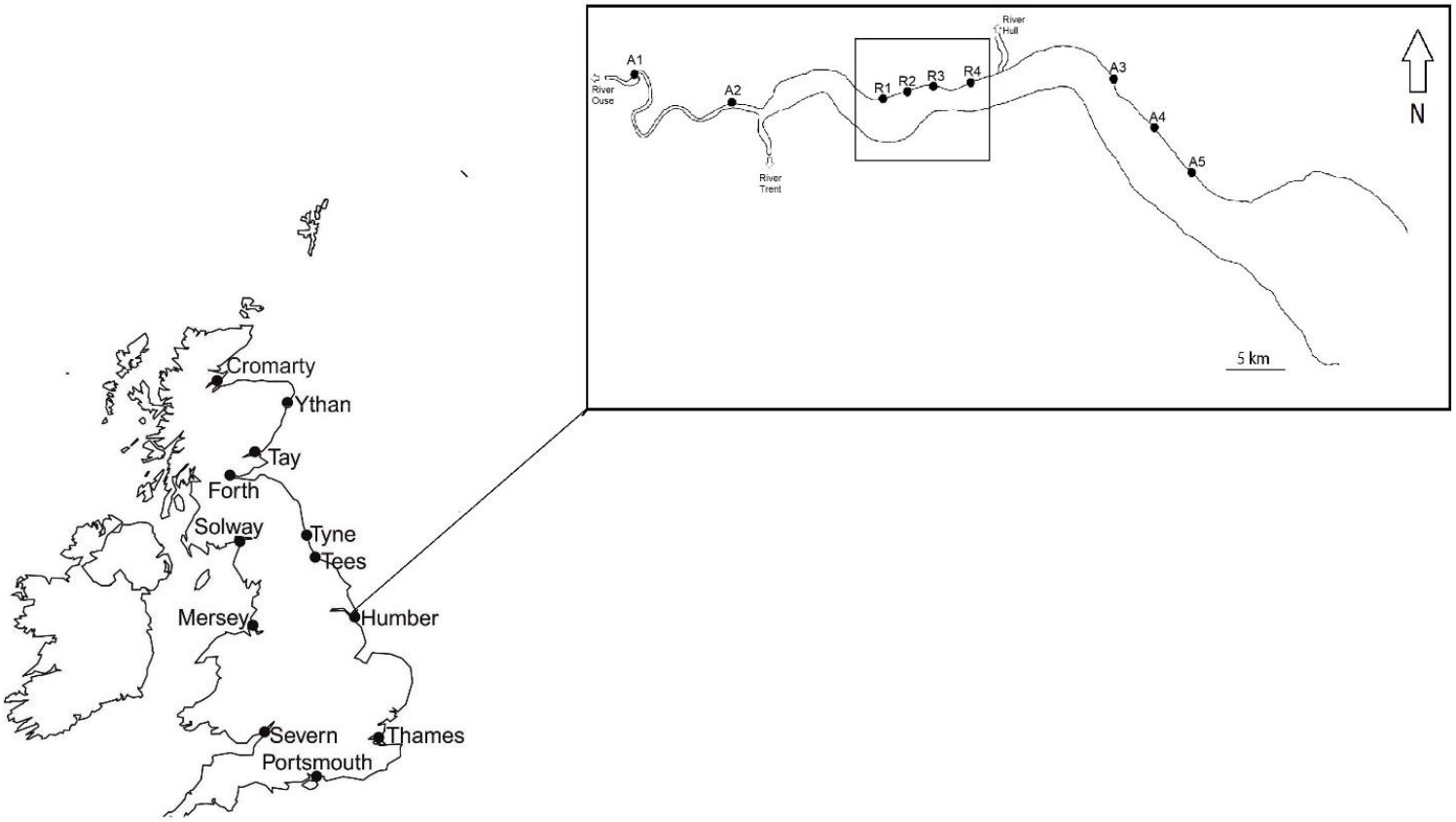


Figure 4.1 Map of field sites for seasonal and UK wide monitoring of selected pharmaceuticals. The sites in the box (R1-R4) indicate those which were sampled every two months. A1-A2 and A3-A5 were sampled every four months

4.2.3 Chemical Analysis

4.2.3.1 Study Compounds

Five study compounds — ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram, were chosen for monitoring (Table 4.3). Standards of diclofenac sodium (≥98.5), acetaminophen (≥99%), citalopram (≥98), ibuprofen (≥98%), and trimethoprim (≥98%) were supplied by Sigma-Aldrich Ltd. (Dorset, UK).

Table 4.3: Physico-chemical characteristics of the study compounds. Physico-chemical data obtained from USEPA (2019). Prescription data obtained from (National Health Service 2019; Appendix 4.3).

Compound	Therapeutic Use	Prescriptions (kg year^{-1})	Water Solubility (mg l^{-1})	Log$_{KOW}$	Molecular Weight	pKa
Ibuprofen	NSAID	82,756	21	3.79	206.29	4.9
Paracetamol	Painkiller	2,169,244	14,000	0.9	151.16	9.9
Diclofenac	NSAID	5459	2.37	4.51	296.15	4.2
Trimethoprim	Antibiotic	8444	400	0.7	290.32	7.1
Citalopram	Antidepressant	9204	31	3.74	324.39	9.4

4.2.3.2 Solid Phase Extraction

A composite sample was made, by combining the 3 x 1L surface water samples collected from each site during seasonal monitoring, or from each of the estuaries during the UK-wide survey; they were added together in a 5 L beaker and stirred vigorously for two minutes. A 500 mL subsample was taken and filtered through a 0.45 µm cellulose filter (Scientific Laboratory Supplies, Hessle, UK) under vacuum. Solid phase extraction was performed on the filtered water samples using Oasis HLB cartridges (Waters Corporation, Massachusetts, USA), which were conditioned with 5 mL 100% methanol followed by 5 mL deionised water at a rate of 1 mL min^{-1}. The sample was loaded on to

the cartridge at a rate of 10 mL min^{-1}, during which care was taken not to let the sorbent material dry out. The cartridges were then rinsed with 5 mL deionised water. The sorbent was dried under vacuum for 15 minutes to remove excess water prior to elution. Elution was performed with 5 mL 0.1% TFA in methanol, followed by a further 5 mL. The eluent was evaporated to dryness using a rotary evaporator (40ºC, speed 7) and reconstituted with methanol: water (10:90).

SPE recovery was evaluated by spiking known concentrations (100, 200, and 1000 ng l^{-1}) of all study compounds into three replicates each of artificial seawater made up to 20 ppt in deionised water (Appendix 4.4). The mean recovery across all concentrations was used to correct the measured environmental concentration (Table 4.4).

Table 4.4: Mean method detection limits (± standard deviation), mean method quantification levels (± standard deviation) and mean recovery (± standard deviation) of target compounds.

Compound	MDL (ng l^{-1})	MQL (ng l^{-1})	Recovery (%)
Citalopram	0.34 (0.25)	1.18 (0.85)	43 (5.5)
Diclofenac	1.77 (1.35)	5.91 (4.49)	20 (11.0)
Ibuprofen	1.45 (0.41)	4.83 (1.38)	73 (34.0)
Paracetamol	3.28 (1.82)	10.93 (6.07)	86 (34.1)
Trimethoprim	0.07 (0.04)	0.24 (0.12)	63 (10.6)

4.2.3.3 UltraperformanceTM-ESI-(QqLIT) MS/MS analysis

Analysis was carried out according to Gros et al. (2012). Briefly, chromatographic separations were performed with a Waters Acquity Ultra-Performance liquid chromatograph system equipped with two binary pumps systems (Milford, Massachusetts, USA), and coupled to a 5500 QTRAP hybrid quadrupole-linear ion trap mass spectrometer with a turbo ion spray source (Applied Biosystems, Foster Systems, Foster City, CA, USA). Citalopram and trimethoprim were analysed under positive electrospray ionisation (PI) using an Acquity HSS T_3 column (50 mm x 2.1 mm, 1.8µm particle size) and ibuprofen, paracetamol and diclofenac were analysed under negative ion (NI) electrospray using an Acquity BEH C_{18} column (5 mm x 2.1 mm, 1.7 µm particle size), both from Waters Corporation.

All data acquisition was performed in Analyst 2.1 software. Quantification of analytes was performed by selective reaction monitoring (SRM), monitoring two transitions for

each compound as described in Gros et al. (2012). Method detection limits (MDL) and Quantification levels (MQL) were determined for each of the compounds based on a signal-to-noise ratio of 3 and 10, respectively (Table 4.4).

4.2.4 Statistical Analysis

Statistical analysis was performed in R 3.3.1. In order to determine if there was a difference in the occurrence of pharmaceutical between sampling months, concentrations from Melton, North Ferriby, Hessle East and Hessle West were grouped together, as these sites were sampled during all of the sampling periods. A Friedman's Test followed by a Nemenyi post-hoc test were conducted using the PMCMR package (Pohlert 2014). Relationships between pharmaceutical concentrations and site-specific physico-chemical properties (Salinity, pH and dissolved oxygen) were investigated using a linear model. All data is presented in graphs created by the ggplot2 package (Wickham 2016).

4.3 Results

4.3.1 Humber Estuary

Pharmaceuticals were frequently detected (58 - 97% of samples for individual study compounds) in the Humber Estuary (Table 4.5) and concentrations followed the order of ibuprofen>paracetamol>diclofenac>trimethoprim>citalopram. Whilst mean concentrations were in the order of 100 ng l^{-1} or below, maximum concentrations were approximately 5 to 10 times higher (Table 4.5; Appendix 4.5). Maximum levels of ibuprofen and paracetamol detected in the Humber are the highest concentrations reported in estuaries to date (Table 4.1). Furthermore, this is the first study to detect citalopram in the estuarine environment (Table 4.1).

Table 4.5: Pharmaceutical concentrations (ng l^{-1}) in surface water in the Humber Estuary (n=38) during a 12 month sampling campaign. Values were corrected based on mean recovery values (Table 3). Max = maximum concentration, SD = standard deviation. Detection rate is the amount of samples above the method quantification limit (MQL).

Compound	Detection Rate (%)	Max (ng l^{-1})	Mean (ng l^{-1})	SD
Ibuprofen	97.37	6297.14	665.58	1481.49
Paracetamol	73.68	916.88	88.65	163.66
Diclofenac	57.89	250.8	51.44	68.29
Trimethoprim	92.11	247.02	27.43	54.56
Citalopram	89.47	42.93	6.39	7.66

A general pattern was observed in the occurrence of pharmaceuticals in the Humber surface water, with pharmaceutical concentrations peaking at sampling site R1 (Figures 4.2) and concentrations upstream (sampling sites A1-A2) and downstream (sampling sites R2-A5) of this site were similar to each other. Conversely, this pattern was not consistent in that the chemical concentrations at some of the sampling periods (for instance: paracetamol and diclofenac in June), displayed a reduction in levels downstream (A3-A5). Maximum concentrations were generally seen at sampling site R1 although during some of the sampling periods, they also occurred at sites R2-R4.

Salinity in the Humber Estuary ranged between 0 ppt (sites A1 and A2) to 27 ppt (site A5). Although salinity differed during each sampling period, a general downstream decline was observed (Appendix 4.1). There was not a clear pattern in the pH and dissolved oxygen measurements. The linear regression analysis indicated that there was significant relationship between dissolved oxygen and concentrations of paracetamol (R^2 = 0.15, P = 0.03), diclofenac (R^2 = 0.29, P = 0.001) and trimethoprim (R^2 = 0.22, P = 0.007), with lower dissolved oxygen corresponding with higher concentrations (Figure 4.3). There was also a significant relationship between pH and dissolved oxygen (R^2 = 0.12, P = 0.03). However, this was a very weak relationship with R^2 values less than 0.3 for all compounds. No statistically significant relationship was seen between salinity and any of the compound concentrations (Figure 4.3).

Of the three months where all sites were sampled, February had the highest detection rates and concentrations of pharmaceuticals at downstream sites (A3-A5), whilst many of the compounds were absent at these sites in October and June (Figure 4.2). In contrast, ibuprofen was an exception to this with compounds found at these sites during

all of the sampling periods. Citalopram also showed little decline in downstream concentrations in June, and was present at A3-A5, at concentrations similar to or higher than many of the sites further upstream (Figure 4.2). There appeared to be a relationship between the concentration of pharmaceuticals at R1 and those seen at the other sites; typically, a higher concentration at R1 resulted in a higher presence at sites further downstream (Figure 4.2).

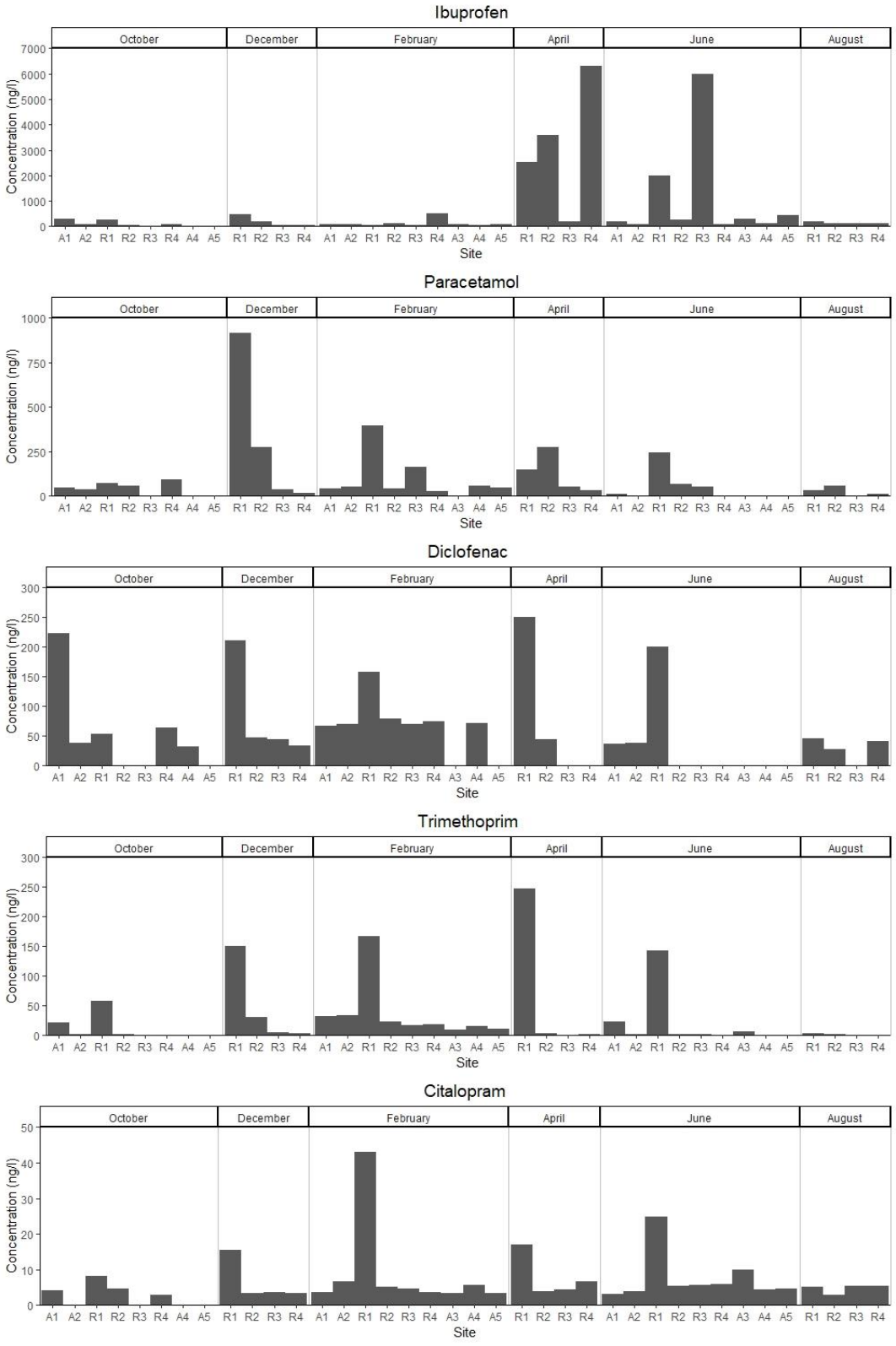


Figure 4.2 Concentrations of target analytes at nine sites in the Humber Estuary. Values were corrected based on mean recovery values (Table 4.3). Sites are listed from furthest upstream (A1) to furthest downstream (A5). R1-R4 were sampled every sampling event, whilst the other sites were only sampled in October, February and June, except for A1 which was not sampled in October.

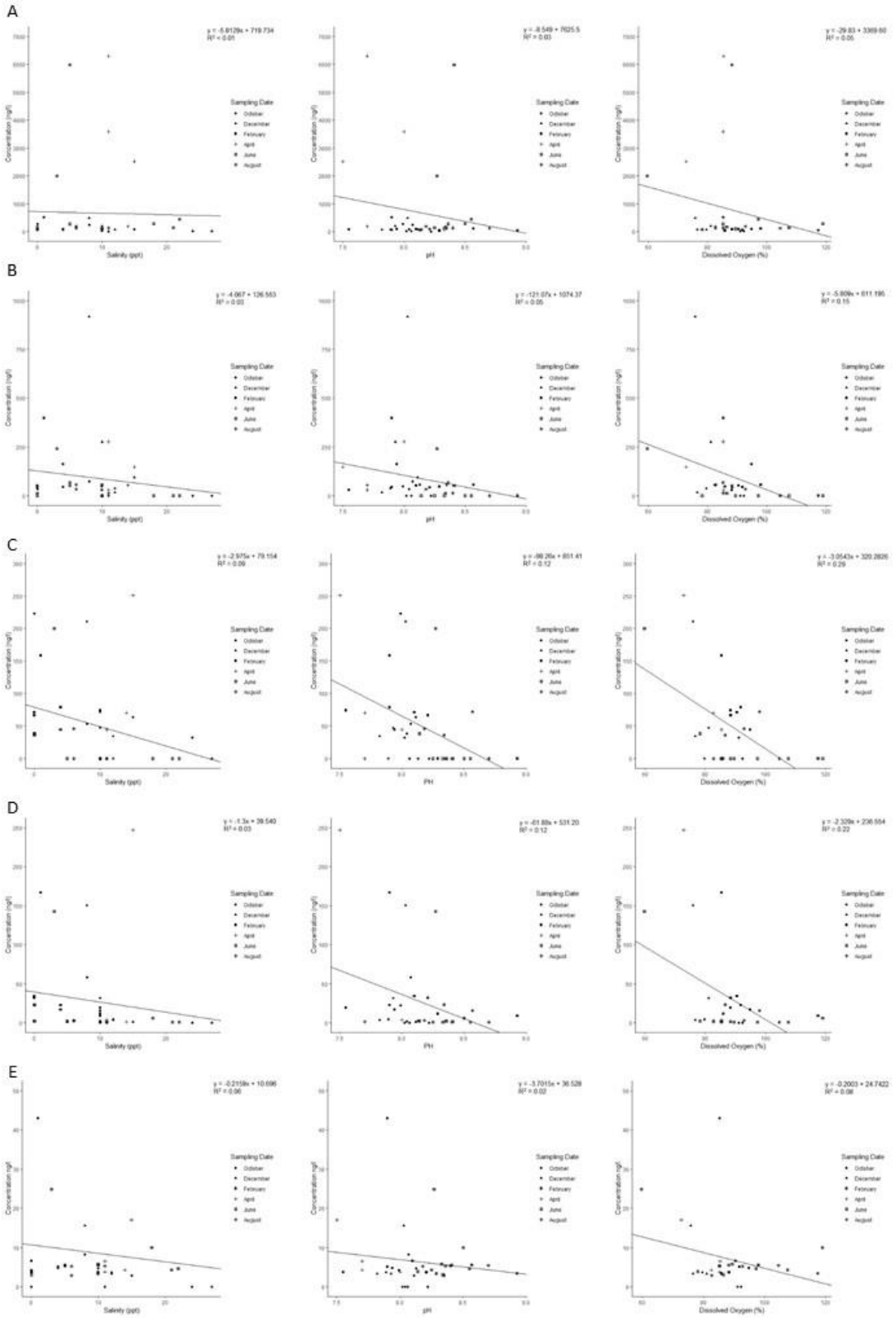


Figure 4.3: Linear regressions of salinity, pH and dissolved oxygen and concentrations of (A) ibuprofen (B) Paracetamol, (C) Diclofenac, (D) Trimethoprim and (E) Citalopram.

Sites R1-R4 were sampled more frequently than the other sites, and trimethoprim was the only compound to show a statistically significant difference between sampling months (Friedman's Test, chi-squared = 14.71, $p < 0.05$) with concentrations, significantly higher in winter (December and February; 3.29 – 166.54 ng l^{-1}), compared to October and the summer months (June and August; 0 – 142 ng l^{-1}; Figure 4.4). Nevertheless, the difference was almost significant for ibuprofen ($p = 0.054$) and citalopram ($p = 0.051$). For citalopram, February had the highest concentrations (3.74 – 42.93 ng l^{-1}), whereas ibuprofen concentrations were higher in April and June (186.37 – 6297.14 ng l^{-1}; Figure 4.3) in comparison to the other sampling periods. All compounds had lowest mean concentrations in August (Figure 4.4), with no peaks seen at sampling site R1 (Figure 4.2).

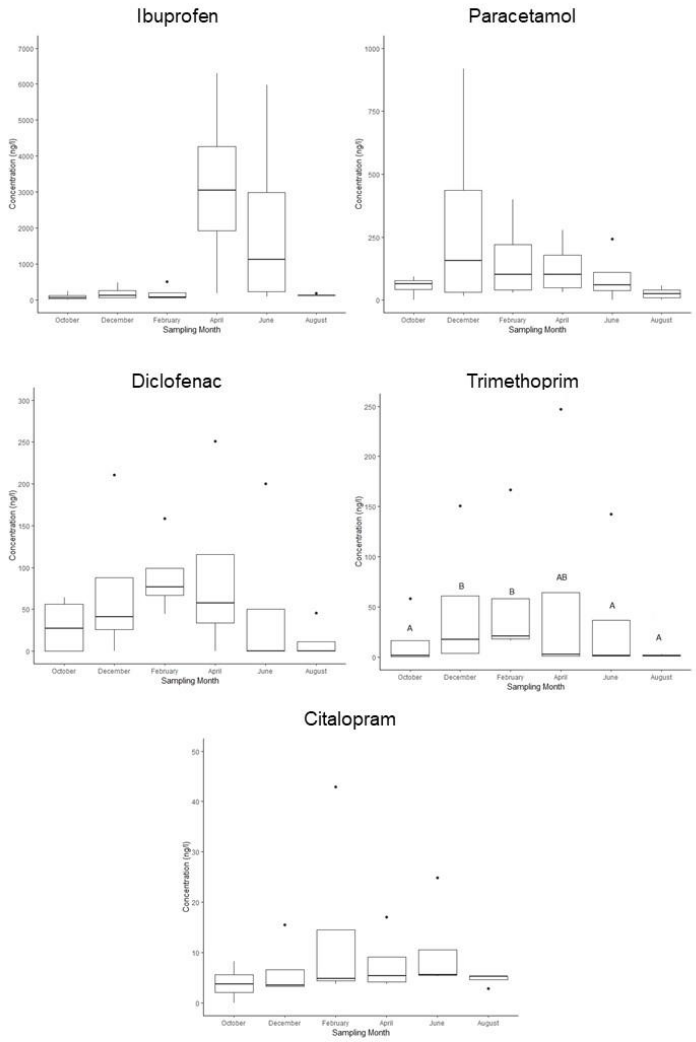


Figure 4.4 Mean bi-monthly concentrations (± one standard deviation) of (A) Ibuprofen (B) Paracetamol (C) Diclofenac (D) Trimethoprim and (E) Citalopram at the four sites monitored most frequently (R1-R4). Values were corrected based on mean recovery values (Table 4.3). Letters denote statistically significant difference (Friedman's Test).

4.3.2 UK-wide Sampling

Pharmaceuticals were detected in all of the estuaries sampled around the UK but only at concentrations in the low ng l^{-1} range and were generally present at concentrations lower than those detected in the Humber Estuary (Figure 4.5). The order of pharmaceuticals were similar to that found in the Humber (ibuprofen>paracetamol>diclofenac>citalopram>trimethoprim), except trimethoprim was found at lowest concentrations (Appendix 4.6). Ibuprofen and trimethoprim were present in all of the estuaries sampled, whereas diclofenac was only detected in two of the other estuaries, the Cromarty and Thames (Figure 4.5). The Thames and Humber were the only estuaries to contain all of the compounds. The Humber had the overall highest concentration of pharmaceuticals, and the Cromarty and Tay were the only other estuaries which had a total concentration of pharmaceuticals over 200 ng l^{-1} (Figure 4.5).

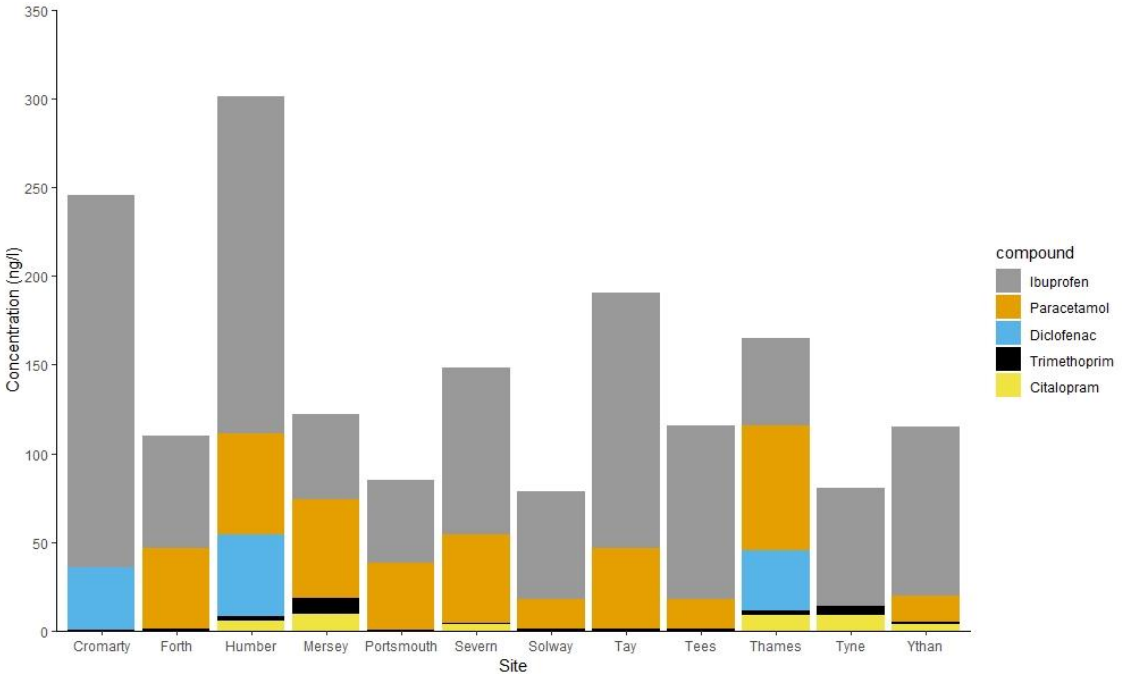


Figure 4.5 Concentrations of citalopram, diclofenac, ibuprofen, paracetamol and trimethoprim across eleven estuaries in the UK. Concentrations have been corrected for recovery (Table 4.3). Concentrations reported for the Humber are maximum concentrations measured in August, when the wider UK survey was undertaken.

4.4. Discussion

Most monitoring studies to date have been carried out in freshwater systems as it was originally thought that estuaries and coastal waters would dilute compounds so that they would be undetectable (Fabbri and Franzellitti 2016). Despite this hypothesis, pharmaceutical contamination was found to be widespread as all of the estuaries monitored contained at least three of the target analytes at levels of a similar magnitude to those found in the freshwater environment, and higher than those measured in many other estuaries (Hughes et al. 2013; Table 4.1). The levels of pharmaceuticals detected in this study, contribute to the overall picture on pharmaceutical pollution and add to the growing evidence that it is a global issue (aus der Beek et al. 2016). Our work indicates that the limited monitoring carried out to date may not have captured peak concentrations that occur in these environment and clearly highlights that further work is needed.

Ibuprofen was detected at the highest concentrations and in all of the estuaries sampled, with its occurrence not only exceeding levels detected in other estuaries (Table 4.1), but also those seen in river water both in the UK (Barbara Kasprzyk-Hordern et al. 2008; Kay et al. 2017; Burns et al. 2017, 2018), as well as globally (Hughes et al. 2013). Ibuprofen has only been measured in 7 estuaries previously, with maximum concentrations all under 100 ng l^{-1} (Table 4.1). Further monitoring studies should include ibuprofen as a priority to determine if high concentrations seen in the UK are similar to those elsewhere.

Concentrations of paracetamol, diclofenac and trimethoprim were similar to those seen in other global estuaries, with mean concentrations less than 100 ng l^{-1} (Table 4.1). Whilst maximum concentrations of paracetamol were similar to those detected in rivers (Barbara Kasprzyk-Hordern et al. 2008; Burns et al. 2017), concentrations of diclofenac and trimethoprim were considerably lower (Hughes et al. 2013; Nakada et al. 2017). In the present study, water samples were collected at high tide, when concentrations would be expected to be lowest, so it is possible that these levels could be higher at other points in the tidal cycle (Yang et al. 2016). This is the first study to measure the occurrence of citalopram, however concentrations were low and did not exceed 50 ng l^{-1}. These low concentrations are in agreement with previous studies which have monitored citalopram in rivers (Hughes et al. 2013). Despite these low concentrations, PNECs for citalopram are below this (Minguez et al. 2016).

Whilst an overall widespread occurrence of pharmaceuticals was seen in the UK, patterns in their spatial and temporal distributions within and between estuaries were observed.

4.4.1 Humber Estuary

4.4.1.1 Spatial Variation

It is generally expected that pharmaceutical concentrations will decrease downstream due to physical processes in an estuary leading to their breakdown and removal (Daughton 2016). The spatial pattern of pharmaceutical occurrence in the Humber Estuary followed this pattern to a degree; peak concentrations were found in the middle of the estuary, particularly at R1, where samples were collected next to an outlet from a WWTP, indicating that they could be a significant source of pharmaceuticals in the Humber Estuary. Input from WWTPs has been attributed as the largest source of pharmaceutical pollution in the aquatic environment (Caldwell 2016). Dissolved oxygen was often lowest at R1, and can explain the relationship observed between diclofenac, paracetamol and trimethoprim. Dissolved oxygen is often lowest at sites where wastewater effluent is discharged, as the result of increased microbial activity and decreased water quality (Igbinosa and Okoh 2009). However, the overall relationship between these variables was weak and could be explained by maximum concentrations seen outside of this site or difference in dissolved oxygen between sampling periods and indicates that other variables are important in determining the concentrations of these compounds. In some cases maximum concentrations were detected outside of this site; in April and June, maximum concentrations for paracetamol and ibuprofen occurred at sites R2-4. It is difficult to determine what caused these peaks as composite sampling can lead to uncertainty in the representativeness of samples in cases such as this, however these sites are within 6km from R1, so it is possible that the large increases seen at these sites are still due to input at R1, and fluctuations of concentrations between these sites are the result of sampling timing or within sample variation (Ort et al. 2010). The site R4, which showed the highest levels (6.2 µg l^{-1}) of ibuprofen was also 7km upstream from the confluence of the River Hull. Transport of pharmaceuticals from this tributary upstream during high tide could also account for the increases seen. The River Trent, located near the confluence with the Ouse (Figure 4.1), will also account for the addition of further pharmaceuticals. Inputs of pharmaceuticals in other studies have also been attributed to other sources such as improper disposal, leaching from landfills or through veterinary usage and subsequent runoff of these compounds into the aquatic environment, which could account for these differences. (Bound and Voulvoulis 2005; Ebele et al. 2017).

Dilution plays a key role in the fate of pharmaceuticals in the aquatic environment and the decrease in concentrations after R1 is presumably caused by dilution away from the input source (Baker and Kasprzyk-Hordern 2013). Decline of pharmaceutical

concentrations downstream the estuary was observed more in some compounds than others, and as a result, is unlikely to be fully explained by dilution. Other studies have seen a negative correlation between pharmaceutical concentrations and salinity, which was not seen in this study, and could partially be explained by the input of pharmaceuticals throughout the estuary or other factors leading to their removal from surface water (Cantwell et al. 2017). Degradation of pharmaceuticals has been found to be a significant factor affecting the fate of pharmaceuticals and could account for these differences (Caracciolo et al. 2015). Citalopram experienced the lowest decrease in concentration downstream, and was typically the same concentration, or higher at A5 than A1, which could be explained by the low degradation which has been observed in other studies (Metcalfe et al. 2010; Styrishave et al. 2011). Ibuprofen, paracetamol and trimethoprim also showed little decline in concentration beyond initial dilution after R1, which is consistent with what has been seen in other studies. These compounds have been found up to 10 km downstream from a WWTP (Bendz et al. 2005, Kay et al. 2017, Burns et al. 2018), and trimethoprim has even been found 200 km downstream from an WWTP (Tamtam et al. 2008). Further WWTPs are located within the estuary (European Environment Agency, 2017) which could also account for this lack in decline. Diclofenac on the other hand, was not detected at A3 or A5 during any of the sampling periods, but was found at A4. The downstream decline of diclofenac has been found to be variable, with some studies finding it to be more persistent than others (Bendz et al. 2005; Wilkinson et al. 2017). Removal of compounds through degradation and sorption to sediment has been found to be highly dependent on environmental conditions, compound properties and sediment type. Linear regressions indicated there was a weak negative relationship between diclofenac concentrations and pH. Diclofenac is an acidic compound (pKa 4.2), and it would be expected that removal as the result of sorption to sediment and uptake by organisms would be higher at lower pH as the result of ionisation (Oh et al. 2016). The pH in the Humber ranged between 7.5 and 8.9, and as a result diclofenac would not be fully protonated at any of the sites. In estuaries, a positive correlation is often seen between pH and salinity, but not in the Humber. However, pH can also fluctuate as the result of mixing, biological activity, water quality and presence of other contaminants (Howland et al. 2000).

4.4.1.2 Temporal Variation

Seasonal differences of pharmaceuticals have been observed in a number of studies and these are often attributed to changes in usage and local environmental conditions (Golovko et al. 2014b; Moreno-González et al. 2014). Trimethoprim was the only compound to show significant temporal differences in concentrations (at sites R1-R4),

with average winter concentrations over double that of those during the summer months. Previous studies have explained the seasonal occurrence of antibiotics in winter due to their higher usage in those months to treat seasonal infections (Verlicchi and Zambello 2016). The temporal differences seen in the occurrence of trimethoprim in the Humber Estuary appeared to follow this pattern, as prescriptions were highest in October 2016 to March 2017 and lowest in August 2017 (Appendix 4.4). Trimethoprim has been observed to have higher winter concentrations in some studies (Golovko et al. 2014b) but not in others (Burns et al. 2018). Burns et al. (2018) found higher levels of trimethoprim during spring in the Ouse (upstream from A1), which was attributed to hydrological differences seen between the seasons sampled. As a result, it is likely that the temporal differences in trimethoprim are the result of different site specific conditions or daily variations. Temporal variations in other studies have also been explained by lower temperatures, leading to lower degradation (Golovko et al. 2014a), however, input at R1 was highest in April. The other target compounds have exhibited temporal differences in other locations, but did not in the Humber. Paracetamol, for instance, has been detected at high concentrations in spring in some rivers but winter in others, whilst other studies found no temporal variations (Paíga et al. 2016; Ma et al. 2017; Burns et al. 2018).

Temporal variations in the downstream pattern of pharmaceuticals were also observed, with the greatest variation seen at the sites furthest downstream (A3-A5). Pharmaceuticals were mostly absent from these sites in October, with the exception of ibuprofen, where concentrations were reduced. Sampling at high tide could account for the absence of these pharmaceuticals downstream as the result of increased dilution or transport of contaminants upstream (Munro et al. 2019). Pharmaceutical concentrations often fluctuate diurnally as the result of timing of effluent discharges from WWTPs and combined sewer overflows (CSOs), as well as variations in wastewater as the result of consumption patterns (Xu et al. 2007). To an extent, there was a pattern in the presence of compounds at R1 consistent with those seen downstream the estuary, so it is possible that the temporal variations could be the result of these daily variations, instead of conditions seen seasonally. The concentration of pharmaceuticals at R1 were lowest in October and the low input could, in part, account for the absence of compounds seen at sites furthest downstream (A3-A5). Likewise, concentrations for the majority of compounds were highest at R1 during February where concentrations were highest at sites furthest downstream (A3-A5). This is further evidence that there is a difference in input from WWTPs. R1 is not the only site at which wastewater is discharged, but if these other sites exhibit the same temporal variations, then it could explain the differences

observed in concentrations at A3-A5. WWTP removal has been found to be less efficient during the winter time due to lower temperatures and decreased biodegradation, leading to higher concentrations in effluent (Vieno et al. 2005). At R1, concentrations for all compounds were lowest in August when temperatures were warmest (Appendix 4.1).

4.4.3 UK Estuaries

The Humber Estuary was shown to represent a worst case scenario in terms of pharmaceutical pollution, with all five pharmaceuticals present at relatively high concentrations. Of the estuaries sampled, it was the second highest impacted by WWTPs, with a PE of approximately 13.7 million people. The Thames, which was the most impacted, was the only other estuary to contain all five compounds. A higher presence of pharmaceuticals is frequently seen in large urban areas due to their increased usage (Hong et al. 2018). With the exception of both the Humber and the Thames estuaries, there was no apparent relationship between the number of WWTP and concentrations (Table 2). The Cromarty Firth, which was the receiving water of only 3 WWTPs (15,600 PE), exhibited similar levels of pharmaceuticals to the Humber. This could be explained by differences in WWTP efficiency, as technology used in WWTPs can greatly affect the removal of pharmaceuticals. For example, ibuprofen removal has been reported to be between 7% and 99% at different WWTPs (Radjenovic et al. 2007; Jelic et al. 2015). It is possible that the removal efficiency of WWTPs could differ between areas, with rural areas being less efficient as they are serving smaller populations. Rural areas are more likely to have a higher occurrence of septic tanks, which could contribute to the elevated levels seen in the Cromarty (Hanamoto et al. 2018). Whilst the Humber experienced the lowest concentration in August, it is possible that seasonal variations in population in areas like the Scottish Highlands (a tourist destination), where the Cromarty is located, could be responsible for these higher concentrations, increasing pressure on WWTPs. Pharmaceuticals in a Portuguese river have previously shown higher concentrations which was thought to be the result of increased summer populations (Rocha et al. 2014).

The presence of pharmaceuticals is greatly influenced by environmental conditions and proximity of the sampling site to input sources, possibly accounting for some of the apparent differences in concentrations observed between estuaries. Water samples from different locations in the estuary were mixed together and a subsample was taken to obtain a snapshot of the presence of pharmaceuticals, and it is likely that these concentrations will vary depending on these factors. This could possibly explain the absence of diclofenac, which in the Humber study was frequently undetected in sites

downstream the estuary. Citalopram also had a low detection (50%) in estuaries, however, it was detected in estuaries which have the highest PE.

There are also likely to be more complex interactions in play which further affect the occurrence of pharmaceuticals in estuaries and can help to explain the spatial differences seen. Differences in site specific conditions such as salinity profiles and hydrology can affect sorption processes, degradation and dilution. Undoubtedly, these processes, in conjunction with daily variations in rainfall and temperature, are likely to be responsible for differences in concentrations in estuaries between sampling periods, yet it is still clear that pharmaceutical pollution is a ubiquitous problem in estuaries (Tamtam et al. 2008).

Ibuprofen, paracetamol, diclofenac and trimethoprim were previously monitored in the Mersey, Thames, Tees and Tyne estuaries (as well as Belfast Lough) in 2002 (Thomas and Hilton, 2004). It was also found that ibuprofen was present at highest concentrations. Paracetamol, however, was not detected in any of the estuaries sampled in 2002, which indicates that the occurrence of this compound could be rising. A rise in pharmaceuticals would be consistent with what has been found in other areas. For example, analysis of sediment cores in the Bay of Jamaica showed an overall rise in pharmaceutical concentrations over time, with these concentrations doubling over the last decade (Lara-Martin et al. 2015). This highlights the importance of establishing baseline measurements of pharmaceuticals, in order to determine areas most at risk and therefore require continued monitoring. The Humber Estuary likely poses the greatest risk, particularly due to the high level concentrations of ibuprofen. Other large urban estuaries (such as the Thames and Severn) may also warrant a further detailed study. However, as seen with the Cromarty, focus on monitoring should be extended to rural areas as well.

4.5 Conclusion

All five target analytes — ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram were detected in twelve estuaries in the UK. Diclofenac is a compound that has been highlighted as a potential concern, yet paracetamol and ibuprofen were consistently detected at higher concentrations and at levels which could be toxic to aquatic organisms (Vestel et al. 2016). In particular, the concentrations of ibuprofen measured indicates that the limited monitoring of pharmaceuticals in estuaries around the globe to date has not accurately quantified peak concentrations. Whilst trimethoprim was detected in every sample it was only present at concentrations in the low ng l^{-1} range. Citalopram was present at lowest concentrations, but also showed the least change in

concentration downstream the estuary. A more intensive monitoring regime of the Humber Estuary showed that pharmaceutical input from WWTPs is a significant source and could explain the overall higher concentrations of pharmaceuticals in large urban estuaries. Despite this, a rural estuary had the highest concentration of ibuprofen which may be due to lower removal at smaller rural sewage works. More detailed studies need to be undertaken in order to understand the complex interactions taking place in estuaries which could affect the fate of pharmaceuticals.

Whilst there was little significant variation of pharmaceutical concentrations between sampling periods in the Humber Estuary, August typically had the lowest input from WWTPs and overall lowest concentrations, which is when samples were taken from estuaries throughout the UK. Consequently, it could be expected that pharmaceutical concentrations may exceed those measured. Additionally, samples were taken on a high tide when it would be expected that concentrations are lowest due to dilution. This study provides an important baseline of pharmaceutical measurements in the UK, and highlights ibuprofen as a compound which may warrant further assessment. This work provides further evidence to the growing problem of pharmaceutical pollution, highlighting that it is not only an urban and localised issue.

4.6 References

Arpin-Pont L, Bueno MJM, Gomez E, Fenet H (2014) Occurrence of PPCPs in the marine environment: a review. Environ Sci Pollut R. 23(6):4978-91 doi: 10.1007/s11356-014-3617-x

Aus der Beek T, Weber F, Bergmann A, Hickmann S, Ebert I, Hein A, Kuster A (2016) Pharmaceuticals in the environment - Global occurrences and perspectives. Environ. Toxicol. Chem. 35(4): 823-835 doi: 10.1002/etc.3339

Baker DR, Kasprzyk-Hordern B (2013) Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: New developments. Sci Total Environ 454–455: 442–456. doi: 10.1016/j.scitotenv.2013.03.043

Bendz D, Paxéus NA, Ginn TR, Loge FJ (2005) Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden. J Hazard Mat 122: 195–204. doi: 10.1016/j.jhazmat.2005.03.012

Benotti MJ, Brownawell BJ (2007) Distriutions of Pharmaceuticals in an Urban Estuary during both Dry and Wet-Weather Conditions. Environ Sci Technol 41 (16) 5795-5802 doi: 10.1021/es0629965

Birch GF, Drage DS, Thompson K, Eaglesham G, Mueller JF (2015) Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary, Australia. Mar Pollut Bull 97(1-2): 56-66

Bound JP, Voulvoulis N (2005) Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environ Health Perspect 113: 1705–1711. doi: 10.1289/ehp.8315

Buck AL (1997) An inventory of UK estuaries - Volume 5. Eastern England Joint Nature Conservation Committee, Peterborough

Burns EE, Carter LJ, Kolpin DW, Thomas-Oates J, Boxall ABA (2018) Temporal and spatial variation in pharmaceutical concentrations in an urban river system. Wat Res doi: 10.1016/j.watres.2018.02.066

Burns EE, Thomas-Oates J, Kolpin DW, Furlong ET, Boxall ABA (2017) Are exposure predictions, used for the prioritization of pharmaceuticals in the environment, fit for purpose? Environ Toxicol Chem 36: 2823–2832. doi: 10.1002/etc.3842

Caldwell DJ (2016) Sources of Pharmaceutical Residues in the Environment and their Control. Issues in Environmental Science and Technology 41: 92–119. doi: 10.1039/9781782622345-00092

Cantwell MG, Katz DR, Sullivan JC, Ho Kay, Burgess RM (2017) Temporal and spatial behaviour of pharmaceuticals in Narragansett Bay, Rhode Island, United States. Environ Chem 36 (7) 1846-1855 doi: 10.1002/etc.3710

Cantwell MG, Katz DR, Sullivan JC, Shapley D, Lipscomb J, Epstein J, Juhl AR, Knudson C, O'Mullan GD (2018) Spatial patterns of pharmaceuticals and wastewater tracers in the Hudson River Estuary. Wat Res 137: 335–343. doi: 10.1016/J.WATRES.2017.12.044

Caracciolo AB, Topp E, Grenni P (2015) Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities: A review. J Pharm Biomed Anal 106: 25–36. doi: 10.1016/j.jpba.2014.11.040

Chen H, Liu S, Xu X, Zhou G, Liu S, Yue W, Sun K, Ying G (2015) Antibiotics in the coastal environment of the Hailing Bay region, South China Sea: spatial distribution, source analysis and ecological risks. Mar Pollut Bull. 95(1) 365-373 doi:10.1016/j.marpolbul.2015.04.025

Cui Y, Wang Y, Pan C, Li R, Xue R, Guo J, Zhang R (2019) Spatiotemporal distributions,

source apportionment and potential risks of 15 pharmaceuticals and personal care products (PPCPs) in Qinzhou Bay, South China. Mar Pollut Bull 141: 104-111 doi: 10.1016/J.MARPOLBUL.2019.02.012

Davidson NC, Laffoley DD, Doody JP, Way LS, Gordon J, Key R, Pienkowski MW, Mitchell R, Duff KL (1991) Nature conservation and estuaries in Great Britain. Nature Conservancy Council, Perborough

Daughton CG (2016) Pharmaceuticals and the Environment (PiE): Evolution and impact of the published literature revealed by bibliometric analysis. Sci Total Environ 562: 391–426. doi: 10.1016/j.scitotenv.2016.03.109

Dong H, Yuan X, Wang W, Qiang Z (2016) Occurrence and removal of antibiotics in ecological and conventional wastewater treatment processes: A field study. J Environ Manage 178: 11–19. doi: 10.1016/j.jenvman.2016.04.037

Eades C, Waring CP (2010) The effects of diclofenac on the physiology of the green shore crab Carcinus maenas. Mar Environ Res 69: S46–S48. doi: 10.1016/j.marenvres.2009.11.001

Ebele AJ, Abou-Elwafa Abdallah M, Harrad S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerging Contaminants 3: 1–16. doi: 10.1016/J.EMCON.2016.12.004

European Environment Agency (2017) Urban Waste Water Treatment Map. Available from: https://www.eea.europa.eu/themes/water/european-waters/water-use-and-environmental-pressures/uwwtd/interactive-maps/urban-waste-water-treatment-maps. (March 15, 2019)

Fabbri E, Franzellitti S (2016) Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. Environ Toxicol Chem 35: 799–812. doi: 10.1002/etc.3131

Franzellitti S, Buratti S, Valbonesi P, Fabbri E (2013) The mode of action (MOA) approach reveals interactive effects of environmental pharmaceuticals on Mytilus galloprovincialis. Aquat Toxicol 140–141: 249–256. doi: 10.1016/j.aquatox.2013.06.005

Gaw S, Thomas K V, Hutchinson TH (2014) Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philosoph Trans Royal Soc B 369. doi: 10.1098/rstb.2013.0572

Godoy AA, Kummrow F, Pamplin PAZ (2015) Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment - A review. Chemosphere 138: 281–291. doi: 10.1016/j.chemosphere.2015.06.024

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014a) Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular pharmaceuticals and UV filters in wastewater treatment plant. Environ Sci Pollut R 21: 7578-7585 doi:10.1007/s11356-014-2654-9

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014b) Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111: 418–426. doi: 10.1016/j.chemosphere.2014.03.132

Gonzalez-Rey M, Tapie N, Menach KL, Dévier MH, Budzinski H, Bebianno MJ (2015) Occurrence of pharmaceutical compounds and pesticides in aquatic systems. 96(1-2): 384-400 doi:10.1016/j.marpolbul.2015.04.029

Gros M, Rodríguez-Mozaz S, Barceló D (2012) Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. Jo Chromat A 1248: 104–121. doi: 10.1016/j.chroma.2012.05.084

Guo J, Sinclair CJ, Selby K, Boxall ABA (2016) Toxicological and ecotoxicological risk-based prioritization of pharmaceuticals in the natural environment. Environ Toxicol Chem 35 (6): 1550-1559 doi: 10.1002/etc.3319

Hanamoto S, Nakada N, Yamashita N, Tanaka H (2018) Source estimation of pharmaceuticals based on catchment population and in-stream attentuation in Yodo River Watershed, Japan. Sci Total Environ 615: 964-971 doi: 10.1016/J.SCITOTENV.2017.10.013

Hong B, Lin Q, Yu S, Chen Y, Chen Y, Chiang P (2018) Urbanization gradient of selected pharmaceuticals in surface water at a watershed scale. Sci Total Environ 634: 448–458. doi: 10.1016/J.SCITOTENV.2018.03.392

Howland RJM, Tappin AD, Uncles RJ, Plummer DH, Bloomer NJ (2000) Distributions and seasonal variability of pH and alkalinity in the Tween Estuary, UK. Sci Total

Environ 251-252: 125-138 doi: 10.1016/S0048-9697(00)00406-X

Hughes SR, Kay P, Brown LE (2013) Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ Sci Technol 47: 661–677. doi: 10.1021/es3030148

Igbinosa EO, Okoh AI (2009) Impact of discharge watewater effluents on the physico-chemical qualities of a receiving watershed in a typical rural community. Int J Environ Sci Tech 6(2): 175-182 doi: 10.1007/BF03327619

Jelic A, Rodriguez-Mozaz S, Barceló D, Gutierrez O (2015) Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Wat Res 68: 98–108. doi: 10.1016/j.watres.2014.09.033

Kantor ED, Rehm CD, Haas JS, Chan AT, Giovannucci EL (2015) Trends in Prescription Drug Use Among Adults in the United States From 1999-2012. JAMA 314: 1818. doi: 10.1001/jama.2015.13766

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2007) Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. J Chromat A 1161: 132–145. doi: 10.1016/J.CHROMA.2007.05.074

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2008) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Wat Res 42 (13): 3498-3518 doi: 10.1016/j.watres.2008.10.047

Kay P, Hughes SR, Ault JR, Ashcroft AE, Brown LE (2017) Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. Environ Pollut 220: 1447-1455 doi: 10.1016/j.envpol.2016.10.087

Klosterhaus SL, Grace R, Hamilton MC, Yee D (2013) Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ Int 54: 92-99

Kunkel U, Radke M (2012) Fate of pharmaceuticals in rivers: Deriving a benchmark dataset at favorable attenuation conditions. Wat Res. 46(17): 5551-5565 doi: 10.1016/J.WATRES.2012.07.033

Lara-Martín PA, González-Mazo E, Petrovic M, Barceló B (2014) Occurrence,

distribution and partitioning of nonionic surfactants and pharmaceuticals in the urbanized Long Island Sound Estuary (NY). Mar Pollut Bull 85: 710-719 doi: 10.1016/J.MARPOLBUL.2014.01.022

Lara-Martín PA, Renfro A, Cochran J, Brownawell B (2015) Geochronologies of pharmaceuticals in a sewage-impacted estuarine urban setting (Jamaica Bay, New York). Environ Sci Technol. 49(10) 5948-5955 doi: 10.1021/es506009v

Li WC (2014) Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. Environ Pollut 187: 193–201. doi: 10.1016/j.envpol.2014.01.015

Ma R, Wang B, Yin L, Zhang Y, Deng S, Huang J, Wang Y, Yu G (2017) Characterization of pharmaceutically active compounds in Beijing, China: Occurrence pattern, spatiotemporal distribution and its environmental implication. J Hazard Mat 323: 147–155. doi: 10.1016/J.JHAZMAT.2016.05.030

Madureira TV, Barreiro JC, Rocha MJ, Rocha E, Cass QB, Tiritan ME (2010) Spatiotemporal distribution of pharmaceuticals in the Douro river estuary. Sci Total Environ 408(22):5513-5520

Mavragani A, Sypsa K, Sampri A, Tsagarakis K (2016) Quantifying the UK Online Interest in Substances of the EU Watchlist for Water Monitoring: Diclofenac, Estradiol, and the Macrolide Antibiotics. Water 8: 542. doi: 10.3390/w8110542

Metcalfe CD, Chu S, Judt C, Li H, Oakes KD, Servos MR, Andrews DM (2010) Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. Environ Toxicol Chem 29: 79–89. doi: 10.1002/etc.27

Mijangos L, Ziarrusta H, Ros O, Kortazar L, Fernández LA, Olivares M, Zuloaga O, Prieto A, Extebarria N (2018) Occurrence of emerging pollutants in estuaries of the Basque Country: analysis of sources and distribution, and assessment of the environmental risk. Wat Res. 147: 152-163 doi:10.1016/j.watres.2018.09.033

Minguez L, Pedelucq J, Farcy E, Ballandonne C, Budzinski H, Halm-Lemeille M-P (2016) Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environ Sci Pollut R Int 23(6): 4992-5001 doi: 10.1007/s11356-014-3662-5

Moreno-González R, Rodríguez-Mozaz S, Gros M, Pérez-Cánovas E, Barceló D, León VM (2014) Input of pharmaceuticals through coastal surface watercourses into a Mediterranean lagoon (Mar Menor, SE Spain): Sources and seasonal variations.

Sci Total Environ 490: 59–72. doi: 10.1016/j.scitotenv.2014.04.097

Munro K, Martina CPB, Loewenthal M, Comber S, Cowan DA, Pereira L, Barron LP (2019) Evaluation of combined sewer overflow impacts on short-term pharmaceutical and illicit drug occurrence in a heavily urbanised tidal river catchment (London, UK). Sci Total Environ 657: 1099-1111 doi: 10.1016/j.scitotenv.2018.12.08

Nakada N, Hanamoto S, Jürgens MD, Johnson AC, Bowes MJ, Tanaka H (2017) Assessing the population equivalent and performance of wastewater treatment through the ratios of pharmaceuticals and personal care products present in a river basin: Application to the River Thames basin, UK. Sci Total Environ 575: 1100–1108. doi: 10.1016/J.scitotenv.2016.09.180

National Health Service (2017) Prescriptions Dispensed in the Community - Statistics for England, 2006-2016. Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescriptions-dispensed-in-the-community/prescriptions-dispensed-in-the-community-statistics-for-england-2006-2016-pas (January 1, 2019).

National Health Service (2019) Prescriptions Dispensed in the Community - NHS Digital. Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescriptions-dispensed-in-the-community (January 8, 2019).

Oh S, Shin WS, Kim HT (2016) Effects of pH, dissolved organic matter, and salinity on ibuprofen sorption on sediment Environ Sci Pollut 23(22): 22882-22889 doi: 10.1007/s11356-016-7503-6

Ort C, Lawrence MG, Reungoat J, Mueller JF (2010) Sampling for PPCPs in Wastewater Systems: Comparison of Different Sampling Modes and Optimization Strategies. Environ Sci Technol 44: 6289-6296 doi: 10.1021/es100778d

Paíga P, Santos LHMLM, Ramos S, Jorge S, Silva JG, Delerue-Matos C (2016) Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Sci Total Environ 573: 164–177. doi: 10.1016/J.SCITOTENV.2016.08.089

Pohlert T (2014) The pairwise multiple comparison of mean ranks package (PMCMR). Available from: https://cran.r-project.org/package=PMCMR (October 12, 2018).

Radjenovic J, Petrovic M, Barceló D (2007) Analysis of pharmaceuticals in wastewater

and removal using a membrane bioreactor. Anal Bioanal Chem 387: 1365–1377. doi: 10.1007/s00216-006-0883-6

Redshaw CH, Stahl-Timmins WM, Fleming LE, Davidson I, Depledge MH (2013) Potential Changes in Disease Patterns and Pharmaceutical Use in Response to Climate Change. J Toxicol Environ Health B 16: 285–320. doi: 10.1080/10937404.2013.802265

Reis-Santos P, Pais M, Duarte B, Cacador I, Freitas, A, Pouca ASV, Barbosa J, Leston S, Rosa J, Ramos F, Cabral HN, Gillanders BM, Fronseca VF (2018) Screening of human and veterinary pharmaceuticals in estuarine waters: A baseline assessment for the Tejo estuary. Mar Pollut Bull 135: 1079-1084. doi:10.1016/j.marpollbul.2018.08.036

Ridgway J, Shimmield G (2002) Estuaries as Repositories of Historical Contamination and their Impact on Shelf Seas. Estuar Coast Shelf Sci 55: 903–928. doi: 10.1006/ECSS.2002.1035

Roberts PH, Thomas K V. (2006) The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ 356: 143–153. doi: 10.1016/j.scitotenv.2005.04.031

Roos V, Gunnarsson L, Fick J, Larsson DGJ, Rudén C (2012) Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Sci Total Environ 421 (102-110) doi: 10.1016/j.scitotenv.2012.01.039.

Santos LHMLM, Araújo AN, Fachini A, Matos-Delerue PC, Montenegro MCBSM (2010) Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mat. 175: 45-95 doi: 10.1016/j.jhazmat.2009.10.100

Styrishave B, Halling-Sørensen B, Ingerslev F (2011) Environmental risk assessment of three selective serotonin reuptake inhibitors in the aquatic environment: a case study including cocktail scenario. Environ Toxicol Chem 30: 254–261. Available from: 10.1002/etc.372.

Sun Q, Li Y, Li M, Ashfaq M, Lv M, Wang H, Hu A, Yu C (2016) PPCPs in Jiulong river estuary (China): spatiotemporal distributions, fate and their use as chemical markers of wastewater. Chemosphere 150: 596-604. doi:10/1016/j.chemosphere.2016.02.036

Tamtam F, Mercier F, Le Bot B, Eurin J, Tuc Dinh Q, Clement M, Chevreuil M (2008)

Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci Total Environ 393 (1): 84–95. doi:10.1016/j.scitotenv.2007.12.009

Thomas K V, Hilton MJ (2004) The occurrence of selected human pharmaceutical compounds in UK estuaries. Mar Pollut Bull 49: 436–444. doi: 10.1016/j.marpolbul.2004.02.028

USEPA (2019) Estimation Programs Interface Suite™ fr Microsoft® Windows [v1.11] United States Environmental Protection Agency, Washington, DC, USA

Verlicchi P, Zambello E (2016) Predicted and measured concentrations of pharmaceuticals in hospital effluents. Examination of the strengths and weaknesses of the two approaches through the analysis of a case study. Sci Total Environ 565: 82–94. doi: 10.1016/j.scitotenv.2016.04.165

Vestel J, Caldwell DJ, Constantine L, D'Aco VJ, Davidson T, Dolan DG, Millard SP, Murray-Smith R, Parke NJ, Ryan JJ, Straub JO, Wilson P (2016) Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environ Toxicol Chem 35: 1201–1212. doi: 10.1002/etc.3260

Vieno NM, Tuhkanen T, Kronberg L (2005) Seasonal Variation in the Occurrence of Pharmaceuticals in Effluents from a Sewage Treatment Plant and in the Recipient Water. Environ Sci Technol 39: 8220–8226. doi: 10.1021/es051124k

Weigel S, Kuhlmann J, Hühnerfuss H (2002) Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. Sci Total Environ 295(1-3): 131-141. doi:10.1016/S0048=9697(02)0064-5

Wickham H (2016) ggplot2; Elegant Graphics for Data Analysis. Available from: http://ggplot2.org.

Wilkinson JL, Hooda PS, Swinden J, Barker J, Barton S (2017) Spatial distribution of organic contaminants in three rivers of Southern England bound to suspended particulate material and dissolved in water. Sci Total Environ 593–594: 487–497. doi: 10.1016/J.SCITOTENV.2017.03.167

Xu W, Zhang G, Zhou S, Li X, Liu Y (2007) Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. Environ. Poll. 145: 672-679 doi: 10.1016/j.envpol.2006.05.038

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL (2011) Occurrence and phase distribution of selected pharmaceuticals in the Yangtze estuary and its coastal zone. J Hazard Mat 190(1-3): 588-596 doi;10.1016/jhazmat.2011.03.092

Yang Y, Cheng Q, Lin L, Wang X, Chen B, Luan T, Tam N (2016) Partitions and vertical profiles of 9 endocrine disrupting chemicals in an estuarine environment: Effect of tide, particle size and salinity. Environ Pollut. 211: 58-66 doi: 10.1016/j.watres.2016.05.048

Zhang R, Zhang G, Zheng Q, Tang J, Chen Y, Xu W, Zou Y, Chen X (2012) Occurrence and risks of antibiotis in the Laizhou Bay, China: impacts of river discharge. Ecotox Environ Safe 80: 208-215 doi:10.1016/j.ecoenv.2012.03.002

Zhao H, Zhou JL, Zhang J (2015) Tidal impact on the dynamic behaviour of dissolved pharmaceuticals in the Yangtze estuary, China. Sci Total Environ. 536: 946-954 doi:10.1016/j.scitotenv.2015.06.055

Chapter 5: Effects of metformin and diclofenac on the ragworm, *Hediste diversicolor*

Sarah Letsinger[1], Paul Kay[1], Jeanette M. Rotchell[2]

[1] School of Geography, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT

[2] School of Environmental Sciences, University of Hull, Cottingham Road, Hull, HU6, 7RX

Abstract

Diclofenac and metformin are two pharmaceuticals of particular environmental concern due to their widespread usage and presence in the aquatic environment at high concentrations. Estuaries have the potential to act as a sink for emerging contaminants such as these, putting resident and visiting biota at risk. Despite the ecological and commercial importance of the estuarine polychaete, *Hediste diversicolor*, little is known about the effects of pharmaceutical contamination on this species. This study investigates the effects of diclofenac and metformin on energy status, by evaluating mRNA expression of ATP synthase and c-amp activated protein kinase. *H. diversicolor* were exposed to either 100 ng l^{-1} or 1µg l^{-1} of either diclofenac or metformin for 7 days. ATP synthase expression was significantly higher in individuals exposed to the higher level of metformin than the other treatments. No other significant differences were seen in any of the other treatments. This study reveals that environmentally relevant waterborne concentrations (1 µg l^{-1}) of metformin have the potential to induce environmental stress in *H. diversicolor* individuals and the requirement to sustain high energy levels could have long term consequences on physiological processes.

5.1 Introduction

Many compounds are bioavailable to aquatic organisms and some compounds (such as diclofenac and ibuprofen) have been found in the tissues of aquatic organisms, whilst others have not. Both of these compounds were present in the tissues of mussels *(Mytilus galloprovincialis*) sampled from the field and after exposure under laboratory conditions, whilst ketoprofen and paracetamol were not (Mezzelani et al. 2016). Pharmaceuticals may pose a risk to non-target organisms, as they are designed to be biologically active, and have the potential to illicit a response in non-target organisms which possess conserved drug targets (Gunnarsson et al. 2008). In some species, these targets may have a different physiological role, causing effects not seen in humans. An example of this is diclofenac, which caused the decline of vultures (*Gyps coprotheres*) in

Pakistan as the result of renal toxicity, despite being used in veterinary medicine without the same effects (Oaks et al. 2004). This increased sensitivity is thought to be due to the differences in cytochrome P450 enzymic pathways (Naidoo et al. 2010). Diclofenac is a widely available over the counter (OTC) non-steroidal anti-inflammatory drug (NSAID), which is frequently detected in surface water at hundreds of ng l^{-1}, with peak concentrations as high as 18 µg l^{-1} (Hughes et al. 2013). In vertebrates, diclofenac inhibits cyclooxygenase (COX), which is responsible for the formation of prostanoids (Gan 2010). There are two isoforms in vertebrates, COX I which is responsible for the baseline levels of prostaglandins involved in processes such as thermoregulation, ovulation, sexual behaviour, homeostasis, ion transport and kidney filtration required for physiological processes, and COX II which produces prostaglandins at the point of an injury (Gan 2010). These isoforms are also present in invertebrates, and there is evidence that many of these functions are conserved (Ruggeri and Thoroughgood, 1985).

Metformin is among the top 10 drugs prescribed with annual prescriptions in the millions in USA and Europe (Marshall 2017). It is of environmental concern, because of the amount consumed, its increasing usage, and the fact that it is not heavily metabolised and is excreted via urine relatively unchanged (Oosterhuis et al. 2013; Xu et al. 2018). As a result, it should be considered a priority substance. Recent studies have detected metformin at high concentrations in wastewater effluent (21 µg l^{-1}; Scheurer et al. 2009) and surface water (2.5 µg l^{-1}; Bradley et al. 2016; Burns et al. 2018). Metformin is prescribed for type II diabetes, and is used to regulate glucose levels through the activation of c-AMP activated protein kinase (*AMPK*) leading to inhibition of hepatic glucogenesis and increased glucose uptake in muscles (Joshi 2005).

H. diversicolor are polychaetes, which are a key species ubiquitously present in estuaries globally (Scaps 2002). They are one of the most important prey items in estuaries, providing food for a variety water birds, such as the grey plover (*Pluvialis squatarola*) and fish species such as sole (*Solea solea*; Cobra et al. 2000; Rosa et al. 2008). *H. diversicolor* are also of commercial interest and are harvested from estuarine sediment and sold as fishing bait (Virgilio and Abbiati 2004). To the authors' knowledge, only two studies (Maranho et al. 2014, 2015) have previously studied the effects of pharmaceuticals on this species, however, they are well studied for other groups of substances such as metals (He et al. 2019), nanoparticles (Buffet et al. 2014) and pesticides (Scaps et al. 1997). Additionally, they are easily maintained in the laboratory, and sensitive to contaminants, which could make them a useful bioindicator of sublethal pharmaceutical pollution in estuaries (Scaps et al. 2002; Maranho et al. 2014).

Few studies have looked at the effects of pharmaceuticals in estuarine or marine species, and studies are often limited to short exposures and standard endpoints (Gaw et al. 2014; Fabbri and Franzellitti 2016). The objective of this study was to assess the effects of diclofenac and metformin at environmentally relevant concentrations on *H. diversicolor* energy status, through the evaluation of ATP Synthase (*ATPS*) and *AMPK* mRNA expression.

Energy status has previously been used an indicator of environmental stress and energy reserves have been found to be lower in *H. diversicolor* in contaminated estuaries (Durou et al. 2007). Energy levels have been found to naturally vary in this species as they often live at the edge of their tolerance zone, and lower temperatures, pH and salinity can lead to increased metabolic rate (Barrick et al. 2016; Freitas et al. 2016). ATP is an important source for normal physiological functions such as growth and reproduction, and as a result, energy stores are often high in mature individuals, particularly close to spawning (Durou and Mouneyrac, 2007). As a result, exposure to environmental stressors which lead to increased ATP expenditure can lead to a reduction in these processes, which are essential for survival. It is therefore an important endpoint for assessment as exposure to pharmaceuticals could potentially impact *H. diversicolor* physiology.

5.2. Materials and Methods

5.2.1 Sample collection and maintenance

H. diversicolor individuals were collected during low tide at Paull, East Riding of Yorkshire, U.K. (53°43’ North, 0°14’ West) in October 2016. Worms were kept in sediment until return to the lab, where individuals were rinsed and placed in aquaria containing 2.5 litres artificial seawater (20 ppt; Tropic Marin Synthetic Sea Salt) and coral sand. Coral sand was chosen as a substrate for *H. diversicolor* as it allows them to burrow, whist also ensuring that it is free from environmental contaminants. No more than 15 individuals were placed in each container in order to allow sufficient space and were left for 4 weeks to acclimate. Worms were maintained at a photoperiod 12:12 hours (light: dark), constant temperature (13°C ± 0.6), pH (7.9 ± 0.1), salinity (22 ppt ± 1) and oxygenation level (>89% saturation) under constant aeration. A photoperiod of 12:12 was chosen, as *H. diversicolor* were collected in early October when there is between 11 and 12 hours of daylight (UK Hydrographic Office, 2019). Feeding and water changes were carried out on alternate days; individuals were fed with ZM flake fish food, feeding ceased two days prior to exposure assays.

5.2.2 Exposure assays

Exposure assays were conducted under semi-static conditions for 7 days. Ten individuals (mean length 40.11 mm ± 17.90 SD, mean mass 90.46 mg ± 42.82 SD; no statistically significant difference between treatment) were placed in each treatment: either control, low concentration of metformin or diclofenac (100 ng l^{-1}), or high concentration of metformin or diclofenac (1 µg l^{-1}). These concentrations were chosen as they reflect median and peak concentrations of these compounds measured in surface waters (Yang et al. 2011; Hughes et al. 2013; Meador et al. 2016; Burns et al. 2018). Four replicates of each treatment were maintained at each exposure. A standard solution of metformin hydrochloride (≥98%; Sigma-Aldrich, Dorset, UK) or diclofenac sodium (≥98.5%; Sigma-Aldrich, Dorset, UK) were made up at the beginning of the exposure. Water changes were carried out on days 3 and 5, where 2.5 litres of water from each treatment was renewed with 20 ppt seawater and with the relevant pharmaceutical added to each treatment after each water change. Water quality measurements were carried out daily to ensure temperature, salinity, pH and oxygenation level remained constant. The assays were terminated after 7 days and individuals were removed from the treatments and placed at -80°C to euthanise them. Each individual was divided into thirds; one third was reserved for tissue chemical analysis and two thirds for mRNA expression placed in 0.4 mL RNA*later*® Stabilisation Solution (Thermo Fisher Scientific, Loughborough, UK) prior to storage at -20°C.

5.2.3 mRNA isolation and characterisation

The total RNA was extracted from 10 mg *H. diversicolor* tissue using the High Pure RNA Tissue Kit (Roche, Burgess Hill, UK) including DNase I treatment (180 U per sample) according to manufacturer's instructions. Total RNA was quantified using a Qubit 1.0 Fluorometer (Life Technologies, UK). cDNA synthesis utilised the Transcriptor High Fidelity cDNA Synthesis Kit reagents (Roche, Burgess Hill, UK) and was carried out according to manufacturer's instructions. A total of 20 ng RNA was used in each reaction with 2 µl random hexamer primers (6 µM); following pre-incubation, Transcriptor high fidelity reaction buffer (containing RT reaction buffer, 250 mM Tris/HCl, 150 mM KCl, 40 mM MgCl2), RNase inhibitor (0.02 U) and dNTP mix (100 µM) were added to create a final volume of 20 µl. Conditions for cDNA synthesis were as follows: pre-incubation for 10 min at 65ºC followed by the cDNA reaction for 10 min at 25 °C and 60 min at 50 °C. The samples were frozen at -20 °C until analysis.

5.2.4 Primer design

Primers were designed for the *18S* mRNA gene, to act as a housekeeping gene, from *H. diversicolor* (**KC686629.1**) using the Primer-Blast tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Degenerate primers were then designed for the housekeeping gene, elongation factor 1 (*EF1*), and the targeted genes of interest: *AMPK* and *ATPS* from a nucleotide alignment using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/; Appendix, 5.1). Primer details for all genes can be seen in Table 5.1.

Table 5.1: Primers used for gene isolation of *18S, EF1, AMPK* and *ATPS* from *H. diversicolor*

Gene	Primer	Sequence 5'-3'	Tm (°C)	Expected Amplicon Size (bp)
18S	Forward	GGC CGT TCT TAG TTG GTG GA	59	100
	Reverse	TCT AAG AAG TTG GCG CCC G	58	
EF1	Forward	GAY TTC ATC AAR AAC ATG AT	50	686
	Reverse	ACR TTG AAD CCN ACA TTG TC	52	
AMPK	Forward	GGC TAC AAC AAA GCC GTA	52	277
	Reverse	TAG ATR GCR ATC CAG TC	48	
ATPS	Forward	GAC AAC ATT TTC AGR TTC	45	312
	Reverse	GGG TAR ATA CCC AAY TC	46	

5.2.5 PCR Amplification

All PCR reactions contained 17.25 µl molecular grade water, 0.5 µl 40mM dNTP mix, 0.25 µl (0.005 U) Q5® High Fidelity DNA polymerase (New England BioLabs, Massachusetts, USA), and 5 µl Q5® buffer (containing 2 mM Mg) (New England BioLabs, Massachusetts, USA). Thermal cycling conditions were as follow: 94°C for 30 sec, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 2 min. PCR products were separated and visualised by agarose gel electrophoresis. EZ Seq Sanger sequencing service (Macrogen Europe, Amsterdam, The Netherlands) was used for DNA sequencing.

Sequence data were edited, aligned and formed into sequences using BioEdit (Version 7.0.9.0). Sequence identities were investigated using BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to perform nucleotide sequence comparison (blastn) and to compare the translated nucleotide sequences against the protein database (blastx) to identify protein domains. Sequences were aligned and used to perform phylogenetic analysis of amino acid sequences in Mega 5.2. Phylogenetic analysis consisted of Maximum Likelihood Analysis with the Nearest Neighbour Interchange method (1000 bootstrap replicates).

5.2.6 Total quantitative real-time PCR analysis

5.2.6.1 Amplification using quantitative real-time PCR analysis

The total RNA was extracted from 10 mg *H. diversicolor* tissue from each of the treatments as previously described. RNA concentrations were quantified using a Qubit 1.0 Fluorometer (Life Technologies, UK) and cDNA was generated using 20 ng RNA with Transcriptor High Fidelity cDNA Synthesis Kit reagents (Roche, UK) as previously described. Reactions were performed on a CFX96 Real Time PCR Detection System (BioRad, Hemel Hempstead, UK) and contained 10 µl FastStart Universal SYBR Green Master Mix (PrimerDesign, Camberley, UK), 7 µl of molecular-grade water, 2 µl each primer (Table 5.2) and 1 µl cDNA. All samples were analysed in duplicate and template negative reactions were carried out for each of the reactions.

5.2.6.2 Primer optimisation and assay performance

The optimisation of qPCR assays is required to ensure the validity and accuracy of gene expression evaluation. Primers were designed from the sequences isolated from *H. diversicolor* individuals outlined in section 5.2.5 using the NCBI primer-blast tool, and optimised for qPCR assays as described below. qPCR products were separated and visualised using gel electrophoresis (Figure 5.1) and sent to EZ Seq Sanger sequencing services. Identity of isolated sequences were confirmed through alignment with previously aligned sequences and BLAST searches as described previously.

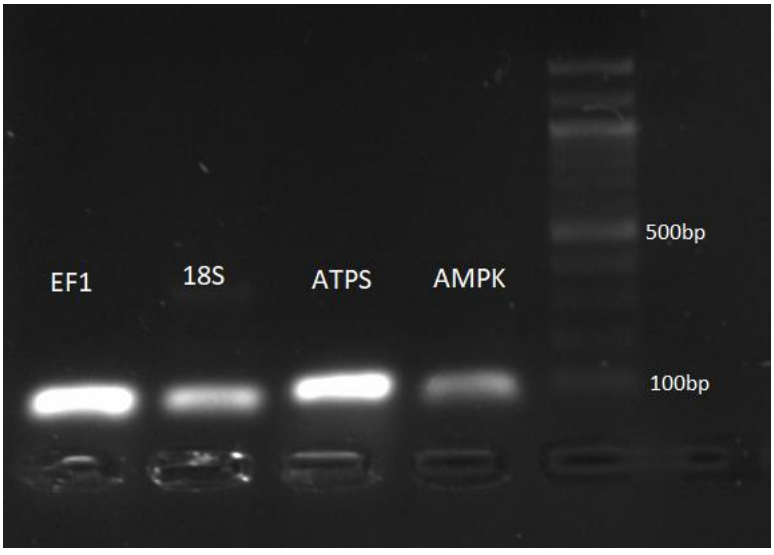


Figure 5.1: Image of a 1% agarose gel electrophoresis of qPCR products. Lane 1, *EF1* housekeeping gene; Lane 2, *18S* housekeeping gene; Lane 3, *ATPS*; Lane 4, *AMPK*; Lane 5, 100 bp ladder. Negative controls were run on a separate agarose gel (Appendix 5.2).

Primer specificity was determined by the melt peaks generated by the reaction, and the absence of other products. Firstly, five different primer concentrations were investigated:

100 nM, 200 nM, 300 nM, 400 nM and 500 nM with the conditions described previously. The primer pair with the lowest Ct (threshold value) and a melt curve only showing a single distinct product was chosen for subsequent qPCR assays (Table 5.2). Ct values greater than 40 were assumed to be due to low efficiency.

To test the efficiency, accuracy and sensitivity of qPCR reactions, a standard curve was performed using a 1:10, 1:5 or 1:2 serial dilution of cDNA, subjected to the same conditions as previously described. To obtain a standard curve, the Ct values of each dilution were plotted against cDNA dilution. Primer efficiencies, assessed from these standard curves and those which had a value between 90–110% were chosen for qPCR assays in accordance with the MIQE guidelines (Bustin et al. 2009). The primers which met these guidelines are outlined in Table 5.2 and were used in qPCR based assays to determine expression of *ATPS and AMPK* of *H. diversicolor* exposed to pharmaceuticals.

Table 5.2: Primers used for qPCR amplification of housekeeping genes and genes of interest from *H. diversicolor*

Gene	Primer	Sequence 5'-3'	Final Concentration (nM)	Efficiency (%)	Expected Amplification Size (bp)
18S	Forward Reverse	GGT GGA GCG ATT TGT CTG GT CGT GCA CGC TGA TTG CTT C	100	90.0	74
EF1	Forward Reverse	CAA CAC CTG GTC CGT CAA GA TGT CCA AGG CAT CGA GAA GG	300	110.4	74
AMPK	Forward Reverse	GTC AAG TCG ACC TGT AGC AGA TGC GCT TCC CAT CTC ACT TT	100	97.1	70
ATPS	Forward Reverse	GCA GGA CGT ATC ACA ACA ACA C TGT AGG GGC AGG ATC TGT CA	200	98.1	93

5.2.7 Statistical Analysis

Statistical analysis was carried out in R studio (1.0.136), using packages PMCMR and ggplot2 (Pohlert 2014; Wickham 2016). In order to determine if there was a statistically significant difference between the size and mass of *H. diversicolor* individuals between treatments, a one-way ANOVA test was used. The stability of housekeeping genes was checked by carrying out ANOVAs, with *18S* selected as a housekeeping gene for data analysis. To evaluate the relative gene expression, the $2^{-\Delta\Delta Ct}$ method was used (Schmittgen and Livak 2008). Normalised values were expressed as fold difference compared to normalised control values, and used to calculate the degree of induction or inhibition. This method was chosen as normalisation to the reference genes can correct and compensate for sample to sample variation of the RNA input. Statistical analysis was carried out on $2^{-\Delta Ct}$ values to determine if there was statistically significant difference in expression between treatments according to Livak and Schmittgen (2001) using

Kruskall-Wallis. A post-hoc Nemenyi test was conducted to determine differences between treatments.

5.3 Results

5.3.1 Isolation and characterisation of genes

5.3.1.1 Target Genes

A partial 199 bp *ATPS* sequence was isolated, sharing 91% similarity with *Nereis vexillosa ATPS* (**DQ087492.1**) sequence. The translated nucleotide sequence showed similarity with protein sequences from other species (Figure 5.2a). Comparison did not identify any specific conserved domain, but comparison of *N. vexillosa* protein sequence identified the ATPase beta subunit binding domain and conserved Walker A and Walker B motifs (Figure 5.2a). Phylogenetic analysis revealed that *H. diversicolor* was clustered with other annelids, *N. vexillosa* (**AAZ30692.1**) and *Nephasoma pellucidum* (**ADW27397**; Figure 5.3a).

A partial 205 bp *AMPK* sequence was isolated, sharing 86% similarity with *Schistosoma japonica AMPK* (**GU130533.1**), and the translated nucleotide showed similarity with protein sequences from other species (Figure 5.2b). A comparison of *H. diversicolor* translated nucleotide sequence identified the serine/threonine protein kinase domain, as well as protein domains of the protein kinase superfamily, of which *AMPK* is a member. Phylogenetic analysis revealed that *H. diversicolor* was clustered with another annelid *Hydroides elegans* (**BAE19914.1**; Figure 5.3b). It is also closely related to vertebrates and arthropod species.

5.3.1.2 Housekeeping Gene

A 511 bp partial *EF1* sequence was isolated, sharing 97% similarity with *Hediste japonica EF1* sequence (**AB003702.1).** The translated nucleotide showed similarity with protein sequences from other species, and elongation factor Tu GTP binding domains (GTP_EFTU) were identified (Figure 5.2c). Protein domains characteristic of *EF1* alpha were also identified. Phylogenetic analysis revealed that the isolated partial *H. diversicolor EF1* amino acid sequence was clustered with another annelid from the same genus *H. japonica* (**BAA25731.1**), but was more distantly related to another annelid, *N. vexillosa* (**ABI13251.1**; Figure 5.3c).

(a) *ATPS*

```
N.pellucidum      VDVQFDEELPPMLNALEVQGRDTRLVLEVAQHLGENTVRTIAMDGTEGLVRGQPVVDTNA      60
N.vexillosa       -------DLPPILNALEVQNRTPRLILEVAQHLGENTVRTIAMDGTEGLVRGQPCYDIGS      53
H.diversicolor    ------------------------------------------------------------      0
O.lemacina        ----------------------------------------IAMDGTEGLVRGQVCVDTGT      20

                            Beta subunit nucleotide binding domain
N.pellucidum      PIRXPVGPETLGRIMNVIGEPIDERGPIPSKAFSGIHQEAPEFTEMSVEXEILETGIKVV      120
N.vexillosa       PISIPVGPETLGRIINVIGEPIDERGPVNARTTAPIHAEAPEFVEMSVEQEILETGIKVV      113
H.diversicolor    ------------------------------------------------------------      0
O.lemacina        PISIPVGPETLGRIINVIGEPIDERGPVNAKTYWGIHQDAPEFVEMSVEQEILETGIKVA      80
                                  Walker A Motif
N.pellucidum      DLLAPYSKGGKIGLFGGAGVGKTVLIMELINNVAKAHGGYSVFAGVGERTREXNDLYHEM      180
N.vexillosa       DLLAPYAKGGKIGLFGGAGVGKTVLIMELINNVAKAHGGYSVFAGVGERTREGNDLYHEM      173
H.diversicolor    ------------------------------------------------------------      0
O.lemacina        DLLAPYAKGGKIGVFGGAGVGKTVLIMELINNVAKAHGGYSVFAGVGERTREGNDLYHEM      140
                                                                Walker B Motif
N.pellucidum      IESGVIXLKDDTSKVSLVYGQMNEPPGARARVALTGLTVAEYFRDQEGQDVLLFIDNIFR      240
N.vexillosa       IEGGVISLKDDTSKVSLVYGQMNEPPGARARVALTGLTVAEYFRDIEGQDVLLFIDNIFR      233
H.diversicolor    ------------------------------------------------------------      0
O.lemacina        IESGVINLKDDSSKVSLVYGQMNEPPGARARVALTGLTVAEYFRDQEGQDVLLFIDNIFR      200

N.pellucidum      FTQAGSEVSALLGRIPSAVGYQPTLATDXGTMQERITTTKKGSITSVQAIYVPADDLTDP      300
N.vexillosa       FTQAGSEVSALLGRIPSAVGYQPTLATDMGAMQERITTTRKGSITSVQAIYVPADDLTDP      293
H.diversicolor    --------------PSAVGNQPTLATDMGTMQERITTTQKGSITSVQAIYVPADDLTDP      45
O.lemacina        FTQAGSEVSALLGRIPSAVGYQPTLATDMGTMQERITTTTKGSITSVQAIYVPADDLTDP      260
                                ***** ******* *:******** ********************

N.pellucidum      APATTFAHLDATTVLSRGIAELGIYPAVDPLDSISRILDPNVVGEEHXNVARAVQKILQD      360
N.vexillosa       APATTFAHLDATTVLSRGIAELGIYPAVDPLDSTSRILDPNIVGAEHYGVARGVQKILQD      353
H.diversicolor    APTTTFAHLXATTVLSRGIAKL---------------------------------------      67
O.lemacina        APATTFAHLDATTVLSRGIAELGIYPAVDPLDSNSRILDKNVVGEEHYTVARGVQKILQD      320
                  **:****** **********:*

N.pellucidum      HKSLQDIIAILGMDXLS----------------------      377
N.vexillosa       YKSLQDIIAILGMDDLSEDDKLTVSRARKIQRFLS----      388
H.diversicolor    ----------------------------------------      67
O.lemacina        NKSLQDIIAILGMDELSEEDKLTVSRARKIQRFLSQPFQ      359
```

(b) *AMPK*

```
L.polyphemus      MGNAATTKKGEHVTESVEKFLAEAKEQFEIKWNNPSKNTSSLDDFDRIKTLGTGSFGRVM      60
L.crocea          ------------------------------------------------------------M      1
S.japonica        MGNAQTAKKGDPA--DVKALLEEAKKEFLEKWEKPAQNTATLDSFDRIKTLGTGSFGRVM      58
H.diversicolor    ------------------------------------------------------------      0
V.jacobsoni       ----------------------------------------MLDDFDRIKTLGTGSFGRVM      20

L.polyphemus      LVQHKNDKDYYAMKILDKQKVVKLKQVEHTLNEKKILQAIDFPFLVKLEFHFKDNSNLYM      120
L.crocea          LVKHKETNQFYAMKILDKQKVVKLKQIEHTLNEKRILQAVSFPFLVRLEYSFKDNSNLYM      61
S.japonica        LVQHKVSKEYYAMKILDKQKVVKLKQVEHTLNEKRILQAISFPFLVRLDYHFKDNSNLYM      118
H.diversicolor    ------------------------------------------------------------      0
V.jacobsoni       LVKHRNAGDYFAMKILDKQKVVKLKQVEHTLNEKKILQAVDFPFLVRLAYHFKDNSNLYM      80

L.polyphemus      VLPYVVGGEMFSHLRKVGRFSEPHSRFYAAQIVLAFQYLHSLDLVYRDLKPENILIDQDG      180
L.crocea          VMEYVPGGEMFSHLRRIGRFSENHARFYAAQIILTFEYLHSLDLIYRDLKPENLLIDQHG      121
S.japonica        VLEFVNGGEMFSHLRRIGRFSESHSRFYASQVVLAFEYLHHLELVYRDLKPENILIDQYG      178
H.diversicolor    ------------------------------------------------------------      0
V.jacobsoni       VLEYVQGGEMFTHLRKVGKFSEPHARFYAAQIVLAFQYLHSLDLIYRDLKPENLLIDHQG      140
                                Serine/Theonine Protein Kinase Domain
L.polyphemus      YIKVTDFGFAKRVKGRTWTLCGTPEYLSPEIILSKGYNKAVDWWALGVLVYEMAAGYPPF      240
L.crocea          YIQVTDFGFAKRVKGRTWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPF      181
S.japonica        YLKITDFGFAKRVKGRTWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLVYEMAAGYPPF      238
H.diversicolor    ----------------------------------------VDWWALGVLVYEMAAGYPPF      20
V.jacobsoni       YIKVTDFGFAKRVKGRTWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLVYEMAAGYPPF      200
                                                          *********:**********

L.polyphemus      FADQPIQIYEKIVSGKVRFPSHFTSDLKDILRNLLQVDLTKRYGNLKNGVNDIKNHKWFA      300
L.crocea          FADQPIQIYEKIVSGKVRFPSHFSSDLKDLLRNLLQVDLTKRFGNLKNGVNDIKNHKWFS      241
S.japonica        FADQPIQIYERIVSGKVRFPSHFSSDLKDLLRNLLQVDLTKRFGNLKNGVNDIKTHKWFA      298
H.diversicolor    FADQPIQIYEKIVSGKVRFPSHFSSDLKDLLKNLLQVDLTKRYGNLKNGVNDII------      74
V.jacobsoni       FADQPIQIYEKIVSGKVRFPSHFTSDLKDLLRNLLQVDLTKRYGNLKNGVNDIKNHRWFA      260
                  **********:***********:*****.*:**********:**********

L.polyphemus      TLDWIAIYQKKVEAPFLPKCKGPGDTSNFDEYEEETLRVSGTEKCVKEFADF       352
L.crocea          TTDWIAIYERKVEAPFLPKCRGPGDTSNFDDYEEEDIHVSQTEKCAKEFADF       293
S.japonica        TTDWFSIFKRDIEAPFTPKCSGAGDASNFDDYEEEPLRIATTEKCAKEFSEF       350
H.diversicolor    ------------------------------------------------------      74
V.jacobsoni       QTDWIAIYRKEIEAPFLPRSAGPGDTSNFDEYEEEPLRISSTEKCAKEFADF       312
```

Figure 5.2: Alignment of *H. diversicolor* translated nucleotide sequence with multiple species. Dashes represent gaps in the alignment and asterisks represent homology. Shaded boxes show conserved domains and grey boxes show other conserved features. Alignments were cropped and are not shown in full. **(a)** *EF1* alignments with *A. aurita*, *O. mykiss* and *H. japonica* (**BAA25731.1**) **(b)** *ATPS* alignments with *N. vexillosa* (**AAZ306902.1**), *N. pellucidum* (**ADW27397.1**), *O. lemacina* (**ADW27401.1**). **(c)** *AMPK* alignments with *H. elegans* and *S. japonica* (**GU130533.1**).

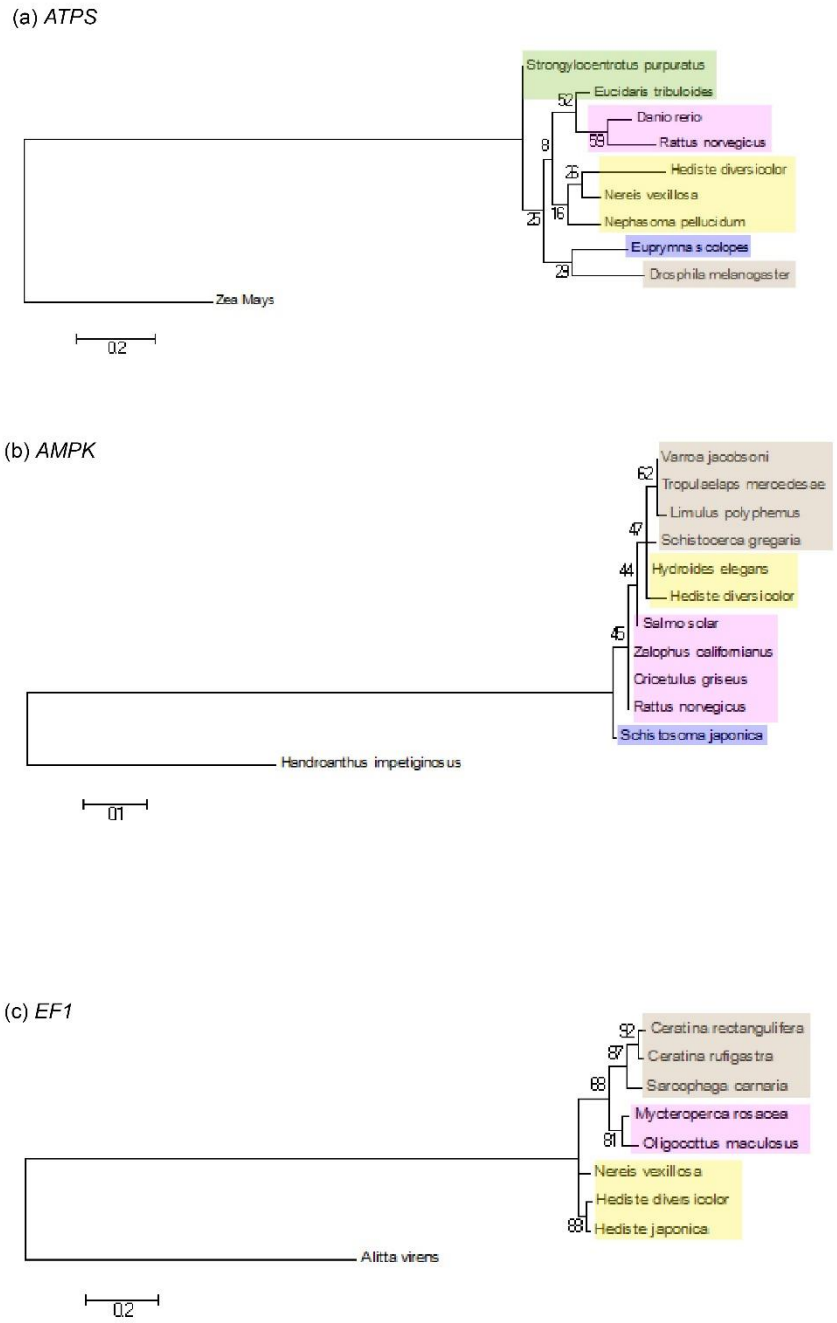


Figure 5.3: Phylogenies of full and partial amino acid sequences for **(a)** *ATPS* **(b)** *AMPK* and **(c)** *EF1* rooted with *Alitta virens* elongation factor 2. Shaded boxes represent species groups: green – echinoderms; pink – vertebrates; yellow – annelids; blue – molluscs; brown – arthropods.

5.3.2 Quantitative real-time PCR optimisation

Five primer concentrations were tested in order to determine the optimal primer concentrations. 100 nM *18S and AMPK,* 200 nM *ATPS*, and 300 nM *EF1* resulted in the lowest Ct value and unique dissociation temperature peak according to melt curves (Figure 5.4ii). PCR amplification efficiency for reference and target genes ranged from 90% (*18S*) to 110% (*EF1*), indicating that all of the primers had high specificity (Figure 5.4iii). The serial dilution of these genes resulted in an $R^2 > 0.96$ for all genes, showing that non-diluted cDNA used in qPCR assays were within this range (Figure 5.4i).

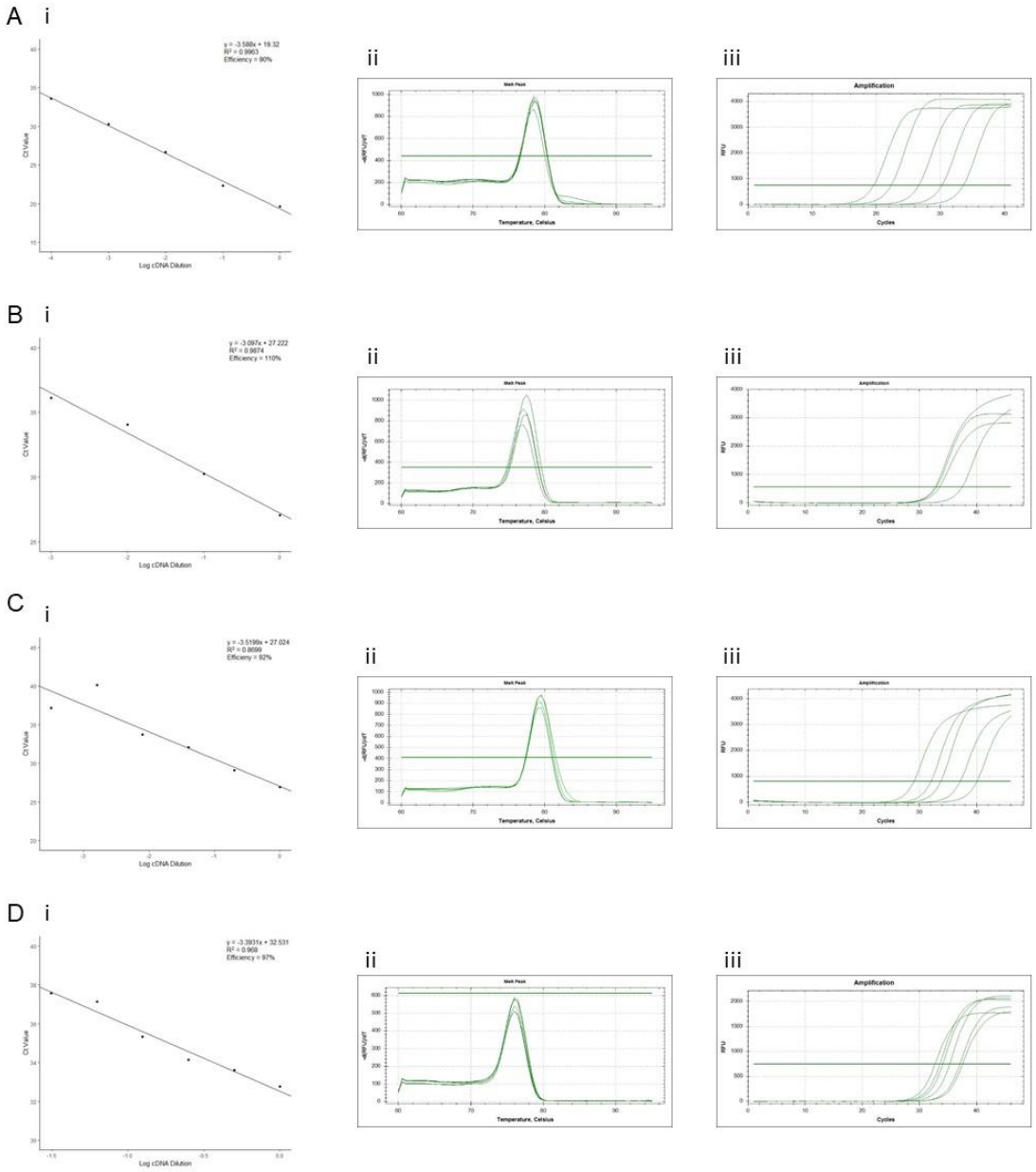


Figure 5.4: (i) Standard curves (ii**)** melt peaks and (iii) qPCR amplification generated from amplification of each gene using diluted cDNA samples as follows: A: *18S*, B: *EF1,* C: *ATPS* and D: *AMPK*

5.3.3 Expression of *ATPS*

After 7 days of exposure, only *H. diversicolor* exposed to the metformin treatment with a nominal concentration of 1 µg l^{-1} showed a significant difference from the control in the expression of *ATPS* (Kruskal-Wallis, $\chi^2 = 19.271$, $p < 0.001$), which led to an increase in expression (Figure 5.4). The relative expression of *ATPS* was also significantly higher in this treatment than the diclofenac treatments, however there was no statistically significant difference between metformin treatments (Figure 5.5).

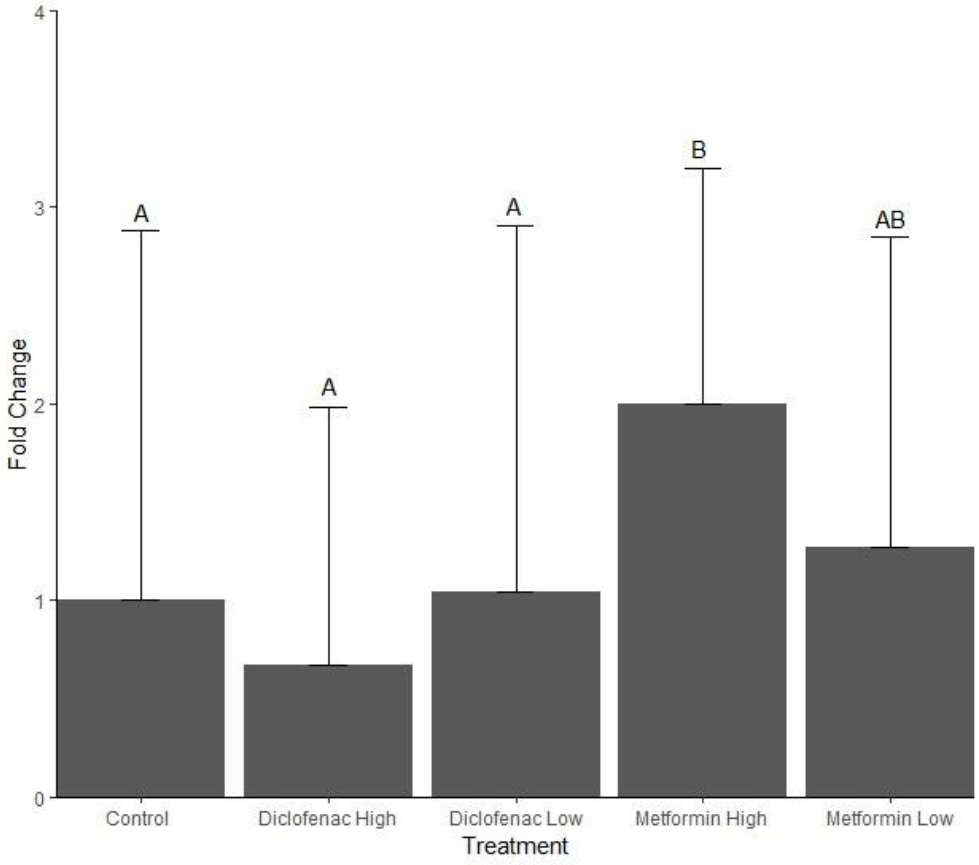


Figure 5.5: Fold change ($2^{-\Delta\Delta Ct}$) in expression of *ATPS* in *H. diversicolor* exposed to diclofenac high (1 µg l^{-1} nominal concentration; n = 22), diclofenac low (100 ng l^{-1}; n = 28), metformin high (1µg l^{-1} nominal concentration; n = 28) or metformin low (100ng l^{-1} nominal concentration; n = 23) relative to control control (n = 28). Error bars represent standard deviation calculated as outlined in Livak and Schmittgen (2001). Different letters denote exposure groups that are significantly different (P>0.05) analysed using Kruskal-Wallis.

5.3.4 Expression of *AMPK*

After 7 days of exposure, there was no significant difference in the expression of *AMPK* between any of the treatments (Kruskal-Wallis $\chi^2 = 2.0641$, $p > 0.05$; Figure 5.6).

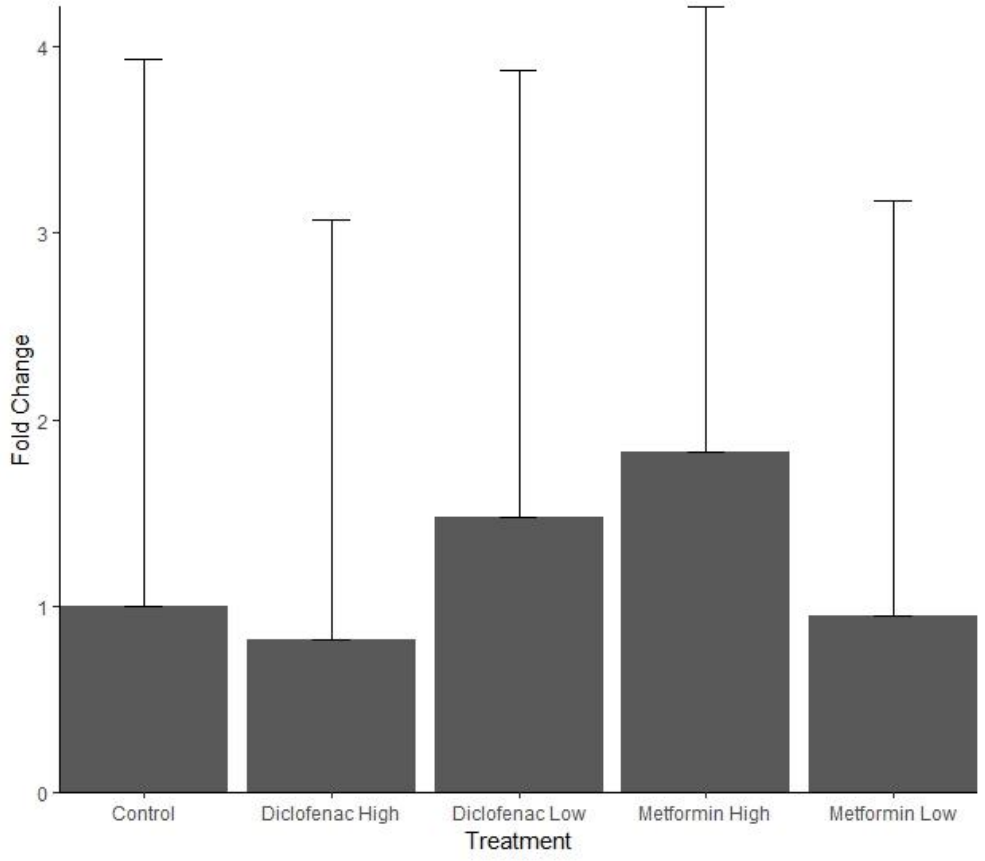


Figure 5.6 Fold change ($2^{-\Delta\Delta Ct}$) in expression of *AMPK* of *H. diversicolo*r exposed to either diclofenac high (1 µg l^{-1} nominal concentration; n = 22), diclofenac low (100 ng l^{-1} nominal concentration; n = 23), metformin high (1µg l^{-1} nominal concentration; n = 27) or metformin low (100ng l^{-1} nominal concentration; n =23) relative to control (n = 21). Error bars represent standard deviation calculated as outlined in Livak and Schmittgen (2001).

5.4 Discussion

5.4.1 RNA isolation and assay optimisation

One partial housekeeping sequence (*EF1*) and two target sequences (*ATPS* and *AMPK*) were isolated from *H. diversicolor* as demonstrated by GenBank database comparisons, multiple species amino acid alignments (Figure 2) and phylogenetic trees (Figure 3). The target genes, *ATPS* and *AMPK* were 91% and 86% similar to other related genes and the phylogenetic analysis showed clustering of the target genes with other annelid species, indicating that they were very likely the homologs of the *ATPS* and *AMPK* genes respectively.

Primer efficiencies for *18S, EF1, ATPS* and *AMPK* were 90 – 110% efficient and concentrations of cDNA fell within the standard curves generated. Analysis of gene transcripts was calculated based on relative change in mRNA expression of a reference and target gene, so it is essential that these are consistent and reliable. RNA quality was not measured and partially degraded RNA could have resulted in poor reactions and unreliable expression results (Vermeulen et al. 2011). However, steps were taken to limit RNA degradation such as appropriate storage of samples in RNA later and storage of RNA at -80ºC. Additionally, two technical replicates were conducted for each of the sample reactions, and those which had a difference in Ct value greater than 0.5 were not included in the final analysis. Ct values of *EF1* differed significantly between treatments and as a result was not a suitable housekeeping gene, and ΔCt values were calculated as relative expression between target gene and *18S.*

5.4.2 Pharmaceutical exposures

The effects of metformin and diclofenac at low nominal (100 ng l^{-1}) and high nominal (1 µg l^{-1}) concentrations on mRNA expression after 7 days of exposure were investigated in the polychaete *H. diversicolor*. Following controlled exposure for 7 days, the high nominal dose (1 µg l^{-1}) of metformin was the only treatment to alter expression of *ATPS*, and none of the treatments had a significant effect on *AMPK*. No mortalities were observed in these exposures, indicating that these compounds only have the potential for sub-lethal toxicity. It was not possible to analyse water samples from exposures for pharmaceutical concentrations, and as a result, it is only possible to express treatment doses as nominal concentrations. It would have been beneficial to take these measurements in order to confirm actual exposure concentrations in order to better interpret the results (Harris et al. 2014). Semi-static exposures were conducted, which could result in a decrease in water concentration between dosing and replenishing water as the result of degradation or in the rise in concentrations as the result of repeated dosing. All exposure media was replenished every other day in order to minimise these effects and try to ensure stable exposure concentration for the duration of the experiment.

Whilst there was no statistically significant difference in the size of *H. diversicolor* individuals between treatments, there was a difference within-treatments, which could account for the high variation seen in the expression of *ATPS* and *AMPK* (Harris et al. 2014). The uptake of pharmaceuticals could differ between individuals of different sizes or maturity, which could lead to this variation in gene expression. Additionally, difference in size indicates differences in maturity which can lead to differences in energy status and can also account for variability (Durou and Mouneyrac 2007). At the end of the

exposures, *H. diversicolor* were divided into thirds, with two thirds reserved for qPCR analysis and one third for tissue analysis. The portion of the worm (i.e. head, middle or tail) was randomly divided for these analyses, which could account for some of the variability seen in gene expression. Different organs will have different metabolic requirements and energy is often partitioned to tissue and organs based on this need which can lead different expression between the anterior and posterior end. In fish, partitioning has of energy has been found to be allocated differently in mature and reproducing individuals, and will also differ between sex (Patterson et al. 2004). The uptake and accumulation of pharmaceuticals has also been found to be tissue-dependent in fish, and if this is the same for *H. diversicolor,* it could help explain these variations. (Zhao et al. 2015)

5.4.3 Metformin

In vertebrates, the primary function of metformin is to reduce glucose output in the liver and secondarily to stimulate glucose uptake in the muscles (Joshi 2005). The primary target of metformin in humans has been debated (Viollet et al. 2012). It was originally thought *AMPK* was the primary target, but it has also been suggested that the activation of *AMPK* is the result of specific inhibition of respiratory chain complex I (Bridges et al. 2014; Fontaine 2014). Metformin has been shown to activate *AMPK* and exert a similar therapeutic effect in non-target vertebrates as humans, causing the inhibition of hepatic glucogenesis in fish (Panserat et al. 2009) and the activation of glucose uptake into fish muscle (Magnoni et al. 2012).

The pathway of metformin in invertebrates is not currently known but there is evidence that it still acts as an *AMPK* activator (Sheng et al. 2012). *AMPK* is highly conserved and has been found to maintain energy budgets in other invertebrates including crustaceans and molluscs (Sokolova et al. 2012). Metformin has also been found to activate *AMPK* in *Daphnia* (Sheng et al. 2012). *AMPK* is responsible for regulating energy budgets in response to environmental or nutritional stress (Bridges et al. 2014). It is activated by limited ATP or increased ATP depletion. Whilst *AMPK* expression did not differ between treatments, *ATP*S expression was higher in the high metformin treatment indicating that metformin is causing stress in *H. diversicolor* leading to depletion of ATP. It is possible that longer exposure or higher concentrations could lead to *AMPK* activation as the result of ATP synthesis not being able to keep up with requirements. *H. diversicolor* individuals were collected from Paull (A1) where other pharmaceuticals were detected in surface water in Chapter 4, and Metformin has been detected in tributaries of the Humber (Burns et al. 2018). It is therefore plausible that metformin may be present at this site, and could

affect the expression of these genes as individuals may already be stressed, or may have acclimatised to the concentrations of these compounds.

The effect of metformin on *ATPS*, a general biomarker for stress could be indicative of other negative effects occurring (Sokolova et al. 2012). Exposure to metformin has also caused reproductive changes including causing lower fecundity in intersex minnows (*P. promelas;* Niemuth and Klaper 2015), as well as increasing vitellogenin in mussels, (*Mytilus edulis*; Koagouw and Ciocan 2018). There are conflicting reports in the literature as to whether this is an expected (Crago et al. 2016) or unexpected (Sumpter et al. 2016) mechanism in non-target organisms. A therapeutic effect of metformin in humans is to reduce the androgen effects of polycystic ovaries to increase ovulation, however, the mechanism of action is poorly understood (Spritzer 2014). It is thought that this use is the result of lowered insulin, but it is also thought that metformin could also directly affect steroidogenesis (Lashen 2010). Sexual steroids also play a vital role in the reproduction of polychaetes including *H. diversicolor*, so this and depleted energy reserves have been shown to affect reproductive abilities in contaminated estuaries (Durou and Mouneyrac 2007). As a result, further investigation into the pathways and effects of metformin on *H. diversicolor* reproduction is warranted.

Many of the reproductive effects seen in experimental studies, were the result of exposure to very high concentrations of metformin to mussels (*M. edulis;* 40 µg $l^{-1;}$;Koagouw and Ciocan, 2018) and fathead minnows, *Pimphelas promelas* (40 µg l^{-1}; Niemuth et al. 2014, Niemuth and Klaper 2015). No differentiation was made in the inclusion of males and females in the present study, and if metformin does impact the reproductive system, it will affect each sex differently. Concentrations of 1 µg l^{-1} were seen to increase vitellogenin expression in juvenile fathead minnows, but no changes were seen at levels up to 100 µg l^{-1} in adults (Crago et al. 2016). This is the only study to investigate the age-dependent effects of metformin and introduces uncertainty surrounding the variability seen in mRNA expression, and whether this is natural variation or as the result of the range of sizes used in the exposures. The effects of metformin on glucose homeostasis in trout (*Oncorhynchus mykiss*) were seen after being fed or injected metformin at doses of approximately 50 mg kg^{-1} (Panserat et al. 2009; Polakof et al. 2009; Polakof et al. 2010). This is reflective of the large quantities of metformin (approximately 2.5 g per day), which are required to have a therapeutic effect in humans (Rena et al. 2013). Although metformin has been detected in surface waters at high concentrations in comparison to other compounds, the concentrations used in these studies (1 – 100 µg l^{-1}) are similar to those seen in wastewater influent (2 – 129 µg l^{-1}) and effluent (1.2 – 100 µg l^{-1}), and those which have been measured in surface water

are much lower (< 3µg l^{-1}; Bradley et al. 2016, Briones et al. 2016, Burns et al. 2018). Metformin has frequently been detected in surface water at approximately 1 µg l^{-1} (Scheurer et al. 2012), but averages are far lower than this (Briones et al. 2016). The effect on *ATPS* expression seen in the current study suggests that metformin could have an effect at peak environmental concentrations. Additionally, the potential role of metformin as an endocrine disruptor are concerning and this highlight the need for further investigation into whether these effects can be seen at concentrations regularly detected in the environment.

5.4.4 Diclofenac

Diclofenac didn't have an effect on *AMPK* or *ATPS* expression at either low nominal (100 ng l^{-1}) or high nominal (1 µg l^{-1}) concentrations. Similarly to mRNA expression in the metformin treatment, there was high within-treatment variability. However, size differences of *H. diversicolor* were non-significant between treatments so it is possible that these endpoints are not affected by diclofenac. These endpoints have not previously been measured in non-target species, however, there is some evidence that diclofenac, and other acidic nonsteroidal anti-inflammatories (NSAIDs) may activate AMPK in humans and mice, and it is thought that this could contribute to the anti-inflammatory and analgesic properties (King et al. 2015). Additionally*, AMPK* has been observed to be activated in mussels exposed to municipal effluent (Goodchild et al. 2015). Although it is a target of metformin, *AMPK* can also be a sign of environmental stress as the result of depleted energy reserves.

Although *H. diversicolor* is a key species in estuarine environments, and has been suggested as a bioindicator of contaminated estuaries, few studies have researched the impact of pharmaceuticals on this or similar species (Catalano et al. 2012; Maranho et al. 2014). *H. diversicolor* exposed to ibuprofen has been found to result in the inhibition COX, leading to increased mitochondrial energy consumption and neuroendocrine effects (Maranho et al. 2015). Diclofenac is generally considered to be more toxic to organisms than other NSAIDs as evidenced by both acute (Sanderson and Thomsen 2009; Vestel et al. 2016) and chronic toxicities (Du et al. 2016). Diclofenac has been shown to inhibit COX activity and prostaglandin synthesis in other aquatic invertebrates such as the mussels *Mytilus galloprovincialis*, and *Perna perna* (Courant et al. 2017; Fontes et al. 2018). As a result, it is also possible that diclofenac could inhibit COX activity in *H. diversicolor*, but this has not been studied in aquatic annelids.

Prostaglandins not only play a role in inflammation response, but also in other physiological processes including osmoregulation, homeostasis and reproduction

(Ruggeri and Thoroughgood 1985). Most invertebrates only have one isoform of this enzyme, which is responsible for all of these processes, and as a result can be affected by diclofenac (Heckmann et al. 2008; Rowley et al. 2005). In vertebrates, diclofenac selectively inhibits COX-II, however, it is unknown how these base-line physiological processes will be affected in *H. diversicolor*. Interruption of these processes could lead to stres and increased ATP demand in order to survive. The variation seen in these experiments, could indicate that the toxicity of diclofenac could be dependent on other factors such as age, sex and size due to differences in metabolic requirements and prior exposure to contaminants as previously discussed.

Diclofenac has been shown to cause oxidative stress in the zebra mussel, *Dreissena polymorpha* (Quinn et al. 2011), alter reproduction in mussels (*Mytilus spp;.* Schmidt et al. 2011), reduce hatching success in *Daphnia magna* (Lee et al. 2011), impact osmoregulation in the edible crab, *Carcinus maenas* (Eades and Waring 2010), and cause neurotoxic effects in *M. galloprovincialis* (Gonzalez-Rey and Bebianno 2012). It has also been found to affect the motility of annelid *Arenicola marina* sperm at concentrations of 100 ng l^{-1} (Mohd Zanuri et al. 2017). *H. diversicolor* reproduce, by males spawning into the water and females bringing sperm into the burrows where they have buried their eggs, diclofenac polluted waters could negatively affect this (Scaps 2002). These studies are evidence that diclofenac can cause biological changes in aquatic organisms, and this has often been demonstrated to occur at concentrations similar to those found in surface water (Acuña et al. 2015). However, there has been a focus on the use of fish and bivalves, and it would be beneficial to improve information to account for the effects of diclofenac outside of these species.

5.5 Conclusion

This is the first study to investigate the effects of metformin or diclofenac on the estuarine polychaete *H.* diversicolor. Metformin was found to increase the expression of *ATPS* at the high nominal concentration (1 µg l^{-1}) and the requirement to sustain high energy levels could have long term consequences in the environment. Metformin failed to alter the expression of *AMPK,* the target of metformin in vertebrates. As a result, there is a need for further investigation of metformin pathways in aquatic invertebrates, particularly at environmentally relevant concentrations. This could help to interpret the results seen in this study and determine how factors such as maturity and sex could affect toxicity of metformin and diclofenac. The potential impact of metformin to biota is particularly concerning, as metformin in now one of the most widely used drugs globally and if recent trends continue, usage will rise leading to higher concentrations in surface water

(Oosterhuis et al. 2013). Diclofenac on the other hand, did not activate *ATPS* or *AMPK* at either concentration, indicating that diclofenac does not affect the energy balance in *H. diversicolor*. There is also a need to further investigate the effects of both these compounds at longer exposures in order to further understand the potential implications to long-term chronic exposures in the environment.

5.6 References

Acuña V, Ginebreda A, Mor JR, Petrovic M, Sabater S, Sumpter J, Barceló D (2015) Balancing the health benefits and environmental risks of pharmaceuticals: Diclofenac as an example. Environ Int 85: 327–333 doi: 10.1016/J.ENVINT.2015.09.023

Barrick A, Chatel A, Marion J-M, Perrein-Ettajani H, Bruneau M, Mouneyrac C (2016) A novel methodology for the determination of biomarker baseline levels in the marine polychaete *Hediste diversicolor.* Mar Pollut Bull 108: 275-280. doi: 10.1016/J.MARPOLBUL.2016.04.056

Bradley PM, Journey CA, Button DT, Carlisle DM, Clark JM, Mahler BJ, Nakagaki N, Qi SL, Waite IR, VanMetre PC (2016) Metformin and Other Pharmaceuticals Widespread in Wadeable Streams of the Southeastern United States. Environ Sci Technol Lett 3(6): 243-249 doi: 10.1021/acs.estlett.6b00170

Bridges HR, Jones AJY, Pollak MN, Hirst J (2014) Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. Biochem J 462:475–487. doi: 10.1042/BJ20140620

Briones RM, Sarmah AK, Padhye LP (2016) A global perspective on the use, occurrence, fate and effects of anti-diabetic drug metformin in natural and engineered ecosystems. Environ Pollut 219L 1007–1020 doi: 10.1016/j.envpol.2016.07.040

Buffet PE, Poirier L, Zalouk-Vergnoux A, Lopes C, Amiard JC, Gaudin P, Risso-de Faverney C, Guibbolini M, Gilliland D, Perrein-Ettajani H, Valsami-Jones E, Mouneyrac C (2014) Biochemical and behavioural responses of the marine polychaete Hediste diversicolor to cadmium sulfide quantum dots (CdS QDs): waterborne and dietary exposure Chemosphere 100: 63-70 doi: 10.1016/j.chemosphere.2013.12.069

Burns EE, Carter LJ, Kolpin DW, Thomas-Oates J, Boxall ABA (2018) Temporal and spatial variation in pharmaceutical concentrations in an urban river system. Wat Res

137: 72-85 doi:10.1016/j.watres.2018.02.066

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele, Wittwer, CT (2009) The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin Chem 55(4): 611-622 doi: 10.1373/clinchem.2008.112797

Catalano B, Moltedo G, Martuccio G, Gastaldi L, Virno-Lamberti C, Lauria A, Ausili A (2012) Can Hediste diversicolor (Nereidae, Polychaete) be considered a good candidate in evaluating PAH contamination? A multimarker approach. Chemosphere 86(9): 875–882 doi: 10.1016/J.CHEMOSPHERE.2011.10.040

Courant F, Arpin-Pont L, Bonnefille B, Vacher S, Picot-Groz M, Gomez E, Fenet H (2017) Exposure of marine mussels to diclofenac: modulation of prostaglandin biosynthesis. Environ Sci Pollut R 25(7): 1–8. doi: 10.1007/s11356-017-9228-6

Crago J, Bui C, Grewal S, Schlenk D (2016) Age-dependent effects in fathead minnows from the anti-diabetic drug metformin Gen Comp Endocrin 232: 185–190 doi: 10.1016/j.ygcen.2015.12.030

Du J, Mei C, Ying G, Xu M (2016) Toxicity Thresholds for Diclofenac, Acetaminophen and Ibuprofen in the Water Flea Daphnia magna. Bull Environ Contam Toxicol 97(1): 84–90. doi: 10.1007/s00128-016-1806-7

Durou C, Mouneyrac C (2007) Linking steroid hormone levels to sexual maturity index and energy reserves in Nereis diversicolor from clean and polluted estuaries. Gen Comp Endocrin 150(1): 106–113 10.1016/j.ygcen.2006.07.019

Durou C, Smith BD, Romeo M, Rainbow PS, Mouneyrac C, Mouloud M, Gnassia-Barelli M, Gillet P, Deutch B, Amiard-Triquet C (2007) From biomarkers to population responses in *Nereis diversicolor*: Assessment of stress in estuarine species. 66: 402-411 doi: 10.1016/J.ECOENV.2006.02.016

Eades C, Waring CP (2010) The effects of diclofenac on the physiology of the green shore crab Carcinus maenas Mar Environ Res 69: S46–S48. doi: 10.1016/j.marenvres.2009.11.001

Fontaine E (2014) Metformin and respiratory chain complex I: the last piece of the puzzle? Biochem J 463(3): e3–e5. doi: 10.1042/bj20141020

Fontes MK, Gusso-Choueri PK, Maranho LA, Abessa DMS, Mazur WA, de Campos BG, Guimarães LL, de Toledo MS, Lebre D, Marques JR, Felicio AA, Cesar A, Almeida

EA, Pereira CDS (2018) A tiered approach to assess effects of diclofenac on the brown mussel Perna perna: A contribution to characterize the hazard. Wat Res 132: 361–370 doi: 10.1016/j.watres.2017.12.077

Freitas R, Pires A, Moreira A, Wrona FJ, Figueira E, Soares AMVM (2016) Biochemical alterations induced in *Hediste diversicolor* under seawater acidification conditions. Mar Environ Res. 117: 75-84 doi: 10.1016/J.MARENVRES.2016.04.003

Gan TJ (2010) Diclofenac: an update on its mechanism of action and safety profile. Curr Med Res Opin 26(7): 1715–1731 doi: 10.1185/03007995.2010.486301

Gonzalez-Rey M, Bebianno MJ (2012) Does non-steroidal anti-inflammatory (NSAID) ibuprofen induce antioxidant stress and endocrine disruption in mussel Mytilus galloprovincialis? Environ Toxicol Pharmacol 33(2): 361–371. doi: 10.1016/j.etap.2011.12.017.

Gunnarsson L, Jauhiainen A, Kristiansson E, Nerman O, Larsson DGJ (2008) Evolutionary Conservation of Human Drug Targets in Organisms used for Environmental Risk Assessments Environ Sci Technol 42(15): 5807–5813. doi: 10.1021/es8005173

Goodchild CG, Frederich M, Zeema SI (2015) AMP-activated protein kinase is a biomarker of energetic status in freshwater mussels explosed to municipal effluents. Sci Total Environ. 512-513: 201-209.

Harris CA, Scott AP, Johnson AC, Panter GH, Sheahan D, Roberts M, Sumpter JP (2014) Principles of sound ecotoxicology Environ Sci Technol 48: 3100-3111 doi: 10.1021/es4047507

He J, Wang Q, He X, Chen X, Liu C, Zhou Y (2019) Distribution of Cu fraction in sediments using Suaeda heteroptera - Nereis succinea combination: A greenhouse study Int J Phytoremediation 21(2): 129-137 doi: 10.1080/15226514.2018.1488807

Heckmann L, Silbly RM, Timmermans MJTN, Callaghan A (2008) Outlining eicosanoid biosynthesis in the crustacean Daphnia. Front Zool 5(1): 11-20 doi: 10.1186/1742-9994-5-11

Hughes SR, Kay P, Brown, LE (2013) Global Synthesis and Critical Evaluation of Pharmaceutical Data Sets Collected from River Systems. Environ Sci Technol 47(2): 661–677 doi: 10.1186/1742-9994-5-11

Joshi SR (2005) Metformin: old wine in new bottle--evolving technology and therapy in

diabetes J Assoc Physicians India 53:963–972 doi: 10.1016/j.phrs.2016.08.035

King TS, Russe O, Möser CV, Ferreirós, Kynast KL, Knothe C, Olbrich K, Geisslinger F, Niederberger E (2015) AMP-activated protein kinase is activated by non-steroidal anti-inflammatory drugs. Eur J Pharmacol 762: 299–305 doi: 10.1016/j.ejphar.2015.06.001

Koagouw W, Ciocan C (2019) Impact of Metformin and Increased Temperature on Blue Mussels Mytilus edulis-Evidence for Synergism. J Shellfish Res 37(3): 467–474 doi: 10.2983/035.037.0301

Lashen H (2010) Role of metformin in the management of polycystic ovary syndrome Ther Adv Endocrinol Metab 1(3): 117-128 doi: 10.1177/2042018810380215

Lee J, Ji K, Kho YL, Kim P, Choi K (2011) Chronic exposure to diclofenac on two freshwater cladocerans and Japanese medaka. Ecotox Environ Safe 74(5): 1216–1225. doi: 10.1016/J.ECOENV.2011.03.014

Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2 C T Method. Nat Meth 25: 402–408. doi: 10.1006/meth.2001.1262

Magnoni LJ, Vraskou Y, Palstra AP, Planas JV (2012) AMP-activated protein kinase plays an important evolutionary conserved role in the regulation of glucose metabolism in fish skeletal muscle cells. PloS one 7(2): p.e31219 doi: 10.1371/journal.pone.0031219

Maranho LA, Baena-Nogueras RM, Lara-Martín PA, DelValls TA, Martín-Díaz ML (2014) Bioavailability, oxidative stress, neurotoxicity and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete Hediste diversicolor as bioindicator speciess Environ Res 134: 353–365. doi: 10.1016/j.envres.2014.08.014

Maranho LA, Garrido-Pérez MC, DelValls TA, Martín-Díaz ML (2015) Toxicological evaluation of sediment samples spiked with human pharmaceutical products: Energy status and neuroendocrine effects in marine polychaetes Hediste diversicolor. Ecotox Environ Safe 118: 27–36. doi: 10.1016/J.ECOENV.2015.04.010

Marshall SM (2017) 60 years of metformin use: a glance at the past and a look to the future. *Diabetologia*, 60(9): 1561–1565. doi: 10.1007/s00125-017-4343-y

McCallum ES, Krutzelmann E, Brodin T, Fick J, Sundelin A, Balshine S (2017) Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals. Sci Total Environ 605-606: 578-588 doi: 10.1016/J.SCITOTENV.2017.06.073

Meador JP, Yeh A, Young G, Gallagher EP (2016) Contaminants of emerging concern in a large temperate estuary Environ Pollut 213: 254-267 doi: 10.1016/J.ENVPOL.2016.01.088

Mezzelani M, Gorbi S, Fattorini D, d'Errico G, Benedetti M, Milan M, Bargelloni L, Regoli F (2016) Transcriptional and cellular effects of non-steroidal anti-inflammatory drugs (NSAIDs) in experimentally exposed musseld, Mytilus galloprovincialis. Aquat toxicol 180: 306-319 doi: 10.1016/j.aquatox.2016.10.006

Mohd Zanuri NB, Bentley MG, Caldwell, GS (2017) Assessing the impact of diclofenac, ibuprofen and sildenafil citrate (Viagra®) on the fertilisation biology of broadcast spawning marine invertebrates. Mar Environ Res 127: 126–136. doi: 10.1016/J.MARENVRES.2017.04.005

Naidoo V, Lenter L, Wolter K, Taggart M, Cuthbert R (2010) The toxicokinetics of ketoprofen in Gyps coprotheres: toxicity due to zero-order metabolism. Arch Toxicol 84(10): 761–766. doi: 10.1007/s00204-010-0521-0

Niemuth NJ, Jordan R, Crago J, Blanksma C, Rodney J, Klamper RD (2014) Metformin exposure at environmentally relevant concentrations causes potential endocrine disruption in adult male fish. Environ Toxicol Chem 34(2): 291–296. doi: 10.1002/etc.2793

Niemuth NJ, Klaper RD 2015. Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. Chemosphere 135: 28–45 doi: 10.1016/J.CHEMOSPHERE.2015.03.060

Oaks JL, Martin G, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad, HL, Ahmed S, Chaudhry MJI, Arshad M, Mahmood S, Ali A, Khan AA (2004) Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 427(6975): 630–633. doi: 10.1038/nature02317

Oosterhuis M, Sacher F, ter Laak TL (2013) Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. Sci Total Environ 442: 380–388. doi: 10.1016/J.SCITOTENV.2012.10.046

Panserat S, Skiba-Cassy S, Seilie I, Lansard M, Plagnes-Juan E, Vachot C, Aguirre P, Larroquet L, Chavernac G, Medale F, Corraze G, Kaushik S, Moon TW (2009) Metformin improves postprandial glucose homeostasis in rainbow trout fed dietary carbohydrates: a link with the induction of hepatic lipogenic capacities? Am J Physiol Regul Integr Comp Physiol 297(3): R707-715 doi: 10.1152/ajpregu.00120.2009

Patterson DA, Macdonald JS, Hinch SG, Healey MC, Farrell AP (2004) The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. J Fish Biol 64: 1039-1059 doi: 10.111/j.1095-8649.2004.00370.x

Pohlert T, (2014) The pairwise multiple comparison of mean ranks package (PMCMR). Available at: https://cran.r-project.org/package=PMCMR [Accessed October 12, 2018].

Polakof S, Skiba-Cassy S, Panserat S (2009) Glucose homeostasis is impaired by a paradoxical interaction between metformin and insulin in carnivorous rainbow trout. Am J Physiol Regul Integr Comp Physiol 297(6): R1769-R1776 doi: 10.1152/ajpregu.00369.2009

Polakof S, Moon TW, Aguirre P, Skiba-Cassey S, Panserat S (2010) Glucose homeostasis in rainbow trout fed a high-carbohydrate diet: metformin and insulin interact in a tissue-dependent manner. Am J Physiol Regul Integr Comp Physiol 300(1). R166-R174 doi: 10.1152/ajpregu.00619.2010

Quinn B, Schmidt W, O'Rourke K, Hernan R (2011) Effects of the pharmaceuticals gemfibrozil and diclofenac on biomarker expression in th zebra mussel (Dreissena polymospha) and their comparison with standardised toxicity tests Chemosphere 84(5): 657-663 doi: 10.1016/j.chemosphere.2011.03.033

Rena G, Pearson ER, Sakamoto K (2013) Molecular mechanism of action of metformin: old or new insights? Diabetologia 56(9): 1898-1906 doi: 10.1007/s00125-013-2991-0

Rosa S, Granadeiro JP, Vinagre C, França S, Cabral HN, Palmeirim JM (2008) Impact of predation on the polychaete Hediste diversicolor in estuarine intertidal flats. Est Coast Shelf Sci 78: 655-664 doi: 10.1016/J.ECSS.2008.02.001

Rowley AF, Vogan CL, Taylor GW, Clare AS (2005) Review Prostaglandins in non-insectan invertebrates: recent insights and unsolved problems J Exp Biol 208: 3–

14 doi: 10.1242/jeb.01275

Ruggeri B, Thoroughgood CA (1985) Prostaglandins in aquatic fauna : a comprehensive review. Tetrahedron 23: 301–306 doi: 10.3354/meps023301

Sanderson H, Thomsen M (2009) Comparative analysis of pharmaceuticals versus industrial chemicals acute aquatic toxicity classification according to the United Nations classification system for chemicals. Assessment of the (Q)SAR predictability of pharmaceuticals acute aquatic toxicity and their predominant acute toxic mode-of-action Toxicol Lett 187(2): 84–93 doi: 10.1016/j.toxlet.2009.02.003

Scaps P, Demuynck S, Descamps M, Dhainaut A (1997) Effects of organophosphate and carbamate pesticides on acetylcholinesterase and choline acetyltranserase acitivites of the polychaete Nereis diversicolor Arch Environ Contam Toxicol 33(2): 203-208 doi: 10.1007/s002449900244

Scaps P (2002) A review of the biology, ecology and potential use of the common ragworm Hediste diversicolor (O.F. Müller) (Annelida: Polychaeta). Hydrobiologia 470: 203–218 doi: 10.1023/A:1015681605656

Scheurer M, Sacher F, Brauch H.-J (2009) Occurrence of the antidiabetic drug metformin in sewage and surface waters in Germany. J Environ Monit 11(9): 1608–1613. doi: 10.1039/b909311g

Scheurer M, Michel A, Brauch H-J, Ruck W, Sacher F (2012) Occurrence and fate of the antidiabetic drug metformin and its metabolite guanylurea in the environment and during drinking water treatment Wat Res 46: 4790–4802. doi: 10.1016/j.watres.2012.06.019

Schmidt W, O'Rourke K, Hernan R, Quinn B (2011) Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (Mytilus spp.) and their comparison with standardized toxicity tests. Mar Pollut Bull 62(7): 1389–1395. doi: 10.1016/j.marpolbul.2011.04.043.

Schmittgen T, Livak K (2008). Analyzing real-time PCR data by the comparative Ct method. Nat Protoc 3(6): 1101–1108 doi: 10.1038/nprot.2008.73

Sheng B, Liu J, Li GH (2012) Metformin preconditioning protects Daphnia pulex from lethal hypoxic insult involving AMPK, HIF and mTOR signaling. Comp Biochem Physiol B Biochem Mol Biol 163(1): 51–58. doi: 10.1016/J.CBPB.2012.04.009

Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis

as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar Environ Res 79:1–15 doi: 10.1016/J.MARENVRES.2012.04.003

Spritzer MP (2014) Polycystic ovary syndrome: reviewing diagnosis and management of metabolic disturbances. Arq Bras Endocrinol Metab 58(2): 182–187. doi: 10.1590/0004-2730000003051

Sumpter JP, Scott AP, Katsiadaki I (2016) Comments on Niemuth, N.J. and Klaper, R.D. 2015. Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. Chemosphere 135:38–45. doi: 10.1016/j.chemosphere.2016.08.049

UK Hydrographic Office (2019) HM Nautical Almanac Office: Miscellanea Available from: http://astro.ukho.gov.uk/nao/miscellanea/birs2.html [Accessed July 3, 2019]

Vermeulen J, de Preter K, Lefever S, Nuytens J, de Vloed F, Derveaux S, Hellemans J, Speleman F, Vandesompele J (2011) Measurable impact of RNA quality on gene expression results from quantitative PCR. Nucleic Acids Res. 39(9): e63 doi: 10.1093/nar/gkr065

Vestel J, Caldwell DJ, Constantine L, D'Aco VJ, Davidson T, Dolan DG, Millard SP, Murray-Smit R, Parke NJ, Ryan JJ, Straub JO, Wilson P (2016) Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environ Toxicol Chem 35(5): 1201–1212. doi: http://doi.wiley.com/10.1002/etc.3260

Viollet B, Guigas B, Sanz GN, Leclerc J, Foretz M, Andreelli F (2012) Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 122(6): 253–270. doi: 10.1042/CS20110386

Virgilio M, Abbiati M (2004) Habitat discontinuity and genetic structure in populations of the estuarine species Hediste diversicolor (Polychaeta: Nereididae). Est Coast Shelf Sci 61(2): 361–367. doi: 10.1111/j.1365-294X.2009.04170.x

Wickham, H., 2016. ggplot2; Elegant Graphics for Data Analysis. Available at: http://ggplot2.org [Accessed October 12, 2018]

Xu G, Liu B, Sun Y, Du Y, Snetselaar LG, Hu FB, Bao W (2018) Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. BMJ 361: k1497 doi: 10.1136/bmj.k1497

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL (2011) Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone J Hazard Mat 190: 588-596 doi: 10.1016/j.jhazmat.2011.03.092

Zhao J-L, Liu Y-S, Liu W-R, Jiang Y-X, Su H-C, Zhang Q-Q, Chen X-W, Yang Y-Y, Chen J, Liu S-S, Pan C-G, Huang G-Y, Ying G-G (2015) Tissue-specific bioaccumulation of human and veterinary antibiotics in bile, liver and muscle tissues of wild fish from a highly urbanised region. Environ Pollut 198: 15-24 doi: 10.1016/J.ENVPOL.2014.12.026

Chapter 6: Discussion

The literature review in Chapter 1 highlights that there has been an increasing interest in the occurrence of pharmaceuticals in the aquatic environment. Reviews have succinctly covered the presence of these compounds in fresh (Hughes et al. 2013) and marine waters (Fabbri and Franzellitti 2016), but studies on the occurrence of pharmaceuticals in estuaries lag behind these environments. Estuaries act as a site of interaction between the freshwater and marine environments and play a role in the fate of pollutants (Ridgway and Shimmield 2002). They provide an important habitat for many organisms and provide commercially important resources such as food, transport and recreation, and as a result the presence of pollutants can have negative consequences (Monserrat et al. 2007). There is evidence that pharmaceuticals are biologically active, and have the potential to impact non-target organisms (Santos et al. 2010). However, there is still uncertainty over the pathways of these drugs and at which levels an effect will occur. The overarching aim of this thesis was to address these gaps, which were further discussed in Chapter 1, and gain a deeper understanding surrounding the occurrence and effects of pharmaceuticals in estuaries, specifically by (1) assessing if prioritisation schemes accurate identify priority compounds (2) identifying pharmaceuticals of environmental concern (3) measuring the concentrations of pharmaceuticals and the spatial and temporal variations in their occurrence, and (4) determining their effects on non-target organisms.

Chapters 3-5 have been separated by research themes, and the results and implications have been discussed within each of the preceding sections. This chapter of the thesis aims to collate the findings from each of these chapters and discuss them as a whole within the context of the original aims.

6.1 Research Synthesis

6.1.1 Evaluation of prioritisation schemes

The prioritisation exercises carried out in Chapters 2 and 3 identified a range of compounds which have the potential to enter the environment and pose a risk to the environment. Chapter 3 included a more detailed assessment, where differences in predicted environmental concentration (PEC) calculations evaluated against each other, measured environmental concentrations (MECs), and the existing literature to determine the efficacy of the different methods in protecting the environment. PEC_A which included

excretion rates and critical environmental concentrations (CECs) were suggested as being the most conservative of the schemes. Whilst these two schemes may provide a useful tool for an initial assessment, they are not appropriate for all situations, and could be further improved to ensure compounds are not overlooked or unnecessarily flagged as a priority when they are not. Whichever methods are used, it is important to take a holistic approach to combine environmental and effects data, as some compounds, such as ethinylestradiol will illicit a biological response at concentrations much lower (less than 10 ng l^{-1}) than that of other compounds (Länge et al. 2010). Despite the limitations of the assessed prioritisation methods, antidepressants, antibiotics, ibuprofen, metformin, allopurinol and candesartan were not only highlighted as compounds of concern in Chapters 2 and 3, but also by the existing literature, emphasising the importance of directing research towards these areas (Linert et al. 2007; Besse et al. 2008, Kostich and Lazorchak 2008, Roos et al. 2012, Daouk et al. 2015).

The largest differences arose in the results between the two chapters due to the inclusion of more compounds in chapter 2. Carbamazepine for instance, was ranked as the 65th most used drug in the UK, so as a result was not included in the assessments carried out in Chapter 3. Carbamazepine is one of the most well-studied compounds in existing freshwater literature and has been found at concentrations up to 11 µg l^{-1}, and thought to cause harm at environmental concentrations (Martin-Diaz et al. 2009; Camacho-Muñoz et al. 2010; Hughes et al. 2013). This shows it is important to consider that the number of compounds included in these schemes, yet a large variation (12 – 3000 compounds) has been seen in the number of compounds included in previous prioritisation exercises (Sanderson et al. 2004; Donnachie et al. 2016). The number of compounds included must be enough to adequately protect the environment, but it also must be within time and financial resources to feasibly conduct the prioritisation exercise. It would therefore appear, that the inclusion of only 50 compounds in a broad assessment such as the one carried out in Chapter 3, does not strike this balance. In the context of this study, the excretion rates, cmax and $\log_{KOW}$ values needed to calculate PEC_A and CECs are relatively easy to obtain, and therefore including more compounds would allow a more robust assessment, whilst also being feasible in terms of time and resources needed.

Whilst the rankings of compounds by PECs appeared to accurately portray relative concentrations, they were largely inaccurate at predicting environmental concentrations. Comparing MECs to PECs can be challenging, as many of these measurements were limited by sample numbers and study locations. As is evidenced by the existing literature and results from the monitoring study in Chapter 4, there are many natural variations in

the occurrence of pharmaceuticals (Paíga et al. 2016; Cantwell et al. 2018; Munro et al. 2019). The variation in these concentrations, highlights the difficulty in including PECs into risk quotients (RQs) and the fish plasma model (FPM). In section *3.4.2.*, the inclusion of local environmental criteria was suggested in order to provide more accurate PECs, however, the variation in these conditions could make this difficult to apply to a large number of compounds. Dilution appears to have been a key factor in determining the occurrence of pharmaceuticals in the aquatic environment, and its inclusion in PECs would be useful (Burns et al. 2018a). PECs in some studies have previously included site specific information on dilution, and have been found to be accurate predictors in some rivers (Burns et al. 2017) and accurate for some compounds, but not others in wastewater treatment plans (WWTPs) (Ferrari et al. 2004).

Other differences arose in the prediction of toxicity of these compounds between the two chapters and existing literature. The main limitations of CECs and the FPM are that they are utilising mammalian data to predict possible concentrations at which compounds are thought to be likely to exert an effect on fish (Huggett et al. 2003). The implications of assessing potential toxicity to one trophic level could fail to identify compounds (such as antibiotics), which have shown greater toxicity to other organisms, and as a result predicted toxicities for multiple trophic levels should be included (Guo et al. 2015). Other assessments have done this through the use of experimental acute and chronic data (Dong et al. 2013), modelling predicted effects (Sangion and Gramatica 2016) and utilising information on pharmacological mode of action (MoA) (Besse et al. 2008). In Chapter 3, it was discussed that FPM and CECs were the most conservative and accurately identified compounds which posed a risk to multiple trophic levels. However, this is likely to fail when there are unexpected ecotoxicological effects of targets non-conserved targets, such as the reproductive toxicity of metformin observed in *P. promelas* and *Mytilus edulis* (Niemuth et al. 2015; Koagouw and Ciocan 2018).

Tiered risk assessments are used in ERAs (Hoyett et al. 2016) and are often utilised in the prioritisation literature (Besse et al. 2008). This could help overcome the limitations discussed previously of providing sufficient detail on a large number of chemicals. The schemes discussed in Chapter 3 (PEC_A and CEC) could provide a useful first tier assessment, but there would need to be further criteria to include assessments on the toxicity of the compounds to other trophic levels, whether this is through acute data or PNECs. The use of a tiered scheme could produce a smaller subset of compounds on which to do a more detailed assessment. Roos et al. (2012) assessed the use of different methods for prioritising pharmaceuticals in a Swedish context. They also found that these criteria accurately predicted the relative importance of several well-studied compounds,

and also suggested the use of QSAR and $\log_{KOW}$ as alternatives. The use of QSAR has been widely debated (Schrieber et al. 2011, Nallani et al. 2016) and discussed in previous sections (*1.1.2* and *3.4.3*). Regardless of which is used, it needs to be chosen based on relevance to the rationale of why the assessment is being carried and to the compounds included, as well as available data.

A first-tier assessment can be used to create a smaller sub-set of compounds upon which a more detailed assessment can be carried out. PECs can be further refined to include local data such as the number of prescriptions, population, WWTP removal, flow, and inputs. There are many different examples of calculations in the literature which include local information on input and flushing rates in rivers (Burns et al. 2017), fate calculations (Oldenkamp et al. 2013), and local usage (Helwig et al. 2016). This could help to provide more reliable data for FPM and RQs.

Other prioritisation schemes have used information on the pharmacological MoA to further assess the potential toxicity of chemicals, and those which had a relevant MoA were placed on a priority list (Besse et al. 2008), or through the use of adverse outcome pathways (Caldwell et al. 2014). The point based ranking system used in Chapter 2 attempted to do this. It was adapted from a prioritisation exercise carried out by Capleton et al. (2006) to prioritise veterinary pharmaceuticals. An assessment like this utilised pharmacological information in addition to data in the existing literature, but was time consuming to carry out on a large number of compounds. Further consideration into the adaption of such a method to a human pharmaceutical context, such as the weighting of different criteria and inclusion of more relevant endpoints could be beneficial. For example, adverse effects to reproduction are important as they could have population level effects and should be weighted appropriately (Ankley et al. 2010). Oxidative stress on the other hand, is an endpoint commonly used in the literature and is useful in determining stress caused by exposure to a pharmaceutical, however, the overall biological significance can be variable and therefore should be weighted differently (Regoli et al. 2002). Additionally, weighting for data quality such as concentrations which induce an effect or sample numbers should be considered

6.1.2 Occurrence of pharmaceuticals in estuaries

To date, relatively few studies have monitored pharmaceuticals in estuaries and those which have, have largely been limited to East Asia (13 studies), North America (10 studies) and Europe (7 studies; Figure 6.1). Only the monitoring study in Chapter 4 and those conducted by Mijangos et al. (2018), Long et al. (2013) and Thomas and Hilton (2004) measured compounds in more than one estuary. Some patterns in the occurrence

of pharmaceuticals have evolved from these studies. Concentrations of pharmaceuticals decrease with increasing salinity, leading to dilution being named as the key factor influencing the fate of these compounds within estuaries (Cantwell et al. 2016, 2017). Other processes such as tides, water flow and rainfall also influence dilution and subsequent pharmaceutical concentrations (Benotti and Brownawell 2007, Mijangos et al. 2018). Sorption of pharmaceuticals to sediment and degradation have also been found to play a role in removal (Yang et al. 2011, Hedgespeth et al. 2012). However, due to the varying concentrations, limited study locations and complex interactions occurring in estuaries, there has been uncertainty over the magnitude of estuarine pharmaceutical pollution (Cantwell et al. 2018). The findings of this thesis help to put the wider problem of pharmaceutical contamination into context, by contributing to the overall picture on the global occurrence of pharmaceuticals, and what is currently known about the spatial and temporal patterns in estuaries. As illustrated by Figure 6.1, further monitoring needs to be conducted in order to fully understand the global scale of this issue.

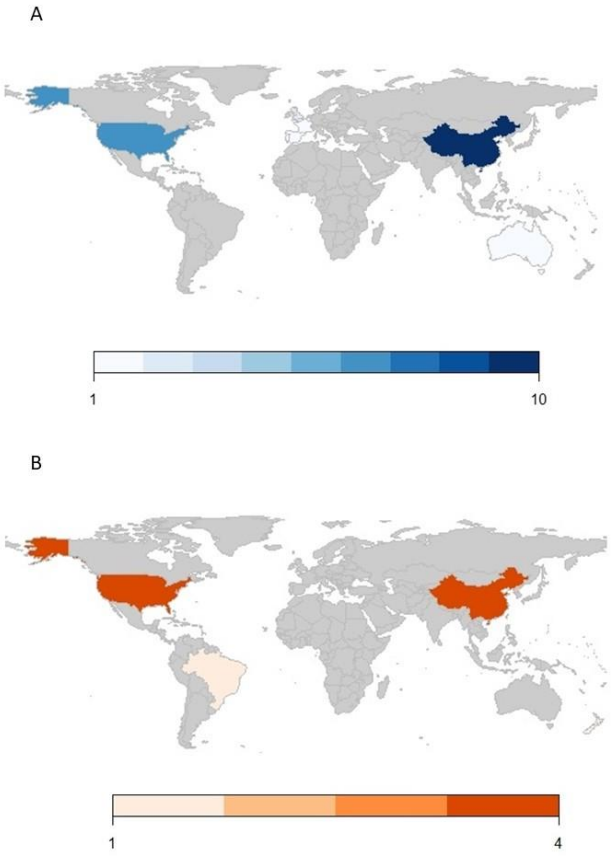


Figure 6.1 Number of studies conducted in each country monitoring pharmaceuticals in (A) surface Water and (B) sediment. Further information on pharmaceuticals monitored and concentrations can be found in appendices 1.1 and 1.2.

In Chapter 4, pharmaceutical pollution was found to be ubiquitous in the UK, which adds to the increasing literature that pharmaceuticals are not just present in freshwater (Fabbri and Franzellitti 2016, Gaw et al. 2016). The ability of these compounds to persist and their occurrence in the downstream most regions of estuaries and therefore likelihood of entering the marine environment shows that pharmaceuticals are widespread within the aquatic environment as a whole (Rocha et al. 2014). Ibuprofen, paracetamol and trimethoprim had the highest detection frequencies (90-100%) across UK estuaries and were present in nearly all of the samples collected. Whilst diclofenac and citalopram were highly detected in the Humber Estuary, they were only present in 25% and 50% of the 12 estuaries sampled, respectively.

In Chapter 3, PECs were calculated for the five compounds measured in these estuaries. These calculations predicted that paracetamol would be found at the highest concentrations (800 – 5740 ng l^{-1}) followed in order by ibuprofen (1484 – 2968 ng l^{-1}), diclofenac (112 – 488 ng l^{-1}), trimethoprim (133 – 488 ng l^{-1}) and citalopram (74 – 223 ng l^{-1}). This order was mostly reflected in the concentrations of pharmaceuticals measured in UK estuaries, although ibuprofen was generally detected at higher concentrations than paracetamol. However, the resulting calculations were less accurate, which is unsurprising due to the temporal and spatial variations that were seen in Chapter 4, as well as in other monitoring studies (Conley et al. 2008, Wilkinson et al. 2017, Cantwell et al. 2018). As a result, it is most important that these schemes are sufficient for predicting the highest concentrations. The calculated PECs mostly underestimated the maximum concentrations by a factor of 2 – 10, but overestimated mean concentrations by a factor of 2 - 113, which was the case for many of the MECs in Chapter 3 (Figures 3.2 and 3.3). Most of the MECs used were measurements taken from rivers, and PEC_A was found to be the most conservative estimate. However, in the estuaries sampled, PEC_D accurately predicted maximum concentrations of paracetamol (916 ng l^{-1}) and diclofenac (42.93 ng l^{-1}) in the Humber Estuary, whilst PEC_B accurately predicted the maximum concentrations of trimethoprim (247 ng l^{-1}). In the context of this thesis, PEC_A still provided the most conservative estimate for the compounds and would be useful to prioritise pharmaceuticals in the aquatic environment as a whole, but lacks detail to provide estimates in specific areas.

The Humber Estuary, which is the second largest estuary in the UK and is the receiving environment for the sewage effluent for approximately 13.7 million population equivalent (PE; European Environment Agency 2017), had the highest overall levels of pharmaceuticals. Concentrations of ibuprofen in the Humber were the highest recorded in an estuary, globally (Table 4.1). Further large estuaries, including the Mersey (3.7

million PE) and Thames (16.5 million PE), also had relatively high concentrations of pharmaceuticals in comparison to the other estuaries sampled (European Environment Agency, 2017). A relationship between the amount of pharmaceuticals and densely populated catchment areas has been seen in other waterbodies; a monitoring study in the Jiulong River, China showed highest concentrations in urban areas in comparison to other land uses (Hong et al. 2018). Pharmaceuticals were also present in rural areas due to their combined usage as veterinary medicines and spreading of sludge and manure, however, pharmaceutical presence in areas with higher forest cover were much lower. A positive correlation between catchment population and pharmaceutical populations was also found in Japanese rivers (Hanamoto et al. 2018). This relationship was not observed in this thesis; the Cromarty Firth (92.3 km^2; PE 156, 000), a relatively secluded estuary in the North of Scotland had the highest level of any pharmaceutical (ibuprofen – 210 ng l^{-1}) measured in the August-September monitoring campaign, and was one of the few estuaries to contain diclofenac. This could, in part be due to differences in WWTP technology resulting in the lower removal of these compounds (Nebot et al. 2015). Septic tanks are likely to be higher in rural areas (which are not included in the calculated PE of each catchment) and have been attributed as a source of pharmaceuticals in rural areas in Canada (Comeau et al. 2008), Sweden (Magnér et al. 2010), and USA (Palmer et al. 2008). As a result countries with growing populations and inefficient or non-existent sewage removal (such as Bangladesh, Pakistan, China and India) could pose the biggest threat to water quality (Rehman et al. 2015). Not only are these countries the highest global consumers of pharmaceuticals, they also house many pharmaceutical manufacturing companies where there is a lack of regulation surrounding the emission of pharmaceutical waste (Ashfaq et al. 2017). Few studies have measured pharmaceuticals in these regions, but diclofenac was found in Pakistan at levels of 0.1 to 4.4 µg l^{-1} (Scheurell et al. 2009), and other pharmaceuticals were frequently detected above 1 µg l^{-1} in India (Mutiyar et al. 2018). These differences in land use, sewage treatment and pharmaceutical consumption in areas such as this can make it difficult to apply findings from this thesis to other countries. Pharmaceuticals have been detected at concentrations up to 500 ng l^{-1} in surface water and up to 87 µg l^{-1} in effluent in sub-arctic locations (Faroe Islands, Iceland and Greenland), which have low populations (50,000 – 329,000 people), which shows the potential wide-reaching impacts of pharmaceuticals pollution and that monitoring studies shouldn't be limited to urban areas (Huber et al. 2016).

Geochronological sampling of estuarine sediment in New York has revealed that most pharmaceutical concentrations have increased over the last 50 years, with

concentrations doubling in the last ten years, a higher increase in comparison to previous decades (Lara-Martín et al. 2015). Four of the estuaries sampled in Chapter 4 (Mersey, Thames, Tees and Tyne) were previously sampled in 2002 (Thomas and Hilton 2004). It can be difficult to directly compare concentrations in these estuaries, due to seasonal and temporal variations, however, detection frequencies of ibuprofen, trimethoprim and paracetamol were higher than previously sampled. Particularly concerning, is the occurrence of paracetamol, which was completely absent in these estuaries, but is now the second most occurring compound 15 years later. Diclofenac concentrations measured in this thesis were similar and even lower than these previous measurements, however, due to low recovery, higher method quantification limit (MQL) and potential temporal variations of this compound, it may be too early to say concentrations are declining, particularly as it has recently been found in the Ouse (a tributary of the Humber) at concentrations up to 2.8 µg l^{-1} (Kay et al. 2017). In the UK, prescription rates of diclofenac have declined in recent years, and only low doses are available over the counter (OTC), and as a result a decline in concentrations could be expected (National Health Service 2014). As for trimethoprim and ibuprofen, concentrations were higher in the Mersey (by a factor of 10) in 2002, but similar to the other estuaries sampled. Baseline data on the occurrence of pharmaceuticals in estuaries (or many other waterbodies) does not exist (Figure 6.1), yet is essential to determine if these levels are in fact rising.

This variation in pharmaceutical levels could be the result of site selection (and distance to input sources), as well as variations in seasonal or diurnal concentrations as opposed to an overall decline. Thomas and Hilton (2004) collected samples in November, whereas the UK wide survey in Chapter 4 was conducted in August, when concentrations would be expected to be lowest. In the Humber, trimethoprim was the only compound to show seasonal difference, with highest concentrations in February, when highest overall pharmaceutical concentrations occurred, and December. Other studies have showed highest concentrations of pharmaceuticals, such as hydrochlorothiazide (which showed 50% higher detection frequency) to be highest in winter as the result of colder temperatures, when degradation is lower, resulting in higher input, higher concentrations and more persistence (Cantwell et al. 2017). Previous studies have also identified the flow rate of an estuary to be an important factor in the fate of pharmaceuticals (Cantwell et al. 2016). This was not accounted for in the Humber or UK wide monitoring, but rainfall recorded in Yorkshire the previous years showed highest levels in June and August, which could also account for the low concentrations observed at sites furthest downstream (A3-A5) during these months (Tanguy et al. 2016). These complex

interactions between the input of pharmaceuticals, their removal and transport can make it difficult to predict these variations, as they can vary daily as well as seasonally.

Total prescription numbers of the most commonly used pharmaceuticals in the UK have risen by a factor of 1.6 between 2005 and 2015 (National Health Service 2006, 2016), and if this increase remains constant, then over 1 billion prescriptions of these compound classes could be dispensed in 2025 (Figure 6.2). Further understanding of consumption patterns and prediction of environmental concentrations is needed in order to understand if this same increase could be reflected in surface water. The Lara-Martin et al. (2015) study showed higher increases in sediment over a ten year period, and as a result it isn't unfeasible. If little is done to curb the rise of pharmaceutical levels, then they have the potential to become a problem in the future. According to our current understanding of ecotoxicology, few pharmaceuticals pose a risk at the levels currently found in the environment, however detrimental effects could be seen if concentrations continue to increase at this rate (Taylor and Senac 2014).

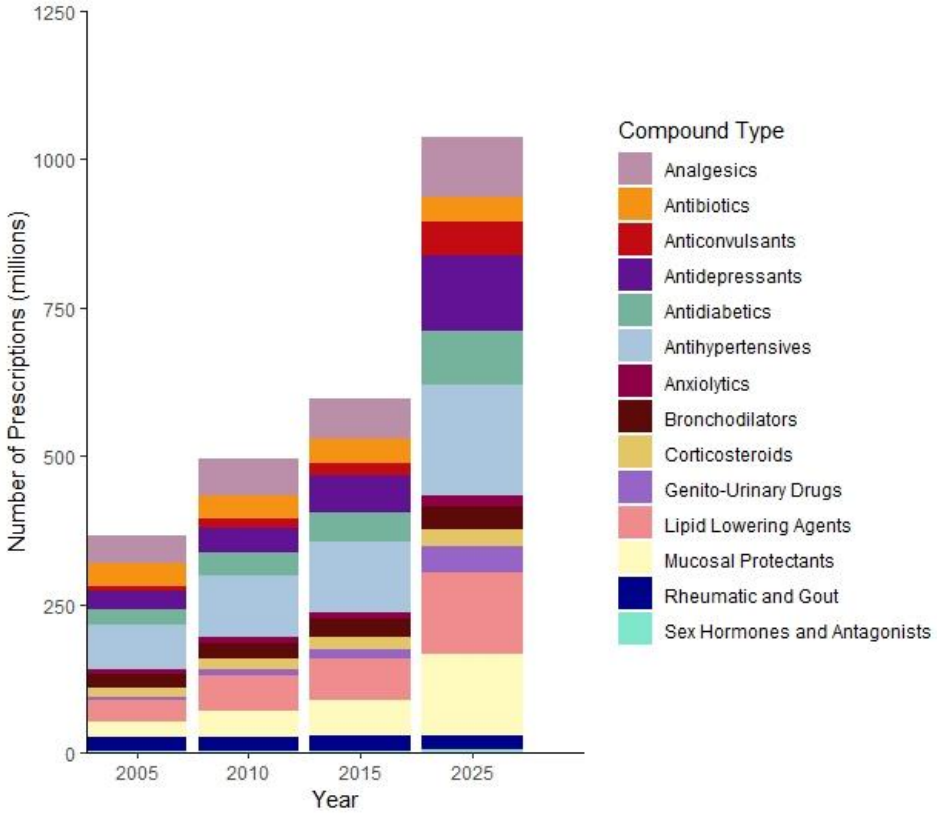


Figure 6.2 Annual prescription numbers for the most prescribed pharmaceuticals for 2005, 2010 and 2015 taken from National Health Service (2006, 2011, 2016). Projected prescription numbers for 2025 calculated based on change between 2005 and 2015.

A better understanding of the spatial and temporal differences of pharmaceuticals and the factors which influence their fate can help to identify areas and organisms which could be most at risk from this pollution. In the Humber, the highest concentrations were observed in the 6 km section between R1 and R4. This was the likely the result of effluent discharged at R1, and sustained concentrations from this source. Other peak concentrations seen at R3 and R4 could have been the result of compounds transported from the River Hull. There are eight other WWTPs which discharge effluent into the Humber, the largest of which serves a PE of 500,000 (European Environment Agency 2017). Improper disposal, agriculture and aquaculture have also been identified as sources of pharmaceuticals, as a result the spatial distribution of some compounds may differ depending on land use (Godoy et al. 2015). For instance, trimethoprim, which is also used in fish farming, has been found to be present at concentrations 2-3 times higher in seawater where farming was present than other areas (Kim et al. 2016). It is essential that monitoring campaigns are designed to include areas where concentrations are highest in order to gain a better understanding of risk in that area. An understanding of consumption patterns is also important when determining which pharmaceuticals to monitor; prescriptions of lipid lowering agents, antidepressants, antihypertensives, mucosal protectants and antidiabetic drugs have experienced the highest rate of increase, doubling between 2005 and 2015 (Figure 6.2), and as a result, may be of interest for future monitoring work. However, as discussed previously in Chapter 2, it is essential to have an understanding on the effects of pharmaceuticals, when determining those which may be a priority.

6.1.3 Biological effects of pharmaceuticals in estuaries

Many pharmaceuticals exhibit temporal variations in their occurrence, and as a result, some pharmaceuticals may pose a greater risk at certain times of the year (Conley et al. 2008). This can have implications for migratory species or biological processes which occur at certain times of the year. Reproductive processes often exhibit seasonal patterns, which could leave species more vulnerable during these periods (Milligan et al. 2009). For example, recruitment for the ragworm, *Hediste diversicolor* occurs at different times of the year, depending on the population (Scaps 2002). In the Humber, populations near sites R1-R4 spawn around June (coinciding with highest levels of ibuprofen), whereas for populations near A3-A5, this occurs around February, when downstream concentrations of pharmaceuticals are highest. Diclofenac has been found to decrease sperm motility in the lugworm (*Arenicola marina*) at environmentally relevant concentrations (100 ng l^{-1}), and high concentrations coinciding with spawning events could have implications on population numbers (Mohd Zanuri et al. 2017). Many other

pharmaceuticals (such as antidepressants) have been observed to negatively impact reproduction in fish (Overturf et al. 2015) and invertebrates (Fong and Ford 2014) in laboratory exposures. The timing of reproductive events are often coordinated with events of other species. For instance, the recruitment periods of *H. diversicolor,* coincide with the highest feeding periods of one of their predators, sole (*Solea solea*) juveniles (Cabral 2000). This could result in increased pressure on the prey item (in this case, *H. diversicolor*), leading to population reductions, which would in turn impact the predator (in this case, *S. solea*).

In chapter 5, *H. diversicolor* were exposed to two different concentrations of metformin and diclofenac (100ng l^{-1}, 1µg l^{-1}) for 7 days. Only the highest level of metformin was seen to alter *ATPS* after 7 days, and no effect was seen for *AMPK* in any treatment. However, the variation in gene expression in response to metformin exposure introduces uncertainty as to the full extent of the effect on energy status. Levels of metformin have been found to be high, with concentrations in tributaries of the Humber (Rivers Ouse and Foss) found at 2.3 µg l^{-1} in surface water and 6.1 µg l^{-1} in effluent, and a detection rate of 100% (Burns et al. 2017, 2018b). Globally concentrations are up to 1 µg l^{-1} in estuaries, 3 µg l^{-1} in freshwater and 10 µg l^{-1} in effluent (Briones et al. 2016, Meador et al. 2017, Burns et al. 2018b). It is therefore plausible that concentrations could reach 1µg l^{-1} in the Humber, which could result in an increase of *ATPS* in *H. diversicolor.* However, these are likely to encompass peak concentrations in estuaries, and as a result are unlikely to continually be exposed at the levels.

H. diversicolor are polychaetes, which are ubiquitous, and they are a key species in estuarine sediment in Europe and North America (Coelho et al. 2008). Closely related relatives, such as *Hediste japonica* and *Hediste limnicola,* are abundant in other regions, and it is possible that pathways in these species could be similar due to conserved targets, however differences in toxicity could also occur (Fong and Garthwaite 1994, Fabbri 2015). Additionally, *H. diversicolor* have been suggested as good indicators of estuarine pollution due to their susceptibility to effects of pollutants in estuaries (Scaps 2002, Kalaman et al. 2009, Maranho et al. 2014). Due to the dynamic process in estuaries, these species often live at the edge of their tolerance zone for pH, salinity and dissolved oxygen, so further stress caused by contaminants has a greater impact on their physiology. Further to metformin, ibuprofen has been showed to have an effect on energy metabolism in *H. diversicolor* exposed to sediment spiked with 5 ng g^{-1} (Maranho et al. 2015). Organisms have limited energy for processes such as movement, reproduction and growth, so prolonged stress and increased energy requirements

caused by pharmaceuticals could prohibit them from sustaining these processes (Goodchild et al. 2015).

Not only are *H. diversicolor* a commercially important species for bait, but effects to their populations can impact other important species (Rosa et al. 2008). *H. diversicolor* are an important prey for other species of commercial value such as the edible crab, *Carcinus maenas* and *S. solea* (Cabral 2000, Baeta et al. 2006). They are also an important food source for water birds such as, dunlin (*Calidris alpina*), black headed gull (*larus ridbundus*), grey plover (*Pluvialis squatarola*) and the bar tailed godwit (*Limosa lapponica*; Rosa et al. 2008). These species also feed on bivalves such as the clam, *Scrobicularia plana*, which have been found to accumulate carbamazepine (Almeida et al. 2017). The Humber Estuary is of ecological significance to many species of water birds, supporting approximately 150,000 individuals, including those mentioned previously, which rely on *H. diversicolor* as a prey item (Mander et al. 2007; Austin et al. 2008). These species have accounted for 2% (*C. alpina*) to 13% (red knot, *Calidris canutus*) of the international population, however, many of these species have experienced a 25-50% of decline in population numbers between 1991 and 2006, and reduction in their food sources could add further pressure (Buck 1997; Stillman et al. 2005; Austin et al. 2008). This trend is not seen in all species, and populations of some, such as *L. lapponica* have increased. The reduction in water bird species has been attributed to other anthropogenic threats such as habitat loss and decline in water quality, and the contamination of pharmaceuticals or other contaminants could add to this threat (Norris et al. 2004).

There is evidence that many other emerging contaminants, such as flame retardants and plasticisers, transfer through the food chain (Nilsen et al. 2019). Laboratory and environmental studies on the trophic dynamics of pharmaceuticals are limited, however, there is little evidence for biomagnification of pharmaceuticals, and the main route of entry appears to be environmental exposure (Du et al. 2014, Boström et al. 2017, Haddad et al. 2018). Nonetheless, pharmaceutical pollution can have an effect on food chains, particularly as these pollutants have been found to be more bioavailable to the lower trophic organisms (Vernouillet et al. 2010, Lagesson et al. 2016). Reduction of lower trophic species will have a knock on effect on those which have an effect further up the food chain (Lagesson et al. 2016).

The CECs calculated in Chapter 3, were exceeded by ibuprofen, diclofenac and citalopram in all estuaries sampled in Chapter 4. CECs are a prediction that these compounds will be taken up by fish, and not that they will necessarily cause an effect in

that organism (Huggett et al. 2003; Fick et al. 2010). Considering the large size of the Humber, it has a relatively small fish population (~28,000 individuals), compared to the Severn (~172,000) and Thames (~103,000), so these predictions may be more important for some estuaries than others (Environment Agency 2019) The CECs for ibuprofen (0.2 ng l^{-1}), diclofenac (2.2 ng l^{-1}), and citalopram (0.4 ng l^{-1}), were low, yet there is currently no evidence that these compounds illicit a biological effect at these concentrations. Of the compounds assessed in the prioritisation scheme, 19 out of 50 had lowest LC_{50} values for algae, showing they were most acutely sensitive to pharmaceuticals (Appendix 3.4). This shows the importance in using multiple trophic levels in risk assessments and prioritisation schemes. Whilst trimethoprim had a high CEC (1.6 µg l^{-1}), bacteria and algae are most sensitive to this drug (Vestel et al. 2016) which is further evidence that use of CECs alone in prioritisation schemes are not adequate predictors of environmental toxicity.

Whilst diclofenac did not have an effect on *H. diversicolor* energy metabolism, as measured by *ATPS* and *AMPK* expression in Chapter 5, it has been shown to cause oxidative stress in tilapia (*Oreochromis niloticus*) and mussels (*Mytilus galloprovincialis*), reduce osmoregulation in shore crabs (*C. maenas*), and cause liver and kidney damage in trout (*Salmo trutta*) at concentrations under 1 µg l^{-1} (Hoeger et al. 2005, Eades and Waring 2010, Gonzalez-Rey and Bebianno 2014, Gröner et al. 2017). In 2013, diclofenac was placed on the watch list under the EU Water Framework Directive (WFD), with maximum allowable concentrations of 0.01 µg l^{-1} in marine waters; these levels were exceeded in the Humber, Thames and Cromarty (Lonappan et al. 2016). However, there is no evidence that negative effects will occur at concentrations this low, and in 2018 its removal from the watch list was recommended (Loos et al. 2018).

The CECs for paracetamol were 35 µg l^{-1}, however, there is evidence that aquatic invertebrates and fish accumulate and are affected by paracetamol at levels lower than this. Paracetamol was detected in the estuaries sampled in Chapter 4 at 13 - 916 ng l^{-1}, which are consistent with levels that have been observed to cause adverse effects in freshwater species, such as neurotoxicity in the planarian worm (*Dugesia japonica*) and oxidative stress in *Daphnia magna* (Parolini et al. 2010, Wu and Li 2015). Many of the toxicity tests of paracetamol, focus on acute exposures, and due to its ubiquitous presence in the aquatic environment, further studies are needed for chronic low level exposures (Kim et al. 2007, Antunes et al. 2013). The pathways of paracetamol are thought to be similar in vertebrates as humans, and chronic exposures to moderate levels of paracetamol have cause hepatic toxicity in fish (*Rhamdia quelen*) exposed to 250 ng l^{-1} for 21 days (Guiloski et al. 2017).

6.2 Future direction for the management of pharmaceuticals in the environment

If pharmaceutical concentrations in the environment continue to increase it is possible that they could become a global problem. Globally, rivers, estuaries and seas are facing growing pressure as the result of pollution, climate change and other anthropogenic pressures, and as a result could become more sensitive to contaminants such as pharmaceuticals (Chapman 2016). Therefore, the continued monitoring and investigation on pharmaceuticals is important. The previous sections have identified the potential ecological implications of pharmaceutical contamination, and whilst most pharmaceutical concentrations seen in the environment are too low to illicit biological effects seen in laboratory exposures, their impact in not yet fully understood. The WFD watch list was reviewed in 2018, and currently contains the following pharmaceuticals: 17α-ethinylestradiol, 17β-estradiol, estrone, erythromycin, clarithromycin, azithromycin (Loos et al. 2018). Ibuprofen may also be of interest to regulators as the result of high levels found in this study, and the potential effects at these concentrations. There are further compounds which have been identified in the previous chapters, which have the potential to also pose a considerable risk. These include metformin, candesartan, allopurinol, antibiotics (particularly amoxicillin, flucloxacillin) and antidepressants (particularly citalopram, fluoxetine and amitriptyline). Due to the limited number of compounds included in the prioritisation exercise, this list will not be exhaustive, and if prescription numbers continue to rise, other compounds may become more of a priority. Additionally, this list will need to be adapted to different geographical areas based on consumption patterns and identification of potential sources. More research on these and other compounds is needed to determine the severity of the risk.

The usage of some pharmaceuticals, particularly antidepressants and antibiotics, are growing in the UK (as evidenced by Figure 6.2) as well as globally, which can have implications on the environment (OECD, 2017). This growth is even more pronounced in areas such as Brazil, China or India which have growing populations and highest rate of antibiotic usage (Van Boeckel et al. 2014). ERAs for veterinary pharmaceuticals contain a risk-benefit analysis, which is not feasible for those used in human medicine, as human health will always be seen as overriding benefit (Pereira et al. 2017). Whilst not a complete solution in itself, awareness of the environmental effects of pharmaceuticals within the communities as well as to prescribers could help to lower the usage of some pharmaceuticals (Daughton and Ruhoy 2014). There are currently campaigns to reduce the unnecessary prescription of antibiotics to try and prevent resistance, and similar initiatives could be used for other pharmaceuticals (Edgar et al. 2009). Regulation of

OTC drugs could also reduce the environmental risk, but equally could have implication on an already overstretched medical system, resulting in an increased need for appointments and prescriptions (Daughton and Ruhoy 2014). Additionally, pharmaceuticals are often disposed of inappropriately in household waste or down toilets and increased awareness of alternative disposal methods could help prevent this (Bound and Voulvoulis 2005).

WWTPs have been found to remove pharmaceuticals with variable efficiency, and since a primary route of pharmaceuticals into estuaries is through wastewater improving this technology would be an important step to regulating the input of pharmaceuticals (Valdés et al. 2014, Munro et al. 2019). A significant amount of research has been put into the removal of not only pharmaceuticals, but other emerging contaminants, and the wide range of different types of compounds provides a challenge (Gavrilescu et al. 2015). Whilst this wouldn't stop the input of all compounds, it could be an important step for effluent dominated estuaries. In some regions, there also needs to be improved regulation and infrastructure to prevent the discharge of untreated sewage into the environment.

6.3 Limitations and future research

6.3.1 Prioritisation of pharmaceuticals

There were limitations in the studies conducted in chapters 3-5, and it is important to take these into consideration when interpreting the results. The schemes included in the assessment were not exhaustive of all those used in the literature, however those which were not included (such as QSARs and PBT assessments) have been covered in other prioritisation studies (Roos et al. 2012; Donnachie et al. 2016). A limitation of this study, was the number of compounds included. Whilst a smaller set of compounds made it easier to compare results, it may not have accurately identified all compounds which pose a risk to the environment. The limitations of the specific calculations have been discussed in previous section (*3.4* and *6.1.1*), and this highlights where further research needs to be done in order to increase accuracy. The study in Chapter 3 was conducted using prescription data to calculate PECs for the UK as a whole, and did not look at regional differences. Additionally, comparison with MECs were made across a large temporal and spatial scale. Further comparison with localised parameters and carefully designed sampling could help to provide further insight into how PECs could be improved. Consumption patterns have an influence on these calculations and concentrations seen in the environment, and it would be beneficial to further understand the trends behind this. Whilst overall prescription levels are increasing, the patterns of

some compounds are decreasing, and it may be a better use of resources to fund research into those which pose a greater risk in the future.

Future research into improving predictions on the toxicity of pharmaceuticals to aquatic organisms needs to be conducted. As the FPM has been found to be a useful method in determining toxicity to fish, it would be useful to determine how to better read across this data to invertebrates (Roos et al. 2012). There is evidence that algae may be sensitive to some compounds such as antibiotics (Guo et al. 2015), statins (Brain et al. 2008) and allopurinol (Clode et al. 2009). Due to the importance of algae in aquatic ecosystems, a better method needs to be developed to predict the effects of pharmaceuticals to these organisms. In comparison to vertebrates and invertebrates, far less in known about impacts on marine algae, and this warrants further investigation. Future research on the occurrence of pharmaceuticals in estuaries and their biological effects are discussed in sections 6.3.2 and 6.3.3, and this knowledge is essential to improving prioritisation schemes.

6.3.2 Occurrence of pharmaceuticals in estuaries

The monitoring study was limited by the time taken to collect samples, which meant that all samples were taken from the North side of the Humber and from only one of the tributaries. In order to have a better understanding of the source of pharmaceuticals, monitoring studies should include as many points of input (such as tributaries, CSOs and areas where effluent is discharged) as possible. Estuaries undergo mixing between freshwater and marine, which will differ between estuaries and can determine the fate of compounds (Mijangos et al. 2018). All samples from this study were taken from the surface of the water column, close to the shore. Further studies should include samples from other compartments including sediment, within the water column and the middle of the estuary in order to understand the full exposure of organisms to these chemicals. Samples were collected at high tide partly to compare these concentrations, but also because most sites were inaccessible at low tide. It potentially could have accounted for low concentrations at the sites furthest downstream (A3-A5), and sampling at other times in the tidal cycle would help determine if the concentrations are observed are representative of this site as a whole. Salinity, pH and temperature measurements were taken with each of the samples. There was an attempt to collect information on turbidity, however, due to the limited access to sites, this was not possible. In order to gain a better understanding of the spatial and temporal variations, it would also be beneficial to collect information on water flow and rainfall to determine differences in dilution between sampling periods.

This study was also limited by equipment difficulties and cost of external analysis, which led to a restriction on the number of samples which could be analysed. As a result sample replication was low, and peak concentrations in the estuaries sampled may not have been captured. Under optimum circumstances, replicates would be taken from each site during each sampling period in order to increase confidence in the concentrations of pharmaceuticals observed. Additionally, recoveries from spiked water samples were low and variable between samples, which could have accounted for some of the variability observed. Adjusting for recovery enables a more accurate overview on the levels of pharmaceuticals, however increasing recovery reduces uncertainty seen in any unexpected results. The low recovery of some compounds, particularly diclofenac, may have accounted for its low detection frequency. Differences in the recovery of compounds from solid phase extraction (SPE) occurred between Chapters 2 and 4. This could be explained by a change in the reconstitution of samples in methanol: water (10:90) instead of 100% methanol. The addition of TFA to the samples in Chapter 2 improved recovery, however further acidification of acidic compounds such as diclofenac and ibuprofen may have affected their solubility.

6.3.3 Biological effects of pharmaceuticals

Adverse reactions have been seen in many organisms, but the pathways for many pharmaceuticals are poorly understood (Fabbri 2015). This is partially due to the endpoints chosen such as mortality, growth and oxidative stress, which are important in determining the effects of these compounds, but a deeper understanding on specific pathways is needed. Endpoints should be chosen based on the information on MoA in humans and applied to knowledge on the biology of the non-target species. Exposure experiments need to be more environmentally relevant in terms of treatment concentrations, and using multiple compounds. Even though some compounds may not illicit an adverse reaction in single ecotoxicity tests, they are present in the environment with other pollutants and anthropogenic pressures, which could make them more toxic (Di Poi et al. 2018). In other cases, pharmaceuticals have been observed to have a positive effect, metformin was found to have a protective effect on *Daphnia* against hypoxia (Sheng et al. 2012). The use of pharmaceutical mixtures in effects based studies has been increasing, but still little is known about these effects (Backhaus and Faust 2012). However, there is evidence that pharmaceutical mixtures are toxic at levels where single substances are not, and as a result this is an important gap in the literature. (Cleuvers 2004). Most exposure experiments only include a single test species, when in reality, there are complex interactions between species and effects on one species, could have indirect implications on others. Few studies have determined the effect of

pharmaceuticals in mesocosms, which could provide further information on the potential effects of pharmaceuticals to an ecosystem (von der Ohe et al. 2011).

In the environment, pharmaceuticals undergo a number of processes, which result in the formation of metabolites (Celiz et al. 2009). Often, these metabolites are inert, but some are pharmacologically active and have the potential to be more toxic than the parent compound, but relatively little is known about their occurrence, fate or effects (García-Cambero et al. 2015). Many metabolites are found in the environment at concentrations in the same order of magnitude or higher than that of the parent compound, but their overall environmental relevance is not known (López-Serna et al. 2012). For example, 10,11-Epoxi carbamazepine a biologically active metabolite was found at concentrations 15 times higher than its parent compound carbamazepine in a river in Spain (López-Serna et al. 2012). Many metabolites have the same MoA as their parent compound, so if both are present in a mixture, the effect may be amplified (Besse et al. 2008)

In order to be more environmentally relevant, exposures need to use a range of concentrations which include those found in the environment. It is also beneficial to have long-term as well as short-term studies, as exposure to pharmaceuticals is likely to be to low levels over a sustained period of time (Godoy et al. 2015). A time-dependent increase in effects has been seen in some exposures, for instance, Japanese medaka (*Oryzias latipes*) exposed to 1 µg l^{-1} diclofenac showed increased vitellogenin expression after 4 days of exposure, but not beforehand (Hong et al. 2007). A longer exposure to metformin in Chapter 5 could help to determine if *ATPS* increase was the result of permanent stress and could lead to the activation of *AMPK* or if they will be able to adapt to the stress over time. *H. diversicolor* are sediment dwelling organisms, and as a result, it is likely they could also uptake pharmaceuticals from sediment, where concentrations of pharmaceuticals are likely to be found at lower concentrations than surface water. Environmental relevance could have been improved through the use of sediment found in the estuarine environment and appropriate dosing.

There was variation seen in the relative gene expression within each of the treatments, which introduced uncertainty as to whether it was only metformin that was causing increased expression of *ATPS*, or another variable that wasn't accounted for. Salinity, dissolved oxygen, temperature and pH were controlled for, however, the size of organisms varied between treatments. Confirmation of pharmaceutical concentrations in the exposure water or *H. diversicolor* could help to reduce this uncertainty. This information is often missing from ecotoxicology studies and could help to quantify bioaccumulation of compounds, which can help to put the effects seen into context and

help to extrapolate the effects to other species (Harris et al. 2014). Additionally, there are still gaps over the routes of uptake, bioaccumulation and transfer of pharmaceuticals to other trophic levels. Reproducibility of results is often a concern in ecotoxicological studies, and was a limit of the one carried out in Chapter 4, and the need for repeated studies have been highlighted by previous authors, and it could help reduce uncertainty over variable results (Sumpter et al. 2016).

6.4 Conclusion

This thesis has demonstrated that pharmaceuticals may pose a risk to estuaries. It quantified the concentrations of five pharmaceuticals – ibuprofen, paracetamol, diclofenac, trimethoprim and citalopram in twelve estuaries, which provides an important baseline on levels in the UK. Their presence in all of the estuaries sampled shows that they are not only present in large urban catchments, but also in rural estuaries, and as a result the implications of their presence could be wide reaching. Ibuprofen was found at levels up to 6.2 µg l^{-1}, which to date is the highest level found in any estuary globally. The results from this thesis also show that based on current knowledge on the biological effects of pharmaceuticals that most pharmaceuticals are currently not present at concentrations high enough to cause a detrimental effects at a population level. Despite this, concentrations of pharmaceutical are high enough in some estuaries to be biologically active in organisms, but the overall implications are not fully understood. Metformin was found to increase the exposure of *H. diversicolor* at 1 µg l^{-1}, which demonstrates that peak concentrations seen in surface waters have the potential to illicit this effect. Laboratory exposures are limited in length and the sustained long-term exposure of pharmaceuticals particularly when they are present in mixtures with other contaminants are relatively unknown.

There is some evidence that pharmaceuticals levels are increasing with time, and as a result, concentrations need to be monitored and inputs reduced in order to prevent serious implications in the future. Pharmaceuticals exhibit spatio-temporal variations in their occurrence as the result of complex environmental interactions. The patterns seen in the Humber Estuary follow some patterns exhibited in other regions; wastewater effluent is a major source of pharmaceuticals in estuaries, and input through this route plays a role in the fate of pharmaceuticals. As a result, improvement of removal during wastewater treatment is an important step in reducing environmental concentrations.

Prioritisation schemes can be useful tools in determining the relative exposure of pharmaceuticals in the aquative environment, but can not adequately protect the environment as a whole. Exposure predictions could be improved by including localised

information on usage, removal and environmental conditions. Additionally, many of the predictors of toxicity used in prioritisation schemes (CEC, FPM, LC_{50} and Log_{KOW}) could not individually predict toxicity of pharmaceuticals. Futher understanding on the uptake, bioaccumulation and effects of pharmaceuticals at multiple trophic levels is needed to better inform these models An inclusive approach of multiple schemes and comparison with the experimental work highlighted metformin, antibiotics and antidepressants as a priority for research. Further to these compounds, this thesis identified allopurinol, anti-hypertensives (candesartan and losartan) and lipid lower drugs (atorvastatin and simvastatin), which are largely absent from the literature. Further to these compounds, Chapter 3, identified ibuprofen as a compounds of potential interest to regulators as the result of its ranking across prioritisation schemes. The possible implications of this drug were further emphasised by the monitoring work, which observed some of the highest concentrations of ibuprofen observed in estuaries.

6.5 References

Almeida Â, Calisto V, Domingues MRM, Esteves VI, Schneider RJ, Soares AMVM, Figueira E, Freitas R (2017) Comparison of the toxicological impacts of carbamazepine and a mixture of its photodegradation products in Scrobicularia plana. J Hazard Mat 323: 220–232. doi: 10.1016/J.JHAZMAT.2016.05.009

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL (2010) Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chemi 29: 730–741. doi: 10.1002/etc.34

Antunes SC, Freitas R, Figueira E, Gonçalves F, Nunes B (2013) Biochemical effects of acetaminophen in aquatic species: Edible clams Venerupis decussata and Venerupis philippinarum. Environ Sci Pollut R 20: 6658–6666. doi: 10.1007/s11356-013-1784-9

Ashfaq M, Nawaz Khan K, Saif Ur Rehman M, Mustafa G, Faizan Nazar M, Sun Q, Iqbal J, Mulla SI, Yu CP (2017) Ecological risk assessment of pharmaceuticals in the receiving environment of pharmaceutical wastewater in Pakistan. Ecotoxicology and Environmental Safety 136: 31–39. doi: 10.1016/j.ecoenv.2016.10.029

Austin GE, Calbrade NA, REhfisch MR, Wright LJ (2008) Humber estaury SPA waterbird populations: trend analyses by count sector Available from:

https://www.bto.org/research-data-services/publications/research-reports/2008/humber-estuary-spa-waterbird-populations-t [Accessed April 14, 2019]

Backhaus T, Faust M (2012) Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ. Sci. Technol. 46: 2564–2573. doi: 10.1021/es2034125

Baeta A, Cabral HN, Marques JC, Pardal MÂ (2006) Feeding ecology of the green crab, carcinus maenas (L., 1758) in a temperate estuary, Portugal. Crustaceana 79: 1181–1193 doi: 10.1163/156854006778859506

Benotti MJ, Brownawell BJ (2007) Distributions of Pharmaceuticals in an Urban Estuary during both Dry- and Wet-Weather Conditions. Environ Sci Technol. 41(16): 5795-5802 doi: 10.1021/ES0629965

Besse J-P, Kausch-Barreto C, Garric J (2008) Exposure Assessment of Pharmaceuticals and Their Metabolites in the Aquatic Environment: Application to the French Situation and Preliminary Prioritization. Hum Ecol Risk Assess 14: 665–695. doi: 10.1080/10807030802235078

Brain RA, Hanson M, Solomon K, Brooks BW (2008) Aquatic plants exposed to pharmaceuticals: Effects and risks 192: 67-115

Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R (2014) Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis 14: 742–750. doi: 10.1016/S1473-3099(14)70780-7

Boström ML, Ugge G, Jönsson JÅ, Berglund O (2017) Bioaccumulation and trophodynamics of the antidepressants sertraline and fluoxetine in laboratory-constructed, 3-level aquatic food chains. Environ Toxicol Chem 36: 1029–1037. doi: 10.1002/etc.3637

Bound JP, Voulvoulis N (2005) Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environ Health Perspect 113: 1705–1711. doi: 10.1289/ehp.8315

Briones RM, Sarmah AK, Padhye LP (2016) A global perspective on the use, occurrence, fate and effects of anti-diabetic drug metformin in natural and engineered ecosystems. Environ Pollut 219: 1007–1020. doi: 10.1016/j.envpol.2016.07.040

Buck AL (1997) An inventory of UK estuaries - Volume 5. Eastern England Joint Nature Conservation Committee, Peterborough

Burns EE, Thomas-Oates J, Kolpin DW, Furlong ET, Boxall ABA (2017) Are exposure predictions, used for the prioritization of pharmaceuticals in the environment, fit for purpose? Environ Toxicol Chem 36: 2823–2832. doi: 10.1002/etc.3842

Burns EE, Carter LJ, Snape JR, Thomas-Oates J, Boxall ABA (2018a) Application of prioritization approaches to optimize environmental monitoring and testing of pharmaceuticals. J Toxicol Environ Health B Crit Rev 21(3): 115-141 doi: 10.1080/10937404.1465873

Burns EE, Carter LJ, Kolpin DW, Thomas-Oates J, Boxall ABA (2018b) Temporal and spatial variation in pharmaceutical concentrations in an urban river system. Wat Res. doi: 10.1016/j.watres.2018.02.066

Cabral HN (2000) Comparative feeding ecology of sympatric Solea solea and S. senegalensis, within the nursery areas of the Tagus estuary, Portugal. J Fish Biol 57: 1550–1562. doi: 10.1111/j.1095-8649.2000.tb02231.x

Caldwell DJ, Mastrocco F, Margiotta-Casaluci L, Brooks BW (2014) An integrated approach for prioritizing pharmaceuticals found in the environment for risk assessment, monitoring and advanced research. Chemosphere 115: 4–12. doi: 10.1016/j.chemosphere.2014.01.021

Camacho-Muñoz D, Martín J, Santos JL, Aparicio I, Alonso E (2010) Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Doñana Park (Spain). J Hazard Mat 183: 602–608. doi: 10.1016/J.JHAZMAT.2010.07.067

Cantwell MG, Katz DR, Sullivan JC, Ho K, Burgess RM, Cashman M (2016) Selected pharmaceuticals entering an estuary: Concentrations, temporal trends, partitioning, and fluxes. Environ Toxicol Chem 35: 2665–2673. doi: 10.1002/etc.3452

Cantwell MG, Katz DR, Sullivan JC, Ho K, Burgess RM (2017) Temporal and spatial behavior of pharmaceuticals in Narragansett Bay, Rhode Island, United States. Environ Toxicol Chem. doi: 10.1002/etc.3710

Cantwell MG, Katz DR, Sullivan JC, Shapley D, Lipscomb J, Epstein J, Juhl AR, Knudson C, O'Mullan GD (2018) Spatial patterns of pharmaceuticals and wastewater tracers in the Hudson River Estuary. Wat Res 137: 335–343. doi: 10.1016/J.WATRES.2017.12.044

Capleton AC, Courage C, Rumsby P, Holmes P, Stutt E, Boxall ABA, Levy LS (2006) Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. Toxicol Lett 163: 213–223. doi: 10.1016/j.toxlet.2005.10.023

Celiz MD, Tso J, Aga DS (2009) Pharmaceutical metabolites in the environment: analytical challenges and ecological risks. Environ Toxicol Chem 28: 2473. doi: 10.1897/09-173.1

Chapman PM (2016) Assessing and managing stressors in a changing marine environment. Mar Pollut Bull. 130(1): 192-199 doi: 10.1016/j.marpolbul.2016.10.039

Cleuvers M (2004) Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotox Environ Safe 59: 309–315. doi: 10.1016/S0147-6513(03)00141-6

Clode PL, Saunders M, Maker G, Ludwig M, Atkins CA (2009) Uric acid deposits in symbiotic marine algae. Plant Cell Environ. 32(2): 170-177 doi: 10.1111/j.1365-3040-2008-01909.x

Coelho JP, Nunes M, Dolbeth M, Pereira ME, Duarte AC, Pardal MA (2008) The role of two sediment-dwelling invertebrates on the mercury transfer from sediments to the estuarine trophic web. Estuar Coast Shelf Sci 78: 505–512. doi: 10.1016/J.ECSS.2008.01.017

Comeau F, Surette C, Brun GL, Losier R (2008) The occurrence of acidic drugs and caffeine in sewage effluents and receiving waters from three coastal watersheds in Atlantic Canada. Sci Total Environ 396: 132–146. doi: 10.1016/j.scitotenv.2008.02.031

Conley JM, Symes SJ, Schorr MS, Richards SM (2008) Spatial and temporal analysis of pharmaceutical concentrations in the upper Tennessee River basin. Chemosphere 73: 1178–1187. doi: 10.1016/j.chemosphere.2008.07.062

Daouk S, Chèvre N, Vernaz N, Bonnabry P, Dayer P, Daali Y, Fleury-Souverain S (2015) Prioritization methodology for the monitoring of active pharmaceutical ingredients in hospital effluents. J Environ Manage 160: 324–32. doi: 10.1016/j.jenvman.2015.06.037

Daughton CG, Ruhoy IS (2014) Green pharmacy and pharmEcovigilance: prescribing and the planet. Expert Rev Clin Pharmacol. 4(2): 211-232 doi: 10.1586/ecp.11.6

Dong Z, Senn DB, Moran RE, Shine JP (2013) Prioritizing environmental risk of prescription pharmaceuticals. Regul Toxicol Pharmacol 65: 60–67. doi: 10.1016/j.yrtph.2012.07.003

Donnachie RL, Johnson AC, Sumpter JP (2016) A rational approach to selecting and ranking some pharmaceuticals of concern for the aquatic environment and their relative importance compared with other chemicals. Environ Toxicol Chem 35: 1021–1027. doi: 10.1002/etc.3165

Du B, Haddad SP, Luek A, Scott WC, Saari GN, Kristofco LA, Connors KA, Rash C, Rasmussen JB, Chambliss CK, Brooks BW (2014) Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. Philos Trans Royal Soc B Biol Sci 369: 20140058–20140058. doi: 10.1098/rstb.2014.0058

Eades C, Waring CP (2010) The effects of diclofenac on the physiology of the green shore crab Carcinus maenas. Mar Environ Res 69: S46–S48. doi: 10.1016/j.marenvres.2009.11.001

Edgar T, Boyd SD, Palame MJ (2008) Sustainability for behaviour change in the fight against antibiotic resistance: a social marketing framework. J Antimicrob Chemother 63: 230–237. doi: 10.1093/jac/dkn508

Environment Agency (2019) TraC Fish Counts for all Species for all Estuaries and all years. Available from: https://data.gov.uk/dataset/41308817-191b-459d-aa39-788f74c76623/trac-fish-counts-for-all-species-for-all-estuaries-and-all-years. [Accessed March 20, 2019]

European Environment Agency (2017) Urban Waste water Treatment Map. Available from: https://eea.maps.arcgis.com/apps/MapJournal/index.html?appid=7fa4f0267d8249888b077803714e39fe&embed=true# [March 15, 2019]

Fabbri E (2015) Pharmaceuticals in the environment: Expected and unexpected effects on aquatic fauna. Annals NY Acad Sci 1340: 20–28. doi: 10.1111/nyas.12605

Fabbri E, Franzellitti S (2016) Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. Environ Toxicol Chem 35: 799–812. doi: 10.1002/etc.3131

Ferrari B, Mons R, Vollat B, Fraysse B, Paxéus N, Lo Giudice R, Pollio A, Garric J (2004) Environmental risk assessment of six human pharmaceuticals: are the current

environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environ Toxicol Chem 23: 1344. doi: 10.1897/03-246

Fick J, Lindberg RH, Tysklind M, Larsson DGJ (2010) Predicted critical environmental concentrations for 500 pharmaceuticals. Regul Toxicol Pharmacol 58: 516–523. doi: 10.1016/j.yrtph.2010.08.025

Fong PP, Garthwaite RL (1994) Allozyme electrophoretic analysis of the Hediste limnicola - H. diversicolor - H. japonica species complex (Polychaeta: Nereididae). Mar Biol 118: 463–470. doi: 10.1007/BF00350303

Fong PP, Ford AT (2014) The biological effects of antidepressants on the molluscs and crustaceans: A review. Aquat Toxicol 151: 4–13. doi: 10.1016/j.aquatox.2013.12.003

García-Cambero J., García-Cortés H, Valcárcel Y, Catalá M (2015) Environmental concentrations of the cocaine metabolite benzoylecgonine induced sublethal toxicity in the development of plants but not in a zebrafish embryo–larval model. J Hazard Mat 300: 866–872. doi: 10.1016/j.jhazmat.2015.08.019

Gavrilescu M, Demnerová K, Aamand J, Agathos S, Fava F (2015) Emerging pollutants in the environment: Present and future challenges in biomonitoring, ecological risks and bioremediation. New Biotechnol 32: 147–156. doi: 10.1016/j.nbt.2014.01.001

Gaw S, Thomas K, Hutchinson TH (2016) Pharmaceuticals in the marine environment. Iss Environ Sci Technol 41 . doi: 10.1039/9781782622345-00070

Godoy AA, Kummrow F, Pamplin PAZ (2015) Occurrence, ecotoxicological effects and risk assessment of antihypertensive pharmaceutical residues in the aquatic environment - A review. Chemosphere 138: 281–291. doi: 10.1016/j.chemosphere.2015.06.024

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014a) Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/ cardiovascular pharmaceuticals and UV filters in wastewater treatment plant. Environ Sci Pollut R 21: 7578-7585 doi: 10.1007/s11356-014-2654-9

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014b) Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111: 418–426. doi: 10.1016/j.chemosphere.2014.03.132

Gonzalez-Rey M, Bebianno MJ (2014) Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel Mytilus galloprovincialis. Aquat Toxicol 148: 221–230. doi: 10.1016/J.AQUATOX.2014.01.011

Goodchild CG, Frederich M, Zeeman SI (2015) Bering Ecosystem Project (BEST) View project AMP-activated protein kinase is a biomarker of energetic status in freshwater mussels exposed to municipal effluents. Sci Total Environ 512-513: 201-209 doi: 10.1016/j.scitotenv.2015.01.065

Gröner F, Höhne C, Kleiner W, Kloas W (2017) Chronic diclofenac exposure affects gill integrity and pituitary gene expression and displays estrogenic activity in nile tilapia (Oreochromis niloticus). Chemosphere 166: 473–481. doi: 10.1016/j.chemosphere.2016.09.116

Guiloski IC, Stein Piancini LD, Dagostim AC, de Morais Calado SL, Fávaro LF, Boschen SL, Cestari MM, da Cunha C, Silva de Assis HC (2017) Effects of environmentally relevant concentrations of the anti-inflammatory drug diclofenac in freshwater fish Rhamdia quelen. Ecotoxicol Environmental Safe 139: 291–300. doi: 10.1016/j.ecoenv.2017.01.053

Guo J, Boxall A, Selby K (2015) Do Pharmaceuticals Pose a Threat to Primary Producers? Crit Rev Environ Sci Technol 45: 2565–2610. doi: 10.1080/10643389.2015.1061873

Haddad SP, Luek A, Scott WC, Saari GN, Burket SR, Kristofco LA, Corrales J, Rasmussen JB, Chambliss CK, Luers M, Rogers C, Brooks BW (2018) Spatio-temporal bioaccumulation and trophic transfer of ionizable pharmaceuticals in a semi-arid urban river influenced by snowmelt. J Hazard Mat 359: 231-240 doi: 10.1016/j.jhazmat.2018.07.063

Hanamoto S, Nakada N, Yamashita N, Tanaka H (2018) Source estimation of pharmaceuticals based on catchment population and in-stream attenuation in Yodo River watershed, Japan. Sci Total Environ 615: 964–971. doi: 10.1016/J.SCITOTENV.2017.10.013

Harris CA, Scott AP, Johnson AC, Panter GH, Sheahan D, Roberts M, Sumpter JP (2014) Principles of Sound Ecotoxicology. Environ Sci Technol 48: 3100–3111. doi: 10.1021/es4047507

Hedgespeth ML, Sapozhnikova Y, Pennington P, Clum A, Fairey A, Wirth E (2012) Pharmaceuticals and personal care products (PPCPs) in treated wastewater

discharges into Charleston Harbor, South Carolina. Sci Total Environ 437: 1–9. doi: 10.1016/j.scitotenv.2012.07.076

Helwig K, Hunter C, McNaughtan M, Roberts J, Pahl O (2016) Ranking prescribed pharmaceuticals in terms of environmental risk: Inclusion of hospital data and the importance of regular review. Environ Toxicol Chem 35: 1043–1050. doi: 10.1002/etc.3302

Hoeger B, Köllner B, Dietrich DR, Hitzfeld B (2005) Water-borne diclofenac affects kidney and gill integrity and selected immune parameters in brown trout (Salmo trutta f. fario). Aquat Toxicol 75: 53–64. doi: 10.1016/j.aquatox.2005.07.006

Hong B, Lin Q, Yu S, Chen Y, Chen Y, Chiang P (2018) Urbanization gradient of selected pharmaceuticals in surface water at a watershed scale. Sci Total Environ 634: 448–458. doi: 10.1016/J.SCITOTENV.2018.03.392

Hong HN, Kim HN, Park KS, Lee S-K, Gu MB (2007) Analysis of the effects diclofenac has on Japanese medaka (Oryzias latipes) using real-time PCR. Chemosphere 67: 2115–21. doi: 10.1016/j.chemosphere.2006.12.090

Hoyett Z, Owens MA, Clark CJ, Abazinge M (2016) A comparative evaluation of environmental risk assessment strategies for pharmaceuticals and personal care products. Ocean Coast Manage 127: 74–80. doi: 10.1016/j.ocecoaman.2016.04.013

Huber S, Remberger M, Kaj L, Schlabach M, Jörundsdóttir HT, Vester J, Arnórsson M, Mortensen I, Schwartson R, Dam M (2016) A first screening and risk assessment of pharmaceuticals and additives in personal care products in waste water, sludge, recipient water and sediment from Faroe Islands, Iceland and Greenland. Sci Total Environ 562: 13–25. doi: 10.1016/j.scitotenv.2016.03.063

Huggett DB, Cook JC, Ericson JF, Williams RT (2003) A Theoretical Model for Utilizing Mammalian Pharmacology and Safety Data to Prioritize Potential Impacts of Human Pharmaceuticals to Fish. Hum Ecol Risk Asses 9: 1789–1799. doi: 10.1080/714044797

Hughes SR, Kay P, Brown LE (2013) Global Synthesis and Critical Evaluation of Pharmaceutical Data Sets Collected from River Systems. Environ Sci Technol 47: 661–677. doi: 10.1021/es3030148

Kalaman J, Palais F, Amiard JC, Moneyrac C, Muntz A, Blasco J, Riba I, Amirad-Triquet C (2009) assessment of the health status of populations of the ragworm Nereis

diversicolor using biomarkets at different levels of biological organisation. Mar Ecol Progr Ser 393: 55–67. doi: 10.3354/meps08239

Kay P, Hughes SR, Ault JR, Ashcroft AE, Brown LE (2017) Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters. Environ Pollut 220: 1447-1455 doi: 10.1016/j.envpol.2016.10.087

Kim H-Y, Lee I-S, Oh J-E (2016) Human and veterinary pharmaceuticals in the marine environment including fish farms in Korea. Sci Total Environ. 579: 940-949 doi: 10.1016/j.scitotenv.2016.10.039

Kim Y, Choi K, Jung J, Park S, Kim PG, Park J (2007) Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. Environ Inter 33: 370–375. doi: 10.1016/j.envint.2006.11.017

Kostich MS, Lazorchak JM (2008) Risks to aquatic organisms posed by human pharmaceutical use. Sci Total Environ 389: 329–339. doi: 10.1016/j.scitotenv.2007.09.008

Lagesson A, Fahlman J, Brodin T, Fick J, Jonsson M, Byström P, Klaminder J (2016) Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web - Insights from a field experiment. Sci Total Environ 568: 208–215. doi: 10.1016/j.scitotenv.2016.05.206

Länge R, Hutchinson TH, Croudace CP, Siegmund F, Schweinfurth H, Hampe P, Panter GH, Sumpter JP (2010) Effects of the synthetic estrogen 17α-ethinylestradiol on the life-cycle of the fathead minnow (Pimephales promelas). Environ Toxicol Chem 20: 1216–1227. doi: 10.1002/etc.5620200610

Lara-Martín PA, Renfro AA, Cochran JK, Brownawell BJ (2015) Geochronologies of Pharmaceuticals in a Sewage-Impacted Estuarine Urban Setting (Jamaica Bay, New York). Environ Sci Technol 49: 5948–5955. doi: 10.1021/es506009v

Linert J, Güdel K, Escher BI (2007) Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. Environ Sci Technol 41(12): 4471-4478 doi: 10.1021/es0627693

Lonappan L, Kaur Brar S, Kumar Das R, Verma M, Surampalli RY (2016) Diclofenac and its transformation products : Environmental occurrence and toxicity - A review. Environ Int 96: 127-138 doi: 10.1016/j.envint.2016.09.014

Long E, Dutch M, Weakland S, Chadramouli B, Benskin JP (2013) Quantification of pharmaceuticals, personal care products, and perfluoroalkyl substances in the marine sediments of puget sound, Washington, USA. Environ Chem 32(8): 1701-1710

Loos R, Marinov D, Sanseverino I, Napierska D, Lettieri T (2018) Review of the 1 st Watch List under the Water Framework Directive and recommendations for the 2 nd Watch List. Environ Pollut 157(2): 561-568 doi: 10.2760/614367

López-Serna R, Petrović M, Barceló D (2012) Occurrence and distribution of multi-class pharmaceuticals and their active metabolites and transformation products in the Ebro River basin (NE Spain). Sci Total Environ 440: 280–289. doi: 10.1016/j.scitotenv.2012.06.027

Magnér J, Filipovic M, Alsberg T (2010) Application of a novel solid-phase-extraction sampler and ultra-performance liquid chromatography quadrupole-time-of-flight mass spectrometry for determination of pharmaceutical residues in surface sea water. Chemosphere 80: 1255–1260. doi: 10.1016/j.chemosphere.2010.06.065

Mander L, Cutts ND, Allen J, Mazik K (2007) Assessing the development of newly created habitat for wintering estuarine birds. Estuar Coast Shelf Sci 75: 163–174. doi: 10.1016/J.ECSS.2007.04.028

Mansour F, Al-Hindi M, Saad W, Salam D (2016) Environmental risk analysis and prioritization of pharmaceuticals in a developing world context. Sci Total Environ 557: 31–43. doi: 10.1016/j.scitotenv.2016.03.023

Maranho LA, Baena-Nogueras RM, Lara-Martín PA, DelValls TA, Martín-Díaz ML (2014) Bioavailability, oxidative stress, neurotoxicity and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete Hediste diversicolor as bioindicator species. Environ Res 134: 353–365. doi: 10.1016/j.envres.2014.08.014

Maranho LA, André C, DelValls TA, Gagné F, Martín-Díaz ML (2015) Toxicological evaluation of sediment samples spiked with human pharmaceutical products: Energy status and neuroendocrine effects in marine polychaetes Hediste diversicolor. Ecotox Environ Safe 118: 27–36. doi: 10.1016/j.ecoenv.2015.04.010

Martin-Diaz L, Franzellitti S, Buratti S, Valbonesi P, Capuzzo A, Fabbri E (2009) Effects of environmental concentrations of the antiepilectic drug carbamazepine on biomarkers and cAMP-mediated cell signaling in the mussel Mytilus galloprovincialis. Aquat Toxicol 94: 177–185. doi:

10.1016/J.AQUATOX.2009.06.015

Meador JP, Yeh A, Gallagher EP (2017) Determining potential adverse effects in marine fish exposed to pharmaceuticals and personal care products with the fish plasma model and whole-body tissue concentrations. Environ Pollut 230: 1018–1029. doi: 10.1016/j.envpol.2017.07.047

Mijangos L, Ziarrusta H, Ros O, Kortazar L, Fernández LA, Olivares M, Zuloaga O, Prieto A, Etxebarria N (2018) Occurrence of emerging pollutants in estuaries of the Basque Country: Analysis of sources and distribution, and assessment of the environmental risk. Wat Res 147: 152–163. doi: 10.1016/J.WATRES.2018.09.033

Milligan SR, Holt W V, Lloyd R (2009) Impacts of climate change and environmental factors on reproduction and development in wildlife. Philosoph Transa Royal Soc Lond B Biol Sci 364: 3313–9. doi: 10.1098/rstb.2009.0175

Mohapatra S, Huang C-H, Mukherji S, Padhye LP (2016) Occurrence and fate of pharmaceuticals in WWTPs in India and comparison with a similar study in the United States. Chemosphere 159: 526–535. doi: 10.1016/J.CHEMOSPHERE.2016.06.047

Mohd Zanuri NB, Bentley MG, Caldwell, GS (2017) Assessing the impact of diclofenac, ibuprofen and sildenafil citrate (Viagra®) on the fertilisation biology of broadcast spawning marine invertebrates. Mar Environ Res 127: 126–136. doi: 10.1016/J.MARENVRES.2017.04.005

Monserrat JM, Martínez PE, Geracitano LA, Lund Amado L, Martinez Gaspar Martins C, Lopes Leães Pinho G, Soares Chaves I, Ferreira-Cravo M, Ventura-Lima J, Bianchini A (2007) Pollution biomarkers in estuarine animals: Critical review and new perspectives. Comp Biochem Physiol C Toxicol Pharmacol 146: 221–234. doi: 10.1016/J.CBPC.2006.08.012

Munro K, Martins CPB, Loewenthal M, Comber S, Cowan DA, Pereira L, Barron LP (2019) Evaluation of combined sewer overflow impacts on short-term pharmaceutical and illicit drug occurrence in a heavily urbanised tidal river catchment (London, UK). Sci Total Environ 657: 1099–1111. doi: 10.1016/J.SCITOTENV.2018.12.108

Mutiyar PK, Gupta SK, Mittal AK (2018) Fate of pharmaceutical active compounds (PhACs) from River Yamuna, India: An ecotoxicological risk assessment approach. Ecotox Environ Safe 150: 297–304. doi: 10.1016/J.ECOENV.2017.12.041

Nallani G, Venables B, Constantine L, Huggett D (2016) Comparison of Measured and Predicted Bioconcentration Estimates of Pharmaceuticals in Fish Plasma and Prediction of Chronic Risk. Bull Environ Contam Toxicol 96: 580–584. doi: 10.1007/s00128-016-1782-y

National Health Service (2006) Prescriptions dispensed in the community, 1995-2005 Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescriptions-dispensed-in-the-community/prescriptions-dispensed-in-the-community-statistics-for-england-2005 [Accessed April 18, 2019]

National Health Service (2011) Prescriptions dispensed in the community, 2000-2010 Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescriptions-dispensed-in-the-community/prescriptions-dispensed-in-the-community-statistics-for-england-2000-2010 [Accessed April 18, 2019]

National Health Service (2014) Prescriptions dispensed in the community, 2004-2014. Available from: http://wales.gov.uk/topics/statistics/headlines/health2013/prescriptions-dispensed-community-2012/?lang=en. [Accessed February 10, 2019]

National Health Service (2016) Prescriptions dispensed in the community, 2005-2015 Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescriptions-dispensed-in-the-community/prescriptions-dispensed-in-the-community-statistics-for-england-2005-2015 [Accessed April 18, 2019]

National Health Service (2017) Prescriptions Dispensed in the Community - Statistics for England, 2006-2016. Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescriptions-dispensed-in-the-community/prescriptions-dispensed-in-the-community-statistics-for-england-2006-2016-pas [January 1, 2019].

Nebot C, Falcon R, Boyd KG, Gibb SW (2015) Introduction of human pharmaceuticals from wastewater treatment plants into the aquatic environment: a rural perspective. Environ Sci Pollut R 22: 10559–10568. doi: 10.1007/s11356-015-4234-z

Nilsen E, Smalling KL, Ahrens L, Gros M, Miglioranza KSB, Picó Y, Schoenfuss HL (2019) Critical review: Grand challenges in assessing the adverse effects of contaminants of emerging concern on aquatic food webs. Environ Toxicol Chem

38: 46–60. doi: 10.1002/etc.4290

Norris K, Atkinson PW, Gill JA (2004) Climate change and coastal waterbird populations - past declines and future impacts. Ibis 146: 82–89. doi: 10.1111/j.1474-919X.2004.00331.x

Oldenkamp R, Huijbregts MAJ, Hollander A, Versporten A, Goossens H, Ragas AMJ (2013) Spatially explicit prioritization of human antibiotics and antineoplastics in Europe. Environ Int 51: 13–26. doi: 10.1016/J.ENVINT.2012.09.010

OECD. (2017) Health at a glance 2017: OECD indicators: organization HEALTH AT A GLANCE 2017 : oecd indicators. Available from: https://read.oecd-ilibrary.org/social-issues-migration-health/health-at-a-glance-2017_health_glance-2017-en#page1 [Accessed April 19, 2019]

Overturf MD, Anderson JC, Pandelides Z, Beyger L, Holdway D a (2015) Pharmaceuticals and personal care products: A critical review of the impacts on fish reproduction. Crit Rev Toxicol 8444: 1–23. doi: 10.3109/10408444.2015.1038499

Paíga P, Santos LHMLM, Ramos S, Jorge S, Silva JG, Delerue-Matos C (2016) Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Sci Total Environ 573: 164–177. doi: 10.1016/j.scitotenv.2016.08.089

Pal A, Gin KYH, Lin AYC, Reinhard M (2010) Impacts of emerging organic contaminants on freshwater resources: Review of recent occurrences, sources, fate and effects. Sci Total Environ 408: 6062–6069. doi: 10.1016/j.scitotenv.2010.09.026

Palmer PM, Wilson LR, O'Keefe P, Sheridan R, King T, Chen C-Y (2008) Sources of pharmaceutical pollution in the New York City Watershed. Sci Total Environ 394: 90–102. doi: 10.1016/J.SCITOTENV.2008.01.011

Parolini M, Binelli A, Cogni D, Provini A (2010) Multi-biomarker approach for the evaluation of the cyto-genotoxicity of paracetamol on the zebra mussel (Dreissena polymorpha). Chemosphere 79: 489–498. doi: 10.1016/j.chemosphere.2010.02.053

Pereira AMPT, Silva LJG, Lino CM, Meisel LM, Pena A (2017) A critical evaluation of different parameters for estimating pharmaceutical exposure seeking an improved environmental risk assessment. Sci Total Environ 603: 226–236. doi: 10.1016/j.scitotenv.2017.06.022

Di Poi C, Costil K, Bouchart V, Halm-Lemeille M-P (2018) Toxicity assessment of five

emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. Environ Sci Pollut R 25: 6122–6134. doi: 10.1007/s11356-017-9306-9

Regoli F, Gorbi S, Frenzilli G, Nigro M, Corsi I, Focardi S, Winston G. (2002) Oxidative stress in ecotoxicology: from the analysis of individual antioxidants to a more integrated approach. Mar Environ Res 54: 419–423. doi: 10.1016/S0141-1136(02)00146-0

Rehman MSU, Rashid N, Ashfaq M, Saif A, Ahmad N, Han J-I (2015) Global risk of pharmaceutical contamination from highly populated developing countries. Chemosphere 138: 1045–1055. doi: 10.1016/J.CHEMOSPHERE.2013.02.036

Ridgway J, Shimmield G (2002) Estuaries as Repositories of Historical Contamination and their Impact on Shelf Seas. Estuar Coast Shelf Sci 55: 903–928. doi: 10.1006/ECSS.2002.1035

Roberts PH, Thomas K V. (2006) The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ 143–153. doi: 10.1016/j.scitotenv.2005.04.031

Rocha MJ, Cruzeiro C, Reis M, Pardal MÂ, Rocha E (2014) Spatial and seasonal distribution of 17 endocrine disruptor compounds in an urban estuary (Mondego River, Portugal): Evaluation of the estrogenic load of the area. Environ Monit Assess 186: 3337–3350. doi: 10.1007/s10661-014-3621-0

Roos V, Gunnarsson L, Fick J, Larsson DGJ, Rudén C (2012) Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Sci Total Environ 421–422: 102–110. doi: 10.1016/j.scitotenv.2012.01.039

Rosa S, Granadeiro JP, Vinagre C, França S, Cabral HN, Palmeirim JM (2008) Impact of predation on the polychaete Hediste diversicolor in estuarine intertidal flats. Estuar Coast Shelf Sci 78: 655–664. doi: 10.1016/J.ECSS.2008.02.001

Sanderson H, Johnson DJ, Reitsma T, Brain RA, Wilson CJ, Solomon KR (2004) Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regula Toxicol Pharmacol 39: 158–183. doi: 10.1016/j.yrtph.2003.12.006

Sangion A, Gramatica P (2016) Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity. Environ Int doi: 10.1016/j.envint.2016.08.008

Santos LHMLM, Araújo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MCBSM (2010) Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mat 175: 45–95. doi: 10.1016/j.jhazmat.2009.10.100

Scaps P (2002) A review of the biology, ecology and potential use of the common ragworm Hediste diversicolor (O.F. Müller) (Annelida: Polychaeta). Hydrobiologia 470: 203–218. doi: 10.1023/A:1015681605656

Scheurell M, Franke S, Shah RM, Hühnerfuss H (2009) Occurrence of diclofenac and its metabolites in surface water and effluent samples from Karachi, Pakistan. Chemosphere 77: 870–876. doi: 10.1016/J.CHEMOSPHERE.2009.07.066

Schrieber R, Gundel U, Franz S, Kuster A, Rechenberg B, Attenburger R (2011) Using the fish plasma model for comparative hazard identification for pharmaceuticals in the environment by extrapolation from human therapeutic data. Regul Toxicol Pharmacol 61: 261–275. doi: 10.1016/J.YRTPH.2011.08.006

Sheng B, Liu J, Li GH (2012) Metformin preconditioning protects Daphnia pulex from lethal hypoxic insult involving AMPK, HIF and mTOR signaling. Comp Biochem Physiol B Biochem Mol Biol 163: 51–58. doi: 10.1016/J.CBPB.2012.04.009

Stillman RA, West AD, Goss-Custard JD, McGroty S, Frost NJ, Morrisey DJ, Kenny AJ, Drewitt AL (2005) Predicting site quality for shorebird communities: a case study on the Humber Estuary, UK. Mar Ecoly Progr Ser 305: 203–217. Available from: www.int-res.com (March 10, 2019).

Tanguy M, Dixon H, Prosdocimi I, Morris DG, Keller VDJ (2016). Gridded estimates of daily and monthly areal rainfall for the United Kingdom (1890-2015) [CEH-GEAR]. NERC Environmental Information Data Centre. doi: 10.5285/33604ea0-c238-4488-813d-0ad9ab7c51ca

Taylor D, Senac T (2014) Human pharmaceutical products in the environment – The “problem” in perspective. Chemosphere 115: 95–99. doi: 10.1016/J.CHEMOSPHERE.2014.01.011

Thomas K V., Hilton MJ (2004) The occurrence of selected human pharmaceutical compounds in UK estuaries. Mar Pollut Bull 49: 436–444. doi: 10.1016/j.marpolbul.2004.02.028

Valdés ME, Amé MV, Bistoni M de los A, Wunderlin DA (2014) Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquía River basin (Córdoba, Argentina). Sci Total Environ 472: 389–396. doi:

10.1016/j.scitotenv.2013.10.124

Vernouillet G, Eullaffroy P, Lajeunesse A, Blaise C, Gagné F, Juneau P (2010) Toxic effects and bioaccumulation of carbamazepine evaluated by biomarkers measured in organisms of different trophic levels. Chemosphere 80: 1062–1068. doi: 10.1016/j.chemosphere.2010.05.010

Vestel J, Caldwell DJ, Constantine L, D'Aco VJ, Davidson T, Dolan DG, Millard SP, Murray-Smith R, Parke NJ, Ryan JJ, Straub JO, Wilson P (2016) Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environ Toxicol Chem 35: 1201–1212. doi: 10.1002/etc.3260

von der Ohe PC, Dulio V, Slobodnik J, de Deckere E, Kühne R, Ebert R-U, Ginebreda A, de Cooman W, Schüürmann G, Brack W (2011) A new risk assessment approach for the prioritisation of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the Euopean Water Framework Directive. Sci Total Environ 409: 2064-2077

Wilkinson JL, Hooda PS, Swinden J, Barker J, Barton S (2017) Spatial distribution of organic contaminants in three rivers of Southern England bound to suspended particulate material and dissolved in water. Sci Total Environ 593–594: 487–497. doi: 10.1016/J.SCITOTENV.2017.03.167

Wu JP, Li MH (2015) Inhibitory effects of pain relief drugs on neurological enzymes: Implications on their potential neurotoxicity to aquatic animals. Environ Toxicol Pharmacol 39: 898–905. doi: 10.1016/j.etap.2015.02.022

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL (2011) Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone. J Hazard Mat 190: 588–596. doi: 10.1016/J.JHAZMAT.2011.03.092

Appendices

A1 Supporting information for Chapter 1

A1.1 Summary of the occurrence of pharmaceutical in estuarine surface water

Table A1.1: Median (ng l-1) and maximum (ng l-1) concentrations of pharmaceuticals in estuarine surface waters globally, and number of estuaries sampled in each study. Detection frequency is the number of positive detections/number of samples analysed across studies.

Class	Compound	Median ($ng\ l^{-1}$)	Maximum ($ng\ l^{-1}$)	Detection Frequency (%)	Number of Estuaries Sampled	Reference
Antibiotic	Azithromycin	0	2.2	15	5	Zhang et al. (2012, 2013), Meador et al. (2016), Reis-Santos et al. (2018)
	Benzylpenicillin	1.89	13.4	87.1	1	Reis-Santos et al. (2018)
	Chloramphenicol	3.5	23	90	3	Yan et al. (2013), Zhao et al. (2015), Munro et al. (2019)
	Chlortetracycline	0.08	3.5	25	2	Yan et al. (2013), Reis-Santos et al. (2018)
	Ciprofloxacin	1	540	22.5	8	Tamtam et al. (2008), Zhang et al. (2012), Yan et al. (2013), Mijangos et al. (2018), Reis-Santos et al. (2018), Guo et al. (2019)
	Clarithromycin	0	17.6	20.9	5	Zhang et al. (2012, 2013), Klosterhaus et al. (2013), Chen et al. (2015), Sun et al. (2016)
	Danofloxacin	0	19	1.7	2	Tamtam et al. (2008), Reis-Santos et al. (2018)
	Difloxacin	0	<MQL	3.4	1	Tamtam et al. (2008)
	Enoxacin	29.5	209	44.5	2	Zhang et al. (2012), Reis-Santos et al. (2018)
	Enrofloxacin	0	56.7	12.4	9	Tamtam et al. (2008), Zheng et al. (2011), Zhang et al. (2012), Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Meador et al. (2016), Sun et al. (2016), Reis-Santos et al. (2018)
	Erythromycin	0	45.4	45.2	12	Thomas and Hilton (2004), Zhang et al. (2012, 2013), Yan et al. (2013), Zhao et al. (2015), Reis-Santos et al. (2018), Guo et al. (2019)
	Florfenicol	8.84	89.5	80	3	Zheng et al. (2011), Yan et al. (2013), Zhao et al. (2015)
	Flumequine	0	32	22.8	3	Tamtam et al. (2008), Meador et al. (2016), Reis-Santos et al. (2018)

Table A1.1: *Continued.*

Class	Compound	Median ($ng\ l^{-1}$)	Maximum ($ng\ l^{-1}$)	Detection Freq. (%)	Estuaries Sampled	Reference
Antibiotic	Lincomycin	0	23.4	4.3	1	Chen et al. (2015)
	Methacycline	2.05	2.45	82.6	1	Chen et al. (2015)
	Nalidixic Acid	0	<MQL	3.6	2	Tamtam et al. (2008), Reis-Santos et al. (2018)
	Norfloxacin	5	163	29.6	10	Tamtam et al. (2008), Zhang et al. (2012), Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Meador et al. (2016), Mijangos et al. (2018), Reis-Santos et al. (2018)
	Oflaxacin	0	55	30.4	9	Tamtam et al. (2008), Zheng et al. (2011), Zhang et al. (2012), Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018), Guo et al. (2019)
	Ornidazole	0	58	15.8	1	Tamtam et al. (2008)
	Oxolinic Acid	0	19	3.1	3	Tamtam et al. (2008), Meador et al. (2016), Reis-Santos et al. (2018)
	Oxytetracycline	0	15163	24.8	5	Yan et al. (2013), Chen et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018), Guo et al. (2019)
	Roxithromycin	0	8.2	27.9	8	Yang et al. (2011), Zhang et al. (2012, 2013), Liang et al. (2013), Yan et al. (2013), Zhao et al. (2015), Guo et al. (2019)
	Salinomycin	6.67	12.9	100	1	Yang et al. (2011)
	Sarafloxacin	0	10	1.3	3	Tamtam et al. (2008), Meador et al. (2016), Sun et al. (2016)
	Sulfademethoxine	0	0.46	33.3	3	Chen et al. (2015), Meador et al. (2016), Reis-Santos et al. (2018)
	Sulfadiazine	0.93	71.8	49.4	14	Zheng et al. (2011), Zhang et al. (2012), Liang et al. (2013), Yan et al. (2013), Zhang et al. (2013), Chen et al. (2015), Zhao et al. (2015), Mijangos et al. (2018), Reis-Santos et al. (2018), Guo et al. (2019)
	Sulfadimidine	0	0.35	3.8	2	Zhang et al. (2013)
	Sulfamerazine	0	0.16	50	2	Zhao et al. (2015), Sun et al. (2016)
	Sulfameter	0	2.05	14.4	2	Zheng et al. (2011), Chen et al. (2015)

Table A1.1: *Continued.*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
	Sulfamethazine	0	89.1	44.9	11	Tamtam et al. (2008), Zheng et al. (2011), Zhang et al. (2012), Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Zhao et al. (2015), Meador et al. (2016), Sun et al. (2016), Reis-Santos et al. (2018), Munro et al. (2019)
	Sulfamethizole	0	15.6	6.7	3	Klosterhaus et al. (2013), Meador et al. (2016), Reis-Santos et al. (2018)
	Sulfamethoxazole	5.15	765	59.4	27	Thomas and Hilton (2004), Benotti and Brownawell (2007), Tamtam et al. (2008), Yang et al. (2011), Zheng et al. (2011), Zhang et al. (2012, 2013), Klosterhaus et al. (2013), Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Zhao et al. (2015), Cantwell et al. (2016), Meador et al. (2016) Sun et al. (2016), Cantwell et al. (2017, 2018), Mijangos et al. (2018), Reis-Santos et al. (2018), Guo et al. (2019)
	Sulfamonomethoxine	2.1	31.1	33.8	2	Zheng et al. (2011), Chen et al. (2015)
	Sulfapyridine	0	9.1	45.9	5	Zheng et al. (2011), Yan et al. (2013), Zhao et al. (2015), Reis-Santos et al. (2018), Munro et al. (2019)
	Sulfathiazole	0	5.23	65.7	3	Yan et al. (2015), Zhao et al. (2015), Reis-Santos et al. (2018)
	Sulfaquinoxaline	0.19	23.5	51.7	4	Yan et al. (2013), Chen et al. (2015), Zhao et al. (2015), Reis-Santos et al. (2018)
	Thiamphenicol	2.97	19.50	100	1	Zhao et al. (2015)
	Tetracycline	0	2305	18.6	6	Liang et al. (2013), Yan et al. (2013), Chen et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018), Guo et al. (2019)
	Trimethoprim	4.12	2046	66.3	20	Thomas and Hilton (2004), Benotti and Brownawell (2007), Tamtam et al. (2008), Zhang et al. (2012, 2013), Klosterhaus et al. (2013), Chen et al. (2015), Cantwell et al. (2016, 2017, 2018), Mijangos et al. (2018), Reis-Santos et al. (2018), Munro et al. (2019)
N.D: Amoxicillin, Ceftiofur, Cefotaxime, Cephalexin, Cinoxacin, Cloxacillin, Doxycycline, Enalapril, Lomefloxacin, Marbofloxacin, Narasin, Oxacillin, Pipemidic Acid, Penicillin G, Penicillin V, Spiramycin, Sulfacetamide, Sulfachloropyridazine, Sulfadoxine, Sulfamerazine, Sulfanilamide, Sulfasomidine, Sulfisoxazole						Tamtam et al. (2008), Klosterhaus et al. (2013), Zhang et al. (2013), Chen et al. (2015), Meador et al. (2016), Sun et al. (2016), Reis-Santos et al. (2018)

Table A1.1: *Continued.*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Anticonvulsant	Alprazolan	0	<MQL	3.2	1	Reis-Santos et al. (2018)
	Carbamazepine	4.35	675	67.9	16	Benotti and Brownawell (2007), Yang et al. (2011), Klosterhaus et al. (2013), Birch et al. (2015), Gonzalez-Rey et al. (2015), Zhao et al. (2015), Sun et al. (2016), Cantwell et al. (2016, 2017, 2018), Reis-Santos et al. (2018), Mijangos et al. (2018), Cui et al. (2019), Munro et al. (2019)
	Gabapentin	0.08	1.66	67.7	1	Reis-Santos et al. (2018)
	Phenytoin	9	1401	18.7	3	Mijangos et al. (2018)
	Topiramate	0	<MQL	25.8	1	Reis-Santos et al. (2018)
Antidepressant	Amitriptyline	0	100	29.4	6	Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015), Mijangos et al. (2018), Munro et al. (2019)
	Clopiramine	0	2	1	3	Mijangos et al. (2018)
	Fluoxetine	0	596	36.1	6	Benotti and Brownawell (2007), Birch et al. (2015), Gonzalez-Rey et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018), Munro et al. (2019)
	Nortriptyline	0	6	8.3	4	Mijangos et al. (2018), Munro et al. (2019)
	Sertraline	0	304	3.3	2	Klosterhaus et al. (2013), Reis-Santos et al. (2018)
	Thiamphenicol	18.3	110	100	1	Yan et al. (2013)
	Venlafaxine	0.33	44.7	55.6	2	Birch et al. (2015), Reis-Santos et al. (2018)
	N.D: Doxepin, Impiramine, Lofepramine					Thomas and Hilton (2004), Gonzalez-Rey et al. (2015), Mijangos et al. (2018)
Antidiabetic	Metformin	234.25	832	61.3	2	Benotti and Brownawell (2007), Meador et al. (2016)
	N.D. Glibenclamide					Sun et al. (2016)
Antifungal	Clotrimazole	5.5	100	63.3	5	Thomas and Hilton (2004)
	N.D. Miconazole					Sun et al. (2016)
Antihistamine	Diphenhydramine	1.2	1.9	80	2	Klosterhaus et al. (2013), Meador et al. (2016)
	N.D.: Loratadine					Sun et al. (2016)
Antihypertensive	Acebutolol	0.3	0.8	21.5	1	Cantwell et al. (2018)

Table A1.1: *Continued.*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Antihypertensive	Atenolol	7.23	38.34	82.4	6	Klosterhaus et al. (2013), Cantwell et al. (2016, 2017, 2018), Meador et al. (2016), Reis-Santos et al. (2018)
	Bisoprolol	0.49	0.97	100	1	Reis-Santos et al. (2018)
	Carvediol	0	0.49	12.9	1	Reis-Santos et al. (2018)
	Diltiazem	0.61	23.5	88.0	6	Benotti and Brownawell (2007), Klosterhaus et al. (2013), Cantwell et al. (2016, 2017, 2018), Meador et al. (2016)
	Eprosartan	14	183	33.7	3	Mijangos et al. (2018)
	Furosemide	0	44.9	3.9	3	Cantwell et al. (2017, 2018), Reis-Santos et al. (2018)
	Indapamide	0	4.67	9.7	1	Reis-Santos et al. (2018)
	Irbesartan	12.6	494	77	4	Mijangos et al. (2018), Reis-Santos et al. (2018)
	Losartan	12.3	183	51.9	6	Sun et al. (2016), Cantwell et al. (2018), Mijangos et al. (2018), Reis-Santos et al. (2018)
	Metoprolol	18.75	312.5	93.3	6	Klosterhaus et al. (2013), Cantwell et al. (2016, 2017, 2018), Sun et al. (2016), Munro et al. (2019)
	Nimodipine	0	0.21	8	1	Zhao et al. (2015)
	Nifedipine	2.5	899	35.6	2	Benotti and Brownawell (2007), Munro et al. (2019)
	Propranolol	0.73	142	44.3	17	Thomas and Hilton (2004), Yang et al. (2011), Birch et al. (2015), Cantwell et al. (2016, 2017, 2018), Sun et al. (2016), Mijangos et al. (2018), Reis-Santos et al. (2018), Cui et al. (2019), Munro et al. (2019)
	Telmisartan	9	969	71.7	3	Mijangos et al. (2018)
	Valsartan	47.4	248	76.1	7	Klosterhaus et al. (2013), Cantwell et al. (2016, 2017, 2018), Mijangos et al. (2018)
	Verapamil	0.9	2.6	58.5	3	Cantwell et al. (2016, 2017, 2018)
	N.D. Clonidine, Digoxin, Sotalol					Zheng et al. (2011), Meador et al. (2016)
Antiparasitic	Crotamiton	0	1.08	30	1	Sun et al. (2016)
	Thiabendazole	0	2.5	10	2	Klosterhaus et al. (2013), Sun et al. (2016)

Table A1.1: *Continued.*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Antispasmodic	Mebeverine	0	154	16.7	2	Yang et al. (2011), Cui et al. (2019)
Anxiolytic	Alpraxolam		1		1	Gonzalez-Rey et al. (2015)
	Diazepam	0	2.61	42.3	3	Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015), Zhao et al. (2015), Sun et al. (2016)
	Lorazepam	0	<MQL	6.5	1	Reis-Santos et al. (2018)
	Meprobamate	21.5	36.1	100	1	Klosterhaus et al. (2013)
	Nordiazepam		3	100	1	Gonzalez-Rey et al. (2015)
	N.D. Bromazepam					Gonzalez-Rey et al. (2015)
Blood Thinner	Warfarin	1	5.26	51.6	2	Benotti and Brownawell (2007, Munro et al. (2019)
Bronchodilator	Salbutamol	0	1440	32.5	3	Benotti and Brownawell (2007), Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015)
	Theophylline	0	186	50	2	Gonzalez-Rey et al. (2015), Meador et al. (2016)
	N.D. Bethamethasone, Clenbuterol, Fluticasone Proprionate					Gonzalez-Rey et al. (2015), Meador et al. (2016)
Diuretic	Hydrochlorothiazide	26.77	277.34	75.6	2	Cantwell et al. (2016, 2017)
	Triamterene	2.3	9.6	100	1	Klosterhaus et al. (2013)
Chemotherapy Agent	N.D. Cyclophosphamide, Paclitaxel					Zhao et al. (2015), Sun et al. (2016)
Decongestant	N.D. Dextromethorphan					Munro et al. (2019)
Erectile Dysfunction	Sildenafil	0	0.448	2.3	1	Sun et al. (2016)
Hormone	Estradiol	3.78	18	66.7	2	Noppe et al. (2007), Rocha et al. (2014)
	Estrone	4.5	14	100	2	Noppe et al. (2007), Rocha et al. (2014)
	Ethinylestradiol	0.08	11	50	3	Ferguson et al. (2013), Rocha et al. (2014), Nie et al. (2015)
	Tamoxifen	0	224	40.4	8	Thomas and Hilton (2004), Yang et al. (2011), Zhao et al. (2015), Cui et al. (2019)

Table A1.1: *Continued.*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Hormone	Testosterone	0	1.9	25	4	Meador et al. (2016), Mijangos et al. (2018)
	N.D. Norgestimate, Progesterone					Meador et al. (2016), Mijangos et al. (2018)
Lipid Lowering Agent	Atorvastatin	0	<MQL	3.2	1	Reis-Santos et al. (2018)
	Bezafibrate	3.5	67	49.8	6	Mijangos et al. (2018, Reis-Santos et al. (2018), Cui et al. (2019), Munro et al. (2019)
	Fenofibrate	0	241	36.6	3	Benotti and Brownawell (2007), Zhao et al. (2015), Reis-Santos et al. (2018)
	Gemofibrozil	1.77	76.22	72.2	10	Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015), Zhao et al. (2015), Cantwell et al. (2016, 2017, 2018), Meador et al. (2016), Sun et al. (2016), Reis-Santos et al. (2018), Cui et al. (2019)
	N.D. Simvastatin					Zhao et al. (2015), Reis-Santos et al. (2018)
Metabolite	10-Hydroxy-amitriptyline	0.1	0.3	40	2	Klosterhaus et al. (2013), Meador et al. (2016)
	Benzoylecgonine	12	20	100	2	Klosterhaus et al. (2013), Munro et al. (2019)
	Dehydronifedipine	0.7	1.3	80	1	Klosterhaus et al. (2013)
	Desmethyldiltiazem	0	1.7	40	1	Klosterhaus et al. (2013)
	Erythromycin-H_2O	2.85	12.1	100	2	Meador et al. (2016), Meador et al. (2016)
	Norfluoxetine	6.59	/	2.8	1	Hedgespeth et al. (2012)
	Temazepam	16	19	100	1	Munro et al. (2019)
	N.D. Epi-chloratetracycline, Epi-oxytetracycline, Epi-tetracycline					Reis-Santos et al. (2018)
Mucosal Protectant	Cimetidine	0.28	67.2	88.7	2	Benotti and Brownawell (2007), Zhao et al. (2015)
	Omeprazole	0	0.37	73	1	Zhao et al. (2015)
	Pirenzepine	0	0.432	2.275	1	Sun et al. (2016)
	Ranitidine	0	13.22	25.1	5	Benotti and Brownawell (2007), Klosterhaus et al. (2013), Cantwell et al. (2016, 2017, 2018)

Table A1.1: *Continued.*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
NSAID	Diclofenac	3.1	650	48.2	14	Thomas and Hilton (2004), Wiegel et al. (2004), Yang et al. (2011), Gonzalez-Rey et al. (2015), Sun et al. (2016), Mijangos et al. (2018), Reis-Santos et al. (2018), Cui et al. (2019)
	Fenoprofen	/	241	72.8	1	Sun et al. (2016)
	Ibuprofen	0.6	928	47.3	11	Thomas and Hilton (2004), Wiegel et al. (2004), Hedgespeth et al. (2012), Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015), Sun et al. (2016), Reis-Santos et al. (2018)
	Indomethacin	0	979	65.4	4	Chen et al. (2011), Zhao et al. (2015), Sun et al. (2016), Cui et al. (2019)
	Ketoprofen	0	57	10.1	8	Gonzalez-Rey et al. (2015), Sun et al. (2016), Cantwell et al. (2018), Mijangos et al. (2018), Cui et al. (2019), Munro et al. (2019)
	Meclofenamic Acid	217.5	679	66.7	1	Yang et al. (2011)
	Mefenamic Acid	0	125	28.5	6	Thomas and Hilton (2004), Sun et al. (2016)
	Naproxen	0	17	42.9	4	Klosterhaus et al. (2013), Gonzalez-Rey et al. (2015), Sun et al. (2016), Cui et al. (2019)
	Nimesulide	0	0	0	2	Reis-Santos et al. (2018), Munro et al. (2019)
	N.D. Nimesulide, Propyphenazone					Sun et al. (2016), Reis-Santos et al. (2018), Munro et al. (2019)
Pain Killer	Antipyrine	0	10	15	3	Benotti and Brownawell (2007), Sun et al. (2016), Munro et al. (2019)
	Codeine	2.49	105	44.8	3	Benotti and Brownawell (2007), Birch et al. (2015), Sun et al. (2016)
	Dextropropoxyphene	0	80	10	5	Thomas and Hilton (2004)
	Ketamine	10	14	53.8	1	Munro et al. (2019)
	Hydrocodone	0.81	20.8	47.1	2	Benotti and Brownawell (2007), Klosterhaus et al. (2013)
	Paracetamol	1.23	440	45.74	19	Thomas and Hilton (2004), Benotti and Brownawell (2007), Hedgespeth et al. (2012), Birch et al. (2015), Gonzalez-Rey et al. (2015), Zhao et al. (2015), Cantwell et al. (2016, 2017, 2018), Meador et al. (2016), Sun et al. (2016), Mijangos et al. (2018), Reis-Santos et al. (2018)
	Propoxyphene	0	0.7	40	1	Klosterhaus et al. (2013)

Table A1.1: *Continued.*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Pain Killer	Tramadol	82.5	192	53.5	2	Birch et al. (2015), Munro et al. (2019)
	N.D. Ethenzamide					Sun et al. (2016)
Steroid	N.D. Digoxigenin, Methylprednisolone, Prednisolone, Prednisone, Trembolone, Trembolone Acetate					Klosterhaus et al. (2013), Meador et al. (2016)
Stimulant	Amphetamine	7.8	29	70	2	Klosterhaus et al. (2013), Meador et al. (2016)
	Cocaine	9.3	19	90	2	Klosterhaus et al. (2013), Munro et al. (2019)
X-Ray Contrast	Iopromide	3.9	12.5	93.1	1	Birch et al. (2015)

A1.2 Summary of the occurrence of pharmaceutical in estuarine sediment

Table A1.2: Median (ng g-1) and maximum (ng g-1) concentrations of pharmaceuticals in estuarine sediments globally and number of estuaries sampled in each study. Detection frequency is the number of positive detections/number of samples analysed across studies

Class	Compound	Median (ng g^{-1})	Maximum (ng g^{-1})	Detection Frequency (%)	Number of Estuaries Sampled	Reference
Antibiotic	Azithromycin	/	1.6	7.5	1	Long et al. (2013)
	Chloramphenicol	0	<MQL	96.4	1	Shi et al. (2014)
	Chloratetracycline	0.41	184	52.1	2	Shi et al. (2014), Chen et al. (2015)
	Ciprofloxacin	3.1	42.9	46.7	3	Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
	Clarithromycin	0.75	10.7	52.1	4	Klosterhaus et al. (2013), Lara-Martin et al. (2014), Stewart et al. (2014)
	Doxycycline	0	18.6	100	1	Shi et al. (2014), Chen et al. (2015)
	Enrofloxacin	0.30	4.84	46.6	3	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015)
	Erythromycin	0.24	51.5	41.9	3	Beretta et al. (2014), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
	Florfenicol	0	<MQL	100	1	Shi et al. (2014)
	Flumequine	1.2	2.9	100	1	Lara-Martin et al. (2015)
	Narasin	0	7.71	8.4	1	Chen et al. (2015)
	Norfloxacin	3.36	69.3	64.7	3	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015)
	Novobiocin	0	0.56	4.2	1	Chen et al. (2015)
	Ofloxacin	2.59	458.2	54.5	4	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
	Oxytetracycline	0.84	176	37.8	4	Long et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
	Pefloxacin	0	1.95	4.2	1	Chen et al. (2015)
	Roxithromycin	0.9	13.5	32.1	4	Liang et al. (2013), Shi et al. (2014), Guo et al. (2019), Stewart et al. (2014)
	Salinomycin	0	10.02	29.2	1	Chen et al. (2015)
	Sulfadiazine	0	1.7	9.1	4	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)

Table A1.2: *Continued*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Antibiotic	Sulfamerazine	25	0.41	25	1	Shi et al. (2014)
	Sulfamethazine	0.56	4.84	27.4	4	Liang et al. (2013), Stewart et al. (2014), Shi et al. (2014), Chen et al. (2015)
	Sulfamethoxazole	0	1.13	9.7	5	Klosterhaus et al. (2013), Liang et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
	Sulfapyridine	0.53	9.12	33.5	2	Shi et al. (2014), Chen et al. (2015)
	Sulfaquinoxaline	0.14	0.96	50	2	Shi et al. (2014), Chen et al. (2015)
	Sulfathiazole	0	<MQL	12.5	2	Klosterhaus et al. (2013), Shi et al. (2014)
	Tetracycline	0.81	14.6	37.7	4	Liang et al. (2013), Shi et al. (2014), Chen et al. (2015), Guo et al. (2019)
	Thiamphenicol	0	<MQL	100	1	Shi et al. (2014)
	Trimethoprim	0	18.2	26.7	5	Klosterhaus et al. (2013), Stewart et al. (2014), Chen et al. (2015), Lara-Martín et al. (2015), Cantwell et al. (2017)
	N.D. Enalapril, Lincomycin, Methacycline, Sulfamonomethoxine					Klosterhaus et al. (2013), Chen et al. (2015)
Anticonvulsant	Carbamazepine	0.41	4.81	36.2	5	Klosterhaus et al. (2013), Beretta et al. (2014), Stewart et al. (2014), Lara-Martín et al. (2015), Cantwell et al. (2017)
Antidepressant	Amitriptyline	0	0.45	1.25	1	Lara-Martín et al. (2015)
	Fluoxetine	0	14.5	22.2	1	Lara-Martín et al. (2015)
	Paroxetine	0	21.5	11.1	1	Klosterhaus et al. (2013)
	N.D. Sertraline					Beretta et al. (2014)
Antidiabetic	Galaxolide	9.17	14.54	100	1	Beretta et al. (2014)
Antifungal	Miconazole	0	1.5	2.5	1	Long et al. (2013)
Antihistamine	Diphenhydramine	0	4.81	43.8	2	Klosterhaus et al. (2013), Long et al. (2013)
	Loratidine	0.8	1.8	88.9	1	Lara-Martín et al. (2015)
Antihypertensive	Atenolol	0	7.83	37.3	3	Klosterhaus et al. (2013), Beretta et al. (2014), Cantwell et al. (2017)
	Metoprolol	2.11	44	54.7	4	Klosterhaus et al. (2013), Stewart et al. (2014), Lara-Martín et al. (2015), Cantwell et al. (2017)

Table A1.2: *Continued*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Antihypertensive	Nadolol	0.3	/	/	1	Stewart et al. (2014)
	Pindolol	0.4	/	/	1	Stewart et al. (2014)
	Propranolol	0.3	6.7	74	2	Lara-Martín et al. (2015), Cantwell et al. (2017)
	Sotalol	0.9	/	/		Stewart et al. (2014)
	Timolol	0.8	0.8	/	1	Stewart et al. (2014)
	Verapamil	0.6	14	39.7	2	Long et al. (2013), Cantwell et al. (2017)
	N.D. Diltiazem, Valsartan					Klosterhaus et al. (2013)
Antiparasitic	Thiabendazole	0	9.1	40	1	Klosterhaus et al. (2013)
Anxiolytic	Diazepam	0.20	0.64	35.5	2	Klosterhaus et al. (2013), Beretta et al. (2014)
	N.D. Meprobamate					Klosterhaus et al. (2013)
Bronchodilator	Salbutamol	0.25	0.5	/	2	Klosterhaus et al. (2013), Stewart et al. (2014)
Diuretic	Hydrochlorothiazide	0.2	1.1	11.1	2	Stewart et al. (2014), Lara-Martín et al. (2015)
	Triamterene	0.55	10.8	74.2	2	Klosterhaus et al. (2013), Long et al. (2013)
Hormone	Tamoxifen	2.8	11.2	77.8	1	Lara-Martín et al. (2015)
Lipid Lowering Agent	Bezafibrate	0.1	/	/	1	Stewart et al. (2014)
	Fenofibrate	1.6	/	/	1	Stewart et al. (2014)
	Gemofibrozil	0.85	4.2	50	2	Klosterhaus et al. (2013), Lara-Martín et al. (2015)
Metabolite	Anhydrochlorotetracycline	0	46.9	2.5	1	Long et al. (2013)
	Epitetracycline	0	6.06	2.5	1	Long et al. (2013)
	Erythromycin-H_2O	1.13	65.33	57.6	2	Klosterhaus et al. (2013), Chen et al. (2015)
	Norverapamil	0	0.17	2.6	1	Long et al. (2013)
	N.D. 10-Hydroxy-amitriptyline, Dehydronifedipine, Desmethyldiltiazem					Klosterhaus et al. (2013)

Table A1.2: *Continued*

Class	Compound	Median (ng l^{-1})	Maximum (ng l^{-1})	Detection Freq. (%)	Estuaries Sampled	Reference
Mucosal Protectant	Cimetidine	0.9	/	/	1	Stewart et al. (2014)
	Famotidine	0.7	0.7	/	1	Stewart et al. (2014)
	Ranitidine	0.01	1.2	/	3	Klosterhaus et al. (2013), Stewart et al. (2014), Cantwell et al. (2017)
NSAID	Diclofenac	0.67	1.5	63.7	3	Beretta et al. (2014), Stewart et al. (2014), Lara-Martín et al. (2015)
	Ibuprofen	1.1	21.7	50.6	4	Klosterhaus et al. (2013), Beretta et al. (2014), Stewart et al. (2014), Lara-Martín et al. (2015)
	Indomethacin	1.2	2.3	77.8	1	Lara-Martín et al. (2015)
	Mefenamic Acid	1.3	1.8	100	1	Lara-Martín et al. (2015)
	Naproxen	2.75	5.5	/	2	Klosterhaus et al. (2013), Stewart et al. (2014)
Pain Killer	Paracetamol	7.69	/	/	1	Stewart et al. (2014)
	Propoxyphene	0	1.74	1.3	2	Klosterhaus et al. (2013), Long et al. (2013)
		N.D.	Hydrocodone			Klosterhaus et al. (2013)
Stimulant	Amphetamine	0	3.3	26.3	2	Klosterhaus et al. (2013), Long et al. (2013)

A2 Supporting Information for Chapter 2

A2.1 Ranking of priority compounds

Table A2.1: Scores of priority compounds based on PECs, wastewater removal, logKOW and potential for toxicity

Score	Compound	Class
17	Fluoxetine	Antidepressant
18	Paracetamol	Pain Killer
19	Bezafibrate	Lipid Lowering Agent
	Citalopram	Antidepressant
	Ibuprofen	NSAID
20	Tamoxifen	Hormone
22	Amoxicillin	Antibiotic
	Atorvastatin	Lipid Lowering Agent
	Diclofenac	NSAID
23	Carbamazepine	Anticonvulsant
	Metformin Hydrochloride	Antidiabetic
24	Erythromycin	Antibiotic
	Flucloxacillin sodium	Antibiotic
	Ketoprofen	NSAID
	Mefenamic Acid	NSAID
	Sodium Valproate	Anticonvulsant
25	Atenolol	Antihypertensive
	Pregabalin	Anticonvulsant
	Ranitidine	Mucosal Protectant
	Trimethoprim	Antibiotic
26	Furosemide	Antihypertensive
	Pravastatin	Lipid Lowering Agent
	Simvastatin	Lipid Lowering Agent
	Valsartan	Antihypertensive
27	Cimetidine	Mucosal Protectant
	Codeine	Pain Killer
	Dextropropoxy-phene	Pain Killer
	Gabapentin	Anticonvulsant
	Lansoprazole	Mucosal Protectant
	Levothyroxine	Hormone
	Mirtazapine	Antidepressant
	Morphine	Pain Killer
	Naproxen	NSAID
	Sulfamethoxazole	Antibiotic
	Tramadol	Pain Killer
28	Bisoprolol	Antihypertensive
	Clotrimazole	Antifungal
	Diltiazem hydrochloride	Antihypertensive
	Lamotrigine	Anticonvulsant

Table A2.1: *Continued*

Score	Compound	Class
28	Mebeverine Hydrochloride	Antispasmodic
	Orlistat	Antidiabetic
	Propranolol	Antihypertensive
	Quetiapine	Anxiolytic
	Sertraline	Antidepressant
	Sotalol	Antihypertensive
29	Abiraterone Actetate	Chemotherapy Agent
	Amitriptyline	Antidepressant
	Aripiprazole	Antipsychotic
	Fluticasone Proprionate	Bronchodilator
	Lisinopril	Antihypertensive
	Mesalazine	Aminosalicylate
	Metronidazole	Antifungal
	Oxytetracycline	Antibiotic
	Phenoxymethyl Penicillin	Antibiotic
	Solifenacin succinate	Antispasmodic
	Sulfasalazine	Antirheumatic
30	Aflibercept	Chemotherapy Agent
	Bendroflumethiazide	Diuretic
	Etanercept	Chemotherapy Agent
	Gilclazide	Antidiabetic
	Imatinib mesilate	Chemotherapy Agent
	Metoprolol	Antihypertensive
	Olanzapine	Aminosalicylate
	Omeprazole	Mucosal Protectant
	Ramipril	Antihypertensive
	Rituximab	Chemotherapy Agent
31	Budesonide	Bronchodilator
	Sitagliptin	Lipid Lowering Agent
	Truvada	Antiretroviral
32	Cyclophosphamide	Chemotherapy Agent
	Enoxaparin Sodium	Blood Thinner
	Lenalidomide	Chemotherapy Drug
	Salbutamol	Bronchodilator
	Tiotropium Bromide	Bronchodilator
	Trastuzumab	Chemotherapy Agent
33	Allopurinol	Gout Treatment
34	Aspirin	NSAID
	Warafin Sodium	Blood Thinner
35	Amlopidine	Antihypertensive

A3 Supporting Information for Chapter 3

A3.1 Prescriptions of pharmaceuticals in 2014

Table A3.1: Amount (kg) of top 50 most prescribed drugs in 2014 by month

Compound	Type	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total (kg)
Alendronic Acid	Other	209.8	190.8	203.1	203.6	211.3	200.8	215.0	200.9	206.6	215.4	193.9	216.4	2467.8
Allopurinol	Other	3034.0	2740.9	2962.2	2982.2	3120.8	2959.7	3160.8	3152.8	3125.5	3262.3	2947.2	3351.7	36800.1
Amitriptyline	Antidepressant	932.9	837.9	905.8	897.5	941.6	893.5	952.9	891.2	932.5	972.9	883.0	999.2	11040.8
Amlodipine	Anti-anginal	490.9	445.8	485.3	480.8	501.5	473.9	506.3	474.2	491.4	512.6	468.2	530.9	5861.9
Amoxicillin	Antibiotic	14659.3	12994.4	13873.3	12500.2	11421.2	10237.3	9754.7	7931.3	10311.3	11625.7	13572.8	21758.3	150639.9
Atenolol	Beta-Blocker	1544.9	1378.9	1544.8	1471.1	1520.9	1420.1	1521.6	1413.8	1442.1	1489.5	1338.3	1512.8	17598.7
Atorvastatin	Lipid Regulating	1712.1	1568.0	1714.2	1728.6	1819.1	1740.4	1888.9	1787.4	1873.0	1978.4	1830.1	2114.6	21755.0
Beclomethasone	Corticosteroid	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	1.6
Bisoprolol	Beta-Blocker	177.6	161.5	174.4	175.9	184.1	175.0	189.2	178.4	184.3	193.1	175.9	201.0	2170.5
Budesonide	Corticosteroid	1.0	0.9	1.0	1.0	1.1	1.0	1.1	1.0	1.1	1.1	1.0	1.1	12.6
Candesartan	Anti-Hypertensive	199.4	181.1	200.6	203.6	214.6	202.7	217.7	204.4	210.0	218.9	199.5	227.6	2480.0
Citalopram	Antidepressant	766.7	684.6	737.6	723.0	757.2	724.1	768.3	711.2	749.7	778.3	712.2	797.7	8910.7
Codeine	Analgesic	4247.2	3716.1	4083.0	4183.2	4229.9	4063.4	4363.9	4065.8	4293.8	4473.4	4036.8	4556.4	50313.0
Diclofenac	NSAID	650.1	562.8	611.7	620.0	640.1	591.8	637.9	591.5	610.2	623.1	550.6	588.2	7278.0
Diltiazem	Anti-anginal	1633.4	1470.4	1580.0	1568.4	1579.8	1526.9	1207.7	1501.9	1569.7	1597.0	1476.7	1626.1	18338.1
Doxazosin	Anti-Hypertensive	87.4	79.2	85.3	84.9	88.6	83.5	89.2	83.4	85.8	89.1	80.9	92.1	1029.4
Ethinylestradiol	Hormone	0.5	1.3	0.5	0.5	0.4	0.5	0.4	0.3	0.4	0.3	0.2	0.4	5.8
Felodipine	Anti-anginal	70.6	67.9	68.7	67.5	70.1	65.9	70.6	66.0	67.5	70.2	59.3	72.6	816.9
Flucloxacillin	Antibiotic	4955.0	4585.8	5136.8	4985.2	5324.7	5773.1	6524.5	5482.7	5697.6	5414.1	4707.8	4826.1	63413.6
Fluoxetine	Antidepressant	475.0	427.0	462.1	454.3	472.0	455.5	484.3	449.7	474.1	492.7	452.1	508.5	5607.3
Fluticasone	Corticosteroid	0.4	0.3	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	5.5
Gabapentin	Anti-Epileptic	11635.5	10567.9	11529.6	11591.8	12143.4	11763.7	12766.4	12044.3	12677.0	13298.1	12153.1	14014.4	146185.2
Gliclazide	Antidiabetic	3110.6	2808.4	3031.4	3025.8	3142.0	2986.1	3189.1	2976.1	3075.8	3172.9	2892.5	3262.1	36672.7
Ibuprofen	NSAID	14725.8	9655.0	10647.3	9429.6	10393.1	9423.1	10467.3	9277.2	10200.3	9844.2	9697.6	10881.5	124641.9
Isosorbide	Anti-Anginal	532.1	469.2	510.2	477.3	522.7	494.7	529.2	489.1	519.4	530.0	481.0	526.2	6081.0
Lansoprazole	Mucosal Protectant	1443.4	1295.5	1401.2	1400.8	1417.2	1398.8	1498.4	1407.6	1470.6	1535.7	1396.6	1586.0	17251.8
Levothyroxine	Hormone	5.6	5.1	5.5	5.5	5.7	5.4	5.8	5.4	5.6	5.8	5.3	6.0	66.8
Lisinopril	Anti-Hypertensive	400.0	361.1	388.7	386.8	402.0	378.6	404.6	378.9	387.4	402.1	363.6	440.4	4694.3
Losartan	Anti-Hypertensive	1493.4	1361.1	1521.6	1560.6	1650.9	1571.0	1680.6	1572.9	1629.0	1685.8	1548.5	1751.1	19026.4
Metformin	Antidiabetic	82787.5	74458.1	81002.7	80278.5	84482.6	80586.8	86095.8	80791.7	84037.2	86507.7	79590.3	90153.8	990772.6

Table A3.1: *Continued*

Compound	Type	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total (kg)
Mirtazapine	Antidepressant	343.7	308.6	336.1	334.7	347.5	334.3	360.2	337.3	355.4	377.0	342.9	389.1	4166.8
Morphine	Analgesic	1124.9	623.1	674.4	680.3	698.8	676.8	733.1	626.0	720.2	838.6	686.4	788.2	8870.8
Naproxen	NSAID	13335.7	11887.6	13137.5	12831.1	13532.2	13219.9	13993.3	12944.7	13851.2	14212.7	12783.6	13618.1	159347.7
Omeprazole	Mucosal Protectant	1804.9	1599.1	1751.7	1742.6	1825.9	1743.3	1862.9	1750.0	1827.8	1910.7	1741.0	1971.8	21531.7
Paracetamol	Analgesic	190602.7	166213.8	185165.9	175841.1	190619.2	180058.6	195087.1	179776.2	190258.0	199765.9	181444.3	206790.1	2241622.9
Perindopril	Anti-Hypertensive	78.1	70.5	75.8	75.5	78.7	73.9	79.0	73.9	76.2	78.5	71.5	81.0	912.5
Prednisolone	Corticosteroid	144.1	116.3	120.6	121.3	121.8	115.1	118.9	107.4	118.6	130.7	121.5	159.1	1495.5
Pregabalin	Anti-Epileptic	2151.9	1959.5	2134.5	2163.2	2263.2	2198.7	2382.0	2250.2	2360.5	2494.4	2280.1	2613.0	27251.2
Propranolol	Anti-Hypertensive	787.3	707.3	773.4	771.5	815.3	781.0	843.1	788.0	840.0	873.4	754.7	894.7	9629.6
Ramipril	Anti-Hypertensive	471.0	426.6	461.1	460.1	479.7	452.7	487.2	456.0	469.3	488.1	444.4	503.8	5600.1
Ranitidine	Mucosal Protectant	3277.9	2948.6	3180.6	3164.6	3291.3	3165.5	3385.4	3166.2	3314.7	3490.4	3193.9	3622.8	39201.9
Salbutamol	Bronchodilator	13.6	12.0	12.7	12.8	13.2	12.7	13.3	12.0	12.5	13.3	12.2	14.7	155.0
Sertraline	Antidepressant	1414.0	1280.2	1408.8	1401.5	1476.6	1440.8	1554.2	1457.3	1561.7	1650.0	1524.0	1733.9	17902.9
Simvastatin	Lipid Regulating	3491.7	3126.7	3340.7	3312.8	3419.1	3199.3	3414.5	3174.6	3247.7	3348.8	3009.4	3384.2	39469.5
Tamsulosin	Genito-Urinary	6.1	5.5	6.0	6.0	6.3	6.4	6.5	6.1	6.3	6.7	6.1	6.9	74.9
Tiotropium	Bronchodilator	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.3	2.9
Tramadol	Analgesic	3728.7	3381.2	3663.9	3649.6	4233.9	3436.8	4046.9	3280.0	3377.5	3570.2	3539.3	3578.8	43486.7
Trimethoprim	Antibiotic	971.3	849.3	910.5	876.7	916.5	885.0	919.6	842.6	932.8	957.8	879.8	923.6	10865.5
Venlafaxine	Antidepressant	990.4	902.8	950.9	955.4	994.9	942.3	1107.4	968.5	1007.2	1044.9	947.8	1084.2	11896.8
Warfarin Sodium	Blood Thinner	102.8	93.4	99.9	100.5	104.2	100.2	107.2	100.4	103.1	78.9	97.5	112.9	1201.2

A3.2 Excretion and removal rates of pharmaceuticals

Table 3.2 Highest excretion rate (%) and lowest removal rate (%) used in PEC calculations

Compound	Highest Excretion Rate (%)	Reference	Lowest Removal Rate (%)	Reference
Alendronic Acid	95	Compendium	0	
Allopurinol	20	Drugbank	64	Bound and Vousvouslis (2006)
Amitriptyline	5	Compendium	63	Wu et al. (2015)
Amlodipine	10	Compendium	54	Jelic et al. (2015)
Amoxicillin	90	Hirsch et al. (1999)	0	Santos et al. (2013)
Atenolol	90	Compendium	20.2	Chen et al. (2011)
Atorvastatin	2	Drugbank	0	
Beclomethasone	10	Drugbank	42	Radjenovic et al. (2007)
Bisoprolol	52	Drugbank	24	Radjenovic et al. (2007)
Budesonide	1	Drugbank	0	
Candesartan	56	Compendium	negative	Jelic et al. (2015)
Citalopram	33	Drugbank	negative	Kasprzyk-Hodern et al. (2009)
Codeine	16	Pubchem	negative	Jelic et al. (2015)
Diclofenac	65	Drugbank	70	Matsuo et al. (2011)
Diltiazem	64	Compendium	negative	Jelic et al. (2015)
Doxazosin	4.8	Drugbank	7	Jelic et al. (2015)
Ethinylestradiol	59	Anderson et al. (2002)	0	
Felodipine	0.5	Compendium	40	Gros et al. (2010)
Flucloxacillin	76.1	Compendium	0	Gurke et al. (20150
Fluoxetine	10	Pharmgkb	negative	Jelic et al. (2015)

Table 3.2: *Continued*

Compound	Highest Excretion Rate (%)	Reference	Lowest Removal Rate (%)	Reference
Fluticasone	1	Compendium	0	
Gabapentin	100	Compendium	negative	Gurke et al. (20150
Gliclazide	1	Compendium	2.3	Ivanova et al. (2017)
Ibuprofen	50	Compendium	38	Kasprzyk-Hodern et al. (2009)
Isosorbide	2	Drugbank	0	
Lansoprazole	1	Drugbank	0	
Levothyroxine	70	Drugbank	0	
Lisinopril	100	Drugbank	0	
Losartan	95	Drugbank	negative	Jelic et al. (2015)
Metformin	100	Compendium	negative	Jelic et al. (2015)
Mirtazapine	75	Drugbank	0	Santos et al. (2013)
Morphine	10	Drugbank	3	Gurke et al. (2015)
Naproxen	95	Compendium	12	Jelic et al. (2015)
Omeprazole	23	Mansour et al. (2016)	0	
Paracetamol	4	Mansour et al. (2016)	negative	Kasprzyk-Hodern et al. (2009)
Perindopril	12	Drugbank	72.8	Baker and Kasprzyk-Hordern (2013)
Prednisolone	80	Habet and Rogers (1989)	negative	Jelic et al. (2015)
Pregabalin	90	Drugbank	negative	Jelic et al. (2015)
Propranolol	1	Pubchem	0	Santos et al. (2013)

Table 3.2: *Continued*

Compound	Highest Excretion Rate (%)	Reference	Lowest Removal Rate (%)	Reference
Ramipril	1.9	Verho et al. (1995)	0	Santos et al. (2013)
Ranitidine	35	Compendium	0	
Salbutamol	28	Drugbank	8.5	Rosal et al. (2010)
Sertraline	0.2	Drugbank	90	Matsuo et al. (2011)
Simvastatin	13	Drugbank	73.6	Sulaiman et al. (2015)
Tamsulosin	10	Drugbank	0	
Tiotropium	74	Drugbank	98.5	Wang et al. (2016)
Tramadol	30	Drugbank	50	Gurke et al. (2015)
Trimethoprim	48	Drugbank	0	Roberts and Thomas (2006)
Venlafaxine	5	Drugbank	0	Gurke et al (2015)
Warfarin	1	Drugbank	81	Golovko et al. (2014)

Table A3.3: Ranking and results of predicted environmental concentrations (PEC; ngl^{-1}), maximum measured environmental concentrations (MEC; $ng\ l^{-1}$) and mean MECs for each of the study compounds in Chapter 3

	PEC_A		PEC_B		PEC_C		PEC_D		MEC	
Compound	**Value**	**Ranking**	**Value**	**Ranking**	**Value**	**Ranking**	**Value**	**Ranking**	**Maximum**	**Mean**
Alendronic Acid	60.0	23.0	63.2	35.0	60.0	22.0	47.5	23.0		
Allopurinol	188.5	15.0	942.3	9.0	188.5	14.0	900.0	10.0		
Amitriptyline	14.1	31.0	104.6	30.0	5.2	31.0	50.0	22.0	71.9	2.6
Amlodipine	15.0	29.0	69.0	33.0	6.9	29.0	4.0	37.0		
Amoxicillin	3471.4	4.0	3078.0	5.0	2770.2	4.0	16740.0	2.0	622.0	125.5
Atenolol	405.6	10.0	450.6	15.0	405.6	8.0	522.0	12.0	560.0	83.0
Atorvastatin	11.1	32.0	557.0	12.0	11.1	27.0	8.0	35.0		
Beclomethasone	0.0	48.0	0.0	49.0	0.0	49.0	0.8	42.0		
Bisoprolol	28.9	26.0	55.6	37.0	28.9	25.0	52.0	21.0		
Budesonide	0.0	49.0	0.3	46.0	0.0	48.0	0.1	49.0		
Candesartan	35.6	24.0	63.5	34.0	35.6	23.0	44.8	25.0		
Citalopram	75.3	22.0	222.9	24.0	73.6	21.0	46.5	24.0	71.4	9.0
Codeine	206.1	14.0	798.7	11.0	127.8	16.0	19.2	28.0	815.0	98.9
Diclofenac	112.0	20.0	172.2	25.0	112.0	19.0	487.5	13.0	2991.0	51.4
Diltiazem	300.5	13.0	413.2	17.0	264.4	12.0	1536.0	8.0	83.6	90.5
Doxazosin	1.3	39.0	26.4	39.0	1.3	38.0	1.2	40.0		
Ethinylestradiol	0.1	46.0	0.1	50.0	0.0	47.0	0.1	48.0	10.2	0.2
Felodipine	0.1	45.0	20.9	41.0	0.1	45.0	0.5	45.0		
Flucloxacillin	1235.6	7.0	941.7	10.0	716.7	6.0	15220.0	3.0		
Fluoxetine	14.4	30.0	109.1	29.0	10.9	28.0	29.1	26.0	13.5	1.1
Fluticasone	0.0	50.0	0.1	47.0	0.0	50.0	0.2	47.0	<LOD	<LOD
Gabapentin	3743.1	3.0	3743.1	4.0	3743.1	3.0	18000.0	1.0	1887.0	388.4
Gliclazide	9.4	33.0	281.7	20.0	2.8	34.0	16.0	30.0		
Ibuprofen	1595.7	6.0	2968.0	6.0	1484.0	5.0	1584.0	7.0	4838.0	193.0
Isosorbide	3.1	35.0	155.7	26.0	3.1	33.0	12.0	33.0		
Lansoprazole	4.4	34.0	441.7	16.0	4.4	32.0	3.0	38.0		
Levothyroxine	1.2	40.0	1.7	44.0	1.2	39.0	0.7	43.0		

Table A3.3: *Continued*

	PEC_A		PEC_B		PEC_C		PEC_D		MEC	
Compound	Value	Ranking	Value	Ranking	Value	Ranking	Value	Ranking	Maximum	Mean
Lisinopril	120.2	19.0	120.2	28.0	120.2	17.0	304.0	15.0		
Losartan	462.8	9.0	487.2	14.0	462.8	7.0	327.8	14.0		
Metformin	25368.6	1.0	253.7	1.0	25368.6	1.0	2720.0	5.0	2595.0	530.1
Mirtazapine	80.0	21.0	103.5	31.0	77.6	20.0	62.4	19.0		
Morphine	22.7	27.0	61.8	36.0	6.2	30.0	90.0	18.0		
Naproxen	3876.1	2.0	4080.1	3.0	3876.1	2.0	5937.5	4.0	683.0	36.3
Omeprazole	126.8	18.0	504.5	13.0	116.0	18.0	13.8	32.0		
Paracetamol	2295.9	5.0	5739.7	2.0	229.6	13.0	800.0	11.0	9822.0	303.0
Perindopril	2.8	36.0	23.4	40.0	2.8	35.0	4.8	36.0		
Prednisolone	30.6	25.0	0.6	45.0	0.5	41.0	160.0	17.0		
Pregabalin	628.0	8.0	348.9	18.0	314.0	11.0	2700.0	6.0		
Propranolol	2.5	38.0	246.6	23.0	2.5	37.0	16.0	29.0	165.0	18.4
Ramipril	2.7	37.0	143.4	27.0	2.7	36.0	1.0	41.0		
Ranitidine	351.3	11.0	1003.8	8.0	351.3	9.0	15.8	31.0	180.0	16.7
Salbutamol	1.1	41.0	4.0	42.0	1.1	40.0	28.0	27.0	268.0	4.3
Sertraline	0.9	42.0	87.1	32.0	0.2	44.0	2.0	39.0	<LOD	<LOD
Simvastatin	131.38	17	266.80	22	34.68	24	9.88	34		
Tamsulosin	0.19	44	1.92	43	0.19	43	0.20	46		
Tiotropium	0.06	47	0.07	48	0.06	46	0.07	50		
Tramadol	334.04	12	1113.47	7	334.04	10	216.00	16	7731	654.97381
Trimethoprim	133.54	16	278.21	21	133.54	15	1440.00	9	427	34.724359
Venlafaxine	15.23	28	304.62	19	15.23	26	56.25	20	85.4	12.197778
Warfarin Sodium	0.31	43	30.76	38	0.31	42	0.50	44	<LOD	<LOD

A3.4 Information used in the calculation of effect criteria

Table A3.4: Cmax, Highest LogP and lowest LC50 values for each of the study compounds in Chapter 3. NA - not available

Compound	cmax (ng ml^{-1})	Reference	Highest LogP	Lowest LC50 (mg l^{-1})	Species	Reference
Alendronic	38.47	Yun et al. (2006)	-4.3	0.50	Algae	Sanderson and Thomsen (2009)
Allopurinol	1697	MHRA	-0.55	0.45	Algae	British Pharmacoepoeia MSDS
Amitriptyline	75	Kostisch and Lazorchak (2008)	4.92	0.16	Algae	Sanderson and Thomsen (2009)
Amlodipine	2600	Kostisch and Lazorchak (2008)	3	75.00	Algae	Pfizer MSDS
Amoxicillin	13800	FDA	0.87	2.20	Daphnia	Sanderson and Thomsen (2009)
Atenolol	320	Schreiber et al. (2011)	0.16	33.40	Algae	Sanderson and Thomsen (2009)
Atorvastatin	3.2	Kostisch and Lazorchak (2008)	5.7	97.00	Algae	Vestel et al. (2016)
Beclomethasone	0.09	FDA	1.3	3.80	Daphnia	ECOSAR
Bisoprolol	9.45	Schreiber et al. (2011)	1.87	90.00	Algae	British Pharmacoepoeia MSDS
Budesonide	1.8	Dilger et al. (2005)	1.9	8.50	Algae	Vestel et al. (2016)
Candesartan	37.53	MHRA	6.1	32.00	Daphnia	Astrazeneca
Citalopram	21.2	Schreiber et al. (2011)	3.5	3.90	Daphnia	Sanderson and Thomsen (2009)
Codeine	142	Kostisch and Lazorchak (2008)	1.19	1.00	Daphnia	ECOSAR
Diclofenac	583	Schreiber et al. (2011)	4.51	22.40	Daphnia	Sanderson and Thomsen (2009)
Diltiazem	174	Kostisch and Lazorchak (2008)	2.8	29.02	Mysid Shrimp	Sangion and Gramatica (2016)
Doxazosin	25.8	FDA	2.1	3.80	Algae	Pfizer MSDS
Ethinylestradiol	0.05	Schreiber et al. (2011)	3.67	0.84	O. mykiss	Sanderson and Thomsen (2009)
Felodipine	27.6	Blychert et al. (1991)	3.86	0.05	Algae	Astrazeneca
Flucloxacillin	16.7	MHRA	2.58	5.60	Algae	ECOSAR
Fluoxetine	10	Kostisch and Lazorchak (2008)	4.05	0.02	Daphnia	Sanderson and Thomsen (2009)
Fluticasone	0.13	Schreiber et al. (2011)	3.4	>0.55	O. mykiss	GSK MSDS
Gabapentin	3387	MHRA	-1.1	50.00	Daphnia	Pfizer MSDS
Gliclazide	2200	Sarkar et al. (2011)	2.6	>150	Algae	Markiewicz et al. (2017)
Ibuprofen	29.99	MHRA	3.97	7.10	Daphnia	Sanderson and Thomsen (2009)
Isosorbide	545.81	MHRA	-0.15	>120	Fish	Astrazeneca
Lansoprazole	909	MHRA	1.9	18.00	Algae	Sanderson and Thomsen (2009)

Table A3.4: *Continued*

Compound	cmax (ng ml^{-1})	Reference	Highest LogP	Lowest LC50 (mg l^{-1})	Species	Reference
Levothyroxine	742	MHRA	4	4.65	Daphnia	ECOSAR
Lisinopril	30.7	Neubeck et al. (1994)	-1.01	5633.49	Algae	ECOSAR
Losartan	201	MHRA	6.1	245.00	Daphnia	Sanderson and Thomsen (2009)
Metformin	300	Kostisch and Lazorchak (2008)	-0.5	64.00	Algae	Vestel et al. (2016)
Mirtazapine	56	MHRA	2.9	3.09	O. mykiss	British Pharmacoepoeia MSDS
Morphine	14.5	Kostisch and Lazorchak (2008)	0.89	3.60	Daphnia	Pfizer MSDS
Naproxen	58.5	MHRA	3.18	43.50	Daphnia	Sanderson and Thomsen (2009)
Omeprazole	276	Mostafavi and Tavakoli (2004)	2.23	88.00	Daphnia	Sanderson and Thomsen (2009)
Paracetamol	10000	Kostisch and Lazorchak (2008)	0.46	9.20	Daphnia	Sanderson and Thomsen (2009)
Perindopril	30	MHRA	2.6	NA*		
Prednisolone	100	Kostisch and Lazorchak (2008)	1.6	31.00	Algae	Sanderson and Thomsen (2009)
Pregabalin	7453	EMA	-1.35	>300	Daphnia	Vestrel
Propranolol	10	Kostisch and Lazorchak (2008)	3.48	2.70	Daphnia	Sanderson and Thomsen (2009)
Ramipril	16	MHRA	2.9	>100	Algae	Astrazeneca
Ranitidine	450	Kostisch and Lazorchak (2008)	0.27	167.00	Daphnia	GSK MSDS
Salbutamol	801	Jiang et al. (2016)	1.4	243.00	Daphnia	Vestrel
Sertraline	10	Kostisch and Lazorchak (2008)	5.1	0.12	Daphnia	Sanderson and Thomsen (2009)
Simvastatin	3.7	MHRA	4.68	5.90	Daphnia	British Pharmacoepoeia MSDS
Tamsulosin	10	FDA	2.3	37.90	Daphnia	GSK MSDS
Tiotropium	0.635	FDA	-2.2	NA*		
Tramadol	125	Kostisch and Lazorchak (2008)	2.4	73.00	Algae	Sanderson and Thomsen (2009)
Trimethoprim	1000	Kostisch and Lazorchak (2008)	0.91	110.00	Algae	Sanderson and Thomsen (2009)
Venlafaxine	37.5	Kostisch and Lazorchak (2008)	3.2	4.80	Daphnia	Pfizer MSDS
Warfarin Sodium	250	Kostisch and Lazorchak (2008)	2.7	17.00	Daphnia	Sanderson and Thomsen (2009)

Table A3.5: Ranking and results of fish plasma model (FPM) using PECa, PECb, PECc and PECd and of critical environmental concentrations (CEC)each of the study compounds in Chapter 3

	FPM_A		FPM_B		FPM_C		FPM_D		CEC	
Compound	**Value**	**Ranking**	**Value**	**Ranking**	**Value**	**Ranking**	**Value**	**Ranking**	**Value**	**Ranking**
Alendronic Acid	6695.14605	50	6360.38874	50	6695.14605	50	8461.13407	50	401903.86833	49
Allopurinol	172.17859	44	34.43572	44	172.17859	44	36.05283	42	32447.54492	46
Amitriptyline	0.01031	11	0.00139	10	0.02786	12	0.00291	9	0.14570	10
Amlodipine	8.48418	35	1.84439	33	18.44387	36	31.91541	41	127.34249	33
Amoxicillin	6.98672	33	7.87976	40	8.75528	33	1.44885	28	24253.76059	45
Atenolol	4.57407	30	4.11667	37	4.57407	29	3.55369	30	1855.02590	41
Atorvastatin	0.00015	3	0.00000	1	0.00015	2	0.00021	4	0.00168	2
Beclomethasone	18.42636	39	3.07106	35	30.71060	37	0.09384	21	0.07507	8
Bisoprolol	0.10702	19	0.05565	22	0.10702	19	0.05947	19	3.09266	23
Budesonide	173.35711	45	1.73357	32	173.35711	45	5.60109	34	0.56011	15
Candesartan	0.00028	5	0.00016	5	0.00028	4	0.00022	5	0.01003	4
Citalopram	0.00595	9	0.00201	12	0.00609	8	0.00964	14	0.44806	14
Codeine	0.70707	23	0.18247	25	1.14044	24	7.59079	35	145.74321	34
Diclofenac	0.02015	13	0.01310	15	0.02015	10	0.00463	10	2.25615	21
Diltiazem	0.03969	14	0.02887	20	0.04510	14	0.00777	13	11.92750	27
Doxazosin	4.53392	29	0.21763	26	4.53392	28	4.81867	33	5.73614	25
Ethinylestradiol	0.00983	10	0.01611	18	0.02730	11	0.00581	11	0.00086	1
Felodipine	3.04535	28	0.01523	17	3.04535	26	0.69617	25	0.31850	13
Flucloxacillin	0.00134	7	0.00176	11	0.00231	7	0.00011	1	1.65698	19
Fluoxetine	0.00584	8	0.00077	8	0.00768	9	0.00288	8	0.08385	9
Fluticasone	2.22844	27	0.02228	19	2.22844	25	0.01612	16	0.00315	3
Gabapentin	43.61024	41	43.61024	45	43.61024	38	9.06865	37	163235.71906	48
Gliclazide	22.47792	40	0.74926	30	74.92639	41	13.19176	39	211.06814	36
Ibuprofen	0.00018	4	0.00010	3	0.00019	3	0.00018	3	0.28766	12
Isosorbide	1710.86422	48	34.21728	43	1710.86422	47	443.97676	48	5327.72118	44
Lansoprazole	64.03338	42	0.64033	28	64.03338	39	94.28501	45	282.85502	37
Levothyroxine	5.65267	31	3.95687	36	5.65267	30	9.66731	38	6.76712	26
Lisinopril	10.58136	36	10.58136	41	10.58136	34	4.18374	32	1271.85727	39

Table A3.5: *Continued*

	FPM_A		FPM_B		FPM_C		FPM_D		CEC	
Compound	Value	Ranking	Value	Ranking	Value	Ranking	Value	Ranking	Value	Ranking
Losartan	0.00012	2	0.00011	4	0.00012	1	0.00016	2	0.05373	7
Metformin	0.20789	20	20.78854	42	0.20789	20	1.93889	29	5273.77084	43
Mirtazapine	0.04055	15	0.03135	21	0.04180	13	0.05197	18	3.24480	24
Morphine	1.08488	25	0.39885	27	3.98855	27	0.27379	22	24.64155	30
Naproxen	0.00055	6	0.00052	7	0.00055	6	0.00036	6	2.11717	20
Omeprazole	0.38894	22	0.09777	23	0.42507	22	3.57380	31	49.31841	31
Paracetamol	15.24945	38	6.09978	39	152.49447	43	43.76329	44	35010.63598	47
Perindopril	1.02657	24	0.12319	24	1.02657	23	0.59963	24	2.87820	22
Prednisolone	1.68185	26	89.69852	46	112.12315	42	0.32202	23	51.52286	32
Pregabalin	870.72151	47	1567.29871	48	1741.44301	48	202.51974	47	546803.29286	50
Propranolol	0.08865	18	0.00089	9	0.08865	18	0.01366	15	0.21857	11
Ramipril	0.34029	21	0.00647	14	0.34029	21	0.97588	26	0.92709	16
Ranitidine	6.17183	32	2.16014	34	6.17183	31	137.66767	46	2168.26577	42
Salbutamol	519.67288	46	145.50841	47	519.67288	46	20.62882	40	577.60709	38
Sertraline	0.01566	12	0.00016	6	0.08241	16	0.00718	12	0.01435	6
Simvastatin	0.00008	1	0.00004	2	0.00031	5	0.00109	7	0.01076	5
Tamsulosin	8.28373	34	0.82837	31	8.28373	32	7.94273	36	1.58855	18
Tiotropium	3520.27244	49	2605.00161	49	3520.27244	49	2919.43961	49	194.43468	35
Tramadol	0.05025	16	0.01507	16	0.05025	15	0.07771	20	16.78456	28
Trimethoprim	12.30513	37	5.90646	38	12.30513	35	1.14114	27	1643.23622	40
Venlafaxine	0.08616	17	0.00431	13	0.08616	17	0.02333	17	1.31229	17
Warfarin Sodium	65.91758	43	0.65918	29	65.91758	40	40.54805	43	20.27403	29

Table A3.6: Compounds exceeding (denoted by "x") FPM Threshold Value of 1000 and RQ value of 1 for each of the PEC schemes.

	FPM > 1000				RQ >1					FPM > 1000				RQ >1			
Compound	**PEC_A**	**PEC_B**	**PEC_C**	**PEC_D**	**PEC_A**	**PEC_B**	**PEC_C**	**PEC_D**	**Compound**	**PEC_A**	**PEC_B**	**PEC_C**	**PEC_D**	**PEC_A**	**PEC_B**	**PEC_C**	**PEC_D**
Alendronic									Lansoprazole	x	x	x	x				
Allopurinol	x	x	x	x		x		x	Levothyroxine	x	x	x	x				
Amitriptyline	x	x	x	x					Lisinopril	x	x	x	x				
Amlodipine	x	x	x	x					Losartan	x	x	x	x				
Amoxicillin	x	x	x	x	x	x	x	x	Metformin	x	x	x	x				
Atenolol	x	x	x	x					Mirtazapine	x	x	x	x				
Atorvastatin	x	x	x	x					Morphine	x	x	x	x				
Beclomethasone	x	x	x	x					Naproxen	x	x	x	x				
Bisoprolol	x	x	x	x					Omeprazole	x	x	x	x				
Budesonide	x	x	x	x					Paracetamol	x	x	x	x				
Candesartan	x	x	x	x					Perindopril	x	x	x	x				
Citalopram	x	x	x	x					Prednisolone	x	x	x	x				
Codeine	x	x	x	x					Pregabalin	x			x				
Diclofenac	x	x	x	x					Propranolol	x	x	x	x				
Diltiazem	x	x	x	x					Ramipril	x	x	x	x				
Doxazosin	x	x	x	x					Ranitidine	x	x	x	x				
Ethinylestradiol	x	x	x	x					Salbutamol	x	x	x	x				
Felodipine	x	x	x	x					Sertraline	x	x	x	x				
Flucloxacillin	x	x	x	x				x	Simvastatin	x	x	x	x				
Fluoxetine	x	x	x	x		x			Tamsulosin	x	x	x	x				
Fluticasone	x	x	x	x					Tiotropium								
Gabapentin	x	x	x	x					Tramadol	x	x	x	x				
Gliclazide	x	x	x	x					Trimethoprim	x	x	x	x				
Ibuprofen	x	x	x	x					Venlafaxine	x	x	x	x				
Isosorbide		x		x					Warfarin Sodium	x	x	x	x				

A4 Supporting Information for Chapter 4

A4.1 Humber Estuary site sampling information

Table A4.1 pH, dissolved oxygen, temperature and salinity taken from each of the sites at each sampling period. Asterik denotes sites where measurements could not be taken

Site	Date	Time	Latitude	Longitude	pH	Dissolved Oxygen (%)	Temperature (°C)	Salinity (ppt)
A1	10/10/2016	14:21	54° 43' 42.02" N	0° 53' 14.62" W	7.99	*	12.7	0
	20/02/2017	14:51			8.21	88.4	10	0
	19/06/2017	13:57			8.34	86.5	25.7	0
A2	10/10/2016	13:10	53° 42' 25.29" N	0° 43' 19.12" W	8.04	*	12.6	0
	20/02/2017	14:15			8.1	90.5	8.5	0
	19/06/2017	12:48			8.14	78.2	22.9	0
R1	10/10/2016	12:33	53° 42' 36.00" N	0° 31' 38.44" W	8.07	*	13.5	8
	06/12/2017	12:09			8.03	75.9	6.9	8
	20/02/2017	13:37			7.9	85.4	9.5	1
	18/04/2017	11:27			7.5	72.8	10.9	15
	19/06/2017	11:48			8.27	59.7	23	3
	07/08/2017	12:17			8.18	92.8	17.7	6
R2	10/10/2016	11:46	53° 42' 48.39" N	0° 30' 25.37" W	8.13	*	13.1	10
	06/12/2017	11:27			7.93	81.1	5.2	10
	20/02/2017	12:57			7.9	91.8	8.7	4
	18/04/2017	10:56			8	85.3	11.5	11
	19/06/2017	11:23			8.36	85.2	25.3	5
	07/08/2017	11:52			8.35	82.7	17.2	6
R3	10/10/2016	11:12	53° 43' 00.95" N	0° 27' 49.25" W	8.22	*	13.4	11
	06/12/2017	10:50			7.89	79.7	4.8	12
	20/02/2017	12:33			7.94	949	8.7	4
	18/04/2017	10:11			7.7	82.6	11	14
	19/06/2017	10:41			8.41	88.3	26.7	5
	07/08/2017	11:06			8.7	104.5	18.4	10
R4	10/10/2016	10:45	53° 42' 58.76" N	0° 26' 02.72" W	8.11	*	13.1	15
	06/12/2017	10:09			7.82	76.6	5.3	12
	20/02/2017	11:15			7.55	88.3	8.4	10
	18/04/2017	09:35			7.7	85.4	9.6	11
	19/06/2017	10:17			8.33	89.5	22.7	10
	07/08/2017	10:35			8.4	85.3	19.9	11
A3	20/02/2017	12:38	53° 43' 21.73" N	0° 14' 05.70" W	8.93	117.4	10.2	10
	19/06/2017	13:50			8.5	118.9	28	18
A4	10/10/2016	12:20	53° 42' 33.81" N	0° 13' 39.54" W	8.02	91.1	13.4	24
	20/02/2017	12:17			8.57	98	11.67	10
	19/06/2017	13:30			8.25	107.5	23.2	21
A5	10/10/2016	11:30	53° 39' 00.25" N	0° 08' 01.94" W	8.06	92.3	12.5	27
	20/02/2017	11:05			8.29	85.9	10.2	10
	19/06/2017	12:05			8.55	97.2	26.1	22

Table A4.2: Locations of sampling sites for UK wide monitoring. pH, temperature and dissolved oxygen were taken at each site. Samples were only taken from two sites at the Severn. Asterik denotes locations where measurements could not be taken.

		DO (%)	Temp	Salinity	pH	Date	Time	Latitude	Longitude	Distance from closest WWTP (km)
Ythan	**Upper**	119.5	17.4	6	7	25/08/2017	11:30	57° 20' 41.52552" N	1° 59' 34.47798" W	5
	Middle	109.2	17.4	10	7.1	25/08/2017	12:20	57° 19' 18.90304" N	1° 59' 40.4416" W	7.5
	Lower	103.7	17.6	35	7.3	25/08/2017	13:25	57° 18' 37.17522" N	1° 59' 32.09089q W	9
Cromarty	**Upper**	101.9	17.1	17	7.7	27/08/2017	12:40	57° 38' 23.0463" N	4° 20' 55.8132" W	<1
	Middle	109.6	18.6	31	7.2	27/08/2017	13:50	57° 41' 23.38559" N	4° 10' 59.40919" W	1.5
	Lower	125.3	17.4	35	8.1	27/08/2017	15:10	57° 40' 50.87993" N	4° 1' 48.84917" W	11
Tay	**Upper**	92.2	17.6	0	*	28/08/2017	11:49	56° 21' 8.75333" N	3° 14' 47.12222" W	<1
	Middle	93	17	7	*	28/08/2017	09:46	56° 27' 4.50598" N	3° 4' 31.86329" W	14.5
	Lower	98.7	15.8	27	7.5	28/08/2017	08:40	56° 28' 44.531" N	2° 49' 9.101" W	29
Forth	**Upper**	93.2	20.1	25	*	28/08/2017	20:17	56° 1' 11.40114" N	3° 36' 27.9382" W	<1
	Middle	111.9	17.7	31	*	28/08/2017	18:41	56° 3' 5.55651" N	3° 17' 47.9945" W	17
	Lower	100.5	17.9	21	*	28/08/2017	19:49	56° 11' 47.2654" N	2° 59' 21.92132" W	1.5
Severn	**Middle**	96.9	20.5	30	8	02/09/2017	16:50	51° 29' 23.9091 N	2° 46' 31.36757 W	4.5
	Lower	106.4	22.8	30	8.1	02/09/2017	14:35	51° 21' 10.87643" N	2° 59' 18.02918" W	5
Tyne	**Upper**	90.89	18.3	19	7.8	09/09/2017	17:38	54° 59' 6.94597" N	1° 29' 8.87698" W	<1
	Middle	96.3	16.2	30	7.9	09/09/2017	16:17	55° 0' 23.07852" N	1° 25' 42.39095" W	4.5
	Lower	96.9	14.9	34	7.8	09/09/2017	14:49	55° 0' 40.14806" N	1° 25' 55.84051"W	5
Mersey	**Upper**	89.2	15.7	22	7.7	16/09/2017	09:33	53° 19' 37.62426" N	2° 57' 12.00625" W	<1
	Middle	88.3	14.9	24	7.7	16/09/2017	08:30	53° 22' 1.481" N	2° 59' 39.851" W	4
	Lower	93.5	16.2	30	7.6	16/09/2017	07:36	53° 26' 4.47324" N	3° 2' 4.20162" W	1.5
Solway	**Upper**	9.27	14.1	4	7.8	17/09/2017	11:30	54° 56' 58.2202" N	3° 11' 18.573" W	5.5
	Middle	96.3	13.8	9	7.9	17/09/2017	10:45	54° 57' 8.587" N	3° 13' 9.414" W	2.5
	Lower	91.6	12.7	25	7.8	17/09/2017	09:38	54° 52' 2.859" N	3° 23' 20.832" W	12.5
Tees	**Upper**	*	*	35	*	23/09/2017	13:17	54° 37 57.41976" N	1° 10' 32.62998" W	1.5
	Middle	*	*	34	*	23/09/2017	13:48	54° 38' 9.74126" N	1° 9' 30.14204" W	2.5
	Lower	*	*	35	*	23/09/2017	14:03	54° 38' 24.44939" N	1° 9' 51.68286	3

Table A4.2: *Continued*

		DO (%)	Temp	Salinity	pH	Date	Time	Latitude	Longitude	Distance from closest WWTP (km)
Thames	**Upper**	141.37	14.8	30	8.2	30/09/2017	09:10	51° 30' 38.9038" N	0° 33' 11.05682" E	2.5
	Middle	153.8	16.7	30	8.5	30/09/2017	12:00	51° 32' 19.52167" N	0° 39' 40.59515" E	6
	Lower	111.8	17.8	25	8.1	30/09/2017	12:55	51° 31' 51.14078" N	0° 43' 33.4879" E	10.5
Portsmouth	**Site 1**	100.1	16	25	8	01/10/2017	10:15	50° 50' 42.68094" N	1° 6' 10.28707" W	11.5
	Site 2	119.6	16.3	30	8.1	01/10/2017	10:55	50° 50' 29.47186" N	1° 8' 44.43662" W	7.5
	Site 3	156.9	17.6	25	8.4	01/10/2017	12:02	50° 48' 46.434" N	1° 8' 1.571" W	10.5

A4.3 2016 prescription information

Table A4.3: Amount (kg) of compound prescribed in the UK per month from October 2016 - September 2017. Information was calculated from National Health Service (2019).

Compound	Oct-16	Nov-16	Dec-16	Jan-17	Feb-17	Mar-17	Apr-17	May-17	Jun-17	Jul-17	Aug-17	Sep-17	Total
Citalopram	759.9	783.5	797.6	763.1	710.2	813.0	716.4	790.5	784.0	765.6	763.3	757.0	9204.0
Diclofenac	472.3	478.8	477.5	449.5	406.9	485.4	428.0	463.0	462.0	444.9	449.1	442.6	5460.0
Ibuprofen	7513.9	7856.4	7723.1	7330.6	6649.0	7568.9	6240.7	6746.4	6482.1	6314.6	6163.0	6167.7	82756.4
Paracetamol	183766.9	189974.6	196122.2	183211.7	153487.8	193153.1	170049.6	183850.4	183749.8	176850.2	179018.5	176008.7	2169243.6
Trimethoprim	794.4	805.0	793.1	788.7	712.7	791.6	639.0	682.5	657.6	638.8	520.4	621.0	8444.8

A4.4 Solid phase extraction

Table A4.4: Recovery of compounds (%) from spiked seawater samples after solid phase extraction

Concentration (ng/l)	Citalopram	Trimethoprim	Paracetamol	Ibuprofen	Diclofenac
100	44 ± 2.84	68 ± 4.98	112 ± 16.86	94 ± 39.71	28 ± 0.55
200	43 ± 3.18	64 ± 12.72	98 ± 37.44	84 ± 25.88	22 ± 10.37
1000	43 ± 10.02	56 ± 12.05	51 ± 6.20	43 ± 13.69	9 ± 8.51

Table A4.5 Mean and Corrected values (ng/l) of pharmaceuticals detected at each of the sites

		Ibuprofen		Paracetamol		Diclofenac		Trimethoprim		Citalopram	
Site	**Date**	**Measured**	**Corrected**	**Measured**	**Corrected**	**Measured**	**Corrected**	**Measured**	**Corrected**	**Measured**	**Corrected**
A1	October 10th, 2016	203.22	278.38	41.17	47.87	44.63	223.15	13.90	22.07	1.80	4.19
	February 20th, 2017	62.72	85.92	36.01	41.87	13.32	66.60	20.04	31.81	1.58	3.67
	June 19th, 2017	126.35	173.08	11.60	13.49	7.23	36.15	14.68	23.30	1.33	3.09
A2	October 10th, 2016	57.87	79.27	29.88	34.74	7.63	38.15	1.27	2.01	<MQL	<MQL
	February 20th, 2017	67.73	92.78	43.86	51.00	14.18	70.90	21.33	33.86	2.82	6.56
	June 19th, 2017	56.10	76.85	<MQL	<MQL	7.68	38.40	1.44	2.29	1.68	3.91
R1	October 10th, 2016	181.14	248.14	62.83	73.06	10.75	53.75	36.66	58.18	3.56	8.28
	December 6th, 2016	356.29	488.07	788.52	916.88	42.13	210.65	94.89	150.62	6.70	15.58
	February 20th, 2017	370.79	507.93	342.29	398.01	31.71	158.55	104.92	166.54	18.46	42.93
	April 18th, 2017	1836.53	2515.79	126.70	147.33	50.16	250.80	155.62	247.02	7.32	17.02
	June 19th, 2017	1452.35	1989.52	207.83	241.66	40.01	200.05	89.73	142.43	10.70	24.88
	August 7th, 2017	138.70	190.00	29.17	33.92	9.20	46.00	1.97	3.13	2.25	5.23
R2	October 10th, 2016	40.20	55.07	48.96	56.93	<MQL	<MQL	1.52	2.41	2.03	4.71
	December 6th, 2016	146.34	200.47	237.02	275.60	9.42	47.10	19.68	31.24	1.41	3.28
	February 20th, 2017	45.86	62.82	37.97	44.15	15.79	78.95	14.28	22.67	2.19	5.09
	April 18th, 2017	2615.75	3583.22	238.22	277.00	8.95	44.75	2.35	3.73	1.64	3.81
	June 19th, 2017	200.83	275.11	58.62	68.16	<MQL	<MQL	1.31	2.08	2.28	5.30
	August 7th, 2017	90.93	124.56	48.95	56.92	<MQL	<MQL	1.49	2.37	1.24	2.88
R3	October 10th, 2016	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	December 6th, 2016	45.46	62.27	30.80	35.81	<MQL	<MQL	2.72	4.32	1.59	3.70
	February 20th, 2017	69.28	94.90	138.65	161.22	8.85	44.25	10.59	16.81	2.03	4.72
	April 18th, 2017	136.05	186.37	46.72	54.33	14.04	70.20	0.69	1.10	1.84	4.28
	June 19th, 2017	4364.36	5978.58	43.74	50.86	<MQL	<MQL	1.04	1.65	2.38	5.53
	August 7th, 2017	89.59	122.73	<MQL	<MQL	<MQL	<MQL	0.45	0.71	2.34	5.44
R4	October 10th, 2016	62.65	85.82	80.28	93.35	12.80	64.00	0.60	0.95	1.21	2.82
	December 6th, 2016	43.28	59.29	14.20	16.51	6.89	34.45	2.07	3.29	1.44	3.35
	February 20th, 2017	50.39	69.03	25.13	29.22	14.82	74.10	12.04	19.11	1.61	3.74
	April 18th, 2017	4596.91	6297.14	26.04	30.28	<MQL	<MQL	0.96	1.52	2.81	6.53

Table A4.5 *Continued*

		Ibuprofen		Paracetamol		Diclofenac		Trimethoprim		Citalopram	
Site	Date	Measured	Corrected	Measured	Corrected	Measured	Corrected	Measured	Corrected	Measured	Corrected
R4	June 19th, 2017	71.96	98.58	<MQL	<MQL	<MQL	<MQL	0.44	0.70	2.50	5.81
	August 14th, 2017	93.76	128.44	11.71	13.62	<MQL	<MQL	0.73	1.16	2.29	5.33
A3	February 20th, 2017	27.67	37.90	<MQL	<MQL	<MQL	<MQL	5.61	8.90	1.47	3.42
	June 19th, 2017	209.94	287.59	<MQL	<MQL	<MQL	<MQL	3.74	5.94	4.29	9.98
A4	October 10th, 2016	18.20	24.93	<MQL	<MQL	6.47	32.35	<MQL	<MQL	<MQL	<MQL
	February 20th, 2017	76.56	104.88	49.00	56.98	14.30	71.50	9.49	15.06	2.40	5.58
	June 19th, 2017	98.36	134.74	<MQL	<MQL	<MQL	<MQL	0.66	1.05	1.84	4.28
A5	October 10th, 2016	13.75	18.84	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
	February 20th, 2017	24.39	33.41	41.09	47.78	<MQL	<MQL	7.27	11.54	1.43	3.33
	June 19th, 2017	321.01	439.74	<MQL	<MQL	<MQL	<MQL	0.56	0.89	1.99	4.63
	Maximum (ng/l)	4596.91	6297.14	788.52	916.88	50.16	250.80	155.62	247.02	18.46	42.93
	Mean (ng/l)	499.01	665.58	76.24	88.65	10.29	51.44	17.28	27.43	2.75	6.39
	Standard Deviation	1070.15	1481.49	139.43	163.66	13.58	68.29	34.04	54.56	3.28	7.66
	Detection Frequency (%)	97.37	97.37	73.68	73.68	57.89	57.89	92.11	92.11	89.47	89.47

A4.6 Levels of pharmaceuticals from UK wide sampling

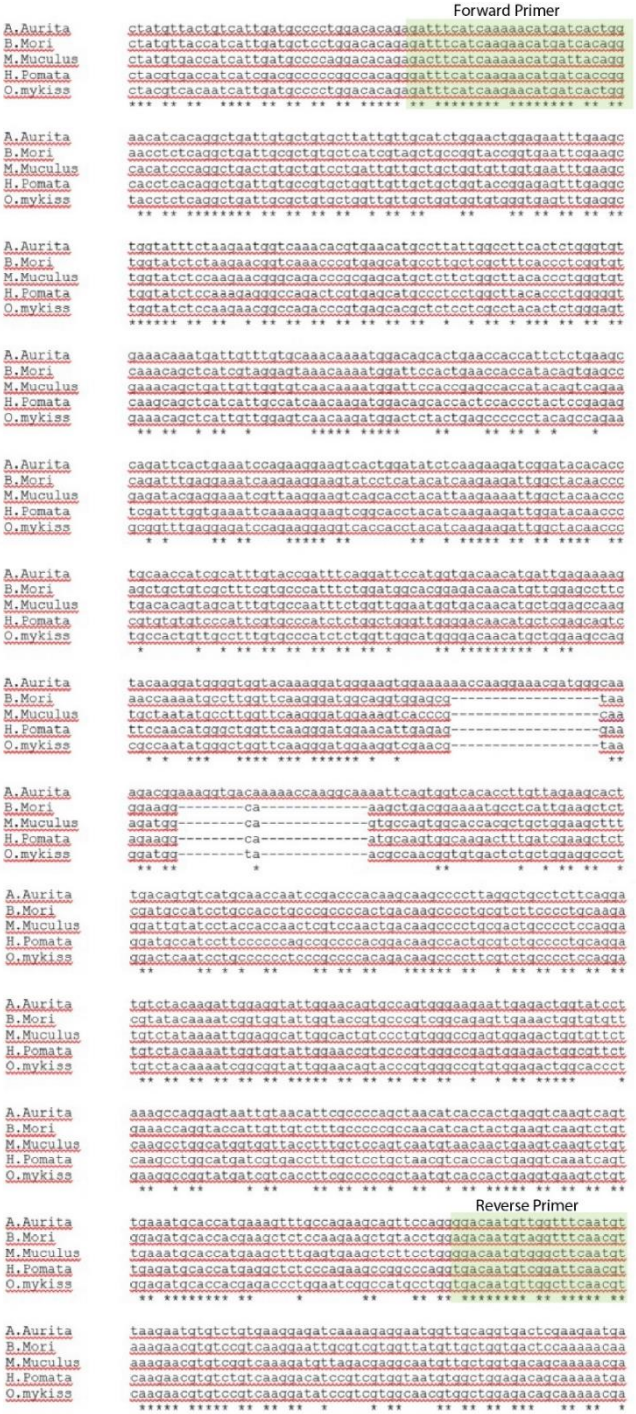
Table A4.6: Mean and corrected values (ng l^{-1}) of pharmaceuticals detected in UK estuaries

	Ibuprofen		Paracetamol		Diclofenac		Trimethoprim		Citalopram	
	Measured	Corrected	Measured	Corrected	Measured	Corrected	Measured	Corrected	Measured	Corrected
Cromarty	153.01	209.60	<MQL	<MQL	7.07	35.35	0.28	0.45	<MQL	<MQL
Forth	46.50	63.70	39.09	45.45	<MQL	<MQL	0.60	0.96	<MQL	0.00
Mersey	34.83	47.72	48.02	55.83	<MQL	<MQL	5.82	9.24	3.99	9.27
Portsmouth	34.40	47.12	32.09	37.32	<MQL	<MQL	0.52	0.82	<MQL	<MQL
Severn	68.59	93.97	42.72	49.68	<MQL	<MQL	0.57	0.90	1.60	3.72
Solway	44.14	60.47	14.45	16.80	<MQL	<MQL	0.75	1.19	<MQL	0.00
Tay	104.74	143.48	39.11	45.48	<MQL	<MQL	0.88	1.40	<MQL	0.00
Tees	71.42	97.83	14.22	16.53	<MQL	<MQL	0.71	1.12	<MQL	<MQL
Thames	36.32	49.75	60.29	70.10	6.81	34.06	1.67	2.65	3.73	8.68
Tyne	48.14	65.95	<MQL	0.00	<MQL	<MQL	3.53	5.61	3.75	8.72
Ythan	69.77	95.57	12.39	14.41	<MQL	<MQL	0.83	1.31	1.65	3.83

A5 Supporting Information for Chapter 5

A5.1 Primer design

(a) Elongation factor 1



(b) ATP Synthase

(c) cAMP-activation protein kinase

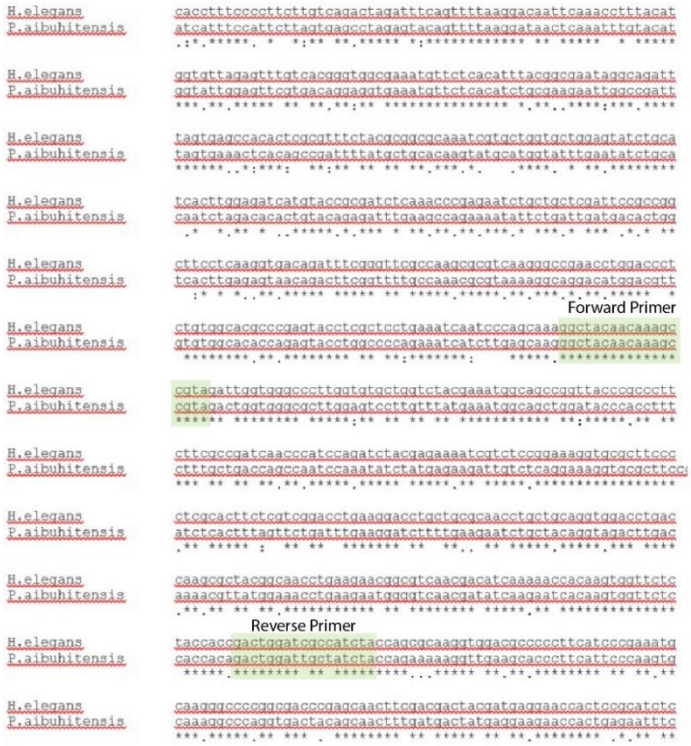


Figure A5.1: Alignment of multiple sequences for designing degenerate primers (green shaded boxes). Dashes represent gaps in the alignment and asterisks represent homology. Alignments were cropped and are not shown in full. **(a)** Elongation factor 1 (*EF1*) alignments with *Aurelia aurita* (GenBank Accession **KC341734.1**), *Bombyx mori* (**NM_001044045.1**), *Mus musculus* (**BC050124.1**), *Helix pomatia* (**KX384883.1**) and *Oncorhynchus mykiss* (**NM_01124339.1**). **(b)** ATP synthase (*ATPS*) alignments with *Nephasoma pellucidum* (**GU592847.1**), *Nereis vexillosa* (**DQ087492.1**), *Ophelia limacina* (**GU592851.1**) and *Erobdella octoculata* (**GU592848.1**). **(c)** cAMP-activation protein kinase (*AMPK*) alignments with *Hydroides elegans* (**AB232160.1**) and *Perinereis aibuhitensis.*

A5.2 PCR Gel Electrophoresis

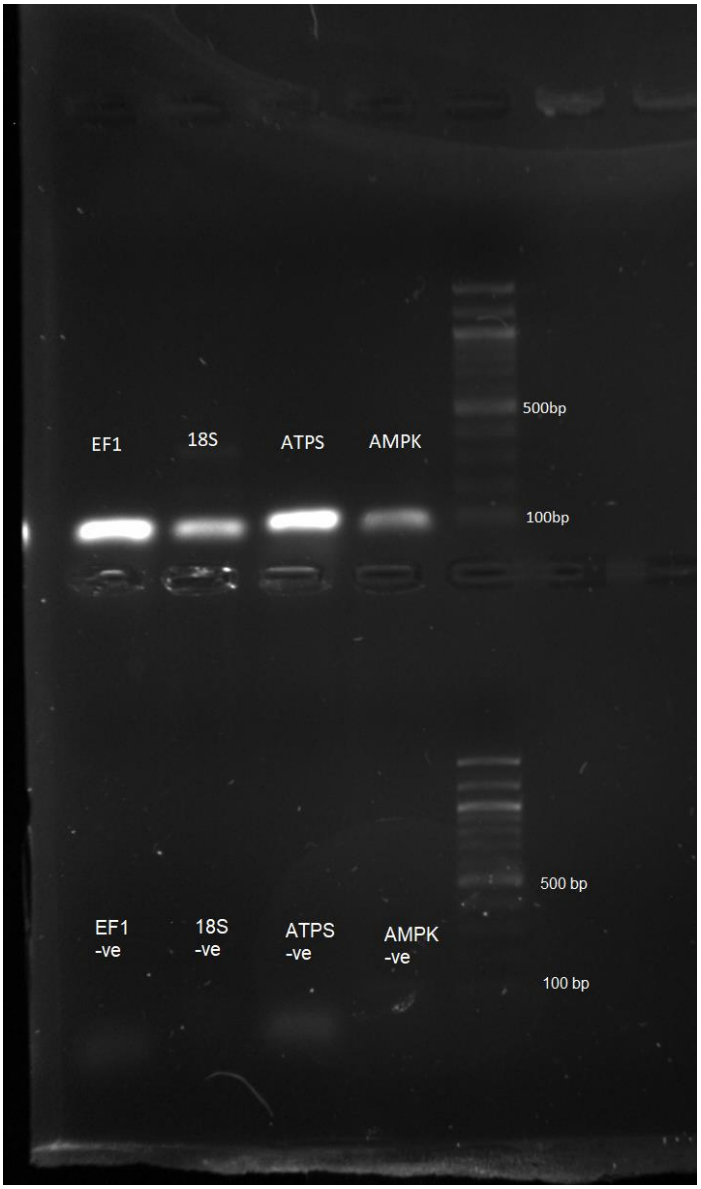


Figure A5.2: Image of a 1% agarose gel electrophoresis of PCR products. Top Row - Lane 1, *EF1* housekeeping gene; Lane 2, *18S* housekeeping gene; Lane 3, *ATPS*; Lane 4, *AMPK*; Lane 5, 100 bp ladder. Bottom Row - Lane 1, *EF1* negative; Lane 2, *18S* negative; Lane 3, *ATPS* negative; Lane 4, *AMPK* negative; Lane 5, 100 bp

A6 References

Anderson PO, Knoben JE, Troutman WG (2002) Handbook of clinical drug data 10th ed. McGraw-Hill

Baker DR, Kasprzyk-Hordern B (2013) Spatial and temporal occurrence of pharmaceuticals and illicity drugs in the aqueous environment and during wastewater treatment: new developments. Sci Total Environ 454-455:442-456Benotti MJ, Brownawell BJ (2007) Distributions of pharmaceuticals in an urban estuary during both dry- and wet-weather conditions. Environ Sci and Technol 41: 5795–5802. doi: 10.1021/es0629965

Beretta M, Britto V, Tavares TM, da Silva SMT, Pletsch AL (2014) Occurrence of pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. J Soils Sed 14: 1278–1286. doi: 10.1007/s11368-014-0884-6

Birch GF, Drage DS, Thompson K, Eaglesham G, Mueller JF (2015) Emerging contaminants (pharmaceuticals, personal care products, a food additive and pesticides) in waters of Sydney estuary, Australia. Mar Pollut Bull 97: 56–66. doi: 10.1016/J.MARPOLBUL.2015.06.038

Blychert E, Edgar B, Elmfeldt D, Hender T (1991) A population study of the pharmacokinetis of felodipine. Br J Clin Pharmac 31:15-24 doi: 10.1111/j.1365-2125.1991.tb03852.x

Bound JP, Voulvoulis N (2006) Predicted and measured concentrations for selected pharmaceuticals in UK rivers: Implications for risk assessment. Wat Res 40: 2885-2892 doi: j.watres.2006.05.036

British Pharmaciepoeia MSDS Available from https://www.pharmacopoeia.com/ [Accessed April 26, 2018]

Cantwell MG, Katz DR, Sullivan JC, Ho K, Burgess RM, Cashman M (2016) Selected pharmaceuticals entering an estuary: Concentrations, temporal trends, partitioning, and fluxes. Environ Toxicol Chem 35: 2665–2673. doi: 10.1002/etc.3452

Cantwell MG, Katz DR, Sullivan JC, Ho K, Burgess RM (2017) Temporal and spatial behavior of pharmaceuticals in Narragansett Bay, Rhode Island, United States. Environ Toxicol Chem. 36(7): 1846-1855 doi: 10.1002/etc.3710

Cantwell MG, Katz DR, Sullivan JC, Shapley D, Lipscomb J, Epstein J, Juhl AR, Knudson C, O'Mullan GD (2018) Spatial patterns of pharmaceuticals and wastewater tracers

in the Hudson River Estuary. Wat Res 137: 335–343. doi: 10.1016/j.watres.2017.12.044

Chen Z, Wang H, Ren N, Cui M, Shukai N, Dongzue H (2011) Simultaneous removal and evaluation of organic substrates and NH3-N by a novel combined process in treating chemical synthesis-based pharmaceutical wastewater. J Hazard Mater 197:49-59 doi: 10.1016/j.jhazmat.2011.09.053

Chen H, Liu S, Xu XR, Zhou GJ, Liu SS, Yue WZ, Sun KF, Ying GG (2015) Antibiotics in the coastal environment of the Hailing Bay region, South China Sea: Spatial distribution, source analysis and ecological risks. Mar Pollut Bull 95: 365–373. doi: 10.1016/j.marpolbul.2015.04.025

Cui Y, Wang Y, Pan C, Li R, Xue R, Guo J, Zhang R (2019) Spatiotemporal distributions, source apportionment and potential risks of 15 pharmaceuticals and personal care products (PPCPs) in Qinzhou Bay, South China. Mar Pollut Bull 141: 104–111. doi: 10.1016/j.marpolbul.2019.02.012

Compendium Available from: http://compendium.ch/home/de?Platform=Desktop [Accessed 25 July 2018]

Dilger K, Alberer M, Busch A, Enningers A, Behrens R, Koletzko S, Stern M, Beckmann C, Gleiter CH (2005) Pharmokinetics and pharmacodynamic action of budesonide in children with Crohn's disease. Aliment Pharmacol Ther 23:387-395 doi: 10.1111/j.1365-2036.2006.027771.x

Drug Bank Available from: http://www.drugbank.ca [Accessed: May 5, 2018]

Ferguson EM, Allinson M, Allinson G, Swearer SE, Hassell KL (2013) Fluctuations in natural and synthetic estrogen concentrations in a tidal estuary in south-eastern Australia. Wat Res 47: 1604–1615. doi: 10.1016/j.watres.2012.12.020

Golovko O, Kumar V, Fedorova G, Randak R, Grabic R (2014) Seasonal changes in antiobiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111:418-426 doi: 10.1016/j.chemosphere.2014.03.132

Gonzalez-Rey M, Tapie N, Le Menach K, Dévier M-H, Budzinski H, Bebianno MJ (2015) Occurrence of pharmaceutical compounds and pesticides in aquatic systems. Mar Pollut Bull 96: 384–400. doi: 10.1016/J.MARPOLBUL.2015.04.029

Gros M, Petrovic M, Ginebreda A, Barcelo D (2010) Removal of pharmaceuticals during

wastewater treatment and environmental risk assessment using hazard indexes Environ Int 36:15-26 doi: 10.1016/j.envint.2009.09.002

Guo X, Feng C, Gu E, Tian C, Shen Z (2019) Spatial distribution, source apportionment and risk assessment of antibiotics in the surface water and sediments of the Yangtze Estuary. Sci Total Environ 671: 548–557. doi: 10.1016/j.scitotenv.2019.03.393

Gurke R, Roβler M, Marx C, Diamond S, Schubert S, Oertel R, Fauler J (2015) Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci Total Environ 532:762-770

Hedgespeth ML, Sapozhnikova Y, Pennington P, Clum A, Fairey A, Wirth E (2012) Pharmaceuticals and personal care products (PPCPs) in treated wastewater discharges into Charleston Harbor, South Carolina. Sci Total Environ 437: 1–9. doi: 10.1016/J.SCITOTENV.2012.07.076

Ivanova L, Faberova M, Mackul'ak T, Grabic R, Bodik I (2017) Estimation of amount of selected pharmaceuticals sorbed onto digested sludge from wastewater treatment plant Bratislava-Petrzalka. Environ Res 155:31-35 doi: 10.1016/j.envres.2017.02.001

Jelic A, Rodriguez-Mozaz S, Barcelo D, Gutierrez O (2015) Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Res 68:98-103 doi: 10.1016/j.watres2014.09.033

Jiang B, Ruan Z, Chen J, Lou H, Shao R, Jin F, Shen H (2016) Pharmacokinetic properties and bioequivalence of orally inhaled salbutamol in healthy chinese volunteers. Drug Dev Ind Pharm 42(9): 1476-1481 doi: doi: 10.3109/03639045.2016.1151027

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res 43(2): 363-380 doi: 10.1016/j.watres.2008.10.047

Klosterhaus SL, Grace R, Hamilton MC, Yee D (2013) Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ Int 54: 92–99. doi: 10.1016/j.envint.2013.01.009

Kostisch MS, Lazorchak JM (2008) Risks to aquatic organisms posed by human pharmaceutical use. Sci Total Environ 389(2-3):329-339 doi: 10.1016/j.scitotenv.2010.06.015

Lara-Martín PA, González-Mazo E, Petrovic M, Barceló D, Brownawell BJ (2014) Occurrence, distribution and partitioning of nonionic surfactants and pharmaceuticals in the urbanized Long Island Sound Estuary (NY). Mar Pollut Bull 85: 710–719. doi: 10.1016/J.MARPOLBUL.2014.01.022

Lara-Martín PA, Renfro AA, Cochran JK, Brownawell BJ (2015) Geochronologies of Pharmaceuticals in a Sewage-Impacted Estuarine Urban Setting (Jamaica Bay, New York). Environ Sci Technol 49: 5948–5955. doi: 10.1021/es506009v

Liang X, Chen B, Nie X, Shi Z, Huang X, Li X (2013) The distribution and partitioning of common antibiotics in water and sediment of the Pearl River Estuary, South China. Chemosphere 92: 1410–1416. doi: 10.1016/j.chemosphere.2013.03.044

Long ER, Dutch M, Weakland S, Chandramouli B, Benskin JP (2013) Quantification of pharmaceuticals, personal care products, and perfluoroalkyl substances in the marine sediments of Puget Sound, Washington, USA. Environ Toxicol Chem 32: 1701–1710. doi: 10.1002/etc.2281

Markiewicz M, Jungnickel C, Stolte S, Bialk-Bielinska A, Kumirska J, Mrozik W (2017) Ultimate biodegrability and eotoxicity of orally administered antidiabetic drugs. J Hazard Mater 333 154-161 doi: 10.1016/J.JHAZMAT.2017.03.030

Matsuo H, Sakamoto H, Arizono K, Shinohara R (2011) Behaviour of pharmaceuticals in waste water treatment plant in Japan. Bull Environ Contam Toxicol 87:31-35 doi: 10.1007/s00128-011-0299-7

Meador JP, Yeh A, Young G, Gallagher EP (2016) Contaminants of emerging concern in a large temperate estuary. Environ Pollut 213: 254–267. doi: 10.1016/J.ENVPOL.2016.01.088

MHRA (2018) Medicines & Healthcare produts regulatory agency http://www.mhra.gov.uk/spc-pil/ [Accessed April 18, 2018]

Mijangos L, Ziarrusta H, Ros O, Kortazar L, Fernández LA, Olivares M, Zuloaga O, Prieto A, Etxebarria N (2018) Occurrence of emerging pollutants in estuaries of the Basque Country: Analysis of sources and distribution, and assessment of the environmental risk. Wat Res 147: 152–163. doi: 10.1016/J.WATRES.2018.09.033

Mostafavi SA, Tavakoli N (2004) Relative bioavailability of omeprazole capsules after oral dosing. DARU J Pharm Sci 12(4):146-151 doi: 10.1111/jvim.14779

Munro K, Martins CPB, Loewenthal M, Comber S, Cowan DA, Pereira L, Barron LP (2019) Evaluation of combined sewer overflow impacts on short-term pharmaceutical and illicit drug occurrence in a heavily urbanised tidal river catchment (London, UK). Sci Total Environ 657: 1099–1111. doi: 10.1016/J.SCITOTENV.2018.12.108

Neubeck M, Fliser D, Pritsch M, Weisser K, Fliser M, Nussberger J, Ritz E, Mutschler E (1994) Pharmacokinetics and pharmacodynamics of lisinopril in advanced renal failure. Consequence of dose adjustment. Eur J Clin Pharmacol 46(6)537-543 doi: 10.1007/BF00196112

Nie M, Yan C, Dong W, Liu M, Zhou J, Yang Y (2015) Occurrence, distribution and risk assessment of estrogens in surface water, suspended particulate matter, and sediments of the Yangtze Estuary. Chemosphere 127: 109–116. doi: 10.1016/J.CHEMOSPHERE.2015.01.021

Noppe H, Verslycke T, De Wulf E, Verheyden K, Monteyne E, Van Caeter P, Janssen CR, De brabander HF (2007) Occurrence of estrogens in the Scheldt estuary: A 2-year survey. Ecotox Environ Safe 66: 1–8. doi: 10.1016/J.ECOENV.2006.04.005

Radjenovic J, Petrovic M, Barcelo D (2007) Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. Anal Bioanal Chem 387:1365-1377 doi: 10.1007/s00216-006-0883-

Reis-Santos P, Pais M, Duarte B, Caçador I, Freitas A, Vila Pouca AS, Barbosa J, Leston S, Rosa J, Ramos F, Cabral HN, Gillanders BM, Fonseca VF (2018) Screening of human and veterinary pharmaceuticals in estuarine waters: A baseline assessment for the Tejo estuary. Mar Pollut Bull 135: 1079–1084. doi: 10.1016/J.MARPOLBUL.2018.08.036

Roberts PH, Thomas KV (2006) The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower tyne catchment. Sci Total Environ 143-153 doi: 10.1016/j.scitotenv.2005.04.031

Rocha MJ, Cruzeiro C, Reis M, Pardal M ??ngelo, Rocha E (2014) Spatial and seasonal distribution of 17 endocrine disruptor compounds in an urban estuary (Mondego River, Portugal): Evaluation of the estrogenic load of the area. Environ Monit Assess 186: 3337–3350. doi: 10.1007/s10661-014-3621-0

Rosal R, Rodea-Palomares I, Boltes K, Fernandez-Pinas F, Leganes F, Gonzalo S, Petre A (2010) Ecotoxicity assessment of lipid regulators in water and biologically treated wastewater using three aquatic organisms. Environ Sci Pollut Res 17:135-144 doi: 10.1007/s11356-009-0137-1

Sanderson H, Thomsen M (2009) Comparative analysis of pharmaceuticals versus industrial chemicals acute aquatic toxicity classification according to the United Nations classification system for chemicals. Assessment of the (Q)SAR predictability of pharmaceuticals acute aquatic toxicity and their predominant acute toxic mode-of-action. Toxicol Lett 84-93 doi: 10.1016/j.toxlet.2009.02.003

Santos LHMLM, Gros M, Rodriguez-Mozaz S, Delerue-Matos C, Pena A, Barcelo D, Conceicao M, Montenegro BSM (2013) Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. Sci Total Environ 461-462:302-316 doi: 10.1016/j.scitotenv.2013.04.077

Sarkar A, Tiwari A, Bhasin PS, Mitra M (2011) Pharmacological and pharmaceutical profile of gliclazide: a review. J Appl Pharm Sci 1(9):11-19

Schreiber R, Gündel U, Franz S, Küster A, Rechenberg B, Altenburger R (2011) Using the fish plasma model for comparative hazard identifiation for pharmaceuticals in the environment by extrapolation from human therapeuti data. Regul Toxicol Pharmacol 61(3):261-275 doi: 10.1016/j.yrtph.2011.08.006

Shi H, Yang Y, Liu M, Yan C, Yue H, Zhou J (2014) Occurrence and distribution of antibiotics in the surface sediments of the Yangtze Estuary and nearby coastal areas. Mar Pollut Bull 83: 317–323. doi: 10.1016/j.marpolbul.2014.04.034

Stewart AM, Grossman L, Nguyen M, Maximino C, Rosemberg DB, Echevarria DJ, Kalueff A V. (2014) Aquatic toxicology of fluoxetine: Understanding the knowns and the unknowns. Aquat Toxicol 156: 269–273. doi: 10.1016/j.aquatox.2014.08.014

Stewart M, Olsen G, Hickey CW, Ferreira B, Jelić A, Petrović M, Barcelo D (2014) A survey of emerging contaminants in the estuarine receiving environment around Auckland, New Zealand. Sci Total Environ 468–469: 202–210. doi: 10.1016/J.SCITOTENV.2013.08.039

Sulaiman S, Khamis M, Nir S, Lelario F, Scrano L, Bufo SA, Mecca G, Karaman R (2015) Stability and removal of atorvastatin, rosuvastatin and simvastatin from wastewater. Environ Technol 36(24):3232-3242 doi: 10.1080/09593330.2015.1058422

Sun Q, Li Y, Li M, Ashfaq M, Lv M, Wang H, Hu A, Yu C-P (2016) PPCPs in Jiulong River estuary (China): Spatiotemporal distributions, fate, and their use as chemical markers of wastewater. Chemosphere 150: 596–604. doi: 10.1016/J.CHEMOSPHERE.2016.02.036

Tamtam F, Mercier F, Le Bot B, Eurin J, Tuc Dinh Q, Clement M, Chevreuil M (2008) Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci Total Environ 393: 84–95. Doi: 10.1016/j.scitotenv.2007.12.009

Thomas K V., Hilton MJ (2004) The occurrence of selected human pharmaceutical compounds in UK estuaries. Mar Pollut Bull 49: 436–444. doi: 10.1016/j.marpolbul.2004.02.028

Wiegel S, Aulinger A, Brockmeyer R, Harms H, Löffler J, Reincke H, Schmidt R, Stachel B, Von Tümpling W, Wanke A (2004) Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57: 107–126. doi: 10.1016/j.chemosphere.2004.05.017

Verho M, Luck C, Stelter WJ, Rangoonwala B, Bender N (1995) Pharmacokinetics, metabolism and biliary and urinary excretion of oral ramipril in man Curr Med Res Opin 13(5)264-273 doi: 10.1185/03007999509111551

Vestel J, Caldwell, DJ, Constantine L, D'Aco VJ, Davidson T, Dolan DG, Millard SP, Murray-Smith R, Parke NJ, Ryan JJ, Straub JO, Wilson P (2016) Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environ Toxicol Chem 35(5):1201-1212 doi: 10.1002/etc.3260

Wang X, Shen J, Chen Z, Zhao X, Xu H (2016) Removal of pharmaceuticals from synthetic wastewater in an aerobic granular sludge membrane bioreactor and determination of the bioreactor microbial diversity. Appl Microbiol Biotechnol 100:8213-8223

Wu M, Xiang J, Que C, Chen F, Xu G (2015) Occurrence and fate of psychiatric pharmaceuticals in the urban water system of Shanghai, China. Chemosphere 138:486-493 doi: 10.1016/j.chemosphere.2015.07.002

Yan C, Yang Y, Zhou J, Liu M, Nie M, Shi H, Gu L (2013) Antibiotics in the surface water of the Yangtze Estuary: Occurrence, distribution and risk assessment. Environ Pollut 175: 22–29. doi: 10.1016/j.envpol.2012.12.008

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL (2011) Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone. J Hazard Mat 190: 588–596. doi: 10.1016/J.JHAZMAT.2011.03.092

Yun MH, Woo JS, Kwon KI (2006) Bioequivalence and pharmokinetics of 70mg alendronate sodium tablets by measuring alendronate in plasma. Arch Pharm Res 29(4):328-332

Zhang R, Zhang G, Zheng Q, Tang J, Chen Y, Xu W, Zou Y, Chen X (2012) Occurrence and risks of antibiotics in the Laizhou Bay, China: Impacts of river discharge. Ecotox Environ Safe 80: 208–215. doi: 10.1016/J.ECOENV.2012.03.002

Zhang R, Tang J, Li J, Cheng Z, Chaemfa C, Liu D, Zheng Q, Song M, Luo C, Zhang G (2013) Occurrence and risks of antibiotics in the coastal aquatic environment of the Yellow Sea, North China. Sci Total Environ 450–451: 197–204. doi: 10.1016/J.SCITOTENV.2013.02.024

Zhao H, Zhou JL, Zhang J (2015) Tidal impact on the dynamic behavior of dissolved pharmaceuticals in the Yangtze Estuary, China. Sci Total Environ 536: 946–954. doi: 10.1016/j.scitotenv.2015.06.055

Zheng S, Qiu X, Chen B, Yu X, Liu Z, Zhong G, Li H, Chen M, Sun G, Huang H, Yu W, Freestone D (2011) Antibiotics pollution in Jiulong River estuary: Source, distribution and bacterial resistance. Chemosphere 84: 1677–1685. doi: 10.1016/j.chemosphere.2011.04.076