Occurrence of chemicals of emerging concern in Irish rivers, with a focus on pesticide contaminants

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A thesis submitted for the award of PhD

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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I am so very lucky to have many people in my life which deserve thanks for getting me to this point, so this will probably be a long one!

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i) Conference Poster Presentations

Title: EPA Research Project "IMPACT" - Innovative Monitoring to Prioritise Contaminants of Emerging Concern (CECs) for Ireland
Authors: Imogen Hands, Helena Rapp Wright, Blánaid White, Fiona Regan
Conference: EPA-HSE Environment, Health and Well Being Annual Conference, Dublin, 7th November 2018

Title: EPA Research Project "IMPACT" - Innovative Monitoring to Prioritise Contaminants of Emerging Concern (CECs) for Ireland
Authors: Imogen Hands, Helena Rapp Wright, Blánaid White, Fiona Regan
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Title: Pyrethroid pesticide occurrence in surface water environments
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Title: Pyrethroid pesticide occurrence in surface water environments
Authors: Imogen Hands, Helena Rapp Wright, Matthew R. Jacobs, Leon Barron, Blánaid White, Fiona Regan
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Title: Assessment of Watch List Chemical Occurrence in Surface Water during a 4-Year Monitoring Programme
Authors: Imogen Hands, Blánaid White, Fiona Regan
Conference: SETAC Europe, Copenhagen, Denmark 15th -19th May 2022

ii) Conference Oral Presentations

Title: Determination of Watch List chemicals in Irish surface waters: Chemical occurrence and analytical method challenges
Authors: Imogen Hands, Helena Rapp-Wright, Dylan O'Flynn, Mathavan Vickneswaran, Catherine Allen, Matthew R. Jacobs, Leon P. Barron, Blánaid White and Fiona Regan
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Title: Method development and monitoring of Watch List chemicals in Irish surface waters over a three year period
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Title: Determination of Watch List chemicals in Irish surface waters: Chemical occurrence and analytical method challenges.
Authors: Imogen Hands, Helena Rapp-Wright, Dylan O'Flynn, Mathavan Vickneswaran, Catherine Allen, Matthew R. Jacobs, Leon P. Barron, Blánaid White and Fiona Regan
Conference: International Conference on Environmental Science & Technology (CEST), Athens, Greece, 2nd September 2021

List of Abbreviations and Acronyms

AMPA	Aminomethylphosphonic Acid
AMR	Anti-Microbial Resistance
ACN	Acetonitrile
CECs	Contaminants of Emerging Concern
EDCs	Endocrine-Disrupting Compounds
DI	Deionized Water
EDTA	Ethylenediaminetetraacetic Acid
EQS	Environmental Quality Standards
GC	Gas Chromatography
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
Incl.	Including
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MeOH	Methanol
MRLS	Maximum Residue Levels
MS	Mass Spectrometry
NaOH	Sodium Hydroxide
PCCPs	Personal Care and Cosmetics Products
RP	Reversed-Phase
RRF	Relative Retention Factor
SPE	Solid Phase Extraction
UPLC	Ultra-High Performance Liquid Chromatography
UV	Ultraviolet
WFD	Water Framework Directive
WL	Watch List
WWTP	Wastewater Treatment Plant

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Thesis Overview and Chapter Author Contributions

This thesis was guided by a primary research question and a primary research aim. The driving question of this project was to assess the chemical water quality of Irish surface waters relating to contaminants of emerging concern (CECs). This was coupled with an end goal of generating monitoring data that is fed to legislators which in turn will ultimately have an impact on improving water quality on a wider EU scale. The subsequent research questions which arose, and the overall flow of the approach taken in this thesis is shown in the below schematic (Figure 1):

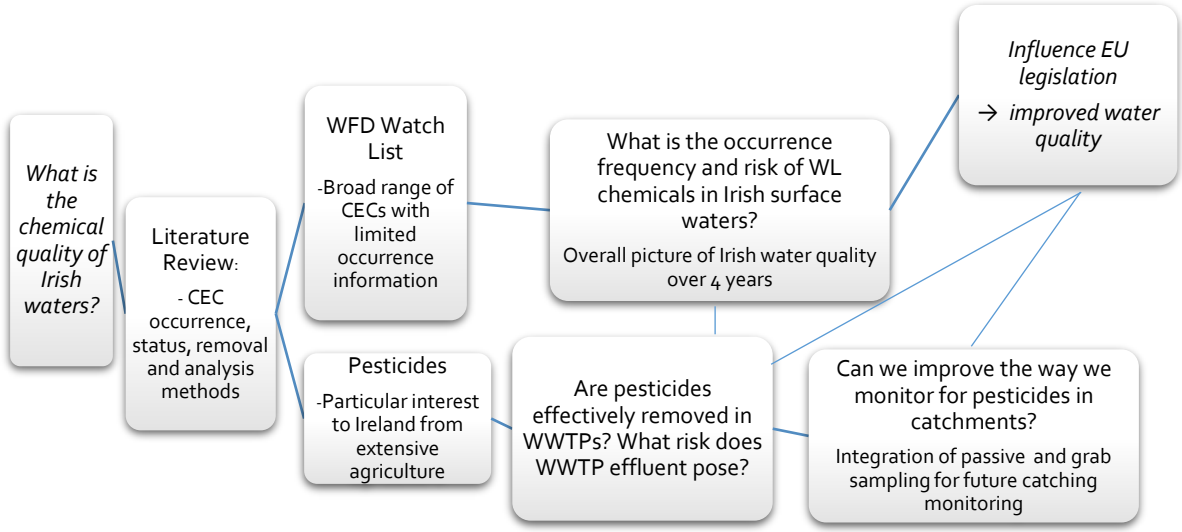


Figure 1. Schematic showing the overall flow and approach taken in this thesis.

Each chapter of this thesis was influenced by these questions, and chapters are laid out generally following this schematic. A brief overview of each chapter along with a breakdown of author contributions is given here.

Chapter 1:

Chapter 1 introduces the topic of chemical water quality through a review of the relevant literature. Literature research and writing of the chapter was completed by Imogen Hands. Proofing of the chapter was performed by Dr. Matthew R. Jacobs, Assistant Prof. Blánaid White and Prof. Fiona Regan.

Chapter 2:

Chapter 2 shows the analytical method development undertaken in order to examine CECs in the aquatic environment. Multiple analytical methods were developed and used in this thesis to examine different targeted groups. The 'estrogens method' applied to 2nd Watch List analysis was developed primarily by Dr. Helena Rapp Wright and was included in this manuscript with her permission. All other method development was performed by Imogen Hands, as well as all sample processing, data generation and data analysis. LC-MS training and advice was given by Dr. Catherine Allen and Dr. Matthew R. Jacobs. Assoc. Prof. Blánaid White and Prof. Fiona Regan reviewed the chapter.

Chapter 3:

Chapter 3 presents four years of Watch List (WL) monitoring data for the island of Ireland from 2018 - 2022. Experimental work, analysis and writing of the chapter was completed by Imogen Hands, with support from Dr. Helena Rapp Wright on the 2nd Watch List analysis. Some assistance in sample extraction was performed by Dylan O'Flynn and Mathavan Vickneswaran. Proofing of the chapter was performed by Assoc. Prof. Blánaid White and Prof. Fiona Regan.

Chapter 4:

Chapter 4 is a temporal study of pesticide contaminants in the influent, effluent and receiving waters of two WWTPs. All experimental work and writing of the chapter was completed by Imogen Hands. Proofing of the chapter was performed by Assoc. Prof. Blánaid White and Prof. Fiona Regan.

Chapter 5:

Chapter 5 is an investigation into the use of multiple sampling approaches for pesticide monitoring in catchments. All experimental work and writing of the chapter was completed by Imogen Hands. Proofing of the chapter was performed by Assoc. Prof. Blánaid White and Prof. Fiona Regan.

Chapter 6:

Chapter 6 highlights the key conclusions, contributions and recommendations arising from this research. Writing of the chapter was completed by Imogen Hands. Proofing of the chapter was performed by Assoc. Prof. Blánaid White and Prof. Fiona Regan.

Abstract

Occurrence of chemicals of emerging concern in Irish rivers, with a focus on pesticide contaminants – Imogen Hands

Water quality is impacted by chemical compounds from a range of anthropogenic sources. Some chemicals are routinely monitored because their risk is well known, while many others called contaminants of emerging concern (CECs) are not. This thesis includes the development and application of LC-MS/MS methods for monitoring groups of CECs, including Watch List chemicals, and selected pesticides.

The first comprehensive investigation into Watch List chemicals in Ireland, spanning four years and two Watch Lists, is presented. Occurrence frequencies for 14 out of 34 compounds studied was ≥50%, showing widespread contamination of CECs in surface waters. Risk quotient determination showed high risk occurrences of neonicotinoid pesticides, estrogen hormones and antidepressant venlafaxine. These results are fed straight to the EU and has a direct influence on EU policy decisions.

In Ireland >67% of the land mass is agricultural, making pesticides a group of interest. A year-long study was conducted to examine pesticides through water treatment in two areas. This is the first study of this kind targeting pesticides to be executed in Ireland. It was indicated that only 4 studied analytes were removed with an average efficiency of ≥50%, highlighting the need for improved treatment. Acid herbicides were determined predominantly in receiving waters only, indicating alternative pollution sources than WWTP effluent.

A catchment-based approach for pesticide monitoring using multiple sampling methods was performed. Catchments identified as at-risk for agricultural pressures were studied during pesticide spraying season by passive and grab sampling. Passive samplers identified analytes which were undetected in grab samples, demonstrating the benefit of employing multiple sampling methods. A catchment previously not thought to be at risk for herbicide pollution was found to contain high (>100 ng L^{-1}) levels of acid herbicides in grab samples.

The findings of these studies contributes to informing future policy, and aids in protecting waters internationally.

Chapter 1:

Introduction

1.1. Contaminants of Emerging Concern (CECs)

1.1.1. Contaminants of Emerging Concern in Water

The term contaminant of emerging concern (CEC) refers to a synthetic or naturally occurring chemical, or any microorganism, that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects [1]. CECs are generally present in waters at trace concentrations, usually ng L^{-1} or µg L^{-1} [2]. Prominent categories of CECs are pharmaceuticals, personal care and cosmetics products (PCCPs), industrial chemicals, flame retardants, endocrine-disrupting compounds (EDCs) and pesticides, however much remains unknown about these CECs as these categories still encompass a large array of contaminants [3]. This combination of trace concentration and the diversity of CECs makes detection, analysis and knowledge of their lifecycle in water systems a challenge [2]. The prevalence of these substances in our society means that these compounds are continually entered into the environment, frequently via run off as well as the wastewater system. Occurrence of CECs in aquatic systems has long been linked with significant adverse effects on the ecosystem, including chronic toxicity in aquatic organisms and antibiotic resistance [4]. The current methods of treating wastewater are designed primarily to remove degradable carbon and nutrients, not CECs [5]. The current infrastructure only partially removes or degrades many of these CECs, leading to their release into surface waters system in wastewater effluent [3].

1.1.2 Sources of CECs

There are many sources of CECs in water and the pathways largely depend on the class of contaminant in question. A 2014 review by Luo *et al.* [2] efficiently summarised the major sources of CECs entering in water systems, which mostly include domestic and industrial wastewater, industrial manufacturing, and agricultural run-off, reproduced in Table 1.

Table 1. Categories, notable subclasses and typical sources of CECs in the aquatic environment [2]

Category	Important subclasses	Major sources	
		Distinct	**Nonexclusive**
Pharmaceuticals	NSAIDs, lipid regulator, anticonvulsants, antibiotics, β-blockers, and stimulants	Domestic wastewater (from excretion) Hospital effluents Run-off from CAFOs[a] and aquaculture	Sources that are not exclusive to individual categories include: Industrial wastewater (from product manufacturing discharges) Landfill leachate (from improper disposal of used, defective or expired items)
Personal care products	Fragrances, disinfectants, UV filters, and insect repellents	Domestic wastewater (from bathing, shaving, spraying, swimming etc.)	
Pesticides	Insecticides, insecticides, herbicides and fungicides	Domestic wastewater (from improper cleaning, run-off from gardens, lawns and roadways etc.) Agricultural runoff	

[a] CAFOs: Concentrated Animal Feeding Operations

Sources of CECs can also be thought of as cyclic, as they are continually being reintroduced into the environment by these various sources laid out above. This flow diagram reproduced from work by Petrovic *et al*. demonstrates this cycle well (Figure. 2).

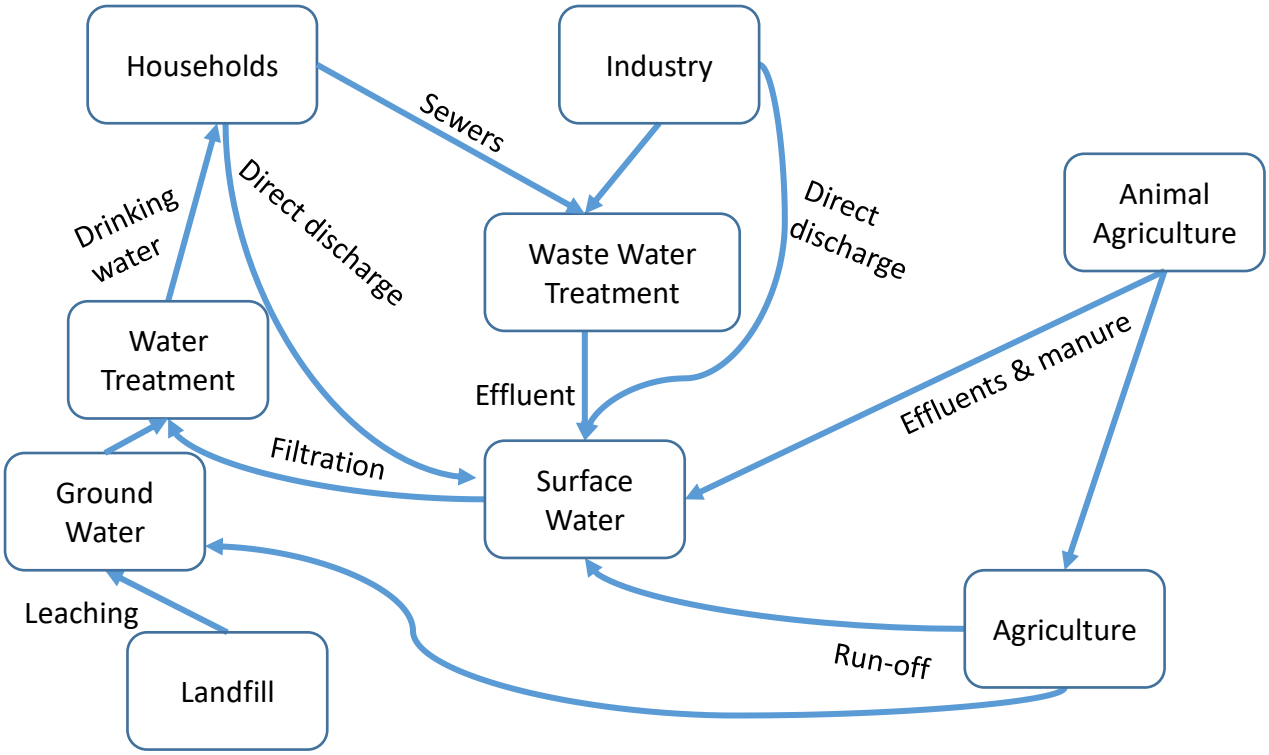


Figure 2. Cyclic nature of sources of CECs in the environment including wastewater treatment, industry, and agriculture (reproduced from [6])

Agricultural runoff is a common source of pesticides in surface and ground water. Run off can be thought of as the movement of contaminants across the surface of the soil where a pesticide is sprayed. Certain factors are known to increase the likelihood of run off, such as the weather conditions, the slope of the spraying site, and the vegetation present on the site. Guidelines for best spraying practices are put in place in Ireland by organisations such as Teagasc or the Pesticide Regulation and Control Division to attempt to minimise run off. Examples of such guidelines include not spraying within 10 metres of a water body, if rain is forecast within the next 48 hours, or during windy conditions [7]. Concentration of the pesticide class of CECs found in surface waters are likely to go through peaks and troughs with the seasons, as the agricultural practice of spraying pesticides is done in the spring/summer (pre-emergence) [8] and autumn (post-emergence) [9] usually, meaning higher likelihood of finding pesticides in water systems during these times than in winter.

As can be seen from Table 1, domestic wastewater is the 1st major source listed for every class of contaminant, namely due to the incomplete removal of CECs via the water treatment

process. However, discharges from industrial manufacturing processes are listed as another major source not confined to any one category of CEC. To try and combat pollution emissions to air and land as well as water, the EPA in Ireland has been licencing certain discharge activities since 1994 [10]. An Integrated Pollution Control (IPC) licence is a single licence granted to a facility which encompasses all emissions which that company is permitted to release into the environment. Emissions produced by a facility must be proven to not cause significant adverse environmental effects for the facility to be granted an IPC license. Some categories of facilities which meet conditions requiring IPC licences are: Chemicals, Food and Drink, Textiles and Leather, Intensive Agriculture, Energy, Fossil Fuels, Cement, Waste, Surface Coatings and a miscellaneous category listed as Other Activities [11]. All these activities have the potential to produce CECs, however currently the EPA nor the EU denote a maximum CEC concentration allowable in emissions into the environment. The only discharges to water parameters currently included in IPC licences are temperature, pH, toxicity, BOD, COD, suspended solids, total nitrogen, ammonia, total phosphorus, and fats, oil and grease [10].

1.1.3 Stability of CECs in the Environment

Once present in the environment most CECs will interact with their surroundings. The nature of this interaction depends on the characteristics of the contaminant. Hydrolysis, biodegradation, volatilization, photo-degradation, oxidation, dilution conversion to metabolites and desorption from particulate matter are just some examples of the possible degradation pathways [2,12,13]. Pathways found to be common in the pesticide class of pollutants include biodegradation and photo-degradation. Desorption to particulate matter is particularly common with non-polar pesticides such as pyrethroids [14]. Whether or not a contaminant will degrade at all depends on numerous conditions involving both the specific analyte in question, and the environment it is present in. Physico-chemical properties of the pollutant such as log K_{ow} and photo-stability, combined with environmental factors such as pH of the matrix and weather conditions are all variables involved with potential degradation pathways of a contaminant [15]. Changes in weather patterns caused by climate change are also likely to have an impact of the stability and behaviour of CECs in the environment [16]. Such a large quantity of variables makes it extremely difficult to predict potential behaviours of CECs in water bodies, and this makes analysis of specific contaminants necessary.

1.1.4 Wastewater Treatment Processes

In Ireland, there are currently approximately 1,100 wastewater treatment facilities (WWTPs) that treat over 1 billion litres of wastewater collected every day via around 30,000 km of sewers. This treated water is then released back into the environment into various water bodies such as rivers, lakes and coastal waters. According to the EPA 2016 Urban Wastewater Report, before being discharged back into the environment, 3% of wastewater undergoes no or preliminary treatment, 1% receives primary treatment, 69% goes through secondary treatment and 27% undergoes secondary treatment as well as nutrient removal (sometimes referred to as tertiary treatment) [17].

Standards are set for a WWTP in the areas of collection, treatment, and discharge of wastewater into the environment [18]. However, it was found that in 2016, out of 185 urban WWTPs in Ireland, only 135 of them were found to be compliant with all standards set by the EU Urban Wastewater Treatment Directive. These 50 WWTPs accounted for 64% of the national wastewater load collected in all large urban areas [17]. Storm water overflows, in which increased rainfall causes a sewer system to become overwhelmed requiring release of excess storm and untreated wastewater into the environment, have also been known to occur in Irish plants [19,20]. This is likely to have a large impact on surface water ecosystems, as well as threatening many of Ireland's water bodies ability to meet the EU Water Framework Directive's requirements for 'good' water status [21].

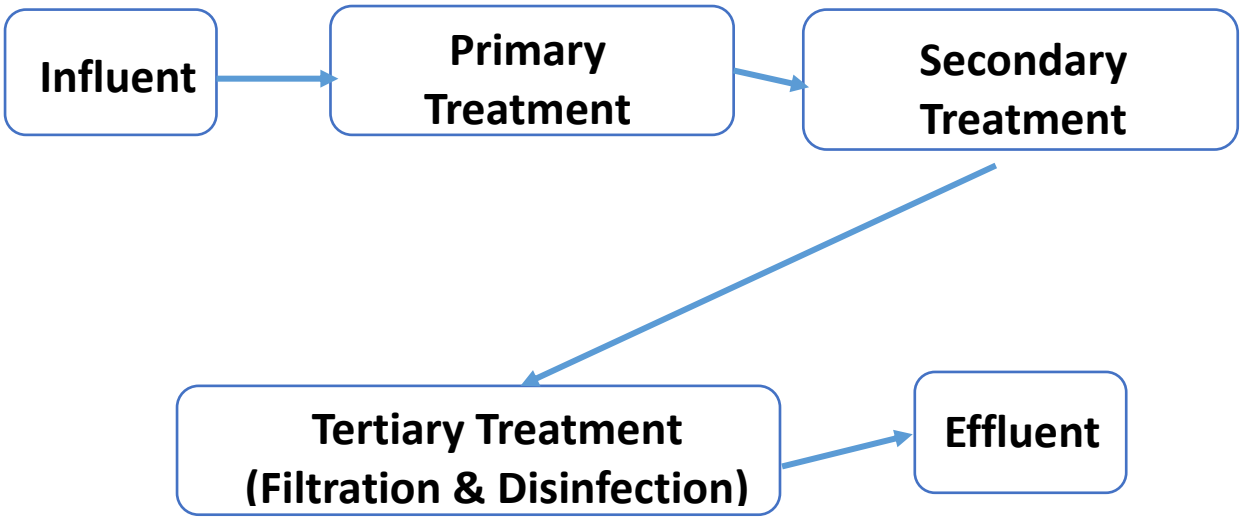


Figure 3. Schematic showing typical wastewater treatment process including primary, secondary and tertiary treatment [22, 23, 24,25,26].

There are three stages to the wastewater treatment process which is outlined in brief in Figure 3. The majority of WWTPs use only primary and secondary treatment, with some larger plants using tertiary as well. Primary treatment involves settlement in sedimentation tanks, while secondary treatment employs the use of aeration and biological processes to degrade substances such as sugars, fats and short chain carbons [27]. A review by Luo *et al.* found large discrepancies between the percentage removal of different contaminants through WW treatment [2]. For example, it was found that the analgesic painkiller paracetamol had a removal rate between 98.7-100% based on 5 different studies[15,28–31]. However, some pollutants had consistently low rates of removal, such as the herbicide atrazine, which had a range of 0-25% removal based on 6 different studies – considerably lower than paracetamol [15,32–36]. Not only are certain compounds being removed consistently poorly or consistently efficiently but the majority of CECs mentioned in this review had an exceedingly wide range of removals, the largest being the surfactant octylphenol, with a percentage removal range from <0-96.7% based on 7 studies [13,15,35,37–40]. This huge variance between removals makes it increasingly difficult to evaluate a plants efficiency without specific studies. Therefore, investigation into the fate of CECs through the WW system is of great importance.

1.1.5 Ecotoxicological Impact

Due to the incomplete removal of CECs from wastewater, CECs can enter the ecosystem via the effluent released into the receiving waters. The concentration levels, in the ng L^{-1} to μL^{-1},

have been reported to have extremely detrimental effects on aquatic organisms. Concentrations at this level are known to cause both acute and chronic toxicity in aquatic organisms [41–43]. A 2015 study on the toxic effects of pyrethroid pesticides on non-target aquatic organisms by Antwi and Reddy found that a number of non-target groups were detrimentally affected by introduction to pyrethroid pesticides. These include the *Ephemeroptera, Plecoptera, Odonata, Hemiptera, Coleoptera and Trichoptera* [44]. In another study by Tsui and Chu investigating the toxic effects of the herbicide glyphosate, they found that glyphosate had acute toxicity in the low µg L^{-1} - mg L^{-1} range to Microtox® bacterium (*Vibrio fischeri*), microalgae (*Selenastrum capricornutum* and *Skeletonema costatum*), protozoa (*Tetrahymena pyriformis* and *Euplotes vannus*) and crustaceans (*Ceriodaphnia dubia* and *Acartia tonsa*) [45].

CECs with a higher $LogK_{ow}$ have the potential to bioaccumulate in fish which is a cause for concern due to the high probability for bioconcentration up the food chain [46,47].

1.1.6 Monitoring and Legislation

The EU Water Framework Directive (WFD) was first established in 2000, with the goal to protect and improve water quality in all EU Member States so to achieve 'good' ecological status on all waters. In Annex VIII of the WFD, biocides and plant protection products are specifically listed as main pollutants [21]. The list of priority substances listed in the WFD has been changed an updated as additional data has become available over the years. Of the 33 substances or groups of priority substances contained in Directive [48], 18 are pesticides or substances used to make pesticides in the case of trichloro-benzenes. These compounds have environmental quality standards (EQS) values set in the low µgL^{-1} range [48]. Later amendments to legislation involved creation of a 'Watch List' (WL) of contaminants that are indicated as pollutants but have limited monitoring data available [49]. The WL has been through 3 iterations since its conception in 2015. The original WL (1st Watch List) contained 10 suspected CECs or groups of CECs, totalling 17 individual chemicals [49]. The update in 2018 (2nd Watch List) removed 5 CECs from the original list and added 3 new ones [50]. Most recently in 2020, the list was updated again (3rd Watch List), which removed 12 compounds from the 2nd WL, and added 16 new ones [51]. Thus far, there has been no studies conducted in Ireland which specifically collect information on Watch List chemicals.

Other initiatives to improve water quality have been developed in conjunction with the WFD, such as the REACH programme. REACH, led by Oxford University funded with UK aid from the UK Government, is a global initiative with partners internationally. Its goal is to 'improve water security for the poor by delivering world-class science that transforms policy and practice [52].

1.2 Pesticides

Ireland is a predominantly agricultural country with 67.6% of the total land reported as being used for agricultural practices [53]. Pesticide use is part of the growing intensification of agricultural practices. Therefore, pesticides are of particular concern and were selected for further investigation within this project.

Pesticide is the umbrella term which includes both biocides and plant protection products (PPPs). The difference between a biocide and a PPP is down to the purpose the substance is being used for, and the distinction is made primarily for legislation purposes. A biocide can be described as a chemical or microorganism intended to control unwanted organisms that are harmful to human or animal health, including such organisms as bacteria, mould, insects, rats and mice [54]. Plant protection products are substances used to control unwanted organisms harmful to cultivated plants [55]. There is the potential for some crossover between these two classifications for certain active substances depending on their intended use. For example, the insecticide cypermethrin is both used as a PPP in agricultural production in products such as Talisma EC for the control of stored grain pests, and as a biocide in the forestry industry in products such as Cuprinol 5 Star Complete Wood Treatment for the control of pine weevils in restocked conifers [56,57]. Although containing the same active ingredient, these two products are available under different legislation, namely Regulation (EU) No. 528/2012 for biocides and Regulation (EU) No. 1107/2009 for PPPs [58,59].

1.2.1 Analyte selection

Pesticides chosen for investigation in this project were selected either due to their indication in the River Basin Management Plan For Ireland 2018-2021, inclusion on either the 2nd or 3rd Watch List, or due to the increasing amount of recent literature which indicates them as a potential contaminant of emerging concern for influent or effluent in European wastewater treatment plants, or associated receiving waters [60]. EQS standards have not been set for the majority of the compounds included in this work as there is limited data is available on their occurrence. EQS values are different from the EU Drinking Water Directive value which sets a maximum limit of 0.1 µg/l for individual pesticide residues in a sample, and 0.5 µg/L for total pesticide concentration [61].

There is currently limited recent data available on the usage of pesticides in Ireland, with the most recent report being published by the Pesticide Registration and Control Division, Department of Agriculture in 2014 [62,63]. Some examples of currently available usage data is presented below for some of the analytes included in this project, with Table 2 showing the weight in kilograms of single pesticide and Table 3 showing the weight in kilograms of a pesticide mixture applied in a particular year for a certain crop.

Table 2. Irish pesticide usage data 2011-2014 for individual pesticides

Pesticide	**Grassland and Fodder Crops, 2013 (kg)** [64]	**Arable Crops, 2012 (kg)** [65]	**Top Fruit Crops, 2014 (kg)** [63]	**Soft Fruit Crops, 2014 (kg)** [62]	**Vegetable Crops, 2011 (kg)** [66]
2,4-D	21,572	N/A	28	N/A	N/A
Mecoprop-p	10,090	42,501	N/A	N/A	N/A
MCPA	247,377	4,625	N/A	N/A	N/A
Glyphosate	116,173	98,788	546	22.57	1,358.1
Cypermethrin	22	3,334	0	N/A	22.4
Permethrin	N/A	N/A	N/A	N/A	N/A
Deltamethrin	N/A	47	0	0.01	6.35
Bifenthrin	N/A	N/A	N/A	N/A	N/A
Esfenvalerate	2	517	N/A	N/A	1.08

Table 3. Pesticide usage data for Ireland 2011-2014 for relevant pesticide mixtures

Pesticide combination	Grassland and Fodder Crops, 2013 (kg) [64]	Arable Crops, 2012 (kg) [65]	Top Fruit Crops, 2014 (kg) [63]	Soft Fruit Crops, 2014 (kg) [62]	Vegetable Crops, 2011 (kg) [66]
2,4-D/ Dicamba/ Triclopyr	26,479	N/A	N/A	N/A	N/A
2,4-D/MCPA	7,241	N/A	N/A	N/A	N/A
2,4-D/ Triclopyr	206	N/A	N/A	N/A	N/A
2,4-DB/MCPA	10,817	N/A	N/A	N/A	N/A
2,4-DB/ Mecoprop-P	3,614	N/A	N/A	N/A	N/A
Dicamba/MCPA/ Mecoprop-p	8,397	950	N/A	N/A	N/A
Dicamba/ Mecoprop-p	19,965	1,798	N/A	N/A	N/A
Dichlorprop-P/ MCPA /Mecoprop-P	8,020	10,587	15	N/A	N/A
2,4-D/Dicamba/Fluroxypyr	N/A	664	N/A	N/A	N/A
Bromoxynil/ioxynil/ Mecoprop-p	N/A	10	N/A	N/A	N/A

There is more recent data available in the form of EU sales data. Figure 4 shows the trends in pesticides sales from the period 2011-2016. As can be seen from the graph, there is a notable decline in pesticide sales across three categories of pesticides between 2011-2012. This could potentially linked to the introduction of the European Communities (Sustainable Use of Pesticides) Regulations 2012 statute [67].

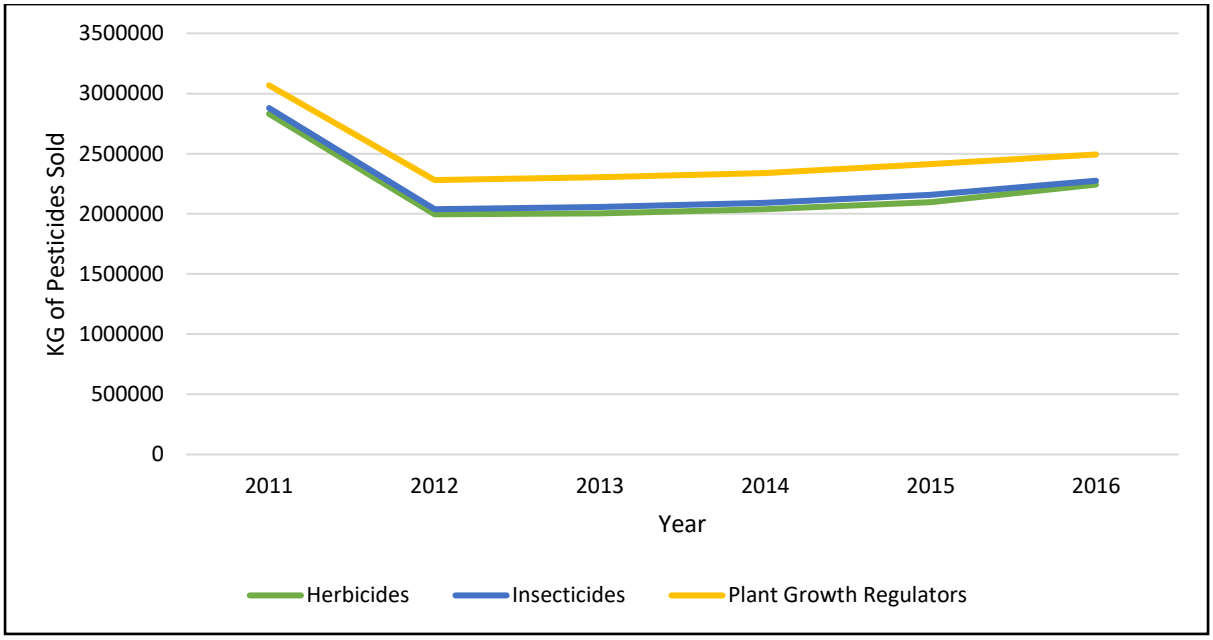


Figure 4. Line graph of pesticide sales data for Ireland for period 2011-2016 [68], showing decrease in 2012 with the introduction of the EC Sustainable Use of Pesticides statute.

In comparison to other EU countries, Ireland appears to contribute less to the overall quantity of pesticides sold as seen in Figure 5. However, the relative landmass of Ireland when compared to countries like France and Germany is a notable factor. France purchased over 30 million kg of pesticides in 2016, while Ireland purchased over 2 million Kg. France has approximately 6 times more agricultural land than Ireland, making the pesticide usage per kilometre more comparable [69,70].

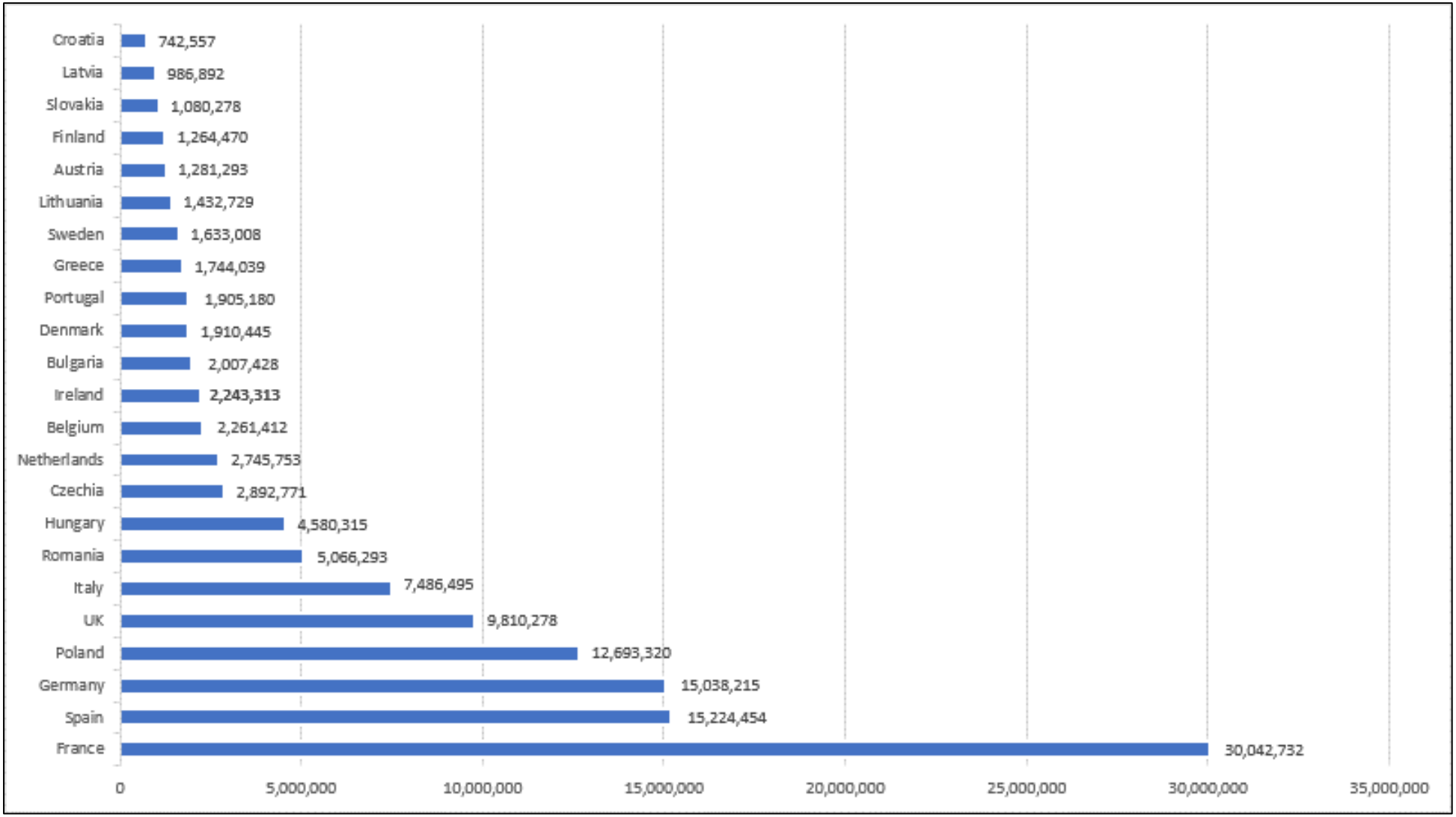


Figure 5. Comparison of EU member state herbicide sales data in kg sold for the year 2016 showing purchase of >2 million kg for Ireland

1.2.2 Acid Herbicides

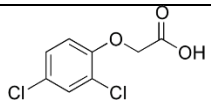
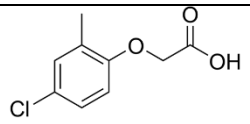
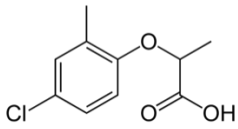
1.2.2.1 Group chemistry, general uses, and mode of action

Phenoxy acid or phenoxyacetic acid herbicides are synthetic analogues of plant growth hormones called auxins. The general structure for this group of herbicides is that of an aromatic ring with a carboxylic acid side chain. While the basic form of this group of herbicides is a carboxylic acid, they are often applied in an ester, amine or salt form due to various manufacturing processes [71].

Phenoxy acid herbicides are manufactured a family of selective herbicides - meaning they target only specific target pests- which are used to remove broad leaf weeds in mainly grassland as well as some cereal crops [72]. Their mode of action is via mimicking of the plant growth hormone indole acetic acid (IAA), causing uncontrolled growth in the weed leading it to 'grow itself to death' -.

Acid herbicides are general quite polar in nature, with log K_{ow} values ranging from 2.8 to 3.25 (Table 4) [74–76]. This means that within the environment and wastewater treatment systems, they have a higher affinity for the aqueous phase rather than any solid particulates such as soil or organic matter. This causes them to persist throughout water treatment [77], with MCPA accounting for over 80% of exceedances over the 0.1 µg/L limit for drinking water over the past few years [78]. Acid herbicides can be analysed by Liquid Chromatography – Mass Spectrometry (LC-MS) [79], however they are also often efficiently analysed by Gas Chromatography – Mass Spectrometry (GC-MS), requiring a derivatisation step into their methyl ester form [80]. There are three acid herbicides to be investigated in this study, 2, 4-D, MCPA and Mecoprop.

Table 4. Physico-chemical properties of acid herbicides

Compound	Chemical Formula	CAS No.	LogKow	Molecular Weight	Structure
2,4-D	$C_8H_6Cl_2O_3$	94-75-7	2.81	221.033	
MCPA	$C_9H_9ClO_3$	94-74-6	3.25/2.8	200.618	
Mecoprop	$C_{10}H_{11}ClO_3$	93-65-2	3.13	214.645	

2,4-dichlorophenoxyacetic acid, more commonly known as 2,4-D is one of the oldest acid herbicides on the market after first being made available in 1942 [81]. The chemical is approved for use in the EU until 2030 under Reg. (EU) 1107/2009 [59]. There are over 35 products listed on the Irish Department of Agriculture; Food and the Marine website that contain 2,4-D as an active ingredient which are available in Ireland [56]. Examples of such products include Vitax Lawn Clear 2, Dicophar and Herboxone.

MCPA is the most used pesticide in Ireland with over 247,000 kg of the chemical applied to Grassland and Fodder crops in 2013 57. It is available in over 55 commercial products on the Irish market 46 including Agritox, Dicophar and Vitax Lawn Clear 2. MCPA is approved for use in the EU under Reg. 1107/2009 until the 31st of October 2019 49. MCPA has been found to be moderately toxic to wildfowl such as pheasants and mallards with an LD50 value of 377 mg/kg 74. It is also mildly toxic to fish with an LC50 value of 90 mg/L 55.

Methylchlorophenoxypropanoic acid, MCPP, or most commonly known as Mecoprop is an acid herbicide available in two forms. Mecoprop, which is a mixture of two stereoisomers, and Mecoprop-p which purely contains the R-enantiomer 75. Only Mecoprop-p is approved for use in the EU under Reg. (EC) No 1107/2009 49. There are over 58 products listed on the Irish Department of Agriculture website as containing Mecoprop as an active ingredient 46, including Dicophar, GreenForce Lawn Weedkiller and Longbow.

1.2.3 Glyphosate

Glyphosate is an organophosphorus containing chemical product commonly used as a pesticide [82]. The general basic formula of an organophosphorus compound contains a central phosphorus atom which is double bonded to an oxygen and single bonded to three other oxygens, each of which are bonded to an R group [82]. The structure can be seen in Figure 6.

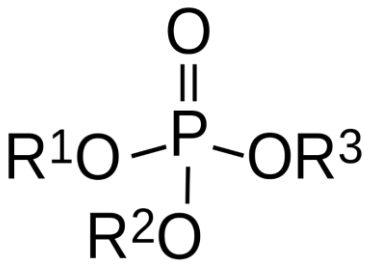


Figure 6. Base structure of organophosphorus compounds

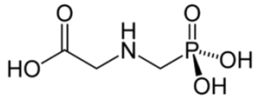
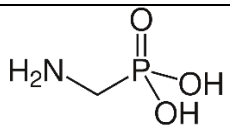
Most organophosphorus pesticides act as insecticides, causing acetylcholinesterase inhibition in the target pests [83]. However, certain organophosphorus compounds have been known to display herbicidal properties, one such chemical being glyphosate and its primary metabolite aminomethylphosphonic acid (AMPA), the only organophosphorus compounds to be studied within this project.

Glyphosate, or N-(phosphonomethyl)glycine is a nonselective, post emergence herbicide used to control the growth of broadleaf weeds [84]. It is the most widely used herbicide in the world [84], with over 115,000 kg of glyphosate applied to Irish Grassland and Fodder crops in 2013 [64]. Ireland purchases a significant quantity of herbicides and plant growth regulators, including glyphosate, in comparison to other countries by land mass. Glyphosate is listed as an active ingredient in over 150 commercial products sold in Ireland, the most popular being Roundup and Roundup related products. Glyphosate is approved for use in the EU under Reg. (EC) No 1107/2009 until 2022 [59]. This approval was renewed in 2017 to some controversy, due to the classification of glyphosate as a probable human carcinogen [84].

Due to its extremely low $\log K_{ow}$ of -3.3, glyphosate is highly soluble in water and has been known to be stable in water at varying pH and temperatures, as well as under sustained

natural sunlight [85]. Glyphosate's major degradation product is aminomethylphosphonic acid (AMPA), and generally when investigating the persistence of glyphosate in the environment, the presence of AMPA is tested for too as it has been known to be similarly environmentally active [86–88]. The compound's main degradation pathway is through microbial-mediated processes, along with other chemical, physical and biological factors [89]. Structure and physico-chemical information for these compounds can be seen in Table 5.

Table 5. Physico-chemical properties of glyphosate and AMPA [90]

Compound	**Chemical Formula**	**CAS No**	**LogK_{ow}**	**Molecular Weight**	**Structure**
Glyphosate	$C_3H_8NO_5P$	1071-83-6	-3.4	169.073	
AMPA	CH_6NO_3P	1066-51-9	-4.7 *	111.04	

* Computed XLogP3-AA value

1.2.4 Pyrethroids

1.2.4.1 Structure and Chemistry

Pyrethroids are non-polar artificial compounds with a molecular structure derived from pyrethrins (Figure. 7), which are found in Chrysanthemum cinerariaefolium flowers [54]. The structure of pyrethrin resembles chrysanthemic acid, a carboxylic acid moiety bonded to a cyclopropane ring and linked to an aromatic alcohol via ester linkage [91]. Naturally occurring pyrethrins are effective pesticides, however they are relatively unstable in sunlight, moisture and air, making their use as a commercial pesticide product limited. This spurred on the development of synthetic pyrethroid products based on pyrethrins that are more environmentally stable and therefore useable as commercial pesticides. These compounds can have degradation rates of over 200 days in environmental matrices [92] .Replacement of the cyclopentenolone groups with alcohol components increased stability somewhat, and further altering of the base compound by substituting the cyclopropane ring with acid functional groups and modifications to the ester linkages eventually produced the pyrethroid compounds in use today [93]. At this early stage, the alcohol group was usually a primary or secondary alcohol, and the acid was based in chrysanthemic acid with various halogenated and non-halogenated substituents. Many pyrethroids have a cyano group bound to the alcohol moiety which increases toxicity, these are referred to type II pyrethroids, an example being cypermethrin. Those without this cyano group are known as type I, such as permethrin [91]. A pyrethroid's stereochemistry plays an important role in its toxicity, and therefore both its use as an effective pesticide, and it's potential for environmental harm. The orientation of the substituent on the 3rd carbon in the cyclopropane ring in relation to the carboxylic acid functional group denotes the cis- and trans- forms of the compound. Pyrethroids with the cis configuration, such as deltamethrin, are less likely to be hydrolysed by esterases, and are more likely to have higher mammalian toxicity than those with the trans conformation and a primary alcohol, such as trans-permethrin [94].

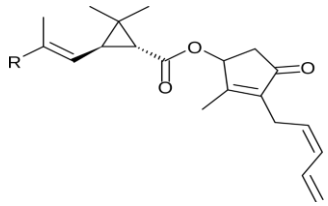


Figure 7. Chemical structure of pyrethrin

1.2.4.2 Modes of action, toxicity and environmental implications

Pyrethroids share a similar mode of action to that of other highly non-polar pesticides such as DDT and DDT related analogues. Pyrethroids have been toxicologically categorised into two classes arising from the initiation of whole body tremors (known as T syndrome) or a whole body tremors developing into choreoathetosis or 'sinuous writhing' with salivation (known as CS syndrome) [94]. T syndrome has been linked with type I pyrethroids and CS syndrome is linked with type II pyrethroids. Type II compounds are known to decrease core body temperature, giving rise to the excessive salivation seen in dosed organisms [95]. The main mode of action of pyrethroids is via inhibition of the voltage gated sodium channels within the neuron. When functioning normally, the voltage-gated sodium channel is stimulated and causes a depolarisation of the membrane, which changes the nerve cell's permeability to the Na^+ cation and K^+ cation. With the excited membrane now permeable to the sodium ion, the Na^+ ions carry a current inward through the membrane, this current is known as the 'action potential'. The migration of the sodium cation causes the membrane potential to change and 'overshoot', with the inside becoming positive relative to the outside of the membrane surface. During this spike the membrane is fixed, and no greater stimulus can cause the gates to open further or for a greater amount of sodium ions to pass inward. The neuron remains fixed for a few milliseconds after the spike and only a strong stimulus will cause a new response. This whole process lasts only 2 to 3 milliseconds at a time [94]. Type 1 pyrethroids alter the sodium channels so that they slightly extend the time that they're open, approximately 20 milliseconds in total, causing multiple long action potentials. Type II pyrethroids prolong the time the channel is open, from 200 ms to min, causing an increased resting membrane potential and a depolarisation dependant block of action potentials [96]. The toxic action is the prevention of the closing of the gate after membrane depolarisation. This causes the disruption of the negative after potential of the nerve due to the leaking of sodium

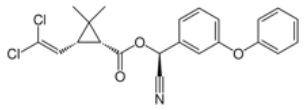
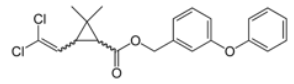
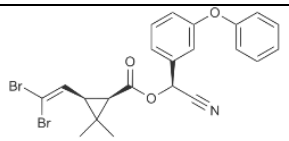
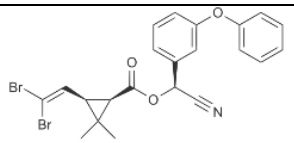
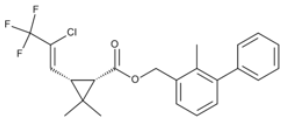
ions through the membrane, causing hyperactivity by prolonging the closing sodium channels, allowing for a persistent inward current to flow after the action potential, leading to repetitive discharges that can occur either concurrently or from a single stimulus [97].

The reason pyrethroids show low mammalian toxicity can be attributed to the enzyme carboxylesterase, which is responsible for the hydrolysis and metabolism of pyrethroids which mammals possess, and the target organisms do not [98]. However, it is not only the target pests which do not possess the necessary enzyme required to break down pyrethroids, non-target organisms such as various species of fish [41,42,99] and aquatic invertebrates black fly *Simulium vitattum*, caddisfly *Hydropsyche*, mayfly Heptagenidae, damselfly *Enellagma* as well as terrestrial based insects such as European corn borer *Ostrinia nubilalis*, housefly *Musca domestica* and Convergent lady beetle *Hippodamia convergens* have all been found to be harmed by pyrethroid pesticides [42,100]. Due to pyrethroids being highly lipophilic, they are rapidly absorbed onto the gills of fish and have a tendency to accumulate in their adipose cells, causing bioaccumulation and eventually death [42].

Pyrethroids are generally used in agricultural production for control of insects, however, they have also been used for the purposes of sheep dipping - submerging the animal in a diluted mixture of the insecticide to control ticks - as well as being used to control sea lice in salmon farming and as a biocide in wood preservative. The use of synthetic pyrethroid insecticides has greatly increased in recent years due to the prohibition of organochlorine pesticides such as diazinon and chlorpyrifos. One quarter of the world insecticide market is made up of pyrethroids [101]. Due to their non-polar nature and high degree of lipophilicity, pyrethroids tend to sorb to the solid particulates in wastewaters. It has been found that although the majority (≤90%) of the pyrethroid concentration is removed with secondary treatment, the approximate 10% that remains in the water has a high enough concentration to be acutely toxic to certain sensitive species [102]. A study by Parry and Young in 2013 found that only 27% to 40% of the remaining pyrethroid concentration in effluent could be removed by increased settling of solids, they felt this indicated that increased settling time in the wastewater treatment process would be unlikely to completely alleviate pyrethroid discharges [103]. It has been suggested that tertiary treatment using biologically aerated flooded filtration and rapid

gravity filtration could potentially further remove pyrethroids from wastewater, however the study did not produce statistically significant results and so further investigation is required [104]. Five pyrethroid pesticides were selected for inclusion in this study, Shown in Table 6.

Table 6. Physico-chemical properties of pyrethroid insecticides[105]

Compound	Chemical Formula	CAS No	LogK$_{ow}$	Molecular Weight	Structure
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	52315-07-8	6	416.298	
Permethrin	$C_{21}H_{20}Cl_2O_3$	52645-53-1	6.5	391.288	
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	52918-63-5	6.2	505.206	
Esfenvalerate	$C_{25}H_{22}ClNO_3$	66230-04-4	6.22	419.905	
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	99267-18-2	6	422.872	

Cypermethrin or [Cyano-(3-phenoxyphenyl)methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate is a type II pyrethroid [106] that was first produced by the addition of an α- cyano group onto the previously synthesised permethrin compound. This cyano group improved the degree of insecticidal properties displayed in the compound, as well as reducing the rate of hydrolysis making for a more stable compound for agricultural use [107]. This combination of high stability and high potency has made cypermethrin one of the most popular pyrethroid insecticides in use. Cypermethrin, alpha cypermethrin and zeta-cypermethrin are approved for use under Reg. (EC) No 1107/2009 [59] however the isomer beta-cypermethrin is not approved under the dossier Reg. (EU) 2017/1526 [108]. Cypermethrin is currently available in 20 commercial products in Ireland [56] including amateur use products such as Cythrin Caterpillar Spray and Doff Rose Sheild Bug & Fungus Killer, as well as

professional use products including Talisma EC which is used to control various pests such as grain weevils, lesser brain borers and mites.

Permethrin, also known as 3-Phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl) -2,2-dimethylcyclopropanecarboxylate, is a type I pyrethroid [106] and was the first synthesised pyrethroid which was photo stable enough to be used as an agricultural pesticide. The increased resistance to light was achieved through changes in the structure of the alcohol functional group and halogenation of the acid moiety [97]. Permethrin was found to be over 100 time more stable to sunlight than the previously synthesised pyrethroid resmethrin, whilst also displaying the same high level of insect and low level of mammalian toxicity [91]. Permethrin is approved as a biocide under Reg. (EU) No. 528/2012 [58]. It is available in 145 commercial biocidal products including multiple 'bug spray' type products that target wasps, fleas and ants (Doff, Insectrol, Nippon, Pestsheild) and multiple wood preserver products (Ronseal, Lignum, Everbuild, Palace) [57].

Deltamethrin or [(S)-cyano-(3-phenoxyphenyl) methyl] (1R, 3R)-3-(2, 2-dibromoethenyl)-2, 2-dimethylcyclopropane-1-carboxylate, was formed from the same process as cypermethrin, involving the inclusion of a cyano- substituent within the alcohol functional group. Further altering of the compound by substituting two bromine atoms for the chlorines in the alcohol side chain lead to the final molecule known as deltamethrin today [107]. Deltamethrin is approved for use in the EU under Reg. (EC) No 1107/2009 until October 31st 2019 when it must be either renewed or its approval lifted [109]. It is available in 9 commercial plant protection products available in Ireland including Multirose Concentrate 2, Polux and Decis [56].

Esfenvalerate is the commonly used name for (αS)-α-cyano-3-phenoxybenzyl (2S)-2-(4-chlorophenyl)-3-methylbutyrate. It is the most insecticidally active isomer of the synthetic pyrethroid insecticide fenvalerate. It is the 2-S alpha enantiomer of fenvalerate. Esfenvalerate is approved for use in the EU under Reg. (EC) No 1107/2009 until the 31st of December 2022 [59]. According to the Pesticide Registration and Control Division, it is only available in 1 commercial plant protection product in Ireland, called Sumi-Alpha [56].

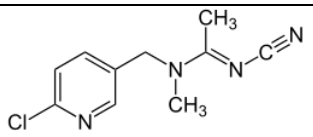
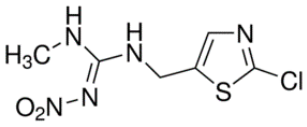
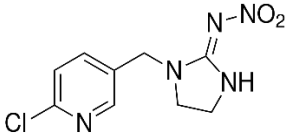
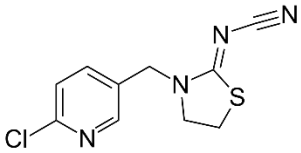
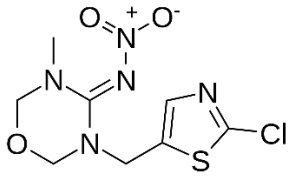
Bifenthrin, or 2-Methyl-3-phenylphenyl) methyl (1S, 3S)-3-[(Z)-2-chloro-3, 3, 3-trifluoroprop-1-enyl] - 2, 2-dimethylcyclopropane-1-carboxylate, is a relatively stable type I pyrethroid insecticide. Bifenthrin is an ester with alcoholic components whose activity was found not to be enhanced by an alpha-cyano substituent [91].

1.2.5 Neonicotinoids

Neonicotinoids are a group of insecticides which are chemically similar to nicotine. Neonicotinoids, in particular Imidacloprid, are the most widely used group of insecticides in the world [110]. The first neonicotinoid was approved for use in the EU in 2005 [111]. Neonicotinoids are environmentally persistent polar compounds which readily move into the water column and break down slowly. They have been shown to have a photodegredation half-life of 34 days [112]. However, in conditions without sunlight such as in soil they can persist for >1000 days, making their presence in water matrices through run off a cause for concern [112]. Physico-chemical properties of these compounds can be seen in Table 7.

Their mode of action is by binding to the cellular nicotinic acetylcholine receptors in the central nervous system in insects, eventually causing paralysis and death. They have low mammalian toxicity historically making them an attractive choice for agricultural practices [110]. However, in recent years they have been shown to be a non-targeted pesticide and have caused immense issues with essential pollinator colonies around the world [113]. Due to this, restrictions on neonicotinoids increased from 2013 and were banned for all outdoor use in the EU in April 2018. However usage still persists else ware such as the US and parts of Asia [114–116].

Table 7. Physico-chemical properties of neonicotinoid insecticides

Compound	Chemical Formula	CAS No	$LogK_{ow}$	Molecular Weight	Structure
Acetamiprid	$C_{10}H_{11}ClN_4$	160430-64-8	0.8	222.67	
Clothianidin	$C_6H_8ClN_5O_2S$	210880-92-5	0.7	249.68	
Imidacloprid	$C_9H_{10}ClN_5O_2$	138261-41-3	0.57	255.66	
Thiacloprid	$C_{10}H_9ClN_4S$	111988-49-9	1.26	252.72	
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	153719-23-4	-0.13	291.72	

1.2.6 Azoles

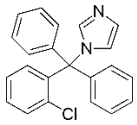
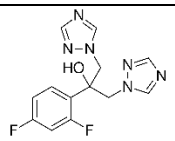
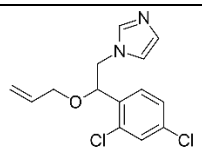
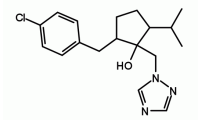
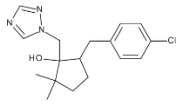
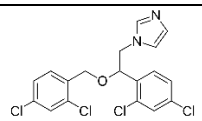
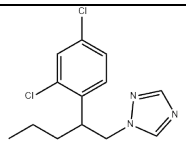
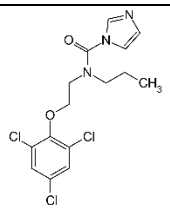
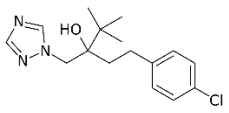
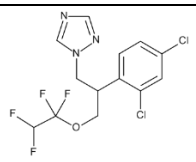
Azoles are a group of compounds characterised by their azole ring which possess antifungal properties [117]. They are a very commonly applied fungicide in Irish agriculture, with azole compounds commonly featuring in the top 10 active ingredients used most extensively on a variety of arable crops. The quantity of azole pesticides used in Ireland is estimated to be in the 10s of thousands of kilograms [65]. Ten azole compounds were selected for inclusion in this thesis (Table 8).

Their main mode of action is through inhibition of fungal lanosterol-14α-demethylase and aromatase [118,119] The former enzyme is responsible for the production of meiosis-activating sterols that are involved in germ cell development, and the latter controls the physiologic balance of androgens and estrogens in mammalian animals. These compounds are therefore significant reproductive and fertility hazards [120].

Azole removal from wastewater is under studied, and generally only focuses on azoles with antifungal pharmaceutical applications. One of the only studies performed on these compounds by Kahle *et al*. found that the compounds fluconazole, propioconazole and tebuconazole were predominantly unaffected by wastewater treatment, however clotrimazole was over 80% eliminated [118]. Clotrimazole has been investigated once previously in Irish wastewaters was in samples taken in 2008 by Lacey *et al* [121]. In this study the analyte was found to have negative removal rates in wastewater, meaning higher effluent concentrations than influent.

Azoles are legislated for use in the EU under Regulation (EU) 528/2012 [58].

Table 8. Physico-chemical properties of azole fungicides

Compound	Chemical Formula	CAS No	$LogK_{ow}$	Molecular Weight	Structure
Clotrimazole	$C_{22}H_{17}ClN_2$	23593-75-1	4.1	344.8	
Fluconazole	$C_{13}H_{12}F_2N_6O$	86386-73-4	0.25	306.27	
Imazalil	$C_{14}H_{14}Cl_2N_2O$	35554-44-0	3.82	297.2	
Ipconazole	$C_{18}H_{24}ClN_3O$	125225-28-7	4.21	333.9	
Metconazole	$C_{17}H_{22}ClN_3O$	125116-23-6	3.85	319.8	
Miconazole	$C_{18}H_{14}Cl_4N_2O$	22916-47-8	6.1	416.1	
Penconazole	$C_{13}H_{15}Cl_2N_3$	66246-88-6	3.72	284.18	
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	67747-09-5	4.1	376.7	
Tebuconazole	$C_{16}H_{22}ClN_3O$	107534-96-3	3.7	307.82	
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$	112281-77-3	3.56	372.14	

1.3 Sampling and Extraction Methods

1.3.1 Sampling techniques

The classic method of monitoring pesticides in water is using grab or spot samples, which have several advantages and limitations. Grab sampling is very straightforward and does not require specialist knowledge, which makes it an attractive avenue for more general studies, studies involving laymen such as citizen science campaigns, and studies involving a large array of different sampling sites or matrixes [122]. Disadvantages include grab samples only provide a 'snapshot' of the concentration of the contaminant in the sample matrix in that time. Additionally, when sampling for the purpose of detecting low level contaminants, large sample volumes are required [123]. Following collection, grab samples are typically acidified and either extracted immediately by Solid Phase Extraction (SPE) or stored at -18°C until extraction. A flow diagram of this procedure can be seen in Figure 8.

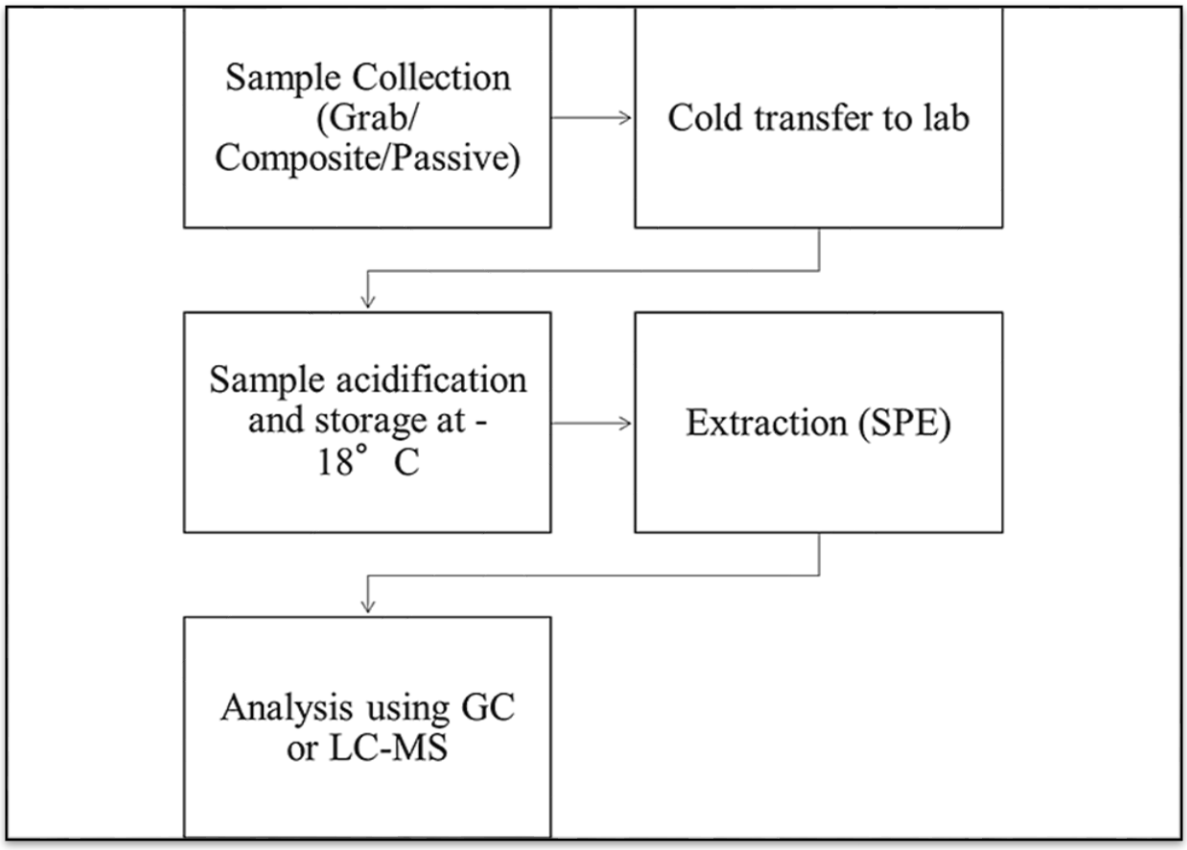


Figure 8. Flow chart showing the steps from sample collection to final analysis for the examination of CECs in aquatic matrices

1.3.2 Passive Sampling

Passive sampling is an umbrella term for any analytical technique that allows for the free transfer of substances in the sample medium (e.g. water, air) into a collector medium [124]. The typical passive sampler consists of a receiving phase that has a strong affinity for the target analyte. This receiving phase can be used alone such as in Low Density Polyethylene Strips (LDPS) and Silicon Rubber (SR) sheets or in conjunction with a diffusion limiting membrane such as in the Chemcatcher or Polar Organic Chemical Integrative Sampler (POCIS). It is possible to tailor the device to for various purposes by changing the combination of receiving phase and diffusion limiting membrane [124]. Passive sampling can considerably improve detection limits of analysis. This is due to the chosen receiving medium having a strong affinity for the analytes of interest, and thus attaining a higher concentration within the receiving medium in comparison to that of the water [125].

There are a broad range of passive sampling devices currently available for water monitoring, however all devices can be categorised into two basic modes of action; absorption which is seen in partition sampling devices, and adsorption, seen in devices in which the target analyte forms bonds with the sampling material. A summary of different types of passive samplers can be seen in Table 9.

Table 9. Passive sampling devices and their characteristics that are used in freshwater monitoring.

Passive sampling device	**Principle of sampler**	**LogK_{ow} range**	**Typical length of deployment**	**Key characteristics**	**Ref**
POCIS	Polymeric receiving phase between two PES membranes	<3	1-12 weeks	Housings can be reused with replacement of the sorbent. Dissolved Organic Matter (DOM) concentration can affect uptake rates meaning discrepancies between lab	126–132

				calibrations and in field uptake.	
Chemcatcher®	Choice of receiving phase depending on Log K_{ow} range studied held within PTFE housing, PES membrane	<3, 3-7	2 weeks	Housings can be reused with replacement of the sorbent. Broad range of analytes can be studied using different sorbent and membrane combinations. Use of PRCs not as effective with this sampler as with others. Limited knowledge of effects of pH, salinity and ionic strength on uptake.	131,131,133–140
SPMD	LDPE tube containing triolein	3-10	1-4 weeks	Often used with PRCS. Single use with relatively laborious sample clean-up. Sorbed analytes have been known to photodegrade if left in sunlight following collection from field trials.	141–145
SR sheets	PDMS sheets supported by stainless steel frame	3-7	Weeks - months	Capable of reuse however this does not appear to be common. Used in conjunction with PRCs as does not reach equilibrium. Soxhlet extraction as part of preparation of the sampler is required to remove presence of oligomers. Preparation is therefore lengthy (>100 hrs).	145,145–157
LDPE	LDPE sheets supported by	3-7	1-8 weeks	Generally not reused as damage can occur to sheets in field.	157–161

	stainless steel frame			Based on similar principle to SPMD so most characteristics are the same.	
SPME	Glass fibre with a polymeric coating	2-7	1-63 days	Reusable however damage may occur in field. Headspace analysis is used so samples cannot be reanalysed. LOQs are generally higher using this device.	[153,162–164]
DGT	Prefilter, diffusive hydrogel and a binding agent contained in a capped plastic piston with hole leaving membrane exposed	-4.5 – 7.5	2-4 weeks	Housings can be reused which replacement of the sorbent. Seemingly less influenced by flow variations. High potential for monitoring a large class of organic pollutants in environment. However, cannot currently reach sensitivity achieved by other passive sampling devices	[165–167]

In passive sampling there are two distinct sampling stages, known as the kinetic and equilibrium stages. The kinetic stage is the initial sampling stage that all types of samplers go through, in which the device can be considered an infinite sink, and the collected amount of the target analyte reflect the time weighted average concentrations throughout the sampling period [168]. Compounds may reach equilibrium concentrations after a long deployment period, usually between 2 weeks to 1 month, however this is not always the case, as the time scale for some analytes to reach equilibrium is not always practical in the field. Time for an analyte to reach equilibrium is often proportional to the sampler-water partition coefficients (Ksw), in that the higher the Ksw the more time is required to attain equilibrium concentrations. Lower Ksw, higher water flow rates, and higher area/volume ratios of the device all contribute to reducing the length of time required to reach equilibrium [125].

Partition samplers can reach equilibrium however, variables such as the characteristics of the sampling device, properties of the target compound and deployment time influence the sampler's ability to do so. Devices based on equilibrium uptake have high exposure times or are given long exposure periods to attain this. Results obtained from passive sampling devices are reflective of the average contamination in the sample matrix over the entire course of time the sampler was deployed for, known as a Time Weighted Average (TWA) [125]. Fluctuations in contaminant levels experienced due to changes in the environment, such as heavy rainfall or periods of intense dry weather, that are usually missed by spot sampling are captured by passive samplers. The use of passive samplers also allows for detection of contaminants at levels typically below the limit of detection in grab samples, allowing for detection of CECs previously thought undetectable [169]. The graphical abstract from Altier *et al.* depicts the relationship between TWA and grab sample concentrations over time effectively (Figure 9).

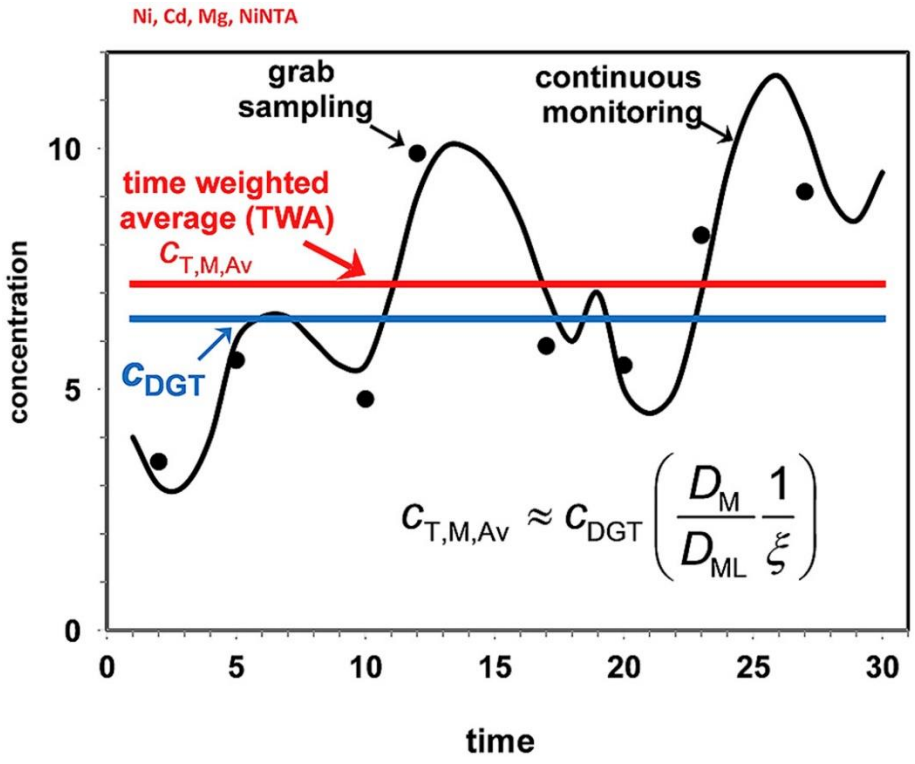


Figure 9. Graphical abstract from Altier *et al.* (2019) showing the relationship between Time Weighted Average (TWA) and grab sampling concentrations over time. Inclusion with copyright permission from Analytica Chemica Acta [170]

The calculation of Time Weighted Averages in passive samplers can be determined using the following equation [124]:

$$C_{TWA} = \frac{n}{R_s t}$$

Equation 1. Calculation for the determination of Time Weighted Average (TWA) Concentrations in passive samplers

Where *n* is the amount of analyte found on the passive sampler disk, R_S is the uptake rate of the analyte onto the disk, and *t* is the deployment time.

Laboratory calibrations are used to calculate the Rs values for the target analyte, however this is limited due to the Rs being affected by turbulence and temperature changes often experienced in the field [171]. The uptake of analytes by adsorption passive samplers is somewhat more complicated in that the viable sampling time is dictated by the capacity of the receiving medium for the target compound. To combat this, Performance Reference Compounds (PRCs) are often used to provide information of in situ sampling rates [172].

Passive samplers can be deployed and then retrieved in just two trips, rather than having to take multiple grab samples over a sampling period [123]. This aids in decreasing the overall cost of a sampling campaign due to less manpower necessary and less travel costs.
While passive sampling has emerged as a very promising technique in environmental monitoring, it is not without a few disadvantages. Because passive sampling creates an average of the concentration of contaminants in a matrix over a period of time, the actual range of concentrations including highest and lowest values present over the sampling period is not produced [173]. Therefore, this technique must be performed alongside spot samples in order to produce this information.

Biofouling can pose a challenge for passive sampling where deployments of > 5 days are carried out. It is not an issue that arises with grab sampling [174,175]. However, the scope of this study does not intend to investigate the impact of biofouling on passive sampling.

1.3.3 Passive sampling of Pesticides

1.3.3.1 Catchment based monitoring studies

Table 10 summarises a range of examples of catchment-based pesticide monitoring studies reported in the past 10 years. These studies are across 10 countries and range in duration from 14 days to 2 years. Key drivers for these studies include the need to meet the requirements of the WFD [176], investigate and assess the levels of pesticide contamination within the studied catchments [177,178] and assess runoff dynamics [179]. Other drivers include correlation of pesticide contamination with agricultural practices [147,180], study spatial and temporal variation of pesticide levels within the catchment [181,182] assess suitability of differing sampling techniques for pesticide monitoring [135,183,184], and capture episodic changes in pesticide occurrences triggered by rainfall events [185].

Table 10. Table showing details of examples of catchment based studies conducted after 2010

Area	Description	Technique	Duration	Time	Analyte	Analysis	Ref
Wark catchment, Northern Luxembourg	Agricultural land, 4 treatment plants, some minor mechanical plants	POCIS with OASIS-HLB sorbent at 6 sites around the catchment, in conjunction with a water level -triggered autosampler.	14 days for PS, full campaign duration for autosampler.	Mid-May – mid-November however only August data discussed	Herbicides: terbuthylazine, flufenacet, metolachlor, bentazone, sulcotrione and isoproturon	LC-ESI-MS/MS LOQ: 5-25 ng L^{-1}	[179]
The Guaporé River catchment, South Brazil	Agricultural land	POCIS with Oasis-HLB sorbent and PES membrane, deployed at 18 sites around the catchment. In conjunction with grab sampling.	14 days for PS. Grab samples taken over a 2 day period.	June	97 pesticides	UPLC-ESI-MS/MS LOD: 1 µg.L^{-1}	[180]
Krom River, Berg River and Hex River catchments, Western Cape, South Africa	1.9 hectares of agricultural land.	Chemcatcher® (SDB-RDP disk and PES membrane), deployed at 3 sites around the catchment. Final total of 21 samples over a 6 month period	14 days for PS deployment	July – January	248 pesticides	LC-HR-MS/MS LOQ: 0-800 ng/disk	[181]
Hartbeespoort Dam catchment, South Africa	Industrialised urban area. Primary sources of pollution : urban population growth & WWTP outfall	Passive sampling using Chemcatcher® with an Oasis-HLB disk	14 days for PS deployment	September-October	Fluconazole, Griseofulvin, Propiconazole, Tolnaftate Prometryn, Sebuthylazine, DEET , Malathion, Pyranocoumarin, Albendazole	UPLC- Q-ToF-MS	[183]
Mimmshall Brook catchment, Hertfordshire, UK	Arable farm land. Crops: oilseed rape, winter wheat, cereals.	Spot sampling, automated bottle sampling and passive sampling using Chemcatcher® with a HLB-L disk and PES membrane	14 days for PS deployment. Grab samples collected weekly during the sampling period and automated samples were collected daily.	October-November	Metaldehyde	LC-ESI-MS/MS Method LOQ: 0.45 ng L^{-1}	[186]

River Lee, Cork, and River Liffey, Dublin catchments, Ireland	Lee is agricultural area, flows towards Cork city (industrialised). Liffey flows through some agricultural but mainly urbanized areas. WWTP identified as primary pressure on Liffey	Grab and passive sampling using POCIS with an OASIS-HLB sorbent	1 month PS deployment, grab samples taken at each site	2013-2014 (Cork), 2014-2015 (Dublin)	Aclonifen, Bifenox, Cybutryn, Dichlorvos, Dicofol, Heptachlor/heptachlor epoxide, Quinoxyfen, Terbutryn	HPLC-Q-TRAP-MS LOD: 0.5-5 $ng.mL^{-1}$	[187]
The River Ugie catchment, Scotland	Peatland. Fisheries present near mouth of river.	Combination of grab and POCIS (Oasis-HLB with PES membrane) passive sampling. Grab samples taken at 10 sites around the catchment and POCIS deployed at 3.	1 month deployment of PS, grab samples were taken monthly for 12 months	July-July	Metaldehyde, Simazine, Atrazine, Isoproturon, Chlorotoluron, Chlorpyrifos, Epoxiconazole, Permethrin, Cypermethrin	GC-MS LOD: 0.02-2.28 $ng\ L^{-1}$	[184]
The River Exe catchment, UK	Agricultural grassland. Presence drinking water treatment plant.	Chemcatcher® with 3M Empore™ anion-SR exchange disks with a PES membrane deployed at 8 sites in field trial 1 and 9 sites in field trial 2.	16 days PS deployment, grab samples taken at each sample location on days 0, 2, 5, 7, 9, 12 and 14 of deployment	May, June-July	2,4-D, dicamba, dichlorprop, fluroxypyr, MCPA, MCPB, Mecoprop and tricolpyr	GC-MS LOD: 1.3-3.2 $ng\ L^{-1}$	[188]
The River Dee and the River Thames catchments, UK	River Dee predominantly agricultural grassland, winter wheat, maize and winter barley. Upper Thames arable agriculture.	Chemcatcher® with a HLB-L disk and PES membrane deployed at 8 sites along the River Dee and 6 sites in the Thames catchment.	14 day deployment for PS , spot samples taken every 7-14 days over 12 months	July-July	Metaldehyde	LC-MS/MS 10 $ng\ L^{-1}$ for grab sampling method and 0.45 $ng\ L^{-1}$	[189]
Lake Naivasha River basin, Kenya	Tropical climate; two dry & two wet seasons. Major agricultural, employing small mixed farming.	Silicone rubber sheets with PRCS and hydrophilic Speedisk samplers deployed at 3 sites around the catchment	1 month deployment of PSDs	June – July	HCH, heptachlor, aldrin, pp-DDE, endrin, dieldrin, α-endosulfan, β-endosulfan, pp-DDD, endrin aldehyde, pp-DDT, methoxychlor	GC-ECD / GC-MS/MS LOD: 1 $\mu g.L^{-1}$	[190]
Auvezere and Aixette	Rural agricultural areas with grassland & arable crops. Downstream	POCIS with Oasis-HLB and PES membrane, deployed at 3 sampling sites in each	14 day deployment of PSDs, with grab	Jan 2012-Dec 2015 in Auvezere.	43 pesticides	LC-MS/MS or UPLC-TOF-MS	[191]

watersheds, France	Aixette catchment a part-urbanized area.	catchment. Grab samples were taken every 2 weeks at each POCIS deployment	samples taken every 14 days	Jan 2015-Oct 2016 in Aixette.		LOQ: 0.1-5 $\mu g.L^{-1}$ (Instrumental)	
Sosiani River catchment, Kenya	Sosiani river flows through both rural and urban areas. Forestry, grassland, cereal crops.	SR sheets in conjunction with PRCs deployed at 7 sampling sites around the catchment.	30-35 day deployment of SR sheets	January-February, December - January	Organochlorine pesticides	GC-MS Time weighted average sampling limits: 0.33-46 $pg.L^{-1}$	[178]
Barratta Creek catchment, Queensland, Australia	Agricultural, large proportion of sugar cane and animal grazing pastures, some legumes.	Grab, time/flow weighted and passive sampling all used. Chemcatcher® -SDB + PES configuration was used along with PDMS strips in order to cover broad range of analytes.	4 week deployment of PSDs. Grab samples were taken during each PS site visit.	24 months, July-July	32 herbicides, 10 insecticides and 1 fungicide	LC-MS/MS and GC-MS Limit of reporting (LOR): 0.01 $\mu g.L^{-1}$	[182]
The Corner Inlet catchment, Victoria, Australia	Includes a national park 'undeveloped wilderness area', and some agricultural land.	Grab and sediment samples were taken from 13 samples from around the catchment. Chemcatcher with an Empore™ SDB-XC disk and PES membrane were deployed at 11 sites in Nov and 10 sites in March.	Spot samples taken over a 2-4 day period of each month sampled. Passive samplers deployed for 28 days.	Nov, Dec, early March, late March and April	10 terzine pesticides including atrazine and its metabolites, 40 polar pesticides including methiocarb, carbaryl, dichlorvos, fenamiphos and methomyl.	Two methods: triazines with LC-MS/MS and multi-residue LC-MS/MS screen for other pesticides. LOR 0.001-0.1 $\mu g.L^{-1}$	[192]
Beaujolais region, France	Morcille River and the Ardières River. Land used for vinyards.	Deployment of 'Twister' passive samplers at 3 sites per river. Concurrent collection of spot samples and weekly average automated samples.	PS devices deployed for 1 month	Spring 2010 and spring 2011	Incl., Azoxystrobin, , 3,4-, Isoproturon, Procymidon, Metolachlor, Fenitrothion, Tebuconazole, Chlorfenvinphos, Acetochlo, Diflufenican, Chlorpyrifos-ethyl, Flufenoxuron	LC-MS/MS LOQ for PS: 0.4-171 ng. L^{-1} LOQ for SPE: 5-20 ng. L^{-1}	[193]
Rhineland-Palatinate region,	Largest vineyard growing region in Germany, extensive pesticide	17 streams in this catchment were monitored using a	Monitoring was based on rainfall-PS and EDS were	July – September	Azoxystrobin, Boscalid, Cyprodinil, Dimethoate, Dimethomorph,	LC-HRMS LOQ for PS: 3-10 ng. L^{-1}	[194]

Southwest Germany	usage, (fungicides). Upstream is a natural conserve forest.	combination of passive sampling using Empore™ SDB disks and event driven water sampling.	deployed 1-2 days prior to expected rainfall. Samplers were retrieved 2-4 days after precipitation event.		Fenhexamid, Fludioxonil, Imidacloprid, Indoxacarb, Iprovalicarb, Kresoxim-methyl, Metalaxyl-M, Metrafenone, Myclobutanil, Pyrimethanil, Quinoxyfen, Tebuconazole, Tebufenpyrad, Tolyfluanid	LOQ for EDS: 1-20 ng. L^{-1}	
Yarra River catchment, Victoria, Australia	Variety of land uses incl. residential, rural residential, industrial, forestry and grassland Three major WWTPs present.	Spot water and sediment samples were collected from 18 sites around the catchment. SPMD and polar Chemcatcher PS devices were deployed in conjunction	Grab and sediment samples taken every 4 weeks. Ps devices deployed for 21-28 days.	September-March	Atrazine, cyanazine, hexazinone, linuron, metribuzin, pendimethalin, prometryn, propyzamide, simazine, terbutryn.	GC-NPD & LC-MS/MS, GC LOQ PS/Grab: 250 µg.L^{-1}/ 1 µg L^{-1}, LC-MS LOQ PS/grab: 0.5-50 µg.L^{-1} /0.002-0.2 µg.L^{-1}	[177]
Four agricultural streams and two rivers – Skivarpsån (S) and Vege å (V), Sweden.	Agriculturally intensive areas with 85-93% and 65-85% arable land in the stream and river catchments. Autumn sown cereals dominant crop.	Composite samples (every 90 min) collected using automated samplers from the four streams. In the two rivers, grab samples were taken over the duration of the sampling campaign.	Composite samples collected weekly for 7 months. Grab samples fortnightly over first 2 months & monthly over final 5.	Duration of the agricultural production period early May – November.	Up to 128 pesticides.	GC-MS, LC-MS and LC-MS/MS dependant on the pesticide 0.1 ng L^{-1} (chlorpyrifos) - 100 ng L^{-1} (AMPA)	[195]
Ythan catchment in North East Scotland, United Kingdom	Arable agriculture and grazing major land use, some small forestry. Pressures from agriculture diffuse pollution under WFD.	Three sites, site 1 at headwaters & sites 2 and 3 at small tributaries. Auto-composite and passive sampling using SR sheets.	PSDs deployed 54 days. Autosamplers continuously for 3 months, composite samples weekly.	October – December	22 Acid herbicides and 47 selected pesticides including atrazine, methiocarb and chlorpyrifos.	GC-MS for 47 & LC-MS for acid herbicides. GC-MS LOD : 4.5- 41.4 ng L^{-1} LC-MS LOD: 1-9 ng L^{-1}	[147]

1.3.3.2 Relationship between land use in catchments and pesticides monitored

Catchment land use is perhaps the biggest deciding factor when it comes contaminant occurrence. The catchments studied for this review contained significant areas of agricultural land. However, a number of papers also referenced areas of industrial or urbanised areas [177,183,187,189]. Studies involving urbanized areas often also include other contaminants in their analyses such as pharmaceuticals and personal care products [183,196]. For the purpose of this review – only pesticides are reported.

The presence of water treatment plants in the catchment can also significantly affect which contaminants enter a water source. It was found by Münze *et al.* that the WWTPs investigated in their study increased the number and concentrations of pesticides in the receiving waters. For instance WWTP effluents added 6 ± 2 analytes to the in-stream pesticide pollution from upstream reaches [196]. In addition, Köck-Schulmeyer *et al.* found that pesticide removal during wastewater treatment was often poor, and sometimes resulted in concentrations in effluent higher than in influent [33].

Knowledge of the particular kind of agricultural practises employed in a catchment can allow for specific targeted studies to be conducted, or particular results from a study to be correlated with land uses in the area. Certain pesticides will be related with certain practices. For instance, atrazine, simazine, and metolachlor were some of the most commonly used pesticides in vinyards prior to their EU ban in 2003 [197]. Although being banned a number of years ago, they are known to be persistent in the environment for decades [198] and so were included in the study conducted by Assoumani *et al.* in 2015 due to the catchment studied consisting of primarily vinyards [193]. The River Exe catchment in the UK consists of predominantly grassland, and thus Townsend *et al.* conducted a study assessing levels of acid herbicides including MCPA which is one of the most popular pesticides for controlling rushes in grasslands [188].

O'Brien *et al*. linked changes in particular pesticide concentration with the sugar cane harvesting season, due to detections of the herbicides imazapic and imazethapyr which are only registered in Australia for use in sugar cane production [182]. The study by Fernanzez *et al.*

selected the specific pesticides they were to monitor based on; information gleaned from a previous study conducted in the area, and spraying recommendations from the local authorities [185]. Guibal *et al*. noted that 60% of the pesticides found in their study were common compounds and their presence could be explained by the surrounding land use for the breeding of Limousin cattle. The high presence of herbicides in their results they also allocated to this extensive cattle breeding, due to the development of self-feeling for local breeders. They also linked a slightly lower occurrences of fungicides in the samples to local apple orchards [191]. Preparation for the monitoring study performed by Curchod *et al* was done by the collection of pesticide spraying records from 38 farms located upstream from the selected sampling points. These records included time, location and the total amount of pesticide sprayed in kg/ha. From these records they were able to identify 96 different pesticides which were applied over a 1 year timeframe [181]. Significant differences in the pesticides used were observed between the three main areas and differing crop types studied, but some of the most commonly occurring pesticides included penconazole, mancozeb, spiroxamine, glyphosate, chlorpyrifos, 2,4-D, bromoxynil and MCPA.

1.3.3.3 Spatial and Temporal Variations in Monitoring Approaches

Spatial variation was taken into account in the majority of studies reviewed, with at least 3 up to 18 sample sites included in most papers included in Table 10. Many studies mapped out the catchment and identified potential pollutant sources, such a presence of WWTPs, industries and farmland [182,188,193]. Most studies included samples taken upstream, downstream and in the middle of the river or stream being studied [180,184,185,195]. Curchod *et al.* observed that the pesticides found in their surface water samples reflected cropping patterns, which is in agreement with that observed by Gilliom [181,199].

Temporal frequency in the studies included monitoring over a period of 14 d to 2 y. Changes in pesticide concentration largely seem to coincide with key periods in agricultural practices, which generally occur in spring/summer time. Strong temporal agreement was found by Curchod *et al.* between pesticide occurrence in samples and spraying events in 11 compounds over the three study areas and 7 sampling rounds (Curchod *et al.,* 2020). In the 2016 study by O'Brien *et al.* samples were taken over a 24 month period allowing for considerable temporal data to be generated. It was found that peak pesticide concentration correlated

with the end of the sugar cane harvest period, July - December. They noted 9 particular pesticides commonly used in sugar cane production were detected at the highest levels during this period [182]. This paper also studied spatial variations in the catchment, and found that pesticide concentrations tended to be lower in the lower catchment estuarine site which they suggested was due to dilution with seawater. Detections of the pesticides bromacil and fluometuron were reported for the west of the catchment which were likely associated with use around urban/industrial buildings or weed management in cotton crops.

When the inclusion of historical or long term data is available this addition can add valuable insights into the status of a catchment over time. In the case of a study conducted by Bundschuh *et al.* long term monitoring data was available from 2002-2011 in addition to the study they conducted in 2014 and thus was included in their analysis. Positively they found that their results indicated a 'rather stable ecotoxicological potential' in the studies streams with no obvious long term trends indicated. However, they did find that the streams did exceed the EU set concentration limits in 2% of the samples taken [195]. Analysis of these studies show the inclusion of greater temporal and/or spatial variation can enhance the quality of a catchment monitoring campaign

1.3.3.4 Precipitation Effects

Previous literature has documented the effect rainfall events have on the introduction to pesticides into a water body, with run-off caused by rain being one of the key pathways [200–202]. It is therefore wise to include a method of accounting for such events in a monitoring campaign. This is of increasing importance in light of climate change and the changes in regular precipitation patterns worldwide.

Some studies made use of water level or flow weighted autosampling in order to account for precipitation effects. For example in the study on the Barratta Creek catchment performed by O' Brien *et al*. in 2016 they used a time/flow weighted autosampler in conjunction with grab and passive sampling. The study period of 2 y this was conducted over included one of the wettest years on record for this catchment, and their results found peaks in pesticide concentrations coinciding with the onset of the first wet-season rains for each year. They also

found that pesticide concentrations rapidly decreased following each subsequent rainfall event during the wet season, only increasing again following crop planting or crop harvest. When rainfall extended later into the winter months this led to a delay in crop harvest, this directly correlated with an increase in TWA concentrations for three pesticides [182].

Fernandez *et al.* made use of Event Driven Sampling (EDS) in order to account for rainfall events. This was executed using a sampling system in which 1L amber glass bottles fixed to a steel bar were placed in the stream with the bottle opening roughly 10-20 cm above regular water level. In order to ensure rainwater was not collected in place of surface water, the bottle caps were secured 1cm above the opening. These samples were collected after the 4 monitored rainfall events. They found that the first and third rainfall events resulted in 2-10 fold higher inputs of pesticides when compared to the second and fourth events [185].

1.3.3.5 Analytical Methods and Detection Limits Achieved

All studies examined used either gas or liquid chromatography for analysis of samples, usually coupled with mass spectrometry. The choice between LC and GC is primarily dictated by the physicochemical properties of the analytes to be studied. The majority of studies favoured LC alone for their analysis method, in contrast to the five studies that chose solely GC. There were four papers that made use of both techniques, effectively allowing for a full suite of pesticides to be sufficiently analysed using whichever method was most practical (Bundschuh *et al.,* 2014; Emelogu *et al*., 2013; O'Brien *et al*., 2016).

The majority of reported studies used mass spectrometry as the detector of choice for their analyses, with most using a targeted approach. A few papers used alternative detectors for their work, for instance in the 2014 study by Allinson *et al.* used GC coupled with a nitrogen phosphorus detector (NPD) for the analysis of volatile nitrogen containing compounds in their samples. This study employed the use of three separate analytical methods in order to analyse a range of compounds. Along with the GC-NPD detector, they also made use of a targeted LC-MS/MS method for the analysis of triazines and a LC-MS/MS screening method for a range of other polar pesticides [177]. In 2018 Abbasi *et al.* made use of a GC-µECD instrument which benefits from being much more cost effective than an MS, however the

drawback is clearly seen when comparing LODs achieved with other studies. The LOD using the µECD was 1µg L^{-1} [190], whereas other detectors achieved LODs in the low ng L^{-1} range.

Very often studies also gathered physico chemical data in conjunction with pesticide monitoring in order to gain a further understanding of the water quality. The most frequent additional parameters monitored were temperature, pH, conductivity Total or Dissolved Organic Carbon (T/DOC) and Total or Dissolved nitrogen [177,182,183,185,188,190,192]. The parameter that was most frequently recorded was pH, most likely as it can readily affect the pesticide behaviour in surface waters. Hydrolysis of pesticides generally begins to occur at a pH of above 7, and so water pH can be an accurate predictor of the likelihood of finding either a pesticide or it's break down products [203]. It is also of particular importance if a method such as passive sampling is being employed, as uptake can be affected by pH. It is also common practice to acidify samples, as many polar pesticides can hydrolyse within the sample and so knowledge of sample pH is needed in order to acidify [204].

1.3.3.6 Notable Pesticide Occurrences in Studied Catchments

Many of the papers reported targeted studies to varying degrees of specificity. For example, Castle *et al.* examined their catchment for only a single analyte, metaldehyde, in two catchments in the UK. They found that the River Dee catchment, had little variability in the concentrations of metaldehyde over the course of the study, with only one occasion where the concentration was above the permitted drinking water value of 100 ng L^{-1}, whereas the River Thames catchment had several exceedances over the permitted value, up to 4180 ng L^{-1} on one occasion [189]. Townsend *et al.* conducted a study with a wider scope than this, in which acid herbicides in the River Exe catchment in the UK were investigated. This area is a notable source of drinking water for the catchment. The found over the course of the 2 week study, Mecoprop exceeded the EU drinking water limit of 100 ng L^{-1} on two occasions, with a spike concentration of 868 ng L^{-1} on the first day of the study. This is concerning due to the highly polar nature of these analytes their likelihood to persist during water treatment is relatively high [205]. A similar range study by Zhang *et al.* looked at 9 different pesticides covering a broad range of $LogK_{ow}$'s from 0.12-6.1. They found that isoproturon had a detection frequency in their samples of 99%, closely followed by chlorotoluron with 98%, and atrazine at 92%. Least

detected were permethrin 2.3% and cypermethrin 3.8%. A potential explanation of this could be the non-polar nature of the pyrethroid pesticides meant they were more likely to sorb to solid particulates rather than remain in the water column. The pesticide with the biggest concentration range from 0.01 to 111.8 ng L^{-1} was chlorotoluron [184]. This study was performed in 2013-2014 so frequency of atrazine is a cause for concern as this is a full 10 years after it was banned. A study by Curchod *et al.* had a scope much larger than either Castle, Townsend or Zhang, in which they used a targeted method for 248 different pesticides, and found occurrences in real samples of 53 of them. The majority of the estimated two week average pesticide concentrations were under 40 ng L^{-1}. However, the insecticides imidacloprid, thiacloprid, chlorpyrifos and acetamiprid and the herbicide terbuthylazine exceeded on at least one occasion their EQS '58-fold (EQS 13 ng L^{-1}), 12-fold (EQS 10 ng L^{-1}), 9-fold (EQS 0.46 ng L^{-1}), 5-fold (EQS 24 ng L^{-1}) and 3-fold (EQS 220 ng L^{-1}), respectively'. Although neonicotinoids are banned in the EU, they are yet to be banned in South Africa where this study was conducted [181]. Similar pesticide contaminants were found by de Castro Lima *et al.* This study found that the herbicides 2, 4-D, atrazine, deethyl-atrazine, and simazine, three fungicides (carbendazim, tebuconazole, andepoxiconazole), and one insecticide (imidacloprid) were the most frequently detected compounds in their catchment. Atrazine and imidacloprid are both banned in the EU but are still legal in Brazil [180].

1.3.3.7 Advantages and Recommendations for Catchment Based Monitoring

From analysis of the literature a number of advantages of using a catchment based approach can be seen. Inclusion of rainfall events has been shown to produce a spike in pesticide concentrations in surface water, thus making inclusion of rainfall data valuable to monitoring practices [194]. Many studies confidently link pesticide detections with agricultural practices in the catchment [180,193]. Examples of large spatial and temporal frequency sampling was shown which allowed for detailed data on spatial and temporal variation within the catchment [182].

From these benefits a number of recommendations for conducting a catchment based monitoring can be elicited.

i. Knowledge of catchment land use is paramount to the design of a monitoring study. Sampling campaigns based on the specific kind of agricultural practices performed

inform which pesticides are anticipated to be found in the area. Research into the specific land use of the catchment should be the first point of call in the design of a study.

ii. Use of another sampling technique along with grab samples is recommended for longer term sampling campaigns to catch potential concentration 'spikes' that can be missed with grab sampling alone, and provide valuable long term data.

iii. Spatial variation should be taken into account in order to achieve a full picture of the catchment's pesticide concentrations. Sampling points should be selected with potential pollutant sources taken into account e.g. taking samples both upstream and downstream from a WWTP, far and close to a stream etc.

iv. Temporal variation should also be accounted for by frequent sampling during spraying or harvesting seasons.

v. Increased rainfall has been linked to spikes in pesticide contamination, and so including rainfall into monitoring data is recommended.

vi. Analysis methods should be chosen based on the pesticides studied, however LC-MS is the method of choice if the choice is available due to a broader range of analytes amenable to it. If it is possible to employ multiple instrumental methods this has advantages.

vii. Analysis of other phys-chem properties should be conducted when possible with pH being particularly important.

Figure 10 shows a flow chart of the recommended process of designing a catchment focussed CEC monitoring campaign, based on the information gleaned from the literature. In Ireland, these is a specified branch of the EPA called the Catchments Unit [206]. This team perform catchment monitoring in a similar way to the approach suggested here but on a broader (i.e. not CEC focussed) scale.

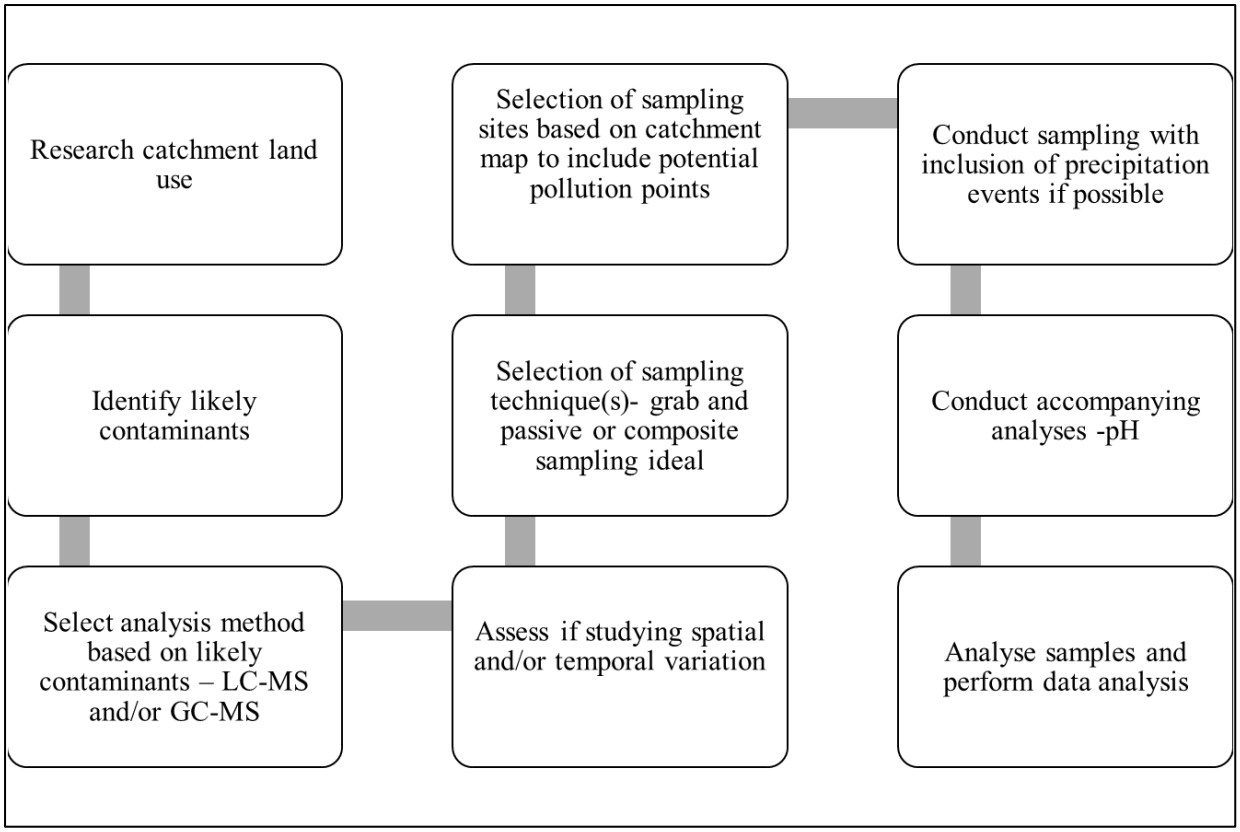


Figure 10. Flow chart of the process of designing a catchment based monitoring study starting from land use assessment through to sample analysis

1.3.3.8 Passive sampling of pesticides of interest

There have been multiple studies on the uses of the Chemcatcher passive sampling device for the detection and monitoring of phenoxyacetic acid herbicides including MCPA, Mecoprop and 2,4-D [72,131,139,207,208]. A paper published in 2018 by Townsend *et al.* describes a new Chemcatcher configuration using a 3M Empore anion-exchange disk and a polyethersulphone (PES) membrane [72]. They found that this new configuration was capable of measuring time weighted average (TWA) concentrations over 6-58 d and has Rs values in the range of 0.044-0.113 per day. The device could sample 0.3-0.8 L of water in a 1-week deployment, comparable to the quantities sampled when employing grab sampling (0.5 - 1L). The method of analysis was by GC-MS which could measure the analytes at 1ng/disk. This involved a derivatisation step to allow for GC-MS analysis. However, while sufficient extraction of most of the target acid herbicides was reported, the strongly acidic herbicides were not efficiently recovered, with the recovery of clopyralid being the lowest at 17%. Additionally, during the calibration studies, only water temperature and turbulence were measured, therefore more

investigation into the effect on uptake of other environmental variables such as dissolved organic carbon (DOC), biofouling and the presence of any other anionic substances in the water are necessary.

Prior to the newer 3M Empore anion-exchange disk, the typical Chemcatcher configurations for acid herbicides employed the use of an SDB-RPS or SDB-XC disk in conjunction with a PES membrane. A study by Tran *et al*. compared these two disks and found the SDB-XC disk had a higher recovery (81-98% excluding dicamba) for acid herbicides compared with SDB-RPS disk for Milli-Q water buffered to pH 3.7 [207]. However, once applied to natural water, both devices performed poorly, in particular for 2, 4-D (21% for SDB-XC and 22% for SDB-RPS) and MCPA (24% for SDB-XC and 26% for SDB-RPS). The SDB-XC disk was chosen as it had overall higher recoveries for most other analytes in this study [209].

In contrast to the majority Chemcatcher based PS studies for phenoxyacetic acid herbicides, an investigation by Guibal *et al.* employed the use of a Diffusive Gradients in Thin films (DGT) device to study 4 pesticides, including Mecoprop, one of the analytes of interest in this thesis [167]. The study involved a comparison of two different receiving phases, Oasis HLB and Oasis Max, and found that the Oasis HLB outperformed the Oasis Max for the accumulation of Mecoprop on the device, with recoveries being 95% and 86% for the Oasis HLB and Max respectively. However, after performing pH studies, it was found that at a higher pH (7-8) the quantification of Mecoprop was significantly altered showing lower pesticide concentrations, with the Oasis HLB being worse effected. This lead to the conclusion that the Oasis Max receiving phase would function better in the field for Mecoprop quantification.

A study by Xue *et al.* used polyethylene film as a passive sampling device for the monitoring of pyrethroid pesticides in water surface water [172]. They found the analytes did not reach equilibrium after 30 d, and so employed the use of isotope labelled pyrethroids as performance reference compounds (PRCs). The introduction of PRCs into the study produced C_{free} measurements that were reproducible in the range of 2-7 d of deployment. Limit of detection was 1ng L, which was noted to be low enough to adequately indicate pyrethroid contamination levels at biologically relevant concentrations. Another paper by Alvarez *et al.* also employed the use of polyethylene strips as a passive sampler for pyrethroids .[210]

In 2014, Moschet *et al.* investigated the use of PDMS, referred to in this paper as SR sheets for the passive sampling of pyrethroids and found them to be very efficient as a PS device [211]. By using passive sampling devices, they found 4 out of 12 analytes investigated exceeded EU AA-EQS standards at least on one occasion, however these exceedances were not detected in the grab samples. It was found that PDMS sheets enrich large amounts of non-polar analytes. Based on their sampling rates, the sampled pollutant collected onto a sheet of dimensions 30 x 10 cm^2 over the course of 2 weeks was equal to the extraction of approximately 500 L of water. Typical grab sample extraction volumes range from 100 mL to 1L, giving passive sampling the potential to improve LODs by a potential factor of 5000.

Neonicotinoids have been effectively monitored through passive sampling using Chemcatcher fitted with styrenedivinylbenzene - reverse phase sulfonated (SDB-RPS) Empore 47 mm disks and PES membrane, as well as through use of the HLB-L disk combined with a PES membrane [212,213] However, the use of passive sampling for monitoring of this group of compounds is still sparse.

There have been relatively limited passive sampling studies specifically targeting azole compounds. A passive sampling screening study for emerging contaminates involving a Chemcatcher with a HLB receiving phase conducted in South Africa detected two azole compounds, fluconazole and propioconazole in multiple sites [214].

A summary of these studies can be seen in Table 11.

Table 11. Passive sampling devices used to monitor pesticides groups

Pollutant	Sampling Method	Analysis Method	Matrix	Ref
Phenoxy acid herbicides	Chemcatcher™ fitted with a 3M Empore™ anion-exchange disk + a polyethersulphone (PES) diffusion membrane	GC-MS	River water	[72]
Phenoxy acid herbicides	Chemcatcher™ fitted with an SDB-XC Empore disk + a PES diffusion membrane	HPLC- UV (230nm)	Mains water	[207]
Mecoprop	DGT with an Oasis® HLB sorbent	HPLC-MS	River water	[167]
Pyrethroids	PRC loaded polyethylene film	GC-MS	River water	[172]
Pyrethroids	Silicon rubber (SR) sheets	GC-MS/MS	River water	[211]
Pyrethroids	Low density polyethylene (LDPE) strips and Solid Phase Micro Extraction (SPME) fibres	GC-MS	Marine water	[210]
Cypermethrin	Ethylene Vinyl Acetate (EVA) polymer	GC-ECD -> GC-MS	Marine water	[215]
Neonicotinoids	Chemcatcher fitted with styrenedivinylbenzene - reverse phase sulfonated (SDB-RPS) Empore 47 mm disks and PES membrane	LC-MS/MS	River water	[213]
Screening method (including azoles)	Chemcatcher fitted with HLB disks and PES membrane	UHPLC-TOF MS	River water	[214]
Pesticides screening method (including azoles and neonics)	Chemcatcher fitted with HLB disks and PES membrane	UHPLC-TOF MS	River water	[212]

1.3.4 Extraction techniques

1.3.4.1 Solid Phase Extraction (SPE)

Solid phase extraction (SPE), is an extraction method used for sample clean-up and enrichment before further analysis. It is often used in order to minimise a matrix effect [216]. The benefits of the SPE technique over other extraction methods such as liquid/liquid extraction involve lower sample and solvent consumption, better reproducibility, potential for automation, as well as preconcentration abilities [217]. SPE cartridges are single use to avoid cross contamination, however they are relatively cheap, require less manual labour and due to the lower solvent consumption than liquid/liquid extraction, are cost saving in the long run [218]. SPE cartridges vary in sorbent type, and so the appropriate cartridge must be selected for the analyte to be studied [219]. SPE cartridges are similar to chromatographic techniques in that the sample separation mechanisms also rely on an analytes relative affinity to a stationary phase. Therefore, sorbents on SPE cartridges are categorised in the same way as high-performance liquid chromatography modes of separation. Two of the most popular forms of sorbents are chemically bonded reverse phase silica such as the Bond Elut C18 cartridge by Agilent [220], and functionalised polymers such as the extremely popular Oasis HLB cartridge [221,222].

The steps of SPE are selective extraction, selective washing, and selective elution [218]. The selective washing steps process consists of four main steps: conditioning, loading, washing and eluting [223]. For cartridge conditioning, which is done to activate the sorbent functional groups by solvation in order to be prepared to interact with the sample, an organic solvent, or a mixture containing one, is used [223]. Following the conditioning step, the sample is loaded onto the cartridge. It is important for the optimal pH to be found to guarantee maximum retention in the cartridge [216]. It is not uncommon for a sample to be pre-treated by pH adjustment and filtration before introduction to the cartridge. Following pre-treatment, the sample is loaded onto the cartridge and passed through the packing material, usually under a vacuum with a flow rate between 2 – 5 mL min^{-1}, although for lower sample volumes the sample can be allowed to flow through the cartridge drop-wise under gravity [218]. The cartridge is then washed to remove any impurities. Commonly the packing material is washed with water or a weak eluting solvent [223]. Following this, the sample is now eluted from the

cartridge, usually with a solvent such as methanol. Depending on the requirements of analysis, the sample is often evaporated to dryness and then reconstituted another solvent in a smaller volume to allow for analysis on a particular instrument as well as preconcentration to ensure the sample concentration in within detection limits. Sometimes direct injection of the elute into the analytical instrument is performed if the elution solvent is compatible with the machine [221].

Table 12 shows a selection of cartridges previously used in the literature for the extraction of some of the pesticides discussed in Section 2.

Table 12. SPE methods reported in literature for determination of emerging pesticide contaminants

Analyte & Matrix	Sorbent	pH	Condition	Load (mL)	Wash	Elute	Dry & Recon	% Rec	Ref
Acid Herbicides Ground water	Bond Elut ENV 200 mg/6 mL	2	10 mL MeOH, 10 mL ultra-pure water	500	-	2 × 3.5 mL acetone	Evaporated under nitrogen at 40 °C to dryness, reconstituted in 500 µL ACN / water (50:50, v/v)	20-124	[224]
Acid Herbicides Drinking water	Oasis® HLB 60 mg	2.5	3 mL MeOH, 3 mL H_2O, 3 mL pH 2.5 H_2O	100	3mL H_2O	2 x 500 µl DCM + 2 x 500 H_2Ol MeOH	1000 µl evaporated under N_2 , reconstituted in 1000µl mobile phase for LC-MS analysis	70 – 117	[225]
Acid Herbicides Ground water	Strata-X 100 mg/6 mL	2	10 mL MeOH, 10 mL ultra-pure water	500	-	2 × 3.5 mL acetone	Evaporated under nitrogen at 40 °C to Dryness, then reconstituted in 500 µL ACN / water (50:50, v/v)	15-86	[224]
Acid Herbicides Ground water	Oasis® HLB 200 mg/6 mL	2	10 mL MeOH, 10 mL ultra-pure water	500	-	2 × 3.5 mL acetone	Evaporated under nitrogen at 40 °C to Dryness, then reconstituted in 500 µL ACN / water (50:50, v/v)	71-118	[224]
Acid Herbicides Surface water	Bond Elut 1000 mg/6 mL	1	5 mL acetone, 5 mL MeOH, 10 mL pH1 H_2O	500	-	MeOH	Evaporated under N_2 to dryness, reconstituted in mobile phase	76 – 90	[226]
Pyrethroids Drinking water	Oasis® HLB 60 mg	2.5	3 mL MeOH, 3 mL H_2O, 3 mL pH 2.5 H_2O	100	3 mL H_2O	2 x 500 µl DCM + 2 x 500 H_2Ol MeOH	100 µl directly used for GC-MS analysis, 900 µl evaporated under N_2 & reconstituted in 100µl DCM: MeOH (1:1) for GC-MS analysis.	70 - 117	[225]
Pyrethroids River water	500 mg of neutral Al_2O_3 in a 3 mL cartridge	-	5 mL n-hexane, 5 mL acetone	100	5mL DCM : H_2O (1:50 , v/v)	4mL acetone : hexane (3:2 v/v)	N_2 dryness & reconstituted in 0.5mL MeOH. Mixed 40 µl C_2Cl_4 & 5mL water. Centrifuged, bottom layer re-evaporated. Final reconstitution in 30 µl n-hexane for GC analysis	90-97	[227]
Pyrethroids Ground water	C18	-	5 mL MeOH, 5 mL n-hexane, 5 mL MeOH, 5 mL ultrapure water	800	-	7 mL n-hexane	Evaporated under N_2, then reconstituted in 0.5 mL of ACN/water (70:30) v/v	72 - 110	[228]

1.3.5 Separation Techniques

1.3.5.1 Chromatography

Two of the most common types of chromatography used for CEC analysis, are Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC). The instrument is then fit with an appropriate detector, such as an FID detector for GC or a UV detector for HPLC. One of the most popular detectors is the mass spectrometer. Mass spectrometry is based on the principal that when a molecule is ionized in a vacuum, a characteristic group of ions of varying masses are formed [229]. The MS can then separate these ions based on the mass to charge (m/z) ratio and plot the output as a mass spectrum showing relative abundance against ionic mass. There are many forms of MS detectors, the most common being a quadrupole, and others include ion trap, time of flight and magnetic sector [230].

In GC, the instrument separates analytes in a mixture using heat based on their relative affinity to a stationary phase as mentioned before, as well as their boiling point [231]. In GC the stationary phase is usually a liquid immobilised onto the surface of an inert solid support within a column. Selection of the correct type of column for the desired analytes is vital to successful separations [230]. Polar stationary phases work well for molecules containing polar moieties such as alcohols, acids and amines. Non-polar phases successfully separate non-polar analytes like hydrocarbons [232].The sample is moved through the column via an inert carrier gas, usually helium or nitrogen. The temperature programme within the GC can be adjusted in order to optimise the analytes separation [232]. Samples can sometimes require derivatisation for successful analysis by GC-MS depending on the analytes of interest. If the analyte has poor volatility or is insufficiently thermally stable it may exhibit unreproducible peak areas, heights and shapes [233].

In HPLC the mobile phase consists of a liquid. Reverse phase HPLC (RP-HPLC), in which the mobile phase is polar and the stationary phase is non-polar, makes up 75% of all separations, due to its reproducibility and broad applicability [234]. The most polar sample component is eluted first due to its stronger affinity to the mobile phase, and the least polar component is eluted last. Common stationary phases of RP-HPLC are the non-polar functional groups octadecyl (ODS) C18 and octyl C8 [235]. These are considered ‘general application’ columns and

are used for a wide variety of analytes. Although more commonly needed for GC-MS analysis, derivatisation is also sometimes required for effect LC-MS analysis when analysing particularly non-volatile or extremely polar compounds. Typical derivatisation techniques include FMOC and FMOC-CL [236,237].

1.3.5.2 Analysis methods for analytes of interest

Acid herbicides require derivatisation in order to be efficiently analysed by GS-MS [72,80]. Diazomethane is one of the most efficient and common derivatising agents in use for acid herbicide derivatisation [238] however in recent years a somewhat less dangerous alternative, trimethylsilyldiazomethane (TMSD) emerged [239]. Glyphosate also requires derivatisation for analysis, which has previously been achieved by using N-methyl.N.(tert-butyldimethylsilyl) trifluoroacetamide at 80 °C for 30 min [240]. LC-MS has been shown consistently to be a preferred analysis method of choice for these groups of compounds, along with azoles and neonicotinoids, with examples of methods detailed in Table 13. Recent advances in column development has allowed for the Hydrophilic Interaction Liquid Chromatography (HILIC) -MS analysis of glyphosate and its metabolite AMPA without FMOC derivatisation. The use of a polyvinyl alcohol base material modified with quaternary ammonium column allows for the successful retention of these highly polar compounds [241].

GC-MS is historically the analysis method of choice frequently used for the determination of pyrethroid pesticides due to their volatility. However, recent literature has showed further investigation into the use of LC-MS for the analysis of these compounds, but further research is required [91]. However, the ability to analyse these compounds in a singular LC-MS method alongside other pesticides would be hugely advantageous as it would significantly reduce analysis and sample prep time.

Tables 13 and 14 below show a sample of the range of different methods used for the determination of the analytes of interest.

Table 13. Analysis methods reported in literature for determination of pesticides

Analyte & *Matrix*	Column	Inlet	Injection mode	Injection volume	Temperature program	Transfer line temp	LOD	Ref
Pyrethroids *Water*	DB-5ms 30m x 0.25 mm x 0.25 µm	275°C	Splitless	1 µl	80 ºC (1 min), 10 ºC/min to 300 ºC, (10 min)	280°C	2-5 ng L^{-1}	[242]
Pyrethroids *Surface water*	DB-XLB 15 m x 0.25 mm x 0.25µm	240°C	Splitless	2 µl	150°C (0 min) 30 ****	300°C	0.01-0.1 ng L^{-1}	[243]
Pyrethroids *Surface water*	Zebron ZB-5MS (15 m x 0.25 mm x 0.25 µm)	55°C	PTV with baffle liner	3 µl	55°C (1 min), 30°C /min to 140°C, 2°C /min to 252°C	240°C	3-60 ng/ 300cm^3 SR	[211]
Pyrethroids *Surface water*	DB-5 fused silica 30 m x 0.25 mm x 0.25 µm	250°C	n/a	n/a	120°C (1 min), 30°C /min to 220°C (1 min), 5°C /min to 300°C (5 min)	280°C	0.5 to 2 µg L^{-1}	[172]
Pyrethroids *Rice*	HP-5 ms 30 m x 0.25 mm x 0.25 µm	250°C	Splitless	1µl	70°C (2 min), 25°C/min to 150°C, 3°C/min to 200°C, 8°C/min to 280°C (10 min), postrun 320°C (5 min)	280°C		[244]
Glyphosate *Human serum*	DB-5 fused silica 15 m x 0.25mm x 0.25µm	300°C	Splitless	1 µl	80°C (2 min), 15°C /min to 300°C (5 min)	280°C	10 pg L^{-1}	[240]
Glyphosate *DI water*	DB-5 ms 30 m x 0.25 mm x 0.25 µm	280°C	Splitless	1 µl	100°C (0 min), 8°C /min to 300°C,	300°C	-	[245]
Acid herbicides *River water*	DB-5ms 30m x 0.25mm x 0.25µm	n/a	n/a	1 µl	55°C (1 min), 15°C/ min to 180°C, 10°C/ min to 250°C (8.7 min)	280°C	7-8 ng L^{-1}	[72]
Acid herbicides *Pond water*	Restek Rtx™-5 40 m x 0.18 mm x 0.2 µm	250°C	Splitless	2 µl	100 °C (1 min), 10 °C/min to 300 °C (4 min)	250°C	n/a	[246]

Table 14. LC-MS methods used for analytes of interest

Analyte & *Matrix*	Column	Mobile Phase	Elution	Flow mL min^{-1}	Inj. (µl)	MS	LOD µg L^{-1}	Ref
Acid Herbicides *Ground water*	BEH analytical column (2.1 mm × 100 mm, particle size 1.7 µm) and a 2.1 mm × 10 mm guard column of the same material	A: 0.01% formic acid in ultra-pure water B: 0.01% formic acid in ACN	Gradient: 99.9% A to 1min, at 2 min 85% A, at 7 min 40% A, at 8.5 min 0.1% A, at 9.0 min 0.1% A, from 9.1 to 11.9 min 99.9% A.	0.6	20	QQQ MS, SRM	0.00008 to 0.0047	[224]
Glyphosate *Breast milk*	Dionex Ionpac AS 11 (2 × 250 mm) and AG-11 guard column (2 × 50 mm)	A: water B: water with 1 mM citric acid	Gradient: 100% A to 2min, 5.5 min linear to 25% B, 2.5 min linear to 50% B, held for 4 min. Return to 100% in 0.5 min, re-equilibrated for 7.5 min.	0.4	25	QQQ MS, - ion mode, MRM	0.5	[247]
Acid Herbicides *Kidney tissue*	Atlantis dC18, 50 mm 9 2.1 mm i.d., 3 um particle size + C18 Security Guard column cartridge, 4 mm 9 2 mm	A: 1mM ammonium acetate in water, B: 100% MeOH	85% A, linear gradient to 10% A over 4.5 min, isocratic for 7.7 min before re-equilibration to initial composition.	0.15 for 13 min, 250 for 7 min	7	QQQ MS, - ion mode, MRM	0.2	[248]
Acid herbicides *Drinking water*	UPS Pursuit C18 with 50 × 3.0 mm (i.d.) and 2.4 µm of particle size.	A: 5 mmol L-1 ammonium formate in water B: MeOH	Gradient: 0 to 3 min 90% (solvent A) decreasing to 50% (solvent A) at 4 min; to 5% (solvent A) at 8 min and to 2% (solvent A) at 11 min until 13 min, returning to the initial condition until 15 min.	0.15	10	QQQ MS, SRM	0.02 - 0.05	[225]
Glyphosate *Surface water*	XBridge column (Waters, C18, 3.5 µm,	A: water with 5 mM ammonium	From 0 to 3 min, a linear increase of B from 10 to 25%; isocratic from 3 to 6 min (75% A: 25% B); from 6 to 15	0.2	20	QQQ MS, SRM	0.0002 – 0.0006	[249]

	30 mm×2 mm i.d., PEEK-lined)	acetate at pH 9 B: 100% MeOH	min, a linear increase of B from 25 to 90%; isocratic 90% B from 15 to 17 min.					
Glyphosate *Solvent (DI), tap water*	ShodexTM HILICpakTM VT-50 2D	(50:50) 50 mM HNH4 CO3 aq. : CH3CN	Isocratic	0.3 mL/ min	50	ESI-MS, MRM (-)	10	[241]
Neonicotinoids *Groundwater*	Phenomenex Synergi 4u Fusion-RP 80A column (100 mm, 2.0 mm, 4 mm particle size)	A = Water, B = Methanol	Gradient: 1 min 90% A, hold for 1.1 min, down to 90% A from 1.1 min to 9 min, hold at 10% A until 16 min. Return to starting conditions at 16.1 min and hold til 21 min.	0.3 mL/ min	10	Sciex QTrap 5500 MS	0.09 (LOQ)	[250]
Azoles *Blood Serum*	Accucore RP-MS, 50 × 2.1 mm, 2.6 µm	A = 0.1% (v/v) formic acid in ultrapure water B = 0.1% (v/v) formic acid in acetonitrile	Gradient was ramped from 40% B to 60% B over 20 s, held at 60% B for 30 s, and finally equilibrated back at 40% B for 60 s	0.8 mL/ min	20	Triple quadrupole MS, MRM mode	0.3 (LOQ)	[251]
Pyrethroids *Ground water*	250 mm × 4.6 mm i.d. Waters Symmetry C_{18} column (5 µm particle size).	A: ACN B: (Ammonium formate 50 mM, 5% ACN acidified to pH 3.5 with formic acid).	Gradient: 3min 70% A, 17 min linear to 80% A, 10 min linear to 100% A, 3 min 100% A, returned to initial conditions in 4 min.	1	20	Q MS, + ion mode, MRM	0.3 - 0.5	[228]

1.3.6 Conclusions

This chapter addresses and evaluates the topic of contaminants of emerging concern, their impact in water quality and some key legislation surrounding them. These compounds are used extensively and have been frequently found in environmental waters worldwide. There have been limited studies conducted in Ireland on CEC occurrence thus far, and none focussing on Watch List chemicals, indicating more research is required. The pesticide group of contaminants was examined further and their status in Ireland was investigated. An overview of pesticide usage and their ecotoxicological impact was given. Pesticides are generally diffuse source pollutants and enter waterways mainly through surface run off. Occurrences of these compounds have been shown to have harmful effects to aquatic life even at low (ng or μg L^{-1}) levels. Many of these analytes are insufficiently removed from water treatment creating another point source of pollution for rivers. This class of compounds is generally under investigated in water treatment systems.

Analysis of the literature showed pesticide monitoring using a combination of grab and an accompanying kind of long term sampling as beneficial for catchment based studies. Land use across all catchments in the papers reviewed was agricultural, with some studies also including some industrial areas. Spring and summer time were the most common seasons to sample, with many studies conducting longer term temporal studies over a number of months. Spatial variation was taken into account by most papers, with the majority including at least 3 sampling sites around the catchment. From this information, recommendations for future monitoring campaigns were given and a flow chart of the process of designing a monitoring study was produced.

The analytical methods available for monitoring of CECs in the environment were introduced. LC-MS was the analysis method of choice for the majority of studies, achieving LODs in the low ng L^{-1} range.

There is currently limited Irish data available for many groups of CECs, and therefore the main aim of this thesis is to gather data on the occurrence and fate of these contaminants in the Irish environment.

The main objectives are:

1) Develop analytical methods for the monitoring CECs in Irish surface waters.
2) Generate chemical water quality data for Ireland for a variety of CECs including pesticides.
3) Investigate methods of catchment monitoring.
4) Inform future policy through the data gathered in this project to better protect surface waters in Ireland and the EU.

Chapter 2:
Development of analytical methods for the monitoring of contaminants of emerging concern

2.1 Introduction

Currently, there is limited data available for the chemical water quality of Irish aquatic environments [121,205,252–254]. To date, there has been no studies conducted in Ireland targeting the entire suite of chemicals listed in the EU Watch Lists. In order to fill this gap in knowledge and produce some of the first Irish data for these compounds, analytical methods capable of their analysis is required.

This chemical variability of analytes contained in the EU Watch Lists can make analysis of surface water samples increasingly complicated [255,256]. Because of the low levels at which WL chemicals may occur in surface waters, in the parts per trillion (ppt) range, reaching the detection and quantification limits needed to monitor these CECs presents a challenge. Often large volumes of sample are required to reach these detection limits, which makes national-scale monitoring programmes difficult to perform logistically and at high frequency. For water, extraction tends to predominantly use off-/online solid phase extraction (SPE) as they allow for pre-concentration and clean-up of the sample matrices [257]. Choice of SPE sorbent material is highly dependent on the specific chemistries of the analytes to be extracted. Analyte polarity plays an important role in SPE cartridge selection [258]. With such a broad range of polarities represented in the WLs (Table 15), a cartridge with the capability of retaining both hydrophilic and lipophilic analytes is ideal. Gas chromatography – mass Spectrometry (GC-MS) or liquid chromatography – mass spectrometry (LC-MS), and often in combination, have been the analysis methods of choice for the suite of analytes contained in the WL [255,257,259]. While a combination of chromatographic techniques has its benefits, many laboratories do not have the facilities to employ both methods. Therefore, an approach which minimises multiple instruments and differing consumables such as analytical columns and SPE cartridges would be of great benefit. This chapter outlines the development of SPE-LC-MS/MS methods for the analysis of Watch List compounds using the same cartridges, column and LC-MS system. A general approach to an SPE-LC-MS/MS procedure is shown in Figure 11, which the developed methods were modelled on.

Pesticide contamination is a cause for concern in particular for Ireland due to the country's majority agricultural land use, with >67% of the land mass being agricultural. While there has been some research into pesticide contamination in the country, information on surface and wastewaters is limited as many of the available studies focus on drinking or groundwater [205,224,260,261]. Targeted pesticides highlighted in the literature review presented in Chapter 1 were selected for examination in Irish waters including neonicotinoids, azoles, acid herbicides, and pyrethroids (Table 15). Therefore analytical methods capable of their analysis was required. LC-MS is generally the chromatographic method of choice for all the groups examined bar pyrethroids. GC-MS is historically the analysis method of choice used for the determination of pyrethroid pesticides due to their volatility [91,262]. The use of LC-MS for the analysis of pyrethroid pesticides is a novel approach with limited studies conducted using this instrument, and even less of which applied it to water samples [91,263–268]. The ability to analyse these all of these groups of pesticides in a singular LC-MS method alongside other pesticide groups would be novel and hugely advantageous approach, significantly reduce analysis and sample prep time. The process of developing this method is also discussed in this chapter.

Lastly, review of the literature indicated the herbicide glyphosate and its metabolite AMPA as analytes of interest for monitoring. The analysis of these compounds presents a unique challenge as they are unable to be retained by most SPE cartridges, nor a regular C18 column unless undergoing derivatisation due to their highly polar nature. However, advances in column development has allowed for the Hydrophilic Interaction Liquid Chromatography (HILIC) -MS analysis of glyphosate and AMPA without FMOC derivatisation [269,270]. This chapter also outlines the analytical method development of a direct injection HILIC method for these compounds.

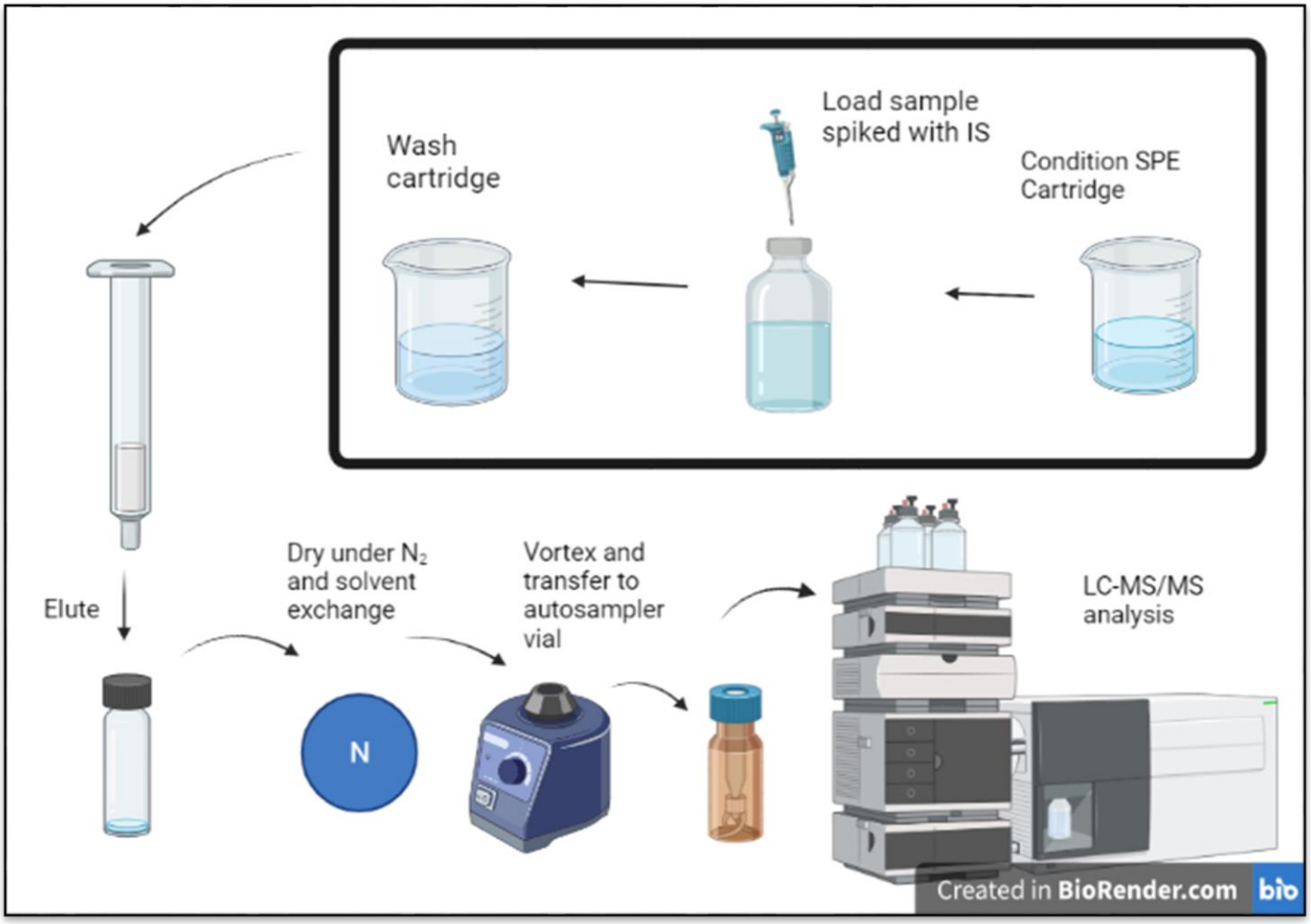
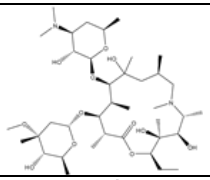
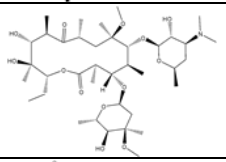
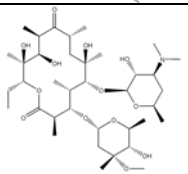
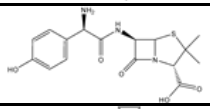
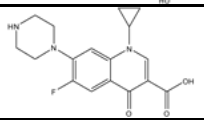
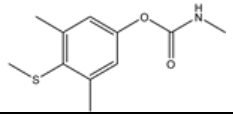
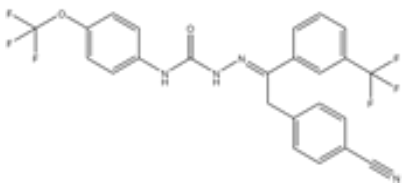
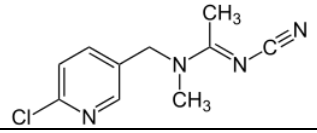
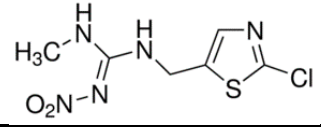
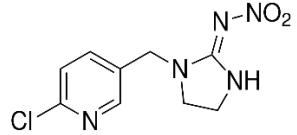
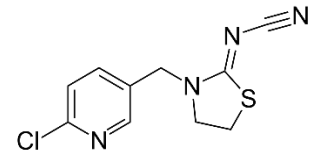
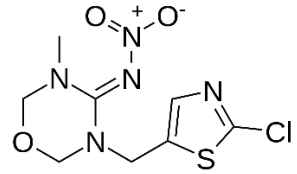
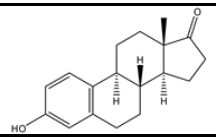
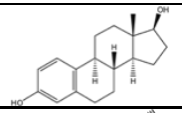
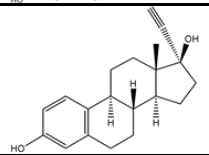
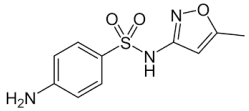
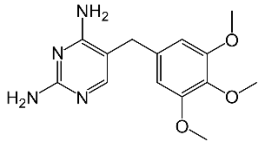
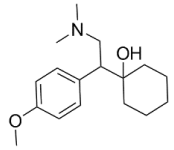
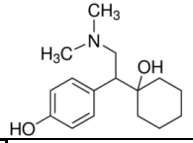
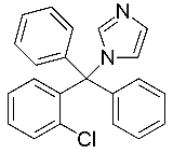


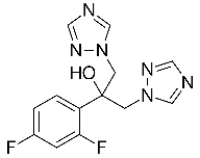
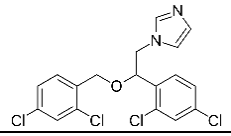
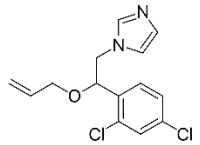
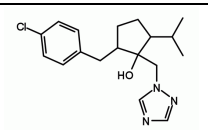
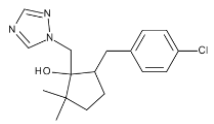
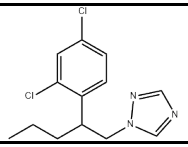
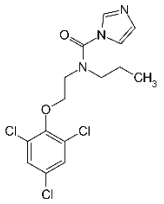
Figure 11. General approach to SPE-LC-MS/MS procedures employed in this thesis. Created using Biorender

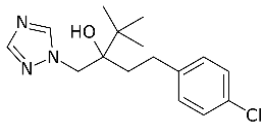
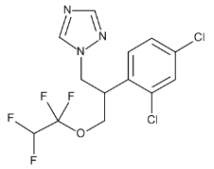
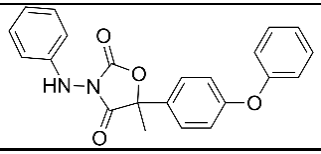
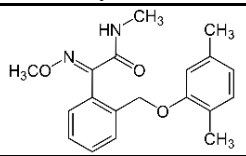
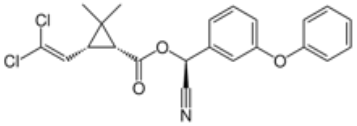
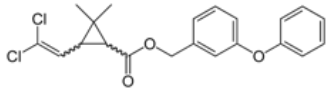
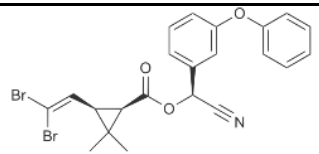
Table 15. Table of physico-chemical properties of targeted analytes, information acquired from PubChem [271]

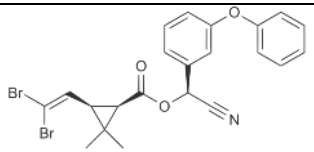
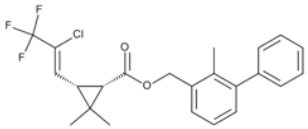
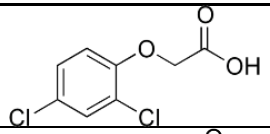
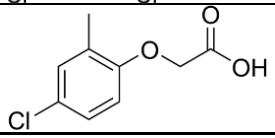
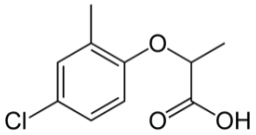
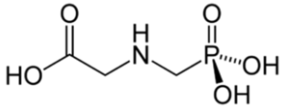
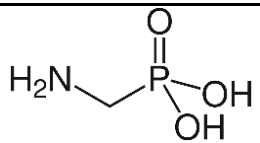
Targeted method group	Group	Class	Name	Structure	Formula	LogK$_{ow}$	CAS no
	Antibiotics	Macrolides	*Azithromycin*		$C_{38}H_{72}N_2O_{12}$	4.02	83905-01-5
			Clarithromycin		$C_{38}H_{69}NO_{13}$	3.16	81103-11-9
			Erythromycin		$C_{37}H_{67}NO_{13}$	3.06	114-07-8
		Penicillin	*Amoxicillin*		$C_{16}H_{19}N_3O_5S$	0.87	26787-78-0
		Fluoroquinolone	*Ciprofloxacin*		$C_{17}H_{18}FN_3O_3$	0.28	85721-33-1
	Pesticides	Carbamate	*Methiocarb*		$C_{11}H_{15}NO_2S$	2.92	2032-65-7

		Semicarbazone	*Metaflumizone*		$C_{24}H_{16}F_6N_4O_2$	4.4-5.1 (Z-E isomer)	139968-49-3
			Acetamiprid		$C_{10}H_{11}ClN_4$	0.8	135410-20-7
			Clothianidin		$C_6H_8ClN_5O_2S$	0.7	210880-92-5
			Imidacloprid		$C_9H_{10}ClN_5O_2$	0.57	138261-41-3
			Thiacloprid		$C_{10}H_9ClN_4S$	1.26	111988-49-9
			Thiamethoxam		$C_8H_{10}ClN_5O_3S$	-0.13	153719-23-4
	Hormones	Estrogens	*Estrone*		$C_{18}H_{22}O_2$	3.13	53-16-7

			17-β-estradiol (E2)		$C_{18}H_{24}O_2$	4.01	50-28-2
			17-α-ethinylestradiol (EE2)		$C_{20}H_{24}O_2$	3.67	57-63-6
3rd Watch List	**Antibiotics**	Sulphonamide	*Sulfamethoxazole*		C10H11N3O3S	0.89	723-46-6
		Diaminopyrimidine	*Trimethoprim*		C14H18N4O3	0.91	738-70-5
	Antidepressant	Phenethylamine	*Venlafaxine*		C17H27NO2	3.2	93413-69-5
			o-desmethylvenlafaxine		C16H25NO2	2.6	93413-62-8
	Antifungal Pharmaceutical	Azole	*Clotrimazole*		C22H17ClN2	4.1	23593-75-1

		Fluconazole		C13H12F2N6O	0.25	86386-73-4
		Miconazole		C18H14Cl4N2O	6.1	22916-47-8
	Pesticides	*Imazalil*		C14H14Cl2N2O	3.82	35554-44-0
		Ipconazole		C18H24ClN3O	4.21	125225-28-7
		Metconazole		C17H22ClN3O	3.85	125116-23-6
		Penconazole		C13H15Cl2N3	3.72	66246-88-6
		Prochloraz		C15H16Cl3N3O2	4.1	67747-09-5

			Tebuconazole		C16H22ClN3O	3.7	107534-96-3
			Tetraconazole		C13H11Cl2F4N3O	3.56	112281-77-3
		Oxazolidinones	*Famoxadone*		C22H18N2O4	4.65	131807-57-3
		Monocarboxylic acid amide	*Dimoxystrobin*		$C_{19}H_{22}N_2O_3$	3.9	149961-52-4
Pesticides Reverse Phase LC-MS	Pesticides	Pyrethroid	*Cypermethrin*		$C_{22}H_{19}Cl_2NO_3$	6	52315-07-8
			Permethrin		$C_{21}H_{20}Cl_2O_3$	6.5	52645-53-1
			Deltamethrin		$C_{22}H_{19}Br_2NO_3$	6.2	52918-63-5

			Esfenvalerate		$C_{25}H_{22}ClNO_3$	6.22	66230-04-4
			Bifenthrin		$C_{23}H_{22}ClF_3O_2$	6	99267-18-2
		Acid herbicide	*2,4-D*		$C_8H_6Cl_2O_3$	2.81	94-75-7
			MCPA		$C_9H_9ClO_3$	3.25/2.8	94-74-6
			Mecoprop		$C_{10}H_{11}ClO_3$	3.13	93-65-2
HILIC LC-MS		Organophosphorus herbicide	*Glyphosate*		$C_3H_8NO_5P$	-3.4	1071-83-6
			AMPA		CH_6NO_3P	-4.7	1066-51-9

Aims and Objectives

The aim of this chapter was to develop analytical methods capable of measuring environmentally relevant concentrations of targeted contaminants of emerging concern in Irish waters.

The main objectives are to:

- Highlight key aspects of the literature methods detailed in Chapter 1 that were used to guide method development;
- Outline the final methods achieved;
- Describe the steps taken to optimize the methods;
- Assess the developed methods for their performance.

2.2 Materials and Methods

2.2.1 Chemicals, consumables and reagents

Honeywell CHROMASOLV™ LC-MS grade acetonitrile and methanol were obtained from Fisher Scientific (Dublin, Ireland). Formic acid, EDTA and LC-MS grade dichloromethane were acquired from Sigma–Aldrich (Arklow, Ireland). Nylon Whatman filters with 0.45 µm pore sizes were supplied by Sigma–Aldrich (Arklow, Ireland). 0.2 µm nylon membrane syringe filters were acquired from VWR, (Dublin, Ireland). Sulphuric acid was purchased from Fisher Scientific (Dublin, Ireland). Ultrapure water (resistance of 18.2 MΩ cm) was generated from a PURELAB® Ultra water purification system (ELGA, Labwater, and High Wycombe, United Kingdom). Analytical reference standards of ≥98% purity for 17-alpha-ethinylestradiol, 17-beta-estradiol, 2.4-D, MCPA, acetamiprid, aminomethylphosphonic acid (AMPA), amoxicillin, azithromycin, bifenthrin, ciprofloxacin, clarithromycin, clothianidin, clotrimazole, cypermethrin, deltamethrin, erythromycin, esfenvalerate, estrone, famoxadone, fluconazole, glyphosate, imazalil, imidacloprid, Mecoprop, metaflumizone, metaflumizone, metconazole, methiocarb, miconazole, o-desmethylvenlafaxine, penconazole, permethrin, prochloraz, sulfamethoxazole, tebuconazole, tetraconazole, dimoxystrobin, thiacloprid, thiamethoxam, trimethoprim and venlafaxine, as well as surrogate standards acetamiprid-d3, aminomethylphosphonic acid-^{13}C,^{15}N,D clothianidin-d3, cypermethrin –(phenoxy-d5), fluconazole-13C3, glyphosate-2-^{13}C,^{15}N, imazalil-(allyl-d5), imidacloprid–d4, MCPA-d6, methiocarb–d3, prochloraz-(ethylene-d4), thiacloprid–d4, thiamethoxam–d3, sulfamethoxazole-13C6 and trimethoprim-d9 were all obtained from Sigma–Aldrich (Arklow, Ireland). Ipconazole was obtained from LGC standards (Teddington, UK). Azithromycin–d3 was obtained from Toronto Research Chemicals (Toronto, Canada). All standards were prepared in methanol (1 mg mL^{-1}) except for amoxicillin, AMPA, glyphosate and venlafaxine which were prepared in water at the same concentration. All stocks were stored in amber glass vials (14 mL) at -18°C. Ciprofloxacin and amoxicillin required the addition of a small amount (>3 µL) formic acid to fully dissolve in solution. Due to rapid breakdown, amoxicillin, ciprofloxacin and famoxadone stocks were prepared fresh every day. Field samples were extracted using Oasis hydrophilic-lipophilic balanced (HLB) 6 cc, 200 mg bed mass, 30 µm

particle size, SPE cartridges obtained from Waters Chromatography Ireland Ltd. (Dublin, Ireland). Liquid chromatography was performed using an Agilent HPLC instrument equipped with a 1290 Infinity II LC multisampler, binary pump and multiple column thermostatted compartment (Agilent, Cheadle, UK). Chromatographic separation was achieved using a 2.1 × 150 mm, 1.9 µm particle size InfinityLab Poroshell 120 EC-C18 column and corresponding guard (Agilent Technologies, Cheadle, UK) or a Shodex 150 x 2mm, 5 µm particle size HILICpakTM VT-50 2D column with corresponding guard (Apex Scientific Ltd, Kildare, Ireland) depending on the method.

2.2.2 Silanisation of glassware

In order to prevent analyte binding to the surface of glassware, particularly during the dry down step of SPE, all vials used were silanised using the procedure shown in Figure 12. Triplicate washes of each step were performed.

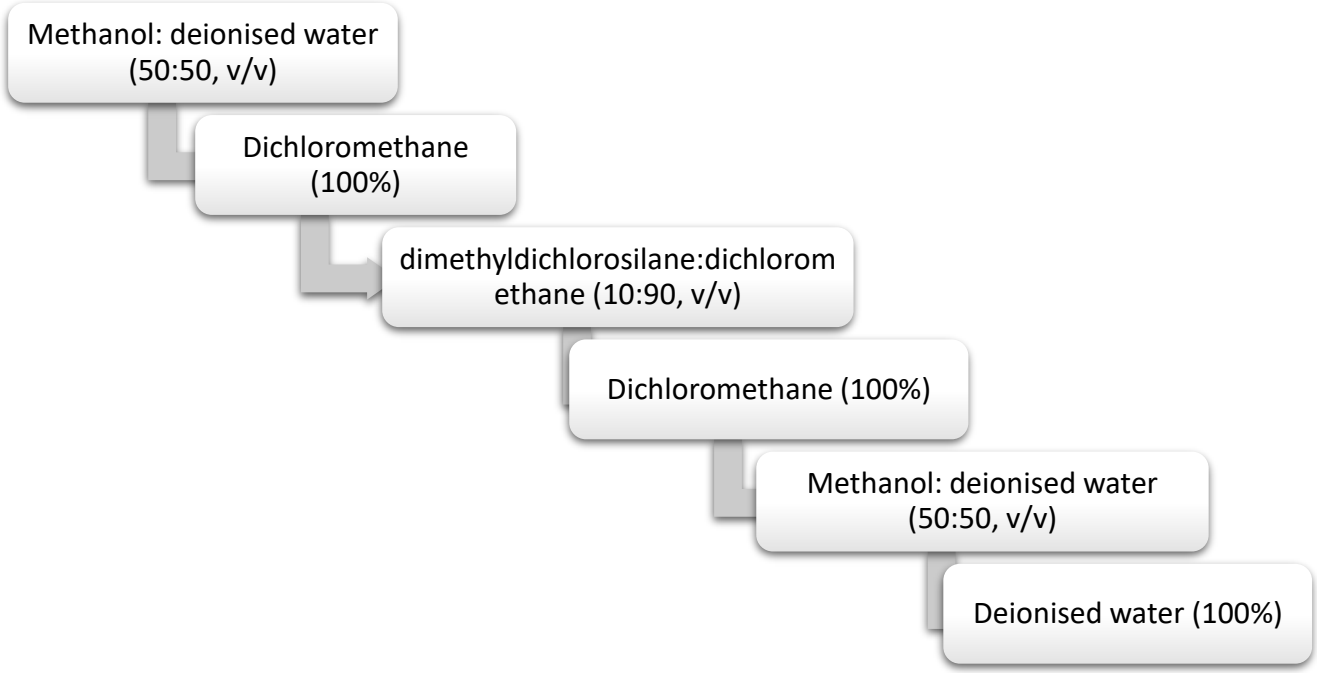


Figure 12. Stepwise procedure of silanisation process to prevent binding of the analytes to the surface of glassware.

2.2.3 Watch List Analysis

2.2.3.1 Field sample collection and preparation

Field samples were taken from five sites located around Ireland. Time periods sampled were December 2018, July and August 2019, and September and October 2020 for 2nd Watch List analytes. For the 3rd 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 1L Nalgene™ Amber HDPE bottles. One litre was taken for General Chemistry analysis performed in the EPA laboratory, Clonskeagh, while the rest was taken for WL analysis and were preserved by acidification to pH 3 using sulphuric acid. Samples were taken during the stated periods stated from the River Annalee in Co. Cavan, the River Nore in Co. Kilkenny (Inistioge), the River Suir in Co. Tipperary (Kilsheelan), and the River Liffey in Co. Dublin (Lucan), site map and further site information are detailed in Chapter 3. One sample was also taken from the River Shannon in Co. Clare (Killaloe) in December 2018.

Field measurements of temperature, dissolved oxygen (DO), 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 shortly after sample collection. For the 3rd 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 was 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 prior to spiking using 0.45 µm pore sized nylon filters to remove particulates. From 2020 onwards samples were also spiked with 0.1M EDTA to a final concentration of 0.1%.

2.2.3.2 2nd Watch List

2.2.3.2.1 Solid Phase Extraction and HPLC conditions

Figure 13 outlines the analytical approach undertaken to determine the WL chemicals from sampling through to analysis. One SPE method was used for both the 2018 and 2019 samples (SPE Method 1). In the 2020 analysis, a modified method (SPE Method 2) was used for some compounds in addition to SPE Method 1 to improve extraction efficiency. The SPE methods were based on a literature method for 166 pharmaceutical compounds in both waste and river water [272,273]. Cartridges loaded with sample were stored at -18°C until the time of analysis.

Liquid chromatography was performed using an Agilent HPLC stack equipped with a 1290 Infinity II LC multisampler, binary pump and multiple column thermostatted compartment (Agilent, Cheadle, UK). Chromatographic separation was achieved using a 2.1 × 150 mm, 1.9 µm particle size InfinityLab Poroshell 120 EC-C18 column (Agilent Technologies, Cheadle, UK). Mass spectrometry was performed using a 6470A triple quadrupole mass spectrometer (MS) (Agilent Technologies, Cheadle, UK). Multiple chromatographic methods were used for analysis of the total watch list in order to allow for optimum sensitivity for all analytes. The 'Mix Method' targeted acetamiprid, amoxicillin, azithromycin, ciprofloxacin, clarithromycin, clothianidin, erythromycin, imidacloprid, methiocarb, thiacloprid and thiamethoxam. 'Metaflumizone method' targeted metaflumizone only. The 'Estrogens method' which had been developed in our laboratory previously targeted 17-alpha-ethinylestradiol, 17-beta-estradiol and estrone.

A gradient elution program was used for the LC separation of the Mix method. The composition of the mobile phase was varied from 90 - 50 % formic acid (A) from 0 to 4 min, then varied from 50 - 10 % formic acid (A) from 5 to 5.50 min, then held at 10 % formic acid (A) until 6.00 min, then reduced from 10 - 0 % A at 6.50 min, which was maintained until 8.00 min. Finally, the column was re-equilibrated to starting conditions of 0 - 90 % A at 8.50 min. A postrun of 1 min was used. Total analysis time was 9.5 min.

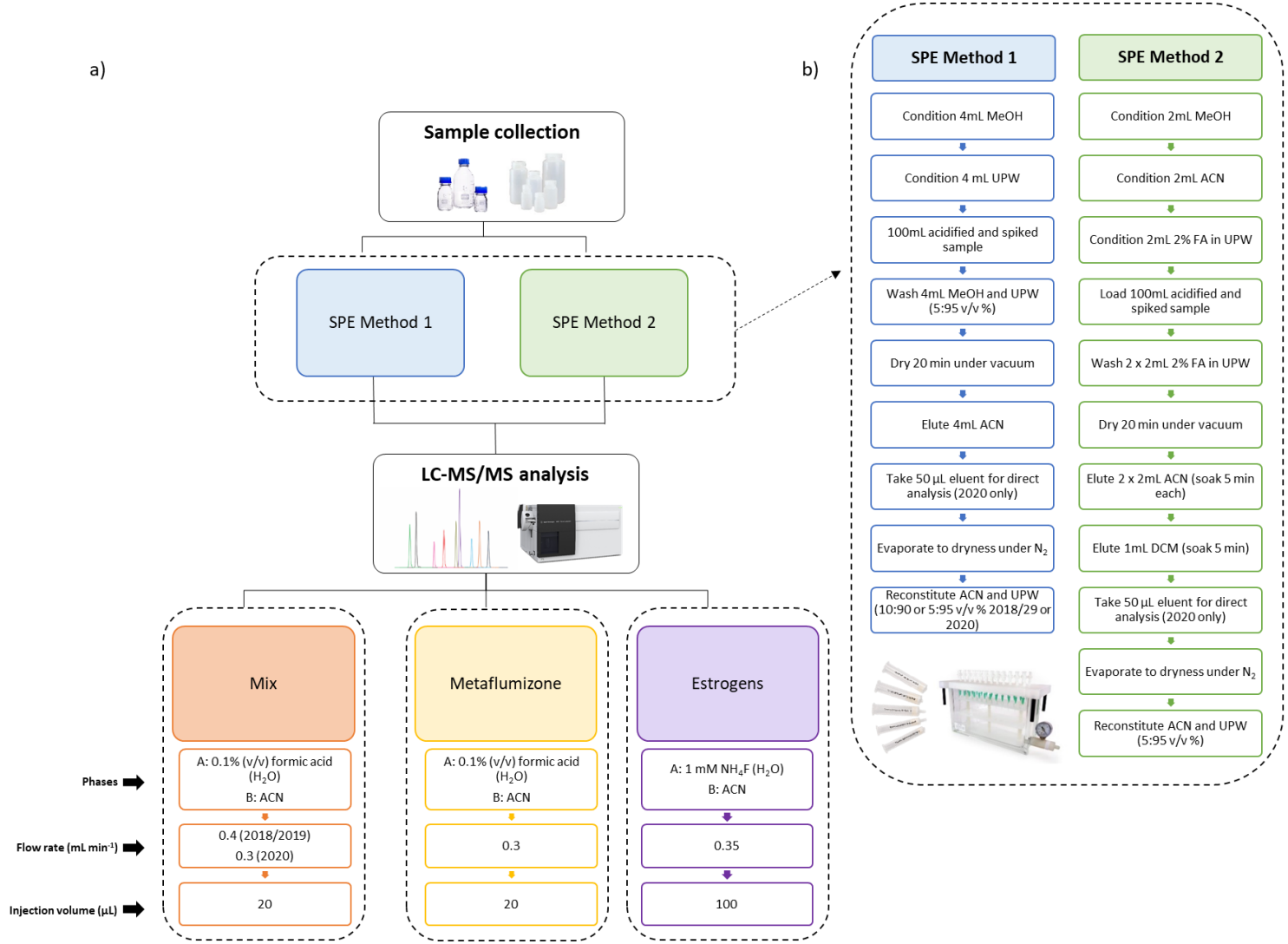


Figure 13. Flow chart of 2nd Watch List analytical method starting from sample collection through to analysis

This was modified slightly for the 2020 analysis in order to further optimize the separation as follows; from 95 - 50 % formic acid (A) from 0 to 4 min, then varied from 50 - 10 % formic acid (A) from 6.5 to 7 min, then held at 10 % formic acid (A) for 1 min, then reduced from 10 - 0 % A from 8 min to 9 min, which was maintained until 9.5 min. Finally, the column was re-equilibrated to starting conditions of 0 - 95 % A at 9.50 min. A postrun of 1 min was used. The injection volume was 20 µL.

A shortened version of the full mix LC separation was used for metaflumizone. This gradient began at 70% B, changing to 100% B at 3 min, holding at 100% B for 1 min and finally returning to 70% B at 5 min. Stoptime was 5 min and there was a 1 min postime. Metaflumizone retention time with this gradient was 3.5 min. All other method parameters remained the same as the full separation method.

For the estrogen separation, a gradient elution program was used for this LC separation, and was modified between analysis for the 2018/19 samples and the 2020 samples. Gradient elution conditions for the estrogen method for the 2018/9 analysis were held from 0 to 0.3 min at 40% B; then it increased to 70% B from 0.3 to 0.8 min; another increase was performed from 3 to 4 min up to 100% B and held until 5.00 min. The gradient was finally reduced to starting conditions of 40% B from 5.0 to 5.5 min and a re-equilibration time of 1 min was added at the end giving a total analysis time of 6.5 min. For the 2020 analysis optimised gradient elution conditions for this method were isocratic from 0 to 2.20 min at 70% B; then it increased to 100% B from 2.20 min to 2.70; this was held for 1 min until 3.70 min. The gradient was returned to starting conditions of 70% B at 4.00 min and held for 1 min until 5.00 min. The stop time was 5.50 min giving a total analysis time of 5.5 min. The injection volume was 100 µL.

2.2.3.2.2 Mass Spectrometry

Analysis was performed in multiple reaction monitoring (MRM) mode using both positive and negative modes for Method 1. Only negative mode was used for Method 2. Both MS1 (quadrupole 1) and MS2 (quadrupole 3) were set to wide mode in order to enhance

sensitivity. MRM optimization of each analyte was performed using the Agilent Optimizer software package (Agilent Technologies, Cheadle, UK). All MRM parameters including product ion *m/z*, collision energy (CE) and fragmentor voltage were optimised directly on the mass spectrometer by bypassing the analytical column. Optimisation of MRM parameters was achieved using standards of individual analytes in ultrapure water or methanol depending on the analyte at either 100 or 50 ng mL^{-1}. A minimum of two transitions for each analyte were selected for inclusion in the MRM method, with the transition producing the highest signal being chosen for quantitation. Only one transition was used for deuterated internal standards. QQQ conditions are summarised in the results section of this chapter. MS source parameters were optimized using the Agilent Source Optimizer software package (Agilent Technologies, Cheadle, UK), with final source conditions shown in Table 16.

Table 16. Table of MS source parameters for 2nd Watch List compounds

Gas Temperature (°C)	340	
Gas Flow (L / min)	8	
Nebulizer (psi)	40	
Sheath Gas Temperature (°C)	350	
Sheathe Gas Flow (L / min)	12	
	Positive	Negative
Capillary (V)	3000	3000
Nozzle Voltage (V)	500	1500

2.2.3.2.3 Method Performance

Examination of method performance was studied based on ICH guidelines [274].

Instrumental LOD/Qs were observed by running low level calibration standards starting at 1 ng L^{-1} prepared in solvent (i.e. un-extracted standards prepared in starting mobile phase), with the inclusion of multiple solvent blanks. A signal to noise ratio (S/N) of 3 for LOD and 10 for LOQ was used to establish instrumental LOD/Q.

Overall method performance in sample matrix was then evaluated using composite of surface water samples. A composite of 13 surface water samples from the 5 sites was prepared for validation of the analytical method for 2018/9. For 2020 a composite of 4 samples from each site were used for each batch in September and then October. Calibration linearity, matrix effects, analyte recoveries, method dynamic range, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) were measured. Calibration was performed using the composite sample matrix by adding the required concentrations of the compounds before extraction (For 2018/9 calibration range was from 10-5000 ng L^{-1}, for 2020 Method 1 the range was 10-2000 ng L^{1} and for Method 2 it was 3-500 ng L^{-1}). The concentration range for the hormones method was lower as concentrations found in samples were expected to be significantly lower. Unspiked matrix (n = 3) was extracted and used for the subtractions of the compounds already present in the calibration samples. Calculated method LOD and LOQ values were determined as 3.3 and 10 times the standard error and the slope of the matrix matched calibration curve, as seen in equation 2:

$$LOD = 3.3(^{Syx}/_{b}) \quad LOQ = 10\left(^{Syx}/_{b}\right)$$

where b = slope, S_{yx} = standard error of the matrix matched calibration curve.

Equation 2. Equation for the determination of method limit of detection and limit of quantitation

LOD and LOQ values based off the matrix matched calibration curve were used for quantitation and reporting purposes. Recovery was determined by comparison of the peak area ratio responses of composite matrix samples spiked post extraction (n = 3) to the responses of composite matrix samples spiked pre-extraction (n=3) at the same concentration of 500 ng L^{-1} in 2018/19 batch and 1000 ng L^{-1} for Method 1 and 100 ng L^{-1} for Method 2 in the 2020 batch. To examine for matrix effects, peak area ratio responses of samples prepared using deionised water (n=3) were compared with the peak area ratio responses of matrix-matched standards (n=3) with the same concentration of 500 ng L^{-1} in 2018/19 and 1000 or 100 ng L^{-1} in 2020 for the mix method, and estrogens at a concentration of 300 ng L^{-1}. Repeatability was assessed through triplicate injections at the same concentration. All calculations were performed using peak area ratios normalised to internal standards added prior to extraction.

2.2.3.3 3rd Watch List

2.2.3.3.1 Solid Phase Extraction and HPLC Conditions

The overall approach to the 3rd Watch List analytical method is shown in Figure 14. The SPE method used 6cc Oasis (HLB) cartridges. The cartridges are conditioned with 4 mL of methanol followed by 4 mL of ultrapure water acidified to pH 3 with formic acid. 100 mL of acidified sample was spiked with internal standard mix and 0.1M EDTA. The 100mL sample was then loaded onto the cartridge at an approximate flow rate of 1mL/min. Cartridges are washed with 4 mL ultrapure water acidified to pH 3 with formic acid before drying the cartridge under vacuum for 20 min. Elution was performed with 4 mL of acetonitrile which was left to soak on the cartridge for 15 min prior to elution.

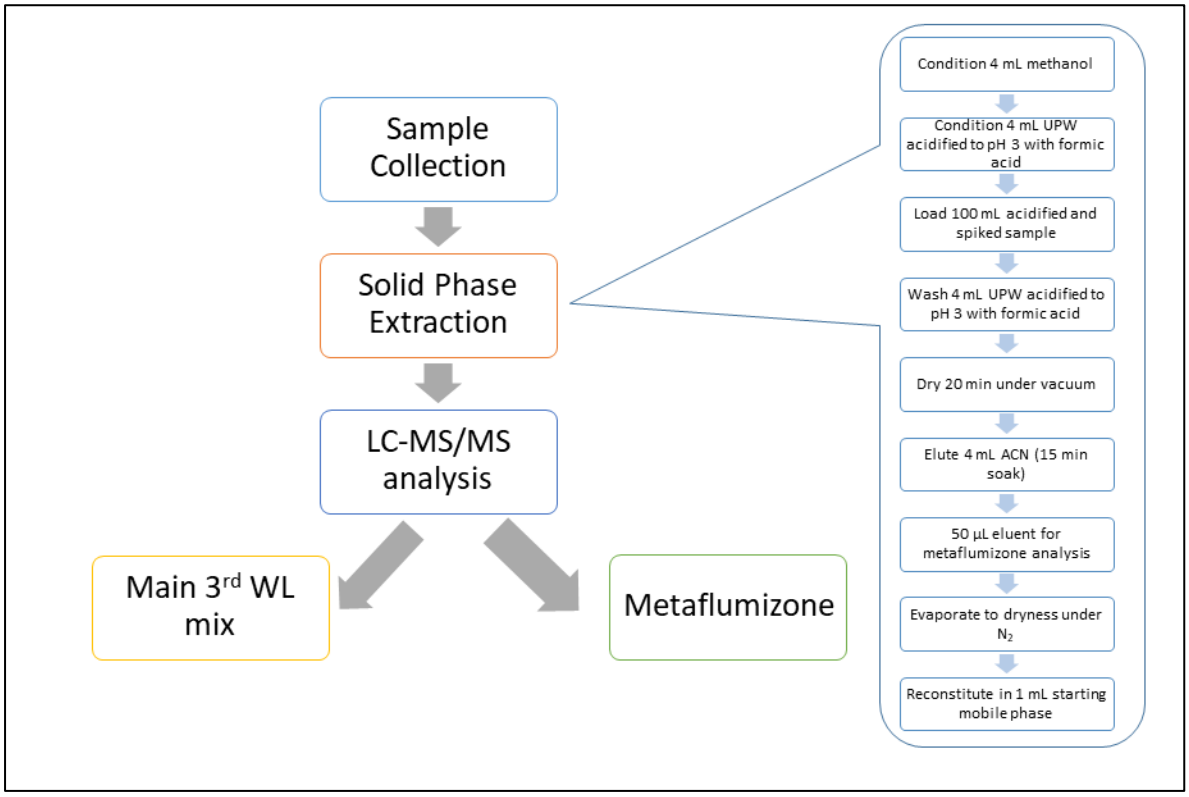


Figure 14. Flow chart of the analytical method for 3rd Watch List compounds from sample collection to analysis.

A 50 µL aliquot of the eluent was taken off for direct metaflumizone analysis, as this compound did not survive the dry down procedure. This aliquot was placed in 4.6 mm I.D vial

inserts for 2 mL autosampler vials. The remaining eluent was evaporated under a gentle stream of nitrogen to complete dryness before reconstitution in 1 mL of starting mobile phase (5:95 mobile phase A: B). Reconstituted samples were vortexed for 30 s, sonicated for 10 min, then vortexed again for an additional 30 s before finally being transferred into 2 mL amber autosampler vials for analysis.

Liquid chromatography was kept constant as possible across the 2nd and 3rd WL analysis by use of the same column and LC-MS/MS. Mobile phases consisted of 5 mM ammonium formate and 0.1% formic acid in ultrapure water (A) and 5 mM ammonium formate and 0.1% formic acid in methanol (B). A gradient elution program was used for the LC separation. A table of the gradient elution conditions are in Table 17. The injection volume was 30 µL and the flow rate was 0.25 $mL.min^{-1.}$

A shortened version of the full mix LC separation was used for metaflumizone similarly to the method developed for the previous Watch List. This gradient began at 50% a, changing to 10% A at 3 min, then 0% A at 3.5 min, holding at 0% A for 1 min and finally returning to 50% A at 5.5 min. Stoptime was 6 min and there was a 1 min postime. All other method parameters remained the same as the full separation method.

Table 17 LC-MS gradient conditions for 3rd Watch List compounds

Time (min)	% A	% B
0.00	95	5
3.00	50	50
14.00	20	80
19.00	5	95
20.00	0	100
21.00	0	100
22.00	95	5

As with the 2nd Watch List, a shortened version of the full mix LC separation was used for metaflumizone similarly to the method developed in Chapter 2. This gradient began at 50% a, changing to 10% A at 3 min, then 0% A at 3.5 min, holding at 0% A for 1 min and finally

returning to 50% A at 5.5 min. Stoptime was 6 min and there was a 1 min postime. All other method parameters remained the same as the full separation method.

2.2.3.3.2 Mass Spectrometry

MRM optimization of additional analytes added from the 3rd WL was performed using the Agilent Optimizer software package used for the prior list (Agilent Technologies, Cheadle, UK). Analysis was performed in dynamic multiple reaction monitoring (dMRM) mode using both positive and negative modes. The use of dMRM allows for optimum dwell times to be achieved for all analytes in the method, and for retention times to be established in the acquisition software to ensure method performance. dMRM cycle times of 500 ms were used with dwell times set between 20 – 50 ms. Both MS1 (quadrupole 1) and MS2 (quadrupole 3) were set to unit mode for all analytes aside from amoxicillin and famoxadone which were set to wide in order to enhance sensitivity. At least two transitions for each analyte were selected for inclusion in the MRM method, with the transition producing the highest signal being chosen for quantitation. Generally, only one transition was used for deuterated internal standards. QQQ conditions are summarised in the results section of this chapter. MS source parameters were optimized using the Agilent Source Optimizer software package (Agilent Technologies, Cheadle, UK), with final source conditions shown in Table 18.

Table 18. LC-MS source conditions for 3rd watch list method

Gas Temperature (°C)	340	
Gas Flow (L / min)	8	
Nebulizer (psi)	35	
Sheath Gas Temperature (°C)	300	
Sheath Gas Flow (L/ min)	12	
	Positive	Negative
Capillary (V)	3500	3000
Nozzle Voltage (V)	500	1500

2.2.3.3.3 Method Performance

The same approach to examination of method performance was used as with the 2nd Watch List. Extracted calibration curves in the range of 1-1000 ng L^{-1} were prepared, and investigation of analyte recovery and matrix effects were performed at a concentration of 750 ng L^{-1}.

2.2.4 Pesticide Analysis

2.2.4.1 Field sample collection and preparation

For the samples corresponding to the study presented in Chapter 4, samples of influent, effluent and receiving waters were collected from two Wastewater Treatment Plants around Ireland. The sites correspond to a rural and an urban area with population equivalents of approximately 3,200 and 130,000 respectively. Due to a confidentiality agreement, any further information including exact locations of the sampling sites cannot be disclosed. Grab samples were collected on 1 sampling day per month for 12 months from the period October 2018 – September 2019 for each site in 1L Nalgene™ Amber HDPE bottles. All samples were preserved by acidification to pH 2 using hydrochloric acid.

For analysis of glyphosate and AMPA using direct injection, samples were prepared by further filtering using 0.2 µm nylon membrane syringe filters before taking a 950µL aliquot of each sample and placing in a 1.5mL autosampler vial. Samples were then spiked with 50µL of internal standard solution to a final volume of 1 mL.

For the samples relating to Chapter 5, surface water field samples were collected from three sites located in Dublin, Ireland and five sites located around County Donegal, Ireland from June-September 2021. Grab field samples were collected for each site in 1L Nalgene™ Amber HDPE bottles. Sampling dates and sites are presented in Table 19.

Table 19. Table of sampling sites and frequency for Chapter 5 catchment study

Area	Site name	Coordinates	Sampling Occurring
Kildare	Upstream	53.230595, -6.721749	June, July
	WWTP	53.229894, -6.698771	June, July
	Downstream	53.287735, -6.679648	June, July
Donegal	National Park	54.989532, -8.051088	July, August, September
	Glen site A	55.126480, -7.814827	July, August, September
	Glen site B	55.114266, -7.785683	July, August, September
	Cranford	55.135288, -7.698086	July, August, September
	Glenadowan	54.801083, -7.973861	July, August, September

Field measurements of temperature, Dissolved Oxygen (DO), turbidity and pH were collected by the YSI EXO3 Multiparameter Water Quality Sonde. All samples were preserved by acidification to pH 3 using sulphuric acid.

Chemcatcher passive sampling devices using two different kinds of sorbent disks were selected for study. Triplicate anion-exchange disks coupled with a PES membrane were deployed at each of the Dublin locations for a period of two weeks. Disk preparation, deployment and extraction was performed using the method described by Khan et al [254]. In brief, Chemcatcher housings were cleaned overnight in a 2% Decon 90 solutions before washing with DI water followed by acetone. PES membranes were soaked for 24 h in methanol to remove potential oligomers and then rinsed with DI before use. The retaining disks were activated by serial rinsing of 50mL each of acetone, methanol, DI, 1M sodium hydroxide and finally DI before assembly. Once assembled, disks were kept wet by adding a small amount of ultrapure water into the well of the sampler before placing the lid for transport. Samplers were transported on ice to sample sites. A field blank was also exposed at each site.

For the Donegal locations, triplicate deployments at each site of C18 disks coupled with LDPE membranes was performed for a period of 5 weeks. Preparation, of the disks was performed using the method described by Vrana et al [275]. LDPE membranes were soaked for 24 h in n-

hexane before use. C18 disks were activated with 10 mL methanol followed by 2 mL of ultrapure water, and then loaded with performance reference compounds (PRCs) by passing through 500 mL of water spiked with PRCs. Disks were then dried under vacuum before placing into the Chemcatcher housing. The disks were topped with one mL of n-octanol in acetone (45% v/v) before allowing the acetone to evaporate off in the fume hood. The LDPE membrane was finally placed on the top of the disk and the retaining ring was put on, ensuring no air bubbles were trapped between the membrane and the disk. Disks were kept on ice during transport to sample sites and field blanks were exposed at each site.

Samples from all studies outlined above were stored in a freezer (-18 °C) until extraction using the final optimized method. Before extraction, each frozen sample was defrosted slowly in a refrigerator (4 °C) and then all samples were filtered prior to spiking using 0.45 µm pore sized nylon filters to remove particulates. Samples were also spiked with 0.1M EDTA to prevent any metal complexation of analytes of interest. Passive sampling disks were also frozen until analysis and defrosted slowly in a refrigerator (4 °C) before extraction.

2.2.4.2 Reverse –Phase Liquid Chromatography

2.2.4.2.1 Solid Phase and Passive Sampler Extraction

The final optimized SPE method from the 3rd Watch List was also applied successfully to the targeted pesticides, without the additional steps required for metaflumizone analysis. As before, 6 cc Oasis (HLB) cartridges were conditioned with 4 mL of methanol followed by 4 mL of ultrapure water acidified to pH 3 with formic acid. A 100 mL of acidified sample spiked with internal standard mix and 250 µL 0.1M EDTA was then loaded on to the cartridge. Cartridges were washed with 4 mL ultrapure water acidified to pH 3 with formic acid before drying under vacuum for 20 min. Cartridges loaded with sample were stored at -18°C until the time of analysis. Elution was performed with 4 mL of acetonitrile which was left to soak on the cartridge for 15 min prior to elution. The eluent was evaporated under a gentle stream of nitrogen to complete dryness before reconstitution in starting mobile phase (65:35 mobile phase A: B).

For passive sampling, the anion exchange disks were extracted in line with the method used by Khan et al., in which the disks were extracted by two aliquots of 25 mL ethyl acetate: formic acid (90:10 v/v) before evaporating to dryness and reconstituting to a final volume of 1mL in methanol [254]. Internal standard solution was added to the reconstituted sample for LC-MS analysis.

For the C18 disks, a modified version of the method by Vrana was used [275]. Disks were defrosted slowly, and placed flat on the bottom of a 100 mL borosilicate bottle. Disks were fully submerged in 8 mL of acetonitrile and sonicated for 10 min. The 8 mL acetonitrile was then collected into a 14 mL amber glass vial, and the disk washed with 1 mL of acetonitrile, which was also collected and combined with the previous 8 mL. The disk was then removed from the bottle and an additional 1 mL of acetonitrile was used to rinse the bottle and ensure minimal analyte loss in solvent transfer. This was combined to make a final volume of 10mL which was evaporated under a gentle stream of nitrogen to complete dryness before reconstitution in starting mobile phase (65:35 mobile phase A: B).

2.2.4.2.2 Liquid Chromatography - Mass Spectrometry

Liquid chromatography was performed using the same Agilent LC-MS/MS fitted with the InfinityLab Poroshell 120 EC-C18 column used for Watch List analysis. The injection volume was 100 µL and the flow rate was 0.25mL.min^{-1}.Mobile phases consisted of 5 mM ammonium formate and 0.1% formic acid in ultrapure water (A) and 5 mM ammonium formate and 0.1% formic acid in methanol (B). A gradient elution program was used for the LC separation, which can be seen in Table 20.

Table 20. Gradient elution LC-MS programme for pesticide analysis on InfinityLab Poroshell 120 EC-C18 column

Time	**%A**	**%B**
0.00	65.00	35.00
6.00	15.00	85.00
18.00	12.50	87.00
20.00	10.00	90.00
21.00	0.00	100.00
22.00	0.00	100.00
23.00	65.00	35.00

Mass spectrometry analysis was performed in dynamic multiple reaction monitoring (dMRM) mode using both positive and negative modes. dMRM cycle times of 500 ms were used with dwell times set between 20 – 50 ms. Both MS1 (quadrupole 1) and MS2 (quadrupole 3) were set to unit mode for all analytes aside from the pyrethroids which were set to wide in order to enhance sensitivity. MRM optimization of each analyte was performed using the Agilent Optimizer software package as previously described (Agilent Technologies, Cheadle, UK). MS source parameters for this method were optimized using the Agilent Source Optimizer software package (Agilent Technologies, Cheadle, UK), with final source conditions shown in Table 21.

Table 21. Optimized source conditions for reverse phase (C18) column pesticide analysis

Gas Temperature (°C)		230
Gas Flow (l/min)		4
Nebulizer (psi)		20
Sheath Gas Flow (l/min)		12
Sheath Gas Temperature (°C)		400
Capillary Voltage (V)	Positive	3500
	Negative	3000
Nozzle Voltage (V)	Positive	500
	Negative	1500

2.2.4.2.3 Method Performance

Examination of method performance was examined as previously with the Watch List work. A composite of 24 water samples for each of the three matrices from the two sites relating to Chapter 4 was prepared for assessment of the analytical method performance. Experiments were performed using a composite of each sample matrix by adding the required concentrations of the compounds before extraction, the calibration ranged from 1 – 1000 ng L^{-1}. Performance of the method was examined in WWTP influent, effluent and receiving waters.

For grab samples relating to Chapter 5, only assessment of matrix effects were required for this study as method performance in river water had been assessed previously. A composite of grab samples from all times and sites (n=24) was prepared to assess this in the same way as outlined above. For passive sampling, only assessment of the C18 disk extraction recoveries was executed due to all other experimental aspects being previously examined in the literature. To assess recovery, disks were spiked in triplicate with known analyte concentrations and put through the extraction procedure. Peak area ratios of the extracted disks were then compared to that of un-extracted standards of the same concentration.

2.2.4.3 HILIC Liquid Chromatography

2.2.4.3.1 Instrumentation Conditions

Liquid chromatography was performed using an Agilent HPLC stack equipped with a 1290 Infinity II LC multisampler, binary pump and multiple column thermostatted compartment (Agilent, Cheadle, UK). Chromatographic separation was achieved using a Shodex 150 x 2mm, 5 µm particle size HILICpak VT-50 2D column with corresponding guard (Apex Scientific Ltd, Kildare, Ireland). Mobile phases consisted of 50 mM ammonium bicarbonate (A) and acetonitrile (B). A gradient elution program was used for the LC separation, which can be seen in Table 22. The injection volume was 30 µL and the flow rate was 0.1 mL min^{-1}.

Table 22. Gradient elution programme for HILIC pesticides method

Time	% A	% B
0.00	80	20
7.50	100	100
15.00	100	100
17.00	80	20

Mass spectrometry was performed in multiple reaction monitoring (MRM) mode using negative mode. Both MS1 (quadrupole 1) and MS2 (quadrupole 3) were set to unit mode for all. MRM optimization of each analyte was performed using the Agilent Optimizer software package (Agilent Technologies, Cheadle, UK). MS QQQ conditions are summarised in the results section of this chapter.

2.2.4.3.2 Method Performance

The composites of influent, effluent and receiving waters relating to Chapter 4 were used to assess this direct injection method. Calibration was performed in the composite sample matrix by adding the required concentrations of the compounds, the calibration ranged from 10 – 1000 ng mL^{-1}.

2.2.5 Calculation of Analyte Concentrations, Occurrence Frequency and Risk Quotient

Analyte occurrences were calculated using the matrix matched calibration lines produced for each analyte (Equation 3).

$$y = mx + c$$

Equation 3. Equation of the line produced from matrix - matched calibration curves used to calculate analyte concentrations

Where y is the measured peak area ratio of an analyte normalised to internal standard, m is the slope of the regression, c is the y intercept and x is the concentration of the analyte in the given sample.

Occurrence frequency for analytes was calculated using the following formula:

$$Percentage\ frequency = \left(\frac{Number\ of\ analyte\ detections}{Number\ of\ samples}\right) x\ 100$$

Equation 4. Calculation used to determine analyte occurrence frequency in surface water samples

Where the number of analyte detections is any detection above the detection limit, and the number of samples is to the amount of samples analysed for a given Watch List.

In order to assess the risk posed on the Irish aquatic environment, the risk quotient (RQ) was calculated for all detected compounds based on the Predicted No-Effect Concentrations (PNEC) for any analytical detections. PNEC values were taken from the values indicated on the relative Watch Lists [51,276]. For unquantifiable detections (i.e. <LOQ), two scenarios were examined, one where the MEC was assumed to be half of the method LOQ value to obtain a worst case scenario, and one where the MEC was assumed to be the method LOD value to obtain a best case scenario. [277]

RQ was estimated as the ratio between the measured environmental concentrations (MEC) and the PNEC values [256], shown in equation 5:

$$RQ = \frac{MEC}{PNEC}$$

Equation 5. Formula for the calculation of Risk Quotient (RQ) to determine risk posed by environmental detections

Resulting RQ values were classified as follows:

$$\text{Low Risk} = \text{RQ} < 0.1$$

$$\text{Moderate Risk} = 0.1 < \text{RQ} < 1$$

$$\text{High Risk} = \text{RQ} > 1$$

2.3 Results and Discussion

2.3.1 Watch List Analysis

2.3.1.1 2nd Watch List

2.3.1.1.1 Optimization of SPE

Oasis HLB cartridges were selected due to the broad range of Log K_{ow} values they are amenable to. The SPE method used for the 2018/19 samples was developed to be applicable to a variety of analytes for initial method development. Further method optimization was completed following this initial analysis. Recovery was aided by the addition of 0.1M EDTA for a final concentration of 0.1% which was included based on enhancements observed in the method used by Mirzaei *et al.* [278]. This was found to improve extraction recoveries for amoxicillin from 54% in the 2018/19 analysis to 70% in the September 2020 analysis. Full summary of recovery results can be found in the method performance section of this chapter. The addition of formic acid to the aqueous condition and wash stages was adapted from the method used by Kasprzyk-Hordern *et al*. also aided in the improvement of amoxicillin recovery [39].

2.3.1.1.2 Optimization of Chromatography

Analytes were first measured using a simple gradient to assess retention time. This preliminary chromatogram included many compounds co-eluting (Figure 15), therefore an adjustment of the LC method was needed to improve their separation.

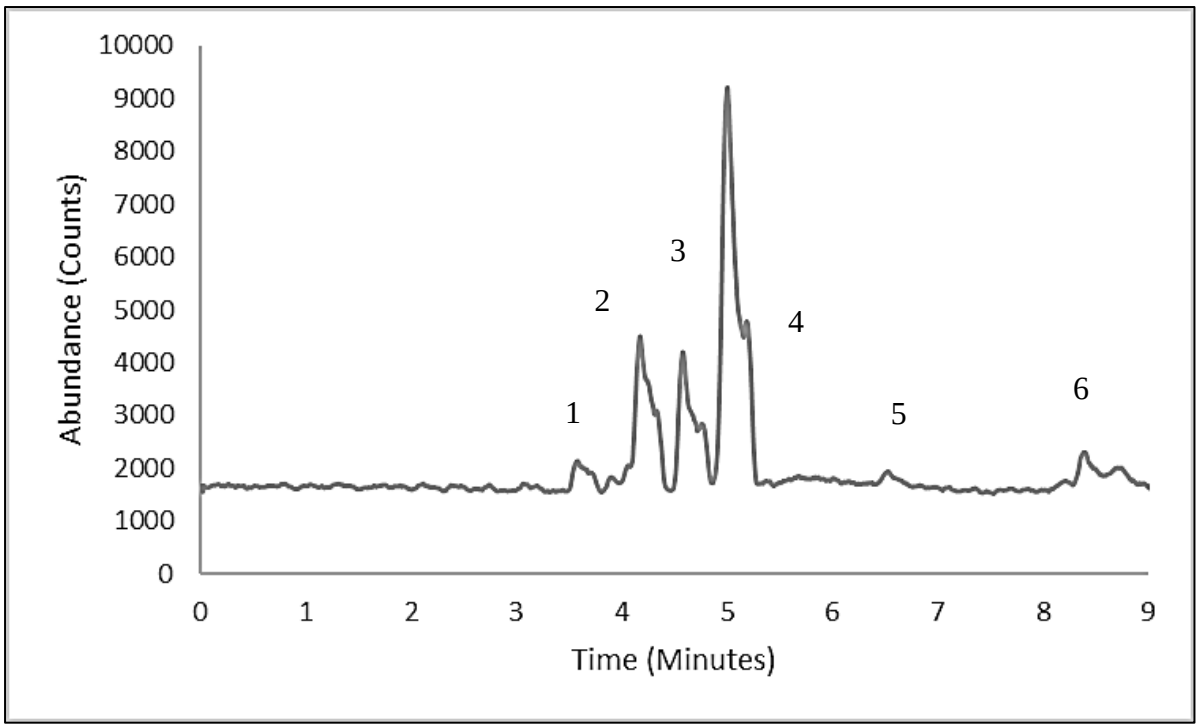


Figure 15. Total ion count (TIC) chromatogram of early method development separation of Watch List chemicals showing multiple co-elutions. 1) Thiamethoxam and ciprofloxacin 2) acetamiprid, imidacloprid and erythromycin 3) thiacloprid 4) azithromycin and clarithromycin 5) methiocarb 6) metaflumizone. (C18 column, 0.6mL/min flow rate, gradient elution of 10% B – 100%B 0-8 min, hold at 100% b for 1, return to start conditions at 9 min. A-10mM AA IN H2O B-ACN)

To improve separation and signal of the analyte mix, multiple mobile phases were trialled. Initially 10 mM ammonium acetate in deionised water was trialled as mobile phase A with acetonitrile as phase B. This method allowed for partial separation of the suite of analytes however the signal was relatively low. Investigation of the use of 0.1 % (v/v) formic acid in deionised water as mobile phase A showed higher signal and so was selected as the aqueous mobile phase going forward. Reduction of flow rate from 0.6 mL/min down to 0.4 mL/min also improved separation. This difference in signal between the two phases can be seen in Figure 16. Formic acid is a mobile phase buffer known to be amenable to a broad range of analytes including many of those included in the watch list [279].

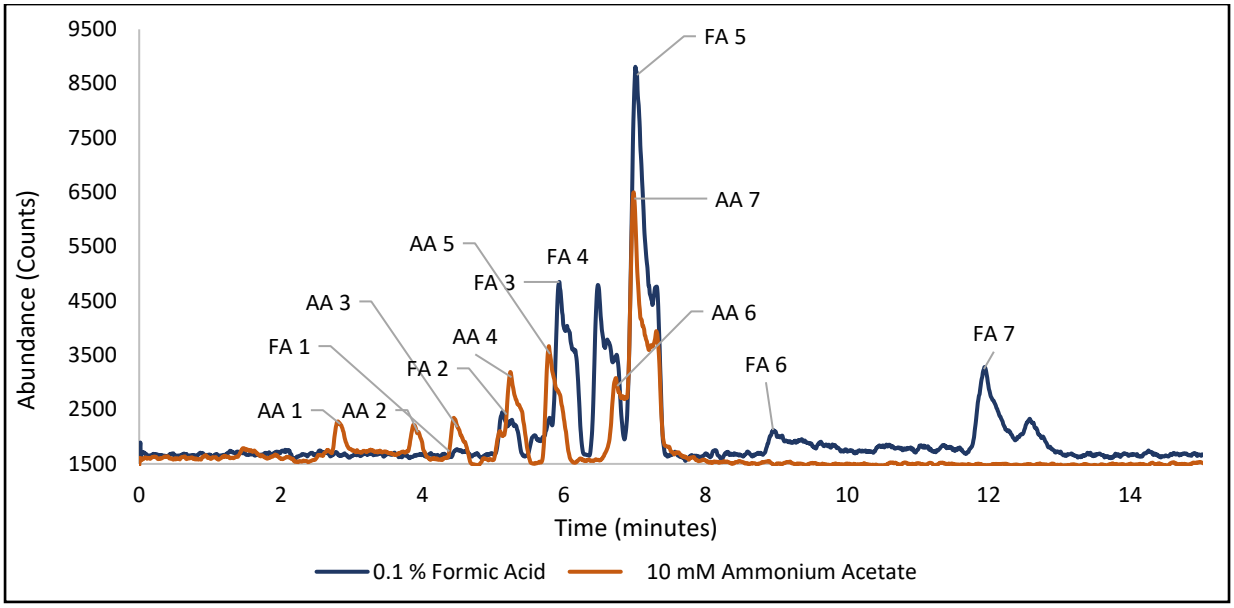


Figure 16. Total ion count (TIC) chromatogram showing difference in overall signal produced by two different mobile phase buffers. FA = formic acid, AA = ammonium acetate. Analyte key FA: 1) ciprofloxacin 2) thiamethoxam 3) acetamiprid, clothianidin and imidacloprid 4) erythromycin and thiacloprid 5) azithromycin and clarithromycin 6) methiocarb 7) metaflumizone. Analyte key AH: 1) amoxicillin 2) ciprofloxacin 3) thiamethoxam 4) acetamiprid, clothianidin and imidacloprid 5) thiacloprid 6) erythromycin 7) azithromycin and clarithromycin. Note additional compounds methiocarb and metaflumizone were added for the FA run. (C18 column, 0.4mL min^{-1} flow rate, gradient elution of 10% B – 100%B 0-8 min, hold at 100% b for 1, return to start conditions at 9 min)

However, azithromycin and clarithromycin were unable to fully separated, but these two compounds can be differentiated by their MS transitions, so this co-elution was deemed acceptable. After the optimisation of the gradient, the gradient was then held at 100 % organic to ensure all compounds had eluted from the column avoiding possible carryover. Time windows were used to ensure retention time reproducibility. The final optimised separation is shown in Figure 17.

Basic mobile phases for analytes monitored in negative mode are quite common. Ammonium additives, such as hydroxide and fluoride, have been previously used for these type of analytes, resulting in sensitivity enhancement with low LOQs achieved [280]. Based on previous work done in our lab, ammonium fluoride additive responses presented higher signals overall, compared to hydroxide. Therefore, ammonium fluoride was selected as the aqueous phase for the estrogens which were monitored in negative mode. Estrogen hormones present similar chemistries, so their elution is expected to be at close retention times or even co-elute. Due to the low LODs required for the study, to achieve higher sensitivities, a full separation was required. This will lead to increased dwell time values when using MRM mode. If

transitions do not overlap over time, responses of the analytes will be higher. Therefore, they were eluted using a gradient elution in order to achieve improved separation. A flow rate of 0.35 mL.min^{-1} using the gradient provided enough separation as seen in the chromatogram in Figure 17. The resultant separation allowed a retention window of 0.5 min between analytes without the overlap of MRM transitions, leading to higher signals increasing sensitivity even further.

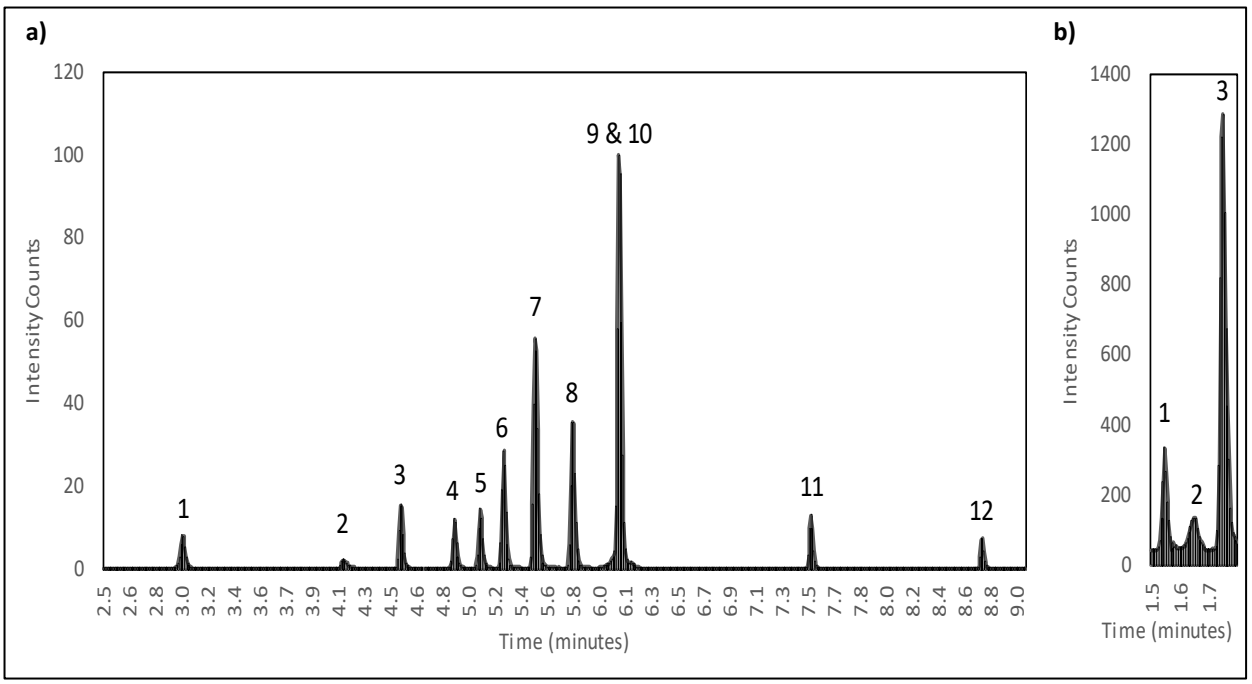


Figure 17 Chromatogram of the final separation of 2nd watch list chemicals acquired is MRM mode using C18 column, 0.4min/mL flow, gradient elution of aq (A) AND ACN (B). Analytes key (A): (1) amoxicillin, (2) ciprofloxacin, (3) thiamethoxam, (4) clothianidin, (5) imidacloprid, (6) acetamiprid, (7) erythromycin, (8) thiacloprid, (9 & 10) clarithromycin and azithromycin, (11) methiocarb, (12) metaflumizone, at a concentration of 2000 ng L $^{-1}$; and B)) analytes key: (1) 17-beta-estradiol, (2) 17-alpha-ethinylestradiol, (3) estrone at a concentration of 100 ng L $^{-1}$

2.3.1.1.3 Optimization of Mass Spectrometry

Initial mass spectra for analytes were produced in MS2 scan mode to assess for potential product ions. Examples of these can be seen in Figures 18 and 19. Optimization of the MS conditions was aided significantly by the Agilent 'Masshunter Optimizer' software. Identification of optimal analysis polarity, product ions, fragmentor voltage and collision energies were selected by using this software package. Optimizer was run for all compounds

in both positive and negative polarities. The software was programmed to search for product ions generated from a precursor ion of $[M+H]^+$ or $[M-H]^-$ for each analyte. A low mass cut off of 40 *m/z* was used for the optimization. Fragmentor voltages were varied in the range of 0 to 180 V in increments of 5 V. Collision energies were varied in the range of 0-50 V. Following the use of the optimizer software, variables still needing to be optimized were MS resolution (MSRes), dwell times, electron multiplier voltage (EMV) and cell accelerator voltage (CAV). Optimized transitions can be seen in Table 23.

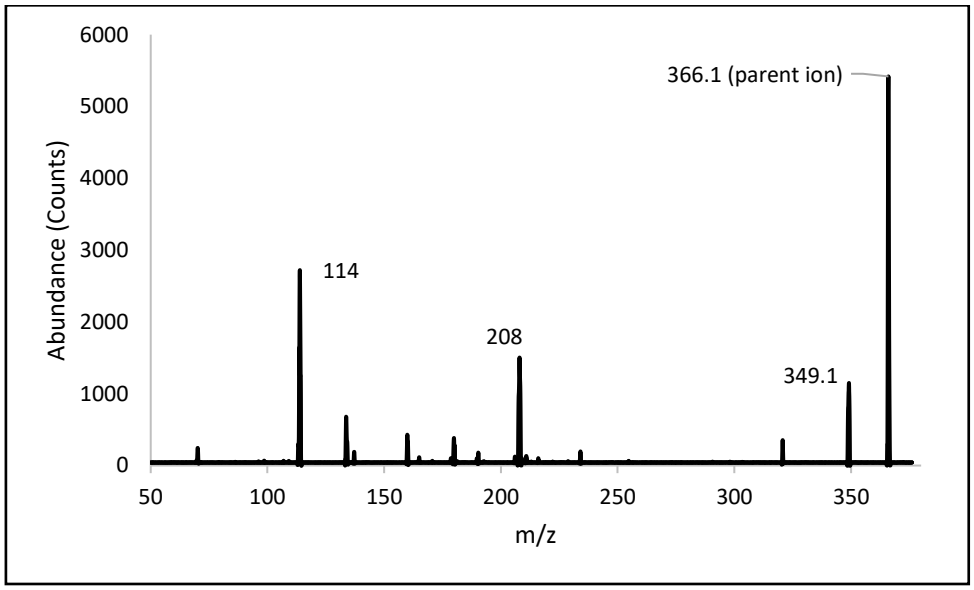


Figure 18 Mass spectrum of amoxicillin (50 ppb standard in ultrapure water) produced by direct infusion onto QQQ MS in scan mode set to scan over m/z ranges 50 – 370.

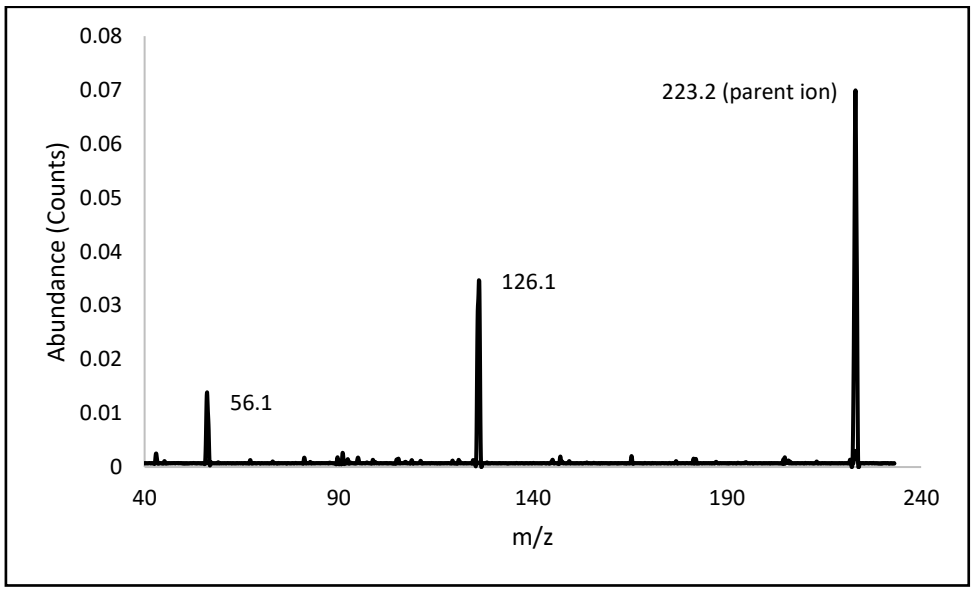


Figure 19 Mass spectrum of acetamiprid (50ppb standard in methanol) produced by direct infusion onto QQQ MS in scan mode set to scan over m/z ranges 40 – 230

Table 23 Table of optimized multiple reaction monitoring (MRM) conditions for 2nd Watch List Substances

Compound Name	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Azithromycin-d_3	752.53	Wide	158.1	Wide	35	205	45	4	Positive
Azithromycin	749.51	Wide	591.4	Wide	35	215	33	4	Positive
Azithromycin	749.51	Wide	158.1	Wide	35	215	45	4	Positive
Clarithromycin	748.48	Wide	590.4	Wide	125	165	17	4	Positive
Clarithromycin	748.48	Wide	158.1	Wide	125	165	33	4	Positive
Erythromycin	734.46	Wide	576.4	Wide	50	145	17	4	Positive
Erythromycin	734.46	Wide	158.1	Wide	50	145	29	4	Positive
Metaflumizone	505.12	Wide	302.1	Wide	125	175	21	4	Negative
Metaflumizone	505.12	Wide	285	Wide	125	175	53	4	Negative
Amoxicillin	366.1	Wide	349.1	Wide	125	80	5	4	Positive
Amoxicillin	366.1	Wide	114	Wide	125	80	21	4	Positive
Amoxicillin	366.1	Wide	208	Wide	125	80	17	4	Positive
Ciprofloxacin	332.13	Wide	288.4	Wide	125	150	17	4	Positive
Ciprofloxacin	332.13	Wide	231.2	Wide	125	150	41	4	Positive

Thiamethoxam-d_3	295	Wide	214	Wide	80	108	20	4	Positive
Thiamethoxam	292.02	Wide	211.1	Wide	80	84	12	4	Positive
Thiamethoxam	292.02	Wide	181	Wide	80	84	24	4	Positive
Imidacloprid-d_4	260	Wide	213	Wide	80	108	20	4	Positive
Thiacloprid-d_4	257.06	Wide	125.8	Wide	50	108	30	4	Positive
Imidacloprid	256.2	Wide	209	Wide	80	84	20	4	Positive
Imidacloprid	256.2	Wide	175.2	Wide	80	84	20	4	Positive
Clothianidin-d_3	253	Wide	172	Wide	35	108	20	4	Positive
Thiacloprid	253	Wide	125.8	Wide	50	114	24	4	Positive
Thiacloprid	253	Wide	90	Wide	50	114	50	4	Positive
Clothianidin	250	Wide	169	Wide	35	84	12	4	Positive
Clothianidin	250	Wide	132	Wide	35	84	12	4	Positive
Methiocarb-d_3	229.1	Wide	169.1	Wide	80	108	20	4	Positive
Methiocarb	226.1	Wide	169.1	Wide	80	108	8	4	Positive
Methiocarb	226.1	Wide	121.1	Wide	80	108	8	4	Positive
Acetamiprid-d_3	226	Wide	126	Wide	35	108	30	4	Positive
Acetamiprid	223.2	Wide	126.1	Wide	80	84	20	4	Positive
Acetamiprid	223.2	Wide	56.1	Wide	80	84	20	4	Positive

Azithromycin-d_3	752.53	Wide	594.4	Wide	35	205	33	4	Positive
17-β-estradiol	271.1	Wide	183	Wide	50	155	49	4	Negative
17-β-estradiol	271.1	Wide	145	Wide	50	155	45	4	Negative
17-α-ethinylestradiol	295.17	Wide	267.1	Wide	50	185	29	4	Negative
17-α-ethinylestradiol	295.17	Wide	145	Wide	50	185	45	4	Negative
Estrone	269.2	Wide	145	Wide	50	165	45	4	Negative
Estrone	269.2	Wide	143	Wide	50	165	60	4	Negative

To assess the impact of MSRes on compound sensitivity, MSRes was varied for both mass scanning quadrupoles (MS1 and MS2). Nine separations were performed on a mixture of all test compounds using differing MSRes conditions on each of MS1 and MS2, either unit, wide, or widest resolution modes which correspond to 0.7 u, 1.2 u and 2.5 u mass resolutions respectively. Conditions which proved to be most favourable were using wide on both MS1 and MS2. Although widest did show enhanced signal compared to the other settings, the trade-off for mass accuracy was too significant to use widest for the final method as there were a number of MRM transitions which were within 2.5 mass units of each other. Retention time windows were set up in the method in order to optimize the dwell times over the span of the run. Dwell times were chosen in order to allow for approximately 500 cycles/sec during each window, depending on the number of analytes which eluted within each window. A number of EMV's were trialled in order to yield optimum sensitivity, either 0, 100 or 200 were trialled for both positive and negative polarities. An EMV of 0 for negative and 200 for positive proved to be the optimum values. Finally, the CAV was investigated by running a standard mix at 3 different CAVs; 2, 4 and 7. CAV is the voltage applied to the collision cell to increase drifting velocity of ions traversing the collision cell [28]. It is an important parameter when it comes to MS optimization as it alters the accumulation time of ions in the collision cell [281]. However, there was minimal difference seen between CAVs and so 4 was chosen to be implemented into the final method, which resulted in the chromatograms and MRM spectra of each target analyte as shown in Figure 20.

Figure 20 MRM chromatograms of 2nd watch list chemicals at either 3 or 10 ng L (lowest calibration standard) in extracted matrix, run using C18 column, 0.4min/mL flow, gradient elution of (A) and (B).

2.3.1.1.4 Evaluation of Method Performance

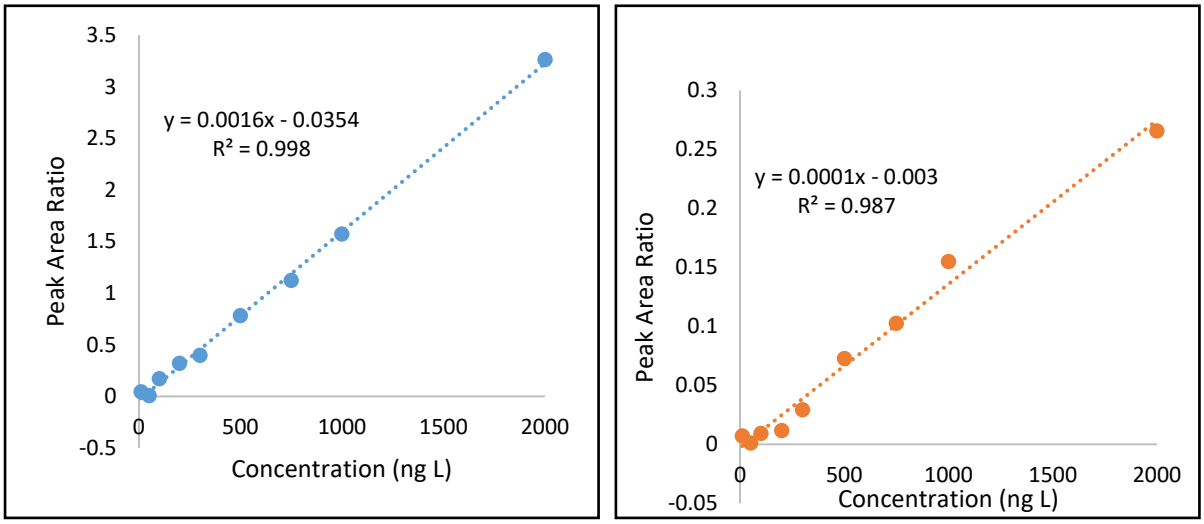


Figure 21 Example of a matrix - matched calibration curves of analytes imidacloprid (left) and erythromycin (right) over the range 10-2000 ng L^{-1} acquired in MRM mode using C18 column, 0.4min/mL flow, gradient elution of (A) AND (B)

Calibration curves were prepared by spiking a composite sample matrix prior to extraction. Examples of matrix matched calibration curves can be seen in Figure 21. For calibration lines in 2018/19, a minimum of 5 points were used except for ciprofloxacin. A calibration of only 4 points was used for this analyte so only semi-quantification was achieved. Metaflumizone and thiamethoxam had no linear regression using a matrix-match calibration line in the 2018/19 analysis, therefore they were prepared as an external calibration line in a 90:10 (v, v) H_2O: ACN reconstitution solvent in order to calculate concentrations in the samples. Issues with quantitation of metaflumizone are likely due to its low recovery achieved of just 10 % (Table 24). Recoveries varied from 10 to 203 % depending on the analyte. However, this was not possible to calculate for ciprofloxacin at the same concentration as it was not detected at 500 ng L^{-1} (meaning 5 ng L^{-1} before SPE concentration factor), and so higher concentrations were used for further analysis.

Further optimization was performed to remedy these issues. The addition of 0.1M EDTA to the samples in 2020 improved the extraction efficiency of ciprofloxacin, and so a matrix-matched calibration curve with ≥ 5 points was achieved. Recovery for ciprofloxacin was 136% in the October 2020 analysis. The average linearity for all analytes across both sampling campaigns was R^2 <0.99. It was found that metaflumizone had been lost during the dry down stage of SPE and so a matrix pre-spike linear regression was achieved by injection of the SPE

eluent before dry down. This enabled an R^2 of 0.9563, a significant improvement from the previous results. Recovery for metaflumizone using this direct eluent method was significantly improved, resulting in a calculated recovery of 104.25%. A 50 µL volume of eluent was taken, of which 20 µL was injected onto the instrument. As the solvent volume was so small, any solvent evaporation would have a substantial effect on the final concentration. In an effort to pre-emptively mitigate solvent loss, a shortened version of the full LC separation was then used where only the gradient was altered in order to elute metaflumizone from the column faster, meaning less time for the sample in the auto sampler between replicates. This gradient began at 70% B, changing to 100% B at 3 min, holding at 100% B for 1 min and finally returning to 70% B at 5 min. Metaflumizone retention time with this gradient was 3.5 min. All other method parameters remained the same as the full separation method. A chromatogram showing the difference in overall signal between this compound pre and post dry down procedure can be seen in Figure 22.

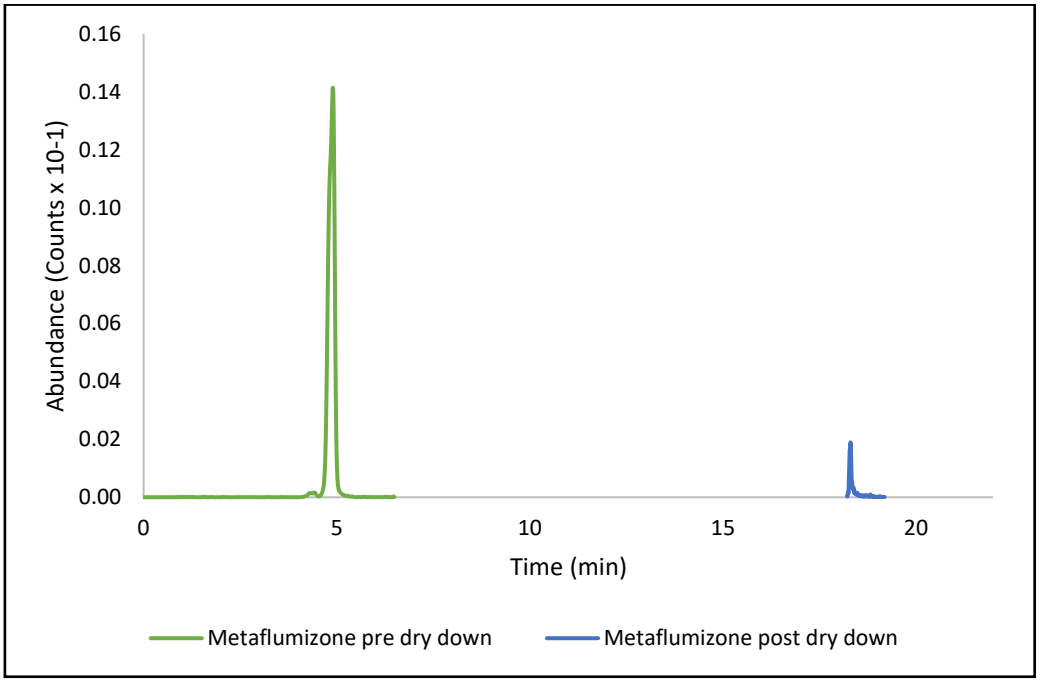


Figure 22. Chromatogram showing metaflumizone pre and post dry down in SPE of 500 ng L^{-1} spiked matrix sample, pre dry down was run with shortened metaflumizone method and post dry down with full mix method causing the difference in RT.

In 2020, thiamethoxam did not exhibit the same matrix effects as previously encountered (652.1%), and the matrix-matched regression achieved during this batch of analysis was

significantly improved with an R^2 of 0.9953. Therefore, we postulate the matrix effects were possibly the cause of the poor regression seen in the earlier batch.

Matrix effects were seen across all analytes in 2018/19, ranging from 10.9% (Erythromycin) to 652.1 % (Thiamethoxam). Values over 100% will correspond to ion enhancement on the MS while values under 100% relate to ion suppression on the instrument. Thiamethoxam presented a very high value that can correlate to the high recovery achieved, this could be due to a really high enhancement caused by a change in the ionization efficiency in the MS [282] that will not happen in solution, therefore being linear in solvent in 2018/19. Due to this, concentrations of thiamethoxam stated for the 2018/19 samples are just a reference as quantification could not be matrix matched.

Significant matrix effects were seen in the 2020 samples, particularly for the antibiotic class of compounds, with amoxicillin exhibiting over 4000% enhancement. Matrix effects are commonly caused by changes in the ionization efficiency of selected analytes due to the co-elution of other substances present in the matrix, and is observed particularly when using ESI [282]. A study conducted by Hong *et al*. found both 'obvious signal enhancement and suppression' for a number of analytes including macrolide antibiotics [283]. The 2020 samples were run and validated in 2 separate analyses, September, and October, with the analysis of the antibiotics and estrogens from the September samples repeated along with the October batches. During 2020 sample collection, field measurements of temperature, dissolved oxygen (DO), turbidity and pH were taken, results of which can be seen in Chapter 3. However it is worth noting at this stage that the turbidity measurements ranged from 0.61-4.48 FNU for all samples taken in September and October with the exception of one, the October River Nore sample. This sample had a turbidity of 21.75 FNU, significantly higher than previously found in the same river. Due to the validations being performed in a composite matrix, this high turbidity is suggested to have had an influence on the matrix effects seen in the 2020 samples, particularly the antibiotics [283,284]. Matrix interferences are more often seen in complicated matrices, and higher turbidity implies the presence of more contaminating substances present in the water sample, leading to a more complicated matrix.

LODs were compared to target values specified in the WL, all the analytes in Method 1 achieved LODs below the target value. However, it was not possible to achieve the targeted LODs for the estrogen analysis. Accurate analysis of these compounds are known to be challenging [285]. This is due to their low concentrations and their rapid degradation. Sensitivity of these compounds depends on different parameters such as the extraction technique, the type of source for analysis, etc. In this study, every effort was taken when developing every step of the method to increase sensitivity. One parameter that was not possible in this study that couple potentially increase sensitivity further is sample SPE volume. If larger volumes are loaded on the SPE cartridge the concentration factor increases which in turn should give higher sensitivity. Although it can be hypothesised that analysing greater volumes of initial sample could result in an increase in sensitivity, the LODs achieved here are comparable to other research which was carried out utilising greater volumes, and so this was not pursued further due to the limited sample volume, and higher preparation and potential degradation times that would accrue. Nevertheless, other studies propose the use of even 1 L, but this leads to higher analysis times, more labour-intensive sampling campaigns and more complex extractions. A compromise between matrix interferences and loading volume will therefore need to be considered, as the mass spectrometer source can be damaged by those matrix interferences [286]. Consequently, LODs and LOQs acquired using only a 100 mL of volume are within the range of other studies using greater volumes and therefore considered acceptable. Validation results including recovery, matrix effect, linearity, LOD and LOQ can be found in Table 24.

Table 24, Table of Calibration and Validation Results for 2nd Watch List Analysis [a]Semi-quantification data as 4 calibration points only [b]Based on an external calibration line prepared in reconstitution solvent [c]Target limit of detection taken from WL substances 05/06/2018 - Value not obtained due to low concentration chosen

	Method LOD ng L^{-1}			**Method LOQ ng L^{-1}**			**Target LOD[c] ng L^{-1}**	**Recovery (%±SD %**			**Matrix Effect (%)**			**Regression**		
	2018/19	Sep-20	Oct-20	2018/19	Sep-20	Oct-20	All	2018/19	Sep-20	Oct-20	2018/19	Sep-20	Oct-20	2018/19	Sep-20	Oct-20
Estradiol	0.977	22.9	22.9	2.96	69.43	69.43	0.03	94 ± 97	175 ± 21	175 ± 21	1846	89	89	0.9868	0.996	0.996
Ethinylestradiol	0.766	41.4	41.4	2.32	125.47	125.47	0.03	192 ± 121	315 ± 7	315 ± 7	4894	4894	4894	0.9939	0.9964	0.9964
Estrone	2.92	1.28	1.28	8.85	3.9	3.9	0.03	35 ± 108	18 ± 21	18 ± 21	-4718	104	104	0.9876	0.9617	0.9617
Acetamiprid	1.2	0.66	0.78	3.7	2.01	2.35	8.3	121 ± 3	105 ± 1	105 ± 1	105	98	99	0.9991	0.9995	0.993
Amoxicillin	22	3.52	8.49	66	10.68	25.72	78	54 ± 39	70 ± 24	26 ± 28	-	1645	**4239**	0.9902	0.9886	0.9534
Azithromycin	5.9	8.48	8.48	18	25.68	25.68	19	100 ± 9	99 ± 3	99 ± 3	1283	722	722	0.9986	0.9867	0.9867
Ciprofloxacin	43	5.46	8.83	130	16.55	26.75	89	-	56 ± 12	137[a]	-	102	75	0.9749[a]	0.9912	0.9247
Clarithromycin	5.3	8.69	8.69	16	26.34	26.34	19	91 ± 32	97 ± 1	97 ± 1	209	786	786	0.9987	0.9821	0.9821
Clothianidin	3.1	1.29	2.55	9.3	3.92	7.72	8.3	132 ± 8	106 ± 7	108 ± 2	120	108	97	0.9944	0.9982	0.994

Erythromycin	6.8	7.96	3.5	21	24.13	10.6	19	97 ± 36	79 ± 43	23 ± 6	10.9	251	18	0.9971	0.9636	0.987
Imidacloprid	3.3	1.8	1.36	10	5.44	4.14	8.3	117 ± 4	98.1 ± 1	106 ± 2	139	116	106	0.9947	0.9965	0.998
Metaflumizone	9 [b]	14.89	14.89	27	45.12	45.12	65	10 ± 57	104 ± 24	104 ± 24	12.4	35	35	0.9627 [b]	0.9563	0.9563
Methiocarb	1	4.04	2.03	3	12.2	6.1	2	119 ± 1	103 ± 19	87 ± 0.4	100	294	254.28	0.9994	0.9827	0.9956
Thiacloprid	1.3	0.72	1.07	4	2.2	3.2	8.3	119 ± 2	99 ± 2	105 ± 3	100	92	104	0.999	0.9994	0.9989
Thiamethoxam	0.8 [b]	1.38	2.05	2.4 [b]	4.18	6.21	8.3	203 ± 15	111 ± 7	116 ± 9	652	135	95	1 [b]	0.998	0.9953

2.3.1.1.5 3rd Watch List

2.3.1.1.5.1 Optimization of Solid Phase Extraction

All SPE method experiments were performed using Oasis HLB cartridges due to their broad applicability to a range of analytes. The first stages of extraction optimization had to take place without 2 compounds (famoxadone and imazalil) as they were not available for initial experiments. In order to investigate if lower LODs were possible by loading more sample onto the cartridge, a volume of 500 mL was initially trialled. Therefore increasing the SPE concentration factor from 100x to 500x. The sample was prepared using 500 mL DI water acidified to pH 3 with sulphuric acid, with the addition of 250 µL of 0.1 M EDTA. The sample was spiked with analyte mix to give a final concentration of 1000 ng L^{-1} following full SPE procedure, and with internal standard mix to give a final concentration of 500 ng L^{-1} ('Sample').

Initially four separate SPE methods were trialled as shown in Figure 23. Method one was selected to be trialled based off Chitescu *et al.* [287] and Method 2 from Casado *et al* [288] as both of these papers investigated at least some of the compounds included in this study. Method 3 was the method used for the 2018-19 set of samples from chapter 2 of this thesis, and Method 4 was the further developed method used for the 2020 samples in that same chapter.

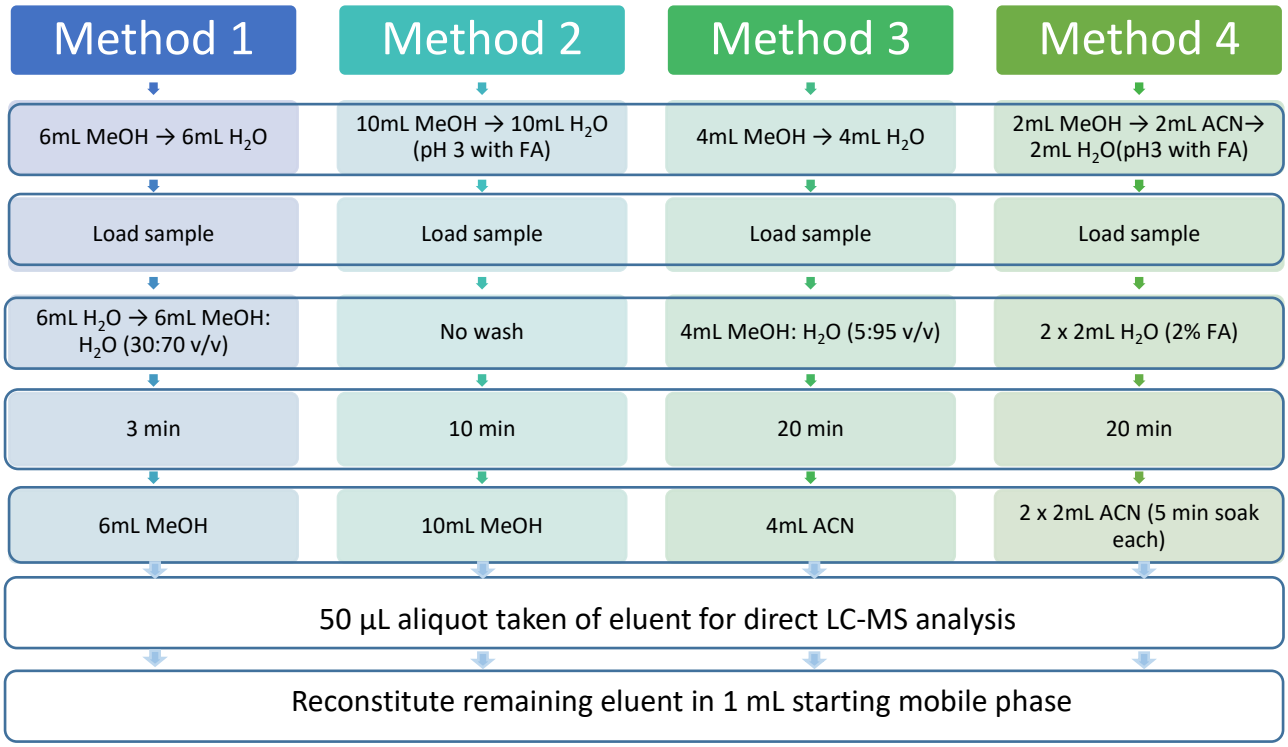


Figure 23. Procedures of four SPE methods trialled for 3rd Watch List compounds including cartridge conditioning, loading, washing and eluting steps.

Each method was performed in triplicate and were compared against triplicate samples spiked after the extraction procedure ('Recovery'). Recoveries were calculated by comparing the average peak area ratio of each compound from each method against the average peak area ratio from the recovery samples. These can be seen in Table 25.

Table 25 Percentage recovery results from the first round of SPE experiments for 3rd watch list compounds

Compound	Percentage Recovery Method 1 (%)	Percentage Recovery Method 2 (%)	Percentage Recovery Method 3 (%)	Percentage Recovery Method 4 (%)
Amoxicillin	0.01	0.01	0.03	0.06
Ciprofloxacin	50.42	255.34	72.10	129.76
Clotrimazole	40.69	17.47	9.96	10.54
Dimoxystrobin	115.21	102.47	26.23	59.77
Fluconazole	1306.50	1672.89	116.95	343.73
Ipconazole	15.09	19.02	3.46	26.13
Metconazole	55.44	45.70	51.80	50.16
Miconazole	2.24	2.17	5.02	3.05
o-desmethylvenlafaxine	39.76	63.91	86.16	54.45
Penconazol	1.04	0.42	1.27	0.76
Prochloraz	18.63	10.31	35.76	62.37
Sulfamethoxazole	2.72	2.41	16.77	19.17
Tebuconazole	47.78	54.51	22.41	27.54
Tetraconazole	751.98	667.00	132.35	231.20
Trimethoprim	38.27	59.06	135.44	295.42
Venlafaxine	10.78	15.29	62.24	84.09
Metaflumizone	27.56	12.90	98.90	134.82

Although varied, results from the first round of trials showed methods 3 and 4 had the most recoveries within or near to the acceptable considered range (50-130 %) for further study. Therefore 4 more methods based off methods 3 and 4 with some modifications were investigated further. Method details are found in Figure 24. The main parameters investigated were the strength of the wash step, the acidification of the DI water used throughout the extraction, and the inclusion of a soak in the elution step.

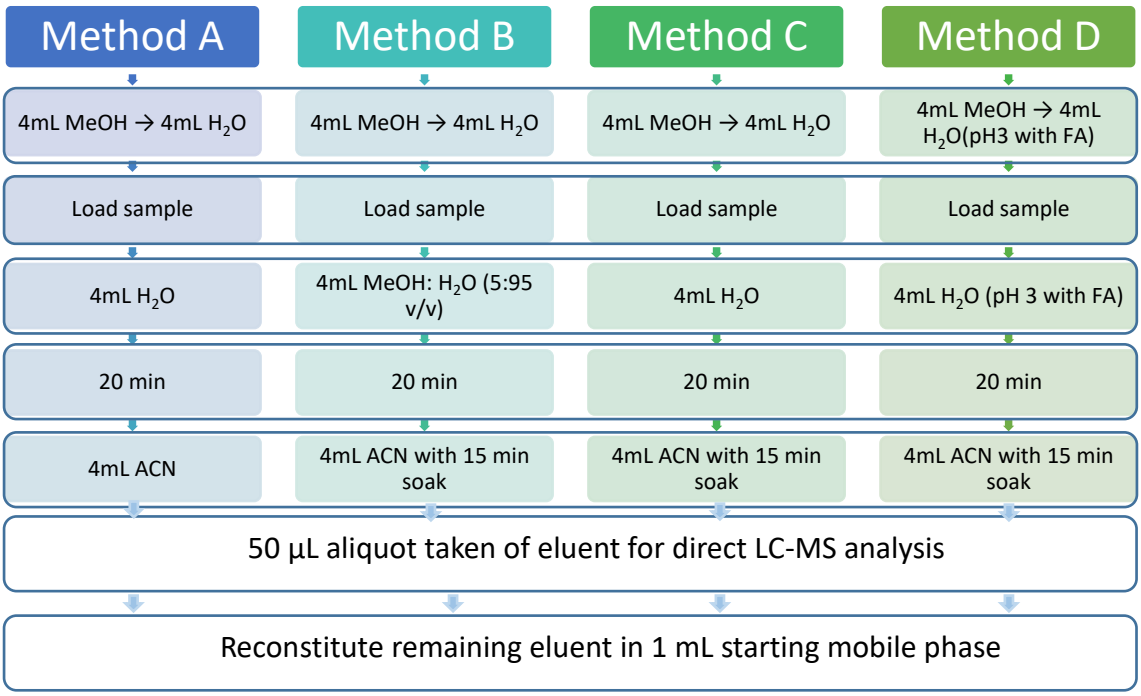


Figure 24. Flow chart of second round SPE trial methods for 3rd Watch List analysis showing steps of condition, wash, load and elute for each of the four procedures trialled.

Recoveries were calculated in the same manner as the previous round of trials and the results are as follows in Table 26.

Table 26 Results from the 2nd round of SPE trials for 3rd Watch List compounds

Compound	Percentage Recovery A (%)	Percentage Recovery B (%)	Percentage Recovery C (%)	Percentage Recovery D (%)
Amoxicillin	68.50	106.35	70.99	107.61
Trimethoprim *	233.65	296.97	204.71	1171.67
Ciprofloxacin	320.03	237.98	255.71	125.43
Dimoxystrobin	344.62	340.32	188.17	110.44
Fluconazole	39.30	44.80	35.19	79.26
Ipconazole	316.01	219.53	150.38	131.31
Metconazole	230.66	411.30	154.37	115.25
Miconazole	161.91	160.30	116.35	58.18
o-desmethylvenlafaxine	389.97	434.43	308.54	88.02
Penconazol	319.42	528.15	264.71	103.92
Prochloraz	395.33	445.10	300.26	100.91
Sulfamethoxazole	489.28	632.99	630.85	106.40
Tebuconazole	373.88	521.13	178.02	130.90
Tetraconazole	250.60	288.72	166.04	122.73
Clotrimazole	45.62	58.96	61.30	76.46
Venlafaxine	429.54	468.52	630.69	125.48
Metaflumizone	198.83	125.72	86.54	98.48

** A preparation error was made with this compound and so the concentration was out by a factor of 10.*

The final method selected was method D from round 2 of SPE trials as this method produced recoveries for all compounds within the acceptable range.

This method was then applied to the remaining missing compounds from the WL upon availability, as well as a repeat of trimethoprim using the correct concentration. All recoveries were within the accepted range, with results shown in Table 27.

Table 27 Results of additional SPE experiments for 3rd Watch List analytes

Compound	% Recovery
Famoxadone	104.32
Trimethoprim	110.03
Imazalil	112.99

Therefore, method D was selected as the final optimized SPE method to be applied to river water matrix.

2.3.1.1.6 Optimization of Separation

Based on literature methods [51,287–290] as well as the work described in the previous section, a mobile phase buffered with formic acid and ammonium formate was evaluated. Two different mobile phases containing differing concentrations of buffer were prepared, one containing 0.1 % FA with 5 mM ammonium formate, and the other containing 2 mm ammonium formate and 0.02 % FA. Higher sensitivity was observed with the 0.1%/ 5 mM phase as seen in Figure 25 and therefore this mobile phase additive was selected for further development. Note that the gradient had not been fully optimized at this stage and so some co-elutions were separated following mobile phase selection. Initial mobile phase B was ACN as used in the previous Watch List, however as one of the compounds formed ammonium adducts in the MS, and the ability to add ammonium formate buffer to the organic phase was also required for optimum sensitivity for this compound. Therefore methanol was chosen for the organic solvent, as ammonium formate is insoluble in ACN but soluble in methanol. The switch to methanol also required a drop in flow rate from 0.35 mL min^{-1} to 0.25 mL min^{-1} to allow for the increase in column backpressure.

Analytes were initially run using a simple gradient to assess retention time. This preliminary separation showed poor resolution and included multiple compounds either part or fully co-eluting, therefore an adjustment of the gradient was required to improve their separation.

The equation used to calculate separation factor α can be seen below (Eqn. 6), where t_{r2} and t_{r1} are the retention times for the analytes in question, and t_0 is the column void time which was determined to be 1.04 min for this method.

$$\alpha = \frac{t_{r2} - t_0}{t_{r1} - t_0}$$

Equation 6. Equation used to calculate separation factor α which measures the method's ability to distinguish between two peaks

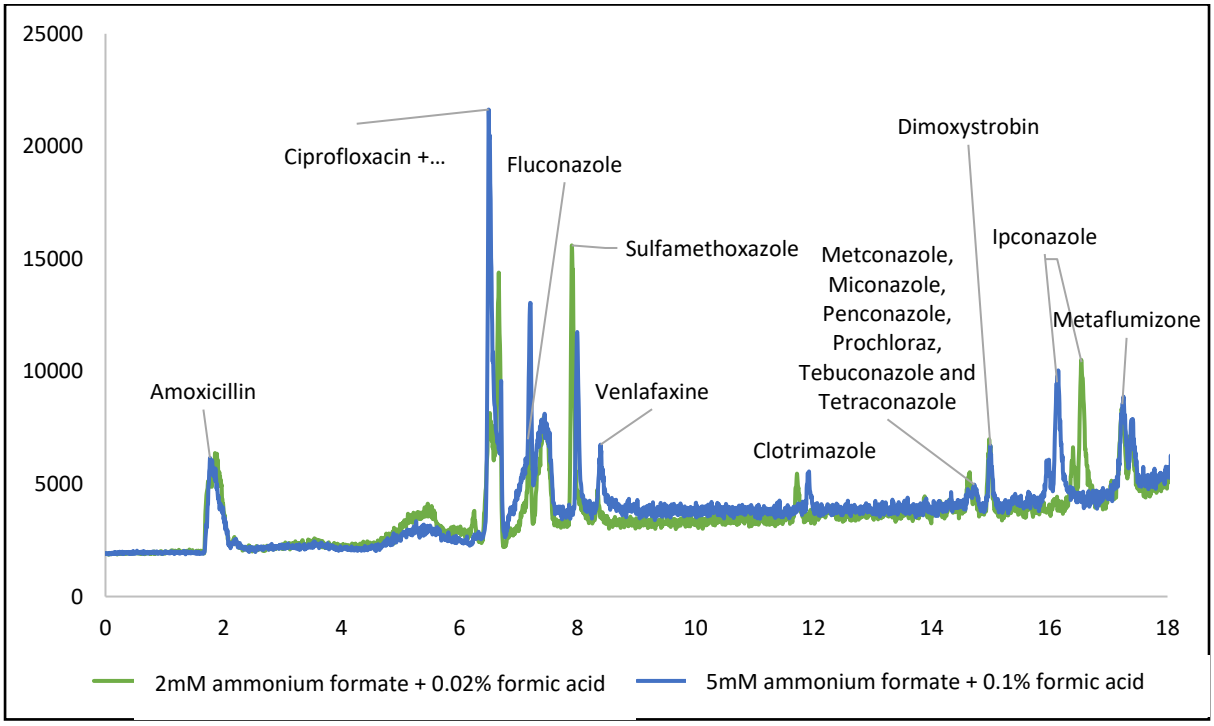


Figure 25. Chromatogram comparing two mobile phase additive concentrations (Acquired using C18 2.1 ✕ 150 mm, 1.9 µm column, 0.35 mL/min, 30uL injection volume, MS - MRM mode)

For example, the calculated separation factor between the two peaks for metconazole and miconazole was 0.997 (Figure 25). An acceptable separation factor is >1 with values ideally ranging from 2-5 [291], indicating that the method can accurately distinguish between the two

peaks. With adjustment of the gradient, the separation factor between these two compounds improved to 1.15 as can be seen in the final separation (Figure 26).

Some co-elutions between azole compounds were unable to be separated such as with prochloraz and penconazole, however due to monitoring of 2-3 different transitions, differentiation was possible in the mass spectrometer. After the optimisation of the gradient, the gradient was then held at 100 % organic to ensure all compounds had eluted from the column avoiding possible carryover. A chromatogram of the final separation achieved can be seen in Figure 26.

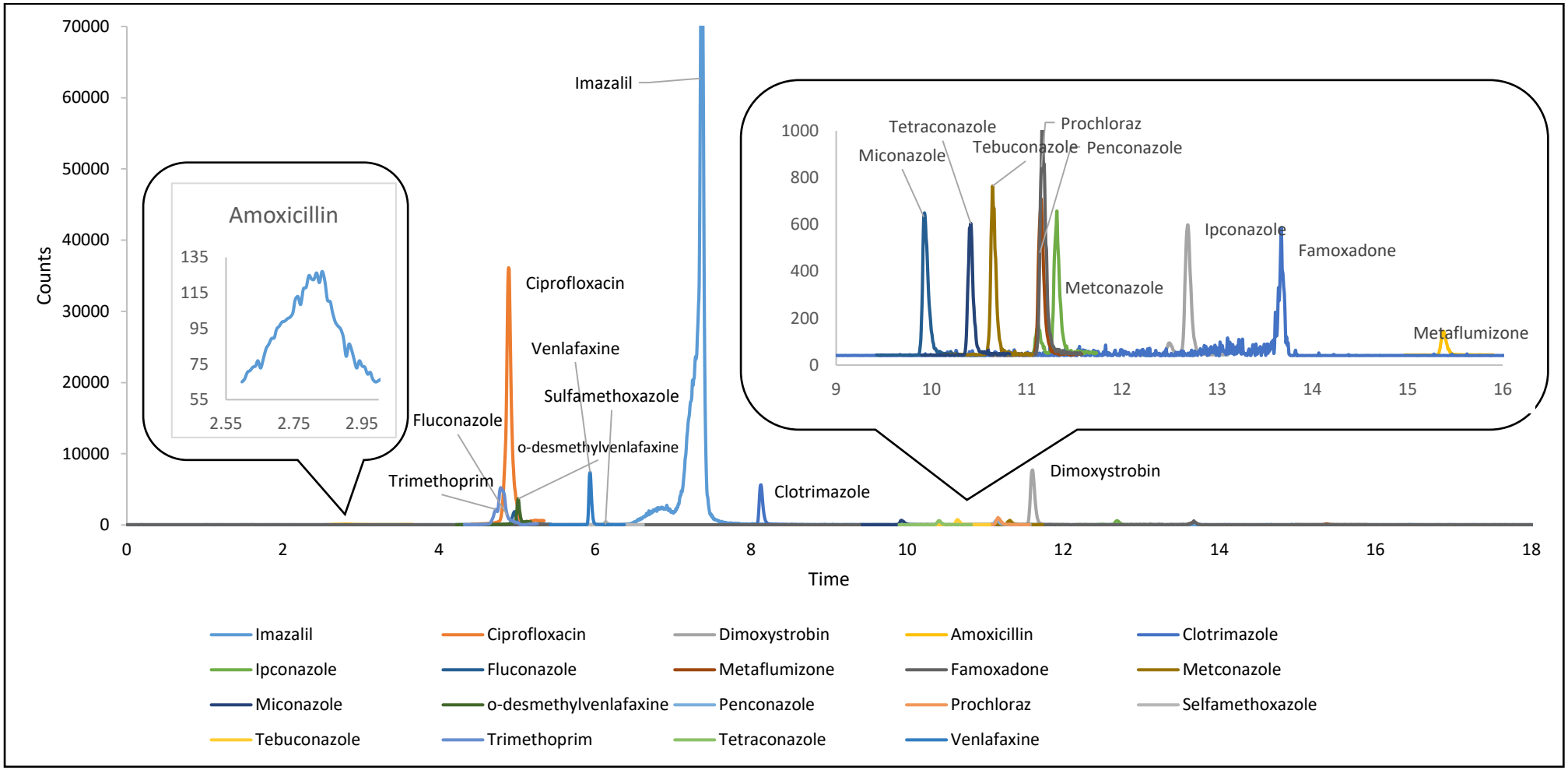


Figure 26. Final chromatographic separation of 3rd Watch List Chemicals, 100ng L^{-1} in solution (Acquired using C18 2.1 ✕ 150 mm, 1.9 µm column, 0.25 mL/min, 30ul injection volume, gradient elution of 0.1% FA + 5mm AF In H_2O (A) and MeOH (B), MS - MRM mode

2.3.1.1.7 Optimization of Mass Spectrometry

Analytes were run individually in MS2 scan mode in order to observe potential product ions. An example MS spectrum obtained can be seen in Figures 27-29. Optimization of the MS conditions was aided significantly by the Agilent 'Masshunter Optimizer' software. Identification of optimal analysis polarity, additional potential product ions, fragmentor voltage and collision energies were selected by using this software package. Optimizer was run for all compounds in both positive and negative polarities. The software was programmed to search for product ions generated from a precursor ion of [M+H] + or [M-H] – for each analyte. A low mass cut off of 40 m/z was used for the optimization. Fragmentor voltages were varied in the range of 0 to 180 V in increments of 5 V. Collision energies were varied in the range of 0-50 V. Final optimized transitions can be seen in Table 28. Delta RT for the dMRM mode was generally set to 1 with the exception of certain compounds which had the potential for peak broadening with analyte breakdown, namely amoxicillin and ciprofloxacin. As these compounds were observed to break down rapidly, a larger delta RT was set for these compounds to avoid wide peaks being cut off by the software.

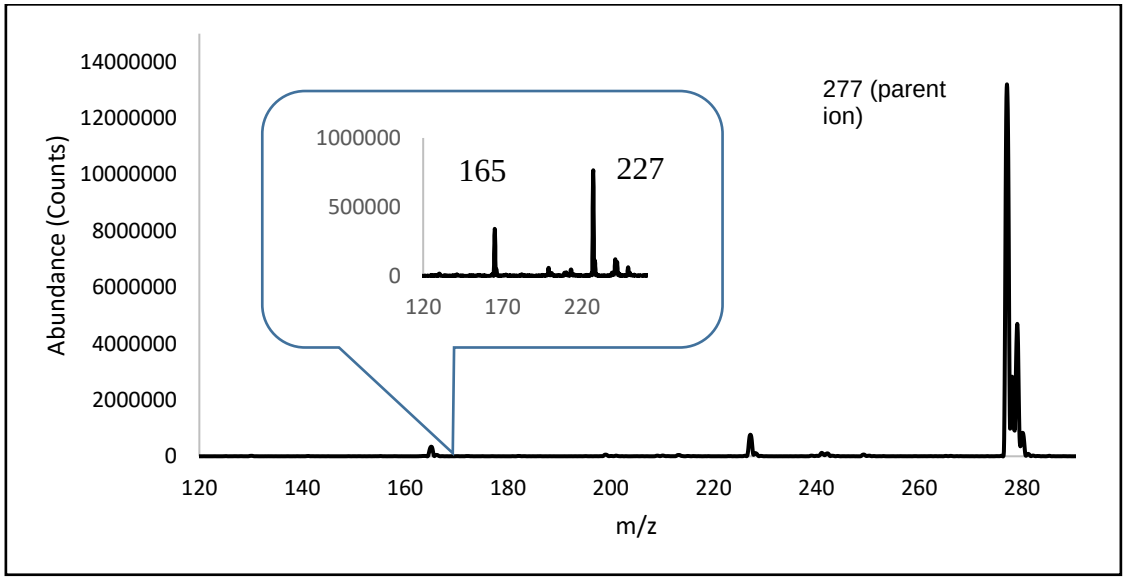


Figure 27. Mass spectrum of clotrimazole analytical standard acquired by direct infusion of standard onto the MS in scan mode.

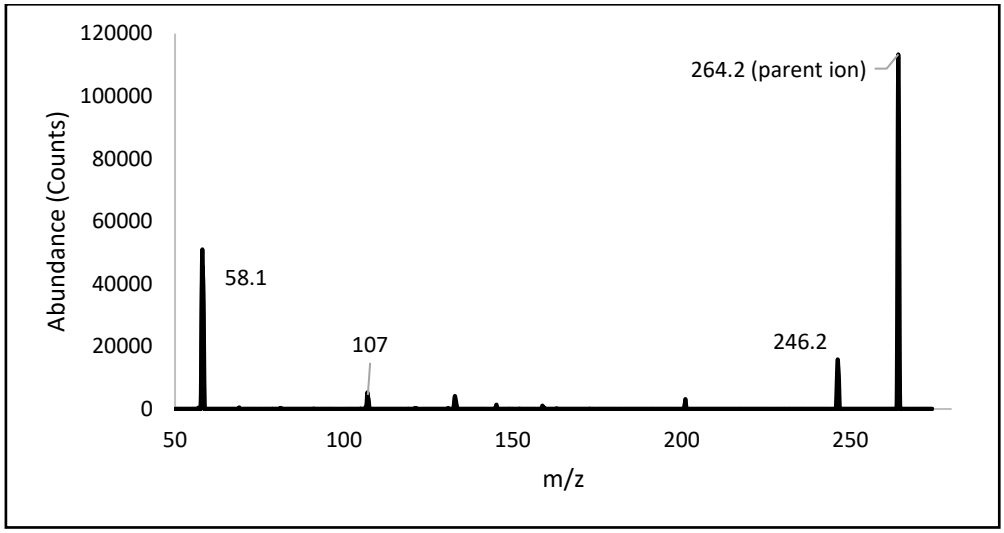


Figure 28. Mass Spectrum Of O-Desmethylvenlafaxine Analytical Standard Acquired By Direct Infusion Of Standard Onto The Ms In Scan Mode.

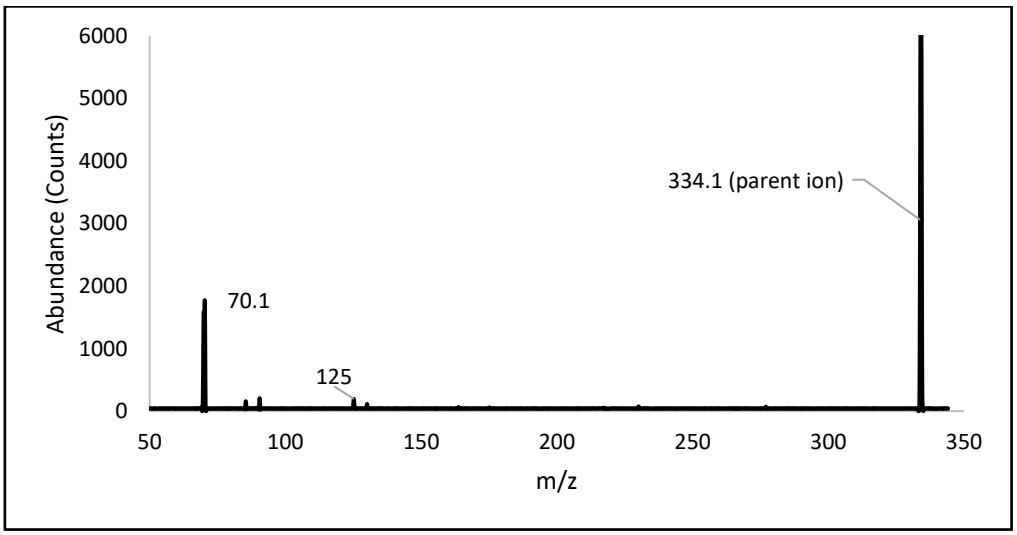


Figure 29. Mass Spectrum Of Ipconazole Analytical Standard Acquired By Direct Infusion Of Standard Onto The Ms In Scan Mode.

Table 28. Optimized MRM conditions for 3rd watch list compounds

Compound Name	Precursor Ion	MS1 Res	Product Ion	MS2 Res	RT	Delta RT	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Amoxicillin	366.1	Wide	114	Wide	2.7	5	80	21	4	Positive
Amoxicillin	366.1	Wide	160	Wide	2.7	5	80	21	4	Positive
Amoxicillin	366.1	Wide	208	Wide	2.7	5	80	5	4	Positive
Amoxicillin	366.1	Wide	349.3	Wide	2.7	5	80	5	4	Positive
Ciprofloxacin	332.13	Unit	231.2	Unit	5.1	1.5	150	41	4	Positive
Ciprofloxacin	332.13	Unit	245	Unit	5.1	1.5	150	17	4	Positive
Ciprofloxacin	332.13	Unit	288.4	Unit	5.1	1.5	150	17	4	Positive
Clotrimazole	277	Unit	165	Unit	8.1	1	120	32	4	Positive
Clotrimazole	277	Unit	241.1	Unit	8.1	1	120	24	4	Positive
Dimoxystrobin	327.2	Unit	116	Unit	11.6	1	90	24	4	Positive
Dimoxystrobin	327.2	Unit	205.1	Unit	11.6	1	90	8	4	Positive
Dimoxystrobin	327.2	Unit	238.1	Unit	11.6	1	90	8	4	Positive
Famoxadone	331	Wide	195	Wide	13.9	2.5	150	24	4	Positive
Famoxadone	331	Wide	238	Wide	13.9	2.5	150	24	4	Positive
Famoxadone	392.2	Wide	238.1	Wide	13.9	2.5	150	24	4	Positive
Famoxadone	392.2	Wide	331.2	Wide	13.9	2.5	150	24	4	Positive
Fluconazole	307.1	Unit	220.06	Unit	5	1	125	20	4	Positive
Fluconazole	307.1	Unit	238.07	Unit	5	1	125	16	4	Positive
Fluconazole-13C3	310.2	Unit	223.1	Unit	5	1	100	25	4	Positive

Imazalil	297.1	Unit	69	Unit	7.3	1.5	125	20	4	Positive
Imazalil	297.1	Unit	159	Unit	7.3	1.5	125	20	4	Positive
Imazalil	297.1	Unit	201	Unit	7.3	1.5	125	20	4	Positive
Imazalil-d5	302.1	Unit	69	Unit	7.3	1.5	130	20	4	Positive
Imazalil-d5	302.1	Unit	158.9	Unit	7.3	1.5	130	24	4	Positive
Ipconazole	334.1	Unit	70.1	Unit	12.9	1	110	22	4	Positive
Ipconazole	334.1	Unit	125	Unit	12.9	1	110	45	4	Positive
Metaflumizone	505.12	Unit	285	Unit	15.5	1	175	53	4	Negative
Metaflumizone	505.12	Unit	303.1	Unit	15.5	1	175	21	4	Negative
Metconazole	320.2	Unit	70	Unit	11.2	1	120	25	4	Positive
Metconazole	320.2	Unit	125	Unit	11.2	1	120	30	4	Positive
Metconazole	320.2	Unit	177.04	Unit	11.2	1	120	30	4	Positive
Miconazole	417	Unit	158.9	Unit	10	1	61	24	4	Positive
Miconazole	417	Unit	160.9	Unit	10	1	61	24	4	Positive
Miconazole	417	Unit	229	Unit	10	1	61	17	4	Positive
o-desmethylvenlafaxine	264.2	Unit	58.1	Unit	5.3	1.5	115	20	4	Positive
o-desmethylvenlafaxine	264.2	Unit	107	Unit	5.3	1.5	115	44	4	Positive
o-desmethylvenlafaxine	264.2	Unit	246.2	Unit	5.3	1.5	115	12	4	Positive
Penconazol	284.1	Unit	70	Unit	11.1	1	120	15	4	Positive
Penconazol	284.1	Unit	159	Unit	11.1	1	120	20	4	Positive
Penconazol	284.1	Unit	173	Unit	11.1	1	120	20	4	Positive
Prochloraz	376	Unit	70.06	Unit	11.1	1	80	10	4	Positive
Prochloraz	376	Unit	266	Unit	11.1	1	80	10	4	Positive
Prochloraz	376	Unit	308	Unit	11.1	1	80	10	4	Positive

Prochloraz-(ethylene-d4)	380.07	Unit	89	Unit	11.1	1	95	20	4	Positive
Sulfamethoxazole	254	Unit	92	Unit	6.1	1	75	28	4	Positive
Sulfamethoxazole	254	Unit	108.4	Unit	6.1	1	75	16	4	Positive
Sulfamethoxazole	254	Unit	156	Unit	6.1	1	75	16	4	Positive
Sulfamethoxazole-13C6	260	Unit	114	Unit	6.1	1	100	20	4	Positive
Sulfamethoxazole-13C6	260	Unit	162	Unit	6.1	1	100	15	4	Positive
Tebuconazole	310	Unit	70	Unit	10.8	1	120	20	4	Positive
Tebuconazole	308.2	Unit	70	Unit	10.8	1	120	20	4	Positive
Tebuconazole	308.2	Unit	125	Unit	10.8	1	120	20	4	Positive
Tebuconazole	308.2	Unit	151	Unit	10.8	1	120	20	4	Positive
Tetraconazole	372	Unit	70.1	Unit	10.4	1	150	16	4	Positive
Tetraconazole	372	Unit	159.1	Unit	10.4	1	150	36	4	Positive
Trimethoprim	291.2	Unit	230.1	Unit	5	1	130	24	4	Positive
Trimethoprim	291.1	Unit	258	Unit	5	1	130	28	4	Positive
Trimethoprim-d9	300.2	Unit	234.1	Unit	5	1	160	28	4	Positive
Trimethoprim-d9	300.2	Unit	264.1	Unit	5	1	160	28	4	Positive
Venlafaxine	278.5	Unit	58.4	Unit	5.9	1	72	18	4	Positive
Venlafaxine	278.5	Unit	121.1	Unit	5.9	1	72	29	4	Positive

2.3.1.1.8 Method Performance

Validation and calibration experiments were performed according to the procedure outlined in section 2.2.3.2.3.

Assessment of initial method performance for the 500 mL extraction volume showed very large standard deviations for both recovery and matrix effects. Although calculated LOD and LOQ values were very low, reliability of the sample data is insufficient due to the extreme variation between replicates for recovery and matrix effects. Method performance results found can be seen in Table 29.

Table 29 Validation experiment results in spiked surface water matrix for 3rd watch list compounds using a 500mL extraction volume

	Matrix Effect (%)	Percentage Recovery %)	R^2	LOD (ng L^{-1})	LOQ (ng L^{-1})
Metaflumizone*	44.39 ± 126.39	118.44 ± 126.39	0.93	0.46	1.4
Ciprofloxacin	41.24 ± 31.21	169.27 ± 31.21	0.810	0.57	1.7
Amoxicillin	44.13 ±104.34	110.51±104.34	0.92	0.66	1.7
Sulfamethoxazole	19.22 ± 94.73	288.03 ± 94.73	0.98	0.1	0.3
Trimethoprim	34.42 ± 73.15	389.38 ± 73.15	0.81	0.94	2.84
Venlafaxine	34.42 ± 121.92	38.28 ± 121.92	0.92	0.19	0.58
O-desmethylvenlafaxine	738.28 ± 68.8	41828 ± 68.8	0.94	0.16	0.48
Clotrimazole	37.6 ± 121.76	150.17 ±121.76	0.99	0.3	0.9
Fluconazole	7.17 ± 71.37	72.22 ± 71.37	0.94	0.17	0.51
Imazalil	96.95 ± 9.33	84.93 ± 9.33	0.97	0.68	2.05
Ipconazole	70.91 ± 145.39	108.14 ± 145.39	0.96	0.36	1.08
Metconazole	25.45 ± 96.94	58.09 ± 96.94	0.95	0.41	1.25
Miconazole	119.72 ± 74.89	85.44 ± 74.89	0.99	0.2	0.6
Penconazole	38.9 ± 137.09	138.75 ± 137.09	0.99	0.1	0.3
Prochloraz	93.19 ± 146.57	161.28 ± 146.6	0.96	0.25	0.75
Tebuconazole	139.37 ± 128.71	143.7 ± 128.71	0.86	0.26	0.79
Tetraconazole	34.28 ± 141.38	140.86 ± 141.38	0.97	0.45	1.36
Dimoxystrobin	17.39 ± 58.47	85.76 ± 58.47	0.92	0.54	1.6
Famoxadone	1089 ± 159.3	313.7 ± 159.3	0.96	0.41	1.22

Results from the validation experiments were likely affected greatly by two main factors; the higher concentration of the matrix causing matrix interference, and the greater sample loading time caused by the higher sample volume. It should be noted that the large sample volume (500 mL) of matrix took significantly longer to load on to the cartridge than the DI water, even after filtering off particulates. There was also great variability between replicates in sample loading time due to the cartridge getting 'stuck', even with full vacuum used, likely contributing to the large standard deviations seen. High amounts of ion suppression and enhancement (7.17 – 1089%) can possibly be attributed to the larger sample volume, which in turn influenced the overall extraction recoveries. Matrix interferences are one of the most frequently encountered difficulties in LC-MS analysis, and much is still not understood about the phenomenon[282,292]. Although field measurements for turbidity were taken, which were shown in the 2nd watch list section to cause matrix interferences previously, they showed relatively low levels compared to prior measurements of the same rivers. However, concentrating up even minimally turbid samples 500x rather than 100x seems to have had a significant effect on method performance.

As the validation experiments at this stage were unsatisfactory, adjustment to the method was necessary in order to be suitable to analysis of field samples. Therefore, the experiments were performed again with a reduced sample volume of 100 mL. All other method parameters remained the same. Results of this experiment are shown in Table 30.

As can be seen from the results shown in Table 30, method performance parameters from reducing the sample volume down to 100 mL showed great improvement. All analyte recoveries were between 70-160% which was considered acceptable for results in the complex sample matrix. Linearity in sample matrix was good with all compounds having R^2 values of ≥0.9. All method LOQs were within target limits set by the EU. Therefore sample analysis was performed using the 100 mL sample volume method.

Table 30. Method performance results from reduced sample volume for 3rd Watch List compounds

Analyte	Method LOD (ng L^{-1})	Method LOQ (ng L^{-1})	Target LOQ (ng L^{-1})	Regression (≤5 calibration points)	Recovery (%)	Matrix Effects %
Amoxicillin	1.03	3.12	78	0.969	118.26 ± 57.18	2537 ± 57.18
Ciprofloxacin	2.36	7.15	89	0.948	126.83 ± 16.41	11.52 ± 16.41
Clotrimazole	1.34	4.07	20	0.994	133.99 ± 24.84	211.6 ± 24.84
Dimoxystrobin	0.86	2.62	32	0.991	104.76 ± 22.39	42.87 ± 22.39
Famoxadone	0.85	2.59	8.5	0.994	76.65 ± 22.64	28.98 ± 22.64
Fluconazole	2.51	7.6	250	0.973	70.1 ± 26.8	88.11 ± 26.8
Imazalil	1.12	3.39	800	0.993	130.2 ± 24.61	73.75 ± 24.61
Ipconazole	2.4	7.37	44	0.981	95.93 ± 31.14	193.73 ± 31.14
Metaflumizone	2.98	9.03	65	0.993	108.18 ± 22.77	99.9 ± 43.99
Metconazole	1.4	4.2	29	0.989	117.34 ± 48.8	143.52 ± 48.8
Miconazole	2.82	8.56	200	0.962	102.76 ± 53.67	64.71 ± 53.67
O-desmethylvenlafaxine	1.92	5.82	6	0.978	134.49 ± 17.24	72.91 ± 17.24
Penconazole	6.94	21.04	1700	0.992	106.03 ± 46.33	171.38 ± 46.33
Prochloraz	1.13	3.41	161	0.996	87.3 ± 21.37	89.61 ± 21.37
Sulfamethoxazole	3.27	9.9	100	0.945	104.85 ± 44.78	79.04 ± 44.78

Tebuconazole	3.98	12.07	240	0.967	89.67 ± 19.88	98.167 ± 19.881
Tetraconazole	1.05	3.17	1900	0.994	121.96 ± 38.96	64.74 ± 38.96
Trimethoprim	3.39	10.26	100	0.967	72.76 ± 16.66	60.72 ± 16.66
Venlafaxine	1.56	4.73	6	0.988	161.16 ± 35.14	30.64 ± 35.14

2.3.2 Pesticide Analysis

2.3.2.1 Reverse Phase LC-MS/MS

2.3.2.1.1 Optimization of SPE and C18 Passive Sampler Extraction

Significant SPE optimization for a broad range of compounds had already been undertaken for the Watch List methods, therefore the final SPE method developed for the 3rd Watch List was applied to the newly included pesticides to assess the methods suitability for the remaining pesticides not included in that list. Analyte recoveries from composite surface water matrix using this method are presented in Table 31.

Table 31. Table of SPE recoveries for RP pesticide method

Compound	% Recovery
2,4-D	107.13 ± 32.59
Acetamiprid	156.64 ± 40.72
Bifenthrin	188.16 ± 6.7
Clothianidin	137.1 ± 20.6
Cypermethrin	123.2 ± 8.25
Deltamethrin	120.29 ± 14.11
Esfenvalerate	135.31 ± 13.35
Imidacloprid	78.77 ± 29.46
MCPA	147.15 ± 45.87
Mecoprop	156.43 ± 12.97
Permethrin	124.1 ± 4.39
Thiacloprid	102.78 ± 4.26
Thiamethoxam	75.1 ± 14.51

As can be seen from this table, the SPE method was found to be effective for all analytes in this method, and was chosen to be applied to all sample matrices of relating to the studies in Chapters 4 and 5 including surface waters, WWTP influent and effluent.

For the C18 passive sampler disk extraction, an investigation into a reduction in the extraction volume used by Vrana was performed. This would reduce solvent consumption and dry down times significantly. Trial extractions were performed in triplicate on PS disks loaded with

reference standards, which were then compared to that of un-extracted standards of the same concentration. The results of this study can be seen in Table 32.

Table 32. Table of analyte recoveries from modified C18 disk extraction procedure

Analyte	Recovery (%)
Bifenthrin	55.01 ± 35.87
Clotrimazole	93.86 ± 29.66
Cypermethrin	107.27 ± 18.25
Deltamethrin	80.9 ± 24.33
Esfenvalerate	102.79 ± 29.69
Imazalil	133.43 ± 11.44
Ipconazole	123.83 ± 64.74
Metconazole	117.29 ± 24.14
Miconazole	132.58 ± 57.71
Penconazol	189.28 ± 14.52
Permethrin	71.4 ± 28.15
Prochloraz	116.91 ± 10.84
Tetraconazole	61.86 ± 46.45
Tebuconazole	151.79 ± 60.58

The extraction efficiency of the modified method was considered acceptable with recoveries in the 55-189 % range for amenable (non-polar) analytes, and so was used for field sample analysis.

2.3.2.1.2 Optimization of Separation

Separation optimization was largely built upon the work done in the previously developed methods. Formic acid had been proven to be an effective mobile phase buffer for both the polar neonicotinoids, and the relatively non polar azoles. Additionally, the use of an ammonium based buffer was indicated for use as pyrethroid pesticides produce good signal when forming ammonium adducts. Ammonium formate was selected for use in the 3rd Watch List - which also contained an adducted analyte - following trials using two different concentrations. Therefore, the mobile phase used in the 3rd Watch List method, 0.1% formic acid and 5mM ammonium formate in water (A) and methanol (B) was chosen for use in this

method. An initial separation using a simple gradient elution going from 20% organic to 100% organic over 20 min at a flow rate of 0.3mL.min^{-1} was performed and can be seen in Figure 30.

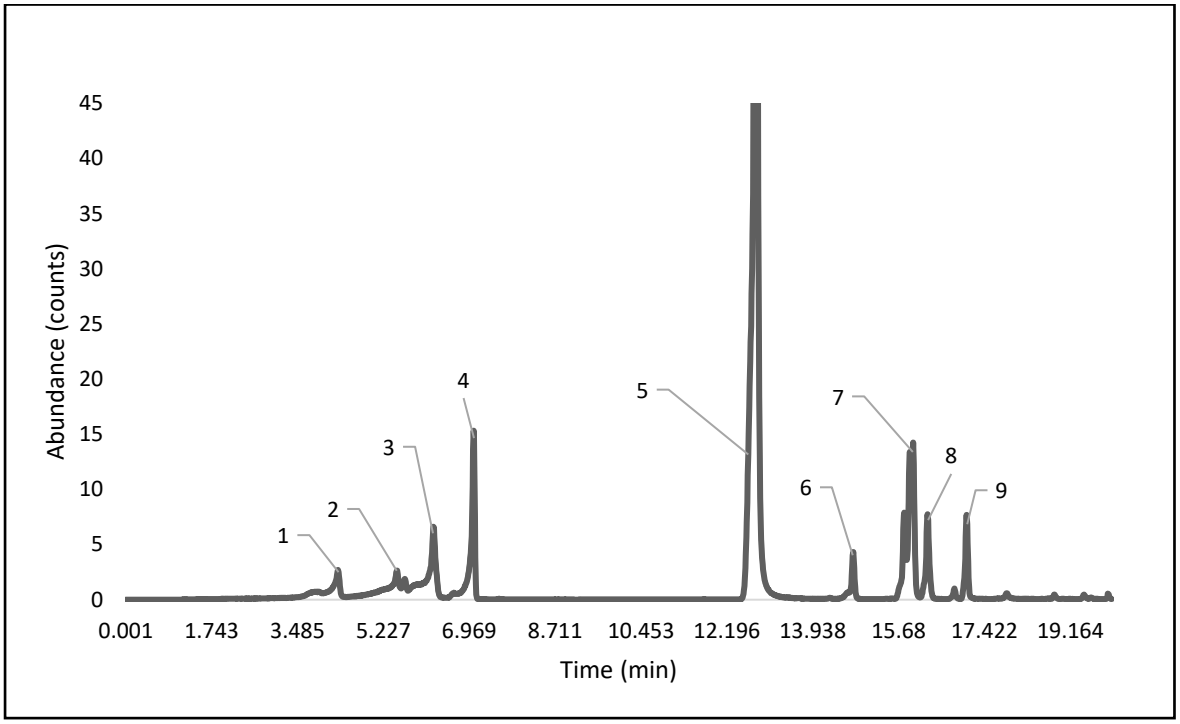


Figure 30. Chromatogram of early stage separation of pesticides method. Key: 1) thiamethoxam 2) acetamiprid, clothianidin, imidacloprid and fluconazole 3)thiacloprid 4)MCPA, 2,4-D and Mecoprop 5) clotrimazole, imazalil and tetraconazole 6)ipconazole, metconazole, miconazole, penconazole, prochloraz and tebuconazole 7)deltamethrin and cypermethrin 8) permethrin and esfenvalerate 9) bifenthrin

As can be seen in the chromatogram above, there were multiple co-elutions observed. To remedy this, the gradient elution was altered in order to allow for a slower gradient over key times in the separation when multiple co-elutions were occurring. A hold at 100% organic was also done to ensure all analytes were fully eluted from the column, before returning to starting conditions. A chromatogram of the final separation achieved can be seen in Figure 31.

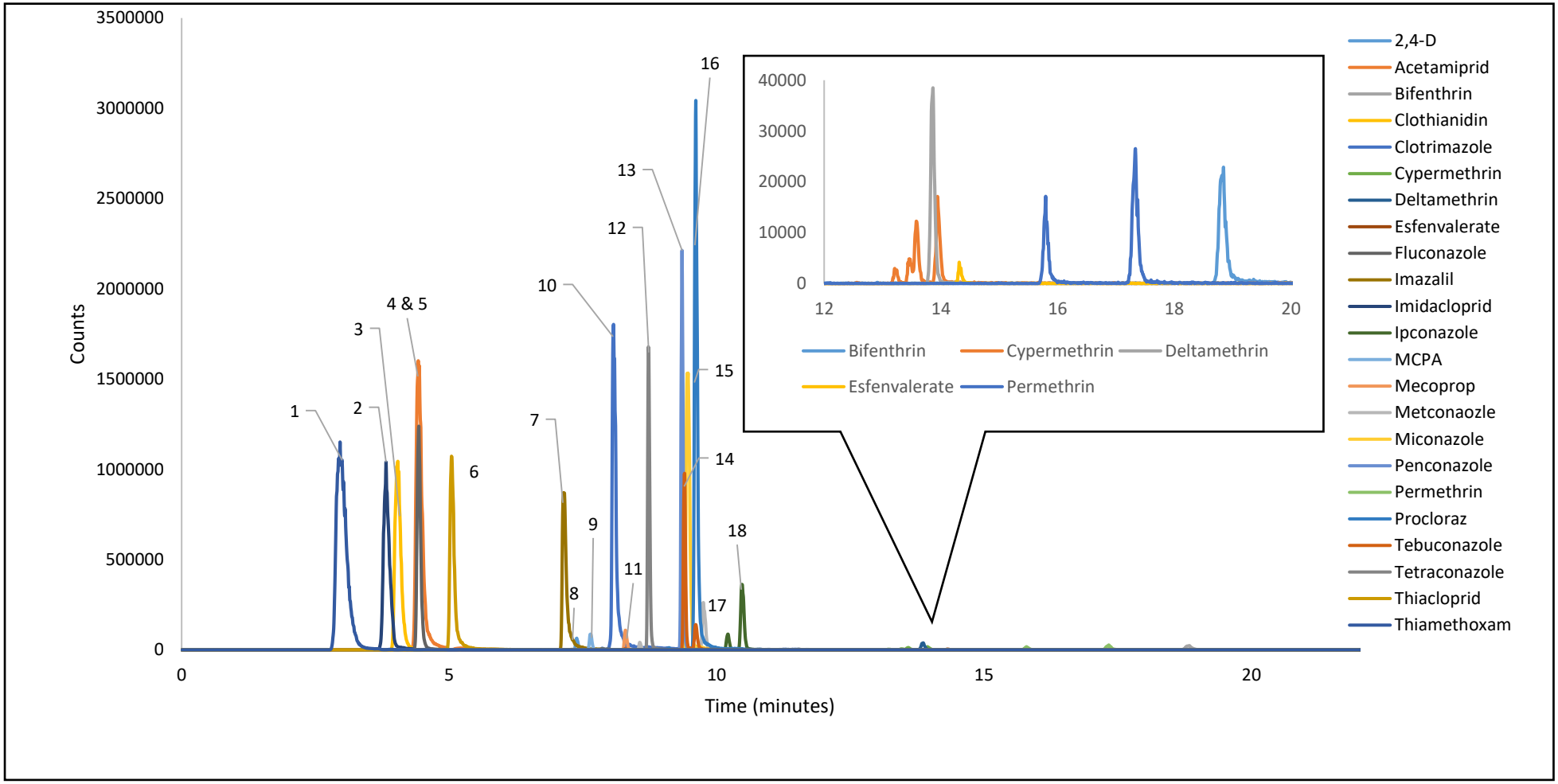


Figure 31. Chromatogram of the final separation for pesticide contaminants achieved using LC-MS (QQQ) run in MRM mode using a c18 2.1 ⨯ 150 mm, 1.9 µm column, 0.25 mL/min, 100 ul injection volume, gradient elution with mobile phase a) 0.1% FA + 5mm ammonium formate in Ultrapure Water b) 0.1% FA + 5mm ammonium formate in MeOH. Analyte Key: 1) Thiamethoxam 2) Imidacloprid 3) Clothianidin 4 & 5) Acetamiprid & Fluconazole 6) Thiacloprid 7) Imazalil 8) 2, 4-D 9) MCPA 10) Clotrimazole 11) Mecoprop12) Tetraconazole 13) Penconazole 14) Tebuconazole 15) Miconazole 16) Prochloraz 17) Metconazole 18) Ipconazole

2.3.2.1.3 Optimization of Mass Spectrometry

From the work done on the previous two Watch Lists, MRM transitions for 10 azoles and 5 neonicotinoids, as well as related internal standards, had already been developed and could be immediately included in the pesticides method. The remaining compounds, 3 acid herbicides and 5 pyrethroid pesticides were therefore requiring optimization. As with previous sections, analytes were run individually in MS2 scan mode in order to observe potential precursor and product ions. An example of this can be seen in Figure 32 showing the mass spectrum for MCPA.

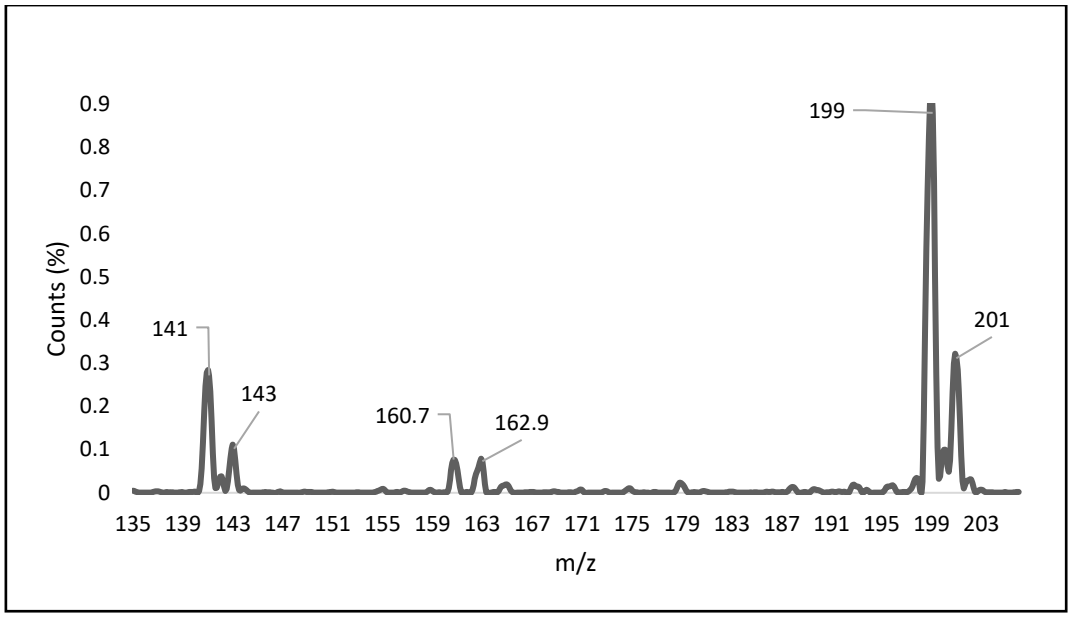


Figure 32. Mass spectrum of 50ppb MCPA standard produced in negative MS2 scan mode.

Optimization of the MS conditions was aided significantly by the Agilent 'Masshunter Optimizer' software. Identification of additional potential product ions, fragmentor voltage and collision energies were selected by using this software package. Optimizer was run for pyrethroids in positive and acid herbicides in negative polarities as indicated by the literature. The software was programmed to search for product ions generated from a precursor ion of [M+H] + or [M-H] – for each acid herbicide. As it has been shown in previous studies that pyrethroids produce greater signal when forming ammonium adducts [293–295], [M + NH_3] + was programmed for these compounds in addition to [M+H] + . A low mass cut off of 40 m/z was

used for the optimization. Fragmentor voltages were varied in the range of 0 to 180 V in increments of 5 V. Collision energies were varied in the range of 0-50 V. As some compounds eluted as multiple peaks due various isomers, delta RT for these compounds was larger to allow for both peaks to be included in the given window. Optimized dMRM conditions can be seen in Table 33.

Table 33. Table of QQQ conditions for reverse phase pesticide method

Compound Name	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
2,4-D	219	Unit	161	Unit	7.3	1	70	12	4	Negative
2,4-D	221	Unit	163	Unit	7.3	1	70	14	4	Negative
Acetamiprid	223.2	Unit	56.1	Unit	4.3	2	84	20	4	Positive
Acetamiprid-d3	226	Unit	126	Unit	4.3	2	108	30	4	Positive
Bifenthrin	442.08	Unit	181.1	Wide	18.8	2.5	76	17	4	Positive
Bifenthrin	440.2	Unit	181.1	Wide	18.8	2.5	95	28	4	Positive
Bifenthrin	439.89	Unit	181.1	Wide	18.8	2.5	100	16	4	Positive
Clothianidin	250	Unit	169	Unit	4	2	84	12	4	Positive
Clothianidin	250	Unit	132	Unit	4	2	84	12	4	Positive
Clothianidin-d3	253	Unit	172	Unit	4	2	108	20	4	Positive
Clotrimazole	277	Unit	241.1	Unit	8.08	1	120	32	4	Positive
Clotrimazole	277	Unit	241.1	Unit	8.08	1	120	32	4	Positive
Clotrimazole	277	Unit	165	Unit	8.08	1	120	24	4	Positive
Cypermethrin	435	Wide	192.9	Widest	13.3	4	110	14	4	Positive
Cypermethrin	433	Wide	191	Widest	13.3	4	110	12	6	Positive
Cypermethrin	417	Wide	191	Widest	13.3	4	110	17	4	Positive
Cypermethrin	417	Wide	190.8	Widest	13.3	4	110	14	4	Positive
Cypermethrin-(phenoxy-d5)	438	Wide	191	Widest	13.2	4	110	17	4	Positive
Deltamethrin	522.9	Unit	506.4	Wide	13.8	4	125	8	4	Positive
Deltamethrin	522.9	Unit	181	Wide	13.8	4	125	50	4	Positive
Deltamethrin	522.8	Unit	280.9	Wide	13.8	4	125	12	4	Positive

Esfenvalerate	437.1	Unit	167.3	Wide	14.3	4	155	12	4	Positive
Esfenvalerate	437.1	Unit	125	Wide	14.3	4	155	50	4	Positive
Fluconazole	307.1	Unit	238.07	Unit	4.5	2	125	16	4	Positive
Fluconazole	307.1	Unit	220.06	Unit	4.5	2	125	20	4	Positive
Fluconazole-13C3	310.2	Unit	223.1	Unit	4.5	2	100	25	4	Positive
Imazalil	297.05	Unit	201	Unit	7.1	1	125	20	4	Positive
Imazalil	297.05	Unit	159	Unit	7.1	1	125	28	4	Positive
Imazalil	297.05	Unit	69	Unit	7.1	1	125	20	4	Positive
Imazalil-d5	302.1	Unit	158.9	Unit	7.1	1	130	24	4	Positive
Imazalil-d5	302.1	Unit	69	Unit	7.1	1	130	20	4	Positive
Imidacloprid	256.2	Unit	209	Unit	3.8	2	84	20	4	Positive
Imidacloprid	256.2	Unit	175.2	Unit	3.8	2	84	20	4	Positive
Imidacloprid-d4	260	Unit	213	Unit	3.8	2	108	20	4	Positive
Ipconazole	334.1	Unit	125	Unit	10.5	1.5	110	45	4	Positive
Ipconazole	334.1	Unit	70.1	Unit	10.5	1.5	110	22	4	Positive
MCPA	201	Unit	143	Unit	7.5	1	90	18	4	Negative
MCPA	199	Unit	141	Unit	7.5	1	90	12	4	Negative
MCPA-d6	205.1	Unit	147	Unit	7.5	1	100	20	4	Negative
Mecoprop	215	Unit	143	Unit	8.2	1	90	14	4	Negative
Mecoprop	213	Unit	141	Unit	8.2	1	90	12	4	Negative
Metconazole	320.2	Unit	177.04	Unit	9.8	1	120	30	4	Positive
Metconazole	320.2	Unit	125	Unit	9.8	1	120	30	4	Positive
Metconazole	320.2	Unit	70	Unit	9.8	1	120	25	4	Positive
Miconazole	417	Unit	229	Unit	9.5	1	61	17	4	Positive

Miconazole	417	Unit	160.9	Unit	9.5	1	61	24	4	Positive
Miconazole	417	Unit	158.9	Unit	9.5	1	61	24	4	Positive
Penconazol	284.1	Unit	173	Unit	9.35	2	120	20	4	Positive
Penconazol	284.1	Unit	159	Unit	9.35	2	120	20	4	Positive
Penconazol	284.1	Unit	70	Unit	9.35	2	120	15	4	Positive
Permethrin	408.1	Unit	355	Unit	16	4	95	10	4	Positive
Permethrin	408.1	Unit	183	Unit	16	4	95	16	4	Positive
Permethrin-(phenoxy-d5)	413.1	Unit	188	Unit	16	4	80	52	4	Positive
Prochloraz	376	Unit	308	Unit	9.6	1	80	10	4	Positive
Prochloraz	376	Unit	266	Unit	9.6	1	80	10	4	Positive
Prochloraz	376	Unit	70.06	Unit	9.6	1	80	10	4	Positive
Prochloraz-(ethylene-d4)	380.07	Unit	89	Unit	9.6	1	95	20	4	Positive
Tebuconazole	310	Unit	70	Unit	9.15	2	120	20	4	Positive
Tebuconazole	308.2	Unit	151	Unit	9.15	2	120	20	4	Positive
Tebuconazole	308.2	Unit	125	Unit	9.15	2	120	20	4	Positive
Tebuconazole	308.2	Unit	70	Unit	9.15	2	120	20	4	Positive
Tetraconazole	372	Unit	159.1	Unit	8.7	1	150	36	4	Positive
Tetraconazole	372	Unit	70.1	Unit	8.7	1	150	16	4	Positive
Thiacloprid	253	Unit	125.8	Unit	5	1.5	114	24	4	Positive
Thiacloprid	253	Unit	90	Unit	5	1.5	114	50	4	Positive
Thiacloprid-d4	257.06	Unit	125.8	Unit	5	1.5	108	30	4	Positive
Thiamethoxam	292.02	Unit	211.1	Unit	3	2	84	12	4	Positive
Thiamethoxam	292.02	Unit	181	Unit	3	2	84	24	4	Positive
Thiamethoxam-d3	295	Unit	214	Unit	3	2	108	20	4	Positive

2.3.2.1.4 Evaluation of Method Performance

Validation and calibration experiments were performed according to the procedure followed for the previous methods in section 2.2.3.2.3.

Serial injection of increasingly lower analyte concentrations showed that the LOD potential for pyrethroid pesticides was not as low as the other pesticide groups, being only visible into the mid ng L^{-1} range. This is not unexpected for these compounds when analysed by LC-MS/MS, and the range still considered environmentally relevant concentrations. This range is can be considered an improvement when compared to the potential range when compared to a GC- single quadrupole MS, as described in the paper by Alder *et al* [267]. With this in mind, in order to achieve the lowest possible detection limits for these analytes, a 100 µL injection volume was used for the final method. The calibration standards for pyrethroids and associated IS were therefore prepared from 500-200000 ng L^{-1} (0.5 – 200 µg.L^{-1}), as well as additional blanks spiked with only IS (n≥3). A chromatogram of an extracted bifenthrin standard at a concentration of 500 ng L^{-1} in river water matrix can be seen in Figure 33. All other standards including neonicotinoids, acid herbicides and azoles were prepared in the range of 1-1000 ng L^{-1}.

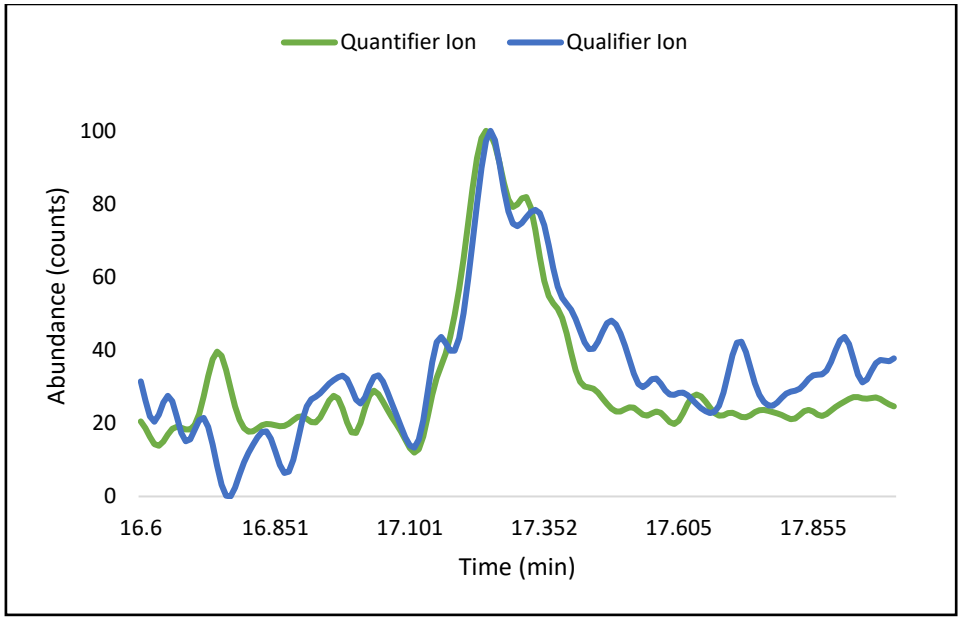


Figure 33. MRM Chromatogram of 500 ng L^{-1} bifenthrin standard in extracted river water matrix.

Linearity across all analytes and each matrix was found to be good, with R^2 values being ≥0.9. Certain analytes calibration curves began to move into a quadratic function over certain concentrations (typically occurring at 1000 ng L^{-1} for the lower concentration analytes and 200 µg.L^{-1} for pyrethroids), and consequently only the linear range was included. This was seen more frequently in the more complex matrices of WWTP influent and effluent, and can therefore be potentially due to the influence of matrix effects on the ionization of certain analytes at higher concentrations.

Calculated LODs based on the extracted matrix matched calibration curves for all pesticides across three matrices were in the ng L^{-1} to low µg.L^{-1} range depending on the analyte, with the lowest being 0.22 ng L^{-1} for clotrimazole in influent and highest being 940.21 ng L^{-1} for esfenvalerate in surface waters. It is interesting to note that LODs for pyrethroid pesticides were lower in wastewater samples (both influent and effluent) in comparison to those achieved in surface waters. Pyrethroids are very non-polar compounds with LogK_{ow} values averaging around 6. This leads to the likelihood of them binding to organic matter within the sample matrix rather than partitioning into the water phase. A possible reason for improved LODs in the more complex matrices could be the greater amount of organic matter in the sample, which could allow for pyrethroid stabilisation within the matrix and thus lower detection limits. A table of all method performance results can be seen in Tables 34-36.

Table 34. Method performance results for SPE-RP LC-MS/MS in river water matrix showing the linearity, range, LOD, LOQ, recovery and matrix effects for each analyte

Group	Analyte	R^2	Range ≥5 points (ng L^{-1})	Method LOD (ng L^{-1})	Method LOQ (ng L^{-1})	Recovery (%)	Matrix Effect (%)
Acid herbicides	**2,4-D**	0.9823	1-1000	2.33	7.05	86.7 ± 15.2	169 ± 15.2
	MCPA	0.907	5 - 1000	6.13	18.58	123.1 ± 3.1	112.5 ± 3.1
	Mecoprop	0.9885	1-1000	1.75	5.31	69.1 ± 32.5	21.3 ± 32.4
Neonicotinoids	**Acetamiprid**	0.9974	1-1000	0.83	2.52	77.4 ± 17.7	70.4 ± 17.7
	Clothianidin	0.9657	1-1000	3.58	10.86	137.1 ± 20.6	55.8 ± 20.6
	Imidacloprid	0.9422	1-750	2.8	8.49	156.4 ± 52.9	122.1 ± 52.9
	Thiacloprid	0.9915	1-1000	1.4	4.25	94.2 ± 8.2	113 ± 8.2
	Thiamethoxam	0.9399	5-1000	4.7	14.25	76.5 ± 7.7	89.4 ± 7.7
Pyrethroids	**Bifenthrin**	0.9902	500 - 75000	141.42	428.53	123.1 ± 33.4	119.4 ± 33.4
	Cypermethrin	0.9824	500-200000	501.08	1518.43	116.6 ± 28.4	105.4 ± 28.4
	Deltamethrin	0.9967	500-175000	782.32	2370.65	142.4 ± 26.4	135.6 ± 26.4
	Esfenvalerate	0.982	500 - 175000	940.21	2849.11	145.8 ± 10.5	106.4 ± 10.5
	Permethrin	0.9801	500 - 175000	396.95	1202.88	95.1 ± 10.5	120 ± 10.5
Azoles	**Fluconazole**	0.9913	1-1000	1.626	4.93	94.7 ± 4.7	139.6 ± 4.7
	Clotrimazole	0.9908	1-750	1.07	3.25	105.9 ± 34.4	354.8 ± 34.4
	Imazalil	0.9971	1-1000	0.89	2.48	99.7 ± 5.7	93.2 ± 5.7
	Ipconazole	0.9924	1-750	0.9	2.74	71.1 ± 17.2	248.7 ± 17.2
	Metconazole	0.991	1-1000	1.37	4.15	96.5 ± 24.8	67.96 ± 24.8
	Miconazole	0.9891	1-300	0.61	1.85	74.8 ± 22.3	302.4 ± 22.3
	Penconazole	0.9921	1-750	0.99	3.01	133.2 ± 33.7	171.6 ± 33.7
	Prochloraz	0.9805	1-1000	2.14	6.47	97.2 ± 15.2	89.6 ± 15.2
	Tebuconazole	0.9957	1-1000	0.99	2.99	125.1 ± 11.1	276.4 ± 11.1
	Tetraconazole	0.9281	5-750	2.98	9.03	104.7 ± 24.8	125.5 ± 24.8

Table 35. Method performance results for SPE-RP LC-MS/MS in effluent matrix showing the linearity range, LOD, LOQ, recovery and matrix effects for each analyte

Group	Analyte	R^2	Range ≥5 points (ng L^{-1})	Method LOD (ng L^{-1})	Method LOQ (ng L^{-1})	Recovery (%)	Matrix effect (%)
Acid herbicides	**2,4-D**	0.9920	1-150	0.26	0.79	112.0 ± 42.5	157.4 ± 42.5
	MCPA	0.9481	1-1000	3.86	11.71	126.6 ± 38.5	283.6 ± 38.5
	Mecoprop	0.9744	1-1000	2.86	8.68	101.9 ± 31.9	134.6 ± 31.9
Neonicotinoids	**Acetamiprid**	0.9846	5-1000	2.03	6.15	80 ± 6.8	116.7 ± 6.8
	Clothianidin	0.9653	25-1000	4.06	12.29	77.8 ± 28.7	84 ± 28.7
	Imidacloprid	0.9810	5-750	1.98	5.99	128.6 ± 12.9	79.4 ± 12.9
	Thiacloprid	0.9903	1-750	1.19	3.61	113 ± 18.3	89.8 ± 18.3
	Thiamethoxam	0.9506	5-1000	4.56	13.82	104.3 ± 26	115.2 ± 26
Pyrethroids	**Bifenthrin**	0.9708	500-75000	282.89	857.22	57.5 ± 10.2	174.2 ± 10.2
	Cypermethrin	0.9851	500 - 150000	313.38	949.65	80.3 ± 20.4	102.9 ± 20.4
	Deltamethrin	0.9868	500 - 150000	294.87	893.55	70.0 ± 22.9	144.6 ± 22.9
	Esfenvalerate	0.9747	500 - 175000	506.11	1533.68	78.3 ± 16.6	155.8 ± 16.6
	Permethrin	0.9956	500 - 75000	100.95	305.9	54.7 ± 20	255.9 ± 20
Azoles	**Fluconazole**	0.9699	1-750	2.5	7.58	106.7 ± 28.9	286.1 ± 28.9
	Clotrimazole	0.9440	5-1000	3.98	12.07	85.7 ± 13.9	215.3 ±13.9
	Imazalil	0.9912	1-1000	1.55	4.71	102.2 ± 12.1	121.8 ± 12.1
	Ipconazole	0.9905	1-750	1.1	3.34	80.7 ± 9.1	192.0 ± 9.1
	Metconazole	0.9989	1-1000	0.5	1.5	94.6 ± 28.9	53.1 ± 28.9
	Miconazole	0.9902	1-1000	1.5	4.56	69.6 ± 5.8	304.6 ± 5.8
	Penconazole	0.9917	1-750	1.03	3.12	85.4 ± 6.6	118.7 ± 6.6
	Prochloraz	0.9982	1-1000	0.65	1.96	94.1 ± 24.4	51.6 ±24.4
	Tebuconazole	0.9928	1-750	0.96	2.91	92.5 ± 13.4	177.2 ± 13.4
	Tetraconazole	0.9946	1-750	0.94	2.8	96.5 ± 14.1	130.99 ± 14.1

Table 36. Method performance results for SPE-RP LC-MS/MS in influent matrix showing the linearity, range, LOD, LOQ, recovery and matrix effects for each analyte

Group	Analyte	R^2	Range ≥5 points (ng L^{-1})	Method LOD (ng L^{-1})	Method LOQ (ng L^{-1})	Recovery (%)	Matrix effect (%)
Acid herbicides	**2,4-D**	0.9296	1-150	0.73	2.08	83.2 ± 31.9	506 ± 31.9
	MCPA	0.9551	1-150	0.57	1.72	60.5 ± 33.7	842.8 ± 33.7
	Mecoprop	0.9666	1-500	1.44	4.37	74.4 ± 20.7	119.9 ± 20.7
Neonicotinoids	**Acetamiprid**	0.9929	1-750	0.95	2.89	126.8 ± 14.7	8.0 ± 14.7
	Clothianidin	0.9791	1-750	1.9	5.75	141.9 ± 63.6	182.3 ± 63.6
	Imidacloprid	0.9527	5-300	1.28	3.88	145.3 ± 13.2	262 ± 13.2
	Thiacloprid	0.9848	1-750	1.4	4.24	129.8 ± 4.7	123 ± 4.7
	Thiamethoxam	0.9383	10-750	3.44	10.42	81.5 ± 28.4	345 ± 28.4
Pyrethroids	**Bifenthrin**	0.9802	500-100000	265.95	805.91	79.4 ± 14.9	127.3 ± 14.9
	Cypermethrin	0.94	500-100000	472.94	1433.16	78.3 ± 28.8	16.7 ± 28.8
	Deltamethrin	0.9637	500-100000	363.07	1100.21	107.5 ± 24	57.1 ± 24
	Esfenvalerate	0.98	500-100000	492.4	1492.13	73.6 ± 10.2	20.5 ± 10.2
	Permethrin	0.9448	500 - 50000	241.97	733.26	108.8 ± 23.1	13.6 ± 23.1
Azoles	**Fluconazole**	0.9586	1-500	1.7	5.16	129.3 ± 19.6	348.2 ± 19.6
	Clotrimazole	0.9816	1-150	0.22	0.66	127.3 ± 18.1	230.8 ± 18.1
	Imazalil	0.9718	1-150	0.51	1.54	96.6 ± 9.6	17.5 ± 9.6
	Ipconazole	0.9819	1-750	1.58	4.78	121.6 ± 18	6.67 ± 17
	Metconazole	0.9033	1-750	5.13	15.55	70.1 ± 13.9	11.3 ± 13.9
	Miconazole	0.9461	5-1000	3.86	11.71	62.6 ± 7.4	12.4 ± 7.4
	Penconazole	0.9891	1-750	1.32	4.01	78.9 ± 14.9	1.95 ± 14.9
	Prochloraz	0.9979	1 - 750	0.52	1.57	72.9 ± 23	2.47 ± 23
	Tebuconazole	0.9233	5-1000	4.68	14.2	79 ± 15.4	2.92 ± 15.4
	Tetraconazole	0.9904	5 - 1000	1.41	4.26	80.2 ± 26	3.28 ± 26

2.3.2.2 HILIC LC-MS/MS

2.3.2.2.1 Column Selection

Use of a HILIC column for the un-derivitized analysis of glyphosate and AMPA has been indicted previously [270]. Initial experiments were carried out on a 150 x 2.1mm, 5µm SEQUANT® ZIC®-HILIC column as this was readily available to trial, and is one of the most common HILIC choices. However, this column showed poor performance for these analytes, with both compounds eluting in the column dead time indicating no analyte retention. Figure 34 shows a chromatogram produced using this column.

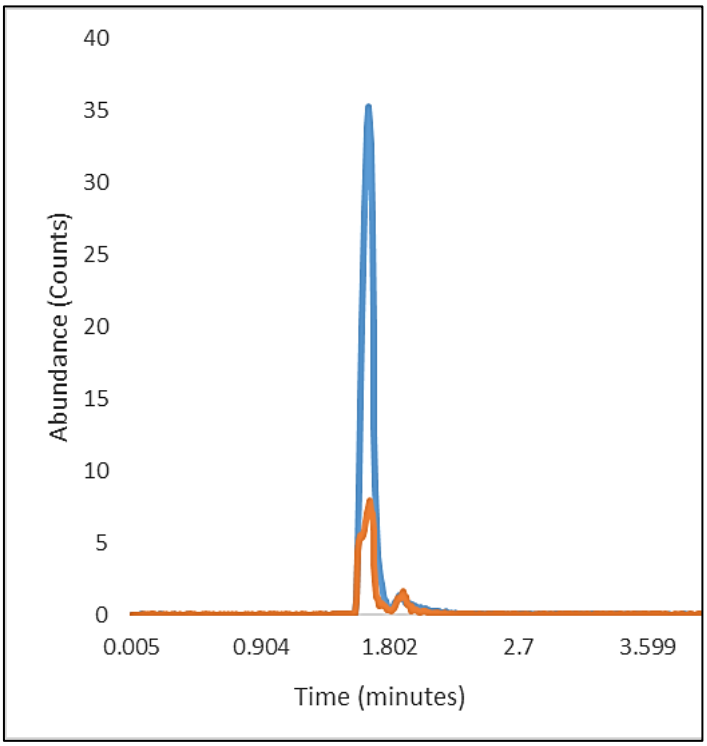


Figure 34. Chromatogram of attempted separation of glyphosate (orange) and primary metabolite AMPA (blue) using ZIC-HILIC column.

Multiple attempts at adjusting instrument parameters including using very low percentage of aqueous mobile phase and a reduced flow rate did not improve retention. Further investigation into the literature suggested that zwitterionic phases, although being indicated for extremely polar analytes which glyphosate and AMPA are, can have poor retention for these specific compounds [270].

Therefore, a different column was required for effective analysis of these pesticides. The use of a polyvinyl alcohol base material modified with quaternary ammonium in a column produced by Shodex has been effectively shown to analyse glyphosate [296], and therefore was selected for use.

Initial optimal separation conditions as indicated in the column technical note were used, the mobile phase consisting of 50mM ammonium bicarbonate in ultrapure water (a) and acetonitrile (b) run isocratically at 50:50. Although the flow rate indicated in the technical note was 0.3 mL min^{-1}, the maximum pressure of the column was 100 bar, which when attempting to run at that flow rate was exceeded on the available LC-MS system. Therefore a flow rate of 0.1 mL min^{-1} was required to ensure the pressure was kept at operating limits, significantly extending the run time needed to elute both compounds. The resulting separation using the isocratic elution and lower flow rate left room for optimization, with the peak for glyphosate eluting very late after 22 min, with a very broad peak shape. This initial separation can be seen in Figure 35.

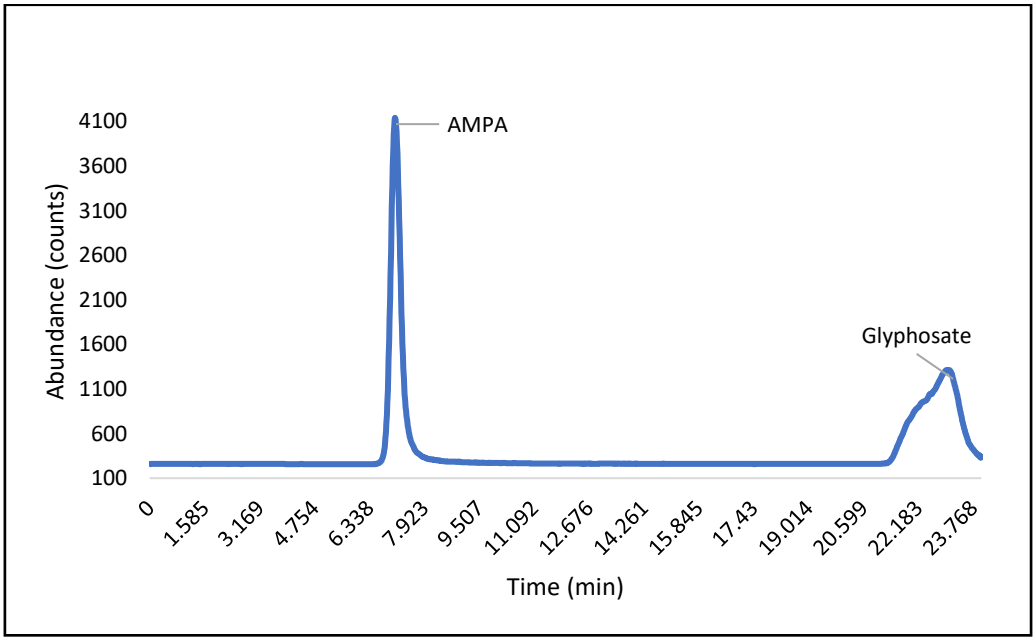


Figure 35. Chromatogram of initial separation of Glyphosate and AMPA on quaternary ammonium HILIC column using an isocratic elution (50:50 A:B)

The first step was switching from an isocratic to a gradient elution to try and reduce the retention time of and improve peak shape for glyphosate, and lower overall run time. With use of a gradient program, the overall run time was reduced to 18 min, both peaks were still

fully separated and glyphosate showed improved peak shape (Figure 36). This method was then used to assess method performance in multiple water matrices.

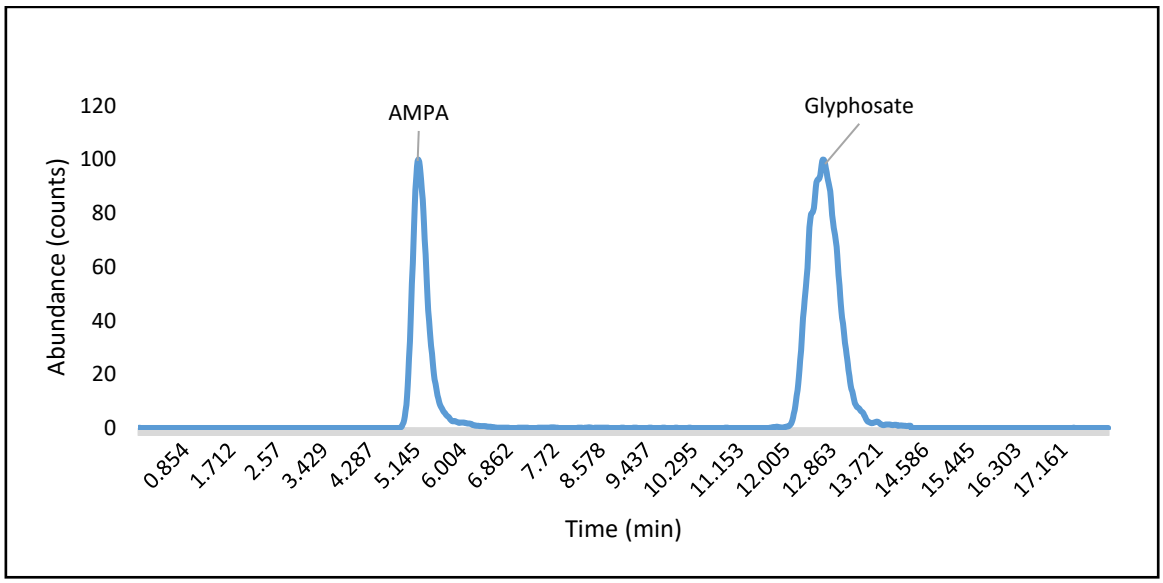


Figure 36. Final chromatographic separation of glyphosate and AMPA on specialised quaternary ammonium HILIC column.

2.3.2.2.2 Optimization of Mass Spectrometry

Analytes were run individually in MS2 scan mode in order to observe potential product ions. An example MS spectrum of glyphosate can be seen in Figure 37. Optimization of the MS conditions was aided significantly by the Agilent 'Masshunter Optimizer' software. Identification of optimal polarities, additional potential product ions, fragmentor voltage and collision energies were selected by using this software package. Optimizer was run in both positive and negative polarities as indicated by the literature. The software was programmed to search for product ions generated from a precursor ion of [M+H] + or [M-H] – for each compound. A low mass cut off of 40 m/z was used for the optimization. Fragmentor voltages were varied in the range of 0 to 180 V in increments of 5 V. Collision energies were varied in the range of 0-50 V. Dwell time were selected by aiming for approximately 500 ms/cycle. Optimized MRM conditions can be seen in Table 37.

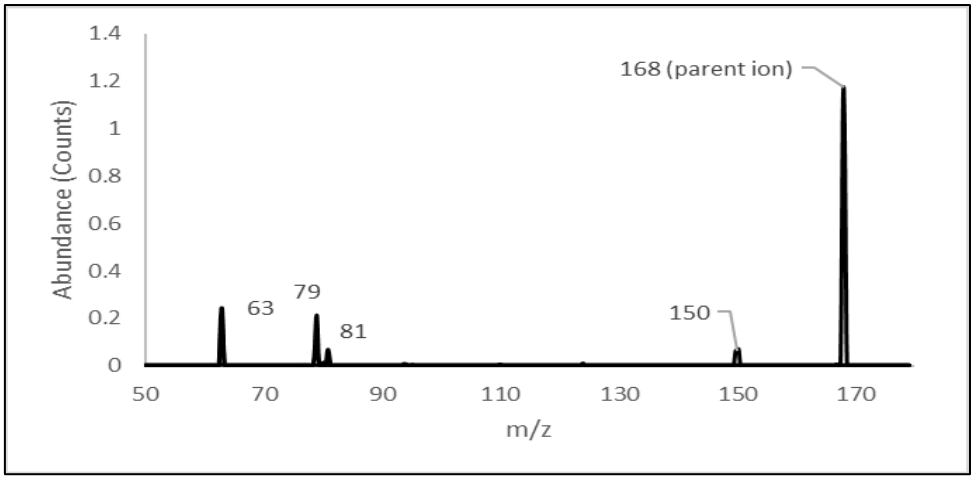


Figure 37 Mass spectrum of glyphosate analytical standard acquired by direct infusion of 500 ppb standard onto the MS in scan mode (negative).

Table 37. Table of QQQ conditions for HILIC method

Compound Name	Pre Ion	MS1 Res	Pro Ion	MS2 Res	Dwell	Frag	Collision Energy	Cell Accelerator Voltage	Polarity
Glyphosate	168	Unit	81	Wide	100	95	16	6	Negative
Glyphosate	168	Unit	79	Wide	100	95	50	6	Negative
Glyphosate	168	Unit	63	Wide	100	95	32	6	Negative
Glyphosate	168	Unit	150	Wide	100	95	10	6	Negative
AMPA	110	Unit	81	Wide	100	100	12	6	Negative
AMPA	110	Unit	79	Wide	100	100	36	6	Negative
AMPA	110	Unit	63	Wide	100	100	28	6	Negative

2.3.2.2.3 Evaluation of Method Performance

Validation and calibration experiments were performed according to the procedure outlined in the experimental section of this chapter for each sample matrix. Repeated injections of matrix blank, matrix spiked with the standard and IS mixes, and DI water spiked the standard and IS mixes were completed for validation. For calibration, matrix samples were for a final

concentration in the range 10-1000 ng.mL^{-1}. Method performance parameters can be seen in Table 38.

Table 38. Table of method performance results for direct injection HILIC method in three different aquatic matrices showing linearity, range, LOD, LOQ and matrix effects

Matrix	**Performance parameter**	**Glyphosate**	**AMPA**
Receiving Waters	*R^2*	0.9807	0.9989
	Dynamic range (µg L^{-1})	10-150	10-300
	LOD	47.1	17.5
	LOQ	142.6	52.9
	Matrix Effects	43.5 ± 57.8	103.9 ± 12.3
Effluent	*R^2*	0.9753	0.9978
	Dynamic range (µg L^{-1})	10-150	10-850
	LOD	53.4	63.3
	LOQ	161.7	191.7
	Matrix Effects	151.7 ± 11	102.2 ± 15.5
Influent	*R^2*	0.9452	0.9827
	Dynamic range (µg L^{-1})	25 - 500	25 - 650
	LOD	137.6	124.3
	LOQ	416.9	376.7
	Matrix Effects	148.8 ± 10.1	152.33 ± 18.89

2.4 Conclusions

The aim of this chapter was to develop new analytical methods capable of the analysis of the full suite of chemicals contained in the 2nd and 3rd Watch Lists, and selected groups of common pesticides, at environmentally relevant concentrations. SPE - LC-MS/MS methods were developed and optimized for the analysis of 15 2nd and 19 3rd watch list chemicals for the monitoring of Irish surface water samples. Performance of the methods was assessed for the 3rd Watch List compounds, an investigation into a higher sample extraction volume was performed however matrix interferences with the method performance made this unsuitable for real sample analysis, and therefore the volume was set at 100 mL.

The use of LC-MS for the analysis of pyrethroid pesticides is still a comparatively novel approach, as historically these compounds have been analysed using GC-MS. However, the ability to monitor these compounds using LC-MS, which is amenable to a much greater variety of chemicals due to the removal of volatility requirements, is of great benefit. A novel LC-MS/MS method which successfully analysed these compounds along with 18 other pesticides was developed. The method was examined for method performance parameters in three aquatic matrices; wastewater influent, effluent and receiving waters. Linearity was good for all compounds with all R^2 values over 0.9. Method detection limits were all in the low ng L^{-1} to µg.L^{-1} range, making the application of this method suitable for environmental concentrations.

Lastly, the monitoring of glyphosate and its metabolite AMPA has been of great interest to the scientific community for a number of years, being the most commonly used pesticide in the world. However, as discussed previously, its analysis is challenging due to its highly polar hydrophilic qualities, typically requiring lengthy and laborious derivatisation procedures in order to be analysed using standard reverse phase LC-MS columns. This chapter presented a direct injection LC-MS method using a specialised HILIC column capable of retaining and analysing these compound. Method performance was investigated in WWTP influent,

effluent and receiving waters. Linearity was good with R^2 values for both analytes over 0.9 in all studied matrices.

The work presented in this chapter shows the steps taken to develop and critically assess the performance of a number of analytical methods for the purpose of monitoring contaminants of emerging concern in a variety of aquatic matrices. The implementation of these methods to field samples allows for some of the first Irish data to be generated for these compounds, which in turn will inform future monitoring practices and environmental policy decisions within both Ireland and the EU.

Chapter 3:
Policy-Driven Monitoring of Contaminants of Emerging Concern in Irish Surface Waters

3.1 Introduction

3.1.1 Policy drivers

Water quality is impacted by a range of anthropogenic sources which contribute to chemical contaminants. Some of these chemicals are monitored because knowledge of their risk is well known. However, chemicals of emerging concern which do not have adequate data on occurrence in the environment are being added to the Watch List. Twenty years ago, the establishment of the Water Framework Directive (WFD) (Directive 2000/60/EC (2000)) changed how water quality was monitored. The aim of the WFD was to achieve good ecological and chemical status of surface waters [21] The WFD was amended to include 33 Priority Substances (PSs) that are recommended for monitoring as specified in Decision 2455/2001/EC (2001) [297]. This list of PSs was updated with Directive 2008/105/EC (2008), which set Environmental Quality Standards (EQS) for each of the substances in surface waters [48]. Following the 2008 amendments, these Priority Substances List further revised in the Directive 2013/39/EU (2013) with an additional 12 PSs, amounting to 45 compounds in total for monitoring [298] .Within Directive 2013/39/EU a mechanism was included to monitor a list of substances for which EU wide monitoring data should be gathered. This was to inform future EU policy decisions and was to be known as the Watch List (WL).

3.1.2 Watch list

The first full WL was established in Decision 2015/495/EU (2015) [49]. The original WL contained 10 suspected CECs or groups of CECs, totalling 17 individual chemicals. The criteria used for inclusion into the WL can be summarised into two key elements. Firstly, the proposed chemical for inclusion must be suspected of presenting a significant risk to the aquatic environment based on well-founded evidence of hazard, and potential exposure to the aquatic ecosystem. The second element is that there is currently insufficient monitoring or modelled exposure data to assess the current EU-wide exposure of the chemical to help decide whether the substance should be prioritised or not [49]. Based on this, the first WL comprised of five neonicotinoid pesticides (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam), three macrolide antibiotics (azithromycin, clarithromycin and erythromycin), two herbicides (oxadiazon and triallate), two natural hormones

(estrone~E1 and 17-β-estradiol~E2), a synthetic estrogen (17-α-ethinylestradiol~ EE2), a carbamate pesticide (methiocarb), an antioxidant (2,6-ditert-butyl-4-methylphenol, BHT), a non-steroidal anti-inflammatory drug (diclofenac) and finally a UV filter (2-ethylhexyl 4-methoxycinnamate~EHMC).

A review of the first WL was published in Decision 2018/840/EU [50] and the following recommendations were made:

- Removal of 5 CECs from the original WL due to sufficient monitoring data collected (diclofenac, oxadiazon, triallate, 2-ethylhexyl-4-methoxycinnamate~EHMC and 2, 6-di-tert-butyl-4-methylphenol~BHT).
- Addition of 3 new CECs to the updated WL, two antibiotics (amoxicillin and ciprofloxacin) and one pesticide (metaflumizone).

With these changes implemented, the 2nd WL consisted of 15 CECs of various chemistries and applications.

In 2020, the 3rd Watch List was published [51]. A stipulation of the WL is that no substance can be kept on it for a period longer than four years, requiring the removal of all compounds aside from metaflumizone, ciprofloxacin and amoxicillin from the previous list. A review of all monitoring data provided by member states is performed to decide whether to add to the compound to the priority substance list after 4 years. However, the review of the 2nd WL has not yet been published and so the fate of the removed 2nd list substance is not yet available. A number of new compounds were added in 2020, to include; two antibiotics sulfamethoxazole and trimethoprim, an antidepressant venlafaxine and its metabolite o-desmethylvenlafaxine, 10 azole compounds used as either pesticides or pharmaceuticals, and finally the fungicides famoxadone and dimoxystrobin.

The new pharmaceuticals added to the list were done so due to their possible contribution to antimicrobial resistance (AMR). AMR has been identified as one of the most serious global public health threats of the century, with potential impacts on both human and economic health [299]. The azole group of fungicides was selected for inclusion due to their prolific use

within the EU, which is considered the dominant market for fungicides with major applications on grains, cereals, fruits and vegetable [300]. Azoles has also been shown to be harmful to a swathe of non-target organisms, making their presence in surface waters a potential issue. Thus far, high quality data on the presence of these substances in surface waters is limited [51]. The new additions, along with the 3 carried over from the previous list, make for a total of 19 different compounds. A summary of all chemicals that have previously or are currently contained on the WFD Watch List are in Table 39, along with their common uses.

Table 39. Table showing all chemicals included for monitoring on each Watch List (2015-2020) and their common uses. Information taken from [49,51,255,301]

Chemical	2015	2018	2020	Common uses
Azithromycin	X	X		Macrolide antibiotic (gram positive bacterial infections e.g. skin, respiratory, sexual transmitted (STI))
Clarithromycin	X	X		Macrolide antibiotic
Erythromycin	X	X		Macrolide antibiotic
Acetamiprid	X	X		Neonicotinoid insecticide (control of aphids and grubs in particular)
Clothianidin	X	X		Insecticide
Imidacloprid	X	X		Neonicotinoid insecticide
Thiacloprid	X	X		Neonicotinoid insecticide
Thiamethoxam	X	X		Neonicotinoid insecticide
Oxadiazon	X			Herbicide (broadleaf weeds)
Triallate	X			Herbicide (grass weeds)
Estrone (E1)	X	X		Estrogenic hormone (menopause, gonadotropic dysfunction, osteoporosis)
17-β-estradiol (E2)	X	X		Estrogenic hormone (contraceptive pill, HRT)
17-α-ethinylestradiol (EE2)	X	X		Estrogenic hormone (contraceptive pill, HRT)
Methiocarb	X	X		Insecticide (control snails, slugs, mites & other insects)
2,6-ditert-butyl-4-methylphenol (BHT)	X			Antioxidant (additive to food, metalworking fluid, cosmetics, pharmaceuticals etc.)
Diclofenac	X			NSAID anti-inflammatory (treat joint & muscle inflammation)
2-ethylhexyl 4-methoxycinnamate (EHMC)	X			UV filter (added to sunscreens)

Amoxicillin		X	X	Antibiotic (typically for respiratory infections)
Ciprofloxacin		X	X	Antibiotic (bacterial infections e.g. respiratory, STI, skin, eye etc.)
Metaflumizone		X	X	Insecticide (control of fleas and ticks)
Sulfamethoxazole			X	Antibiotic (often prescribed in conjunction with trimethoprim)
Trimethoprim			X	Antibiotic (prescribed in conjunction with sulfamethoxazole)
Venlafaxine			X	SNRI anti-depressant (for anxiety and depression)
o-desmethylvenlafaxine			X	Primary metabolite of venlafaxine
Famoxadone			X	Fungicide (used mostly on fruiting vegetables, tomatoes and potato crops)
Dimoxystrobin			X	Fungicide (cereal crops)
Clotrimazole			X	Anti-fungal pharmaceutical (treatment of fungal skin infections)
Fluconazole			X	Anti-fungal pharmaceutical (treatment of more serious fungal skin infections)
Imazalil			X	Fungicide (fruit and vegetable crops)
Ipconazole			X	Fungicide (seed treatment for soil borne disease)
Miconazole			X	Anti-fungal pharmaceutical (treatment of fungal skin infections)
Metconazole			X	Fungicide (grain crops)
Prochloraz			X	Fungicide (gardening and variety of agricultural crops)
Penconazole			X	Fungicide (apples, grapes, other vegetables)
Tebuconazole			X	Fungicide (gardening, landscaping and variety of agricultural crops)
Tetraconazole			X	Fungicide (control leafspot and powdery mildew)

There have been multiple studies conducted in other countries over the years reporting data for compounds contained on the various Watch Lists [256,257,259,302–304], however many countries are limited by cost or available instrumentation to perform routine monitoring [255]. To the authors' knowledge, there is no reported data for the full suite of both 2nd and 3rd WL data for Ireland. Therefore this work presents the first data collected for all WL chemicals from 2018 to 2022, allowing an insight into the chemical water quality of the country and for the assessment of the risk these CECs pose on the aquatic environment. Results from this

monitoring campaign also contributed to the EU wide WL campaign, and consequently have a direct impact on EU policy decisions affecting all member states.

Aims and Objectives

The aim of this work is to provide the first Irish data for the EU watch lists from 2018-2022
The objectives are to:

- Highlight key aspects of the literature methods detailed in Chapter 1 that were used to guide this work;
- Apply the methods developed in Chapter 2 to real field samples;
- Obtain the first Irish data for these compounds, assess their risk and monitor for any trends over the four year period
- Identify compounds of interest for Ireland and examine avenues for future monitoring arising from this work

3.2 Materials and Methods

3.2.1 Reagents, chemicals, consumables

All materials used for sample collection, preparation and analysis are detailed in Chapter 2 section 2.2.1 of this thesis.

3.2.2 Field sample collection and preparation

Full detail of sample collection and preparation procedures are presented in Chapter 2 section 2.2.3.1

3.2.3 Solid Phase Extraction (SPE) and Instrumentation

Method details can be found in Chapter 2 section 2.2.3.2 and 2.2.3.3.

3.3 Results and Discussion

3.3.1 Selection of Studied Catchments

Five catchments or sub-catchments were selected for study over the project period. A catchment is defined as an area of land around a river, lake or other body of water, often comprising of a collection of smaller sub-catchments which are a part of the larger watershed [206]. A map of all sampling sites and general land uses can be found in Figure 38. Catchments were selected to present a representative range of waterbody status and risk levels as indicated by the WFD, with information on all catchments taken from the Irish EPA Catchments website [206].

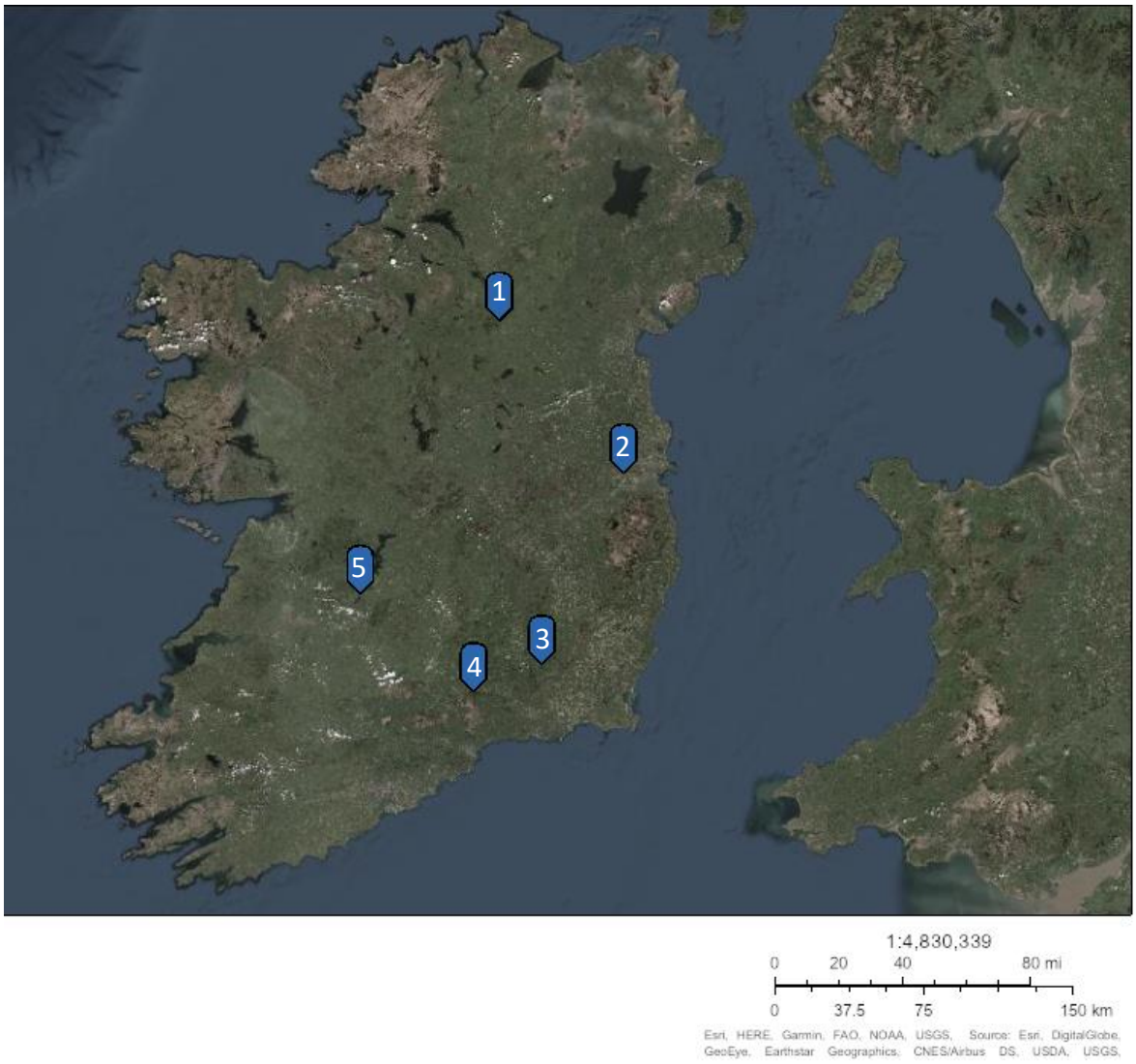


Figure 38. Map of sampling sites for rivers selected for Watch List Monitoring in Ireland from 2018-2022. Site key: (1) Annalee, (2) Liffey, (3), Nore, (4) Suir, (5) Shannon

The River Shannon is the longest river in Ireland, running 360 km due south from the northern part of County Cavan to meet the Atlantic Ocean at its estuary in Limerick. Currently, its status under the WFD is under review. It was sampled once in 2018 at Killaloe Bridge about 30 km from its mouth, however without further knowledge of its risk under the WFD, it was not included for the remainder of the study.

The River Nore (Figure 39) is located in the south-east of the country and flows as such before meeting the Celtic Sea in County Waterford. It is approximately 140 km long and is the only known habitat of the Nore freshwater pearl mussel (*Margaritifera durrovensis)*, a critically endangered species since its decline in population was discovered, and as such much of the surrounding land has undergone conservation efforts [305,306]. Potentially due to these efforts, the Nore sampling point has not been considered at risk under the current (3rd) nor the previous (2nd) WFD Risk Cycle. WFD risk status takes into account a number of chemical and biological parameters to determine status. Latest Q values collected by the EPA were Q4 indicating good water quality. This waterbody was included for study to represent Ireland's not at risk rivers, and to act as a control for the other catchments.

Figure 39. Photograph of the River Nore sampling site at Inistioge Bridge. The SONDE probe used for collection of field measurements, as well as the 1L Nalgene sample bottles used for collection can be seen in the left hand side of the image.

The River Suir (Figure. 40) is also located in the south-east of the country, and joins the Nore at the same sea mouth at Waterford Harbour. It is an estimated 185km long and has approximately double the flow rate of the Nore (76.9 to 42.9 $m^3.s^{-1}$ respectively). While the selected sampling point on the Suir is not currently considered at risk it is projected to move into at risk status, with parameters for ammonia, total oxidised nitrogen and ortho-phosphate levels generally trending upwards in the 2013-2018 monitoring cycle. Latest Q values were 3-4 indicating moderate water quality. This catchment was included for study to represent potential future at risk waters.

Figure 40. Photograph of the River Suir taken from the top of Kilsheelan Bridge where samples were taken from.

The River Annalee (Figure. 41) is located in the northern and western region of the country in County Cavan, and is a sub catchment of the River Erne. It is approximately 67 km long, with its source at Lough Sillan then flowing west before converging with the River Erne to eventually meet the Atlantic Ocean at Ballyshannon in County Donegal. The Annalee is currently considered at risk under the WFD 3rd Cycle, and has been specifically identified as under pressure from agricultural practices. Latest river Q values collected by the EPA here were between Q3-4 indicating moderate water quality. Inclusion in this study was on the basis of an at risk site specifically to examine presence of compounds related to agriculture (i.e. pesticides).

Figure 41. Photograph of the sampling site for the River Annalee in County Cavan

Lastly, the River Liffey (Figure. 42) is located in the east of Ireland, flowing 132km from its source in the Wicklow Mountains northwards before meeting the Irish Sea at Dublin Bay. It supplies water to the majority of the Dublin area, which itself is home to more than a quarter of the total Irish population. The Liffey is currently considered at risk under the WFD, and has been identified for pressures from both urban run-off and urban wastewater. Q values were between Q3-4 indicating moderate water quality. It was chosen for inclusion to represent sites at risk for the presence of contaminants related to urban sources such as pharmaceuticals.

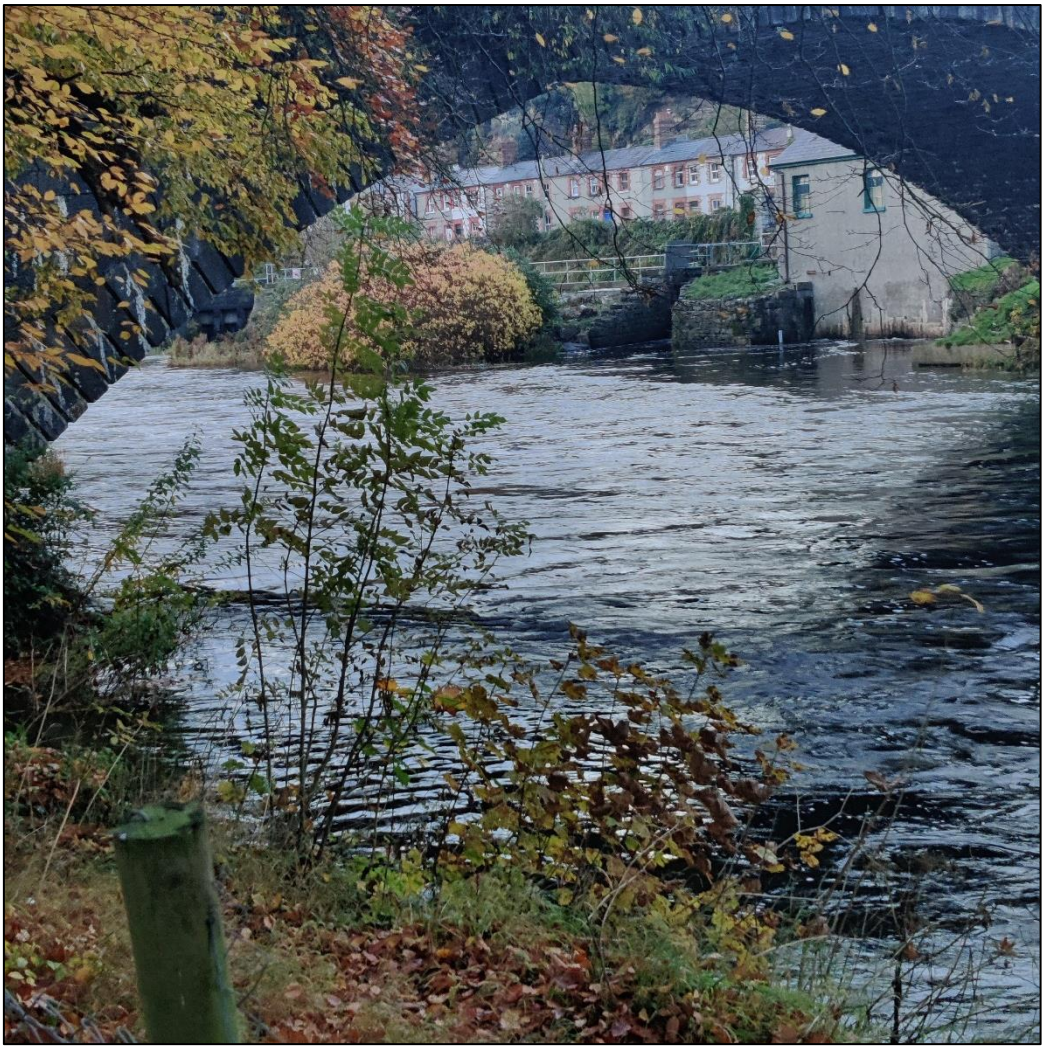


Figure 42. Photograph of the River Liffey at Lucan Bridge where samples for Watch List analysis were collected.

3.3.2 Physicochemical field measurement results and variation

Field measurements for dissolved oxygen (DO), pH, temperature, turbidity and conductivity were taken on all sampling events from 2020 onwards. Physico-chemical factors such as these can have a direct influence on the behaviour and analysis of CECs, making collecting and interpretation of these results important. Full tabulated results of these measurements can be found in Table 40.

Conductivity in all four sites was in the range of approximately 200 – 630 µS/cm, which is considered typical for surface water systems (Figure 43) [307]. Conductivity is a useful parameter for water quality monitoring as it has a direct relationship to the concentration of inorganics in the waterbody, and can give an indication of changes in the introduction of chemicals into

a river if routinely monitored [308]. The Annalee was found to have the lowest overall conductivity levels between the studied sites possibly indicating lower emissions. Each site saw an expected increase in this measurement approaching the summer months when temperatures rise, causing increased solubility of substances, following by the inverse when temperatures begin to drop [308].

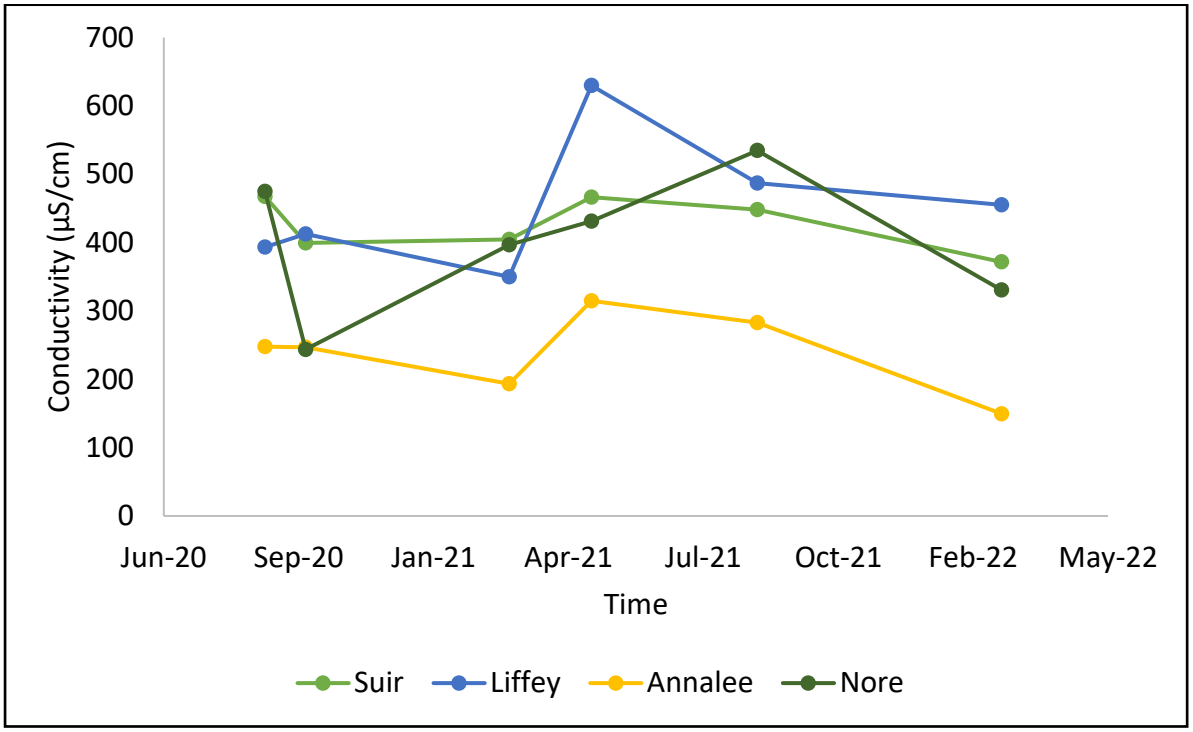


Figure 43 Levels of conductivity recorded in four Irish rivers from 2020-2022.

Levels of pH were also monitored for all four sites from 2020-2022, shown in Figure 44. This parameter was observed to be slightly more alkaline across all sites. This can likely be due to the majority of Ireland being composed of limestone, which in turn influences the levels of Ca and HCO_3 in our rivers, and thus the pH [309]. However, higher variability in the pH of the river Annalee was observed when compared to that of the other sites, indicating that there is possibly more influence from nearby land practices here than at the other locations. The Annalee has been identified as at risk for agricultural point pressures including pasture and forestry, and agricultural practices have been previously linked with changes in surface water pH [310], suggesting a possible causal relationship.

Turbidity was a parameter which showed some of the largest variation over the years, ranging from 0.32 – 21.75 FNU, with both the minimum and the maximum values coming from the river Nore (Figure. 44). A link between levels of turbidity and rainfall events was seen throughout the sampling campaign, where increased rainfall directly related to increases in turbidity. Previous literature has demonstrated a link between rainfall and turbidity [311]. This is likely due to the combination of soil from riverbanks being washed off into the waterbody, and the re-suspension particulates from the riverbed into the water phase. There was below average rainfall documented in September 2020, followed by above average rainfall in October [312,313]. This is particularly well shown in the spike in turbidity seen in the Nore at this point, when it was raining heavily during sample collection. This was again seen in March 2022, when there was heavy rain across Ireland. This was particularly pronounced in the south east of the country causing multiple flooded roads, and can be demonstrated by the high levels of turbidity in both the Suir and the Nore at this time. Turbidity was found to cause significant matrix effects (ME) with certain analytes during the analysis steps. Elevated turbidity levels directly related to more matrix interferences. Matrix effects were shown in the Method Performance section for the relevant methods in Chapter 2 sections 2..3.1.1.4 and 2.3.1.1.8 of this thesis, and ranged from significant suppression (10% ME - Erythromycin) to significant enhancement (4000% ME – amoxicillin). While sample clean-up procedures are employed (filtration and solid phase extraction) to minimise ME, is it impossible to remove all matrix interferences from environmental samples. This has significant implications for future monitoring in light of the changes in rainfall caused by climate change. Increased rainfall and storms are predicted to affect many areas including Ireland [314,315], which in turn will likely cause an increase in matrix interferences. It is therefore recommended for future monitoring campaigns that turbidity measurements be recorded at the time of sampling to help account for matrix effects.

Regular seasonal variation in temperature was seen in all four studied sites, ranging from lows of 6-8 °C in late winter/early spring in March to 15-17 °C in early summer in May (Figure. 44). Variations in DO differed between studied sites, with the Annalee and the Nore exhibiting a relatively similar pattern of lower DO levels following the summer period when there is increased water temperature therefore decreasing the dissolvability of oxygen, followed by

higher DO levels following the inverse after the winter months (Figure 44) [316] . The low of 7.12 $mg.L^{-1}$ in the Annalee in September 2020 is lower than ideal for some more sensitive aquatic organisms [317], however this dip wasn't as pronounced the following year.

The Suir however, showed very little variation in DO during the study period, staying consistently around the 10 mg L^{-1} level, which indicates a healthy level for a river[317]. The Liffey showed variation in DO levels but these were not consistent with the same seasonal pattern as the Suir and Annalee, showing an increase in DO in September 2021 when a decrease was seen at the other sites. A possible cause for elevated DO outside of regular patterns are anthropogenic factors such as urban run-off , which can initially increase oxygen input during the day before eventually leading to lower DO levels through increased oxygen demand from overgrowth of microorganisms. The Liffey was identified as at risk for urban run-off under the WFD, making this a likely explanation.

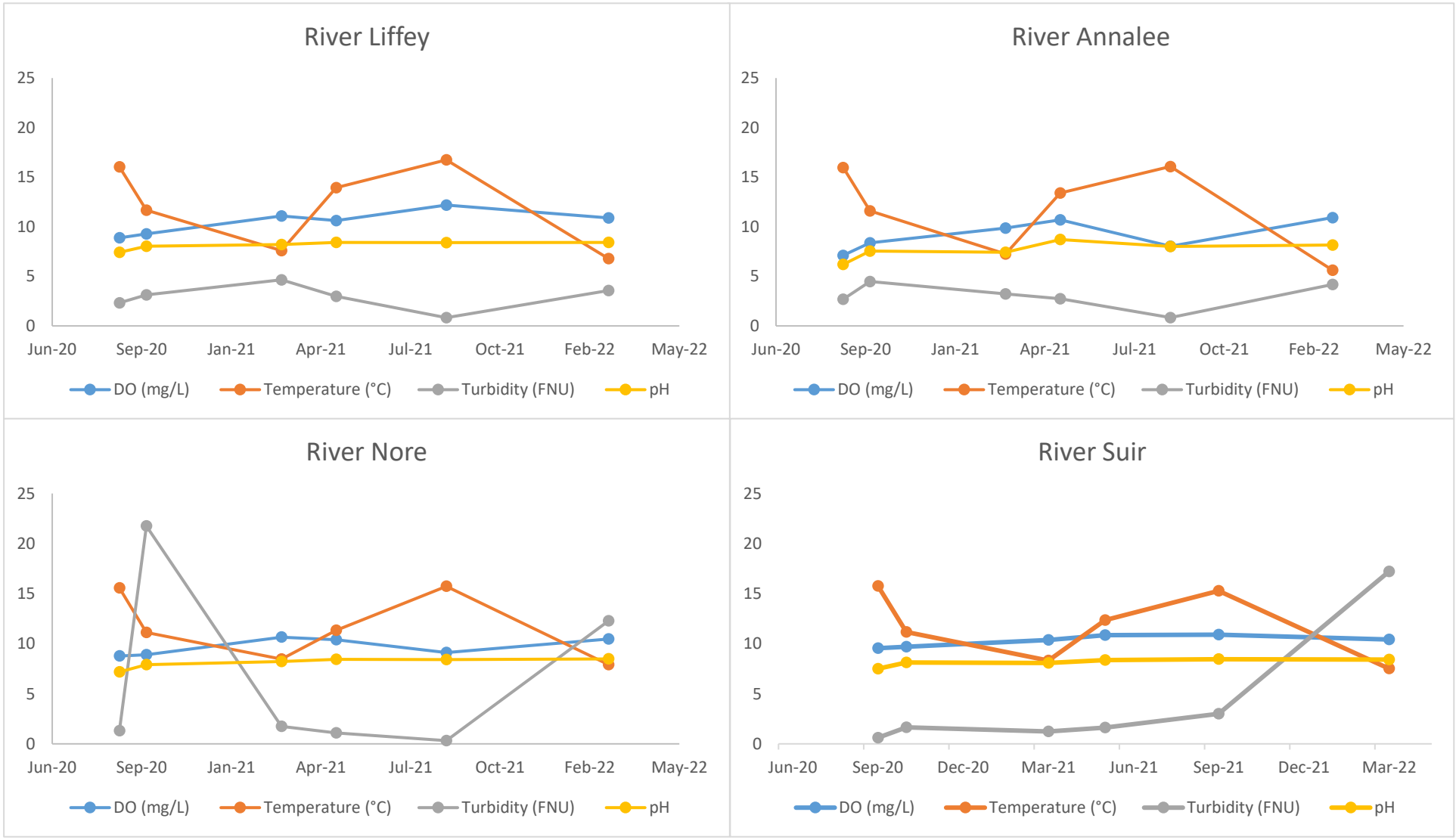


Figure 44. Graph showing seasonal variation in temperature, turbidity, pH and Dissolved Oxygen (DO) levels in four Irish rivers from 2020 - 2022

Table 40. Field measurements for DO, temperature, turbidity, pH and conductivity of four Irish Rivers (Liffey, Suir, Nore and Annalee) from 2020-2022.

	Sample	DO (mg L^{-1})	Temperature (°C)	Turbidity (FNU)	pH	Conductivity (µS cm^{-1})
River Suir	**Sep-20**	9.55	15.77	0.61	7.5	467.5
	Oct-20	9.70	11.18	1.64	8.13	399.3
	Mar-21	10.36	8.32	1.24	8.07	404.8
	May-21	10.86	12.36	1.62	8.36	466.3
	Sep-21	10.90	15.28	3.01	8.45	448.4
	Mar-22	10.41	7.51	17.22	8.42	371.7
	Average	10.30	11.74	4.22	8.16	426.3
	± SD	0.57	3.44	6.42	0.36	39.9
River Liffey	**Sep-20**	8.88	16.05	2.33	7.42	393.5
	Oct-20	9.29	11.68	3.12	8.03	412.4
	Mar-21	11.09	7.60	4.65	8.22	349.9
	May-21	10.62	13.94	2.99	8.42	629.8
	Sep-21	12.19	16.75	0.83	8.41	486.9
	Mar-22	10.90	6.79	3.56	8.42	455.2
	Average	10.50	12.13	2.91	8.15	454.6
	± SD	1.22	4.22	1.28	0.39	98.2
River Annalee	**Sep-20**	7.12	15.97	2.7	6.2	247.9
	Oct-20	8.38	11.60	4.48	7.55	246.8
	Mar-21	9.88	7.25	3.24	7.42	193.6
	May-21	10.71	13.40	2.73	8.71	314.8
	Sep-21	8.04	16.08	0.84	8.02	282.9
	Mar-22	10.93	5.61	4.18	8.16	149.6
	Average	9.18	11.65	3.03	7.68	239.3
	± SD	1.55	4.41	1.30	0.86	59.7
River Nore	**Sep-20**	8.77	15.573	1.31	7.18	475.1
	Oct-20	8.9	11.111	21.75	7.9	243.7
	Mar-21	10.65	8.464	1.75	8.23	396.5
	May-21	10.39	11.34	1.09	8.43	431.3
	Sep-21	9.11	15.727	0.32	8.42	534.6
	Mar-22	10.46	7.91	12.29	8.48	330.8
	Average	9.71	11.69	6.42	8.11	402.0
	± SD	0.87	3.36	8.75	0.50	103.9

3.3.3 Occurrences of Watch List substances

3.3.3.1 2nd Watch List Compounds

The methods for analysis of 2nd 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. Final concentrations detected in these samples is shown in Table 41. Most detections were found to be below quantitation levels. Thiamethoxam, erythromycin and acetamiprid were the CECs which occurred at the biggest individual concentrations in a single sample, with 69 ng L^{-1} of thiamethoxam and 19 ng L^{-1} of acetamiprid (Figure 45) being found in the December 2018 River Suir sample. In the August 2019 River Liffey sample 36 ng L^{-1} of erythromycin was also detected (Figure 46). Despite being banned for all outdoor use in early 2018 alongside other neonicotinoid pesticides clothianidin and imidacloprid [318], thiamethoxam was detected in all 5 samples taken during December 2018.

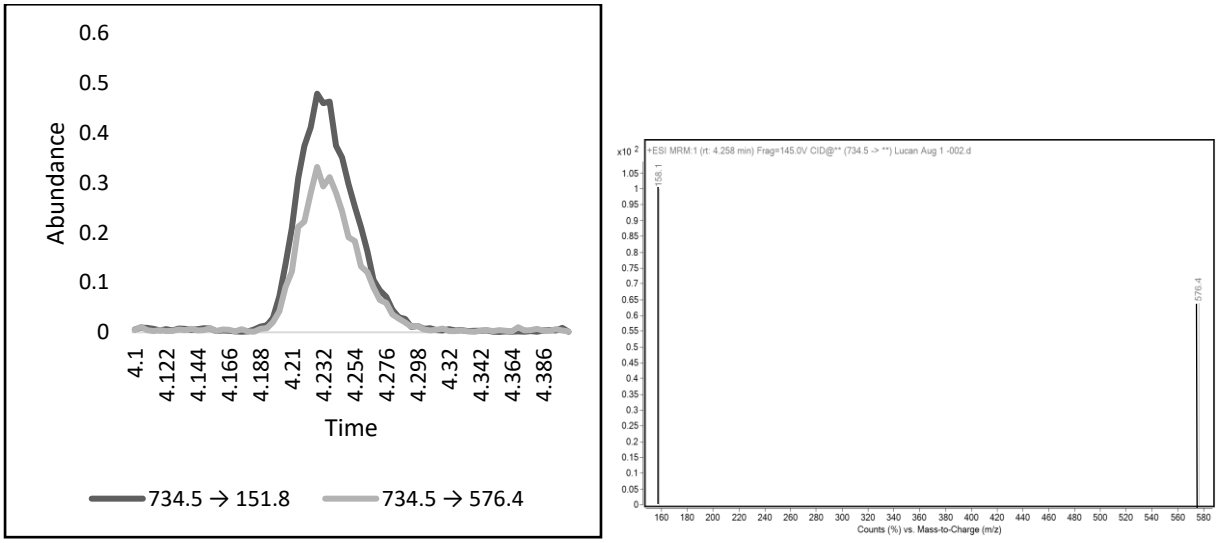


Figure 45. MRM chromatogram (left) and accompanying mass spectrum (right) showing detection of 36 ng L^{-1} of erythromycin in the River Liffey in August 2019

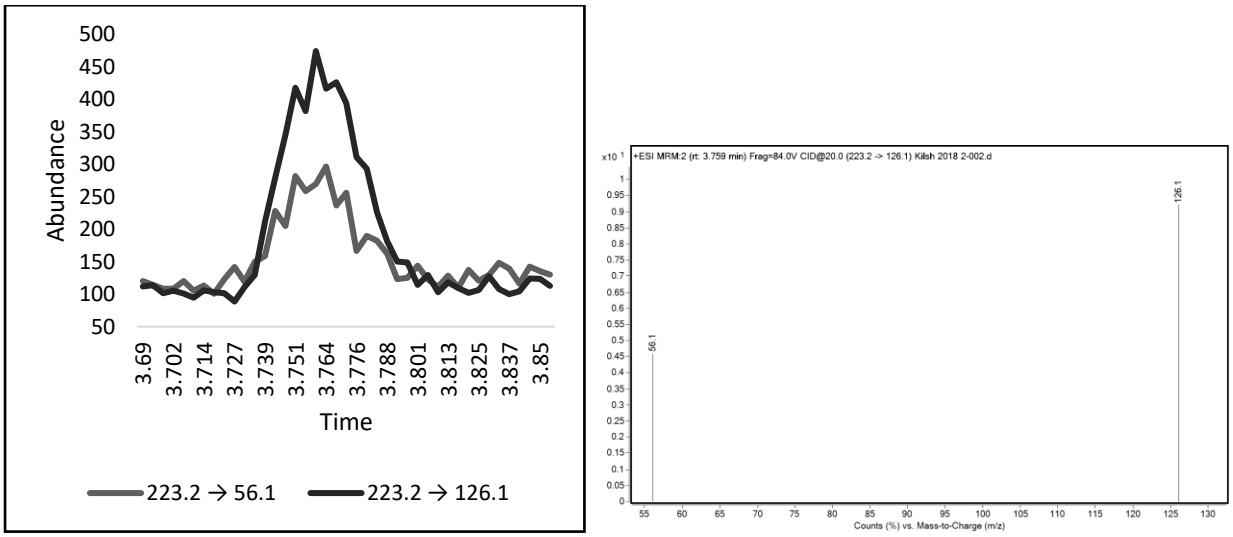


Figure 46. MRM chromatogram (left) and accompanying mass spectrum (right) showing detection of 19 ng L^{-1} of acetamiprid in the River Suir in December 2018

All 15 of the compounds found on the WL were detected in at least four samples over the 3 years. The least frequently detected CECs were amoxicillin, ciprofloxacin, metaflumizone and methiocarb. These results are generally similar to those found by other EU countries as stated in the review of the 1st Watch List, in which clarithromycin and estrone were among the most frequently detected compounds at quantifiable levels, and methiocarb was one of the least. Clarithromycin, estrone and diclofenac had a quantification frequency in samples taken in 25 EU member states of over 50%, whereas methiocarb had a quantification frequency of below 1% [276].

The hormones 17-alpha-ethinylestradiol and 17-beta-estradiol were detected in all 2018 and 2019 samples taken, however the majority of detections were below LOQ. A comparatively very large concentration of 58 ng L^{-1} was detected of 17-alpha-ethinylestradiol in the October 2020 River Suir sample. Estrone was detected in all 21 samples taken over the three-year period however, it was only quantifiable in the Annalee September 2020 sample, as well as the Liffey samples from October and September 2020. The estrogen group of compounds is found in both urban and rural water samples as they are both used for medications such as the contraceptive pill and hormone therapy treatments, as well as commonly used in agriculture as growth-regulators in livestock [319].

The next most frequently detected analyte was clarithromycin which was detected in 17 out of 21 samples, however the majority of these were below the quantitation limits. 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 HSE, so its prevalence is not unexpected, particularly in higher concentrations in urban areas [320]. The other macrolides were the next most frequently detected compounds, with azithromycin and erythromycin both having a frequency of 57% as seen in Figure 47. Antibiotics such as the macrolides are also commonly used in the agricultural sector to prevent diseases spreading through livestock, and so the high frequency of these compounds occurring in rural areas too is also unsurprising. These findings are in line with those found by Sousa *et al.* in which the three macrolide antibiotics were among the most frequently detected compounds in Portuguese rivers [256]. Clarithromycin and erythromycin were also found by Jurado *et al.* to be the most frequently detected compounds in Spanish waters [2].

The neonicotinoids imidacloprid and thiamethoxam were the next most commonly found compounds, with 12 and 10 detections respectively. This is again reflected by Sousa *et al*.'s findings in which imidacloprid and thiamethoxam were frequently detected in their samples [256]. Most of the neonicotinoid detections occurred between in the August and September months, which correlates roughly with spraying and harvesting seasons. This seasonality is particularly well demonstrated by 3 the detections above LOD found of acetamiprid in the September 2020 set of samples compared to the below LOD or non-detects in the October 2020 samples.

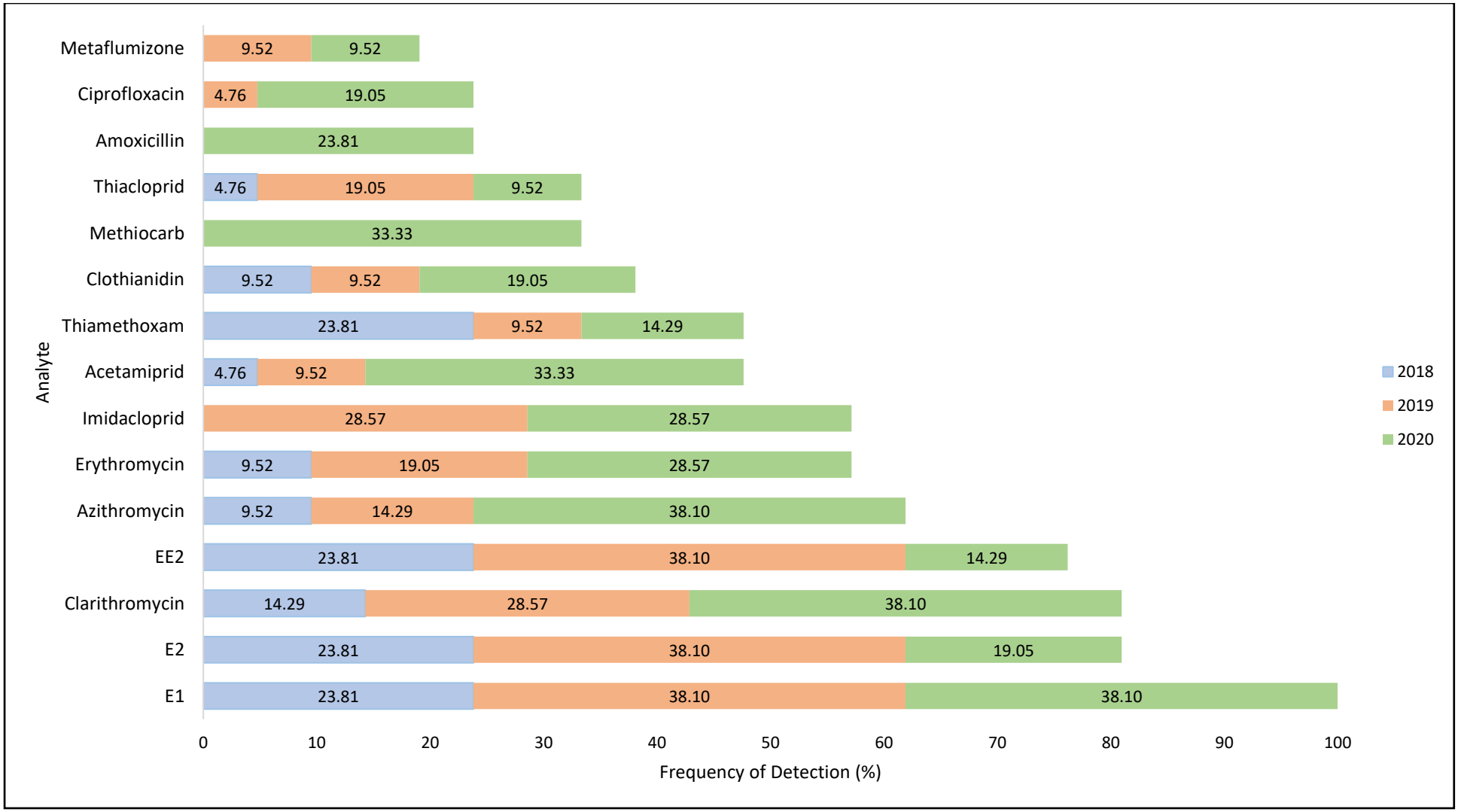


Figure 47 Bar chart showing occurrence frequency of 2nd watch list substances over period 2018-2020

Table 41. Table of sample quantitation results for 2nd watch list substances

Analyte Concentration (ng L^{-1} ± S.D.)*															
Sample	**E2[d]**	**EE2[d]**	**E1**	**Acetamiprid**	**Amoxicillin**	**Azithromycin**	**Ciprofloxacin[a]**	**Clarithromycin**	**Clothianidin**	**Erythromycin**	**Imidacloprid**	**Metaflumizone[b]**	**Methiocarb**	**Thiacloprid**	**Thiamethoxam[b]**
Suir Dec 2018	<LOD	<LOD	<LOD	19 ± 6	ND	ND	ND	ND	ND	17	ND	ND	ND	<LOD	69 ± 22
Liffey Dec 2018	<LOD	<LOD	<LOD	ND	ND	9	ND	12	3 ± 0.5	<LOD	ND	ND	ND	ND	19 ± 14
Annalee Dec 2018	7 ± 11	6 ± 10	<LOD	ND	ND	ND	ND	<LOD	ND	ND	ND	ND	ND	ND	47
Nore Dec 2018	5 ± 7	5 ± 8	<LOD	ND	ND	<LOD	ND	<LOD	5 ± 0.4	ND	ND	ND	ND	ND	41 ± 10
Shannon Dec 2018	<LOD	<LOD	<LOD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	38
Suir Jul 2019	6 ± 3	5 ± 2	<LOD	ND	ND	ND	<LOD	ND	ND	ND	ND	<LOD	ND	<LOD	ND
Liffey Jul 2019	<LOQ	<LOQ	<LOD	2	ND	8 ± 2	ND	<LOD	ND	<LOD	8 ± 4	ND	ND	<LOD	2 ± 1
Annalee Jul 2019	<LOQ	3 ± 4	<LOD	ND	ND	ND	ND	<LOD	ND	ND	6 ± 1	ND	ND	ND	ND
Nore Jul 2019	<LOD	<LOQ	<LOD	ND	ND	<LOD	ND	<LOD	ND	14	<LOD	ND	ND	ND	ND
Suir Aug 2019	<LOD	65 ± 101	<LOD	2	ND	ND	ND	<LOD	<LOD	ND	<LOD	<LOD	ND	<LOD	2 ± 1
Liffey Aug 2019	31 ± 46	19 ± 33	<LOD	ND	ND	<LOD	ND	7 ± 1	<LOD	36 ± 4	8 ± 1	ND	ND	<LOD	ND
Annalee Aug 2019	37 ± 59	3 ± 4	<LOD	ND	ND	ND	ND	<LOD	ND	24	<LOD	ND	ND	ND	ND
Nore Aug 2019	<LOQ	4 ± 4	<LOD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Suir Sept 2020	<LOD	ND	<LOD	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	ND	ND	15 ± 0.2
Liffey Sept 2020	ND	<LOD	5 ± 4	10 ± 1	<LOD	<LOD	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	ND

Annalee Sept 2020	ND	<LOD	4 ± 1	11 ± 5	ND	<LOD	ND	<LOD	ND	ND	4 ± 0.4	ND	<LOD	ND	ND
Nore Sept 2020	<LOD	ND	<LOD	4 ± 2	ND	<LOD	**<LOD	<LOD	<LOD	ND	<LOD	ND	<LOD	ND	ND
Suir Oct 2020	<LOD	58	<LOD	<LOD	<LOD	<LOD	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	ND
Liffey Oct 2020	<LOD	ND	3 ± 4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	8 ± 4	3 ± 1	<LOD	<LOD	ND	ND
Annalee Oct 2020	ND	ND	<LOD	ND	<LOD	<LOD	ND	<LOD	ND	<LOD	ND	ND	<LOD	ND	<LOD
Nore Oct 2020	ND	ND	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	ND	<LOD	<LOD	<LOD

3.3.3.2 3rd Watch List Compounds

The occurrence of 3rd Watch List chemicals was monitored in Irish samples from March 2021 to March 2022, with the total number of samples taken being 16 (with each sample analysed in triplicate). The full results from this sample analysis are presented in Table 42.

The largest individual analyte concentration spikes detected were in the River Suir, where in May 2021 32 ng L^{-1} of trimethoprim and 25 ng L^{-1} of ciprofloxacin were found (Figures 48 and 49). 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 has also been known to be prescribed for other ailments such as chest infections. Ciprofloxacin is a broad application antibiotic used for a range of purposes, also including 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 been continually shown to cause lasting lung damage following even minor infections [321], possibly making recovered patients more susceptible to later bacterial infections and leading to an increase in the use of these antibiotics.

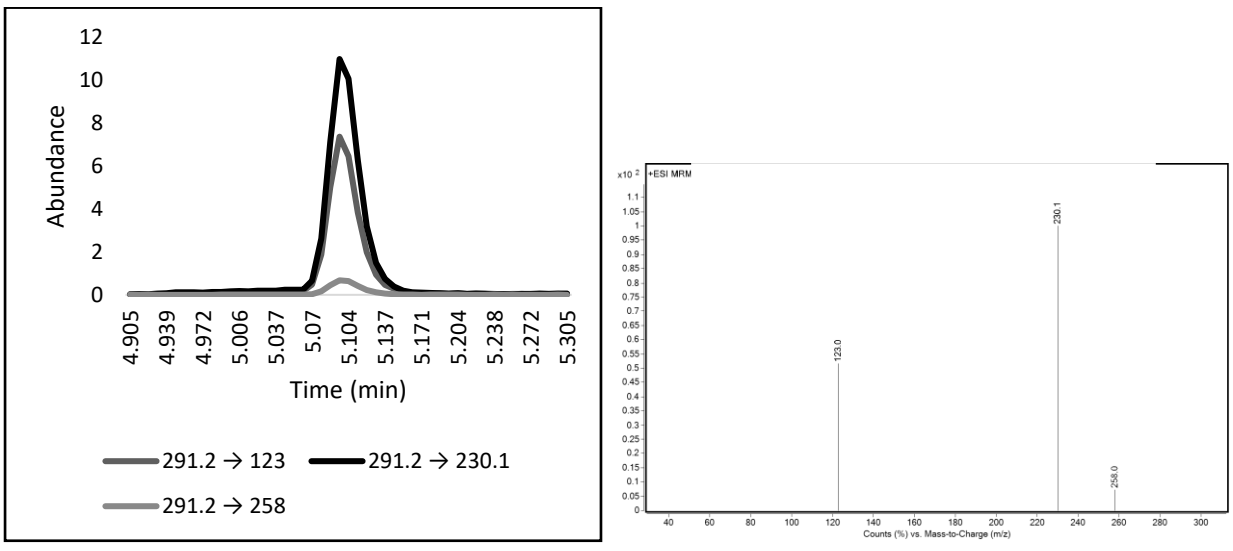


Figure 48. MRM chromatogram (left) and mass spectrum (right) showing detection of 36 ng L^{-1} of trimethoprim in the River Suir in May 2021.

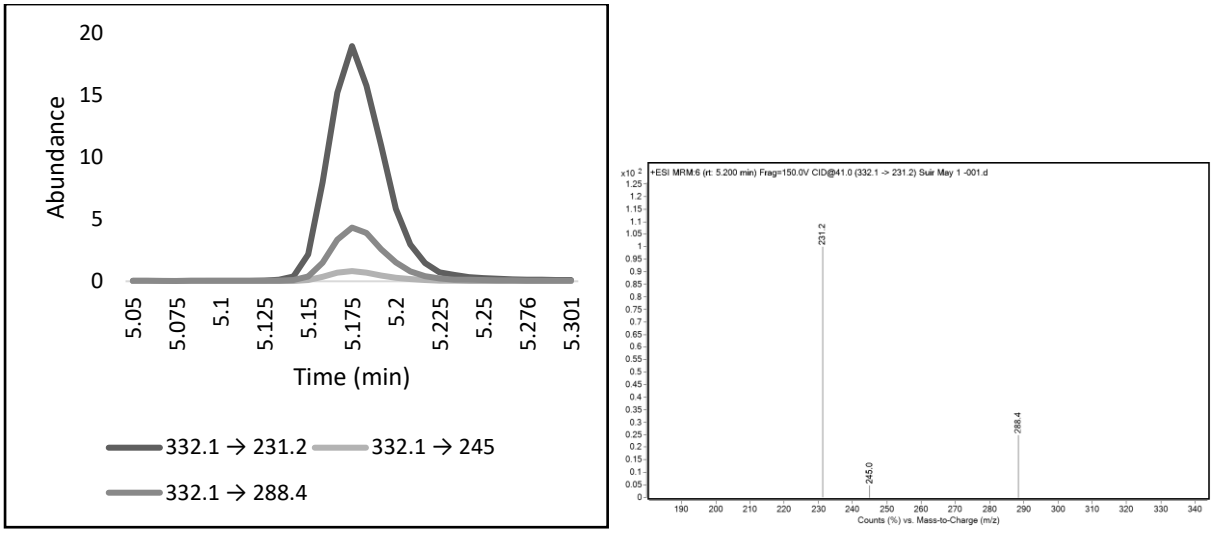


Figure 49. MRM chromatogram (left) and mass spectrum (right) showing detection of 25 ng L^{-1} of ciprofloxacin in the River Suir in May 2021.

The River Suir in fact was the site with the highest number of quantifiable occurrences out of all sites studied over the sampling campaign, showing presence of 27 ng L^{-1} in September and 12 ng L^{-1} in May of prochloraz, as well as 11 ng L^{-1} September and 6 ng L^{-1} in May of tebuconazole. These are both azole compounds used for the prevention of fungal infections in plants. The highest volume of azoles sold worldwide is in Europe, accounting for more than a third of the world's total sales [300]. Both tebuconazole and prochloraz were listed among the top 10 most extensively used active substances in Ireland for specific arable crops, according to a report produced by the Department of Agriculture [65] . Arable crops are generally harvested in the summer to autumn months, making the occurrence of these compounds at quantifiable concentrations in May and September more likely. Increased rainfall in the autumn causing run-off could also be a potential cause for the higher concentrations seen in September for both analytes.

In addition to these occurrences, the presence of 12 ng L^{-1} of venlafaxine in March 2021 and 4.7 ng L^{-1} of clotrimazole in March 2022 were found in the Suir. Clotrimazole was a frequently detected compound occurring in >85% of samples, however, it was only found at quantifiable levels in March 2022. Clotrimazole is also a member of the azole family, however, it is generally used as a pharmaceutical for the treatment of common fungal infections including

athlete's foot, thrush and ringworm. It is available over the counter in Ireland and is one of the most popular of pharmaceutical azole compounds [322].

The Liffey site was found to have the most quantifiable detections, notably 20 ng L^{-1} of sulfamethoxazole and 17 ng L^{-1} of tebuconazole were found in September 2021 (Figures 50 and 51). Sulfamethoxazole is a common sulphonamide 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:trimethoprim [323]. This ratio is interestingly reflected in the ratio of occurrences of these analytes in field samples, where they were found in a ratio of 4.67:1. Sulfamethoxazole has been found on multiple occasions in other EU member states, up to a maximum concentration found in Germany of 469 ng L^{-1}. By comparison, the detected concentration found here is significantly lower, and much more comparative to the level found in Romania (30 ng L^{-1}) [324]. The population density between Ireland and Romania is similar, at 72 and 84 inhabitants per km^2 respectively, whereas the density in Germany is 233 inhabitant's per km^2, which could potentially explain these differences in concentrations [325].

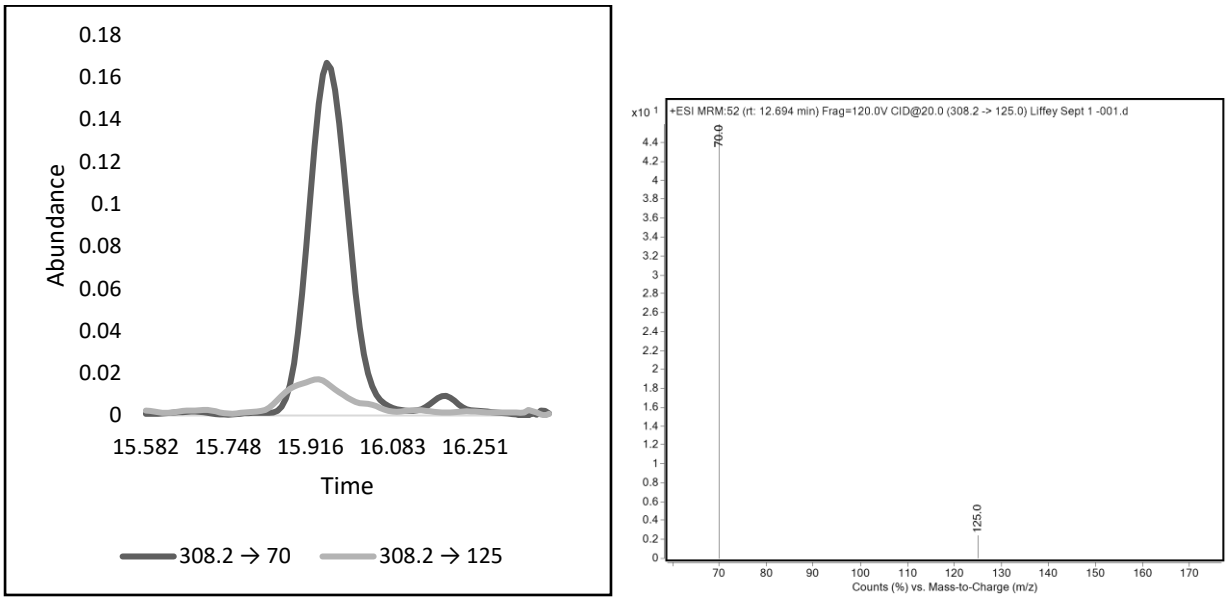


Figure 50 MRM chromatogram (right) and mass spectrum (left) showing detection of 17 ng L^{-1} of tebuconazole and 20 ng L^{-1} of sulfamethoxazole in the River Liffey in September 2021

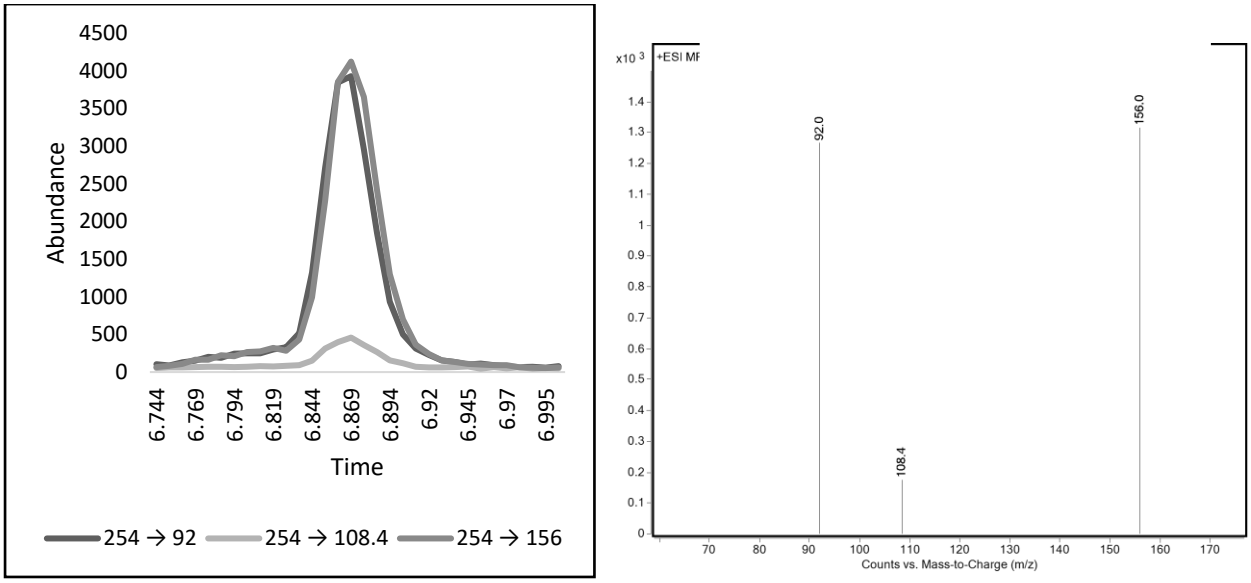


Figure 51. MRM chromatogram (right) and mass spectrum (left) showing detection of 20 ng L^{-1} of sulfamethoxazole in the River Liffey in September 2021

The Annalee and the Nore showed considerably less high-level detections, with both rivers only showing presence of three analytes each above LOQ. These being venlafaxine and amoxicillin for both, and then dimoxystrobin for the Annalee and fluconazole for the Nore.

However, while some notable detections above LOQ were found, most analyte detections in environmental samples were below quantitation levels. Similarly to the previous list, occurrence frequency was again high, with all samples showing presence of CEC contamination. A bar chart showing total analyte occurrence frequency across the study period can be seen in Figure 52. All analytes were detected in at least 3 samples, and 14 out of a total 19 analytes were detected in 50% or more samples.

The most frequently detected analytes were venlafaxine and miconazole, which were detected in every sample aside from the Suir sample taken in March 2022, equating to an occurrence frequency of 93.75% each.

Venlafaxine was also the analyte most frequently detected at quantifiable levels, with occurrences ranging from 8-14 ng L^{-1}. The majority of quantified detections occurred in March 2021. Venlafaxine is a Serotonin and 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 [326]. In addition to this, is it also interesting to consider the possible impact the COVID-19 pandemic has had on the high occurrence frequency of this compound. An article published in January of 2021 by the Irish Examiner in conjunction with the Irish Pharmacy Union showed that more anti-depressant medication was dispensed every month in 2020 than the year before [327]. The likelihood for this trend to have continued into the rest of 2021, and in particular during the first half of the year when Ireland had the most stringent lockdown of any country in the EU [328], is quite high. It is clear from these results that presence of this compound is already ubiquitous in Ireland's water systems, and trends indicate that high level detections are likely to continue without change in prescriptions or policy. Venlafaxine has been found at concentrations above its PNEC value in the aquatic environments of multiple countries within the EU. Concentrations have ranged from <1 ng L^{-1} (UK) to 159 ng L^{-1} (Portugal) [324]. The results found for Irish rivers are in line with the findings from other member states.

Miconazole, while also occurring in all but one sample, was never detected above the LOQ showing a persistent but low level presence across the country. Miconazole is an anti-fungal medication available over the counter in Ireland, and is very frequently used for the treatment of fungal skin infections as well as for treating oral thrush in infants [322]. In addition to this, it is also used to treat fungal infection in animals, making its presence in a variety of waterbodies from urban to rural areas a likely outcome. Miconazole has been found in surface waters on multiple occasions in China, as well as in the UK, Poland and Spain. In these studies it was generally found in the low ng L^{-1} level, reflecting the results seen in Irish rivers [329].

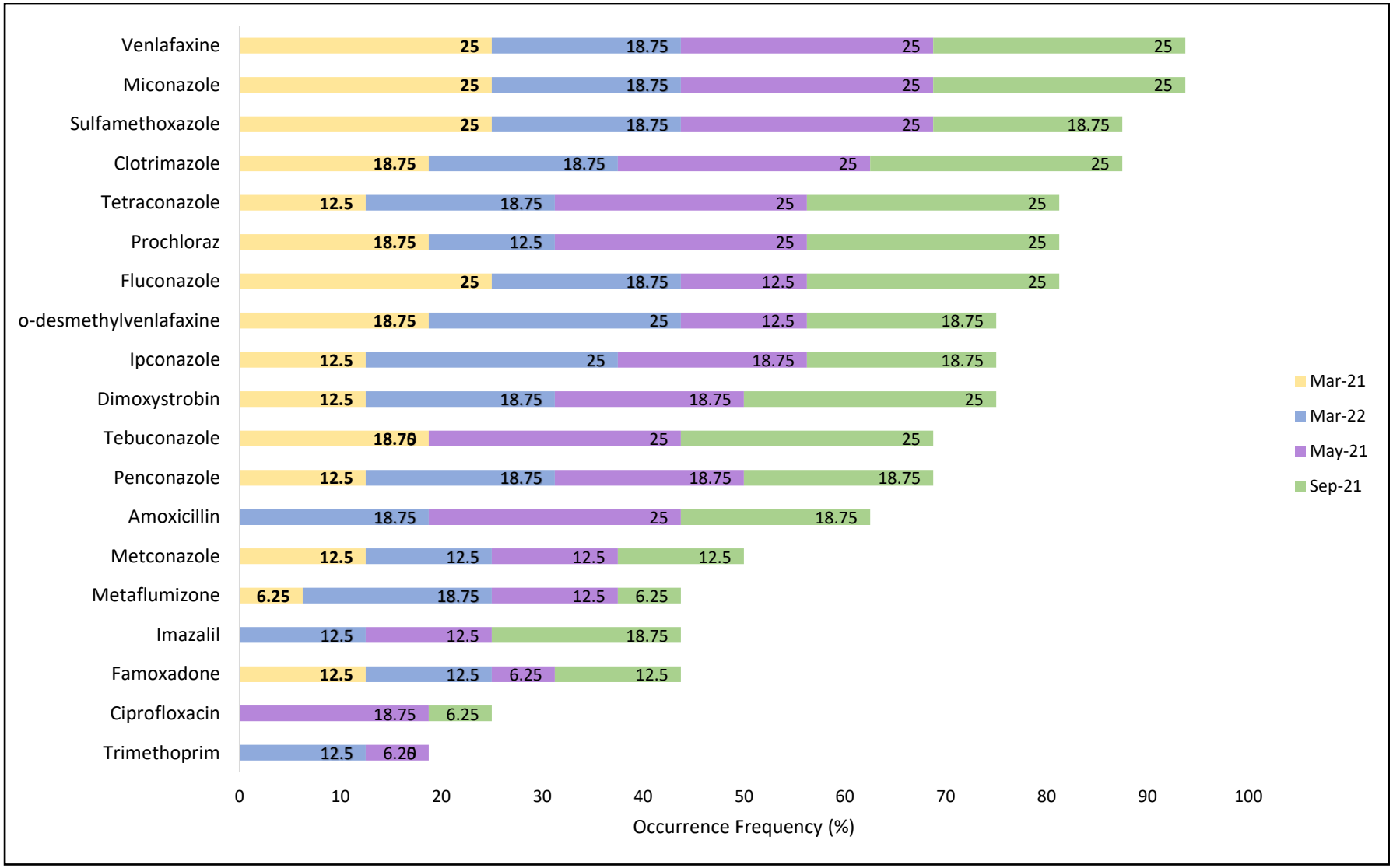


Figure 52. Bar chart showing occurrence frequency of 3rd watch list substances over period 2021-2022

Table 42 Table of sample quantitation results for 3nd watch list substances

	Analyte occurrences (ng L^{-1})															
	March 2021				May 2021				September 2021				March 22			
	A	**L**	**N**	**S**	**A**	**L**	**N**	**S**	**A**	**L**	**N**	**S**	**A**	**L**	**N**	**S**
Amoxicillin	ND	ND	ND	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	**8.9 ± 0.9**	**8.8 ± 3.5**	**9.5 ± 3.2**	ND
Ciprofloxacin	ND	ND	ND	ND	ND	*<LOQ*	*<LOQ*	**25 ± 13**	ND	ND	*<LOQ*	ND	ND	ND	ND	ND
Clotrimazole	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	**4.35 ± 0.1**	ND	**4.69 ± 0.9**
Dimoxystrobin	*<LOQ*	*<LOQ*	ND	ND	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	**3.63 ± 1.9**	**5.8 ± 5.6**	*<LOQ*	ND
Famoxadone	*<LOQ*	ND	ND	*<LOQ*	ND	ND	ND	*<LOQ*	*<LOQ*	*<LOQ*	ND	**ND**	*<LOQ*	ND	ND	*<LOQ*
Fluconazole	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	**23.8 ± 21.5**	*ND*
Imazalil	ND	ND	ND	ND	ND	*<LOQ*	ND	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	ND
Ipconazole	ND	*<LOQ*	ND	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*
Metaflumizone	ND	ND	ND	*<LOQ*	ND	*<LOQ*	ND	*<LOQ*	ND	ND	ND	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*
Metconazole	ND	*<LOQ*	ND	*<LOQ*	ND	*<LOQ*	ND	*<LOQ*	ND	ND	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	ND
Miconazole	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND
O-desmethylvenlafaxine	*<LOQ*	*<LOQ*	*<LOQ*	ND	*<LOQ*	**3 ± 1**	ND	ND	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*
Penconazole	*<LOQ*	*<LOQ*	ND	ND	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	ND	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*

Prochloraz	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	**12 ± 2**	*<LOQ*	*<LOQ*	*<LOQ*	**27 ± 3**	ND	*<LOQ*	ND	*<LOQ*
Sulfamethoxazole	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND	**20 ± 2**	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND	*<LOQ*
Tebuconazole	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	**6 ± 1**	*<LOQ*	**17 ± 6**	*<LOQ*	**11 ± 1**	ND	ND	ND	ND
Tetraconazole	ND	*<LOQ*	*<LOQ*	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND
Trimethoprim	ND	ND	ND	ND	ND	ND	ND	**32 ± 10**	ND	ND	ND	ND	ND	*<LOQ*	ND	*<LOQ*
Venlafaxine	*<LOQ*	**10 ± 6**	**12 ± 0.6**	**12 ± 1**	**14 ± 8**	*<LOQ*	*<LOQ*	*<LOQ*	**12 ± 5**	*<LOQ*	**8 ± 5**	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND

3.3.4 Assessment of risk posed by Watch List Chemicals

Target detection limits are set by the watch list based on the analytes PNEC (Predicted No-Effect Concentration) and therefore individual occurrences below these values are thought to be unlikely to have harmful effects [49]. Therefore, the PNEC values stated in the related Watch Lists were used for determination of risk. As there were multiple detections in field samples over the target limits set by the EU, a risk assessment by calculation of Risk Quotient (RQ) values was performed in order to better understand the potential impact these occurrences may have in Irish waters. Risk in the context of chemical occurrence in environmental matrices can be thought of as the relationship between the ability of a chemical to cause adverse effects, and how much of the chemical is present in the environment [330].

Every analyte contained on both 2nd and 3rd WLs was detected at least once in Irish samples and therefore their risk was assessed. As many detections were only ever at unquantifiable levels, a range of RQ values was generated based on the method LOD and LOQ, to present a best to worst-case scenario for a given analyte. A table of all RQ values generated can be seen in Table 43.

Table 43. Table of RQ values for CECs detected from both the 2nd and 3rd Watch Lists. RQ values generated from the highest Measured Environmental Concentration (MEC) in Irish surface water samples.

Analyte	RQ from highest MEC	Risk category	Occurring in sample
Tetraconazole	0.0005 – 0.008	Low	Multiple <LOQ
Penconazole	0.004 - 0.006	Low	Multiple <LOQ
Imazalil	0.002 – 0.003	Low	Multiple <LOQ
Miconazole	0.01 – 0.015	Low	Multiple <LOQ
Tebuconazole	0.071	Low	Liffey September 2021
Fluconazole	0.095	Low	Nore March 2022
Metaflumizone	0.046 – 0.139	Low - *Moderate*	Multiple <LOQ
Ipconazole	0.047 – 0.142	Low - *Moderate*	Multiple <LOQ
Metconazole	0.073 – 0.221	Low- *Moderate*	Multiple <LOQ
Famoxadone	0.121 – 0.368	*Moderate*	Multiple <LOQ
Amoxicillin	0.122	*Moderate*	Nore March 2022
Thiacloprid	0.157 – 0.482	*Moderate*	Multiple <LOQ
Prochloraz	0.168	*Moderate*	Suir September 2021
Dimoxystrobin	0.181	*Moderate*	Liffey March 2022
Sulfamethoxazole	0.200	*Moderate*	Liffey September 2021
Clotrimazole	0.235	*Moderate*	Suir March 2022
Ciprofloxacin	0.281	*Moderate*	Suir May 2021
Trimethoprim	0.320	*Moderate*	Suir May 2021
Clothianidin	0.361	*Moderate*	Liffey December 2018
Azithromycin	0.474	*Moderate*	Liffey December 2018
O-desmethylvenlafaxine	0.500	*Moderate*	Liffey May 2021
Clarithromycin	0.632	*Moderate*	Liffey December 2018
Imidacloprid	0.964	*Moderate*	Liffey August 2019
Erythromycin	1.895	High	Liffey August 2019
Acetamiprid	2.289	High	Suir December 2018
Venlafaxine	2.333	High	Annalee May 2021
Methiocarb	1.015 - 3.05	High	Multiple <LOQ
Thiamethoxam	8.313	High	Suir December 2018
Estrone (E1)	42.7 - 295	High	Multiple <LOQ
Estradiol (E2)	1233.333	High	Annalee August 2019
Ethinylestradiol (EE2)	2166.667	High	Suir August 2019

Most low and low-moderate risk analytes were found to be the pesticides, making up 88.9% of low and low-moderate risk analytes (Figure 53). This is likely due to the comparatively high PNEC values for some of the azole compounds, which are in the low $\mu g.L^{-1}$ rather than low ng

L^{-1} range of most other chemicals on the lists. However, much is still unknown regarding the effects and associated risks of these compounds occurring in mixtures. In fact, it was suggested by the JRC in the selection of substances for the 3rd WL that it could be prudent to consider these analytes using an accumulative approach for risk assessment [51]. Research in the area of mixture effects is still ongoing, and with no available PNEC value for azole mixtures it is not possible at this time to produce a cumulative RQ value for these compounds in Irish samples. With this in mind, even with most real detections being low, risk it is impossible to confidently conclude that there is no potential for hazardous effects at all.

Out of all analytes for which an RQ value was generated, pharmaceuticals make up over half of the moderate and the majority of high risk chemicals. RQ values in particular for estrogenic hormones were extremely high, making any occurrences of these compounds a cause for concern. The purpose of the Watch List is to collect data for specific analytes in order to assess whether a compound should be routinely monitored as a Priority Pollutant under Annex X of the WFD accompanied with a set EQS value. The majority of compounds were removed from the 2nd list due to the stipulation that no compound can be on the list for more than 4 years [50,51], and so the 3rd list was published before a full review of the 2nd was published [51]. Whether or not the analytes contained on it have been included as Priority Pollutants is currently unknown. However, if findings from other member states are similar to the ones seen in this study, it is likely the synthetic estrogens will be moved to Annex X of the Water Framework Directive (WFD).

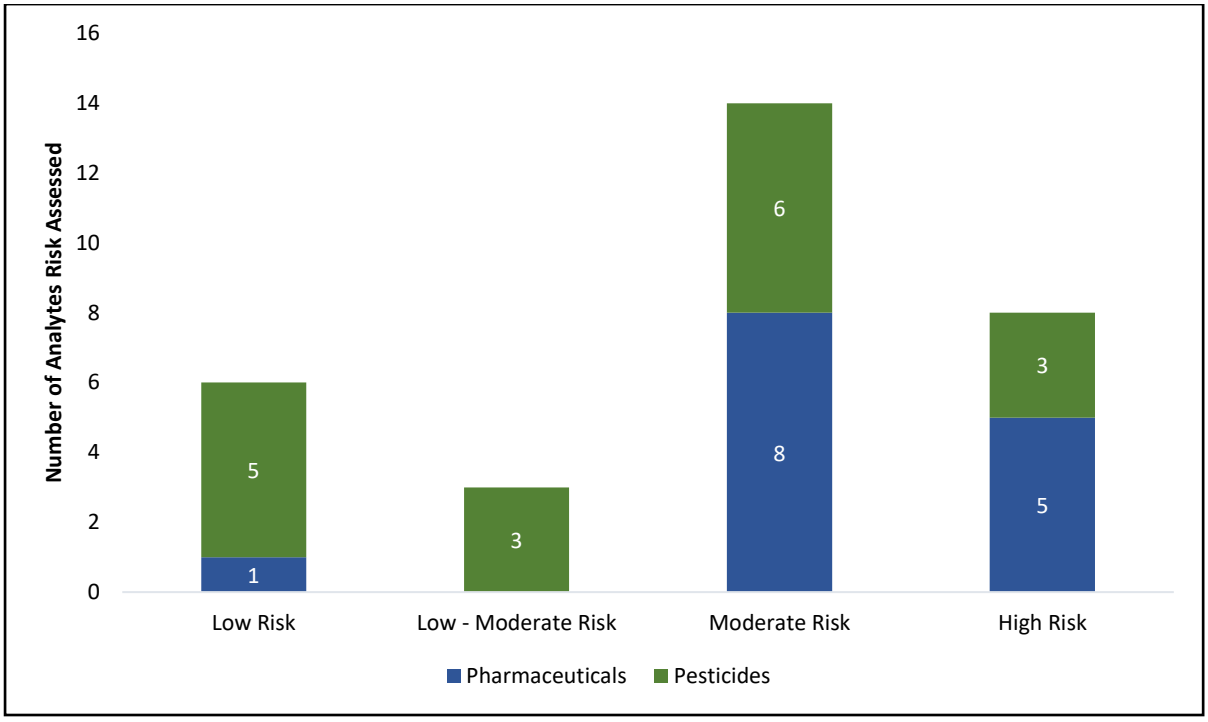


Figure 53. Bar chart of risk categories from generated RQ values for Watch List contaminants, broken down by risk level and analyte group.

Through risk assessment of these chemicals it is possible to identify analytes specifically of concern to Ireland, which in turn can aid in informing future monitoring programmes, and in targeted mitigation tactics. Based on the risk assessment of measured environmental concentrations of WL chemicals, compounds identified as a priority for Ireland include pharmaceuticals E1, E2, EE2, erythromycin and venlafaxine, and pesticides thiamethoxam, methiocarb and acetamiprid. Most of the high risk analytes were from the 2nd Watch List and not carried over to the 3rd, and therefore no longer monitored in the same capacity. It is yet to be seen if these analytes will be added to Annex X of the WFD, however from an Irish perspective it is advisable that monitoring continue in some capacity.

Policy decisions can be a positive driver to affect change and improve water quality, however it is not a complete solution. This is exemplified by the occurrence of neonicotinoid pesticides even in the 2020 set of samples, two full years following their ban for outdoor use. Additionally, while the neonicotinoids may be considered as less of a hazard post EU ban, Ireland is an island affected not only by EU decisions. In March 2022, the UK authorised the use of these compounds again for use on sugar beet crops [331]. Rivers know no political borders

so the possibility for this decision to affect Ireland's waterways is a real one. It is for this reason that a combined approach to improving water quality is vital, through policy changes, continued monitoring and possibly research into alternative approaches to the use of some of these compounds.

3.3.5 Spatiotemporal Variations of Analyte Occurrences

3.3.5.1 Spatial variation

In order to assess whether there was a statistically significant difference in mean analyte concentrations between sample locations, a Kruskal-Wallis test was performed. This test was chosen rather than a One-Way ANOVA test as ANOVA is a parametric test which assumes a normally distributed dataset, however CEC occurrences in surface waters cannot be assumed to follow a normal distribution. Where an analyte occurred <LOQ, the concentration was set to be half of the LOQ value for that analyte. Only analytes which had an occurrence frequency over ≥65% were tested, which included pharmaceuticals clarithromycin, E1, E2 and EE2 from the 2nd watch list, and fluconazole, clotrimazole, sulfamethoxazole, venlafaxine and its metabolite o-desmethylvenlafaxine from the 3rd watch list. The test found that there was a statistically significant difference ($p<0.05$) between the mean concentrations at each location for all compounds selected for testing. A Kruskal-Wallis test was also performed for frequently occurring (≥65%) pesticides. No pesticides contained on the 2nd watch list occurred in more than 65% of samples and therefore were not assessed for spatial variance. Pesticides tested from the 3rd watch list were penconazole, tebuconazole, dimoxystrobin, ipconazole, prochloraz, tetraconazole and miconazole. Similarly to the pharmaceuticals, the test found that there was a statistically significant difference ($p<0.05$) between the mean concentrations at each location for all analytes tested. Therefore it can be concluded that there was statistically significant variation between selected sampling points, making these sites a good representation of differing waterbodies in the country.

The river which had the greatest number of analyte occurrences posing either a moderate or high risk was the River Liffey. This site comprised approximately 44.4% of all high or moderate risk detections. This was closely followed by the River Suir, making up an additional 38.9%.

The remaining high to moderate risk detections were in the River Annalee (11.1%) and River Nore (5.6%). It was shown that the pharmaceutical class made up the majority of the analytes at these risk levels, with the pesticide class making up the majority of lower risk occurrences. This variation of analyte occurrences between sites is indicative of the different land uses and catchment pressures the sites are under. The Liffey was identified as at risk for urban run-off and urban wastewater treatment pressures, showed increased presence of pharmaceutical contaminants. Conversely, the Annalee showed the presence of more pesticides, and was identified as at risk for agricultural pressures.

3.3.5.2 Temporal variation

The majority of above LOQ pesticide detections across both lists occurred in the summer months between May and September, while the majority of higher concentration pharmaceutical occurrences were following the winter period between December and March. Trends such as this are well documented in the literature and anticipated, due to increased farming practices in the warmer months and higher rates of illness in the winter [182,332].

In order to determine any possible trends in the analyte type occurring during the entire study period, CECs were grouped into two categories; pharmaceuticals and pesticides. The percentage of the total number of detections for both of these groups by year was then calculated and graphed as shown in Figure 54. It is important to note at this stage that although proportions of pharmaceuticals and pesticides on both lists are similar, they are not totally equal. Of the 15 compounds on the 2nd list, 7 are pesticides and 8 are pharmaceuticals. Of the 19 chemicals on the 3rd list, 10 are pesticides and 9 are pharmaceuticals. This will influence the proportion of total detections for each group, however some interesting trends can be observed.

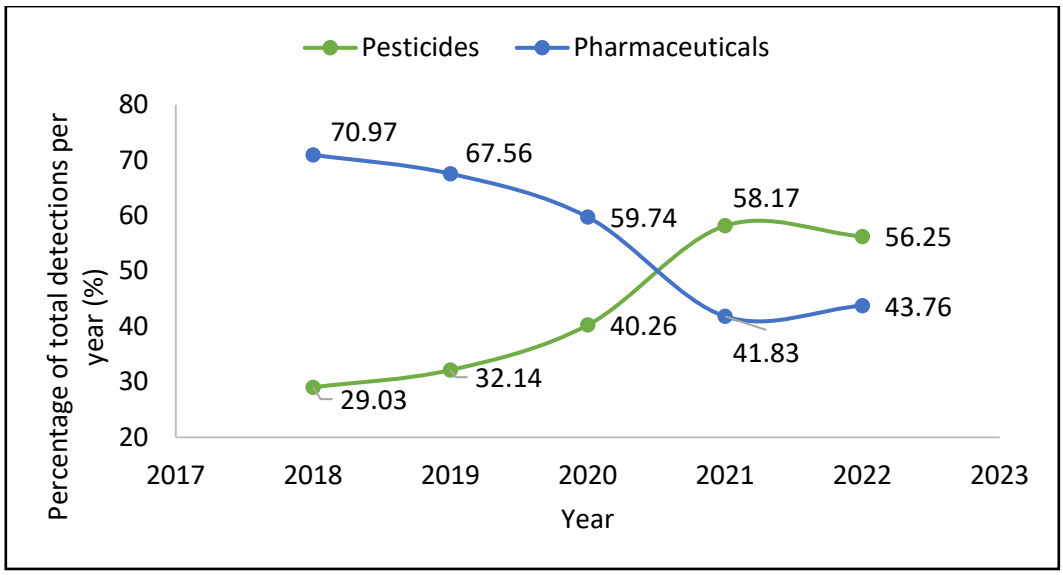


Figure 54. Line graph showing trends in the percentage of pharmaceutical and pesticide contaminant detection frequencies over a four year period

As can be seen from Figure 54, at the start of the project during the 2nd WL monitoring campaign in 2018, the majority of detections were from the pharmaceutical group, accounting for over 70% of total detections. This was seen to trend downwards while pesticides trended up for each year of the 2nd WL campaign, dropping to pharmaceuticals accounting of under 60% of total detections in 2020. EU counties have seen an increase in applications of insecticides, fungicides and chemical-based fertilizers in recent years [333]. This increase in usage is a potential explanation for the trend seen in the occurrence data of this study.

The first samples for the 3rd WL were analysed in 2021, with which the pesticide class overtook pharmaceuticals as the group occurring in the majority of samples. This could be anticipated to an extent since the ratio of pharmaceuticals to pesticides on the 3rd WL is 9:10. However, since pesticide concentrations had been increasing already throughout the 2nd WL campaign, it is interesting that this trend appeared to continue on. It is possible the intensification of pesticide applications in the EU was considered in analyte inclusion onto the 3rd Watch List leading to the higher proportion of pesticides. Initial results from 2022 however, were much more evenly split between the two groups. Further monitoring of the 3rd WL is needed to see if this trend will continue.

3.4 Conclusions

The primary aim of this work was to provide the first comprehensive study on the full suite of both 2nd and 3rd Watch List compounds in Ireland. Currently there is little real environmental occurrence data on these analytes, necessitating their inclusion onto the Watch Lists. Therefore results gathered through this four year monitoring programme have a direct impact on the policy decisions relating to the protection of surface waters throughout the European Union.

Frequently detected analytes from the second WL in Irish samples were the three estrogens, three macrolides antibiotics and the two neonicotinoid pesticides thiamethoxam and imidacloprid. The majority of these detections were below quantifiable levels. All analytes were detected at least four times across all samples, however amoxicillin, ciprofloxacin, metaflumizone and methiocarb were the most infrequently detected, and all detections were below LOQ values.

From the 3rd Watch List, venlafaxine and miconazole were the most often found, being present in over 93% of samples. Venlafaxine in particular was found frequently above the LOQ, and across all sample sites, indicating this compound to be a CEC to investigate further. Similarly to the 2nd Watch List, most detections were below quantitation levels. However the majority of analytes had a detection frequency of over 50%, showing a continued low level presence of CECs in Irish waters.

To assess the risk posed by this continual CEC presence, RQ values were generated for all detected analytes based on the MEC and PNEC values listed in the relevant Watch Lists. This showed a number of moderate and high risk occurrences in field samples, which is a cause for concern for the aquatic ecosystem. These results also allowed for the identification of compounds which are specifically concerning for Ireland including the estrogens, neonicotinoids, macrolides and the antidepressant venlafaxine. This can therefore be used to implement tailored mitigation measures to improve water quality.

The analysis of these compounds is highly sensitive to matrix interferences. This was noticed particularly in samples where very high sample turbidity was present, which was linked with rainfall events. It is recommended therefore that future monitoring campaigns measure turbidity when sampling. Additionally, it is clear that further investigation is needed to assess if it is possible to mitigate the matrix effect phenomenon in the ion source itself.

Chapter 4:

A Temporal Study of Pesticide Contaminants in Irish Wastewater Influent, Effluent and Receiving Waters

4.1 Introduction

While pesticides have been investigated extensively in the surface waters of other countries, there is a distinct lack of literature relating to their occurrence and fate in Wastewater Treatment Plant (WWTP) influents, effluents and receiving waters. In past research, WWTP studies have often focussed on pharmaceutical and personal care and cosmetic products (PCCPs), with few studies focussed on pesticides [334]. Currently, there is limited data available for the chemical water quality of Irish aquatic environments, particularly for the pesticide group of CECs in surface and wastewaters as many of the available studies focus on drinking or groundwater [205,224,260,261]. The pesticide class of contaminants is of particular interest specifically for Ireland, as the majority of the land mass is dedicated to agricultural practice, and there is extensive usage of a number of these compounds as detailed in Chapter 1. This widespread use was further corroborated by the high detection frequencies of a number of pesticides found in the surface waters studied in Chapter 3 of this thesis, which examined catchments from around the country. Many pesticides have been shown to be highly persistent in the environment, again making further investigation of this class needed [333]. Wastewater effluents have been shown to be a considerable source of pesticide contamination into the environment, and therefore further investigation into this from an Irish context would be greatly beneficial [335]. One previous study conducted in Ireland in 2011 on pesticides in wastewater effluent focussed on priority pesticides under the WFD. In this work they found substantial concentrations of pesticides in WWTP effluent [336]. In the other limited studies investigating pesticide removal from WWTP influent, many compounds including neonicotinoids and some azoles have been shown to be inefficiently removed or in even found in higher concentrations in the WWTP effluent [33,334]. The current wastewater treatment process is not designed to removal CECs, and is mainly performed to remove biosolids, sugars, fats, short chain carbons and nutrients such as nitrates. Typical processes include primary treatment where solids are settled in large sedimentation tanks, and secondary treatment where air and microorganisms are introduced. Additional processes such as tertiary treatment including nutrient removal are sometimes employed at larger plants [27].

Insufficient removal from wastewaters can in turn create point pressures on waterbodies, and have an impact on the health of the river. Studies of this kind can aid in identifying specific analytes of interest which are not being removed in treatment, and influence the decisions of both WWTP operators and policy makers to improve water quality.

To gain a greater understanding of the occurrence and removal of pesticides in water treatment, an analysis of the lifecycle of selected pesticide CECs through the WWTP system was performed. Targeted compounds were selected based on their presence on either the 2nd or 3rd EU Watch Lists, their inclusion on the WFD Priority Substances list, or indication in the River Basin Management Plan [51,60,276,298]. This came to a total of 25 compounds, with a differing chemistries, pesticidal applications and usage frequencies as detailed in Chapter 1 and are summarized briefly in Table 44.

Analysis of aquatic samples is often by use of mass spectrometry coupled with a chromatographic method. Historically, gas chromatography (GC) was the method of choice for pesticide analysis, however advances in liquid chromatography (LC) methods over recent years have seen its usage rise steeply. LC-MS/MS has been used extensively for the analysis of the majority of targeted compounds for this study, aside from the pyrethroid pesticides. The use of LC-MS for the analysis of pyrethroid pesticides is a novel approach with very few studies conducted using this instrument, and even less which applied it to aquatic samples [263–267]. Therefore the present study will also present a novel approach to pyrethroid pesticide analysis.

Table 44. Pesticides included in the wastewater study, their chemical group and common uses (Information on usages from [50,51,86,113,118,205,300,301,337])

Group	Compound	Uses
Neonicotinoid	Acetamiprid Clothianidin Imidacloprid Thiacloprid Thiamethoxam	Insecticide for control of aphids and grubs in particular on leafy plants – can be used as alternative to pyrethroid or carbamate pesticides
Acid Herbicide	2,4-D MCPA Mecoprop	Herbicides used for weed control on primarily cereal crops and lawns.
Organophosphorus	Glyphosate AMPA	Herbicide used for control of broadleaf weeds and grasses. Primary metabolite of glyphosate
Azole	Clotrimazole Miconazole Fluconazole	Anti-fungal pharmaceuticals used for treatment of fungal skin infections (fluconazole for more serious infections)
	Imazalil Ipconazole Metconazole Prochloraz Penconazole Tebuconazole Tetraconazole	Fungicides used on a variety of crops including fruit and vegetable, grain and leafy crops. Also used in gardening and landscaping. Ipconazole used as seed treatment for soil borne diseases.
Pyrethroid	Bifenthrin Cypermethrin Deltamethrin Esfenvalerate Permethrin	Insecticides used for the control of multiple pests including weevils, aphids, lice, flies and ants. Used in both agriculture and in commercial bug prays and lice shampoos.

Aims and Objectives

The aim of this work is to examine the lifecycle of selected groups of pesticide contaminants through the Irish Wastewater Treatment System.

The objectives are to:

- Highlight key aspects of the literature methods detailed in Chapter 1 that were used to guide this work;
- Apply the methods developed in Chapter 2 to real field samples of influent, effluent and receiving water;
- Obtain information on the occurrence and removal of these pesticides in the Irish WWTP system to inform future policy and aid WWTP operators in improving treatment practices;
- Calculate Risk Quotient (RQ) values for effluent and receiving water samples to provide an indication of the level of risk posed by detected compounds.

4.2 Materials and Methods

4.2.1 Reagents, chemicals, consumables

All materials used for sample collection, preparation and analysis are detailed in Chapter 2 section 2.2.1 of this thesis.

4.2.2 Field sample collection and preparation

Full sample collection and preparation procedures are detailed in Chapter 2 section 2.2.4. The exact site locations cannot be disclosed due to a confidentiality agreement, and will therefore be referred to throughout this chapter as the 'Urban site' and 'Rural site'. The references to 'urban' and 'rural' are based on the population equivalents of the respective plants. The Urban WWTP has a population equivalent (PE) of approximately 50,000-100,000 and the Rural site has a population equivalent of <2000 (note this is the lowest PE band indicated by Irish Water). According to the Central Statistics Office (CSO), an area with a population of >50,000 is defined as a city, and an area with of population of <1500 is considered rural [338].

4.2.3 Calculation of Removal Rates

Rates of removal of pesticide contaminants through wastewater treatment was calculated using equation 7:

$$Percentage\ removal = \left(\frac{\left(C_{influent} - C_{effluent}\right)}{C_{influent}}\right) x\ 100$$

Equation 7. Formula used for determination of analyte removals from wastewater treatment [339].

Where $C_{imfluent}$ is the environmental concentration determined for a given analyte in influent, and $C_{effluent}$ is the environmental concentration determined for a given analyte in effluent [339]. In the situation where a compound was detected in influent samples but not in effluent, in order to obtain a 'worst case scenario', the method LOD was used as the environmental concentration. In the case where a compound was present in WWTP effluent but not in influent, the LOD was again used. This was done to avoid the establishment of a potentially inaccurate 100% removal rate, as occurrences may have been possible below the limits of detection. For occurrences below LOQ, half the method LOQ was used for environmental concentrations in removal rate calculations [339].

In order to assess the risk posed by detected compounds, the risk quotient (RQ) was calculated based on the Predicted No-Effect Concentrations (PNEC) for any analytical detections. PNEC values were taken from the values indicated on the relative Watch Lists or from the NORMAN network lowest PNEC search function [51,276]. For unquantifiable detections (i.e. <LOQ), two scenarios were examined, one where the MEC was assumed to be half of the method LOQ value to obtain a worst case scenario, and one where the MEC was assumed to be the method LOD value to obtain a best case scenario. [277]

4.3 Results and Discussion

4.3.1 Occurrence of pesticides in WWTP Influent

Occurrence data for 25 different pesticides in influent samples was collected and quantified, with full results shown in Tables 45 and 46. As can be anticipated for studies of this kind, the influent samples had the most analyte detections across all three matrices studied, with 19 out of 25 compounds detected. Overall, there were more total occurrences in the rural site compared to the urban, with the rural site having 109 total detections, and the urban site having 100. As previously mentioned in the introduction to this chapter, studies examining pesticide occurrences in wastewater samples are sparse. Therefore there is limited literature to draw from in order to examine where the results found in this study fit in on wider scale.

Frequently occurring analytes, found in over 50% of influent samples across both urban and rural sites were the azole compounds tetraconazole, tebuconazole, miconazole, imazalil and clotrimazole, the pyrethroid permethrin and neonicotinoid clothianidin. A bar chart showing occurrence frequencies in influent samples across both sites can be seen in Figure 55.

Tetraconazole, an azole fungicide, was found in 100% of rural samples and 92% of urban samples. This compound is used to treat leafspot in susceptible crops including winter wheat, carrots and lettuce, as well as crops grown in other countries including corn, small fruit, low growing berries, pecans, peanuts, soybeans and sugar beet [340]. It is one of the most commonly used azole fungicides in agriculture [341]. To the author's knowledge, there have been no reported studies on the occurrence of this compound in wastewater influent. Finding only indicate that is compound has a distinct presence in the Irish WWTP system, as detections continued in both effluent and receiving water samples. Occurrences in influent ranged from <LOQ to 150 ng L^{-1}, with the maximum detected concentration found in the rural site in June 2019. Higher concentrations in the summer months are anticipated for many analytes in this study, due to the increased agricultural practices typically seen during this period.

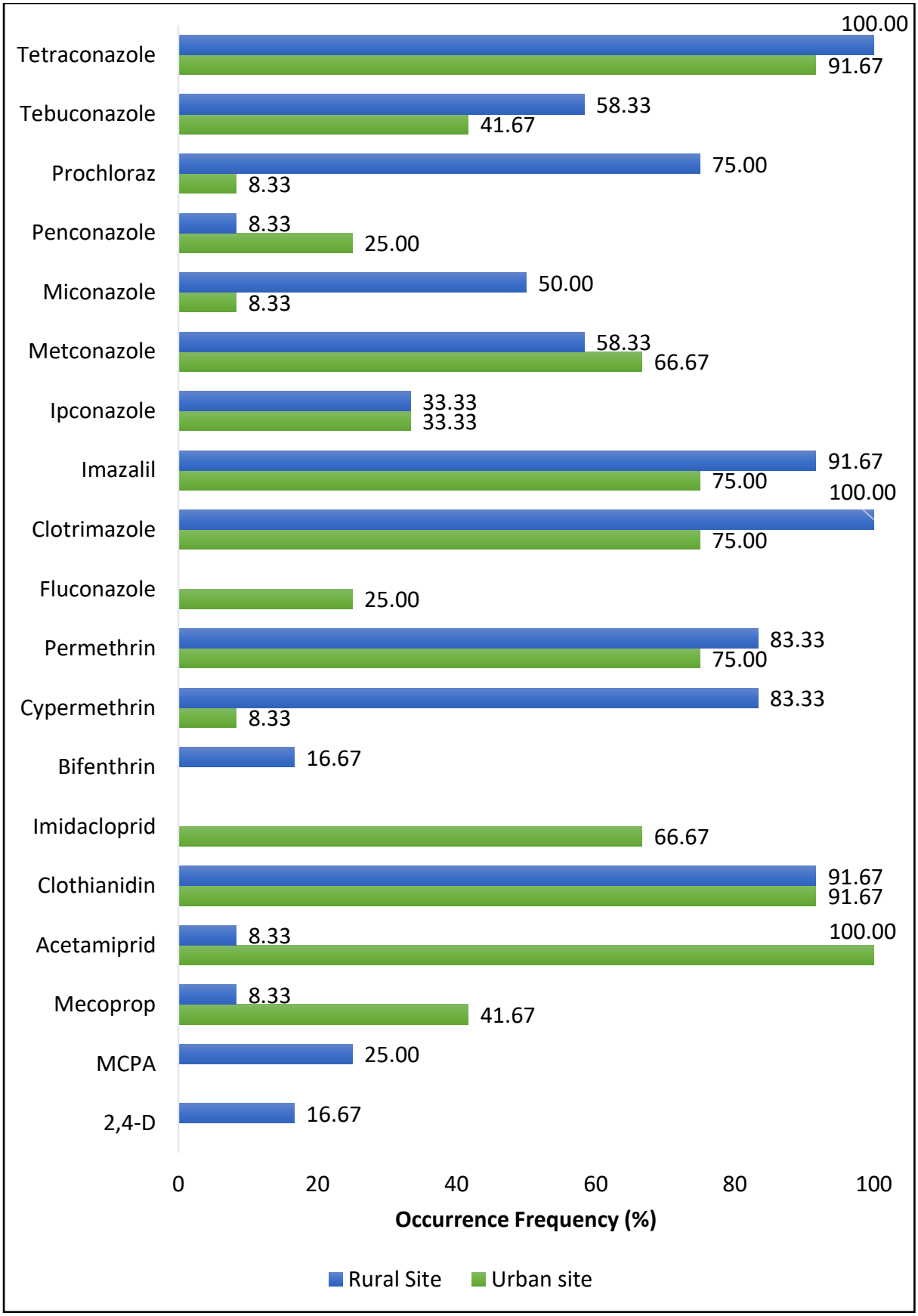


Figure 55. Occurrence frequency of pesticide contaminants in Irish Influent samples over one calendar year from October 2018 – September 2019 (n=24 where 1L sample was collected per month from two sites)

Clotrimazole was also found at a high frequency in both sites, occurring in 100% of rural samples and 75% of urban samples. This compound, which is also an azole compound, is used typically as a topical antifungal pharmaceutical medication. Clotrimazole is one of the compounds included in this study with the most available wastewater occurrence data, however, it has only been studied in Irish WWTP systems once. This study, published in 2012 but concerning samples taken primarily in 2008, found clotrimazole in influent samples at each site studied [121]. The maximum concentration of clotrimazole determined in influent in this study was 900 ng L^{-1}. By comparison the maximum concentration determined in this study of 140 ng L^{-1} found in the rural September sample is considerably lower, possibly indicating lower concentrations entering the WWTP systems. The concentrations found in this study more closely reflect those found in the few other studies performed in Europe on clotrimazole in WWTP samples, all published between 2008 and 2017. In these studies clotrimazole was found in Swiss influent samples in the range of 10-110 ng L^{-1} [118], in Spanish influent in the range of 11-80 ng L^{-1} [342] and in Slovakian samples in the range of 10-90 ng L^{-1} [343]. A more recent study investigating azoles in South Africa was published in 2020, in which average concentrations of clotrimazole in influent was found to be 16.2 ng L^{-1}, considerably lower than those found in European countries. By contrast, the concentrations and occurrence frequencies found in South Africa for the azole fluconazole are up to an order of magnitude higher than those found in the present study, in which fluconazole was only found below LOQ in 25% of urban influent samples. In other influent studies, fluconazole was found at maximum concentrations ranging from 93 – 1710 ng L^{-1} [118,342–344]. This indicates a probable difference in the usage dependence and/or availability of these two anti-fungal compounds from country to country.

Permethrin was the compound detected in the highest concentrations throughout the influent samples, with a maximum concentration found of 42657 ng L^{-1} (42.66 µg.L^{-1}) in the April rural sample. Previous studies have also found permethrin at very high concentrations in WWTP influent. A maximum concentration of 1838 ng L^{-1} of permethrin was found in the summer sampling period of study on Iranian WWTP samples [345]. According to a review published in Chemosphere, pyrethroid pesticides have been found in various aquatic matrices at concentrations ranging from LODs to 13000 µg L^{-1} [346]. This extremely broad range shows

how individual each study or catchment area is when it comes to pyrethroid occurrences, possibly due to the differing uses of this group influencing environmental concentrations.

Clothianidin was found at the same frequency at both sites, occurring in 91% of Irish samples, however, it was generally below the method LOQ. Occurrences above LOQ were only found in rural samples, with a maximum concentration of 14.8 ng L^{-1} in February 2019. Influent concentrations found in US samples for clothianidin were considerably higher than those found here with an average daily concentration of 149.7 ng L^{-1}, a possible cause for this is the EU ban on neonicotinoid use in 2018, whereas no such ban exists in the US [115,347].

Of the three acid herbicides, only Mecoprop was found in the urban area whereas all three were found in the rural site. All acid herbicide occurrences were found between the months of May – September, coinciding with periods of increased agricultural practices. Concentrations in influent for this group of compounds were generally low (<6 ng L^{-1}) across both sites. Occurrences in influent samples of 2,4-D from Seoul, South Korea ranged from 10.2 – 82.5 ng L^{-1}, making the concentrations determined here low by comparison [348]. Where a 24 hour composite influent sample were taken in Denmark and Sweden concentrations of Mecoprop between 31-34 ng L^{-1} were reported, these concentrations are more reflective of the lower levels found in Ireland [349].

Three pyrethroids, bifenthrin, deltamethrin and esfenvalerate, two neonicotinoids thiacloprid and thiamethoxam, and the herbicide glyphosate and its primary metabolite AMPA were not detected in any influent samples over the 12 months in this Irish study. Neonicotinoids were banned for all outdoor use in 2018 [350], and so it is possible the effect of this ban is being seen here. However, the three other neonicotinoids studied, clothianidin, acetamiprid and imidacloprid, were all found in influent sample at varying frequencies across both sites. Additionally, it was seen in Chapter 3 of this thesis that there were multiple detections of neonicotinoids in five surface water catchments around the county in December 2018.

Non-detection of these compounds can mean that the analyte is not present in the environment, however it is also possible there is analyte presence that was unable to be

detected due to analytical limitations. This is particularly the case for glyphosate and AMPA, in which the LODs were in the $\mu g.L^{-1}$ range rather than the ng L^{-1} seen for the other analytes. This limitation was primarily because of the need for direct injection without sample preconcentration. Preconcentration was unable to be performed due to the extremely polar nature of the analytes making them unable to be retained by typical HLB cartridges, and the limited sample volume available not permitting for multiple extractions of the same sample. These factors allowed for the possibility of missed occurrences. Additionally, glyphosate is the most commonly used herbicide in the world, and so 0% occurrence is somewhat unlikely. There have been more recent advances in the area of improving glyphosate retention in SPE by use of alternative cartridge sorbents such as anion exchange resins and molecularly imprinted polymers (MIPs) [351,352]. For future monitoring however, it would be advisable to investigate this method of analysis further in order to minimise the potential for missed occurrences. This being said, LODs in the $\mu g.L^{-1}$ range rather than the ng L^{-1} is not the only explanation for the lack of glyphosate occurrences. A paper published in 2020 on the '*Behaviour of Glyphosate in Wastewater Treatment Plants*', glyphosate was found to strongly adsorb to activated sludge in WWTP treatment, and accumulate in the sludge over time [353]. Therefore, further studies on glyphosate in WWTPs would likely benefit from study of sludge in addition to water samples.

Table 45. Table of pesticide occurrences in Urban Influent samples over 1 year from October 2018 – September 2019 (n=12)

		Analyte Occurrences (ng L^{-1}) in Urban Site Influent											
		Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19
Acid herbicides	2,4-D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	MCPA	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Mecoprop	N.D	<LOQ	N.D	N.D	N.D	N.D	<LOQ	N.D	5.2 ± 0.9	<LOQ	N.D	<LOQ
Neonicotinoids	Acetamiprid	<LOQ	<LOQ	<LOQ	<LOQ	8.21 ± 11.7	<LOQ	<LOQ	<LOQ	4.2 ± 0.5	<LOQ	<LOQ	3.4*
	Clothianidin	N.D	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Imidaclopri d	<LOQ	4.2 ± 2	<LOQ	<LOQ	4.9 ± 4.7	<LOQ	N.D	N.D	N.D	<LOQ	<LOQ	N.D
	Thiaclopri d	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiameth oxam	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Pyrethroids	Bifenthrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Cypermethrin	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D
	Deltamethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Esfenvalerate	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Permethrin	N.D	<LOQ	1579.46 ± 63.96	<LOQ	N.D	N.D	<LOQ	<LOQ	<LOQ	3130.3 ± 2597	2481.3 ± 319	3865 ± 2569
Azoles	Fluconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	<LOQ	N.D	<LOQ

	Clotrimazole	<LOQ	N.D	3.2 ± 0.4	1.1 ± 0.1	<LOQ	N.D	2.3 ± 1.7	<LOQ	<LOQ	N.D	<LOQ	<LOQ
	Imazalil	<LOQ	3.2 ± 5.5	N.D	3.7 ± 3.6	<LOQ	N.D	2.4 ± 1.3	1.7 ± 0.9	<LOQ	N.D	<LOQ	1.8 ± 2.1
	Ipconazole	<LOQ	N.D	N.D	N.D	N.D	N.D	<LOQ	<LOQ	<LOQ	N.D	N.D	N.D
	Metconazole	N.D	N.D	50 ± 7	28.6 ± 6.8	N.D	137.2 ± 11.3	110.7 ± 53.7	61.6 ± 91	<LOQ	29.4 ± 15.7	N.D	100 ± 69.2
	Miconazole	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D
	Penconazole	N.D	37.9 ± 31	N.D	41.6 ± 38.7	N.D	N.D	N.D	N.D	N.D	N.D	N.D	23.5 ± 18.5
	Prochloraz	N.D	N.D	N.D	N.D	N.D	N.D	N.D	1.7 ± 0.1	N.D	N.D	N.D	N.D
	Tebuconazole	N.D	<LOQ	<LOQ	N.D	N.D	N.D	N.D	<LOQ	<LOQ	<LOQ	N.D	N.D
	Tetraconazole	39.8 ± 3	33.8 ± 4.1	21.4 ± 4.9	<LOQ	N.D	9.9 ± 3.7	49.5 ± 28	69 ± 30.2	<LOQ	19.7 ± 12.1	4.8 ± 0.1	29 ± 19.2
Polar herbicides	**Glyphosate**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 46 Table of pesticide occurrences in Rural Influent samples over 1 year from October 2018 – September 2019 (n=12)

		Analyte Occurrences (ng L^{-1}) in Rural Site Influent											
		Oct-18	**Nov-18**	**Dec-18**	**Jan-19**	**Feb-19**	**Mar-19**	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sep-19**
Acid herbicides	**2,4-D**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	<LOQ	N.D	N.D	N.D
	MCPA	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D	4.07 ± 2.63	N.D	<LOQ
	Mecoprop	N.D	N.D	N.D	N.D	N.D	N.D	N.D	5.4 ± 3.7	N.D	N.D	N.D	N.D
Neonicotinoids	**Acetamiprid**	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D
	Clothianidin	<LOQ	N.D	<LOQ	<LOQ	14.8 ± 1	<LOQ	<LOQ	<LOQ	6 ± 3.4	<LOQ	<LOQ	<LOQ
	Imidacloprid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiacloprid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiamethoxam	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Pyrethroids	**Bifenthrin**	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	<LOQ	N.D	N.D	N.D	N.D
	Cypermethrin	4041.77 ± 377.8	N.D	N.D	<LOQ	<LOQ	<LOQ	<LOQ	1764.97 ± 862.5	<LOQ	1604.63 ± 423.7	<LOQ	<LOQ
	Deltamethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Esfenvalerate	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Permethrin	13511 ± 9995	16456 ± 4657	20416 ± 5630	12629 ± 11686	24955 ± 17702	21027 ± 30298	42657 ± 41366	41312 ± 7101	N.D	27061 ± 18121	N.D	37376 ± 6323

Azoles	**Fluconazole**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Clotrimazole	17.5 ± 6.8	<LOQ	63.8 ± 14	17.4 ± 5.6	<LOQ	<LOQ	65.2 ± 0.8	21.3 ± 10	15.4 ± 12.5	2.1 ± 1.7	1 ± 0.2	140 ± 47.6
	Imazalil	46.8 ± 9.9	N.D	51.4 ± 15.4	27.3 ± 15.5	<LOQ	<LOQ	29.8 ± 5.8	23.5 ± 20.7	22.9 ± 18	<LOQ	2.5 ± 0.5	53.4 ± 5.7
	Ipconazole	<LOQ	N.D	<LOQ	<LOQ	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D
	Metconazole	20.9 ± 50.6	N.D	51.6 ± 40.2	81.9 ± 81	N.D	N.D	92.6 ± 17	166 ± 16	128.7 ± 96.5	N.D	N.D	80.2 ± 34.1
	Miconazole	N.D	N.D	12.1 ± 2.2	<LOQ	N.D	N.D	15.2 ± 0.7	<LOQ	<LOQ	N.D	N.D	15 ± 9.4
	Penconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	33.6 ± 22.8	N.D	N.D	N.D
	Prochloraz	<LOQ	<LOQ	<LOQ	1.6 ± 0.9	N.D	N.D	1.8 ± 1.1	N.D	1.8 ± 0.4	<LOQ	<LOQ	<LOQ
	Tebuconazole	17.4 ± 5.6	N.D	21.8 ± 1.6	29.1 ± 20.8	N.D	N.D	30.1 ± 12.2	24.2 ± 5.4	33.6 ± 25.6	N.D	N.D	21.3 ± 7.4
	Tetraconazole	50.9 ± 24.5	4.6 ± 0.1	171.8 ± 73.3	34.5 ± 23.1	4.8 ± 0.3	4.9 ± 0.1	55.8 ± 14.5	68.8 ± 31.2	149.7 ± 112.3	<LOQ	<LOQ	14.7 ± 8.1
Polar herbicides	**Glyphosate**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	AMPA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

4.3.2 Occurrence of pesticides in WWTP Effluent

Of the pesticides tested in influent samples, 17 analytes were also found in the effluent samples. Only three analytes were detected in influent but not in effluent; penconazole, ipconazole and bifenthrin. Interestingly, the pyrethroid deltamethrin was detected <LOQ in the effluent but not in the influent, in a single sample taken from the urban site in October. Generally, individual effluent concentrations were lower than that of influent and were oftentimes below quantitation limits, with a few analytes showing the opposite (i.e. higher effluent concentrations). Full quantitative results can be seen in Tables 47 and 48. Further discussion on the disparity of analyte occurrences and concentrations in WWTP effluent vs influent samples can be found in Section 3.5 on removal rates.

Similar occurrence frequencies for certain analytes were seen in WWTP effluent samples to the influent (Figure 56). Tetraconazole was again the analyte with the highest occurrence frequency across both sites, being detected in 100% of samples at each location. Occurrence concentrations however were considerably lower in effluent than in influent however, indicating at least partial removal through WWTP treatment.

Effluent samples did reveal that a distinct difference between the two sites could be observed in the overall analyte occurrence frequencies. The rural site had only 4 compounds found in ≥50% of samples; tetraconazole, clotrimazole, cypermethrin and MCPA. By contrast, tebuconazole, imazalil, tetraconazole, clotrimazole, cypermethrin, permethrin and imidacloprid were all found to occur in ≥50% of urban effluent samples. Imazalil, imidacloprid and permethrin were found in 100% of urban effluent samples. Tebuconazole in particular was found in 92% of urban effluent samples, albeit at comparatively low levels. Tebuconazole has been seen previously in urban effluent waters in the range of 1-10 ng L^{-1} making the results found here similar [118].

The only compound which was found more frequently in rural than in urban samples was MCPA, which was found at a frequency of 75% and 42% in rural and urban sites, respectively. This is a very interesting result as for the most part occurrence frequencies in WWTP influent

was similar between sites, or if different the rural site showed higher frequencies. This could indicate possible differences in the type of treatment methods used between the plants, however as plant specific information is unavailable this is only a hypothesis and cannot be confirmed.

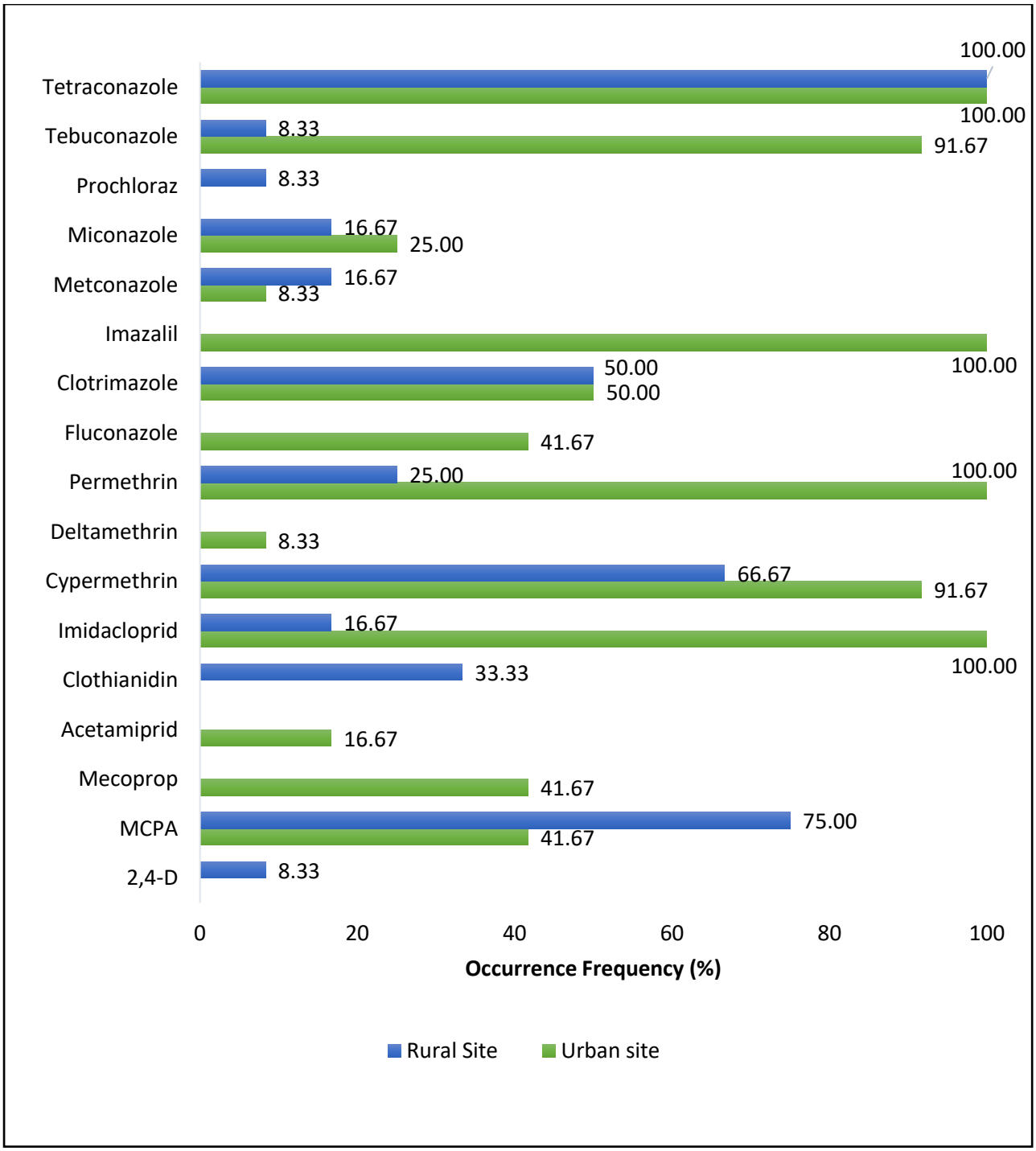


Figure 56 Occurrence frequency of pesticide contaminants in Irish Effluent samples over one calendar year from October 2018 – September 2019 (n=12)

Table 47 Table of pesticide occurrences in Urban Effluent samples over 1 year from October 2018 – September 2019 (n=12)

		Analyte Occurrences (ng L^{-1}) in Urban Site Effluent											
		Oct-18	**Nov-18**	**Dec-18**	**Jan-19**	**Feb-19**	**Mar-19**	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sep-19**
Acid herbicides	**2,4-D**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	MCPA	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	<LOQ	<LOQ	<LOQ	N.D
	Mecoprop	N.D	<LOQ	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	8.98 ± 3.58	8.28 ± 0.96	<LOQ
Neonicotinoids	**Acetamiprid**	N.D	N.D	<LOQ	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D
	Clothianidin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Imidaclorid	<LOQ	9.38 ± 4.42	52.98 ± 34.84	8.71 ± 5.84	14.12 ± 4.84	20.12 ± 9.67	16.44 ± 9.87	13.22 ± 4.48	12.11 ± 4.19	10.61 ± 4.65	6.06 ± 1.07	10.9 ± 8.83
	Thiaclorid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiamethoxam	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Pyrethroids	**Bifenthrin**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Cypermethrin	N.D	<LOQ	<LOQ	1327 ± 228	1522 ± 940	2437 ± 616	<LOQ	<LOQ	<LOQ	<LOQ	1156 ± 790	<LOQ
	Deltamethrin	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Esfenvalerate	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Permethrin	<LOQ	<LOQ	<LOQ	347.7 ± 61.8	<LOQ	474.18 ± 217.8	<LOQ	<LOQ	<LOQ	460.4 ± 117.9	<LOQ	<LOQ
Azoles	**Fluconazole**	N.D	N.D	<LOQ	<LOQ	N.D	N.D	N.D	<LOQ	<LOQ	N.D	N.D	<LOQ

	Clotrimazole	<LOQ	<LOQ	N.D	N.D	N.D	N.D	<LOQ	N.D	<LOQ	<LOQ	<LOQ	N.D
	Imazalil	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Ipconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Metconazole	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Miconazole	N.D	N.D	<LOQ	<LOQ	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Penconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Prochloraz	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Tebuconazole	<LOQ	<LOQ	4.27 ± 0.32	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	N.D	<LOQ	4.77 ± 1.23	<LOQ
	Tetraconazole	<LOQ	<LOQ	<LOQ	3.19 ± 0.2	5.85 ± 4.3	3.76 ± 0.22	3.55 ± 0.6	3.68 ± 0.5	3.2 ± 0.6	3.8 ± 0.5	3.7 ± 0.1	3.3 0.3
Polar herbicides	**Glyphosate**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	AMPA	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Table 48 Table of pesticide occurrences in Rural Effluent samples over 1 year from October 2018 – September 2019 (n=12), * indicated no SD available due to less than n=3 replicates

		Analyte Occurrences (ng L^{-1}) in Rural Site Effluent											
		Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19
Acid herbicides	2,4-D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D
	MCPA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	N.D	N.D	N.D	<LOQ	<LOQ	<LOQ	<LOQ
	Mecoprop	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Neonicotinoids	Acetamiprid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Clothianidin	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	18.1 ± 9.3	<LOQ	N.D	29.7 ± 14.1
	Imidacloprid	N.D	<LOQ	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D
	Thiacloprid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiamethoxam	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Pyrethroids	Bifenthrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Cypermethrin	N.D	1300 ± 238	1230 ± 628	1910 *	1096 ± 409	<LOQ	N.D	N.D	<LOQ	<LOQ	1195 *	<LOQ
	Deltamethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Esfenvalerate	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Permethrin	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	<LOQ	<LOQ	N.D	N.D
Azoles	Fluconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Clotrimazole	<LOQ	<LOQ	<LOQ	N.D	<LOQ	N.D	<LOQ	N.D	<LOQ	N.D	N.D	N.D
	Imazalil	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Ipconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Metconazole	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	<LOQ
	Miconazole	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	<LOQ	N.D	N.D

	Penconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Prochloraz	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D
	Tebuconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D
	Tetraconazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.0 ± 2.0
Polar herbicides	**Glyphosate**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	AMPA	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

4.3.3 Occurrence of pesticides in WWTP Receiving Waters

As can be expected for this kind of study, receiving waters had the least amount of overall analyte detections from the three studies matrices. In total 12 out of 23 compounds were detected in surface waters over the course of the study, and full data can be seen in Tables 49 and 50, and occurrence frequencies can be seen in Figure 57.

As was seen in the effluent samples, it was found that the rural site had fewer overall occurrences of analytes than the urban site. Seven analytes were detected at both sites including tetraconazole, miconazole, fluconazole, acetamiprid, 2, 4-D, MCPA and Mecoprop were all found at both sites. In the urban area, miconazole, acetamiprid and Mecoprop were found more frequently whereas tetraconazole and fluconazole were found more frequently in the rural area. Occurrence frequency was the same across both areas for only two analytes; 2,4-D and MCPA. Many of these analytes have been found previously in surface waters in both urban and rural areas. Chapter 3 of this thesis showed presence of all compounds apart from the acid herbicides listed here in a range of Irish surface waters. Previous studies on acid herbicides have shown considerable presence in aquatic matrices, including within Ireland [61,72,246,254]. Townsend et al. found time weighted average concentrations of acidic herbicides in UK surface waters up to 183 ng L^{-1} over a 16 day deployment period [72]. MCPA has been the most frequently detected compound found in Irish surface waters according to the Environmental Protection Agency, making its detection in this work a likely outcome [354].

Tebuconazole, imazalil, clotrimazole and clothianidin were all found in the urban site but not in the rural. Imidacloprid was the only analyte found in the rural area and not in the urban. Concentrations for all analytes were below quantitation levels for all analytes bar the acid herbicides on multiple occasions, and one off detections of miconazole (urban March) and imidacloprid (rural February). Section 3.4 examines the differences in analyte concentrations found in all studies matrices in greater detail.

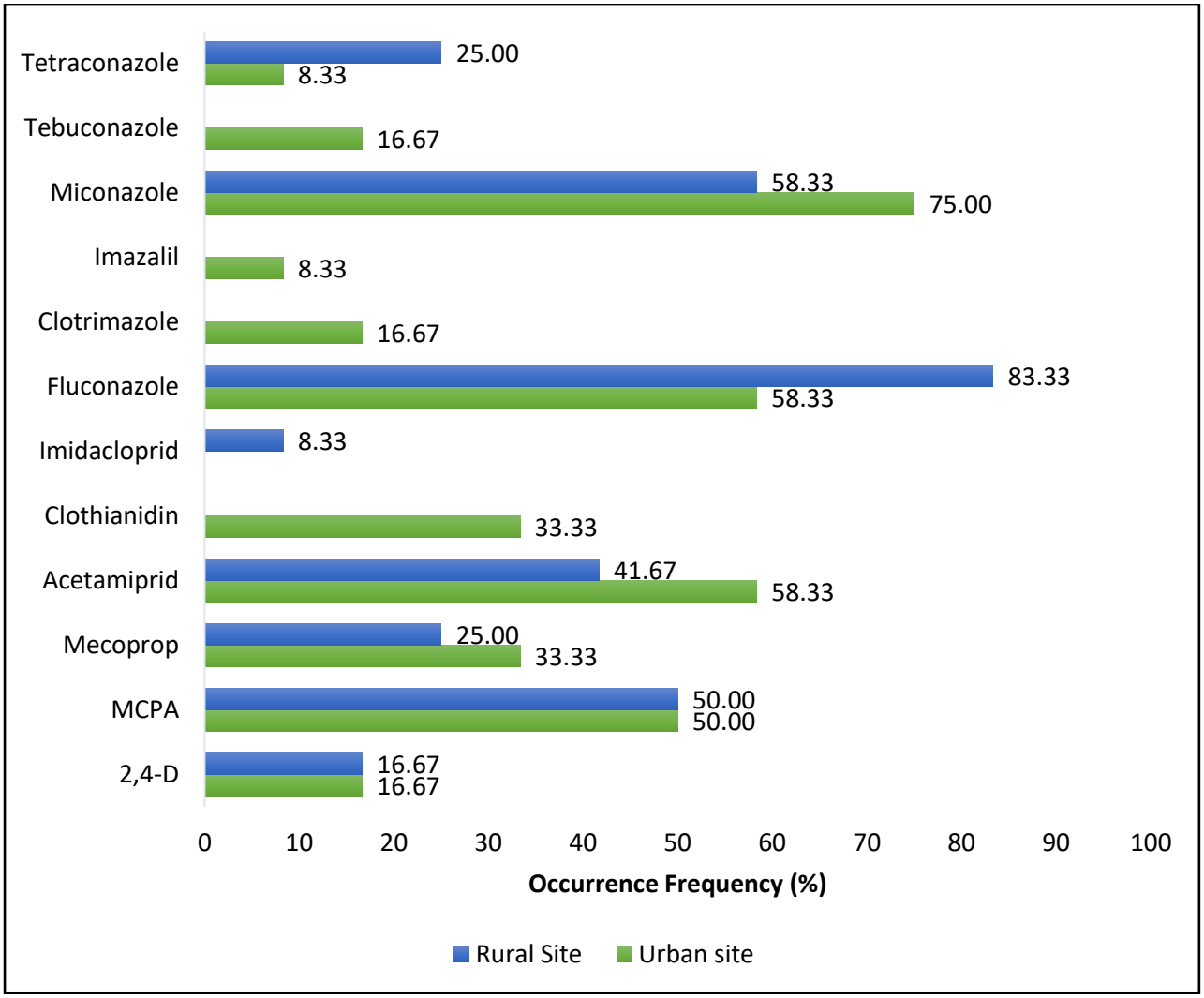


Figure 57 Bar chart showing pesticide occurrence frequency in receiving water samples over 1 year from October 2018 – September 2019 (n-12)

Table 49 Table of pesticide occurrences in Urban Receiving Water samples over 1 year from October 2018 – September 2019 (n=12)

		Analyte Occurrences (ng L^{-1}) in Urban Site Receiving Waters											
		Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19
Acid herbicides	2,4-D	2.83 ± 0.60	N.D	N.D	N.D	N.D	N.D	31.86 ± 7.96	N.D	N.D	N.D	N.D	N.D
	MCPA	N.D	N.D	20.08 ± 0.07	N.D	19.88 ± 0.11	N.D	N.D	19.72 ± 0.19	19.07 ± 0.41	26.17 ± 7.71	N.D	18.59 ± 1.24
	Mecoprop	5.38 ± 4.55	12.98 ± 9.93	N.D	N.D	N.D	<LOQ	7.51 ± 3.46	N.D	N.D	N.D	N.D	N.D
Neonicotinoids	Acetamiprid	N.D	<LOQ	N.D	N.D	<LOQ	<LOQ	N.D	N.D	<LOQ	<LOQ	<LOQ	<LOQ
	Clothianidin	15.7 ± 0.4	<LOQ	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D
	Imidacloprid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiacloprid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiamethoxam	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Pyrethroids	Bifenthrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Cypermethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Deltamethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Esfenvalerate	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Permethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Azoles	Fluconazole	<LOQ	<LOQ	<LOQ	N.D	<LOQ	N.D	<LOQ	<LOQ	N.D	<LOQ	N.D	N.D

	Clotrimazole	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ
	Imazalil	N.D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D
	Ipconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Metconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Miconazole	<LOQ	<LOQ	<LOQ	N.D	<LOQ	4.4 ± 4.6	<LOQ	<LOQ	<LOQ	<LOQ	N.D	N.D
	Penconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Prochloraz	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Tebuconazole	N.D	<LOQ	N.D	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D
	Tetraconazole	<LOQ	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Polar herbicides	**Glyphosate**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	AMPA	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Table 50. Table of pesticide occurrences in Rural Receiving Water samples over 1 year from October 2018 – September 2019 (n=12)

		Analyte Occurrences (ng L^{-1}) in Rural Site Receiving Waters											
		Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19
Acid herbicides	2,4-D	N.D	N.D	N.D	N.D	N.D	<LOQ	N.D	<LOQ	N.D	N.D	N.D	N.D
	MCPA	17.92 ± 0.18	19.88 ± 0.35	N.D	N.D	N.D	16.54 ± 10.56	47.26 ± 2.27	N.D	82.55 ± 5.5	N.D	7.34 ± 3.01	N.D
	Mecoprop	N.D	<LOQ	N.D	N.D	N.D	N.D	<LOQ	<LOQ	N.D	N.D	N.D	N.D
Neonicotinoids	Acetamiprid	N.D	<LOQ	N.D	N.D	<LOQ	N.D	N.D	<LOQ	N.D	N.D	<LOQ	<LOQ
	Clothianidin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Imidacloprid	N.D	N.D	N.D	N.D	10.7 ± 16.8	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiacloprid	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Thiamethoxam	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Pyrethroids	Bifenthrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Cypermethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Deltamethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Esfenvalerate	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Permethrin	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Azoles	Fluconazole	<LOQ	<LOQ	<LOQ	N.D	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	N.D	<LOQ	<LOQ
	Clotrimazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Imazalil	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Ipconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

	Metconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Miconazole	<LOQ	<LOQ	<LOQ	N.D	<LOQ	N.D	<LOQ	<LOQ	N.D	N.D	N.D	<LOQ
	Penconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Prochloraz	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Tebuconazole	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	Tetraconazole	N.D	<LOQ	N.D	N.D	<LOQ	N.D	N.D	<LOQ	N.D	N.D	N.D	N.D
Polar herbicides	**Glyphosate**	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	AMPA	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

4.3.4 Relative composition of pesticide occurrences in Irish samples; spatial and temporal variation

In order to examine spatiotemporal differences in pesticide occurrence, the samples were grouped into four seasons; spring (March-May), summer (June-August), autumn (September-November) and winter (December –February) for each studied site. Cumulative concentrations (in ng L^{-1}) for each analyte at each site were calculated, which was then used to determine the percentage of total pesticide occurrence contributed by each compound. Information on the analytes which comprise significant proportions of cumulative pesticide concentrations can aid in identification of compounds of interest for future monitoring. Analysis of seasonal changes can inform compound use and give an indication of routes of entry into water samples. For detections <LOQ, half of the method LOQ was used to give an indication of the environmental concentration for that compound, and its influence on sample composition. This was performed for each water matrix. The cumulative pollutant concentrations for each matrix are shown in Figure 58. Cumulative concentrations decreased in the order of influent → effluent → receiving waters showing and anticipated reduction in contamination through either water treatment, degradation, or dilution effects [355].

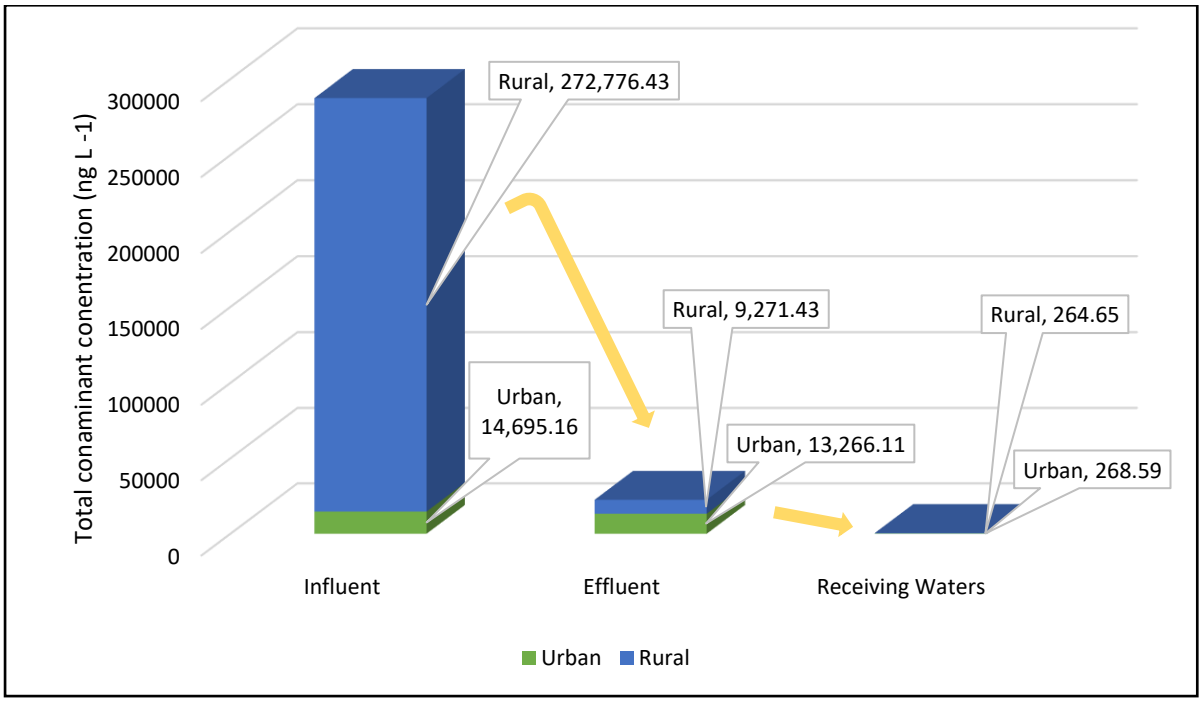


Figure 58. Bar chart showing total cumulative pesticide concentrations in both sites decreasing in the order of influent → effluent → receiving waters.

4.3.4.1 Influent

Total cumulative concentrations by season can be seen in Figure 59. Pesticides are generally diffuse pollutants usually entering river systems from surface runoff. However their presence in wastewater has been documented previously, with suggestions of sources including improper disposal of pesticide containers, equipment washing, and entry into drains via heavy rainfall [335]. There is a stark difference seen between the total pesticide concentrations between the two sites year round. The rural area, as can be anticipated due to the differing land practices associated with these areas, showed significantly higher total concentrations than in the urban area. The spring period coincided with the highest total pesticide loads in the rural area overall, with a cumulative 109623 ng L^{-1} of pesticides quantified over this period. In the urban site, the summer season had the highest cumulative concentration at 6090 ng L^{-1}.

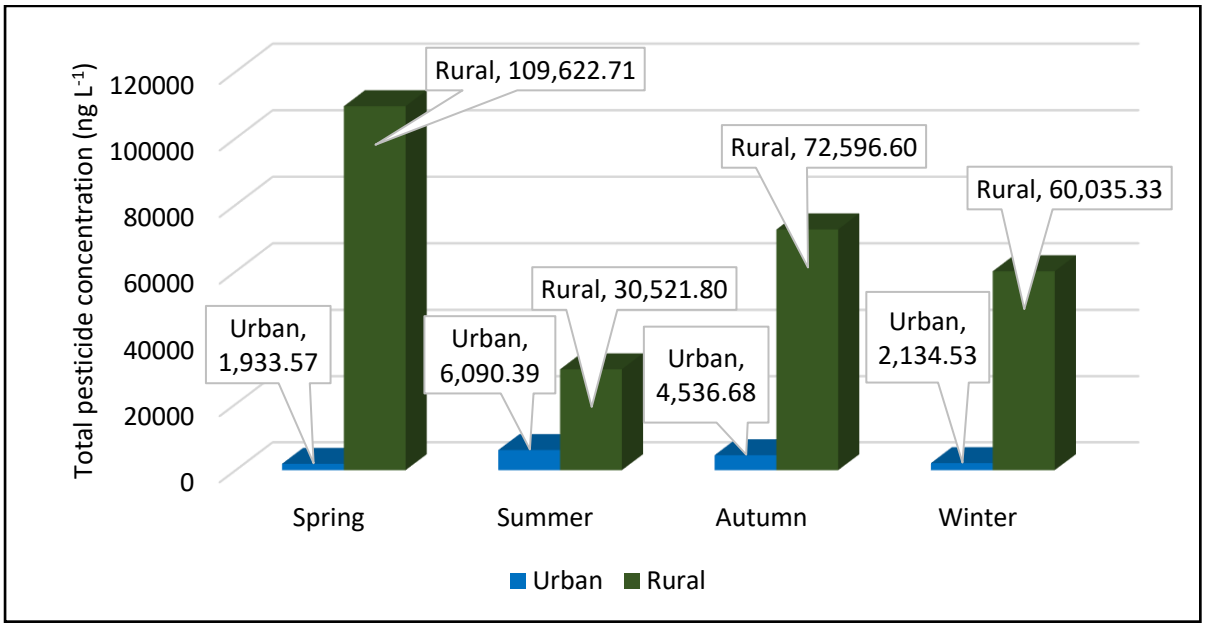


Figure 59. Bar chart showing cumulative pesticide concentrations in influent samples by season and by site.

Pesticide contaminants are generally used more heavily during spring – summer to coincide with agricultural practices. The exact time of spraying can vary depending on the crop type, with some products used pre-emergence and some used post-emergence. Spring would likely be the time of both post-emergence spraying for winter crops, and pre-emergence spraying for later crops, possibly contributing to the increased concentrations at this time. The

differences between the two sites therefore could be an indication into the kind of land practice employed.

Influent pesticide detections from both sites were dominated by the pyrethroid compound permethrin, which accounted for >87% and >94% of the year total cumulative pesticide contamination in rural and urban influent samples respectively. This was followed by the other pyrethroids cypermethrin (5% of yearly total detections at both sites) and to a lesser extent bifenthrin which was only found in spring at the rural site. Occurrences of these analytes in influent was in the high ng L^{-1} to µg L^{-1}. Pie charts showing the relative composition of the yearly influent detections for each site can be seen in Figure 60.

Permethrin has been found previously in other studies in high concentrations in WWTP samples as mentioned in Section 3.1 [33,345], however it is interesting the proportions of the total pesticide contamination this compound is responsible for in Irish samples. Permethrin has uses as a medication, commercial insect repellent and an agricultural insecticide, making its widespread presence in both urban and rural areas unsurprising. Typical medical uses for the treatment of scabies is available over the counter at most pharmacies in the form of a topical cream at concentrations of 5% w/w. Lice treatments can involve shampoos or hair masks at concentrations of 1% w/w. These treatments have a direct route into the wastewater system from being washed off skin or hair. Combined with the other uses as insect repellents and agricultural biocide, high concentrations in influent are very likely.

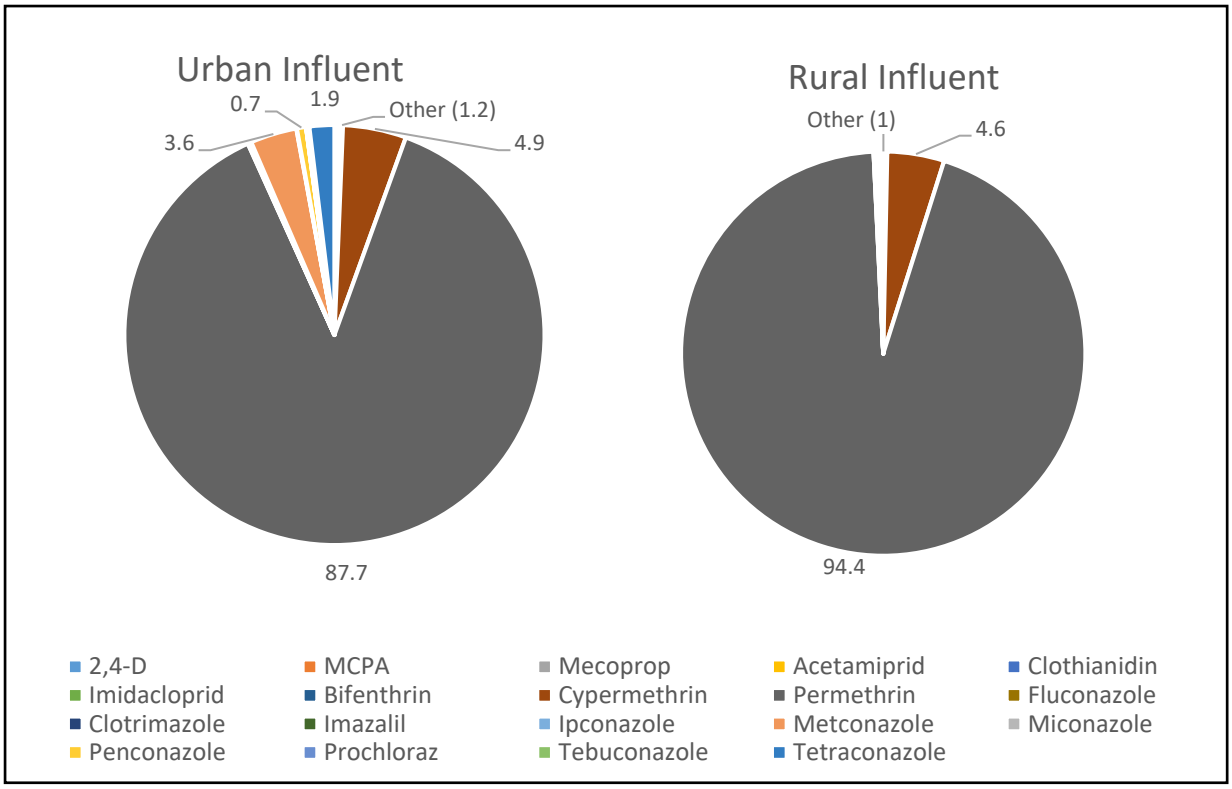


Figure 60. Pie chart showing yearly pesticide contributions to cumulative concentrations in influent samples – with permethrin dominating the data

Due to the dominance of the pyrethroid in influent samples, visualisation of the proportion the other compounds detected was difficult. In order to examine the distribution of the remaining compounds, the pyrethroid compounds were excluded and the total pesticide occurrence for influent samples was recalculated without these analytes. The distribution from this recalculation can be seen in Figure 61.

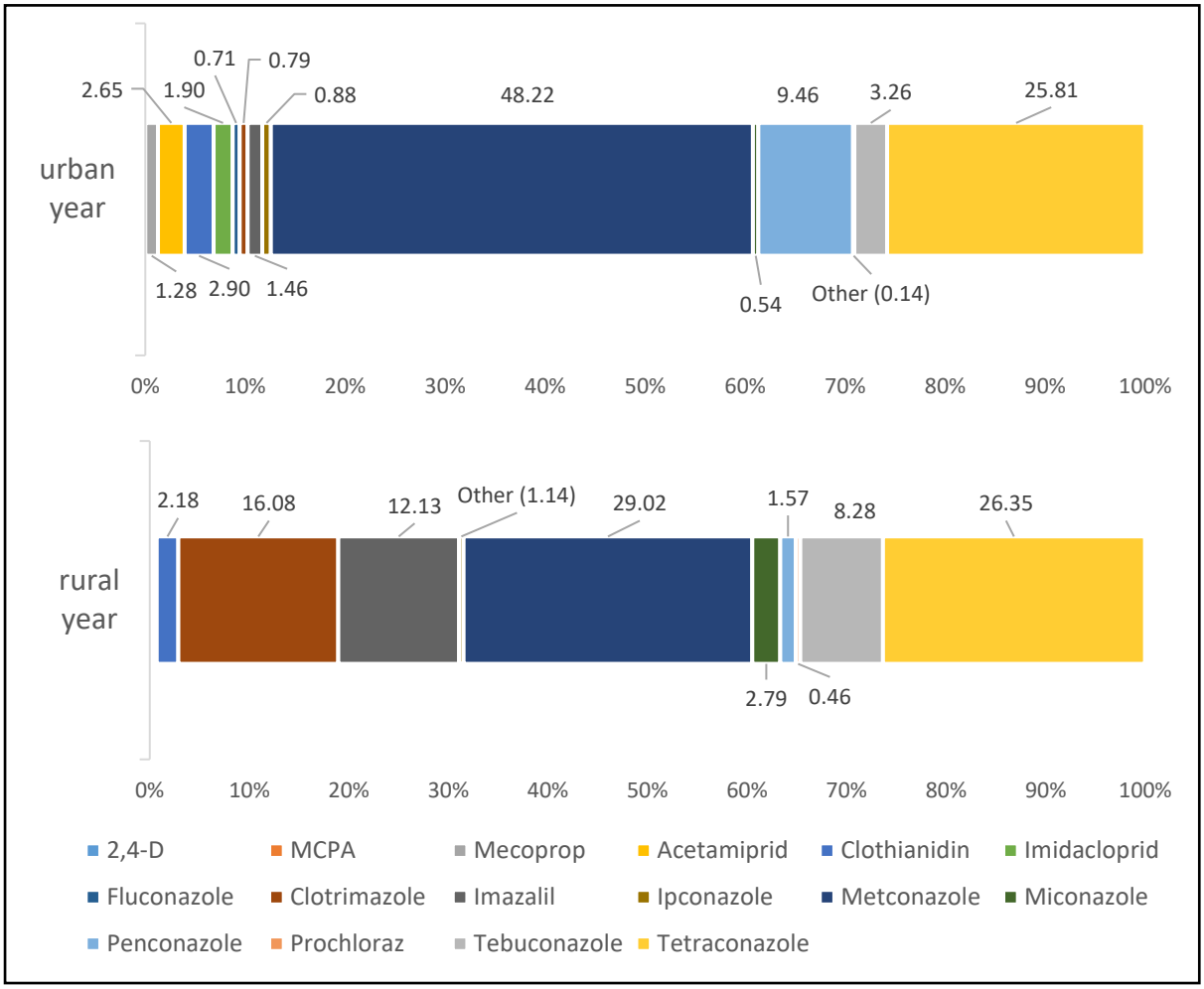


Figure 61.Bar chart showing yearly pesticide contributions to cumulative concentrations in influent samples after excluding pyrethroid compounds

Some key differences were seen between the two sites for the total yearly distribution of the remaining compounds. Metconazole, while making up a significant proportion of the total at both sites, accounted for over 48% of urban and 29% of rural influent concentrations.

Imazalil was seen to compose a significant amount in the rural area but not in the urban. This compound contributed 1.4% of the total urban pesticide load vs 12.2% of the total in the rural area. Imazalil is used on a variety of crops and was found in 17% of fruit and vegetable samples tested by the Irish Department of Agriculture in 2016 [356].This trend was also seen for clotrimazole, which had an even larger difference between sites of 0.79% in the urban area vs. 16.1% in the rural. Tetraconazole composed 25% of the total contaminant concentrations at both sites, reflecting the similar occurrence frequencies seen for this compound.

When examining pesticide contribution by seasons, permethrin remained as the generally highest concentration analyte, accounting for between 89-98% across both sites. The exception to this was a distinct difference seen in urban spring influent, when permethrin was only 38% of the total load. Remaining 37% cypermethrin and 24% other compounds. Compared to rural spring, where 96% permethrin and 3% permethrin and 1% others, shows a marked difference between the two sites at this particular time period. Charts showing this comparison can be seen in Figure 62.

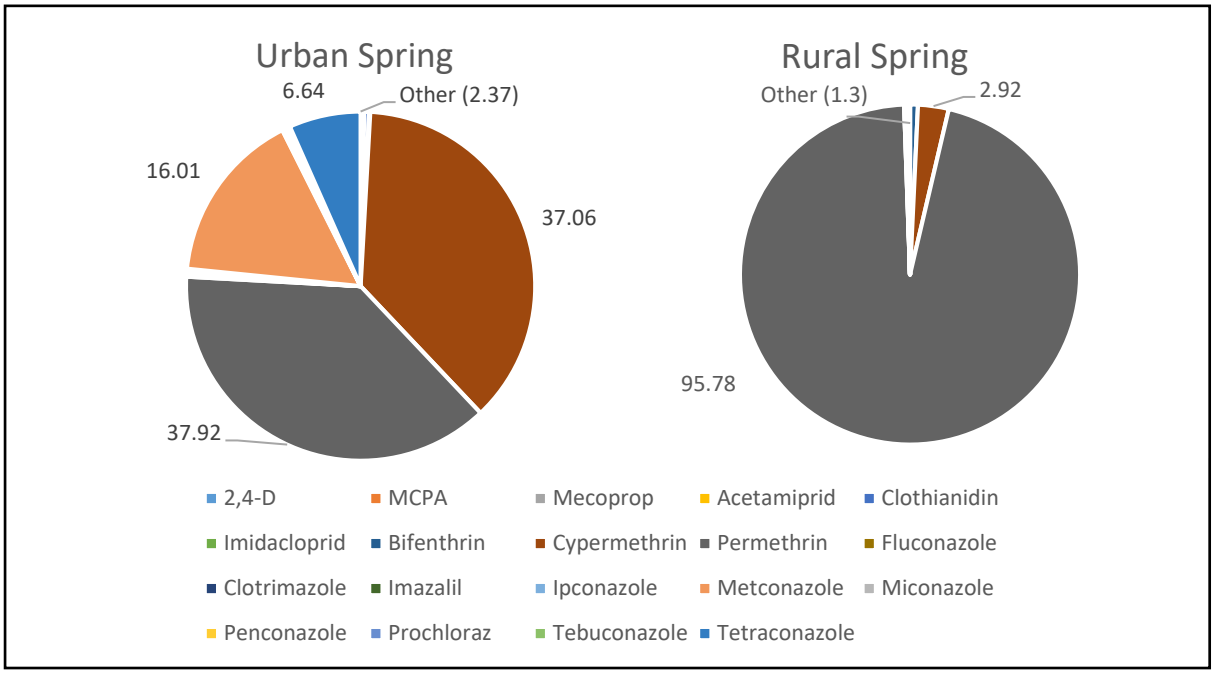


Figure 62. Pie chart showing proportional pesticide contributions to spring influent samples in both rural and urban sites.

Differences between these two sites can also be seen when examining the remaining detections after excluding the pyrethroids. The rural site had more varied analyte composition in comparison to the urban area in which 91% of the total concentration came from just from two analytes. Metconazole made up a large proportion of the total mass in each area, with 41% and 64% rural and urban areas respectively. Tetraconazole accounted for 27% and 21% of the urban and rural areas respectively. The remaining analytes generally contributed less than 2% each of the remaining urban spring totals. In the rural spring period, other significant analytes were tebuconazole, clotrimazole and imazalil at 9%, 14% and 9% respectively. Spring sample compositions (excl. pyrethroids) can be seen in Figure 63.

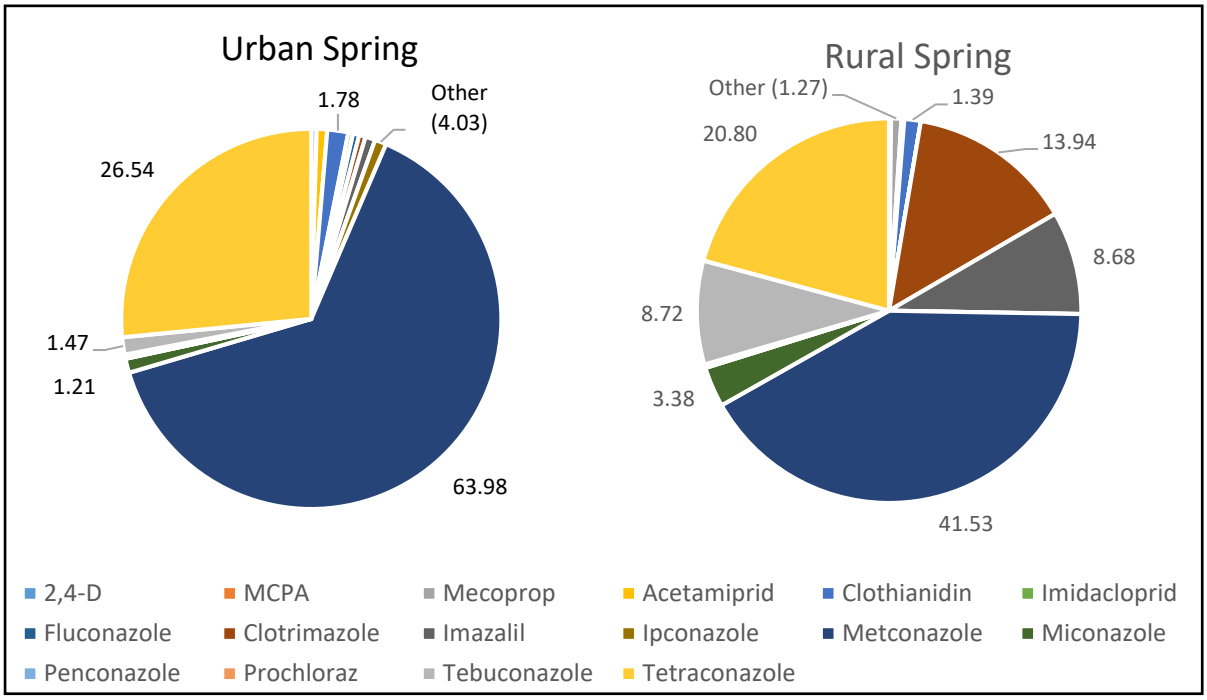


Figure 63. Pie chart showing proportional pesticide contributions to spring influent samples in both sites after excluding pyrethroids

4.3.4.2 Effluent

Similar to the influent samples, effluent samples across the entire year and both sites were dominated by pyrethroids. A highly relevant factor influencing effluent sample compositions are the removal rates of CECs at each WWTP. Removal rates are calculated and discussed in detail later in this chapter. Another potential factor influencing effluent concentrations is storm water overflow in which heavy rainfall causes the WWTP systems to become overwhelmed, and excess flow of untreated water is discharged alongside treated effluent [357].

Total cumulative pesticide concentrations varied greatly both by season and by site (Figure. 64). The highest pesticide loads were found, interestingly, in winter samples for both sites. A possible reason for this could be the increased precipitation during winter months causing run – off from soil bound contaminants, or from pre-emergent spraying of winter crops. Over 4000 ng L^{-1} of contaminants were found at each site during the winter season. Spring saw the greatest difference in total concentrations between sites, where the urban area again had over 4000 ng L^{-1} of pesticide contamination, whereas the rural area had just under 650 ng L^{-1}. Possible explanations for this could be the difference in WWTP removal efficiency both

between sites and between seasons. Additionally, the relative input of certain contaminants between sites and seasons is a contributory factor.

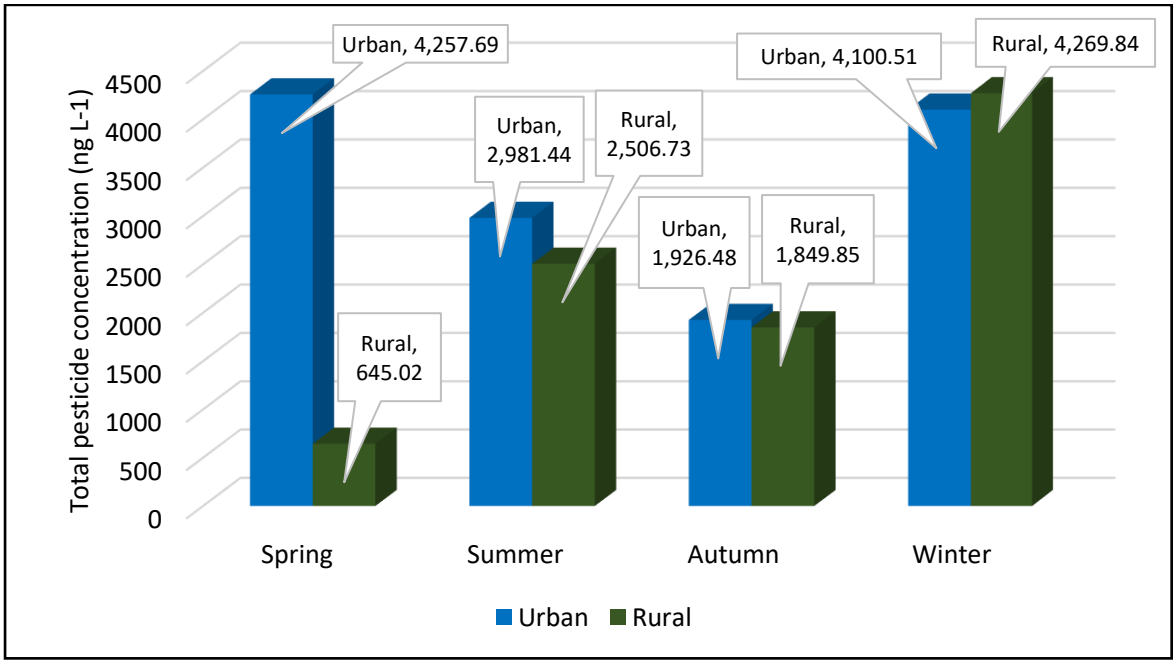


Figure 64. Bar chart showing cumulative pesticide concentrations in effluent samples by season and by site

Similar to the influent samples, pyrethroid pesticides were again the main contributors to effluent samples at both sites (Figure. 65). Interestingly however, the dominant presence changed almost completely from permethrin to cypermethrin. This switch is possibly due to the influence of the treatment processes employed at the studied WWTPs, which is discussed further in the removal rates section. Cypermethrin made up 93% of the total yearly effluent composition at the rural site, and 74% at the urban site. When coupled with the other detected pyrethroids, this analyte group comprised between 94-98% of effluent yearly pesticide loads.

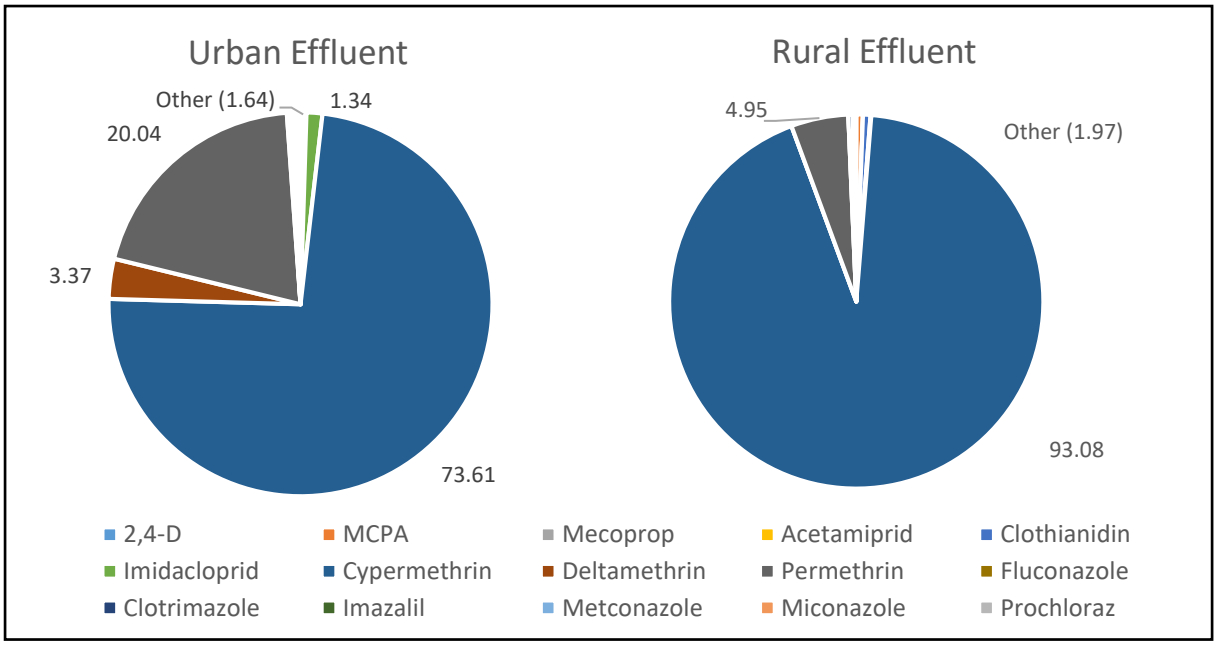


Figure 65. Pie chart showing yearly pesticide contributions to cumulative concentrations in effluent water samples

To examine the composition of this remaining 2-6% of yearly effluent sample detections, the pyrethroid group was excluded and totals recalculated to produce Figure 66. As can be seen, there is again variation between sample compositions between the two sites. In the urban area, imidacloprid comprised a large proportion of the remaining effluent composition. The urban site had more varied distribution of analytes compared to that of the rural site. The remaining total comprised analytes MCPA, Mecoprop, clotrimazole, imazalil and tetraconazole in roughly equal proportions, as well as slightly lower contributions by fluconazole and tebuconazole. In the rural area MCPA, clothianidin, clotrimazole and tetraconazole accounted for the majority of the remaining effluent yearly compositions. The difference in total variation between the two sites again indicates possible differences in removal efficiencies between treatment plants, as greater analyte variation was seen in the rural influent samples in the urban.

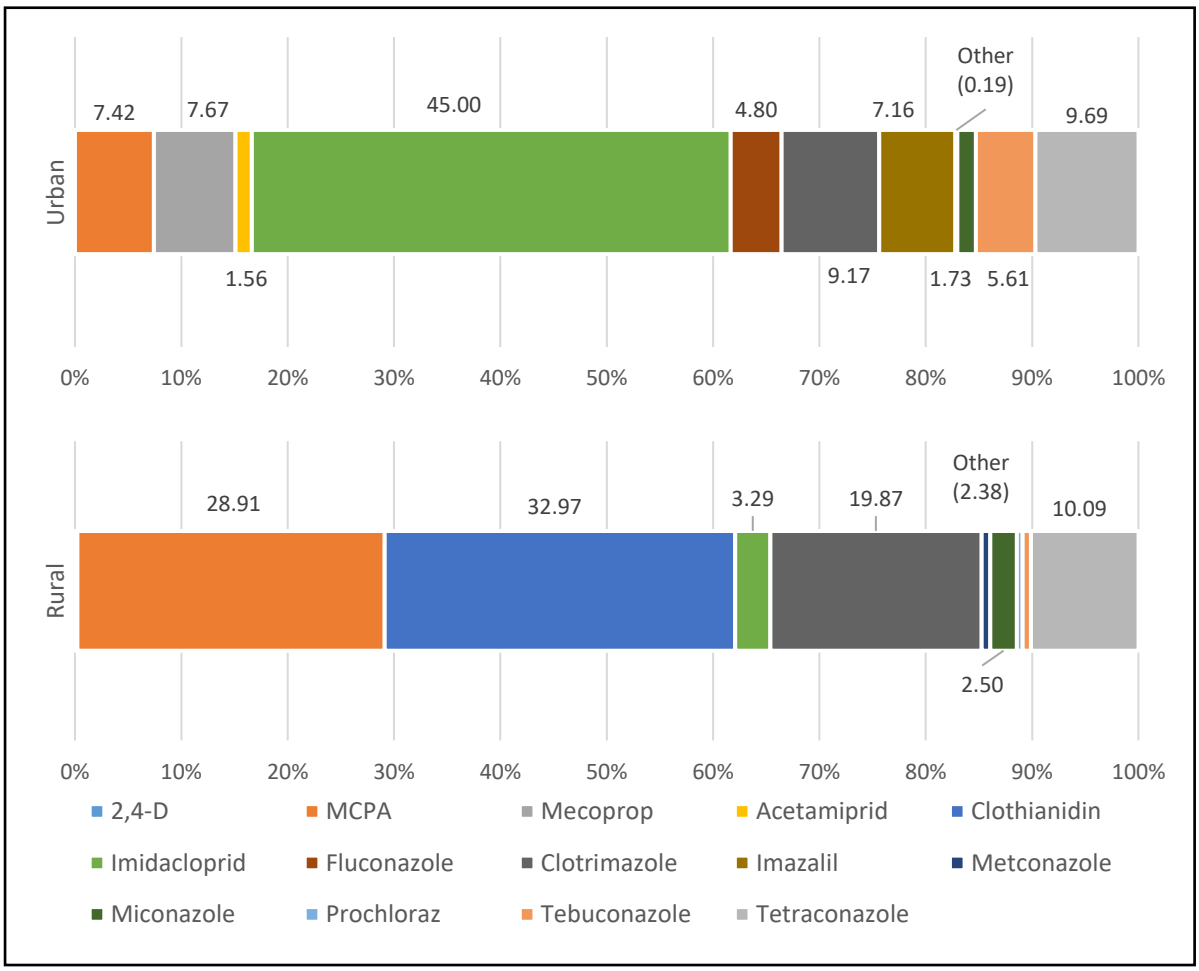


Figure 66. Bar chart showing yearly pesticide contributions to cumulative concentrations in effluent samples after excluding pyrethroid compounds

Examination of seasonal differences in effluent showed similar trends to the influent samples, in which the pyrethroids were main contributors year round. The season showing the greatest difference between the two sites was autumn (Figure 67). In the urban site, the three detected pyrethroids were seen in more equal proportions than in any other season. The remaining autumn urban effluent samples were made up of tebuconazole, tetraconazole and imazalil. By contrast, during the same season in the rural site distribution was limited mainly to cypermethrin, tetraconazole and metconazole.

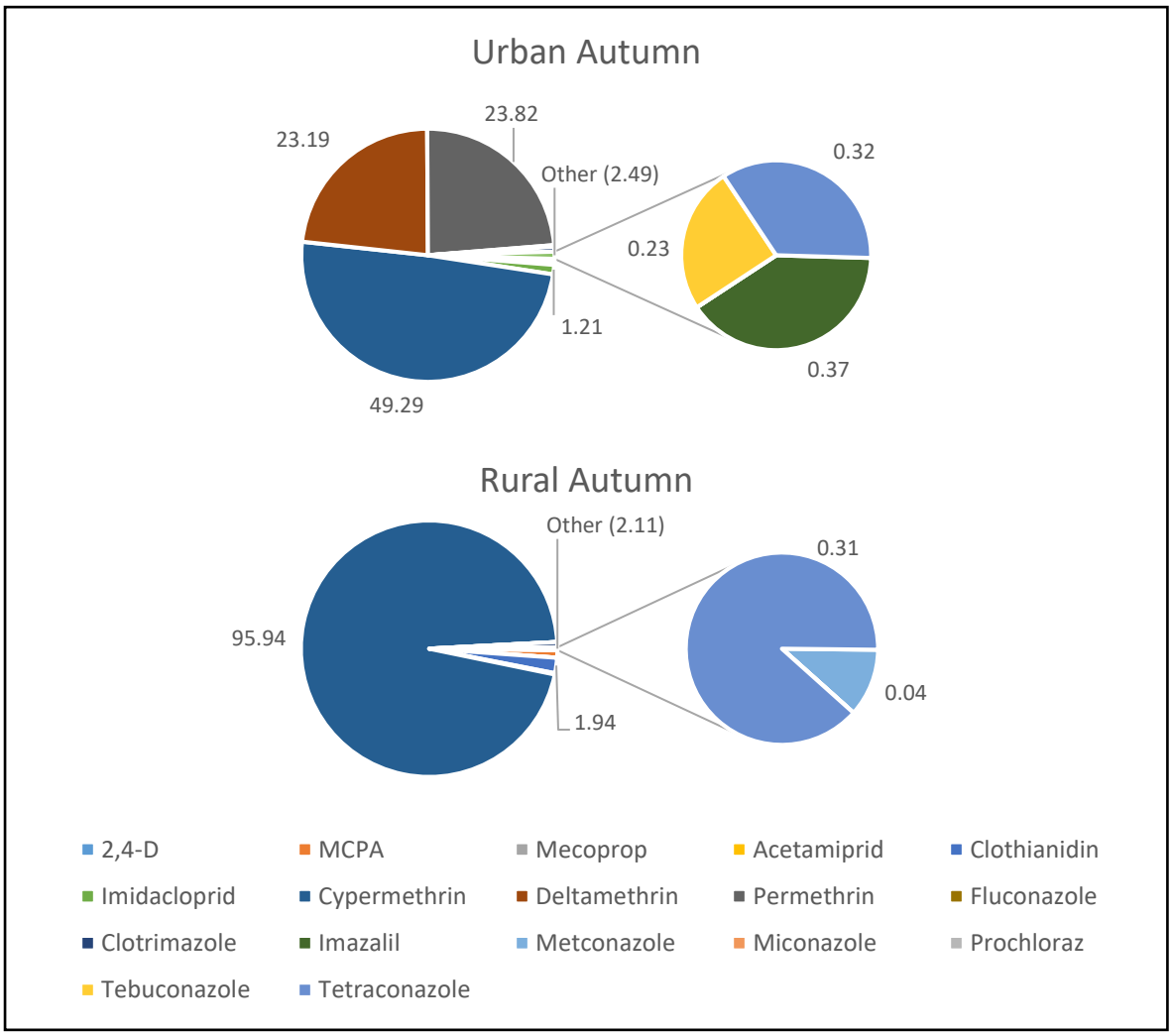


Figure 67. Pie chart showing proportional pesticide contributions to autumn effluent samples

4.3.4.3 Receiving waters

Total receiving water pesticide loads were significantly lower than that of both influent and effluent samples. Cumulative concentrations over the entire 12 month study did not exceed 270 ng L^{-1} at either site, and were overall similar for both areas (268.6 and 264.7 ng L^{-1} in urban and rural areas respectively). Highest concentrations were found during the summer in the rural area and in the autumn in the urban area, differing from the high loads seen in the winter effluent. Concentrations were lowest for both sites in the winter months, indicating differing pollution sources than WWTP effluent. Seasonal variation between total pesticide loads were greatest at the rural site, ranging from 23.26 ng L^{-1} in winter to 96.1 ng L^{-1} in

summer. The urban site had a smaller seasonal change ranging from 53.4 ng L^{-1} in winter to 79.5 ng L^{-1} in autumn. Seasonal changes in cumulative concentrations can be seen in Figure. 68. These differences in distribution throughout the seasons indicates the difference in local land practices between the two areas. Commercial agricultural practices are more tied to seasonal changes than most residential or urban gardening practices, potentially causing this difference [358]. An example of this would be golf course upkeep as a year round activity in more urbanised areas, in contrast to harvesting of spring barley occurring just in August – September.

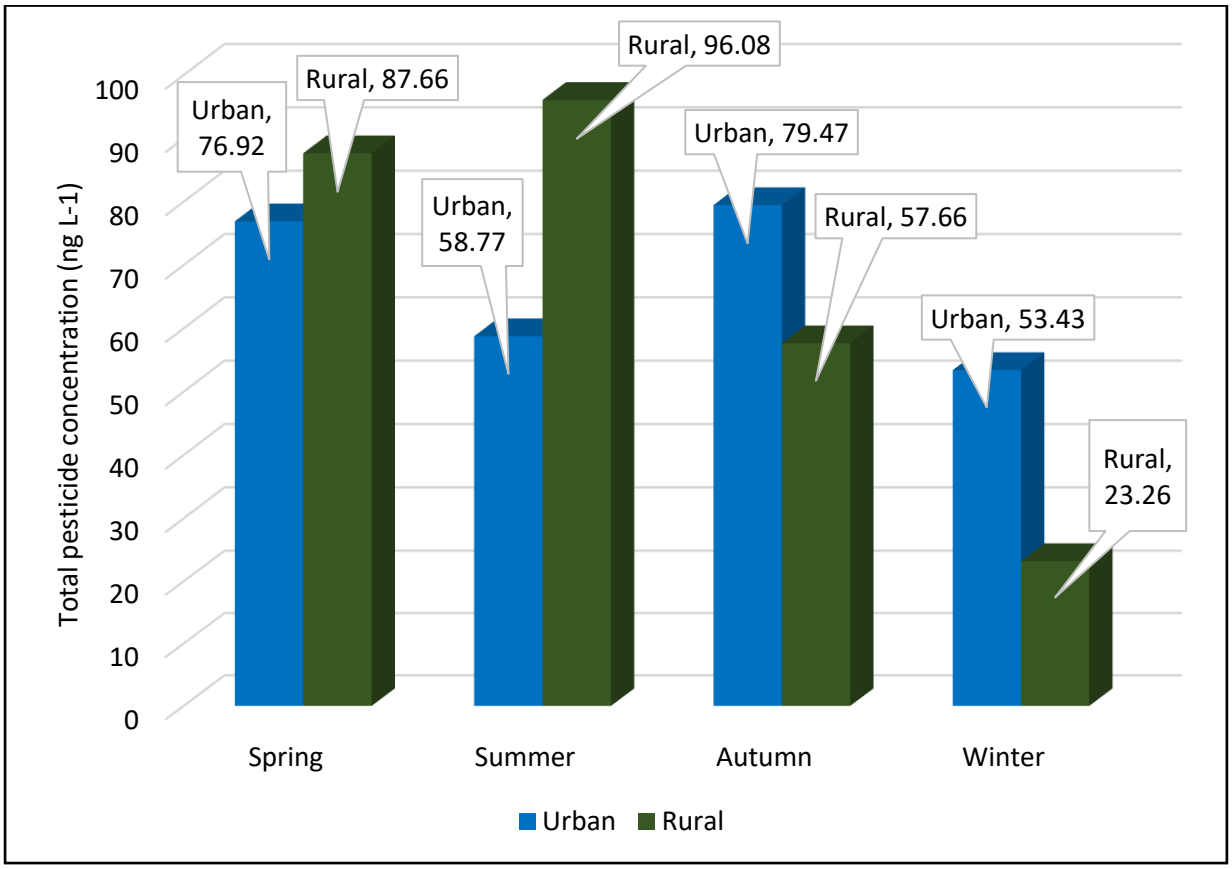


Figure 68. Bar chart showing cumulative pesticide concentrations in receiving water samples by season and by site

Analyte composition in reviewing waters varied greatly from that seen in the influent and effluent samples. Acid herbicides, which contributed a considerably low proportion of the cumulative pesticide concentration in the other two matrices, were considerable presences in receiving water samples. MCPA made up 46% of the yearly total urban detections, and 72% of total rural detections. This difference is a strong indication of an alternative source of MCPA

contamination to the receiving waters other than WWTP effluent. MCPA has been identified previously by the Irish EPA as the most widely observed pesticide in Irish rivers, being detected in approximately two thirds of all studies surface waters [205]. MCPA is used both in agriculture mainly arable and grassland practices, as well as used extensively in lawn care and golf courses, with all of these as possible sources of pollution [205]. Yearly receiving water relative compositions can be seen in Figure 69.

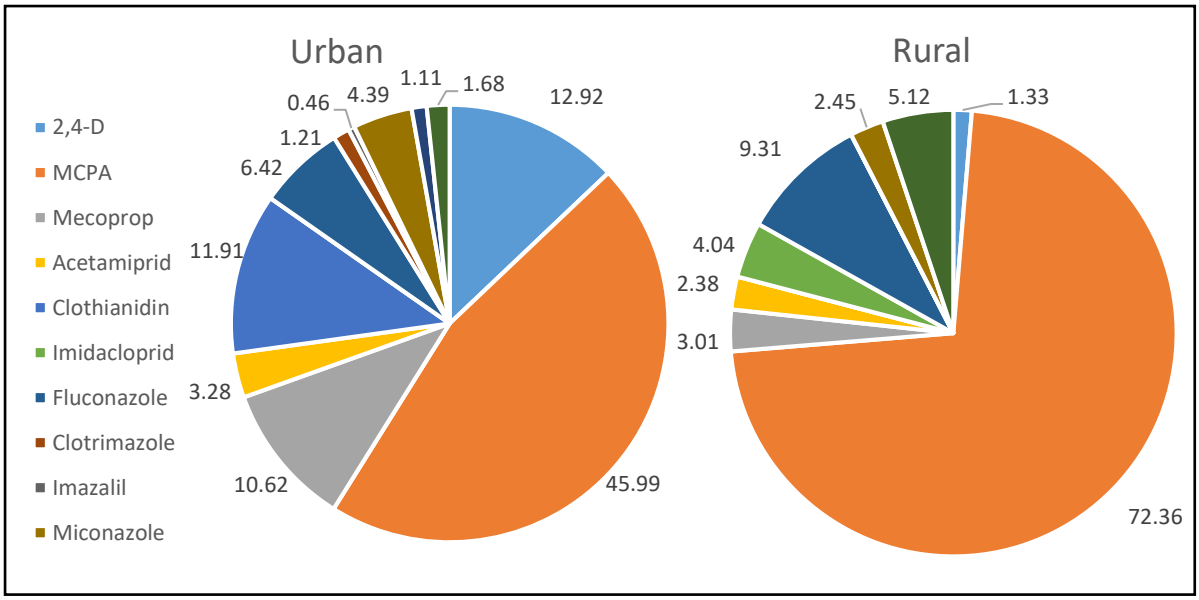


Figure 69. Pie chart showing yearly pesticide contributions to cumulative concentrations in surface water samples

It was found that pesticide compositions varied greatly both by season and by studied area. Full seasonal breakdowns for both sites can be seen in Figures 70 and 71. In the urban area, MCPA was found to be the highest occurring analyte in summer and winter only, accounting for 77 and 75 % of the total pesticide composition respectively. However, in spring and autumn distrubution was much more varied. Spring saw a much higher proportion of 2,4-D than in any other season, as well as higher contributions from azole anti-fungal miconazole. Autumn was the season with most analyte variation than any other. Key contributory analytes were MCPA, clothianidin and Mecoprop. Occurrence of these analytes in surface waters has been well documented in literature, and in particular have been noted to be very susceptible to enter river systems by runoff following precipitation events due to their highly polar nature and affinity to the water phase [205,359]. Autumn in Ireland tends to correlate with an increase in precipation following a dryer summer period, with average rainfall in Spring

and Summer amounting to ~260mm and Autumn and Winter amounting to ~350mm [360]. Therefore higher proportions of these analytes found at this time could be due to this runoff.

In the rural site, the greatest difference in analyte composition can be seen between the winter season compared to all other seasons. From spring through to autumn, MCPA contributed between 66-94% of the total cumulative concentration. However in winter, it was not detected at all. Contributors to winter detections were imidacloprid, fluconazole, tetraconazole, miconazole and acetamiprid. These compounds were found considerably less or not at all during other seasons, showing a very distinct change during this time of year. Two of these compounds, fluconazole and miconazole, have pharmaceutical uses as anti-fungal medications. Previous studies on surface waters have found increased presence of pharmaceutical compound contamination during winter seasons co-inciding with the increase in illness seen in colder months [332]. For example, a previous study by Vieno *et al.* found concentrations of pharamceuticals in aqueous samples 3-5 times higher in winter than in summer [361]. This therefore could explain the significant change in the presence of these analytes at this time.

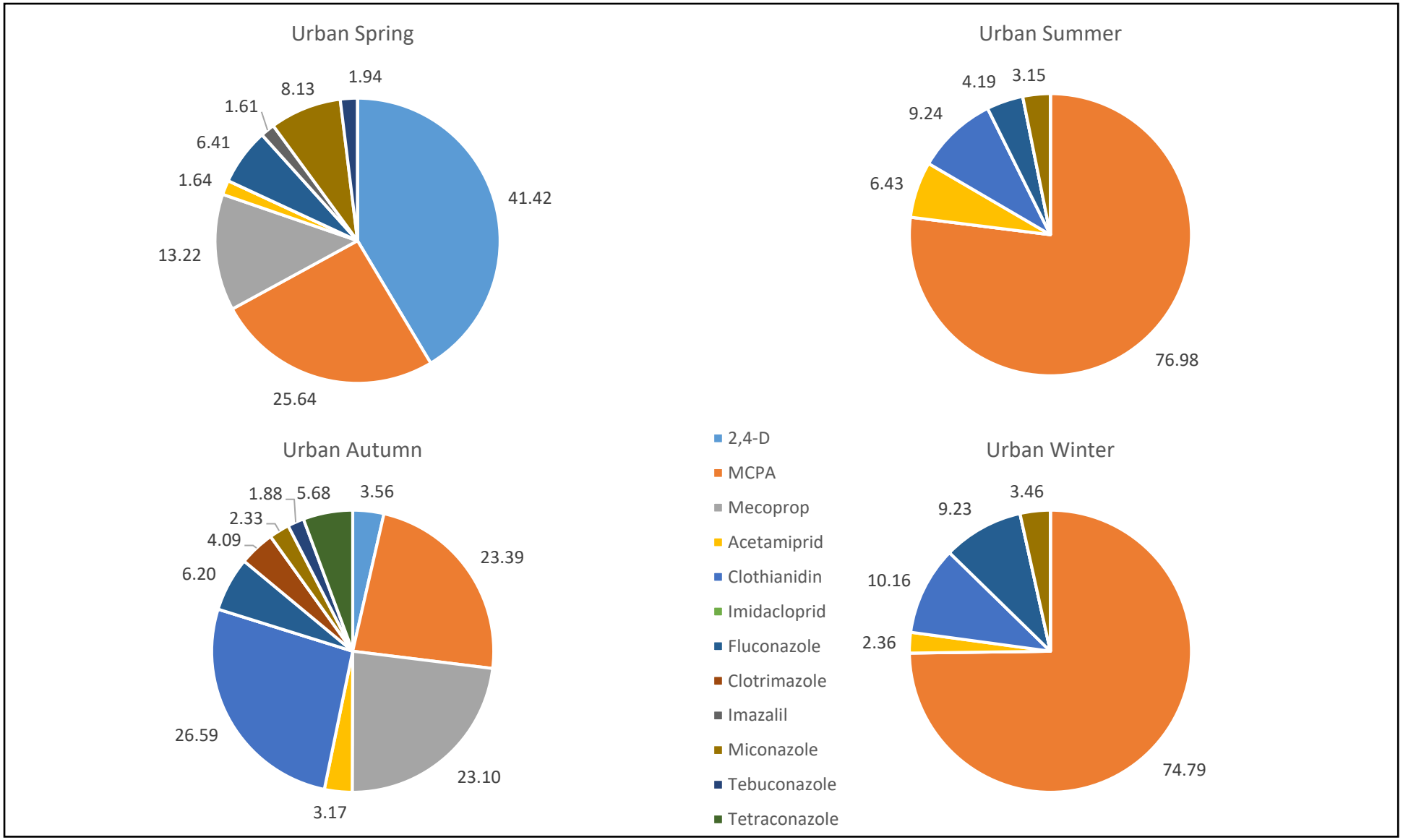


Figure 70. Pie chart showing proportion of total pesticide loads in urban surface water samples by season

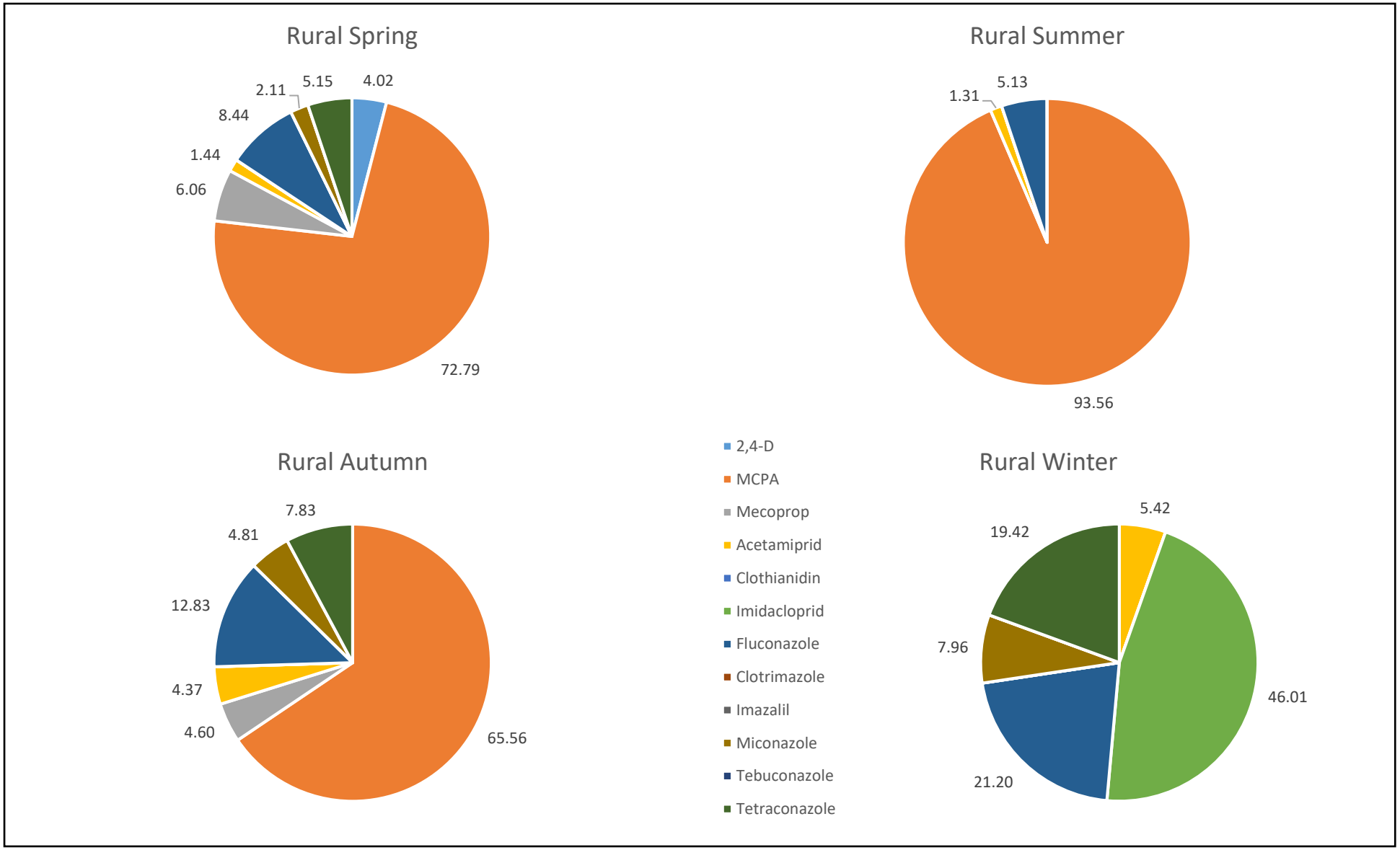


Figure 71. Pie chart showing proportion of total pesticide loads in rural surface water samples by season

4.3.5 Indicated removal of pesticides from wastewater

Removal of CECs through typical WWTPs has been shown in previous studies to be highly variable depending on the analytes' physico-chemical properties as well as the type of treatment employed at a given plant [6,30,31,362,363]. A major factor affecting rates of analyte removal is the hydraulic retention time (HRT) of a treatment plant. HRT is the average amount of time wastewater is kept within a biological reactor at a given plant, directly correlating to the amount of contact time an analyte has with biological organisms and thus influencing the rate of removal [364]. However, due to the confidentiality agreement limiting information on the plants sampled in this study, HRT in this instance was unavailable. Therefore the removals shown in this section are indicative only. A number of the analytes in this study have not been assessed for WWTP removal efficiency before. As seen in other similar studies, removal of analytes from influent in this study varied greatly between analytes, sites, and time periods. Therefore removal was calculated individually for each analyte at each site over the span of 1 year from October 2018-September 2019.

The urban site (Figure. 73) showed the greatest variability in removal both between analytes and the time of year when compared to the rural site (Figure. 74). In the urban site, only 5 compounds were removed >50% on average. These were ipconazole, metconazole, penconazole, prochloraz and tebuconazole, all azole fungicides. In the rural site 8 compounds achieved over 50% removal, which were the azoles ipconazole, metconazole and tebuconazole which were effectively removed at the urban site, as well as acid herbicides 2, 4-D, pyrethroid permethrin and azoles miconazole and tetraconazole. Both metconazole and penconazole were consistently the most efficiently removed compounds at both sites, ranging from 96-97% removal. Analytes effective removal from influent tended to be those with non-polar properties (i.e. higher Log_{Kow} values) with a few exceptions. Previous studies have shown that more non-polar compounds are more efficiently removed in treatment due to their affinity to solids which are removed through various filtration steps. Conversely, more polar analytes tend to remain in the aqueous phase and present more of a challenge [332,365]. However, polarity is not the only factor determining removal, as evidenced by the sufficient removal of 2, 4, D in the rural site and the insufficient removal seen for non-polar analytes

such as cypermethrin at both sites. Other such factors influencing removal are the ability of the compound to be broken down by biological degradation, the present functional groups and associated charge on a given analyte and its molecular weight [362].

As found in Sections 3.1-3.3, tetraconazole was determined to have very high occurrence frequency in influent and effluent samples, and was found in approximately 25% of surface water samples. This compound was generally well removed in the rural site however removal in the urban site was very variable. This is a potential cause for concern given how often it was found in urban WWTP samples, and possibly indicates that the lower occurrence in surface waters was due to dilution effects rather than removal through treatment. Tetraconazole has not been studied previously through water treatment so it is not possible to relate this finding other studies at present, and further studies are needed.

Overall average positive removal for analytes at the urban site were 62%, and 70% at the rural site. However, many of the compounds were found to have negative removal, meaning higher CEC concentrations found in effluent than in influent. Negative removals have frequently been found in previous studies of this kind and a number of potential causes have been proposed [6,30,36,366]. On such possible reason for higher effluent concentrations is the sorption of compounds on bio-materials which then desorb into the aqueous phase during treatment, causing an apparent increase of the compound in the effluent [339]. This is suggested to happen during biological treatment processes. Another potential explanation for negative removal rates is the presence of a compound as a conjugated metabolite which through treatment deconjugates back to its parent compound [121,339,363]. The average negative removals for all analytes were -416% and -175% at the urban and rural sites, respectively. The analyte which saw significant negative removal across both sites was clotrimazole.

Clotrimazole has previously been found to have higher concentrations detected in wastewater effluent than influent in Irish samples. This analyte is a frequently used topical anti-fungal and so can enter water systems through washing off of skin [367]. A study conducted by Lacey *et al*. examined three wastewater treatment plants in the Dublin area for occurrence and removal of selected pharmaceuticals, which included one azole, clotrimazole [121].

Clotrimazole was found to occur at a higher concentration in the effluent than in the corresponding influent more than 70% of the time during the yearlong study. This paper was published in 2012 but samples collected in 2008, exactly 10 years prior to the ones collected for this study. It is interesting to see the results replicated again for this compound after a number of years have elapsed, and to see it occur in both urban and rural areas.

An observation of note and worth exploring further is that of the removal seen for the two pyrethroid pesticides permethrin and cypermethrin. It was seen in the previous section that permethrin comprised the majority of total pesticide loads in influent samples, and cypermethrin that of effluent samples. Upon calculation of removal, it was seen that while permethrin had positive removal between 48-90% depending on the site, cypermethrin had negative removal varying between -72 - -18%. A possible hypothesis for this observation is that permethrin is not in fact being totally removed by treatment but is being transformed into cypermethrin. It has been shown in previous literature that processes employed at WWTPs can cause chemical reactions culminating in the formation of transformation products (TPs) as well as metabolites returning to their parent compound form [339,368,369]. Permethrin and cypermethrin differ only by the presence of a single cyano group (Figure 72).

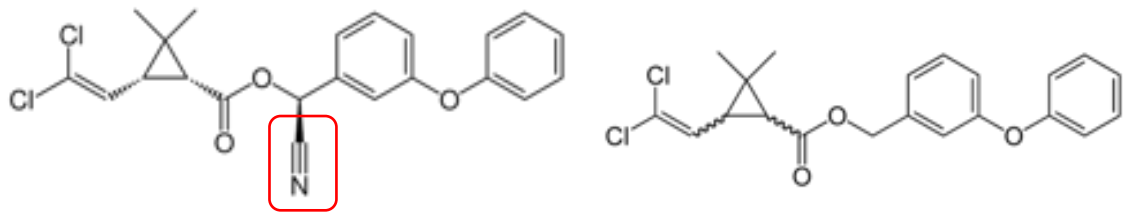


Figure 72. Chemical structures of cypermethrin (left) and permethrin (right) with the differentiating cyano group indicated in red.

Cyanides, including free cyanide (HCN, CN-) can be present in wastewaters as a by-product of industrial processes [370,371]. It could be possible therefore that a reaction between permethrin and free cyanide could be occurring during the treatment process. Permethrin has been found in previous studies to be removed well, with efficiencies between 88-93%, however all of these studies bar one included permethrin only and not cypermethrin [345,372–375]. In the one paper which did include cypermethrin, this compound was undetected in effluent samples. As the details of WWTP processes included in the present study are

unknown, it is possible that different procedures are being used affecting the behaviour of this compound. Therefore further studies would be required to investigate this hypothesis.

Overall, indicated removal efficacy across both studies sites were highly variable and generally poor (Figures 75 and 76), showing only 4 compounds consistently removed at above 50% across both sites. The findings of this study highlight the importance of employing steps in the WWTP process that specifically target the removal of CECs. Processes such as adsorption using activated carbon, ozonation, and filtration using nanofiltration or reverse osmosis membranes have all be shown to effectively remove CECs from wastewaters [362]. Implementation of any of these procedures into the plants would be of great benefit both for WWTP operators and the health of local catchments.

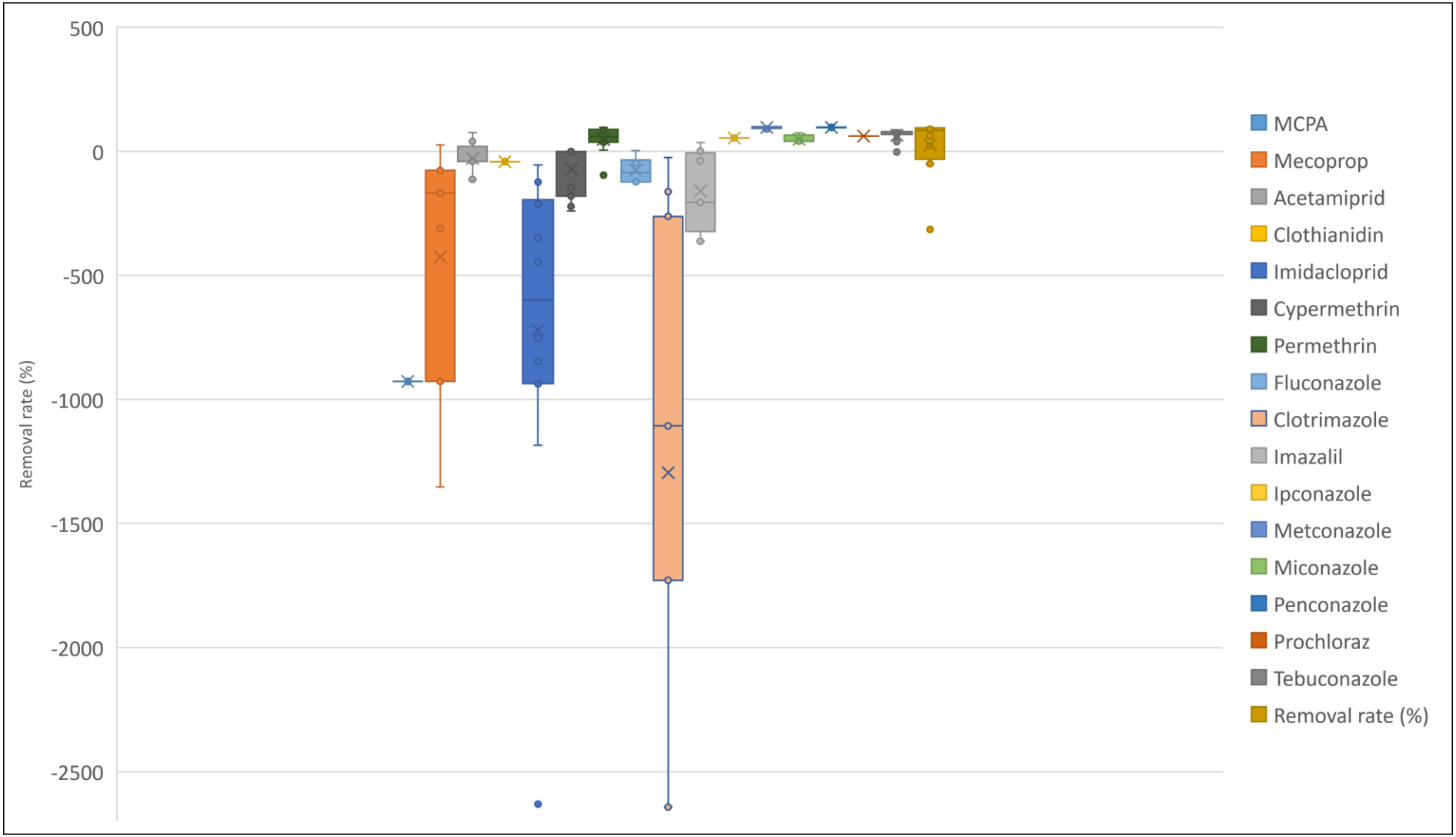


Figure 73. Box plot showing pesticide removal rates in the urban site over 1 year (n-12)

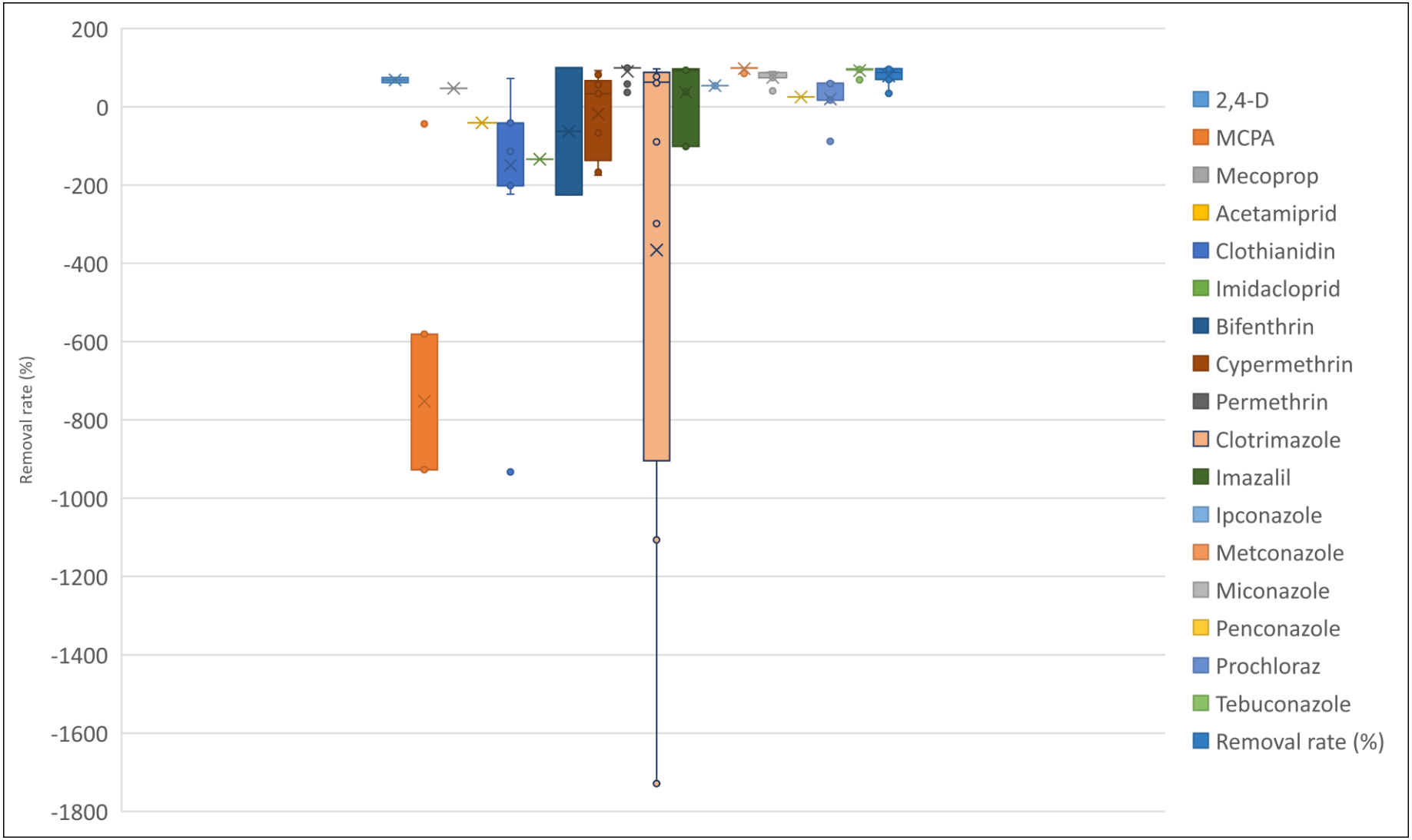


Figure 74. Box plot showing removal rates of pesticide contaminants in the rural site over 1 years (n=12)

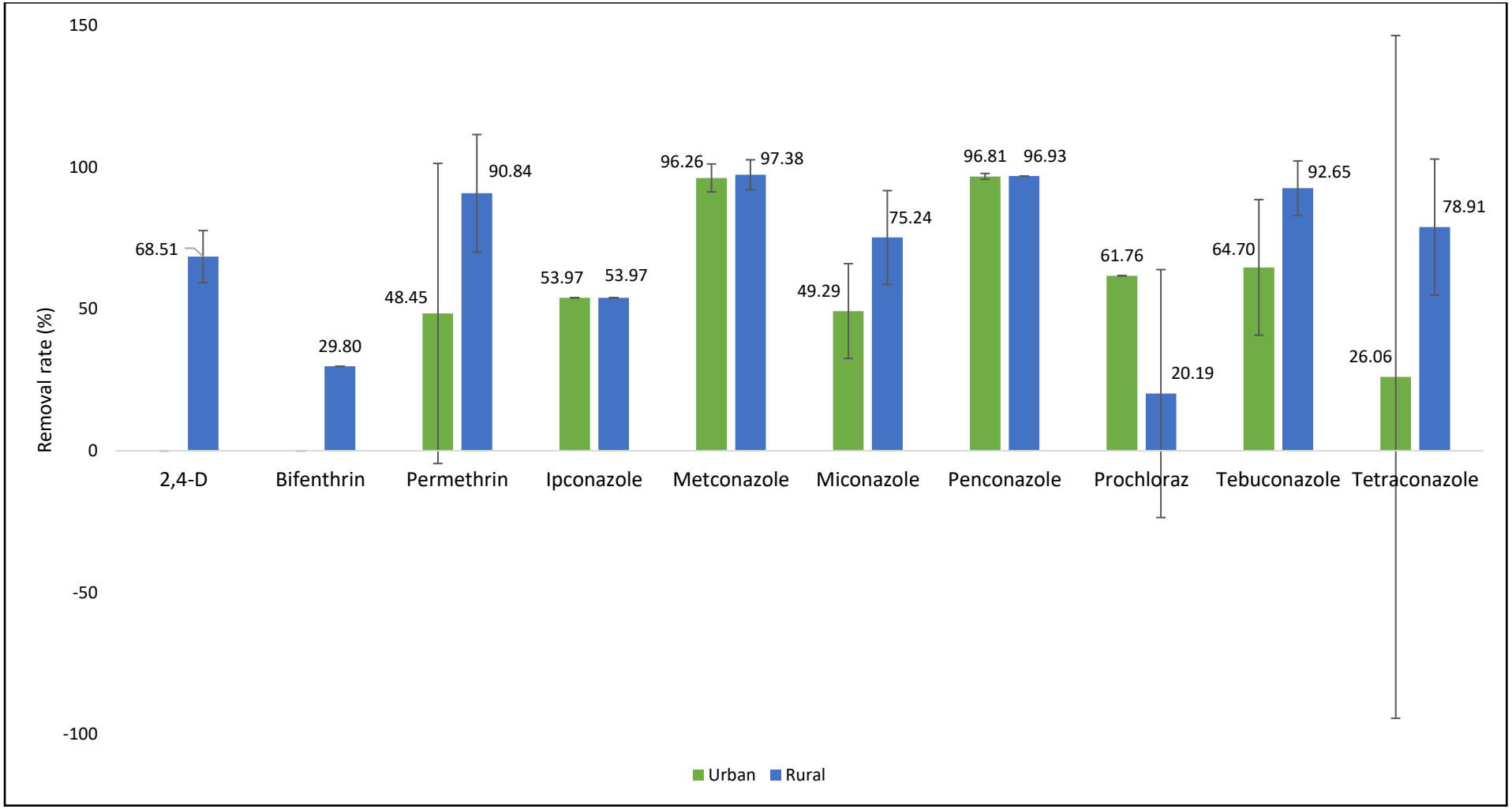


Figure 75. Bar chart showing average positive removal rates from both studied WWTPs taken over 1 year, with error bars showing standard deviation from average removal %

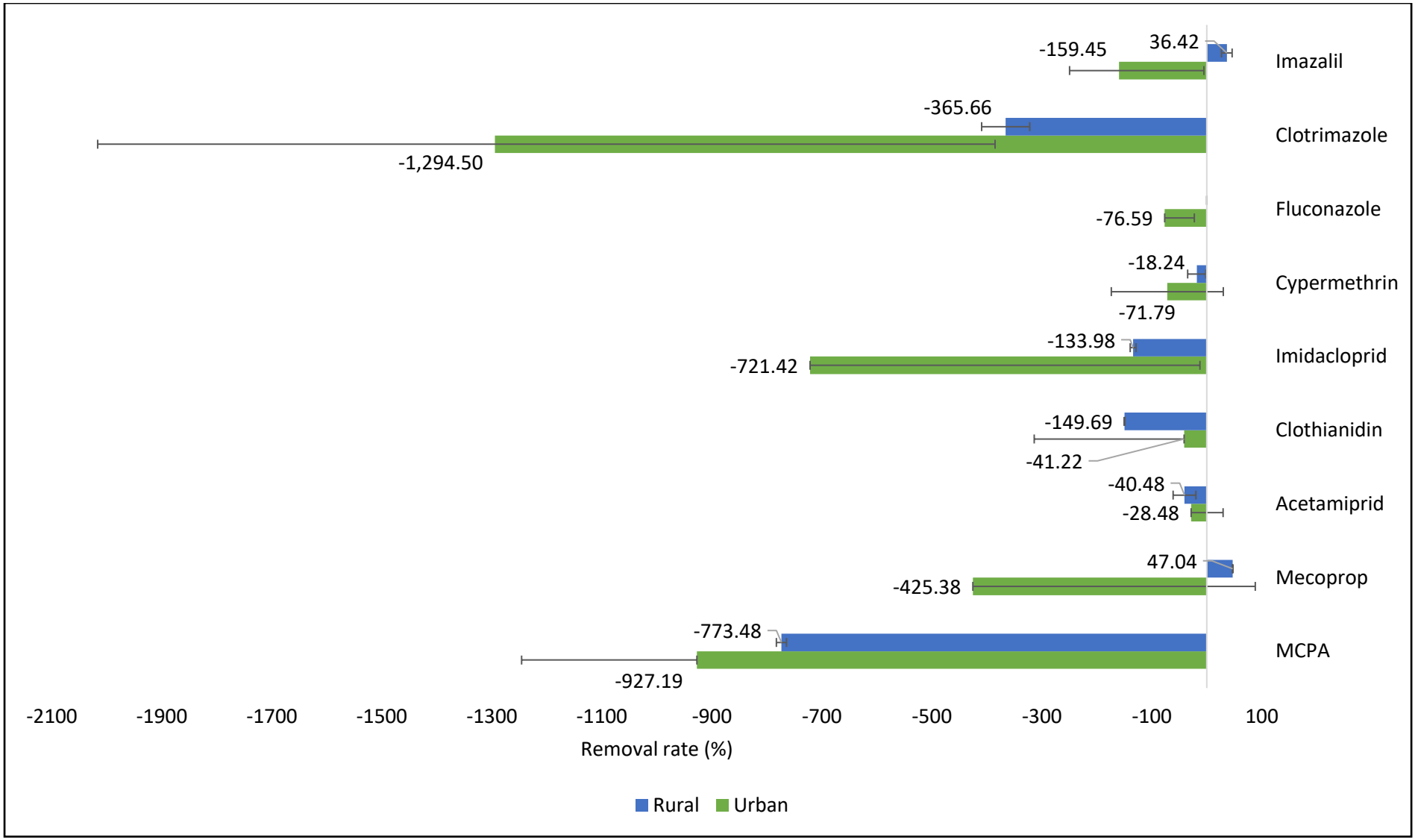


Figure 76. Bar chart showing average negative removal rates from both studied WWTPs taken over 1 year, with error bars showing standard deviation from average removal %

4.3.6 Determination of risk (RQ)

In order to assess the level of risk posed to the aquatic environment, RQ values were determined for occurrences in both receiving and effluent waters. RQ values are generated using PNEC values, and indicate whether environmental concentrations are likely to cause harm to aquatic life. RQ values were not calculated for influent samples as the concentrations found in these samples are not expected to enter the wider environment. To examine the relative risk between sites, the highest MEC was used for each site.

The majority of occurrences found in receiving waters were determined to be low risk for both studied areas. Only four analytes were identified as moderate or high risk in surface waters, one acid herbicide and three neonicotinoids. MCPA was identified as being moderate risk to the rural site due to a maximum measured environmental concentration of 83 ng L^{-1} in June 2019. In the urban site, MCPA was considered low risk.

Of the neonicotinoids, acetamiprid was found to be a moderate risk to both sites, with RQ values in the range of 0.1-0.15. Clothianidin was only found in the urban site with a MEC of 16 ng L^{-1} in October 2018, making for an RQ value of 1.9 and therefore classification as a high risk to the urban site. Conversely, imidacloprid was only found in the rural site with a MEC of 11 ng L^{-1} in February 2019, leading to an RQ value of 1.3 and consequent high risk classification to the rural site. These RQ values share some similarities with those calculated by Solaun *et al,* in which imidacloprid was determined at multiple sampling sites to be moderate or high risk. Interestingly, environmental concentrations for the other neonicotinoids found were all low risk [376]. This study was conducted in the Basque coast in the north of Spain, in an area with a large population and quite urbanised land usage. This is in contrast to the generally more agricultural country of Ireland, potentially influencing the difference in associated risk determined for the same compounds. It is also worth noting that the PNEC values used for the neonicotinoids were taken from the EU Watch Lists and not from the NORMAN network. These values are substantially lower than those stated by NORMAN, allowing for a more conservative estimation of risk. The NORMAN network is a collaboration between reference

laboratories, research centres and related organisations to share information on the monitoring of contaminants of emerging concern [1].

RQ values were also determined for effluent samples as WWTP effluents are released directly into the environment, allowing for any contaminants present in the effluent to potentially pose a risk to aquatic life. As can be anticipated, there were more moderate or high risk RQ values found for WWTP effluent, as concentrations are expected to be higher, and the overall number of analytes found was also higher than in receiving waters.
MCPA was again found in both areas, however it was determined to be low risk in both, with RQ values ranging from 0.005 – 0.008 across both sites. The other acid herbicides were also determined to be of low risk when occurring, with the rural site only showing presence of 2, 4-D and the urban site only showing presence of Mecoprop.

Similarly to the receiving waters, occurrences of neonicotinoids were identified as moderate or high risk to the aquatic environment. Acetamiprid was again identified as moderate risk, and imidacloprid and clothianidin determined to be high risk. However the RQ values for high risk analytes were higher than that of the receiving water, ranging from 3.6 (clothianidin) – 6.4 (imidacloprid).

The azole compounds found in effluent samples were also determined to be low risk, with the exception of clotrimazole. Clotrimazole was determined to be of moderate risk with RQ values ranging from 0.2-0.3 across both sites. All other detected azoles were classed as low risk, with metconazole, miconazole, tebuconazole and tetraconazole found at both sites, fluconazole and imazalil found only at the urban site, and prochloraz found only at the rural site.

Analytes found at considerable concentrations in the effluent were the pyrethroid pesticides cypermethrin, permethrin and deltamethrin. Deltamethrin was only found in one sample <LOQ, at the urban site in October 2018. However, given the extremely low PNEC values for the entire pyrethroid group, this occurrence meant for an RQ range of 2949 – 4468, indicating a very high risk to the urban site. The MEC concentrations of the other two pyrethroids cypermethrin and permethrin also produced RQ values indicating extremely high risk.

Permethrin had RQ values ranging from 505 – 2370 across both sites. Cypermethrin had the highest RQ values, where an occurrence in the urban site of 2437 ng L^{-1} in March 2019 leading to an RQ of 30463, and an occurrence in the rural site of 1910 ng L^{-1} in Jan 2019 leading to an RQ of 23875. These extremely high RQ values indicate that essentially any occurrence of these analytes in environmental waters pose a significant risk, and further work is required to mitigate these risks. These findings are similar to others found in the literature, where the majority of detections of pyrethroid pesticides in water are found to be high risk [377,378].

4.4 Conclusions

Pesticides have been an under investigated CECs class when it comes to wastewater sample occurrence and indicated removal efficiencies. This chapter presented for the first time a comprehensive study of these compounds through the Irish WWTP system.

Total pesticide loads in both WWTP influents and effluents were predominantly composed of pyrethroid compounds permethrin and cypermethrin year round. Permethrin was removed (either fully or partially depending on the site) through water treatment, however cypermethrin was not leading to this compound contributing the highest proportion of the total effluent pesticide concentrations. These analytes were not detected in receiving waters at either sample location, likely due to dilution and sorption to soil particulates.

Acid herbicides were the compounds accounting for the majority of receiving water sample composition. These analysis were either not detected at all or were not significant contributors to influent or effluent pesticide concentrations. This indicates an alternative source contribution of these compounds to the receiving waters than the investigated WWTPs. These compounds have been shown in the literature to be particularly susceptible to surface run off due to their low polarity [379], and are likely entering the waterbody from local land practice.

Removal rates were found to vary hugely between analytes, treatment plants and the time of sampling. Only 4 compounds were found to be consistently removed (>50% removal) across both sites, these being ipconazole, metconazole, penconazole and tebuconazole. Permethrin was calculated to be effectively removed by the rural site but rates varied in the urban area, with an average removal of 48% and a very large standard deviation.

A number of compounds were found to have negative removal rates i.e. a higher concentration in WWTP effluent than in the influent. This was seen most notably with the azole compound clotrimazole, which has been previously shown to exhibit negative removal

in other studies. Two analytes, imazalil and Mecoprop, were found to be partially removed by the rural WWTP but negatively removed by the urban site

In Ireland, the position of Irish Water, the authority responsible for WWTP operations, is that reduction in pesticide contamination is the responsibility of the chemical user at the source rather than through removal from WWTPs. However, this work has demonstrated the occurrence of these compounds in WWTP samples which potentially are coming from a variety of additional sources as well as spraying practices. For example, permethrin as an anti-lice shampoo ingredient. These results further highlight the need for implementation of processes in wastewater treatment which specifically target CEC removal.

Pyrethroid pesticides cypermethrin, permethrin and to a slightly lesser extent deltamethrin were identified as being of extremely high risk to studied sites due to the MEC concentrations in wastewater effluent samples. The azole anti-fungal compound clotrimazole was found to be a moderate risk to both urban and rural sites from WWTP effluent. The acid herbicide MCPA was found to be of moderate risk to rural receiving waters, alongside neonicotinoid pesticides acetamiprid and imidacloprid. This is a cause for concern due to the significant usage of surface waters as sources of drinking water in Ireland. Levels found in this study did not exceed the 0.1 µg L^{-1} drinking water pesticide limit [261], however further monitoring is advisable as concentrations did approach this limit in the summer months.

Through this study, the main aims of identifying for the first time in Ireland both *a)* insufficiently removed analytes from wastewater influent and *b)* analytes posing a moderate or high risk in WWTP effluents and receiving waters have been identified. This knowledge can aid in influencing of future policy decisions to minimise the introduction of these compounds into the environment. Pyrethroid pesticides in particular were found in high levels in both WWTP influents and effluents, indicating sources from both urban practice and agriculture. Since the sampling for this study, further legislative moves have been made to reduce the reliance on pyrethroids by limiting the amount of uses they are licenced for [380]. This is a move in the right direction for these analytes which were determined to be high risk in this study. Additionally, it can assist wastewater treatment operators in selecting possible future

treatment approaches to include alternative mechanisms of CEC removal for the identified problematic compounds. It is recommended from the new information gathered in this study that improvement in the WWTP practice in Ireland is required in order to remove these analytes.

Chapter 5:

Multiple Sampling Approaches to Pesticide Monitoring in Surface Water Catchments

5.1 Introduction

The importance of monitoring aquatic environments for contaminants of emerging concern has become increasingly apparent through the work conducted in this thesis, and the large volume of literature on the topic discussed in Chapter 1. Surface waters are at particular risk for contamination with CECs from a variety of point sources including agricultural and anthropogenic activities as indicated in Chapter 3, and WWTP effluent as demonstrated in Chapter 4. Fresh water is a vital resource and contamination even at the µg L^{-1} or ng L^{-1} level can have very negative effects to human and animal life.

A catchment is defined by the Irish Environmental Protection Agency as an area of land directing rain and groundwater into a river or stream, which leads to all of the water ultimately running off to a single outlet [381,382]. By viewing our water resources not as independent entities but as an entire ecosystem, it allows for effective management of our water resources. Many water monitoring campaigns use a catchment based approach to assess the quality of their water bodies [180,187,254]. By taking into account the entire catchment surrounding a water resource, it is possible to understand the factors affecting the river ecosystem. This approach is particularly applicable to the monitoring of pesticides. Stress factors such as the use of the land surrounding a water body will greatly impact the variety of potential pollutants the water body is exposed to. Land use in the context of water quality monitoring can be loosely broken down into three sectors; agricultural, industrial and household [383]. The practices which can introduce pesticide pollution include arable, pastoral and mixed farming. For example the herbicide MCPA is frequently used to control rushes in grasslands. However, due to its highly polar nature, it is readily soluble in water and thus is extremely sensitive to surface run off which leads to fresh water pollution [205]. However, other potential land-use stress factors should still be considered including the presence of water treatment works, industrial plants, and urban residential areas [303,349]. In addition to this, additional stress factors arising from climate change including drought, flooding and atmospheric deposition of contaminants from changing weather patterns are relevant factors

[16,173,384,385]. With careful consideration of the catchment area, researchers can also strategically plan their sampling campaigns based on the catchment information.

Monitoring of CECs in surface waters has primarily been conducted in previous studies through grab sampling alone. However, the limitations of grab sampling include the possibility for missed contamination events due to only providing a 'snapshot' of the chemical composition of the river at that point in time [171]. In addition to this, the volume of water that can be sampled is limited and in turn affects potential method detection limits [123]. Passive sampling has been suggested as an alternative approach in order to provide data over a given deployment period, and to sequester ultra – trace levels of contaminants by sampling large volumes of water [124].

In the previous chapter, it was found that WWTP effluents containing the pyrethroid pesticides posed a high risk to the studied sites. The EQS values for these compounds is 0.08 ng L^{-1} , which has been shown to be exceedingly difficult to reach by most analytical methods [211]. A previous study by Vorkampt et al in which 12L of water was extracted still failed to reach this EQS [386]. While the method developed to analyse these compounds in this thesis was successful in determining concentrations of pyrethroid pesticides in WWTP influent and effluent due to the significantly higher concentrations in these samples, no presence of pyrethroids were found in surface waters. As the method detection limits were higher than the EQS for surface waters, it is unknown whether these compounds are not actually present at harmful levels in surface waters, or whether the levels are just below the analytical method limitations. In order to assess if these compounds could be found in surface waters, passive sampling was selected for investigation as a tool for additional screening in conjunction with grab sampling.

Aims and Objectives

The aim of this work is to examine pesticide occurrences through multiple sampling approaches to catchment monitoring.

The objectives are to:

- Highlight key aspects of the literature methods detailed in Chapter 1 that were used to guide this work;
- Apply the methods developed in Chapter 2 to grab and passive samples.
- Obtain information on the occurrence of pesticide contaminants in selected catchments.
- Assess the employment of multiple sampling approaches for use in future catchment monitoring practices.

5.2 Materials and Methods

5.2.1 Reagents, chemicals, consumables

All materials used for sample collection, preparation and analysis are detailed in Chapter 2 of this thesis.

5.2.2 Passive Sampler Preparation, Deployment and Extraction

Passive sampler preparation and extraction procedures for both Chemcatcher configurations are described in Chapter 2 sections 2.2.4.3 and 2.2.4.2.1.

Two kinds of deployment set ups were used depending on which was more appropriate for the sampling site. A metal cage which can house up to 6 Chemcatchers which is secured by a length of chain was used where the river bed was sufficiently deep enough to ensure the devices would be fully submerged even during low flow. Where a possibility of insufficient depth was apparent, plastic sheets designed to be used in conjunction with Chemcatcher housing 3 devices per sheet were used and secured using rope. Images of both cages and sheets can be seen in section 5.3.5 of this chapter.

5.2.3 Grab sample collection and preparation

Full sample collection and preparation procedures are detailed in Chapter 2 section 2.2.4.1. Grab samples were taken to coincide with the time of passive sampler deployment, retrieval, and additionally at a mid-point during the C18 disk deployment. Sampling locations can be seen in Figure 77.

5.2.4 Calculation of Time Weighted Average (TWA) concentrations

Time weighted average (TWA) concentrations of each analyte found in the studied catchments over the deployment periods were calculated using the following equation:

$$C_{TWA} = \frac{n}{R_s t}$$

Equation 8. Calculation used for estimation of Time Weighted Average concentrations on passive sampler disks [124].

Where *n* is the amount of analyte found on the passive sampler disk, R_S is the uptake rate of the analyte onto the disk, and *t* is the deployment time. R_S values for analytes in this study were taken from the literature. Direct literature values for uptake of the three acidic herbicides on anion exchange disks, and azole compounds imazalil, penconazole and prochloraz on C18 disks were available [188,387]. Where an R_s for a specific analyte was not available, uptake rates based on previously studied analytes with similar Log_{Kow} values was used as described by Moschet et al. [211]. Due to the increased level of uncertainty associated with usage of estimated values, TWA average concentrations presented in this study for these analytes are considered semi-quantitative and are indicative of the relative concentrations found.

5.3 Results and Discussion

5.3.1 Catchment site selection

For the purposes of this study multi-dimensional sampling was performed in two main areas in Ireland, counties Donegal and Kildare. Site maps of the selected sites can be seen in Figure 77.

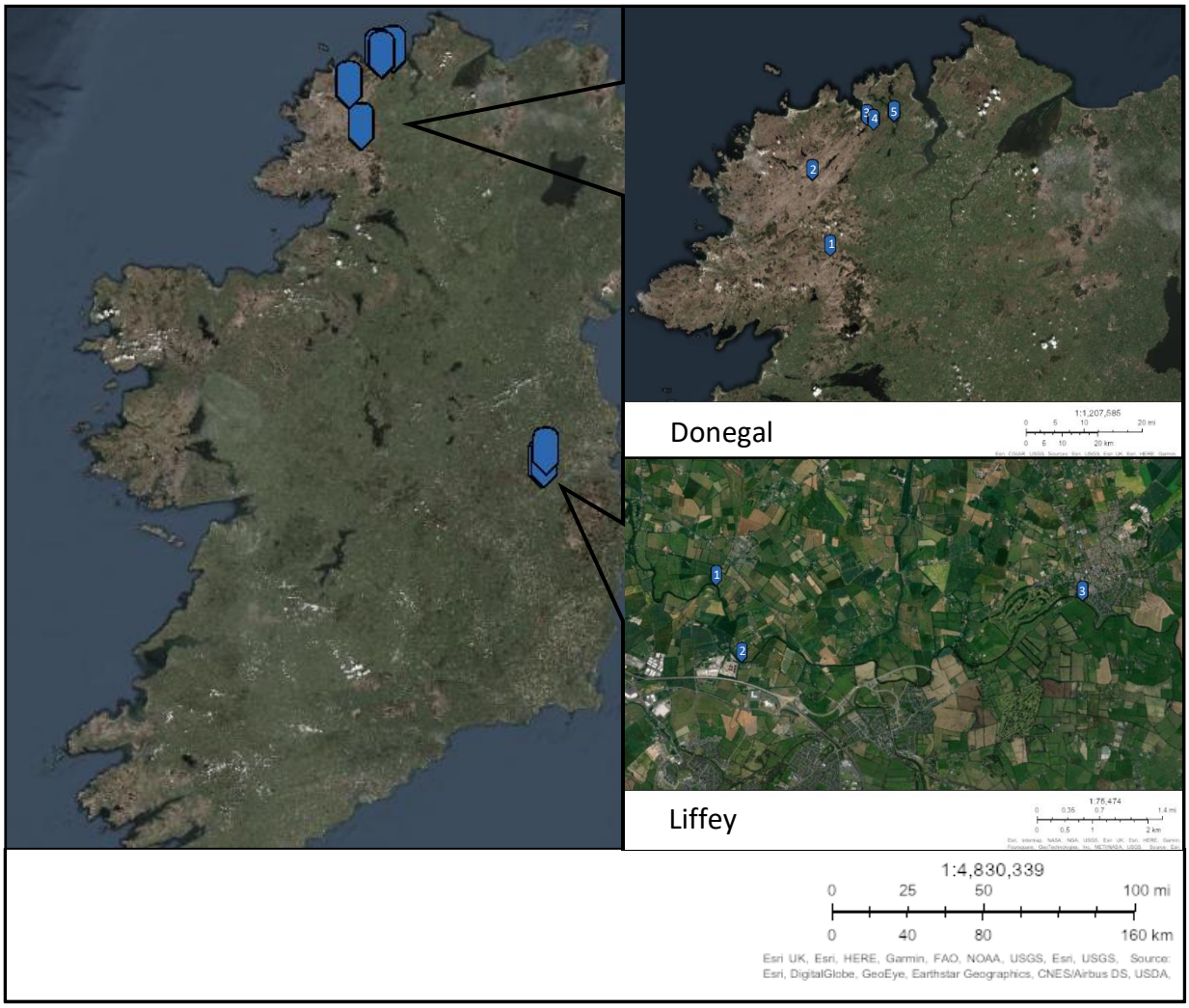


Figure 77. Map of sampling sites selected for study of a combined approach to catchment monitoring. Top right shows the 5 sites selected for study in the Donegal area, site key: (1) Glenadowan (Clogher), (2) National Park, (3) Glen A, (4) Glen B, (5) Cranford (Big Burn). Bottom right shows three sites in Kildare for Liffey study, site key: (1) Liffey Upstream, (2) Liffey Midstream, (3) Liffey Downstream.

County Donegal is in the north-west of Ireland and is a rural area, with the largest population centre of Letterkenny having <20,000 residents. According to the 2016 Census, it is the most rural county in Ireland with over 70% of residents living in rural areas. Previous studies have

shown pesticide contamination throughout the county of Donegal. In particular, the pyrethroid group of compounds have been tied to this area due to the widespread use of these compounds in various agricultural (mainly livestock) and forestry practices. Therefore passive sampling in this area targeted non-polar analytes using C18 disks. Five sites within this area were selected for study to build on the previous studies here by the DCU Water Institute [388]. The Owenveagh River flows through Glenveagh National Park, and has consistently been considered a high status river (Q5) from ecological monitoring performed by the EPA under of the Water Framework Directive (WFD) since 1990. Q values are on a scale from 1-5 and relate to macroinvertebrate bioindicators, and were all accessed through the EPA catchments.ie database [206]. The National Park site was included for study to act as a control catchment within the area. The remaining sites of Cranford (Big Burn), Glenadowan (Clogher) and two sites along the Glen river were identified as being at varying levels of risk for agricultural pollution, and have shown a decline in ecological condition since the late 2000s/early 2010s (currently ranging Q3-4 dependant on site). However, Q values are only determined in this area every 3+ years, with some sites most recent Q values being from 2019 [206].

County Kildare is in the east of Ireland and is home to a large portion of the River Liffey. The Liffey is responsible for the majority of the water supply to the Greater Dublin Area making this waterbody of great importance to a large portion of the Irish population. The south and east part of Ireland is generally used for more arable or grassland agriculture, and kinds of pesticides used tend towards herbicides. The Liffey catchment has been identified as mainly under pressure from anthropogenic sources, including golf courses suggested as pressures [389]. Pesticides, particularly herbicides have been known to be used frequently on golf courses to avoid the growth of weeds. Acid herbicides are some of the most commonly used herbicides in the world, and MCPA in particular is the most frequently detected compound in Irish surface waters according to the EPA[205]. Acidic herbicides have been previously found using passive sampling technologies in the south-east of Ireland [254]. However, the Liffey catchment has not been studied previously for these compounds using a combined approach. Therefore, anion-exchange Chemcatcher disks were selected for passive sampling in this area to predominantly target acidic herbicides [72]. Three points along the upper portion River Liffey

were selected for study, to include the discharge point from a local Wastewater Treatment Plant. This portion of the Liffey was chosen to be studied to avoid Dublin City Proper, in which pesticide pollution was less likely to occur. The WWTP site was incorporated however as WWTP effluent has been shown to be a contributory source of pesticide pollution in previous literature as well as Chapter 4 of this thesis [196]. Q values from the sites studied along the Liffey range from Q4-5, however the upstream site Q value is from 1991 as more recent data was unavailable. Ecological monitoring data from the other two Liffey sites were from 2019 [206].

5.3.2 Field measurements

Field measurements for Dissolved Oxygen (DO), pH, temperature, turbidity and conductivity were collected at every grab sampling event for each site. Field measurements can be found in Table 51. As measurements were only taken over a brief period, temporal trend monitoring for these catchments was unable to be examined. However, some interesting differences between the average measurements at sites during the study period could be seen. This is most clearly demonstrated by the marked difference in conductivity values between some of the rivers in Donegal compared to those in Kildare. Average values along the Liffey ranged from 372-425 µS/cm whereas average values in the National park and Glenadowan (Clogher) sites in Donegal ranged from 51-63 µS/cm. Higher levels of conductivity are an indicator of greater amounts of chemicals in the water such as dissolved salts and other inorganics which directly affect the water's ability to conduct electricity [390,391]. The differences between studied sites is indicative of the influence of differing land uses on water quality parameters. Rapid changes in conductivity can be linked to pollution events [308], and so collecting data for these variables can potentially provide additional information for catchment monitoring.

Table 51. Table of physico-chemical field measurements taken at sampling locations for the combined sampling approach to catchment monitoring study during summer 2021

	Sample	DO (mg/L)	Temperature (°C)	Turbidity (FNU)	pH	Conductivity (µS/cm)
Liffey Upstream	June	8.53	15.077	0.80	8.16	369.2
	July	7.89	15.781	0.84	8.17	375.2
Liffey Midstream	June	8.35	15.201	0.67	8.36	376.0
	July	8.30	15.949	0.60	8.11	384.8
Liffey Downstream	June	8.81	15.868	0.58	8.17	421.2
	July	7.74	16.129	0.69	8.00	428.7
National Park	July	9.22	14.414	6.25	7.96	77.3
	August	9.26	13.155	0.67	6.66	32.8
	September	9.19	14.461	2.45	8.02	77.6
Cranford (Big Burn)	July	8.79	14.354	1.81	7.11	164.7
	August	8.96	13.138	2.14	7.66	187.9
	September	8.96	14.145	4.44	7.88	262.8
Glenadowan (Clogher)	July	9.02	14.931	1.02	6.14	48.2
	August	9.35	14.057	0.69	6.11	30.3
	September	8.98	15.202	2.27	7.76	73.3
Glen A	July	9.00	14.084	4.62	7.49	89.9
	August	9.07	12.700	1.10	7.12	84.8
	September	8.86	13.830	6.85	7.98	173.5
Glen B	July	8.56	14.586	8.56	7.08	81.5
	August	9.18	12.896	1.53	7.45	93.8
	September	8.87	13.579	2.35	7.64	160.5

5.3.3 Occurrence of pesticides in grab samples

Grab samples showed presence of 13 CECs in Irish surface water catchments (Table 52 and Figure. 78). All 13 compounds were found in Donegal, whereas only 9 were found in the Liffey. The top three most frequently occurring compounds were all azoles used for their anti-fungal properties, clotrimazole, fluconazole and miconazole. Clotrimazole was detected in 100% of grab samples however concentrations were always below quantifiable levels. Fluconazole was similarly found below method quantitation limits in all grab samples. However, miconazole was detected above the LOQ on two occasions. Once in the Liffey catchment near the outfall of a WWTP plant in July, and once in Donegal at the Cranford (Big Burn) location

in August. Both detections were at very low levels (3 ng L^{-1}). These results are similar to those found in Chapter 3 of this thesis in which of the azole compounds, the antifungals were among the most frequently found in surface waters but at very low concentrations. This is also consistent with the literature in which these antifungals are frequently found in surface waters [329]. RQ values for these analytes determined in Chapter 3 showed low risk at these levels, which can be extended to the results found in grab samples in this study.

A number of analytes were undetected in grab samples including all 5 pyrethroids, 3 neonicotinoids and 2 azoles. This is similar to the results for receiving waters found in Chapter 4 in which many of the same analytes were not found. Implications of this are that these analytes are not a significant presence in Ireland, or that they are being missed in the grab samples.

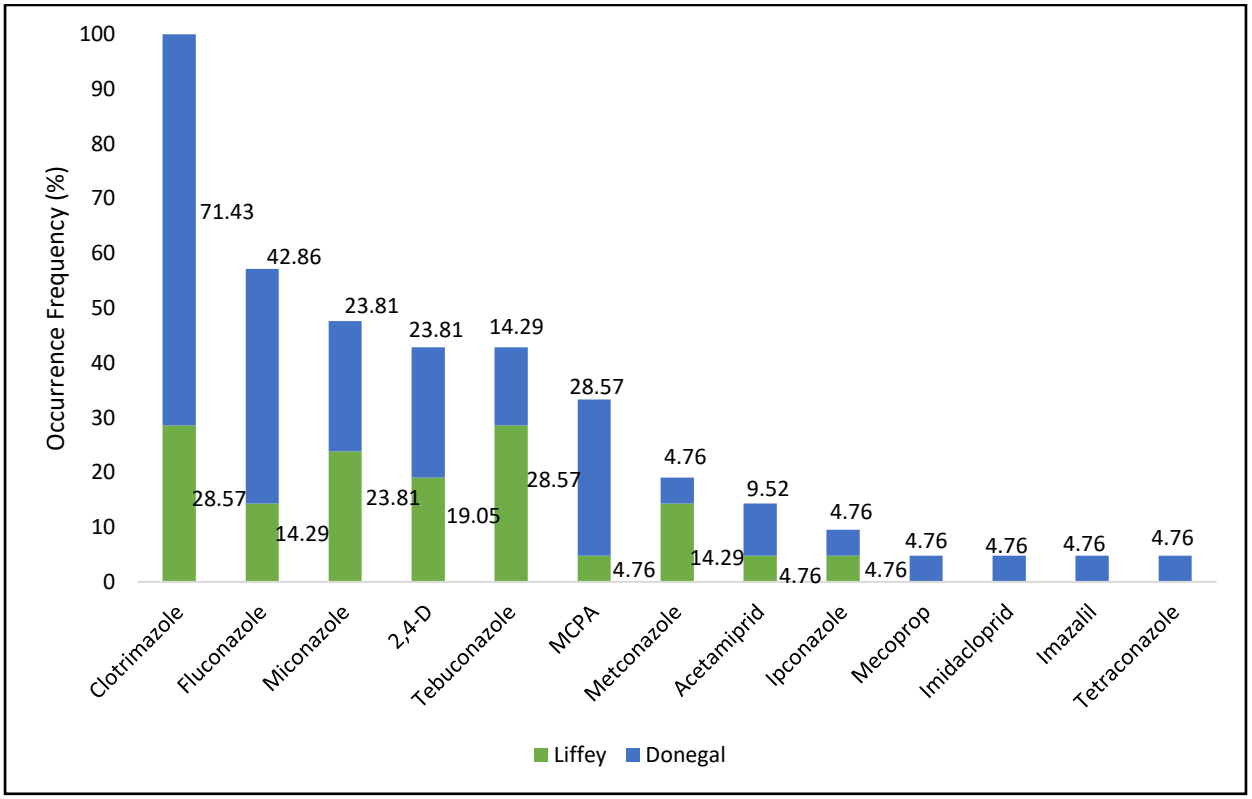


Figure 78. Bar chart of occurrence frequency of analytes found in surface water grab samples during the sampling campaign of summer 2021 for the combined catchment monitoring study (n=21).

In terms of pesticide concentrations in grab samples, the Cranford site showed the highest

cumulative concentrations by a significant margin (Figure 79), with nearly 250 ng L^{-1} found at this location. The majority of this was comprised of acid herbicide MCPA which was detected at levels above LOQ at every sampling event. MCPA was also detected at levels above the LOQ at both the sites along the Glen River. Acid herbicides, predominantly MCPA, were also found in the previous chapter to be make up a significant proportion to river water cumulative CEC concentrations, with 72% of the yearly total cumulative concentration in the rural are coming from this group. From the results gathered in this chapter it is clear that this compound remains a significant presence in Irish samples. This is in line with the literature in which MCPA was the most frequently observed compound detected in freshwater by the EPA [205,354,392]. Their results, published in 2017, indicated that concentrations of this compound were increasing over time. Receiving water samples taken in Chapter 4 of this work showed maximum MCPA concentrations of over 80 ng L^{-1} in June 2019 in the rural area. The grab samples in this chapter found over 117 ng L^{-1} of MCPA in July 2021 in Donegal, which is also rural. Results show an almost 50% increase in the max MCPA concentration determined in Irish samples during this project, confirming the observation by the EPA in 2017. Unfortunately samples from the summer of 2020 were not available for examination of this compound and so a definitive trend over the years could not be established. Countries such as Ireland, Greece, the UK and Bulgaria acquire as much as 65% of their drinking from surface waters and concentrations of this analyte was found at levels exceeding the 0.1 µg L^{-1} limit [261].

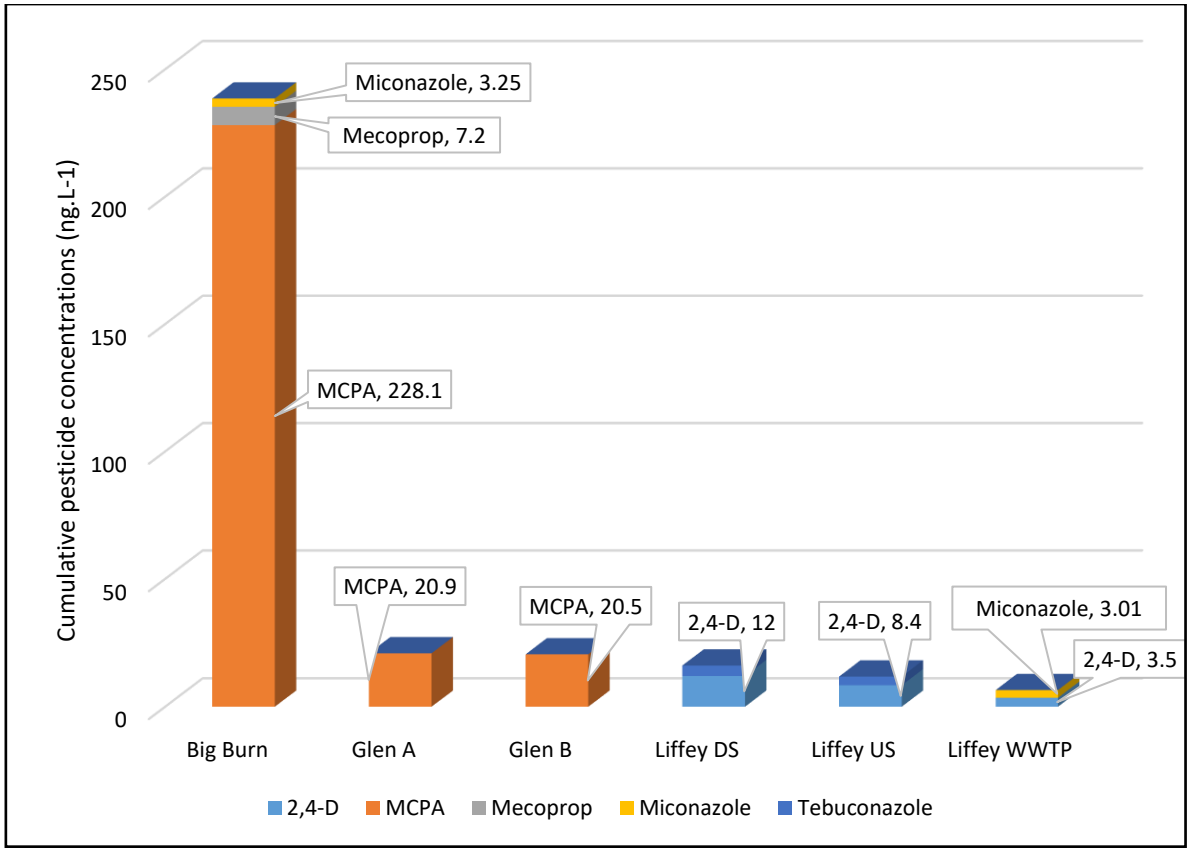


Figure 79. Bar chart showing cumulative CEC concentrations in grab samples for each of the studied sites over the catchment study.

Although a very rural area, Donegal has not historically been linked with intensive herbicidal usage due to the type of land practice dominating in this area being related to sheep farming and forestry [388]. The results found in grab samples here indicate that acid herbicides including MCPA are more of a presence in this area than initially thought, and would benefit from further monitoring.

The National Park and Glenadowan (Clogher) sites were the least contaminated studied areas, in which no detections at quantifiable levels were determined in grab samples. Some detections below LOQ were seen for 2, 4-D and some azole compounds, however occurrences at these concentrations are unlikely to be associated with significant risk as was seen in Chapter 4.

Table 52. Table of grab sample results from the catchment study, results produced by SPE-LC-MS/MS

		Liffey						*Donegal*													
		US		**WWTP**		**DS**		**National Park**			**Cranford (Big Burn)**			**Glenadowan (Clogher)**			**Glen site A**			**Glen site B**		
		Jun	**Jul**	**Jun**	**Jul**	**Jun**	**Jul**	**Jul**	**Aug**	**Sep**	**Jul**	**Aug**	**Sep**	**Jul**	**Aug**	**Sep**	**Jul**	**Aug**	**Sep**	**Jul**	**Aug**	**Sep**
Acid herbicides	**2,4-D**	ND	**8.4 ± 4.4**	*<LOQ*	**3.5 ± 0.5**	ND	**12 ± 2**	*<LOQ*	ND	ND	*<LOQ*	ND	*<LOQ*	*<LOQ*	ND	ND	ND	ND	ND	*<LOQ*	ND	ND
	MCPA	ND	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	**117.7 ± 8.9**	**68.2 ± 11.5**	**42.2 ± 3.8**	ND	ND	ND	**20.9 ± 3.4**	ND	<LOQ	ND	ND	**20.5 ± 0.8**
	Meco	ND	ND	ND	ND	ND	ND	ND	ND	ND	**7.2 ± 0.1**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Neonicotinoids	**Aceta**	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	ND	ND	ND	ND	ND	ND	*<LOQ*	ND
	Cloth	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Imi	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	ND	ND	ND
	Thiac	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Thiam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pyrethroids	**Bif**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Cyper	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Delta	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Esfenv	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Per	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Azoles	**Flucon**	*<LOQ*	ND	*<LOQ*	*<LOQ*	ND	ND	ND	ND	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	ND	*<LOQ*	ND	ND	*<LOQ*	*<LOQ*	ND
	Clotri	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*	*<LOD*
	Imaz	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	ND	ND	ND

	Ipcon	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Metcon	ND	*<LOQ*	N.D	*<LOQ*	ND	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Micon	*<LOQ*	*<LOQ*	*<LOQ*	**3.01***	*<LOQ*	ND	ND	ND	ND	ND	**3.25***	ND	ND	ND	ND	*<LOQ*	*<LOQ*	ND	ND	*<LOQ*	*<LOQ*
	Pencon	ND	ND	ND	ND	ND	ND	ND	ND	ND	N.D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Prochlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Tebucon	*<LOQ*	**3.4 ± 0.9**	*<LOQ*	*<LOQ*	*<LOQ*	**4.1 ± 1.1**	*<LOQ*	ND	ND	ND	ND	ND	*<LOQ*	ND	ND	ND	ND	*<LOQ*	ND	ND	ND
	Tetracon	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	*<LOQ*	ND	ND	ND

5.3.4 Occurrence of pesticides in passive samplers

From the passive sampling conducted in this work a number of analytes were detected in passive sampling devices which were not detected in grab samples. Full details of TWA passive sample concentrations for detected analytes can be found in Table 53.

In this study, all three acidic herbicides included in the method were found at every point along the Liffey using the anion exchange Chemcatcher configuration. The highest TWA concentration was found for 2, 4-D which showed presence in the low µg.L^{-1} range. The highest levels of 2, 4-D were found near the outfall of the WWTP in the Liffey Catchment. This trend was also seen for MCPA, where the levels near the outfall reached a maximum of 427.4 ng L^{-1}. This is an interesting observation as results from Chapter 4 indicated that the acid herbicide pollution in receiving waters was likely coming from an alternative source to the studied WWTPs, further demonstrating the variety of potential point sources for pollution. Examination of the catchment area shows in addition to the WWTP, there is a children's farm (*Caragh Open Farm)* less than 1km from the upstream site which is also a likely source. Passive sampling for acidic herbicides has been performed in recent years in other countries [72,393], and once previously in Ireland. A study published in 2020 by Khan et al. examined two catchments in Wexford in the south east of the country and found continued presence of acidic herbicides year round in Irish surface waters using Chemcatcher passive samplers [254]. They found comparable TWA levels to that found in this Liffey study of up to 262.9 ng L^{-1} of MCPA in the summer months. Interestingly, presence of 2, 4-D was significantly lower in the Wexford study than those found in the Liffey. 2, 4-D is known have broad applications including both agricultural and non-agricultural practices. In the US, the compound was the most widely used herbicide in non-agricultural settings [394]. Its higher presence in the Liffey catchment therefore could likely be related to other anthropogenic activities conducted in this area which are less common as in Wexford.

In contrast to the other two acid herbicides, Mecoprop showed higher PS concentrations at the downstream site, implying a pollution source further downstream than the WWTP for this

analyte. A large golf course *(Millicent Golf Club)* is located less than 1km upstream from the downstream site. Golf courses and the associated lawn care as indicated earlier in the catchment selection section are known to be contributory sources of acid herbicide pollution and so is a likely explanation for this observation [389].

As mentioned previously, the Donegal area has previously been studied and found to have presence of pyrethroid pesticides [388]. Passive sampling in this study employed the use of PDMS and SPMD devices and found levels of cypermethrin between 0.06 – 70 ng L^{-1} in the months of April/May 2014/15. Investigation into these same sites a number of years down the line showed continued presence of these compounds, indicated to be in the µg.L^{-1} range when studied using passive sampling (Table 53). In recent years there has been efforts to phase out the use of pyrethroid pesticides including cypermethrin by restricting its use to limited applications [380], however as has been demonstrated both throughout this thesis and in other studies [395,396], occurrences of controlled substances in environmental matrices is still a frequent finding. This indicated increase in concentration could be due to a number of factors in addition to changes in usage, including the time of year studied and the type of PS device used. For example, SPMD has been known to be susceptible to biofouling which in turn decreases sampling rate and therefore uptake onto the disk [139]. Due to the design of Chemcatcher, fouling on the actual disk is less likely due to the use of the overlaid LDPE membrane. Additionally, another potential factor is a difference in flow velocity of these rivers over time. Flow velocity has been noted in previous studies to have a significant impact on the sampling rates of the C18 Chemcatcher configuration, in which higher flow corresponded with an increase in sampling rates [275,397]. It is therefore possible the Chemcatcher device was able to sequester more of the analyte onto the disk than in previous studies conducted in the area.

Within the Donegal study, the Glen site A showed the most presence of pesticide contamination with presence of 11 analytes detected. It is interesting to note that both sites along the Glen saw a marked increase in conductivity in September, which could possibly relate to a pollution event at this time which was caught by the passive sampler. However, the Glen B and Glenadowan (Clogher) sites showed the least pesticide presence with only 4

analytes found at each, including cypermethrin and tebuconazole found at both sites. The potential link to conductivity could also be an explanation here, as the Glenadowan site had consistently low conductivity during deployment possibly indicating less overall emissions. In regard to the Glen B site it is possible the majority of analytes were sequestered further upstream by the PS device at Glen A, leaving fewer analytes to be collected at site B. Tebuconazole was the only analyte found in all passive samplers in the Donegal area with indicative TWA concentrations in the 100 – 400 ng L^{-1} range. Tebuconazole was found infrequently in the grab samples and always below the LOQ, again demonstrating the benefit of including passive sampling in catchment monitoring. Tebuconazole has been previously found in passive sampling campaigns using Chemcatcher fitted with the HLB-L sorbent at very high detection frequencies in the UK [398].

Table 53. Table of TWA concentrations determined from passive samplers deployed during summer 2021, * denotes semi-quantitative results only.

	TWA concentration per site in ng L^{-1}						
	Liffey			Donegal			
	US	WWTP	DS	Glen A	Glen B	Glenadowan (Clogher)	Cranford (Big Burn)
2,4-D	2923.4 ± 501.5	4144.5 ± 559.6	184 2.7	~	~	~	~
MCPA	280.2 ± 33	427.4 ± 129.8	302. 3	~	~	~	~
Mecoprop	15.04 ± 6.4	15.2 ± 7.02	38.9 4	~	~	~	~
Bifenthrin*	~	~	~	3830.7 ± 429	3575.6	~	~
Cypermethrin *	~	~	~	34947.3	50519. 8	20048.9	~
Deltamethrin*	~	~	~	2156.4 ± 493.8	~	~	~
Permethrin*	~	~	~	25165.5	45259. 5	~	~
Imazalil	~	~	~	41.3 ± 23.7		26.6 ± 11.4	12.6 ± 7.8
Ipconazole*	~	~	~	6.5 ± 2.2	~	~	~
Metconazole*	~	~	~	207.6 ± 155.01	~	~	35
Miconazole*	~	~	~	235.7	~	131.4 ± 85.3	198.5 ± 60
Penconazole	~	~	~	13.7 ± 1.3	~	~	~
Tebuconazole *	~	~	~	217.1 ± 37.7	339.5	115.1 ± 87.2	111.6 ± 82.8
Tetraconazole *	~	~	~	59.3 ± 3.9	~	~	68.2 ± 3.2

5.3.5 Evaluation of a combined sampling approach to catchment monitoring

There are some very evident benefits to using passive sampling technologies for catchment monitoring. The detected occurrences of a number of pesticides in PS disks which were not found in the accompanying grab samples is clear evidence of this. The ability to detect pyrethroid pesticides which were undetected in the grab samples, as well as in the receiving waters of Chapter 4 is of great benefit. The use of these devices can help bridge the gap between analytical method detection limits and the required EQS values for monitoring these substances which has for years presented a challenge. Incorporating PS devices in conjunction with the LC-MS/MS method developed in Chapter 2 presents a promising direction for future monitoring due to its applicability to a broader range of analytes. Although the results

presented in this study were semi-quantitative, qualitative data alone can provide insightful information into the chemical status of a waterbody. Indeed, much of water quality monitoring is incorporating a non-targeted analysis (NTA) approach as a tool for assessment [399,400]. Passive sampling therefore could be a welcome addition to this approach, particularly for compounds which have higher method detection limits. A study recently published in September 2021 by Taylor et al. in which Chemcatcher devices were used in conjunction with suspect screening HRMS-QTOF in order to gather occurrence data on a wide range of pesticides in surface waters [398]. This study found 128 individual pesticides many of which were not typically detected in UK surface waters, further demonstrating the benefit on this approach.

However, passive sampling does present its own unique challenges, including tampering from laypeople, the significant amount of labour required for sampler preparation, and effort involved in deployment and collection. The most major drawback of this approach was seen in the total loss of the Owenveagh set of samplers. It is likely a layperson removed these devices from the river thinking they were litter in the river. Additionally, upon final retrieval of the Clogher disks, the cage was found on river bank, evidently removed and left there by a local person (Figure 80).

Figure 80. Photograph of passive sampler cage which was tampered with at the Glenadowan (Clogher) Donegal site upon retrieval in September 2021.

Replicates from this site produced some results, however the interference with the disks caused issues with producing standard deviations for all analytes as some compounds appeared to be lost/degraded by removal from the river. This tampering must have occurred during the latter half of deployment as the devices were still in place during the mid - point when grab samples were collected. A similar issue was also seen with one of the replicates at the Glen B site in which one of the disks was damaged beyond use (Figure. 81) therefore not allowing for any standard deviations.

Figure 81. Photograph of damaged passive sampler at the Glen B site in Donegal upon retrieval in September 2021.

Usage of the plastic sheets rather than cages for deployment tended to be tampered with or damaged more frequently and so it is recommended that for future campaigns cages be used wherever possible. Although every effort was made to appropriately place and label these devices to avoid interference from passers-by, this is not totally avoidable as was seen by the removal of the cage at the Clogher site. Previous studies using PS have also experienced issues with sampler interference, loss or damage [401,402].

5.4 Conclusions

This study investigated the implementation and suitability of a combined sampling approach to surface water catchment monitoring. Thus far, there have been very limited studies of this kind conducted in Ireland. Two areas of the country were selected for monitoring pesticide contamination by taking into account the local catchment land uses and possible point pressures. Sampling was conducted during the summer season of 2021 to coincide with when pesticide contamination is most likely to be a risk to local waterbodies.

Pesticide contamination was found at all studies sites in both grab and passive samples, aside from the National Park site in which the passive samplers were lost before retrieval. Acid herbicides were some of the most frequently found analytes in this study, and were detected at levels in grab samples that were comparatively very high (>100 ng L^{-1}). The detection of these analytes in areas of Donegal which was not previously thought to be most at risk for these analytes is an interesting discovery and warrants further study. These compounds were also found in passive samplers along the River Liffey, and potential point sources were identified. A cornerstone of a catchment – based approach the involvement of the entire community within a catchment in improving water quality [403]. By identification of anthropogenic activities affecting these rivers such as the golf course, WWTP and children's farm suggested here, it is possible to involve these people in mitigation practices.

One of the objectives of this work was to assess the employment of multiple sampling approaches for catchment monitoring. The findings in this study show that the use of one sampling approach alone is insufficient for creating a detailed picture of water quality in a catchment. As is evidenced here, use of grab samples alone would mean many compounds or pollution events are missed. However use of passive sampling alone is a risk due to the ease of which these devices can be lost or tampered with. There are clear benefits to employing a combined approach to catchment monitoring. Therefore it is recommend that both passive and grab sampling be used in future surface water monitoring campaigns where possible.

Chapter 6: Conclusions

6.1. Key Findings and Contributions

The primary aim of this research was to gather comprehensive data on the chemical status in Irish waters. To paint an overall broader picture of chemical water quality in the country, the Water Framework Directive Watch List was used as a guide for selection of targeted compounds for a four year study. For a more in depth investigation into a class of CECs specifically of interest to Ireland, pesticides were selected for study on their occurrence and removal in Irish WWTPs. Finally, in order to investigate the implementation of additional sampling approaches into future catchment monitoring practices, a study using both passive and grab sampling was conducted in two areas of Ireland. To that end, the key contributions of this thesis were as follows.

Development of new analytical methods for the determination of 2nd and 3rd Watch List chemicals, glyphosate and AMPA, acidic herbicides and a novel approach to pyrethroid pesticide analysis. These methods achieved LODs in the low ng L^{-1} - µg L^{-1} range depending on the analyte, and were assessed for method performance in a variety of aquatic matrices. The ability to include pyrethroid pesticides in an LC-MS method which is more broadly amenable to differing CECs is a significant and positive step forward for CEC monitoring. As vast numbers of new chemicals are synthesised every day, our ability to monitor a variety of analytes in fewer analytical approaches is becoming a requirement. Indeed, many new HRMS methods have been published in recent years which can examine >100 compounds in one analytical run. However, many laboratories do not have access to such instrumentation, and so the need for analysis methods using 'standard' equipment like a C18 column and triple quadrupole LC-MS methods used here remains. EU wide or even global CEC monitoring practices need to be accessible in order to be achievable for a variety of countries with different resources. Therefore the new methods developed here can aid in future monitoring practices on a wider scale. The ability to conduct widespread monitoring effectively and efficiently becomes even more apparent when including the consideration of our changing climate, and the effect this will likely have on CEC occurrences. In areas which will see an increase in rainfall [314,315], more agricultural run-off events will likely introduce greater quantities of CECs into waterbodies. In areas which see higher temperatures or droughts [384] , reduction in river flow will likely cause

increasing concentrations in rivers. Factors such as these highlight the need for effective and achievable monitoring methods.

The first comprehensive Irish study for Watch list chemical occurrence including both 2nd and 3rd Watch List compounds was determined in this work. The findings from this study are fed directly to the EU and as such have an influence on future policy decisions affecting not only Ireland but every member state. With limited Irish occurrence information available, this work presents a significant step forward in CEC monitoring in Ireland. Through this work it was found that contamination of CECs is widespread throughout surface waters in Ireland. Even catchments which are not currently considered 'at risk' under the WFD contained concentrations of CECs which were determined pose a risk to aquatic life. In particular synthetic estrogens, neonicotinoid pesticides and the antidepressant venlafaxine were found to be occurring at high risk levels (RQ >1). A number of azole compounds and macrolide antibiotics were determined to be a moderate risk. Identification of these analytes can aid in establishing a prioritised list of compounds specifically affecting Irish waterbodies, and therefore targeted mitigation practices can be developed to reduce future risks.

The temporal study of WWTP occurrence and removal conducted in this project is one of the few studies of its kind which focuses on pesticide compounds, and was the first of its kind conducted in Ireland. A number of these compounds were determined to be insufficiently removed from WWTP influent. There were only 4 chemicals included in this study which had average removal of >50% in both studied plants - ipconazole, metconazole, penconazole and tebuconazole. Many of the other analytes which were frequently found in samples both in this chapter and chapters 3 and 5 were not being removed by treatment. A number of analytes such as clotrimazole and cypermethrin, were determined to have negative removal. By examination of the occurrence and behaviour of these compounds in water treatment, this can aid WWTP operators in implementing new strategies to effectively remove these analytes. Additionally, conducting of this work provided information of the occurrence and removal of analytes which has never been studied in WWTP samples previously, including the azole anti-fungal tetraconazole, which was found at very high occurrence frequencies in Irish samples.

The catchment monitoring campaign to investigate the use of multiple sampling approaches showed significant presence of pesticides in passive samples which were not found in the grab samples. However, the passive sampling devices were far more susceptible to loss, theft or damage. While every effort was made to minimise this risk, it is not unavoidable. This study demonstrated the benefit in employing both sampling approaches and the limitations of using only one into future catchment monitoring campaigns. Additionally, the identification of high concentrations in the Donegal area was an unexpected finding which can aid in implementing targeted mitigation strategies.

6.2. Recommendations and Future Work

A common finding throughout this body of work was the influence of matrix interferences on LC-MS/MS analysis. For example in the Watch List work, the antibiotic amoxicillin was seen to exhibit high matrix interferences in particularly turbid samples (up to 4000% enhancement). Turbidity has been linked to rainfall [311], and so this parameter also has significant implications related to climate change. Greater matrix interferences could therefore be a point of growing importance as climate change causes increased rainfall in certain areas. While not falling within the scope of the present study, further work in this area would be of huge benefit to the overall scientific community. Literature has been produced into the mechanisms of matrix effects in LC-MS ionization [282,284,292], however there is still a significant knowledge gap of potential remedies to this phenomenon. The relationship between higher sample turbidity, and increased matrix effects for particularly susceptible analytes shown in this thesis could be a potential influencing factor for future investigation.

Although still environmentally relevant levels, the HILIC method to target glyphosate and AMPA was only capable at measuring at the low $\mu g.L^{-1}$ levels, likely due to its difficulty being retained by conventional SPE methods, therefore not allowing for a preconcentration step. However, as seen through monitoring of the various other chemicals examined in this thesis, occurrences in environmental waters, and in particular surface waters, are frequently in the

low ng L^{-1} range. This presents the opportunity for further work to examine whether lower detection limits could be achieved through alternative developments in sample preparation and preconcentration steps. Promising work has been published this area [351,404] and application of these methods to Irish water samples could present further insights into the presence of these compounds.

The WWTPs studied in this project showed a number of compounds were insufficiently removed by the currently used water treatment processes. Further work into implementation of techniques which have been shown to remove CECs such as ozonation and adsorption [362] into WWTPs would be of significant benefit to WWTP operators and citizens alike.

In relation to the passive sampling conducted in Chapter 5, performance of uptake studies for analytes on the C18 disks would allow for the production of fully quantitative results. Although some campaigns employ passive samplers for qualitative screening methods and therefore do not perform uptake studies [212,398], the production of quantitative data is still of great value to both the scientific community and legislators alike. Unexpected determinations of high levels of MCPA in the Donegal area were found although the local land use did not indicate there to be a significant presence of this compound in this area. This finding can inform future investigation in this area, and demonstrated how widespread the reach of these CECs can be.

The growing area of mixture effects and effect based monitoring would fall within the scope for future work relating to this thesis [405,406]. The work presented here found that every sample analysed contained multiple pollutants. For example, The River Suir in September 2021 had positive detections for 17 out of 19 Watch List substances. However the majority of detections were well below their corresponding PNEC values. While this may appear as a relieving result for the health of Irish rivers, much is still unknown about the effects these compounds may have on aquatic life when occurring alongside other CECs, even at very low levels. Additionally, incorporation of effect based monitoring into the kind of sampling campaign performed in Chapter 5 would add another dimension to the catchment based approach. The environment is a highly complex system in which numerous variables are at play. Approaching

assessment from a single perspective allows for the potential for a loss of information. Through employing techniques from multiple disciplines including ecology and ecotoxicology a more complete picture be achieved.

This research has furthered the scientific understanding of contaminants of emerging concern occurrence and behaviour in Irish aquatic environments by performance of a number of extensive monitoring campaigns. Water is one of the Earth's most vital resources, and ensuring its protection is becoming an increasingly important endeavour in an ever changing climate. The results produced in this work have a direct influence on the future policy decisions affecting not only Ireland but the entirety of the EU. By informing policy, the overall impact of CECs on water quality can be reduced.

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