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epa Research

Effect-based Monitoring for Pharmaceutical Pollution in Ireland

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Rialtas na hÉireann
Government of Ireland

Environmental Protection Agency

The EPA is responsible for protecting and improving the environment as a valuable asset for the people of Ireland. We are committed to protecting people and the environment from the harmful effects of radiation and pollution.

The work of the EPA can be divided into three main areas:

Regulation: Implementing regulation and environmental compliance systems to deliver good environmental outcomes and target those who don't comply.

Knowledge: Providing high quality, targeted and timely environmental data, information and assessment to inform decision making.

Advocacy: Working with others to advocate for a clean, productive and well protected environment and for sustainable environmental practices.

Our Responsibilities Include:

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- Large-scale industrial, waste and petrol storage activities;
- Urban waste water discharges;
- The contained use and controlled release of Genetically Modified Organisms;
- Sources of ionising radiation;
- Greenhouse gas emissions from industry and aviation through the EU Emissions Trading Scheme.

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- Prosecute those who flout environmental law and damage the environment.

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- Develop and implement the National Waste Prevention Programme;
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- Support National, EU and UN Climate Science and Policy development activities.

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- Produce the State of Ireland's Environment and Indicator Reports;
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- Collaborate with national and EU environmental research activity.

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- Provide independent evidence-based reporting, advice and guidance to Government, industry and the public on environmental and radiological protection topics;
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- Promote environmental awareness including supporting behaviours for resource efficiency and climate transition;
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- Work with international and national agencies, regional and local authorities, non-governmental organisations, representative bodies and government departments to deliver environmental and radiological protection, research coordination and science-based decision making.

Management and Structure of the EPA

The EPA is managed by a full time Board, consisting of a Director General and five Directors. The work is carried out across five Offices:

1. Office of Environmental Sustainability
2. Office of Environmental Enforcement
3. Office of Evidence and Assessment
4. Office of Radiation Protection and Environmental Monitoring
5. Office of Communications and Corporate Services

The EPA is assisted by advisory committees who meet regularly to discuss issues of concern and provide advice to the Board.

Effect-based Monitoring for Pharmaceutical Pollution in Ireland

Authors: Fiona Regan and Dylan O'Flynn

Lead organisation: Dublin City University

Identifying pressures

Pharmaceutical contamination in Irish surface waters is a growing problem that is intensified by rising pharmaceutical consumption and production. Factors such as geographical location, proximity to waste water treatment plants, seasonal changes, local administration practices and environmental events contribute to the presence of these chemicals in water bodies. The Effect-based Monitoring for Pharmaceutical Pollution in Ireland (EMPIRE) project sought to tackle this issue by identifying sources of pharmaceutical pollution and monitoring Irish surface waters, with a focus on temporal measurements. In addition, the project aimed to assess the effectiveness of ecotoxicology tests in determining the chronic effects of pharmaceuticals and measure toxicity or modes of action using a battery of bioassays on individual pharmaceutical compounds and mixtures. Using this comprehensive approach, the research provided a deeper understanding of pharmaceutical pollution to inform strategies to mitigate its impact on the environment and public health.

Informing policy

The EMPIRE project addressed the significant societal and environmental risks posed by active pharmaceutical ingredients (APIs) in water sources, which can affect wildlife and potentially humans, even at low concentrations. Current risk assessments may not fully account for chronic exposure to low levels of APIs in drinking water, the combined effects of multiple APIs or their impact on vulnerable populations. Urban waste water treatment plants are often unable to remove all APIs, and veterinary pharmaceuticals from manure spread on land can further contaminate surface water and groundwater. The research underscored the need for improved analysis and monitoring techniques, as well as a bioanalytical approach to assessing water safety given the complex mixtures of micropollutants likely to be present in drinking and recycled water. By enhancing our understanding and management of pharmaceutical pollution, this research should inform policy changes, improve public health protections and promote more effective water treatment practices, ultimately safeguarding both human and environmental health.

Developing solutions

The EMPIRE project is the first comprehensive investigation into using effect-based biomonitoring for pharmaceutical pollutants in Irish surface waters. Through a combination of in vivo and in vitro bioassays, the project assessed water quality and highlighted key findings for improving monitoring practices. The research reveals that certain pharmaceutical compounds are consistently present at measurable concentrations, emphasising the need for more frequent monitoring near waste water treatment plants and in surface waters both upstream and downstream. Analytical methods can be influenced by sample conditions such as pH level and storage, requiring careful management of sample matrix interference and the use of suitable internal standards. The project recommends integrating occurrence data with effect-based monitoring to evaluate water toxicity, although standardised bioassays are still needed. In addition, monitoring the effects on ecosystem biodiversity, particularly organism reproduction, is crucial for accurately determining toxicity profiles. The study also stresses the importance of broad chemical screening, noting higher pharmaceutical contamination in urban areas but significant occurrences in rural sites as well.

EPA RESEARCH PROGRAMME 2021–2030

Effect-based Monitoring for Pharmaceutical Pollution in Ireland

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EPA Research Report

Prepared for the Environmental Protection Agency

by

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DISCLAIMER

This report is based on research carried out/data from 2018 to 2023. More recent data may have become available since the research was completed.

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

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Contents

List of Figures

List of Tables

Executive Summary

Consumption of pharmaceuticals is one of the most significant contributors to the environmental load of active pharmaceutical ingredient residues in water among member countries of the Organisation for Economic Co-operation and Development. Human and veterinary pharmaceuticals are classified into two categories: over the counter and prescription. The level of consumption of these pharmaceuticals is the largest influencing factor that will determine the final concentrations in the environment. The behaviour and environmental presence of pharmaceuticals depends on geographical location, proximity to a waste water treatment plant, season, local administration practices (ease of disposal) and environmental factors (temperature, rainfall, sunlight hours and humidity). In the past decade, there has been an increasing effort to address the monitoring of pharmaceuticals in the aquatic environment. In addition, effect-based methods (EBMs) have played an increasingly important role in ecotoxicological assessments of pollutants in surface waters.

The Effect-based Monitoring for Pharmaceutical Pollution in Ireland (EMPIRE) project provides the first comprehensive investigation into the applicability of effect-based biomonitoring for pharmaceutical pollutants, comprising *in vivo* and *in vitro* bioassays to assess the quality of Irish surface waters.

The aims of the EMPIRE project were to:

- determine the sources of pharmaceuticals;
- monitor Irish surface waters for the presence of pharmaceuticals, with a focus on temporal measurements;
- assess the effectiveness of ecotoxicology tests for determining the chronic effects of pharmaceuticals;
- measure toxicity or mode of action based on a battery of bioassays tested on individual pharmaceutical compounds and mixtures.

Each element of the research generated valuable data that will be openly available at the end of the project. The data generated were used to propose an assessment of risk of certain water bodies based on pharmaceutical occurrence/detections and initial EBM data.

The main findings and recommendations arising from the work are as follows:

- Some of the pharmaceutical compounds targeted occurred in every sample at measurable concentrations, for example venlafaxine and sulfamethoxazole. It is recommended that a more frequent monitoring of receiving waters in proximity to waste water treatment plants and surface waters upstream and downstream takes place to establish the burden of this source on surface waters.
- Analytical methods for determining target analytes are available. However, when taking sample matrix into consideration, there can be a significant impact on the quality of quantitative information on certain chemical classes. A thorough assessment of site-specific sample matrix interference and the selection of suitable internal standards for quantitation is strongly recommended.
- Taking occurrence information together with EBMs provides the potential for determining a water sample toxicity profile. There are currently no agreed EBMs that could be harmonised for monitoring. The takeaway observations from the EMPIRE research are that certain compounds can cause increased biological effects in some assays, and that cocktails of chemicals show synergistic effects.
- The biological effects of substances vary. However, if monitoring programmes are to consider effects on ecosystem biodiversity, we recommend the use of ecotoxicology studies involving, for example, invertebrate organism reproduction when screening waters for toxicity.
- The data gathered on pharmaceutical occurrence clearly showed a greater number of detections in the urban site and higher concentrations of analytes. However, results show that pharmaceuticals that may arise from septic tanks or agricultural sources are also found at rural sites.

- EMPIRE results highlight the importance of monitoring surface waters to build up a data set on surface water quality. However, it is recommended that a wide-range suspect screening or a non-target screening be carried out to gather a more comprehensive chemical fingerprint of Irish surface waters.

The EMPIRE data set forms a basis for future monitoring, particularly in advance of the revised Urban Wastewater Treatment Directive.[1] Micropollutant removal is a consideration for certain agglomerations in this proposed legislation. This is a step towards meeting the European Green Deal's zero pollution ambition, and the data and findings from the EMPIRE project can inform how Ireland addresses future pollution arising from anthropogenic sources of pharmaceuticals in surface waters.

1 Proposal for a Directive of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standards in the field of water policy. COM(2022) 540 final, 26.10.2022, Brussels.

1 Introduction

The Organisation for Economic Co-operation and Development (OECD) workshop “Managing contaminants of emerging concern in surface waters: scientific developments and cost-effective policy responses” (February 2018) identified that publicly available (poor-quality) data are available for less than 5% of the 100,000 chemicals in use. Some active pharmaceutical ingredients (APIs) can affect wildlife at concentrations at and below those found in the aquatic environment. Risk assessment approaches (Tahar *et al*., 2017) may need to be improved to consider the possible effects on humans of inadvertent chronic exposure to low levels of APIs in drinking water, also taking into account the potential for combined effects from multiple APIs and the effect on vulnerable sub-populations. Most urban waste water treatment plants (WWTPs) are not able to remove all of each API (Lema and Suarez, 2017). Manure from treated animals (which contains veterinary pharmaceuticals) is usually spread on land, with concentrations of APIs reported for surface waters and groundwaters ranging from below ng/L to above µg/L; the number of exposure scenarios and the potential for bioaccumulation or a “cocktail effect” (combinations of chemicals) are often unknown (Dougherty *et al*., 2010). With better analysis, a higher number of substances are detected, which results in a higher risk estimate. It is virtually impossible to reliably assess water quality with targeted chemical analyses only. The likely presence of a complex mixture of micropollutants in drinking and recycled water emphasises the need for a bioanalytical health-related approach to evaluate water safety.

The broad spectrum of possible pollutants and emission scenarios raises several questions. How do we prioritise chemicals for regulation? When and how do we sample for these compounds? How many chemicals can we measure? In this context, effect-based analysis methods (Triebskorn *et al*., 2014), such as bioassays and bioanalytical methods, have been applied as tools to address some of the above questions. *In vitro* effect-based methods (EBMs) can be valuable as screening tools to reduce the chemical analytical monitoring burden. In the Water Framework Directive (WFD) context, EBMs can be employed:

- as screening tools in the framework of the pressures and impacts assessment to aid in the prioritisation of water bodies;
- to establish early warning systems;
- to prioritise further studies in areas that are not identified as being at risk due to their location;
- to take the effects of chemical mixtures or chemicals that are not analysed into account;
- to provide additional support in water and sediment quality assessments to supplement conventional chemical and ecological monitoring under the WFD.

EBMs are particularly suitable as part of investigative monitoring programmes, where the optimal approach will frequently involve several effect-based tools as well as chemical analysis.

The use of *in vitro* bioassays is increasing for an ethical reason: to reduce animal experimentation. Biomonitoring and EBMs can indicate which, when and where compounds should be monitored, and passive sampling can be used in combination with in situ, *in vivo* and *in vitro* bioassays to assess the impacts on water quality (Clarke *et al*., 2015). The effects on cells of exposure to environmental samples are measured via DNA damage or receptor activation, i.e. at the subcellular level, rather than investigating the tissues or cells of field organisms. This approach is inherently suitable for use with grab samples and passive samplers, as well as many other matrices. Bioassays indicative of adaptive stress responses, such as oxidative stress, have been shown to be excellent potential candidates for drinking water analysis, with *in vitro* assays based on human cell lines providing the best chance of assessment of potential human health effects (Brack *et al*., 2019). The relationship between specific properties of water organisms and their sensitivity to chemical and ecological stressors is also analysed. *In vivo* bioassays generally respond to different types of toxicity from a range of substances, and *Daphnia magna* bioassays are recommended over other models for assessment of aquatic toxicity

because of the increased oral exposure of *D. magna* through filter feeding; they have been utilised previously in the investigation of pharmaceuticals in isolation and in limited combinations (Brack *et al*., 2018). However, the investigation of the health of aquatic environments in relation to hazardous substances is generally based on individual pollutant concentrations, which may not be consistent with ecological quality status assessment.

Examination of field-exposed organisms for markers of stress (molecular, biochemical, cellular and physiological) can identify biomarkers that can be used as an early warning system. Carefully selected sets of biomarkers enable assessment of exposure to and effects of toxic chemicals, as well as the health status of organisms, and, when combined with chemical analysis, identification of toxicant(s). Mechanistic responses on the cellular level can be linked with whole-organism, population, community and potentially ecosystem effects using the concept of "adverse outcome pathways" (Brack *et al*., 2017).

Ecological tools or indices are not predictive of damage, whereas several biomarkers can be used as an early warning system because they can detect effects caused by chemical substances and other environmental stressors at an early stage. Validation of bioassays is critical to enable their adoption into the monitoring framework. This is done by investigating the comparability of methods in line with the quality assurance/quality control requirements of the Water Framework Directive (Lardy-Fontan *et al*., 2016), including EBMs (*in vitro* and *in vivo*), which can be used alongside chemical methods for the evaluation of complex mixtures occurring in different types of aquatic environments. Effect-based trigger values need to be established to differentiate between what is an acceptable or unacceptable response, with a number of techniques available (Altenburger *et al*., 2018).

The Effect-based Monitoring for Pharmaceutical Pollution in Ireland (EMPIRE) project provides the first comprehensive investigation into the applicability of effect-based biomonitoring for pharmaceutical pollutants, comprising *in vivo* and *in vitro* bioassays to assess the quality of Irish surface waters. This research is timely, as the extension of an EBM approach to emerging contaminants has recently been identified as being warranted at the EU level, so the outputs of the research will inform and enable future policy development to protect and enhance the quality of Irish drinking water sources.

2 Pharmaceutical Life Cycle Analysis: A Study of Circular Economy

2.1 Objectives

- To study sources of pharmaceuticals in Ireland and their level of usage.
- To assess the pharmaceutical life cycle.
- To identify target analytes to inform the project.

Understanding the life cycle (Figure 2.1) of these pharmaceuticals plays a significant role in identifying their potential sources and understanding the environmental impact that pharmaceuticals may have on surface waters. The stability and biological activity of these micropollutants can lead to "pseudo-persistence", with ensuing unknown chronic behavioural and health-related effects.

2.2 Introduction

The intentional and unintentional release of APIs into the environment across a variety of point (illegal dumping, industrial waste water, and effluent from hospitals and domestic WWTPs) and diffuse (run-off from agricultural farms and leaching from domestic septic tanks) sources leads to widespread contamination by human and veterinary pharmaceuticals in surface waters across the EU (Gros *et al*., 2012). The concentration of medicines in liquid waste streams emanating from landfill sites can be similar to or even higher concentrations than those found in the effluent from WWTPs (Tischler *et al*., 2013).

Despite their apparent advantages, WWTPs with conventional activated sludge (CAS) are not typically tailored to remove pharmaceuticals or other contaminants of emerging concern (CECs) from waste water, which accounts for the high variability of removal efficiencies among CAS WWTPs (Aerni *et al*., 2004). As a result, a primary source of pharmaceutical pollution in surface waters is effluent water discharged from WWTPs. The continuous release of many APIs is reported to exceed the rate of degradation in WWTPs and in the environment, which leads to pseudo-persistence in surface waters (Fang *et al*., 2012).

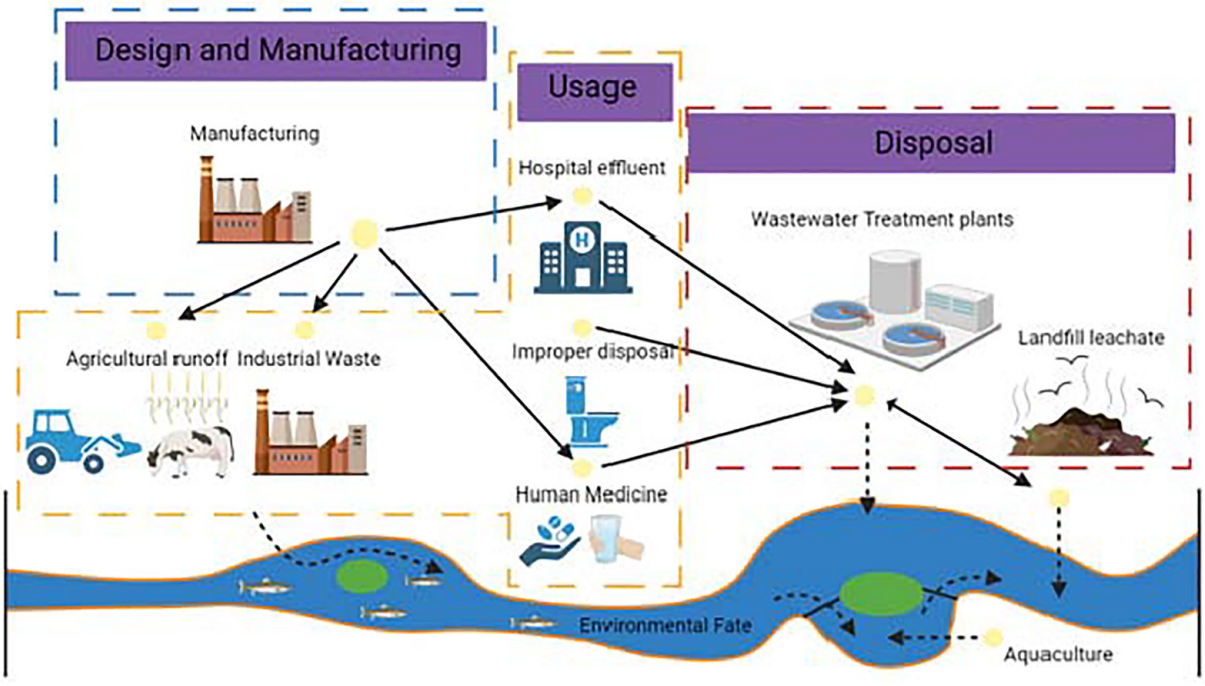

Figure 2.1. The life cycle of a pharmaceutical from its source to final deposition.

Furthermore, the efficiency of single-dwelling septic tanks has also been shown to significantly contribute to the overall pharmaceutical load, particularly in rural areas (Verlicchi *et al*., 2012).

2.2.1 Pharmaceuticals in Ireland

In many European countries, including Ireland, there are limited publicly available data on the consumption and disposal of over-the-counter and prescription medicines, e.g. diclofenac. This leads to an inability to track how pharmaceutical use can affect environmental concentrations. Furthermore, the variety of pharmaceuticals intended for human use is significantly greater than those used for veterinary purposes; the large variety of pharmaceuticals in itself poses a significant challenge to assessing the true level of environmental impact. The rapidly growing and ageing population in developed countries such as Ireland has led to increased use of pharmaceuticals and an increase in the variety of pharmaceuticals being consumed. Ireland uses antimicrobials intensively in livestock and domesticated animals. The total tonnage of veterinary pharmaceuticals used in Ireland was steady from 2013 to 2017 (Health Products Regulatory Authority, 2017). However, the categories of antibiotics that are being used have changed (e.g. there has been an increase in the usage of macrolide antibiotics). Antibiotic use for human medication in Ireland has a greater dependence on penicillin and macrolide antibiotics. However, penicillin is susceptible to a wide variety of degradation pathways, such as hydrolysis, thermolysis, sorption and biodegradation.

The Environmental Protection Agency in Ireland has stated that climate change will increase the level of rainfall in the north and west of Ireland. The increased transport of pharmaceuticals into surface waters is driven by intensifying weather events such as increased rainfall, resulting in stormwater bypassing WWTPs and mobilisation of contaminants in soil and run-off from agricultural land. To provide a holistic view of pharmaceutical pollution, EU legislation must address the contamination of soil, as it is a crucial vector for surface water pollution.

Roughly 50–90% of pharmaceuticals dispensed in the EU are collected via take-back schemes in pharmacies; however, some Member States do not even have take-back schemes in place (European Environment Agency, 2010). This leads to a significant knowledge gap surrounding the disposal of the remaining unused medicines. Furthermore, stockpiling pharmaceuticals is a common practice in many countries: in a study conducted in Ireland, 88% of the 398 respondents reported keeping unused drugs (Peake *et al*., 2016). The same study showed that 72% of respondents had improperly disposed of stored medicines.

2.2.2 The life cycle of pharmaceuticals

Life cycle assessment (LCA) is an inclusive tool that gives the opportunity to measure all inputs, outputs and influencing factors from creation to disposal, and the associated environmental effects as a result of processes (Figure 2.2) (Vellinga *et al*., 2014). LCA additionally expands the discussion on the sources of pharmaceutical pollution by addressing a range of possible inputs, which enables smart decision-making by policymakers and stakeholders. Conducting an LCA of pharmaceuticals not only helps track the pathway of pharmaceuticals into the environment, but it also helps meet the targets of the UN Sustainable Development Goals.

2.2.3 Manufacturing and risk assessment

Pharmaceutical companies are continually developing pharmaceutical products to be more environmentally friendly or “benign by design” (Boxall *et al*., 2012). This is accomplished by reformulating pharmaceuticals to rapidly and totally degrade upon reaching the environment or by changing how pharmaceuticals are administered (creams, tablets, patches, injections). However, a “benign by design” API is not always feasible, as many pharmaceuticals are “discovered” rather than designed. Of all formulations orally administered, pharmaceuticals pose a greater environmental risk, as they have a higher tendency to be excreted out of the body as an active substance (parent compounds and metabolites) into waste water streams (Celiz *et al*., 2009).

2.2.4 Consumption of pharmaceuticals

Consumption of pharmaceuticals (Figure 2.2) is one of the most significant contributors to environmental loads of API residues in water within OECD countries (Abdollahiasl *et al*., 2011). Human and

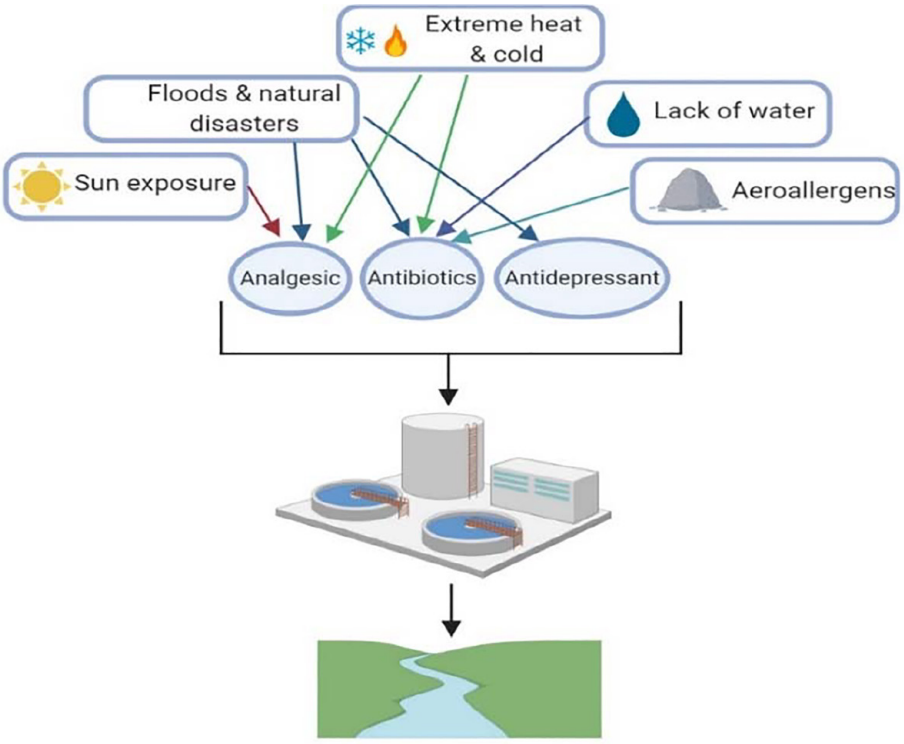

Figure 2.2. Potential uses and sources of groups of pharmaceuticals.

veterinary pharmaceuticals are classified into two categories: over the counter (e.g. diclofenac) and prescription (e.g. venlafaxine, azithromycin, ciprofloxacin, sulfamethoxazole, gemfibrozil) (Grabicova *et al*., 2014). The level of consumption of these pharmaceuticals is the largest influencing factor that will determine the final concentrations in the environment. The lack of public knowledge surrounding the appropriate disposal of unused pharmaceuticals (Pharmaceutical Society of Ireland, 2017) can lead to an increased risk of environmental exposure. Directive 2004/27/EC (relating to medicinal products for human use) introduces an obligation for Member States to implement appropriate collection schemes for unused pharmaceutical products. However, it does not provide guidelines on the implementation of schemes, and a number of studies have pointed to significant differences between Member States. In Ireland, where up until now pharmacies have been liable for all the expenses of the collection scheme, there are reports of pharmacies accepting unused medicines and awaiting a national campaign sponsored by the Health and Safety Executive to get rid of the collected medicines. The current limitations of the EU regulatory frameworks need to be addressed.

Some recommendations for a more environmentally sustainable approach include:

- classifying pharmaceuticals as hazardous waste and promoting or enforcing environmentally sound disposal;
- recognising the environmental risks of pharmaceuticals in the new market authorisations for human medicines and revising them for existing pharmaceuticals;
- harmonising collection schemes in EU Member States;
- developing upstream and downstream measures that avoid emissions of pharmaceuticals into the environment;
- developing infrastructure to improve the efficiency of removal of pharmaceuticals from the waste stream.

The degree to which a pharmaceutical is metabolised can significantly vary, with 30–90% of pharmaceuticals not being metabolised at all; this leads to the excretion of unchanged parent ions and pharmaceutical residues through faeces and urine, which end up in WWTPs and subsequently surface waters. These metabolites in surface waters can be transformed back to their parent compound through microorganisms (Quesada

et al., 2019). Behaviour and environmental presence depend on geographical location, proximity to a WWTP, season, local administration practices (ease of disposal) and environmental factors (temperature, rainfall, sunlight hours and humidity) (Mudgal *et al*., 2013). The Intergovernmental Panel on Climate Change has presented various scenarios on how climate change will increase temperature and humidity (increasing degradation rates and reducing dilution) and precipitation (increasing the dilution rates in rivers) (Nannou *et al*., 2015). Precipitation may also lead to the mobilisation of pharmaceuticals into surface waters from surrounding soil and run-off from agricultural land (Leckie *et al*., 2019).

2.2.5 Impact of pharmaceuticals on human health

The routes of human exposure to pharmaceuticals from environmental pathways are well understood, with the main routes being the consumption of contaminated food and drinking water. However, exposure to pharmaceuticals may also result from exposure to soils and dust, and to contaminated surface/coastal waters when swimming (Boxall, 2018). An indirect consequence of pharmaceutical exposure for human health is associated with exposure to antimicrobial-resistant organisms, as antimicrobial resistance poses a severe threat to both animal and human health (Marti *et al*., 2013). The presence of antibiotics such as sulfamethoxazole and ciprofloxacin in surface waters and soil can lead to the development, maintenance and spread of antimicrobial-resistant bacteria, fungi and biofilms in natural environments (Kaeseberg *et al*., 2018). Furthermore, antimicrobial-resistant bacteria present in fish from aquaculture have been shown to pass this resistance to humans.

2.2.6 The chemical cocktail

The risk of chronic exposure to an individual pharmaceutical is significant; however, a multicomponent mixture of APIs and associated residues can activate multiple biological molecules within an organism (Carpenter *et al*., 2002). A mixture of APIs in an organism can cause synergistic (the effect of the mixture of APIs is greater than the sum of its components), additive (the effect of the mixture is the sum of the effects from the specific APIs) or antagonistic (the mixture of APIs has a lesser effect than the effect of the single compound, e.g. enzyme induction) effects (Yang *et al*., 2018). Multiple chemicals must be tested as the mode of action (MOA), and effects can be unique to a specific chemical cocktail (Altenburger *et al*., 2019). The increasing number of pharmaceutical mixtures, limited occurrence data and tremendous diversity of APIs pose significant challenges to ecotoxicology (Connon *et al*., 2012). To address this knowledge gap, a robust monitoring strategy that includes EBMs with chemical analysis is advised.

2.3 Selection of Pharmaceuticals for Study in EMPIRE

Based on a review of the legislation and literature, 16 pharmaceuticals (Table 2.1) were selected based on their reported consumption globally, poor removal rates in CAS WWTPs, persistence/pseudo-persistence in the aquatic environment and inclusion in prioritisation studies (on the WFD watch list or candidates for the updated watch list).

CECs, such as pharmaceuticals, are frequently found in aquatic ecosystems. Information on sublethal effects of exposure to commonly detected concentrations of CECs is lacking, and the limited availability of toxicity data makes it difficult to interpret the biological significance of occurrence data. However, the ability to evaluate the effects of CECs on aquatic ecosystems is growing in importance as detection frequency increases. Figure 2.3 shows the potential for development of a water toxicity profile based on observed and measured effects, including bioassays and bioanalytical methods with contaminant detections. The information requires both analytical and bioanalytical data to be gathered in real samples.

2.4 Conclusion and Future Perspective

Dealing with a variety of disposal methods frequently creates more waste streams, making waste management a more complicated process. Waste treatment poses a unique challenge when considering pharmaceuticals, as higher treatment costs typically accompany targeted treatment processes that are more efficient. This puts up significant financial barriers, limiting the ability to deal

Table 2.1. Initial selected pharmaceuticals and their predicted no-effect concentration values (PNECs)

Number	Analyte of interest	PNEC (µg/L)	Rationale for selection[a]
1	17β-Estradiol (steroid hormone)	0.0004	PS
2	Estrone (steroid hormone)	0.0036	PS
3	Diclofenac (anti-inflammatory)	0.05	PS
4	Erythromycin (macrolide antibiotic)	0.2	PS
5	Clarithromycin (macrolide antibiotic)	0.12	PS
6	Azithromycin (macrolide antibiotic)	0.019	PS
7	Amoxicillin	0.078	Second and third watch list
8	Ciprofloxacin (fluoroquinolone antibiotic)	0.089	Second and third watch list
9	Trimethoprim (antibiotic)	0.05	Third and fourth watch list
10	Sulfamethoxazole (sulfonamide antibiotic)	0.1	Third and fourth watch list
11	Venlafaxine (antidepressant)	0.0061	Third and fourth watch list
12	O-desvenlafaxine (antidepressant – metabolite)	0.0061	Third and fourth watch list
13	Gemfibrozil (lipid regulators)	0.8519–1.56	Provisional for fifth watch list
14	Gabapentin (anticonvulsant)	10	Provisional for fifth watch list
15	Metformin (biguanide)	10–160	Fourth watch list
16	Carbamazepine (anticonvulsant)	0.5	PS

[a]Watch list under Article 8b of Directive 2013/39/EU expanded under EU Commission Decision 2015/485.
PS, proposed priority substance, included in the proposal amending Directives 2000/60/EC, 2006/118/EC and 2008/105/EC.

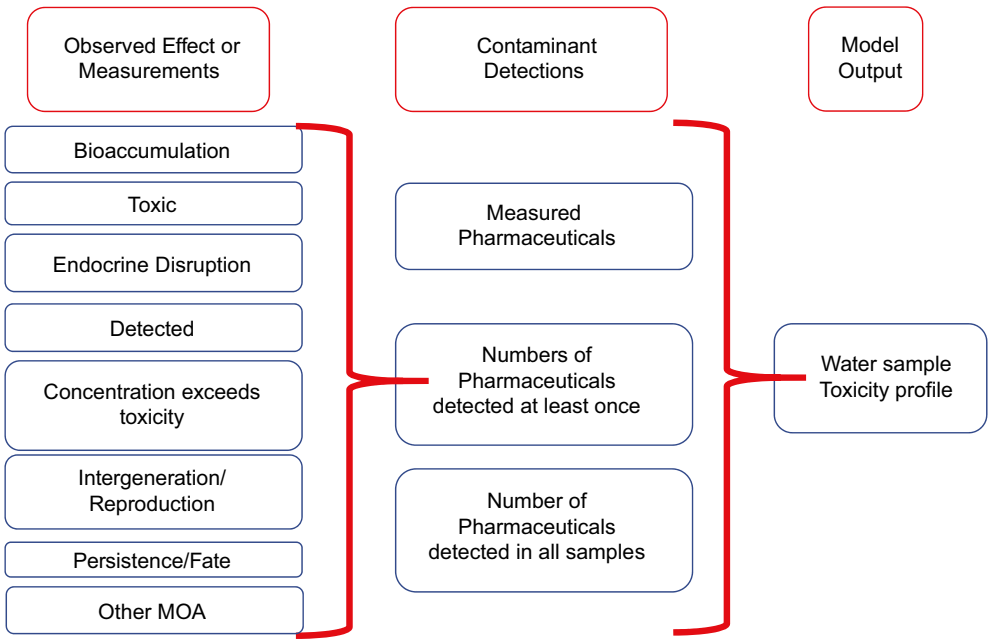

Figure 2.3. Proposed approach to developing a water toxicity profile based on chemical occurrence and biological effects.

with pharmaceuticals in waste water, and increases the need to consider strategies to reduce APIs entering waste water streams. To identify which APIs pose an environmental hazard and therefore need to be prioritised, both novel and targeted monitoring strategies must be developed.

Recommendations for addressing the growing challenge of pharmaceuticals in the Irish aquatic environment include the following:

- A robust monitoring strategy is needed to determine the presence of and associated risks posed by APIs in Irish surface waters.

- The precautionary principle must be used to address the risk of pharmaceutical pollution, as the complexity of risk assessing a multicomponent pharmaceutical mixture may result in underestimating the actual effects.
- There is a need to address each aspect of the life cycle of pharmaceuticals to reduce and manage release into surface waters.
- With the projected increase in demand for pharmaceuticals, associated with climate change-related impacts and COVID-19, source-directed and end-of-pipe measures must be implemented.
- Further research and monitoring campaigns are needed to better inform policymakers and government officials and help create prevention and mitigation strategies, including improving WWTP treatment technologies.
- There is a need for increased funding for public awareness campaigns and pharmacy take-back schemes.

3 Monitoring and Occurrence of Pharmaceuticals in Ireland

3.1 Objectives

- To develop analytical methods for detecting pharmaceuticals.
- To identify compounds for bioassay application.
- To measure concentrations of watch list pharmaceuticals in Irish surface waters.
- To determine temporal variability in pharmaceutical occurrence in selected surface waters.

3.2 Methods

3.2.1 Sampling sites

Samples were taken during the period 2018–2021 from the River Annalee in County Cavan, the River Nore in County Kilkenny (Inistioge), the River Suir in County Tipperary (Kilsheelan) and the River Liffey in County Dublin (Lucan), noted on the site map (Figure 3.1). One sample was also taken from the River Shannon in County Clare (Killaloe)

Figure 3.1. Map of Ireland showing the locations of the four sampling sites.

in December 2018. The project gathered data for watch list (European Commission Joint Research Centre, 2020) monitoring, which included a range of chemicals, including some pharmaceuticals. Sites were selected to represent a range of waterbody statuses and risk levels, as indicated by the WFD, with information on all catchments taken from the EPA catchments website.[2]

3.2.2 Sample analysis

Field sample collection and preparation

Field samples were collected from four sites located around Ireland over 4 years, with an additional fifth site sampled in December 2018 only. Samples for detecting substances in the second watch list were collected during December 2018, July and August 2019, and September and October 2020. For the third watch list, sampling was undertaken in March, May and September 2021 and March 2022. Single grab field samples were collected on 1 sampling day per month for each site in either 1 L clear glass Duran bottles, 2.5 L amber glass bottles or 1 L Nalgene Amber HDPE bottles. Samples were preserved by acidification to pH 3 using sulfuric acid.

Field measurements of temperature, dissolved oxygen, turbidity and pH were collected for the 2020 samples onwards using a YSI EXO3 multiparameter water quality sonde. The samples from 2018 and 2019 were stored in a freezer (–18°C) until extraction, while the 2020 samples were refrigerated and processed as soon as possible following sample collection. For the third watch list, the March and May 2021 samples were frozen until extraction, whereas the September and March 2022 samples were processed immediately.

Samples were divided into triplicate 100 mL aliquots for extraction. Additional equal aliquots of each sample were collected for a composite sample matrix for calibration and validation experiments. Before extraction, each frozen sample was defrosted slowly in a refrigerator (4°C) and then all samples were filtered using nylon filters with a pore size of 0.45 µm prior to spiking with internal standard. From 2020 onwards, samples were also spiked with 0.1 M ethylenediaminetetraacetic acid to a final concentration of 0.1%.

Solid-phase extraction and high-performance liquid chromatography–mass spectrometry conditions

Extraction and analysis methods were modified over the length of the study as the watch list was updated (European Commission Joint Research Centre, 2022). Solid-phase extractions were conducted using Oasis hydrophilic–lipophilic balanced 6 mL, 200 mg bed mass, 30 µm particle cartridges. Liquid chromatography was performed using an Agilent high-performance liquid chromatography stack equipped with a 1290 Infinity II LC multisampler, binary pump and multiple-column thermostatted compartment (Agilent, Cheadle, UK). Chromatographic separation was achieved for all methods using a 2.1 × 150 mm, 1.9 µm particle size Infinity Lab Poroshell 120 EC-C18 column (Agilent, Cheadle, UK). Mass spectrometry was performed using a 6470A triple quadrupole mass spectrometer (Agilent, Cheadle, UK).

3.3 Results and Discussion

During the EMPIRE project, sampling was carried out to (i) determine the pharmaceuticals to select for bioassays and (ii) observe their occurrence and concentration and temporal and spatial variability.

To this end analytical methods were developed and optimised to measure target analytes.

3.3.1 Analytical method for detecting pharmaceuticals in surface waters

To measure the concentration of target analytes it is necessary to optimise analytical methods and determine method robustness. The analytical method limit of quantitation (LOQ) is shown in Table 3.1 for the target analytes. These were found to be below the maximum LOQ legislated by the EU, with the exception of oestrogens, which, it is often noted, can be challenging to analyse in terms of whether they meet the required detection limits. These challenges are a result of their rapid degradation and low environmental concentrations. Furthermore, as quantitation limits are commonly set in accordance

2 https://www.catchments.ie (accessed 5 June 2024).

Table 3.1. Table of calibration and validation results of pharmaceuticals measured in a composite sample made from the Nore/Liffey/Suir/Annalee surface water grab samples

Number	Pharmaceutical	LOD (ng/L)	LOQ (ng/L)	R^2 ($n \geq 5$)
1	Metformin	1.74	5.26	0.982
2	Amoxicillin	6.51	19.72	0.982
3	Gabapentin	2.98	9.03	0.982
4	Trimethoprim	1.37	4.14	0.994
5	Ciprofloxacin	0.77	2.32	0.989
6	Sulfamethoxazole	0.91	2.76	0.997
7	O-desmethylvenlafaxine	0.82	2.48	0.997
8	Venlafaxine	1.58	4.78	0.99
9	Carbamazepine	1.63	4.94	0.989
10	Erythromycin	6.21	18.82	0.973
11	Clarithromycin	2.51	7.61	0.984
12	Azithromycin	2.80	8.50	0.980
13	Diclofenac	1.51	4.59	0.986
14	Gemfibrozil	1.63	4.93	0.984
15	Estrone	2.64	8.01	0.968
16	17β-Estradiol	4.44	13.45	0.99

LOD, limit of detection; R^2, coefficient of determination.

with predicted no-effect concentrations (PNECs), the low LOQ-PNEC criterion set for oestrogens by the WFD (PNEC: 17-alpha-ethinylestradiol = 0.035 ng/L; 17β-estradiol = 0.4 ng/L; estrone = 3.6 ng/L) has highlighted the need for a global effort in improving method sensitivity for these free and conjugated oestrogens. All analytical method details are provided in the PhD thesis of Dylan O'Flynn, where further information on sample analyses can be found (O'Flynn, 2024).

3.3.2 Watch list monitoring

The analytical methods for analysis of the second watch list chemicals were applied to field samples taken from a number of sites around Ireland over the span of 3 years, totalling 21 individual samples. Most detections were found to be below the method quantitation levels shown in Table 3.1. Of the pharmaceuticals measured, erythromycin was the micropollutant that occurred at the highest individual concentration in a single sample: in the August 2019 River Liffey sample, 36 ng/L erythromycin was detected.

The least frequently detected pharmaceuticals were amoxicillin and ciprofloxacin. These results are generally similar to those found by other EU countries, as stated in the review of the first watch list, in which clarithromycin and estrone were among the most frequently detected compounds at quantifiable levels. Clarithromycin, estrone and diclofenac had a quantification frequency of over 50% in samples taken in 25 EU Member States (Barbosa *et al*., 2016).

The analytical challenge with these chemicals relates to the sample matrix effects, which can lead to poor detection or recovery when using sample preparation methods such as solid-phase extraction. The samples collected at the start of the EMPIRE project were refrigerated and then analysed later. Storage conditions may affect analyte integrity, leading to either no detection or reduced concentrations. While this is an appropriate storage method, further work is needed to determine the optimal conditions for a particular sample matrix. The ideal approach is analysis immediately following sample collection.

The hormone 17β-estradiol (E2 in Figure 3.2) was detected in all 2018 and 2019 samples taken; while the majority of detections were below the LOQ (Table 3.1), the detections may be significant. Estrone (E1 in Figure 3.2) was detected in all 21 samples taken over the 3-year period; however, it was only quantifiable in the River Annalee September 2020 sample and the River Liffey samples from October and September 2020.

The oestrogen group of compounds was found in both urban and rural water samples. These compounds are used for medications such as the contraceptive pill and hormone therapy treatments, and are also commonly used in agriculture as growth regulators in livestock. The next most frequently detected analyte was clarithromycin, which was detected in 17 out of 21 samples; however, in the majority of samples it was below the quantitation limit. The two quantifiable detections of clarithromycin were both from River Liffey samples (from December 2018 and August 2019). Clarithromycin is one of the "preferred" antibiotics for use in primary care prescriptions, according to advice published by the Health Service Executive, so its prevalence is not unexpected, particularly in urban areas. The other macrolides were the next most frequently detected antibiotics, as seen in Figure 3.2.

In samples monitored from March 2021 to March 2022 (third watch list; European Commission Joint Research Centre, 2020), the total number of samples was 16 (with each sample analysed in triplicate). The largest individual analyte concentrations detected were in the River Suir, where in May 2021 32 ng/L trimethoprim (Figure 3.3) and 25 ng/L ciprofloxacin were found. These compounds are both pharmaceuticals used for the treatment of bacterial infections.

Trimethoprim is typically used for the treatment of urinary tract infections such as cystitis, although it has also been prescribed for other ailments such as chest infections. Ciprofloxacin is a broad application antibiotic used for a range of purposes (including treating chest infections). One hypothesis for these findings could be related to the high levels of COVID-19 infections in the country in the months preceding this sampling event. COVID-19 has repeatedly been shown to cause lasting lung damage following even minor infections, possibly making recovered patients more susceptible to bacterial infections later and leading to an increase in the use of these antibiotics. In addition to these occurrences, 12 ng/L venlafaxine was found in the River Suir in March 2021. The River Liffey site was found to have the most quantifiable detections; notably, 20 ng/L sulfamethoxazole was found in a September sample. Sulfamethoxazole is a common sulfonamide antibiotic and is often prescribed in conjunction with trimethoprim. These combined medications, also known as co-trimoxazole, are typically administered at a 5:1 ratio of sulfamethoxazole to trimethoprim (Anja Coors *et al.*, 2017). Interestingly, this is similar to the occurrences of these analytes in field samples, where they were found in a ratio of 4.67:1. The Annalee and the Nore rivers showed considerably fewer high-level detections, with both rivers having only two analytes above the LOQ: venlafaxine and amoxicillin.

However, while some notable detections above the LOQ were found, most analyte detections in the environmental samples were below quantitation levels. All analytes were detected in at least three samples, and 14 out of a total 19 analytes were detected in 50% of samples or more.

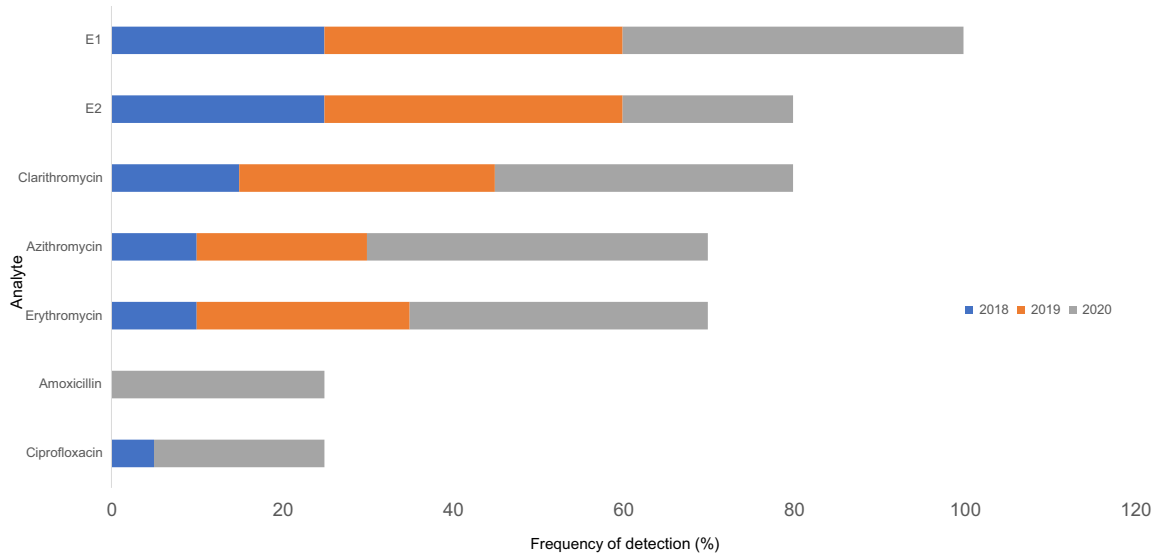

Figure 3.2. Results showing the frequency of detection of pharmaceuticals from the second watch list.

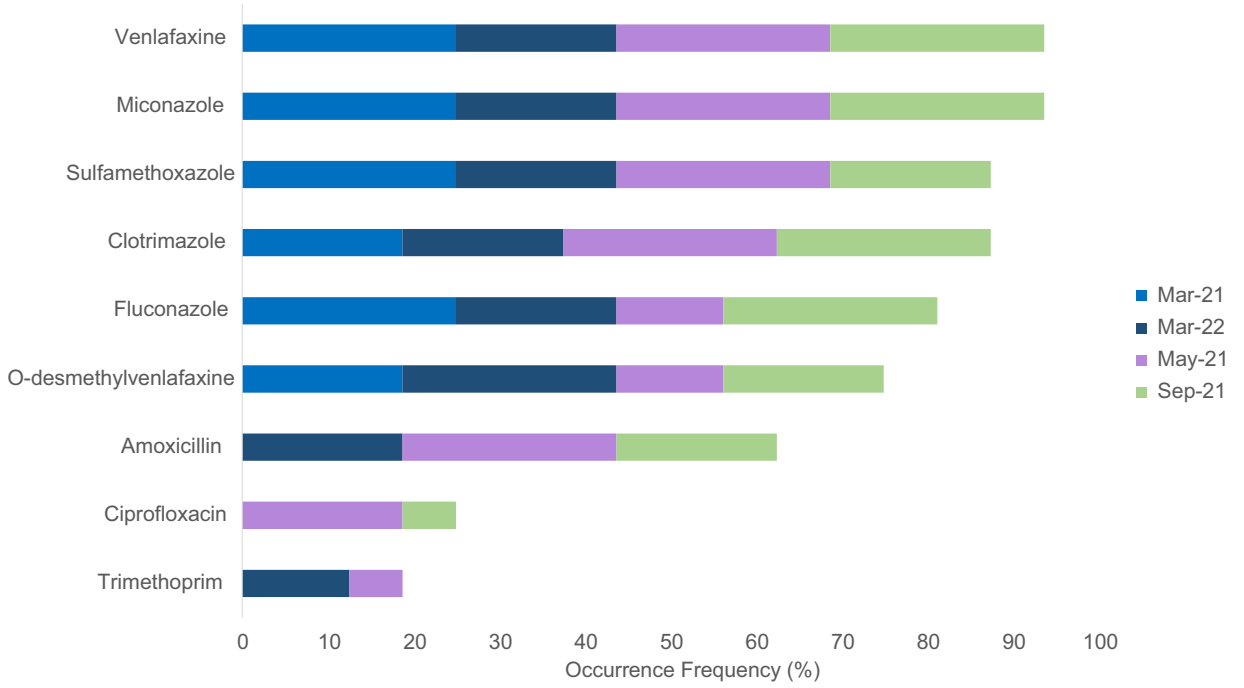

Figure 3.3. Results showing the frequency of detection of watch list pharmaceuticals from the third watch list.

The most frequently detected analyte was venlafaxine, which was detected in every sample aside from the River Suir in March 2022, equating to an occurrence frequency of 93.75%. Venlafaxine was also the analyte most frequently detected at quantifiable levels, at concentrations ranging from 8 ng/L to 14 ng/L. The majority of quantified detections occurred in March 2021. Venlafaxine is a serotonin–noradrenaline reuptake inhibitor (SNRI) prescribed for the treatment of depression and some anxiety disorders. Venlafaxine is Ireland's most common SNRI, and prescriptions for it under the brand name Effexor increased by 48% in the 10 years from 2007 to 2017 (Vethaak *et al*., 2017). It is also interesting to consider the possible impact the COVID-19 pandemic has had on the high occurrence of this compound. Venlafaxine has been found at concentrations above its PNEC value in the aquatic environments of multiple countries within the EU and in the UK. Concentrations have ranged from < 1 ng/L (UK) to 159 ng/L (Portugal) (Johnson *et al*., 2015). The results found for Irish rivers are in line with the findings from other Member States.

Based on these findings, venlafaxine and sulfamethoxazole were selected for tests using different bioassays.

3.3.3 Temporal and spatial monitoring

The sites described above were sampled on six occasions to detect repeat occurrences of the target pharmaceuticals. The results shown in Table 3.2 represent a summary of one of the sites – the River Liffey. Figure 3.4 summarises the occurrence at each site and Figure 3.5 shows a sum total concentration of the target pharmaceuticals over the time period studied. Figure 3.5 illustrates the analytes that were detected in greater or lesser concentrations at each time point, illustrating, for example, an increase in detection of gemfibrozil between March and May 2021. The data show that more frequent monitoring is desirable to determine trends in chemical detections and, critically, the pharmaceuticals that may occur due to increased prescription or health-care demands.

While the sampling periods were infrequent, there is evidence of increased pharmaceutical occurrence in surface waters in early 2021. This may be due to increased use of pharmaceuticals because of the COVID-19 pandemic or due to waste water treatment performance. While it is not possible to confirm this, there is clear evidence of the levels of pharmaceuticals that are reaching surface waters.

Table 3.2. River Liffey summary results showing where substances are quantifiable

Pharmaceutical	March 2022 (ng/L)	September 2021 (ng/L)	May 2021 (ng/L)	March 2021 (ng/L)	October 2020 (ng/L)	September 2020 (ng/L)
Metformin	<LOQ	<LOQ	6.55±0.38	6.46±0.33	5.09±0.34	5.48±0.24
Amoxicillin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Gabapentin	75.87±5.2	50.74±1.8	119.29±4.16	<LOQ	54.40±1.74	45.76±2.33
Trimethoprim	26.72±1.74	13.86±0.23	8.52±0.21	4.62±0.47	9.33±0.25	8.01±0.70
Ciprofloxacin	4.33±0.39	19.29±1.68	4.31±0.23	6.09±0.37	3.20±0.20	11.25±0.41
Venlafaxine	37.27±1.25	64.45±4.90	56.85±0.70	16.13±0.78	20.79±2.58	19.91±0.23
O-desmethylvenlafaxine	58.57±2.02	90.88±5.74	85.55±1.76	20.60±1.22	27.58±0.26	28.04±0.34
Sulfamethoxazole	290.25±2.61	204.78±26.54	102.62±5.23	69.32±1.33	171.05±1.60	151.28±3.90
Carbamazepine	15.94±0.21	26.44±3.14	22.61±0.75	8.48±0.22	7.88±0.10	8.20±0.10
Azithromycin	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD
Clarithromycin	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOD
Erythromycin	<LOD	<LOD	<LOD	55.25±7.27	<LOD	<LOD
Diclofenac	<LOQ	6.76±0.65	6.87±0.08	92.34±2.02	5.83±0.21	6.39±0.58
Estrone	<LOQ	<LOD	5.17±0.21	21.39±0.69	<LOQ	<LOD
17β-Estradiol	7.08; single result	<LOD	<LOD	8.63±0.33	<LOD	<LOD
Gemfibrozil	<LOD	<LOD	85.68±0.88	283.63±4.92	11.24±0.06	5.47±0.008

Note: Similar data are available for all sites.

LOD, limit of detection.

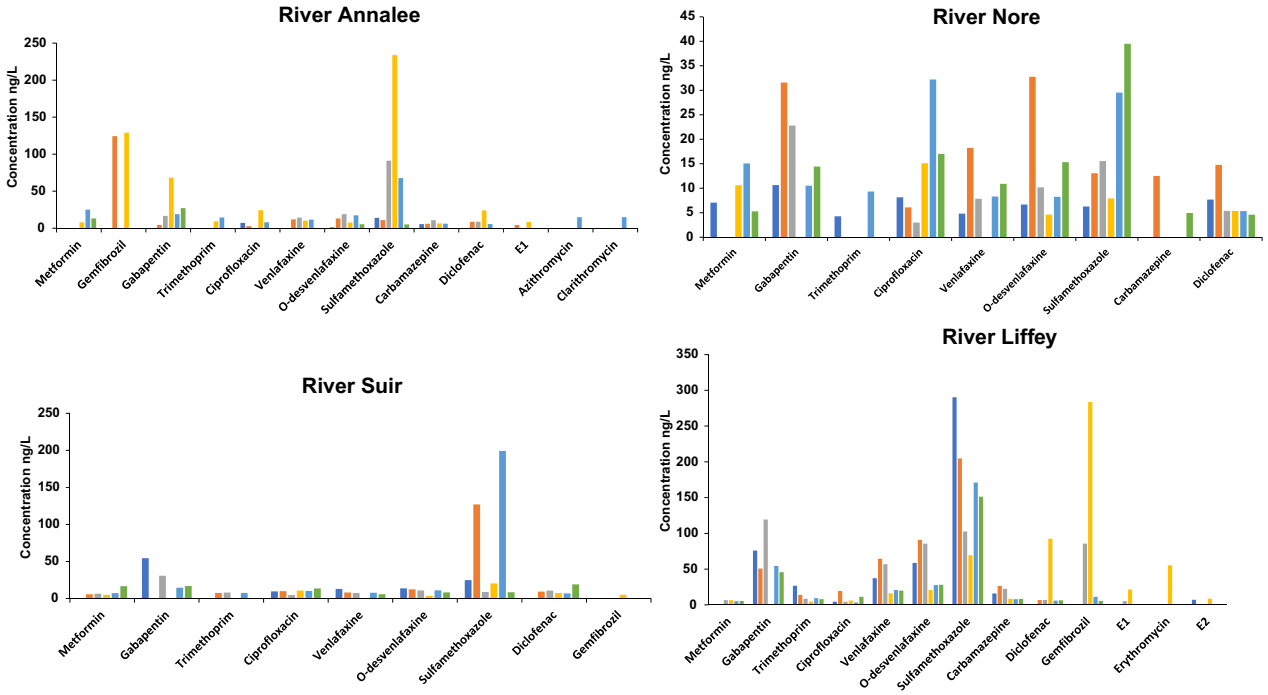

Figure 3.4. Graphs showing pharmaceutical compounds detected in the River Annalee, River Nore, River Suir and River Liffey during six sampling campaigns from 2020 to 2022 (light blue, March 2022; orange, September 2021; grey, May 2021; yellow, March 2021; blue, October 2020; green, September 2020).

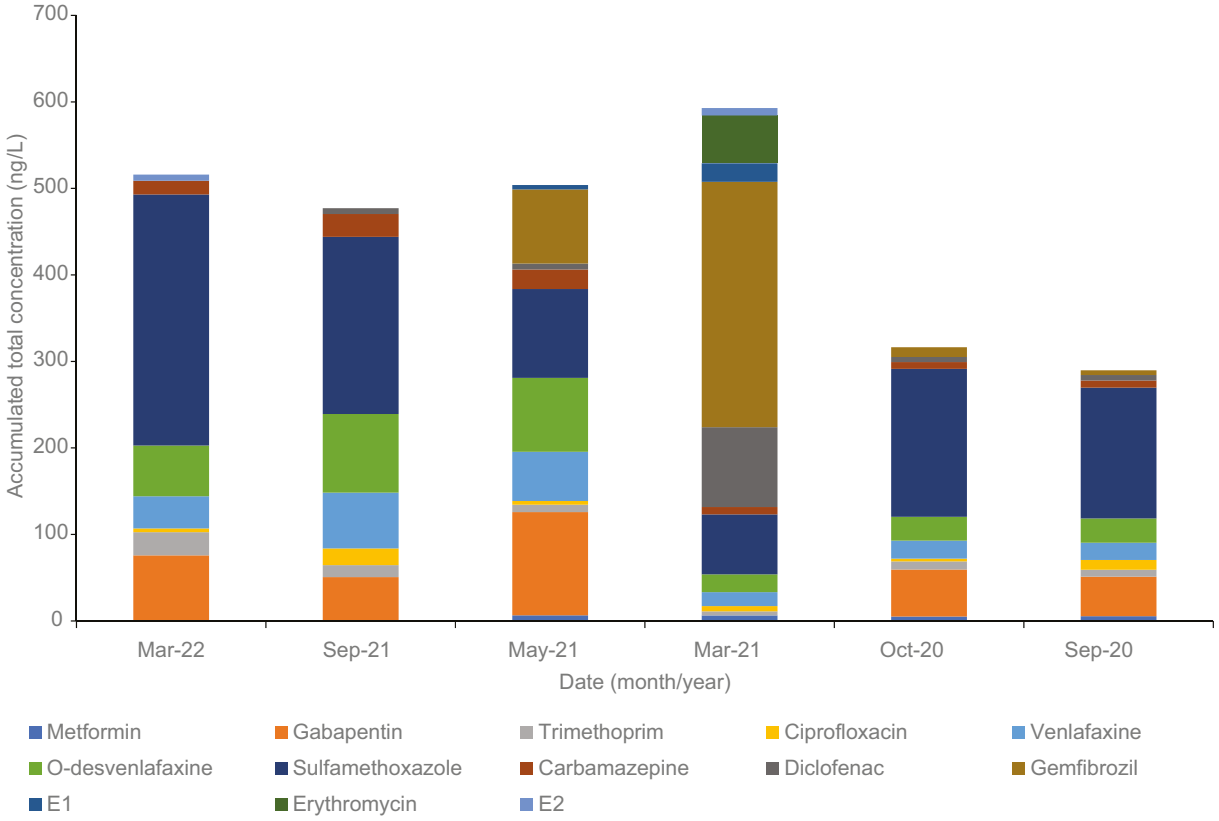

Figure 3.5. Summary of total combined pharmaceutical concentrations measured from samples collected at different times.

3.4 Conclusion

An objective of this chapter was to develop analytical methods for detecting pharmaceuticals in surface waters so that compounds could be selected for application in bioassays. Liquid chromatography–mass spectrometry was used to measure the occurrence of target analytes selected on the basis of prioritisation in legislation and inclusion in watch lists. The analytical methods involved sample collection, solid-phase extraction for analyte enrichment and chromatographic separation. The methods were validated to determine calibration ranges, limit of detection and LOQ values, and the effect of the sample matrix on detections.

A further objective was to measure concentrations of selected pharmaceuticals and to observe temporal variability in their occurrence in Irish surface waters. In addition to data from 2018 to 2020 watch list monitoring, six sampling periods were chosen between September 2020 and March 2022. While the sampling frequency cannot provide a high resolution in temporal variability of pharmaceuticals, it provides an excellent perspective on the repeated occurrence of many pharmaceuticals over the period studied. There is some evidence that levels of some pharmaceuticals, such as venlafaxine and others, increased in samples during the COVID-19 pandemic, indicating that surface water analysis may be a good indicator of human health.

Based on the measured values and number of analyte occurrences it was possible to select individual pharmaceuticals for bioassays and chemicals for mixture analysis studies (Chapter 4), such as sulfamethoxazole, venlafaxine, gemfibrozil, amoxicillin and trimethoprim. The data gathered here are also used to inform the surface water risk assessment (section 6.3).

Further studies on surface water epidemiology are proposed to provide an indication of temporal variation in societal health. The fact that these pharmaceuticals are measured in surface waters indicates that their removal in WWTPs is inadequate. Necessary improvements in WWTPs will aid removal of these micropollutants – and until then more frequent monitoring of surface waters is recommended.

4 Establishment of Effect-based Monitoring Tools for Pharmaceuticals

4.1 Objectives

- To challenge chosen bioassays with target analytes and mixtures.
- To identify model parameters to assess aquatic risk level.

4.2 Introduction

EBMs are currently used globally to monitor the bioactivity of chemical pollution in surface waters. Here, we investigated biological activities of specific pharmaceuticals using EBMs (Figure 4.1) in the context of Ireland's surface waters. EBMs for the determination of water quality can be used to identify potential pollution hotspots using a combination of bioassays and chemical analyses; this provides a significantly better assessment of water quality than what each individually can provide.

The suitability of any particular EBM approach must be evaluated in terms of method, cost, practicality and capability to provide information that can be translated into management practices useful for achieving the monitoring programme objectives.

Three approaches can be considered:

1. bioassays, both *in vitro* and *in vivo*, which measure the toxicity of environmental samples under defined laboratory conditions, on cellular or individual levels, respectively;
2. biomarkers, i.e. biological responses at the cellular or individual levels, measured in field-exposed organisms;

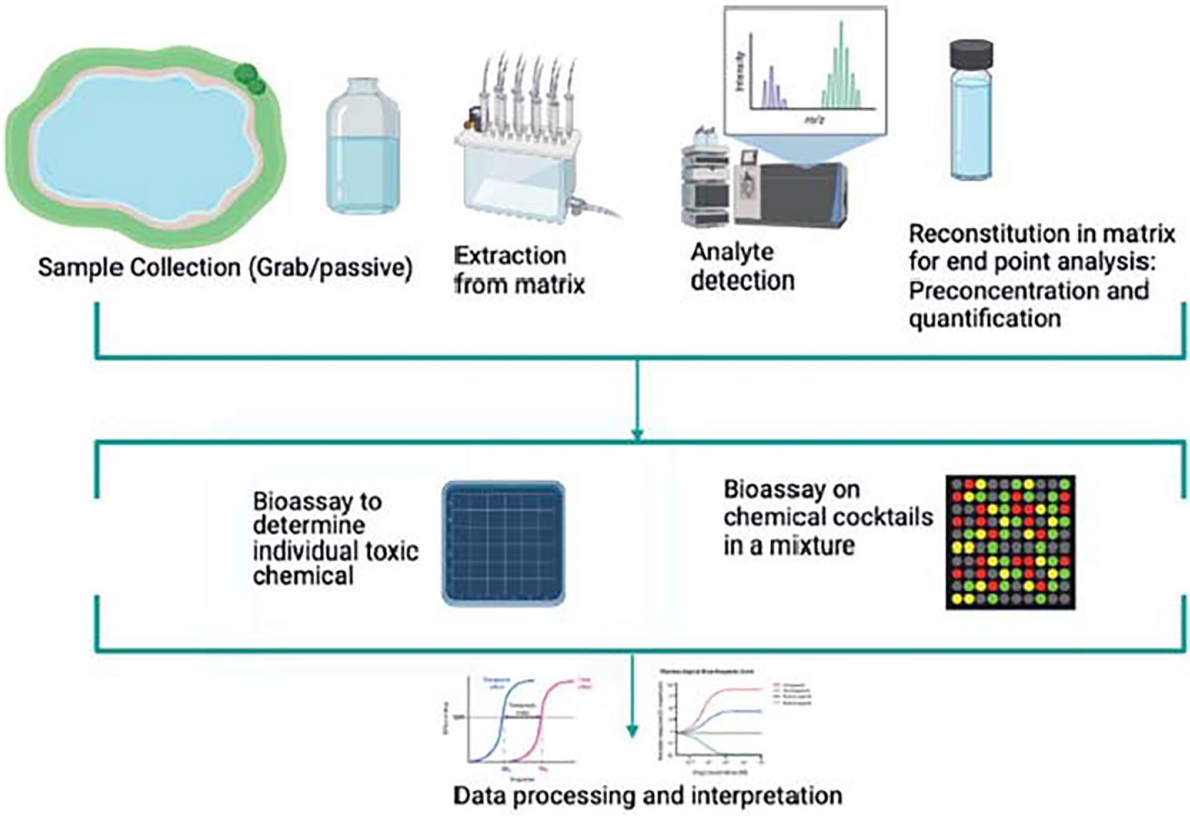

Figure 4.1. A typical workflow for assessment of water samples to determine the biological effects of chemicals.

3. ecological methods, measuring changes observed at higher biological organisation levels, i.e. the population and/or community.

4.3 Bioassays in EMPIRE

In the EMPIRE project, YES and YAS yeast-based bioassays for oestrogenicity and androgenicity, respectively, in addition to an assessment of the growth inhibition of the cyanobacteria *Dolicospermum flos aquae*, were employed to assess bioactivities of eight selected pharmaceuticals. Amoxicillin, carbamazepine, diclofenac, erythromycin, gemfibrozil, sulfamethoxazole and trimethoprim (all 100 mg/L) were used to treat yeast cells in the log phase of growth for 24 h. *D. flos aquae* was also exposed to the same drugs for 72 h for EC_{50} (half-maximal effective concentration) determination and 120 h for mixture effect studies. This was to understand molecular and whole organism toxicity and the bio-effects of these chemical pollutants to highlight the necessity of molecular EBMs for determination and detection of pharmaceutical pollutants in Irish surface waters. The methods used (Table 4.1) have been reported previously (Brack *et al*., 2019). Where concentrations were chosen at the mg/L level, a rapid bioassay effect could be observed. These concentrations were not environmentally relevant (normally ng/L or µg/L); however, they allowed screening of a number of assays for potential application.

4.4 Methods

4.4.1 Battery of bioassays

The agonist controls for the YES and the YAS assays were 17β-estradiol and 4-hydroxytamoxifen, respectively, while the antagonist controls were 5α-dihydrotestosterone and flutamide, respectively.

4.4.2 Growth inhibition of Dolicospermum flos aquae

Algae culture

D. flos aquae was grown in Jaworski's medium (pH 7.8) at 22.5 ± 2.5°C in an incubator equipped with an orbital shaker rotating at 100 rpm and a constant illumination of 76 µmol $m^{-2} s^{-1}$. Culture of the algae was initially maintained in a 250 mL Erlenmeyer flask containing 100 mL of Jaworski's medium and 1 mL of the algae cells. The Erlenmeyer flasks were initially washed in a biodegradable decontaminant before being rinsed with 50 mM hydrochloric acid and then autoclaved at 121°C for 90 min. The cell number was counted using a light microscope and haemocytometer, after which the growth rate was estimated over the course of 9 days to identify the logarithmic phase. Furthermore, the optical density of the algae cells for each day was calculated from the absorption maxima determined by a wavelength scan to be 680 nm.

Toxicity of pharmaceutical cocktail mixture

To assess the toxicity of a pharmaceutical cocktail, eight concentrations around the EC_{50} of the pharmaceutical with the lowest definitive EC_{50} value were selected and the concentrations were in geometric series. The dose–response curve of the growth inhibition of *D. flos aquae* by the pharmaceutical cocktail was then generated based on this. The cells were cultured as described above and exposed to the pharmaceutical cocktail for 96 h

Table 4.1. Battery of bioassays to cover the environmentally relevant modes of action

Test number	Mode of action
1	Oestrogenicity using YES (yeast) kit
2	Androgenicity activity using YAS (yeast) kit
3	Oxidative stress response using 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) dye or Nrf2 reporter system
4	Activation of aryl hydrocarbon receptor using AhR-CALUX or DR-CALUX
5	Genotoxicity using Ames and umuC or p53-CALUX
6	Glucocorticogenic activity using GR-CALUX
7	Activation of the peroxisome proliferator-activated receptor using PPARγ-CALUX

Note: These represent the highest ranked bioassays for testing in EMPIRE.

Source: Data from Brack *et al*. (2019).

and the cell density was measured every 24 h using a UV/Vis (ultraviolet/visible) spectrophotometer. The cell number was calculated from the calibration curve as described above.

4.5 Results and Discussion

4.5.1 Oestrogen receptor activation

To investigate the potential endocrine activity of the selected pharmaceuticals, the YES/YAS assays were utilised. Potential oestrogenic activities of pure samples of the different pharmaceuticals listed in Table 3.1 were assessed by YES assay. Of all the investigated drugs, only erythromycin and gemfibrozil were found to induce expressions of β-galactosidase, which is consequent upon activation of the oestrogen receptor. The maximum concentration resulting in oestrogen receptor activation was 1.4×10^{-4} M or 100 mg/L for erythromycin and 4×10^{-4} M or 100 mg/L for gemfibrozil (Figure 4.2), which were 1.21×10^{-3} µM and 1.14×10^{-3} µM estradiol equivalents, respectively. This amounted to 0.0329 ng estradiol equivalents/L and 0.031 ng estradiol equivalents/L for erythromycin and gemfibrozil, respectively, and these were both below the effect trigger value of 0.4 ng/L for the YES assay. However, none of these pharmaceuticals was found to activate the oestrogen receptor at concentrations below 10 mg/L.

4.5.2 Oestrogen receptor inhibition

To evaluate the possibility of oestrogen receptor antagonism by our pharmaceuticals of interest, yeast cells expressing hERα were co-exposed to

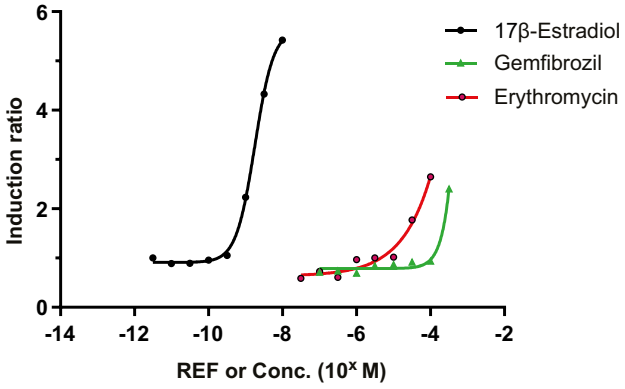

Figure 4.2. Oestrogenicity of erythromycin and gemfibrozil after 24 h exposure to yeast cells.

17β-estradiol and individual pharmaceuticals to establish their activities in inhibiting hERα activation of the *LacZ* operator. Based on the results from this study, these substances can be classified as weakly anti-oestrogenic or strongly anti-oestrogenic compounds. Among the weakly anti-oestrogenic pharmaceuticals were amoxicillin, sulfamethoxazole and trimethoprim, all of which weakly inhibited the oestrogen receptor, as shown in Figure 4.3a. Inhibition of the oestrogen receptor by these compounds was found to be dose dependent, as shown. There was a reduction in the anti-oestrogenic effects of amoxicillin, sulfamethoxazole and trimethoprim when the concentrations increased, as the higher concentrations (up to 10 mg/L) resulted in a plateau in the effects, indicating that higher concentrations may not influence the oestrogen receptor activities further. Gemfibrozil and diclofenac, on the other hand, are strongly anti-oestrogenic compounds, as they both nearly completely inhibited the oestrogen receptor activation in a similar fashion to the tamoxifen control (Figure 4.3b). Gemfibrozil exhibited higher inhibitory activity with a minimum induction ratio of 3.4, which was close to that of the control, tamoxifen (2.8).

The minimum induction ratio of diclofenac, on the other hand, was 5.3 (Table 4.2). The observed mixed agonist–antagonist effect of gemfibrozil seemed to be on the same hERα receptor. This might be possible only if the activity of gemfibrozil is modulated by a change in the conditions of exposure. It is possible that gemfibrozil was acting as a co-repressor that facilitated inhibitory activities of tamoxifen on the hERα. This finding is especially crucial as it highlights the danger of drug mixtures and what potential harm might result from them. As shown above, erythromycin was found to induce some level of oestrogen receptor activation. When it was tested for oestrogen receptor activity inhibition, an inverse inhibition was observed, as shown in Figure 4.3c. This was in contrast to gemfibrozil, and it may indicate that erythromycin acted as a co-activator with tamoxifen, causing it to activate the HERα. Interestingly, this is similar to the effect of estrone on androgen receptor activity in the presence of flutamide (Figure 4.4b). A cocktail of amoxicillin, diclofenac, carbamazepine, sulfamethoxazole and trimethoprim, which were found to have individually no androgenic activities up to 10 mg/L, was also tested, with each of the compounds contributing one-fifth (2 mg/L), and it was found that

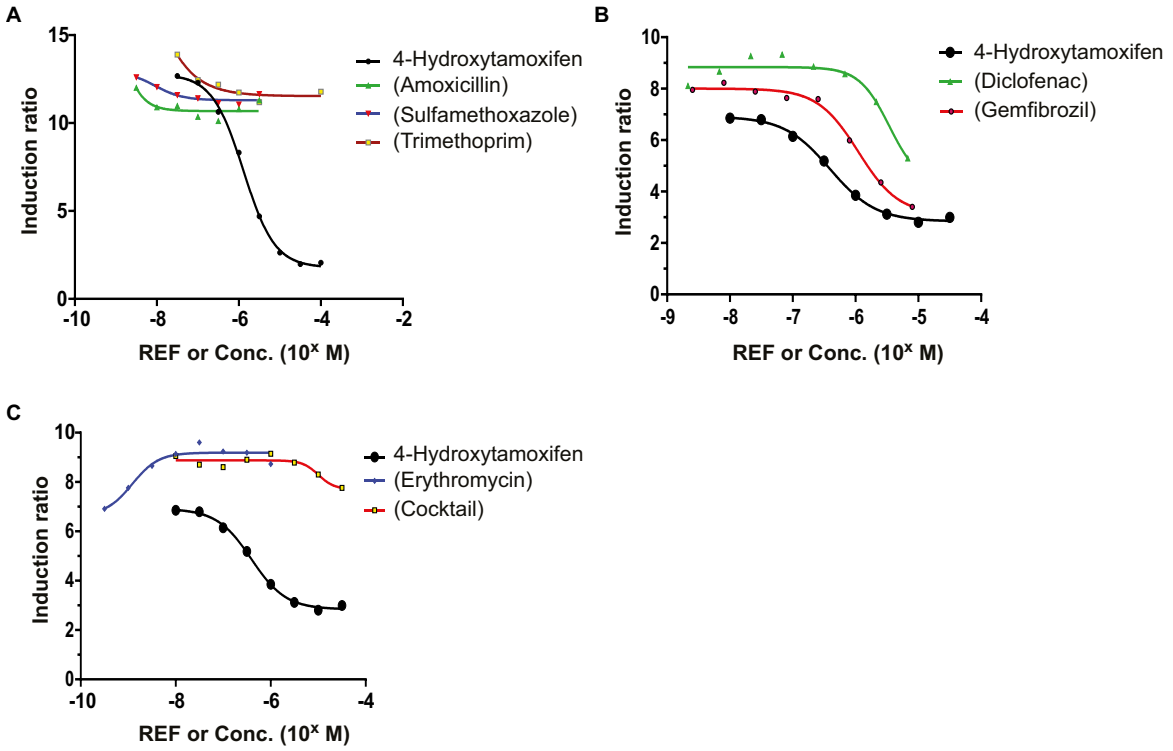

Figure 4.3. Anti-oestrogenicity responses after 24 h yeast cell exposure to pharmaceuticals.

Table 4.2. Anti-oestrogenic activities of pharmaceuticals

YES antagonist assay	IRMin	ECMin (M)	EC_{50} (M)	EC_{50} (mg/L)
Amoxicillin	10.1	8.7×10^{-7}	1.3×10^{-8}	4.75×10^{-3}
Carbamazepine	10.4	1.3×10^{-6}	2.0×10^{-8}	4.7×10^{-6}
Diclofenac	5.3	6.8×10^{-6}	3.5×10^{-7}	10.4
Erythromycin	6.9	7.6×10^{-10}	1.1×10^{-7}	8.07×10^{-2}
Gemfibrozil	3.4	8.0×10^{-6}	4.5×10^{-7}	11.3
Sulfamethoxazole	11.1	3.9×10^{-6}	2.8×10^{-8}	7.09×10^{-3}
Trimethoprim	10.2	1.1×10^{-5}	2.6×10^{-8}	7.55×10^{-3}
Cocktail containing amoxicillin, diclofenac, carbamazepine, sulfamethoxazole and trimethoprim	7.8	2.0×10^{-6}	1.2×10^{-9}	
4-Hydroxytamoxifen (positive control) (tamoxifen)	2.8	3.2×10^{-6}	1.0×10^{-6}	38.8

ECMin, minimum effective concentration; IRMin, minimum induction ratio (IRMin is defined as the lowest induction ratio at a non-toxic concentration for the compound under investigation in the antagonist assay).

this mixture of drugs induced mild androgen receptor inhibition (Figure 4.3c).

4.5.3 Androgen receptor activation and inhibition

Of all the pharmaceuticals that were investigated for possible androgenic activities, only gemfibrozil was found to induce activation of the androgen receptor. The YAS assay determined the 5α-dihydrotestosterone equivalents of samples with a high matrix load. It was found that gemfibrozil at 100 mg/L exhibited 5.89 ng/5α-dihydrotestosterone equivalents/L (Figure 4.4a). This value is also below the effect trigger value for the androgenic receptor, documented to be 217 5α-dihydrotestosterone equivalents/L (Altenburger *et al*., 2018). Similar to erythromycin and gemfibrozil oestrogenic activity, the maximum concentration of gemfibrozil trialled here did not achieve full activation of the androgen receptor to allow calculation of the EC_{50}.

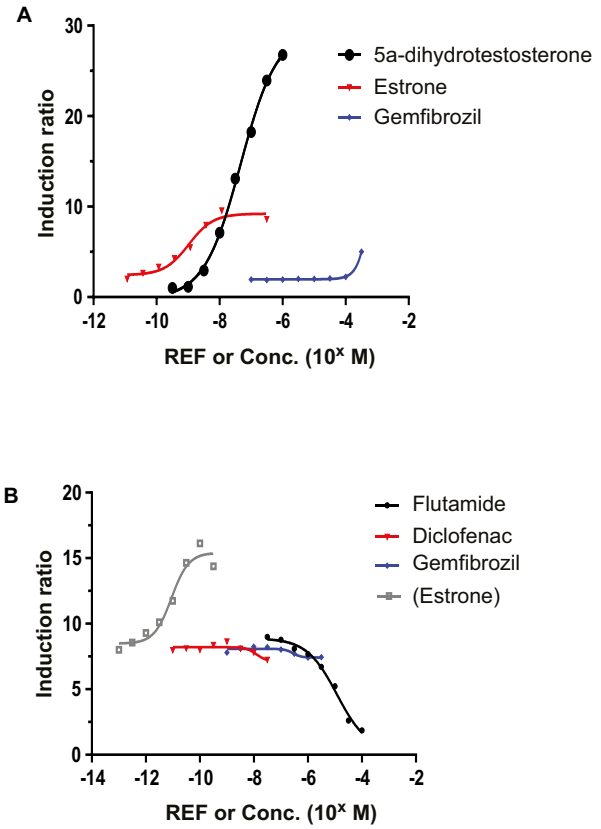

Figure 4.4. (A) Activation of androgen receptor by gemfibrozil and estrone after 24 h of exposure. (B) Mild inhibition of androgen receptor by gemfibrozil and diclofenac, estrone-induced inverse inhibition. Controls were 5α-dihydrotestosterone and flutamide.

4.5.4 Effect of drug mixture on the oestrogen receptor activities

To assess the effect of drug mixtures on the activities of the oestrogen receptor, gemfibrozil and diclofenac, both of which were found to have anti-oestrogenic effects, were selected for treatment of the yeast cells. The result of the yeast cell exposure to the mixture is shown in Figure 4.5a. While the concentration of the mixture was five times lower than the concentration of the individual drugs, the cocktail of gemfibrozil and diclofenac achieved a minimum induction ratio of 1.3, which was higher than that of diclofenac (1.8) alone but almost the same as that of gemfibrozil (1.1). While the concentrations trialled in this study were higher than environmentally relevant concentrations, the findings that a cocktail of the two drugs (gemfibrozil and diclofenac) is more potent at lower concentrations than the single drugs indicates that surface waters containing multiple drugs at very low concentrations through concentration addition may induce biological effects that are not envisaged. Furthermore, this highlights the strength of this bioassay in predicting the bioactivities of different mixtures of pharmaceuticals within the laboratory setting.

4.5.5 Effect of high-concentration mixture on oestrogen receptor: the matrix effect

A surface water sample collected from the River Annalee was used to treat the recombinant yeast cells with or without diclofenac (20 mg/L highest concentration) for 24 h. As shown in Figure 4.5b, the matrix alone induced higher anti-oestrogenic responses, with a minimum induction ratio of 1.3 compared with that of diclofenac alone at 1.8. Interestingly, the diclofenac-spiked matrix achieved a minimum induction ratio of 1.1, highlighting the synergistic anti-oestrogenic effect of diclofenac and the matrix. The reason for the stronger anti-oestrogenic effect of the matrix sample might be the mixture of chemical contaminants in the river sample producing a synergistic inhibitory effect.

When studying the effects on algae, pharmaceuticals tested on the cyanobacteria showed dose-dependent growth inhibition (Figure 4.6). Trimethoprim was found to be the most tolerated chemical. Furthermore, diclofenac was determined to be the driver of toxicity in the mixture containing diclofenac, sulfamethoxazole and trimethoprim.

4.6 Conclusion

There is growing interest in developing a battery of bioassays that can assess the ecological or human impacts of exposure to chemicals of emerging concern, including pharmaceuticals. There is as yet no agreement on an ideal battery of assays that can provide an indicator of toxic and/or mixture effects. The objectives of this chapter were to challenge a chosen selection of bioassays with target analytes and mixtures and to use the information gathered to assist in the identification of model parameters to assess aquatic risk level. A total of seven MOAs were assessed as part of a battery of bioassays. These were applied to a selected list of pharmaceuticals and their mixtures. A second approach to determining biological effects involved an algal inhibition study.

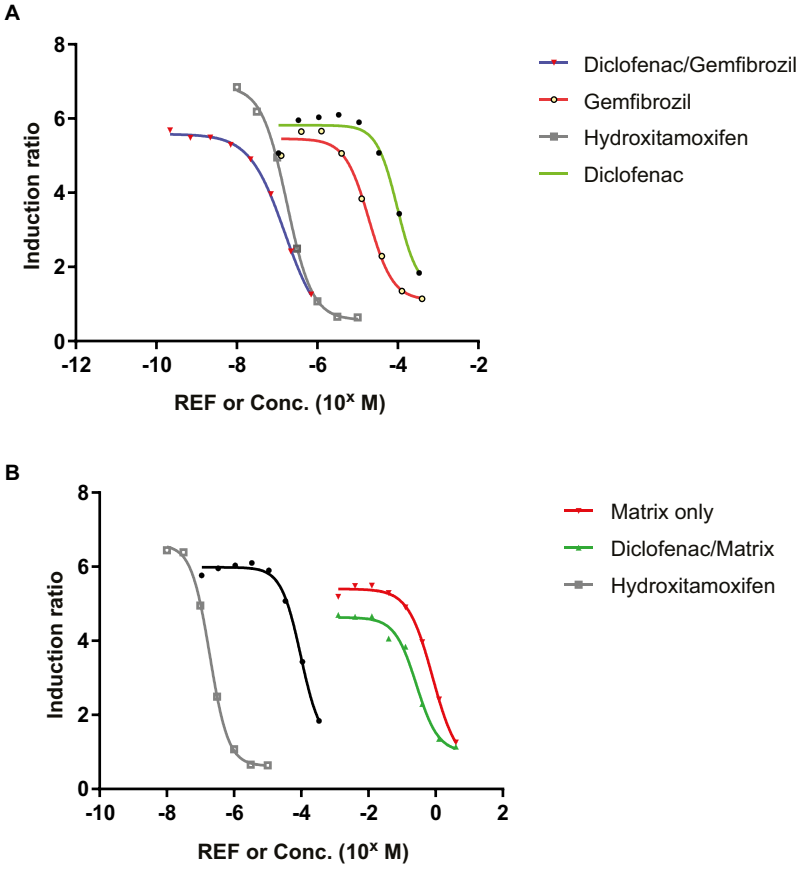

Figure 4.5. Oestrogen receptor: concentration and matrix effect of (A) gemfibrozil and diclofenac, both at 100 mg/L highest concentration for single exposure and 20 mg/L highest concentration in the mixture; and (B) matrix and matrix spiked with 20 mg/L diclofenac on oestrogen receptor inhibition. Control = 4-hydroxytamoxifen. The black line in B denotes induction ratio response data for diclofenac, as are included in A.

In this study, the concentrations used for the MOA EBMs were higher than environmentally relevant concentrations to obtain responses and select potential MOAs for pharmaceutical assessments. When assessing the MOA, it was found that erythromycin and gemfibrozil exhibited the greatest oestrogenic activity of all the pharmaceuticals, while diclofenac showed strong anti-oestrogenic activities. Mixtures containing diclofenac and gemfibrozil showed similar anti-oestrogenic effects. Estrone was the only substance studied that showed an androgenic effect. Diclofenac-spiked and non-spiked water samples from the River Annalee showed an anti-oestrogenic effect in an assessment of sample matrix.

While there is significant effort and cost associated with conducting MOA assessments, as a proof of concept, we have shown that reporter-based systems such as the YES/YAS assays, in combination with toxicity testing of algae species, are useful tools for field applications and can provide useful insights into the biological effects of common pharmaceutical pollutants in Irish surface waters. There is scope for the use of algal assays to determine the toxicity of emerging contaminants and their mixtures and associated environmental matrices to develop threshold values. Thresholds can be designed for receiving waters, and, where they are exceeded, further investigation and compound identification can follow. Our results indicate that the algal studies have potential for use in screening water samples for likely ecological threats or to determine water quality status. A recommendation arising from this research is to utilise algal assessments as a means of evaluating the effectiveness of WWTP processes.

Research on the selection of EBMs continues globally and the recently launched project PARC (Partnership

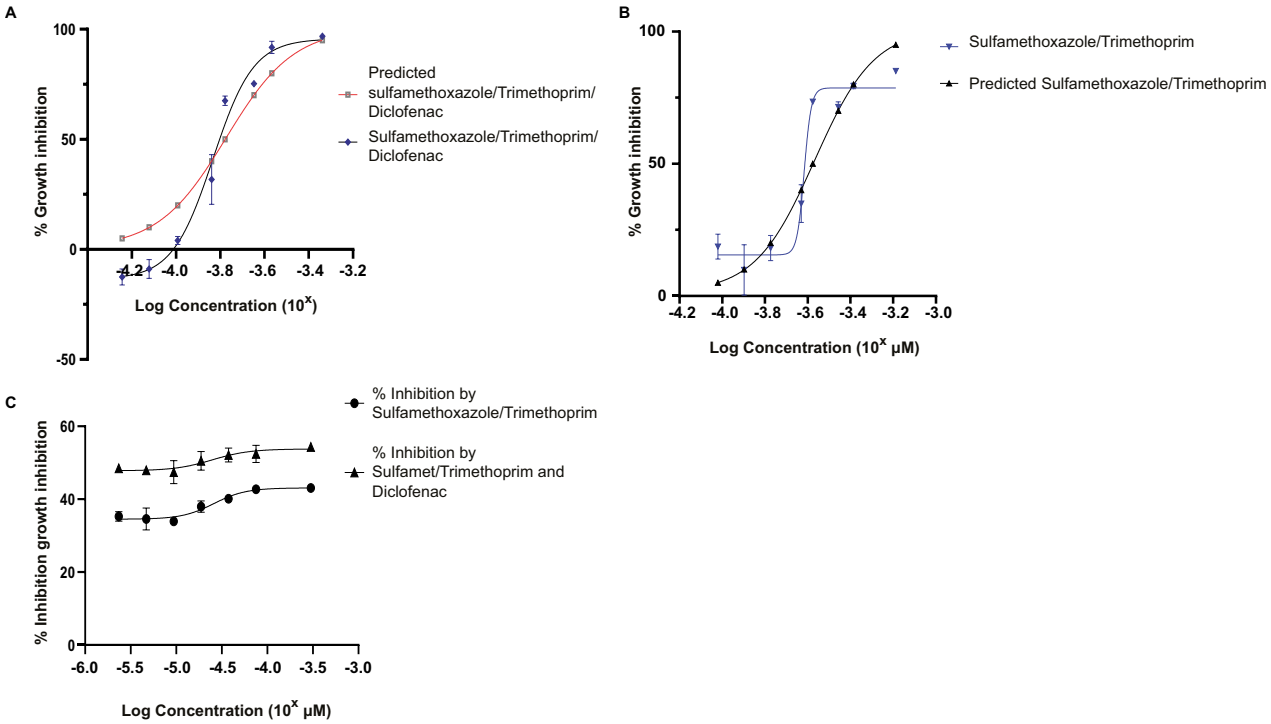

Figure 4.6. Predicted and observed growth inhibition upon exposure of *D. flos aquae* to (A) sulfamethoxazole, trimethoprim and diclofenac and (B) sulfamethoxazole and trimethoprim. (C) Determination of diclofenac toxic burden in mixture containing sulfamethoxazole, trimethoprim and diclofenac. All exposure was carried out over a 120 h period. Data represent mean ± SD for $n = 5$ replicated exposures.

for the Assessment of Risks from Chemicals) aims to develop next-generation chemical risk assessment to protect human health and the environment.[3] The EMPIRE project is very timely and its results contribute to the growing knowledge in this field.

3 https://www.eu-parc.eu (accessed 5 June 2024).

5 Pharmaceutical Ecotoxicology

5.1 Objectives

- To evaluate *D. magna* as a test organism for pharmaceutical assessments.
- To determine pharmaceutical uptake by test organisms.

5.2 Introduction

The uptake of pharmaceuticals into invertebrates such as *D. magna* and *Gammarus pulex* has been traditionally modelled using APIs' physico-chemical properties (log k_{ow} and log P), reflecting the passive diffusion into an organism. *D. magna* is a suitable model organism to investigate the genetic and toxicological effects of SNRIs. Advantages of using *D. magna* are that its genome has been fully coded, it has many neurotransmitters in common with vertebrate organisms that can be affected by neuroactive drugs, and its common presence and role in surface water ecosystems and food webs (Corotto *et al*., 2010).

5.3 Materials and Methods

5.3.1 Animal culture

D. magna was obtained from a parthenogenetic reproduction initiated for a single mother hatched from the ephippium in accordance with the guidelines from Microbiotests Inc. (Belgium). The crustacean culture was maintained in 2.5 L tanks with 2 L of aerated culture medium under a light:dark period of 16:8 h with a constant temperature of 19 ± 1°C. The medium (16 mg of $NaHCO_3$, 100 mg of $CaSO_4 \cdot 2H_2O$, 20 mg of $MgSO_4$ and 3 mg of KCl per litre of deionised water adjusted to a pH of 7.2) was used for all experiments. The number of cultured daphnids was about 30 animals per litre. *D. magna* was fed three times a week with 10 mL per tank of spirulina suspension (10 mg/1 mL). The 21-day-old daphnids were used in the experiment. The study was adapted from *The OECD Guideline for the Testing of Chemicals*, Section 2 – Test No. 211: *D. magna* reproduction test protocol (Figure 5.1; OECD, 2012). Organisms were exposed to a concentration at PNEC or 10 times the PNEC value.

5.3.2 Extraction protocol

Prior to solid-phase extraction, 30 daphnids were placed in a 2 mL Eppendorf tube and 1.5 mL of acrylonitrile (ACN) was added along with glass beads. The samples were then homogenised at 300 rpm for 180 s using a tissueliser. Subsequently, the Eppendorf tube was sonicated for 20 min and centrifuged at 4000 RCF for 5 min. A 1 mL volume of ACN extract was then placed into 100 mL of deionised water. This step was repeated two more times by adding 1 mL of ACN to the Eppendorf tube. The final step involved the removal of the remaining 1.5 mL of ACN in the Eppendorf tube (total volume = 3.5 mL ACN into 100 mL of deionised water). Necessary spiking was carried out directly into the solid matrix with a working standard. Solid-phase extraction was carried out; this was adapted from previous work by Rapp-Wright *et al.* (2023).

5.3.3 Reproduction studies

Young female daphnids, aged less than 24 h at the start of the test (neonates), of the species *D. magna* were exposed to the test substance (pharmaceutical) added to water at a range of concentrations. The test duration was 21 days. At the end of the test, the total number of living offspring produced was assessed. The survival of the parent animals and the time to production of the first brood were recorded.

5.3.4 Heart rate study

In our study we tested concentrations corresponding to levels of PNEC, PNEC × 10 and PNEC × 100. To achieve reproducible results and minimise statistical variation, we used 21-day-old animals. They were maintained at 19°C in the medium based on Ballygowan water and were fed with spirulina powder twice a week.

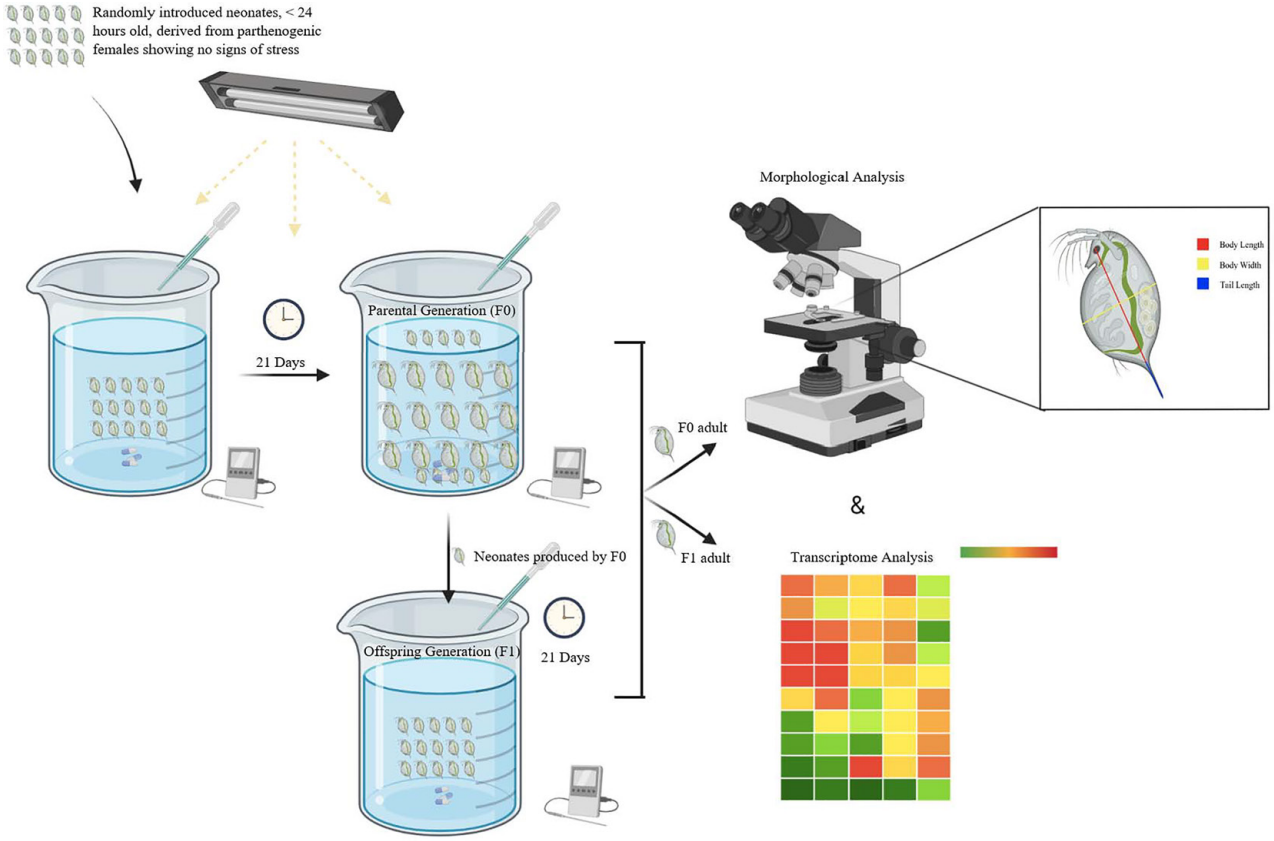

Figure 5.1. Schematic depiction of chronic exposure study methodology for morphological and transcriptome analyses. Parameters were maintained at a temperature of 18 ± 1°C, pH 8 ± 1 and dissolved oxygen of > 3 mg/L. Lighting consisted of a 16:8 light-to-dark cycle of cool white light between 1000 and 1500 lux. Medium changes, as well as feedings, occurred every 2 days. Animals were fed 600 µL of 0.1 g/100 mL spirulina suspension. Objects not to scale. Heat map is not an accurate representation of transcriptome analysis results. *D. magna* neonates < 24 h old were exposed to the PNEC of venlafaxine hydrochloride, 6.1 ng/L, until reaching the adult age of 21 d. Adult animals underwent morphological and transcriptome analyses.

5.4 Results and Discussion

5.4.1 Organism uptake study

Table 5.1 shows the results of three substances that were tested individually on the test organism, and Table 5.2 shows the mixture of multiple pharmaceuticals that organisms were exposed to. This initial test illustrated that chemicals were taken up by organisms, but the variability in results did not allow for concrete conclusions. Further investigation may be required to determine which chemicals are taken up by a test organism based on physico-chemical characteristics of the pharmaceutical. This will relate to the substance polarity and test set-up. It is expected that the uptake of chemicals in a mixture will differ from the results obtained when organisms are exposed to individual chemicals.

Table 5.1. Concentration of pharmaceuticals detected in individual exposure experiments – where the sample contained a value of PNEC × 10 (Table 2.1)

Sample	Venlafaxine (ng/L)	Metformin (ng/L)	Sulfamethoxazole (ng/L)
PNEC × 10	1.30 ± 0.33	7.07 ± 3.19	0.65 ± 0.25

Concentrations relate to 100 mg *D. magna* (*n* = 3).

Table 5.2. Results of measurements following daphnid exposure to a pharmaceutical mixture containing 10 × PNEC concentrations for each analyte

Pharmaceutical at PNEC × 10 mix	ng/L (*n* = 3)
Venlafaxine	0.72 ± 0.26
Metformin	> LOQ
Erythromycin	1.71 ± 0.65
Trimethoprim	16.27 ± 2.36
Sulfamethoxazole	> LOQ
Carbamazepine	29.21 ± 5.84
Clarithromycin	5.58 ± 2.03
Azithromycin	5.41 ± 2.03

The determination of pharmaceuticals from a mixture is shown in Table 5.2. The exposure concentration relates to a value of PNEC × 10. In this study, 30 daphnids were exposed to a mixture of pharmaceuticals.

5.4.2 *Chronic exposure study – morphological*

The offspring (F1) generation was the first production of the parent (F0) generation. It was found that in all cases where organisms were exposed to venlafaxine, morphological features (body length and width and tail length) were smaller in the offspring than in the parents.

5.4.3 *Reproduction effects*

Based on *The OECD Guidelines for the Testing of Chemicals*, the primary objective of this study was the assessment of the effects of chemicals on the reproductive output of *D. magna* (OECD, 2012). Figure 5.2 shows that, when the test organisms

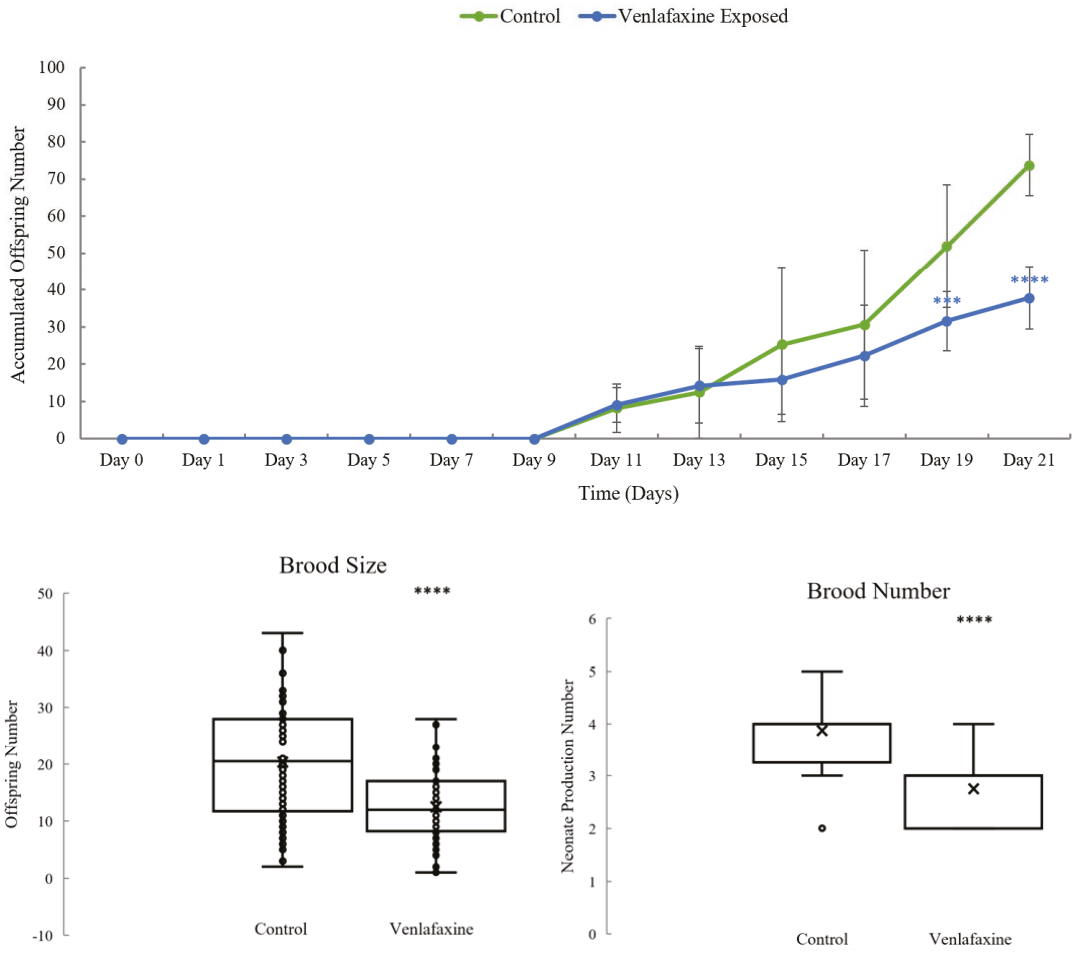

Figure 5.2. Effects of venlafaxine exposure on reproduction of *D. magna* where PNEC values were used. Asterisks denote level of statistical significance. Figure 5.1 shows the methodology for chronic exposure studies.

were exposed to venlafaxine, the brood size and brood number were impacted significantly. These experiments were carried out at very low concentrations and demonstrate the potential effect PNECs can have on reproduction in the environment. This is specific to the laboratory study undertaken but provides a proof of concept for further investigation of reproduction effects.

5.4.4 Heart rate study

There are various physiological end points in ecotoxicological studies (Bownik, 2020). Here, we report effects of venlafaxine, metformin, sulfamethoxazole and gemfibrozil on heart rate in *D. magna*. Each compound was tested at three concentrations after 48 h exposure. The heart is located dorsally and anterior to the brood chamber. The average heart rate according to Corotto *et al.* (2010) is 354 beats per minute (range: 91–521 beats per minute). This parameter is easily affected by temperature: it slows down with decreasing temperatures. However, the variation in heart rate cannot be attributed to variation in daphnid size.

All results (Figure 5.3) from recording were standardised to the unit of beats per minute. This shows the comparison between each concentration of each chemical with controls. Metformin and sulfamethoxazole were found to cause a noticeable concentration-dependent decrease in mean heart rate, while venlafaxine demonstrated an increase in the recorded heart rate under the study conditions.

5.5 Conclusion

The objectives of this chapter were to evaluate *D. magna* as a test organism for pharmaceutical assessments and to determine pharmaceutical uptake by test organisms. The target analytes were selected based on occurrence data and MOA results obtained. A series of studies were carried out: (i) organism chemical uptake, (ii) chronic exposure effects on morphology and reproduction and (iii) effects on heart rate. The results clearly show that, where pharmaceuticals are at PNEC or above, certain substances demonstrate significant effects. Venlafaxine (an antidepressant), sulfamethoxazole

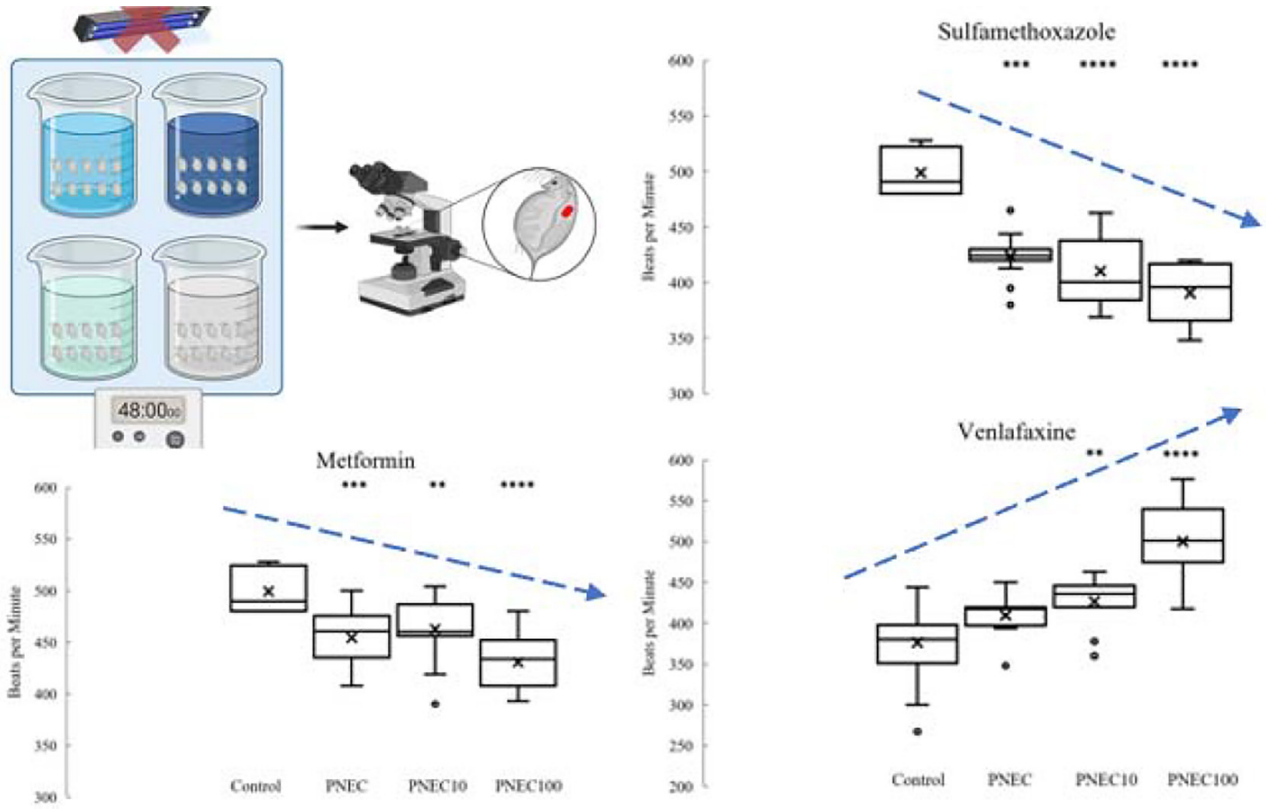

Figure 5.3. Summary of data on heart rate effects of pharmaceutical exposures. Asterisks denote level of statistical significance: ***$p \leq 0.001$; ****$p \leq 0.0001$.

(an antibiotic) and metformin (an antidiabetic drug) were chosen for this investigation.

Venlafaxine hydrochloride has been detected ubiquitously in Irish surface waters, with potential hazardous impacts on ecosystems. The exposure of *D. magna* to the PNEC of the pharmaceutical has been shown to have significant effects, including a reduction in the size of morphological features observed through generations, increases in heart rate with increases in concentrations and a significant decrease in the total offspring number. The observation of effects on reproduction is critical in terms of understanding the potential link with continuous low concentrations of emerging contaminants on ecosystems and biodiversity. While this is a limited study, the results clearly show that both brood size and brood number are affected by chronic exposure. There is a need to expand this work to other chemicals of concern from anthropogenic sources that are known to be present in surface waters and link their occurrence to effects on ecosystems. Specifically, studies should focus on the effects on the life cycles of invertebrates when exposed to chemicals of emerging concern and those on watch lists.

The results confirm that *D. magna* is a good test organism to observe both acute and chronic effects, where the latter represent the greatest value in screening chemicals and also surface waters for chronic toxicity profile.

6 Integration to Develop Effects-based Approach to Monitoring

6.1 Objectives

- To integrate data on chemical occurrence with biological effects.
- To propose a simplified assessment approach for water quality.
- To make recommendations on substance monitoring based on assessment of risk.

6.2 Introduction

6.2.1 Background

In line with the European Green Deal's Zero Pollution Action Plan and the EU biodiversity strategy for 2030, the EMPIRE project aimed to deliver scientific advances that can support "Do no significant harm", specifically relating to the sustainable use and protection of water resources where the use of water is detrimental to its good environmental status. The presence of pharmaceuticals in the environment has so far been mainly described in treated waste water. Current monitoring and assessment of the chemical status of water bodies under the WFD fail to characterise the likelihood that complex mixtures of chemicals affect water quality. The project Solutions (Brack *et al*., 2022) suggested estimating this with EBMs complemented by chemical screening and/or impact modelling to identify the causes of impacts on water quality. EBMs were recommended for WFD monitoring to cover the major modes of action of chemicals to evaluate improvements in water quality upon implementing the programme of measures. The ability to evaluate the effects of CECs on aquatic ecosystems is growing in importance, as detection frequency increases with the advances in mass spectrometry analysis techniques. Effect-directed analysis is based on the biological response that indicates an adverse effect and on the identification of the causative compounds. The effect-directed analysis protocols combine bioassays of environmental samples with their fractionation to reduce the complexity of the matrix before the identification of the active compounds.

6.2.2 Zero pollution vision

Figure 6.1 represents a vision for the assessment of chemical risk in the aquatic environment and it illustrates the potential for developing a risk index, which would require detailed monitoring of chemical occurrence in addition to a bioanalytical assessment. However, a risk index would require that samples include water, biota (algae, invertebrate and fish) and sediment. Sediment is a matrix that is not measured but poses significant risk to surface water quality. Under the zero pollution ambition for a toxin-free environment expressed in the European Green Deal, the European Commission announced in 2020 that it would adopt a Zero Pollution Action Plan for air, water and soil. In Figure 6.1 the vision of the Zero Pollution Action Plan is identified as a threshold level. However, it is a huge challenge to achieve this threshold because of the lack of available monitoring data currently and of the data needed to assess risk.

In Figure 6.1 the left axis highlights the levels and complexity of chemical exposure measurements. The top left reflects the current monitoring under the WFD, while the lower part reflects the required assessments relating to the behaviour of the chemicals in the aquatic environment and within the ecosystem. The right side reflects the analytical approaches – the top part reflecting common current methods and the lower part the effects-based approaches that are needed. Ideal risk assessments would include bioanalytical methods aligned with sediment and biota matrices to gain a better understanding of bioavailability. There is a huge gap in current monitoring that needs to be addressed in order to understand the behaviour of chemicals in the aquatic environment.

6.3 Chemical Occurrence and Risk Assessment

A total of 24 samples were measured in the EMPIRE project for the target group of 17 pharmaceuticals. Figure 6.2 shows the number of detections of certain pharmaceuticals and the risk attributed to the sampled rivers. It is clear that the greatest number of detections

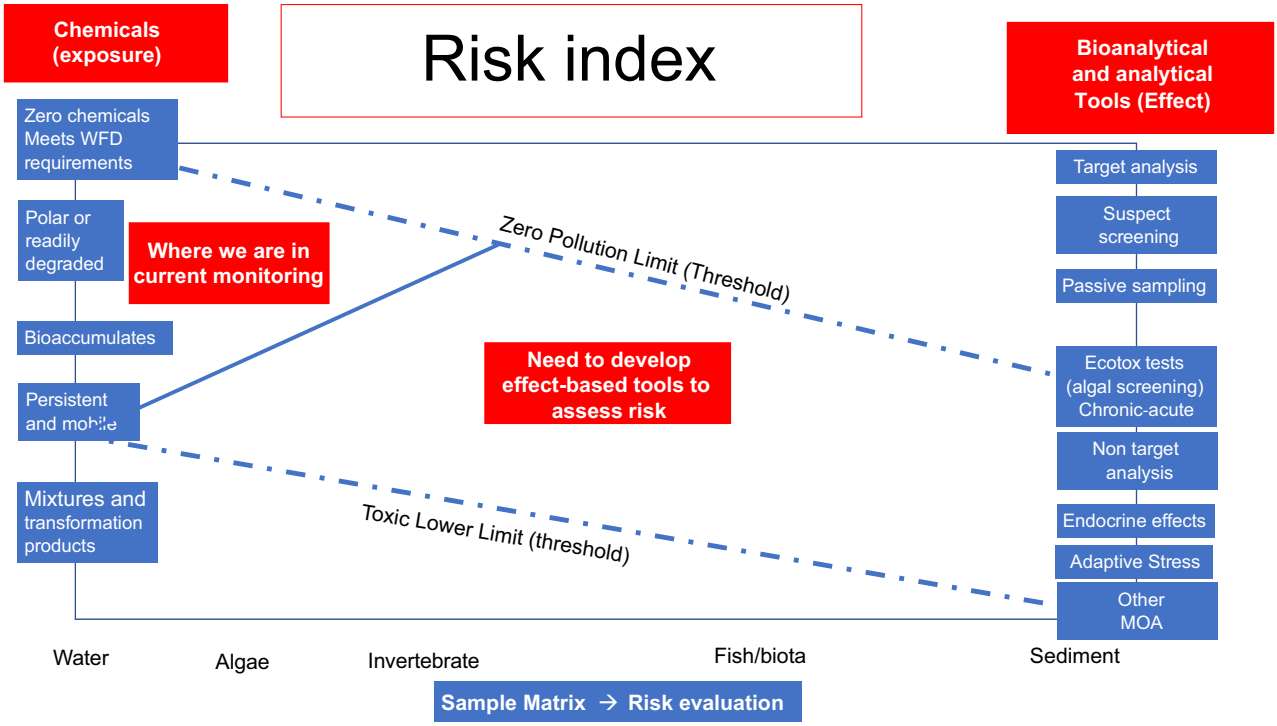

Figure 6.1. A vision for assessment of risk of chemicals in the aquatic environment.

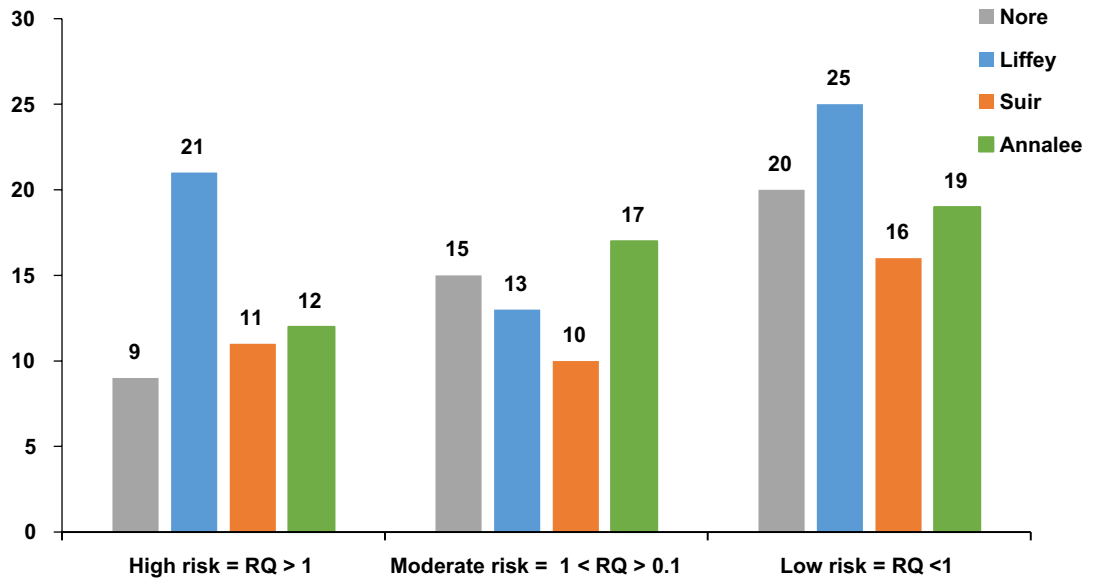

Figure 6.2. Summary of the number of detections of substances at each sampling location. RQ=MEC/PNEC; high risk=RQ>1; moderate risk=0.1<RQ<1; low risk=RQ<0.1. MEC, maximum environmental compound concentration; RQ, risk quotient.

that had a risk quotient (RQ) of >1 (21) occurred in the River Liffey. The detections in the River Liffey demonstrate a water body that has continuous occurrence of pharmaceuticals at levels that may not represent a toxic risk under current measures; however, the results suggest that there is a risk and that continuous monitoring is required to ensure that these levels do not increase further. Similarly, the River Annalee and River Suir have demonstrated a high level of risk on some sampling occasions in relation to certain compounds. There is evidence that venlafaxine and its metabolites are not removed efficiently during waste water treatment. In humans, over 60% of the compound is excreted via urine as metabolites.

Therefore, both the parent compound and metabolites have the potential to be transported in sewage to municipal WWTPs.

Developing this risk assessment further, it is necessary to combine the chemical occurrence data with the bioassay data from Chapter 4. Taking venlafaxine as an example, Figure 6.3 shows how a water toxicity profile might be determined. This example uses venlafaxine and its metabolite to illustrate the value of combining both chemical occurrence and biological effects (e.g. reproduction) as an assessment of effects. Venlafaxine was shown to be present in all River Liffey samples. While concentrations were often very low in all samples, assessments of reproduction have demonstrated that chronic exposure to PNEC values can still have significant effects on offspring. Basing a monitoring programme solely on monitoring occurrence above the environmental quality standard is not adequate to determine the ecosystem effects, according to this study.

This approach to assessment involves both the substance occurrence concentration and the biological effects. This process requires further work to determine the best bioassays to include in the assessment. Initial results from EMPIRE illustrate the importance of including invertebrate studies, such as those using *D. magna*.

6.4 Risk Assessment

As a part of an environmental risk assessment, the pharmaceutical risk to the environment was calculated by the RQ, which is the ratio of their predicted environmental concentration (PEC) to PNEC. To assess the toxicological risks, multitrophic exposure studies could be employed at detected concentrations. The use of RQ, where the measured environmental concentrations (MECs) of pharmaceuticals are ratioed against the PNEC, has been shown to be an effective strategy for predicting risk within a river system. However, as the PEC does not account for compounding exposure as a result of multiple pharmaceuticals with the same APIs, using MEC instead of PEC can help provide a greater outlook on APIs' impact on an aquatic ecosystem and the specific locations where surface waters are monitored. Tables 6.1–6.4 summarise the risk assessment carried out in Irish surface waters. RQs are categorised into four groups – high risk (RQ > 1), moderate risk (1 > RQ > 0.1), low risk (0.1 > RQ > 0.01) and negligible risk (RQ < 0.01) – to give a numerical value to the risk of the environmental impact of the pharmaceuticals present in the rivers. The tables show the substance-specific risk linked to the calculated RQ for each site and each pharmaceutical. It is clear that for each water body studied there are substances that pose the greatest risk. For example, in the River Liffey,

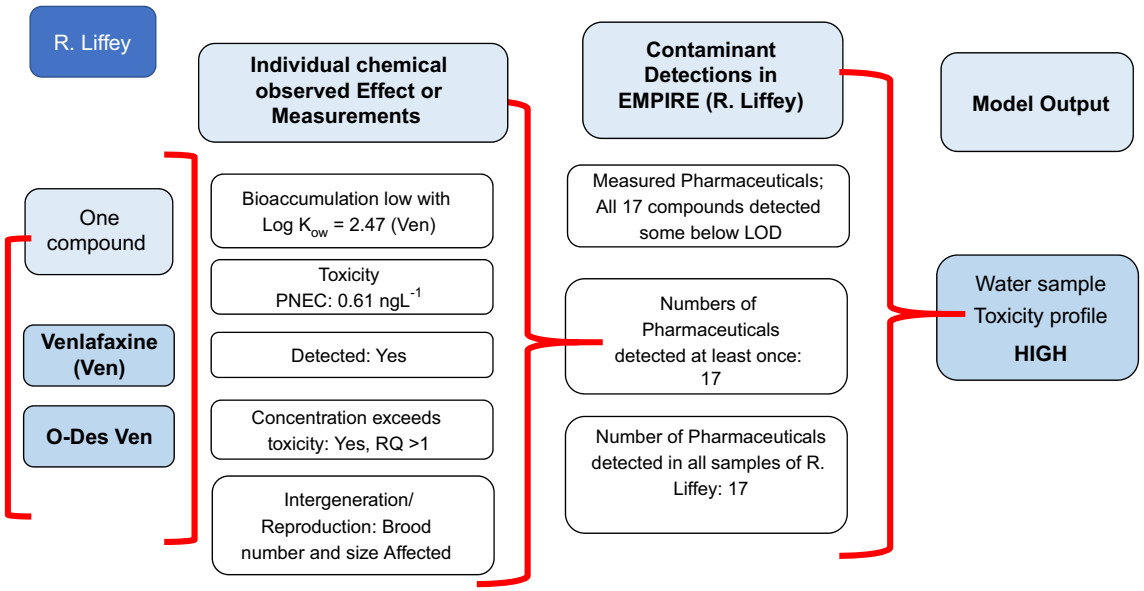

Figure 6.3. Assessment of a water toxicity profile based on combined chemical and bioanalytical assessments, taking the River Liffey and the occurrence of venlafaxine as an example.

Table 6.1. River Liffey evaluation of substances that present no risk (NR), negligible (N), low (L), moderate (M) or high (H) risk based on calculated RQ

Pharmaceutical	March 2022	September 2021	May 2021	March 2021	October 2020	September 2020
Metformin	NR	N	N	N	N	N
Gabapentin	N	N	L	NR	N	N
Trimethoprim	M	M	M	L	M	M
Ciprofloxacin	L	M	L	L	L	M
Venlafaxine	H	H	H	H	H	H
O-desmethylvenlafaxine	H	H	H	H	H	H
Sulfamethoxazole	H	H	H	M	H	H
Carbamazepine	L	L	L	L	L	L
Diclofenac	NR	M	M	H	M	M
Gemfibrozil	NR	NR	M	M	L	N
Estrone	NR	NR	H	H	NR	NR
Erythromycin	NR	NR	NR	M	NR	NR
17β-Estradiol	NR	NR	NR	H	NR	NR

Table 6.2. River Suir evaluation of substances that present no risk (NR), negligible (N), low (L), moderate (M) or high (H) risk based on calculated RQ

Pharmaceutical	March 2022	September 2021	May 2021	March 2021	October 2020	September 2020
Metformin	NR	N	N	N	N	N
Gabapentin	N	NR	N	NR	N	N
Trimethoprim	NR	M	M	NR	M	NR
Ciprofloxacin	M	M	L	M	M	M
Venlafaxine	H	H	H	N	H	M
O-desmethylvenlafaxine	H	H	H	M	H	H
Sulfamethoxazole	M	H	L	M	H	L
Diclofenac	NR	M	M	M	M	M
Gemfibrozil	NR	NR	NR	N	NR	NR

Table 6.3. River Annalee evaluation of substances that present no risk (NR), negligible (N), low (L), moderate (M) or high (H) risk based on calculated RQ

Pharmaceutical	March 2022	September 2021	May 2021	March 2021	October 2020	September 2020
Metformin	NR	NR	NR	N	N	N
Gemfibrozil	NR	M	NR	M	N	NR
Gabapentin	NR	N	N	N	N	N
Trimethoprim	NR	NR	NR	M	M	NR
Ciprofloxacin	L	L	NR	M	L	NR
Venlafaxine	N	H	H	H	H	NR
O-desmethylvenlafaxine	M	H	H	H	H	M
Sulfamethoxazole	M	M	M	H	M	L
Carbamazepine	L	L	L	L	L	NR
Diclofenac	NR	M	M	M	M	NR
Estrone	NR	H	NR	H	NR	NR
Azithromycin	NR	NR	NR	NR	M	NR
Clarithromycin	NR	NR	NR	NR	M	NR
17β-Estradiol	NR	NR	NR	H	NR	NR

Table 6.4. River Nore evaluation of substances that present no risk (NR), negligible (N), low (L), moderate (M) or high (H) risk based on calculated RQ

Pharmaceutical	March 2022	September 2021	May 2021	March 2021	October 2020	September 2020
Metformin	N	NR	NR	N	N	N
Gabapentin	N	N	N	N	N	N
Trimethoprim	L	NR	NR	NR	M	NR
Ciprofloxacin	L	L	L	M	M	M
Venlafaxine	M	H	H	N	H	H
O-desmethylvenlafaxine	H	H	H	M	H	H
Sulfamethoxazole	L	M	M	L	M	M
Carbamazepine	NR	L	NR	NR	NR	N
Diclofenac	M	M	M	M	M	L

venlafaxine, its metabolite O-desmethylvenlafaxine and sulfamethoxazole continuously pose a threat to this surface water. It is likely that the source of these substances is waste water; however, further studies and extensive monitoring are required. These chemicals typically pose a risk for all sites studied, but not continuously. This risk assessment approach is a simple mapping of chemical occurrence, and the RQ allows the determination of the most critical chemicals for continuous monitoring. In each case the risk assessment is based on measured values, which include calculated PNECs but not EBM MOAs.

However, EMPIRE has shown that some of these chemicals, when occurring at very low concentrations, have an effect on aquatic test organisms.

From the study of surface waters in Ireland, the risk quotient frequency (RQf) values for venlafaxine, O-desmethylvenlafaxine, sulfamethoxazole and diclofenac were found to be >0. The RQf allows for the differentiation between pharmaceuticals frequently detected exceeding an RQ of 1 (e.g. O-desmethylvenlafaxine) and pharmaceuticals that only have one exceedance (e.g. diclofenac).

RQf was calculated using the following formula:

$$RQ_f = \frac{MEC}{PNEC} \times \frac{\text{Number of samples where MEC > PNEC}}{\text{Total number of samples}}$$

where RQf values indicate risk in four categories: high risk ($RQf \geq 1$), moderate risk ($1 > RQf \geq 0.1$), limited risk ($0.1 > RQf \geq 0.01$) and negligible risk ($0.01 > RQf > 0$). RQf is a valuable indicator of the performance of a WWTP and should be considered as part of a monitoring programme.

Table 6.5 summarises the compounds that were considered to have the potential to pose a risk. It shows the RQ for chemicals that were suspected to be the most concerning and the modified RQf where detection frequency was taken into consideration.

Table 6.5. List of selected compounds for which the RQf was >0 and detections constituted a minimum of 80% of the tested samples

		Number of samples					
Substance	LOQ/PNEC	Negligible risk: RQ<0.01 (excluding <LOQ)	Low risk: 0.01–0.1 (excluding <LOQ)	Moderate risk: 0.1–1 (excluding <LOQ)	High risk: RQ>1 (excluding <LOQ)	Mean RQ (24 samples including non-detects)	RQf
Sulfamethoxazole	0.028	0	5	11	8	0.80	0.27
Venlafaxine	0.41	0	0	2	18	2.72	2.04
O-desmethylvenlafaxine	0.78	0	0	3	20	3.58	2.98
Diclofenac	0.091	0	1	18	1	0.27	0.011

If the RQf is equal to zero (RQf=0) then no risk is expected at present (safe). These compounds should be prioritised for further work. Compounds in which the RQf=0 are perhaps analysed only in limited sampling sites and the detection frequencies of those compounds are unavailable. RQf values show greater difference in potential environmental risks of the compounds after considering the frequency of MECs exceeding PNECs compared with RQ.

6.5 Conclusion and Recommendations

The EMPIRE project reflects multidisciplinary research involving analytical method development, bioanalytical methods and assessment of biological effects, ecotoxicology assessment of chemicals and mixtures, and a risk assessment. Each element of the research generated valuable data that will be openly available at the end of the project. The data generated have been used to propose an assessment of risk for certain water bodies based on pharmaceutical occurrence/detections and initial effect-based and ecotoxicology data. The main recommendations arising from the work are as follows.

1. In the determination of improvements to monitoring, it is possible to assess the occurrence of chemicals in relation to the concentrations measured and the frequency of detection. The results show that, of the pharmaceutical compounds targeted, some occurred in every sample at measurable concentrations. It is therefore recommended that a more frequent monitoring of receiving waters in proximity to WWTPs and surface waters upstream and downstream takes place to establish the burden of this source on surface waters.

2. Analytical methods for determining target analytes are available; however, when taking the sample matrix into consideration, there can be a significant impact on the quality of quantitative information on certain chemical classes; for example, metformin in pH-adjusted samples behaves differently from in non-pH-adjusted samples. Antibiotics have been found to be significantly affected by storage conditions and sample matrix when compared with other classes of pharmaceutical. Therefore, a thorough assessment of sample matrix interference and the selection of suitable internal standards for quantitation are strongly recommended.

3. Taking occurrence information together with EBMs provides the potential for determination of a water sample toxicity profile. The EMPIRE project has demonstrated that the complex suite of bioassays highlight an array of effects. However, it is not practical for each of these effects to be assessed in a typical monitoring programme. Based on the results obtained, it is recommended that an ideal set of bioassays that can complement the chemical occurrence data be further investigated. The EU PARC project is investigating EBMs and should be consulted for future research in this area. There are currently no agreed EBMs that could be harmonised for monitoring. The takeaway observations from the EMPIRE research are that certain compounds can cause increased biological effects in some assays, and that cocktails of chemicals can show synergistic effects.

4. The biological effects of substances vary; however, if monitoring programmes were to consider effects on ecosystem biodiversity, it would be critical to assess organism reproduction. The initial data in the EMPIRE project suggest that this is a very effective way to determine the toxicity profile of a water body. Therefore, we recommend the use of ecotoxicology studies involving, for example, heart rate and organism reproduction when screening waters for toxicity.

5. The sites were selected for this study on the basis of EPA water quality information and included both urban and rural areas. It is clear from the results that pharmaceuticals occur in these surface waters, with some compounds present in all samples. These results highlight the importance of monitoring surface waters to build up a data set on surface water quality. However, it is recommended that a wide-range suspect screening or non-target screening be carried out to gather a more comprehensive chemical fingerprint of Irish surface waters.

6. The data gathered on pharmaceutical occurrence showed a greater number of detections in the urban site and higher concentrations of analytes. However, results showed that rural sites also have pharmaceutical occurrences that may arise from septic tanks or agricultural sources.

In conclusion, the data gathered during this 4-year assessment of pharmaceuticals in Ireland have greatly contributed to knowledge and provided recommendations for monitoring. The method developments have been shared with the EPA for the transfer of analytical protocols for future monitoring. The data set forms a basis for future monitoring, particularly in advance of the revised waste water treatment regulations. The EU released a proposal for a revised Urban Wastewater Treatment Directive at the end of 2022 in which micropollutant removal is

a consideration for certain agglomerations. This is a step towards meeting the European Green Deal's zero pollution ambition, and the data and findings from the EMPIRE project can inform how Ireland addresses future pollution arising from anthropogenic sources of pharmaceuticals in surface waters.

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Abbreviations

ACN	Acrylonitrile
API	Active pharmaceutical ingredient
CAS	Conventional activated sludge
CEC	Contaminant of emerging concern
EBM	Effect-based method
EC_{50}	Half-maximal effective concentration
LOQ	Limit of quantitation
MEC	Measured environmental concentration
MOA	Mode of action
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentration
PNEC	Predicted no-effect concentration
RQ	Risk quotient
RQf	Risk quotient frequency
SNRI	Serotonin–noradrenaline reuptake inhibitor
WFD	Water Framework Directive
WWTP	Waste water treatment plant

An Ghníomhaireacht Um Chaomhnú Comhshaoil

Tá an GCC freagrach as an gcomhshaol a chosaint agus a fheabhsú, mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ar thionchar díobhálach na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialáil: Rialáil agus córais chomhlíonta comhshaoil éifeachtacha a chur i bhfeidhm, chun dea-thorthaí comhshaoil a bhaint amach agus díriú orthu siúd nach mbíonn ag cloí leo.

Eolas: Sonraí, eolas agus measúnú ardchaighdeáin, spriocdhírithe agus tráthúil a chur ar fáil i leith an chomhshaoil chun bonn eolais a chur faoin gcinnteoireacht.

Abhcóideacht: Ag obair le daoine eile ar son timpeallachta glaine, táirgiúla agus dea-chosanta agus ar son cleachtas inbhuanaithe i dtaobh an chomhshaoil.

I measc ár gcuid freagrachtaí tá:

Ceadúnú

- Gníomhaíochtaí tionscail, dramhaíola agus stórála peitril ar scála mór;
- Sceitheadh fuíolluisce uirbigh;
- Úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe;
- Foinsí radaíochta ianúcháin;
- Astaíochtaí gás ceaptha teasa ó thionscal agus ón eitlíocht trí Scéim an AE um Thrádáil Astaíochtaí.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Iniúchadh agus cigireacht ar shaoráidí a bhfuil ceadúnas acu ón GCC;
- Cur i bhfeidhm an dea-chleachtais a stiúradh i ngníomhaíochtaí agus i saoráidí rialáilte;
- Maoirseacht a dhéanamh ar fhreagrachtaí an údaráis áitiúil as cosaint an chomhshaoil;
- Caighdeán an uisce óil phoiblí a rialáil agus údaruithe um sceitheadh fuíolluisce uirbigh a fhorfheidhmiú
- Caighdeán an uisce óil phoiblí agus phríobháidigh a mheasúnú agus tuairisciú air;
- Comhordú a dhéanamh ar líonra d'eagraíochtaí seirbhíse poiblí chun tacú le gníomhú i gcoinne coireachta comhshaoil;
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Dramhaíola agus Ceimiceáin sa Chomhshaol

- Rialacháin dramhaíola a chur i bhfeidhm agus a fhorfheidhmiú lena n-áirítear saincheisteanna forfheidhmithe náisiúnta;
- Staitisticí dramhaíola náisiúnta a ullmhú agus a fhoilsiú chomh maith leis an bPlean Náisiúnta um Bainistíocht Dramhaíola Guaisí;
- An Clár Náisiúnta um Chosc Dramhaíola a fhorbairt agus a chur i bhfeidhm;
- Reachtaíocht ar rialú ceimiceán sa timpeallacht a chur i bhfeidhm agus tuairisciú ar an reachtaíocht sin.

Bainistíocht Uisce

- Plé le struchtúir náisiúnta agus réigiúnacha rialachais agus oibriúcháin chun an Chreat-treoir Uisce a chur i bhfeidhm;
- Monatóireacht, measúnú agus tuairisciú a dhéanamh ar chaighdeán aibhneacha, lochanna, uiscí idirchreasa agus cósta, uiscí snámha agus screamhuisce chomh maith le tomhas ar leibhéil uisce agus sreabhadh abhann.

Eolaíocht Aeráide & Athrú Aeráide

- Fardail agus réamh-mheastacháin a fhoilsiú um astaíochtaí gás ceaptha teasa na hÉireann;
- Rúnaíocht a chur ar fáil don Chomhairle Chomhairleach ar Athrú Aeráide agus tacaíocht a thabhairt don Idirphlé Náisiúnta ar Ghníomhú ar son na hAeráide;
- Tacú le gníomhaíochtaí forbartha Náisiúnta, AE agus NA um Eolaíocht agus Beartas Aeráide.

Monatóireacht & Measúnú ar an gComhshaol

- Córais náisiúnta um monatóireacht an chomhshaoil a cheapadh agus a chur i bhfeidhm: teicneolaíocht, bainistíocht sonraí, anailís agus réamhaisnéisiú;
- Tuairiscí ar Staid Thimpeallacht na hÉireann agus ar Tháscairí a chur ar fáil;
- Monatóireacht a dhéanamh ar chaighdeán an aeir agus Treoir an AE i leith Aeir Ghlain don Eoraip a chur i bhfeidhm chomh maith leis an gCoinbhinsiún ar Aerthruailliú Fadraoin Trasteorann, agus an Treoir i leith na Teorann Náisiúnta Astaíochtaí;
- Maoirseacht a dhéanamh ar chur i bhfeidhm na Treorach i leith Torainn Timpeallachta;
- Measúnú a dhéanamh ar thionchar pleananna agus clár beartaithe ar chomhshaol na hÉireann.

Taighde agus Forbairt Comhshaoil

- Comhordú a dhéanamh ar ghníomhaíochtaí taighde comhshaoil agus iad a mhaoiniú chun brú a aithint, bonn eolais a chur faoin mbeartas agus réitigh a chur ar fáil;
- Comhoibriú le gníomhaíocht náisiúnta agus AE um thaighde comhshaoil.

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta agus nochtadh an phobail do radaíocht ianúcháin agus do réimsí leictreamaighnéadacha a mheas;
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha;
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta;
- Sainseirbhísí um chosaint ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Ardú Feasachta agus Faisnéis Inrochtana

- Tuairisciú, comhairle agus treoir neamhspleách, fianaise-bhunaithe a chur ar fáil don Rialtas, don tionscal agus don phobal ar ábhair maidir le cosaint comhshaoil agus raideolaíoch;
- An nasc idir sláinte agus folláine, an geilleagar agus timpeallacht ghlan a chur chun cinn;
- Feasacht comhshaoil a chur chun cinn lena n-áirítear tacú le hiompraíocht um éifeachtúlacht acmhainní agus aistriú aeráide;
- Tástáil radóin a chur chun cinn i dtithe agus in ionaid oibre agus feabhsúchán a mholadh áit is gá.

Comhpháirtíocht agus Líonrú

- Oibriú le gníomhaireachtaí idirnáisiúnta agus náisiúnta, údaráis réigiúnacha agus áitiúla, eagraíochtaí neamhrialtais, comhlachtaí ionadaíocha agus ranna rialtais chun cosaint chomhshaoil agus raideolaíoch a chur ar fáil, chomh maith le taighde, comhordú agus cinnteoireacht bunaithe ar an eolaíocht.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an GCC á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóir. Déantar an obair ar fud cúig cinn d'Oifigí:

1. An Oifig um Inbhunaitheacht i leith Cúrsaí Comhshaoil
2. An Oifig Forfheidhmithe i leith Cúrsaí Comhshaoil
3. An Oifig um Fhianaise agus Measúnú
4. An Oifig um Chosaint ar Radaíocht agus Monatóireacht Comhshaoil
5. An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tugann coistí comhairleacha cabhair don Ghníomhaireacht agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

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