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Identifying and Quantifying Environmental Contaminants in Various Matrices using Mass Spectrometry

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Thesis Abstract

Human impact on the environment can be seen in the wide variety of chemicals that are found in our water and soil. Common contaminants arise from insufficient treatment in wastewater treatment plants (WWTPs), and runoff from agriculture. Surface water samples collected in 2017-2020 at 40 different sites in six different watersheds were analyzed to determine if the commonly targeted emerging substances of concern (ESOCs) are present in Ontario and Quebec waterways. The diabetes medication metformin was analyzed more closely alongside its degradation product guanylurea as they have become targets of increasing interest due to their common occurrences and new toxicological data on organisms. Sediment samples at the same sampling sites were also collected. This work is to our knowledge the first long term analysis of metformin and guanylurea in Ontario and Quebec and offers potentially valuable insight into where metformin and guanylurea partition and accumulate in waterways.

Biosolid samples from Ontario WWTPs were also analyzed for the presence of ESOCs to determine if treatment used to remove micropollutants and bacteria is also able to remove common chemical contaminants. Two extraction methods were assessed to determine their efficacy in extracting a wide variety of compounds. The concentrations of extracted compounds were also compared between the untreated biosolid cake and treated fertilizer to determine if a thermal hydrolysis process (THP) could degrade ESOCs. This work serves to validate a widespread biosolid analysis method for use in further ecotoxicological studies.

Keywords

Metformin, guanylurea, mass spectrometry, surface water, sediment, biosolids, triple quadrupole, Orbitrap, WWTP, PPCP

Summary for Lay Audience

We use a wide variety of chemicals in everyday life, whether for medicine, agriculture, or recreational use. Once we have used these chemicals, very few of them degrade and get removed from the ecosystem. In fact, many of them can be found in surface water and soil around the world. Some of these compounds are relatively harmless, while others can be toxic to plants and animals. Even more worryingly, there are many compounds that we have not detected yet, or whose effects we do not know. This makes it a priority in environmental contamination studies to have ways to identify these compounds, and to figure out how much is there. In this work, we use methods to detect these compounds and determine how much is present at various sites in Canada. By doing this, we can ensure that chemicals that are present in high amounts can be regulated to manage their risk. Also, because our samples come from between 2017-2020, we can determine if the amount of chemicals have been increasing in the environment and figure out which compounds are most important to restrict.

To investigate environmental contamination, we examine water, sediment, and biosolids. The water and sediment samples come from rivers, lakes, and streams around Canada, and each site we look at has different things nearby. For example, a sample taken from a river going through a city should contain different compounds than a sample from a river going through farmland. We can identify those compounds in water and sediment to make sure there is nothing toxic or dangerous present. Biosolids are the solid waste produced by humans after it has been treated in a wastewater treatment plant. This waste can be cleaned up and used as fertilizer, but we need to make sure that any harmful chemicals that may have been in the human waste have been removed, otherwise it may impact the farmland it is applied to. These types of samples are all important to investigate to make sure that we are not damaging our environment in any way.

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List of Abbreviations

AAFC – Agriculture and Agri-Food Canada

ABCA – Ausable Bayfield Conservation Authority

ACN - Acetonitrile

AIF – All Ion Fragmentation

C8 – Eight Carbon Alkyl Chain Stationary Phase

C18 - Eighteen Carbon Alkyl Chain Stationary Phase

C-trap – Curved Linear Trap

CEM – Chain Ejection Model

CID – Collision Induced Dissociation

CRM – Charged Residue Model

DAI – Direct Aqueous Injection

DEET - N,N-diethyl-meta-toluamide

DDA – Data Dependent Acquisition

DIA – Data Independent Acquisition

EAWAG – Swiss Federal Institute of Aquatic Science and Technology (German acronym for Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz)

ECCC – Environment and Climate Change Canada

ECETOC - European Centre for Ecotoxicology and Toxicology of Chemicals

EDTA – Ethylenediaminetetraacetic acid

EPA – Environmental Protection Agency

ERCA – Essex Region Conservation Authority

ESOC – Emerging Substance of Concern

FDA – Food and Drug Administration

FWHM – Full Width at Half Maximum

GRCA – Grand River Conservation Authority

H_2O – Water

HC – Health Canada

HCD – Higher-energy C-trap Dissociation

HESI – Heated Electrospray Ionization

HILIC – Hydrophilic Interaction Liquid Chromatography

HLB – Hydrophilic-Lipophilic Balance

HM – High Mass

HPLC – High Performance Liquid Chromatography

HRMS – High Resolution Mass Spectrometry

IEM – Ion Evaporation Model

IS – Internal Standard

LC – Liquid Chromatography

LC-MS/MS – Liquid Chromatography Tandem Mass Spectrometry

LM – Low Mass

LOD – Limit of Detection

LOQ – Limit of Quantification

LRDC – London Research and Development Centre

m/z – Mass-to-charge Ratio

MeOH – Methanol

MM – Medium Mass

MRM – Multiple Reaction Monitoring

MS – Mass Spectrometry

MS/MS – Tandem Mass Spectrometry

NSAID- Nonsteroidal anti-inflammatory drug

OBVT – Organisme de Bassin Versant du Témiscamingue

OMAFRA – Ontario Ministry of Agriculture, Food, and Rural Affairs

PET – Poly-Ethylene Terephthalate

PMRA – Pest Management Regulatory Agency

PCOS – Polycystic Ovary Syndrome

PPCP – Pharmaceutical and Personal Care Product

PPE - Polypropylene

PVDF – Polyvinylidene fluoride

QqQ – Triple Quadrupole Mass Spectrometry

R_E% – Recovery Efficiency

rf - Radiofrequency

SPE – Solid Phase Extraction

SNCA – South Nation Conservation Authority

SRM – Selected Reaction Monitoring

SSRI - Selective Serotonin Reuptake Inhibitor

THP – Thermal Hydrolysis Treatment

UTRCA – Upper Thames Region Conservation Authority

WWTP – Wastewater Treatment Plant

Chapter 1

Introduction

1.1 Environmental Contamination

Human impact on the environment can be observed by the widespread occurrence of human-created chemicals in water,[1–3] soil,[4–6] and plants.[7–9] Compounds are continuously synthesized to treat new illnesses, control pests, and improve chemical processes. As these new compounds are created, they often find their way into our surface water, and can cause untold harm to both the aquatic environment and the organisms that live there.[10,11] It is of great importance to identify and quantify the compounds that contaminate our watersheds to ensure that they are not present in amounts that cause harmful effects. To investigate this environmental contamination, regions can be divided into watersheds, i.e., land areas in which all surrounding water sources drain into a common body of water. Watersheds can vary in size based on the drainage and topography of the area but are a point of accumulation for all water in a large area, and therefore allow for insight into all contamination that is occurring in surface water in a region.

Emerging substances of concern (ESOCs) are common environmental contaminants that have been identified as having possible health risks[12]. Some ESOCs have unknown effects and are common in the environment, while others are found rarely but have severe and known toxic effects. Investigations into ESOCs can therefore vary, as some studies seek to create a method to detect the compounds, some seek to quantify them, and others seek to characterize their toxic effects and at what concentrations they occur. Several regulatory bodies in Canada, including the Pest Management Regulatory Agency (PMRA), Health Canada (HC) and Environment and Climate Change Canada (ECCC), place regulations on ESOCs in a multitude of categories. PMRA is responsible for regulation of pest control products such as herbicides and insecticides, HC manages pharmaceuticals in the environment, while ECCC places regulations on a variety of fields to limit newly identified contaminants and their spread. When regulatory agencies such as these identify a potentially hazardous compound, that contaminant becomes a target for environmental studies to determine how common it is, and if it is hazardous to the environments in which it is found. Once environmental concentrations have been

recorded, toxicology studies can identify if the levels detected are high enough to exhibit toxic effects in aquatic organisms. New regulations can then be put in place if environmental concentrations approach the levels at which adverse effects occur. New compounds are created and identified every year, so this is an ever-repeating pipeline of events to prevent any major deleterious effects as a result of human contamination.

1.1.1 Contamination of Pharmaceuticals

Pharmaceuticals and personal care products (PPCPs) are one class of compounds that includes medications, illicit drugs, and cosmetics. New pharmaceuticals are developed every year to treat both physical and mental illnesses and have a variety of structures and effects. PPCPs can be subdivided into groups based on effects and structures of the compound.[13] Of the many groups of PPCPs, non-steroidal anti-inflammatory drugs (NSAIDs), macrolides, antidepressants, and antipsychotics are some of the commonly observed groups in environmental systems.[14] The compound structure varies based on the target area and the desired effect in the body **(Figure 1)**. Many of these complex pharmaceuticals consumed by humans are metabolically unchanged by the body, and are therefore excreted in their original forms.[15] This human waste ends up in wastewater treatment plants (WWTPs) where many of the compounds cannot be effectively degraded, and are then released into the environment in the treated wastewater effluent.[16–19] Because many of these compounds are designed to have some biological effect on the humans that consume them, there may be undiscovered effects on aquatic organisms in the environments near the effluent pipes. As prescriptions and usage of the drugs increase with higher surrounding populations, there should be higher environmental concentrations found in nearby watersheds.[20] Previous studies have noted a correlation of surface water concentrations of environmental contaminants increasing with population.[21] The highest concentration of these PPCPs in the watershed should be in or near a wastewater effluent pipe, and the compound should dilute as distance from the WWTP increases.[21]

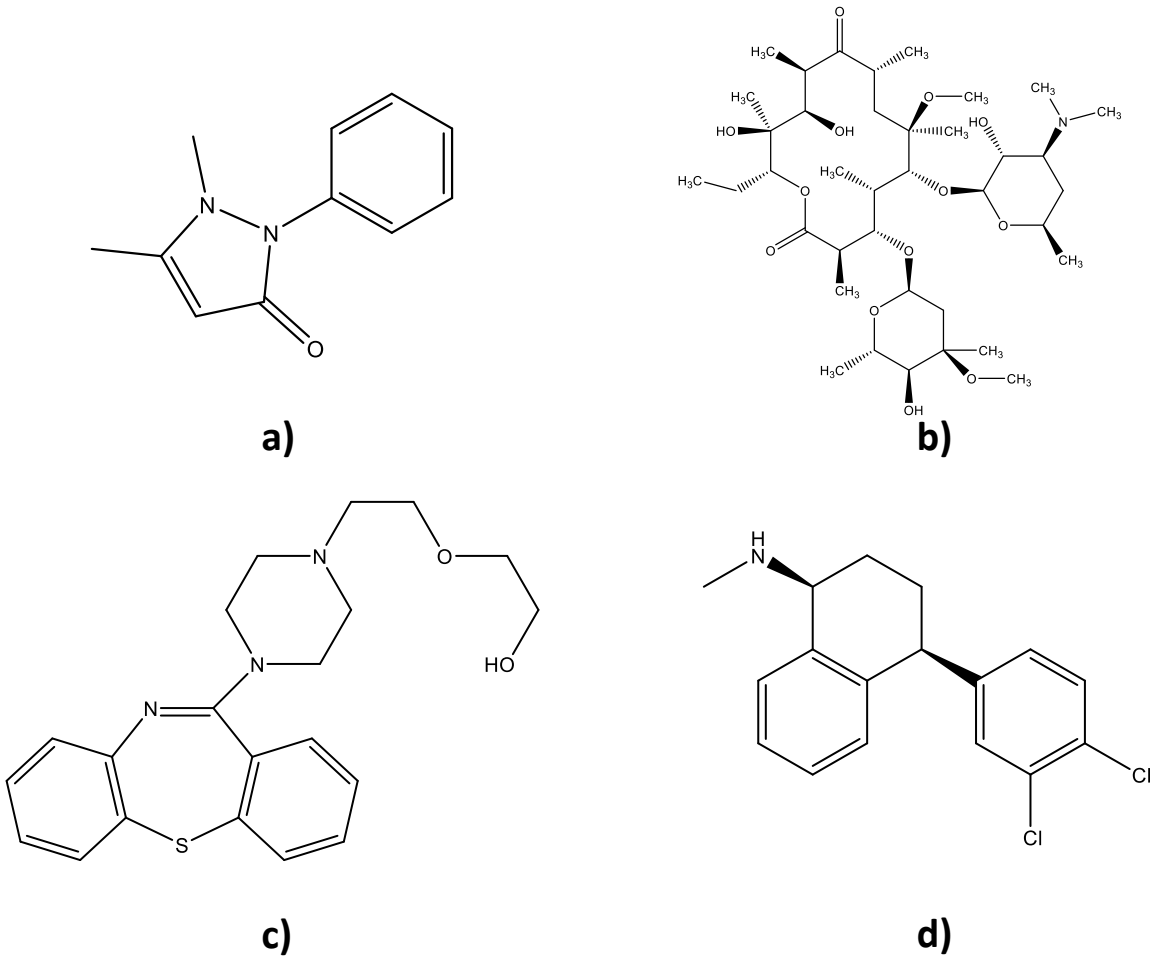


Figure 1: Example of types of pharmaceuticals. a) NSAID antipyrine, b) macrolide clarithromycin, c) antipsychotic quetiapine and d) antidepressant sertraline

1.1.2 Contamination of Pesticides

The next class of ESOCs found commonly in the environment are pesticides, which encompasses herbicides, fungicides, and insecticides.[22–24] Each of these types of pesticides can be further subdivided into classes such as triazines, neonicotinoids, carbamates, organophosphates, etc. based on their molecular structure or mode of action **(Figure 2)**. The wide variety of compounds means that methods of detection and quantification will vary, and the optimal method for one pesticide may not be optimal for another. The widespread contamination of pesticides poses a severe health and environmental issue, as many of these compounds are biologically detrimental to humans

and animals. One study on the children of farmworkers found that prenatal exposure to organophosphate pesticides led to adverse effects on mental development in children.[25] Organophosphates and carbamates both inhibit acetylcholinesterase, which interferes with degradation of acetylcholine in the brain and can cause severe neurological effects.[26,27] Neonicotinoid insecticides are also important to investigate, as their use in recent years has drastically increased. This increase is due to increasing regulations on other pesticides, leaving neonicotinoids as the only financially viable option. Neonicotinoid insecticides were first thought to be less toxic than others, but ongoing toxicological studies have determined there exist a plethora of toxic effects on vertebrates and invertebrates, and both acute and chronic exposure to neonicotinoids can cause extremely toxic or even fatal effects.[28] As there has been increasing interest into effects of these pesticides, it is important to discern how prevalent they are in surface water to determine the severity of the contamination.

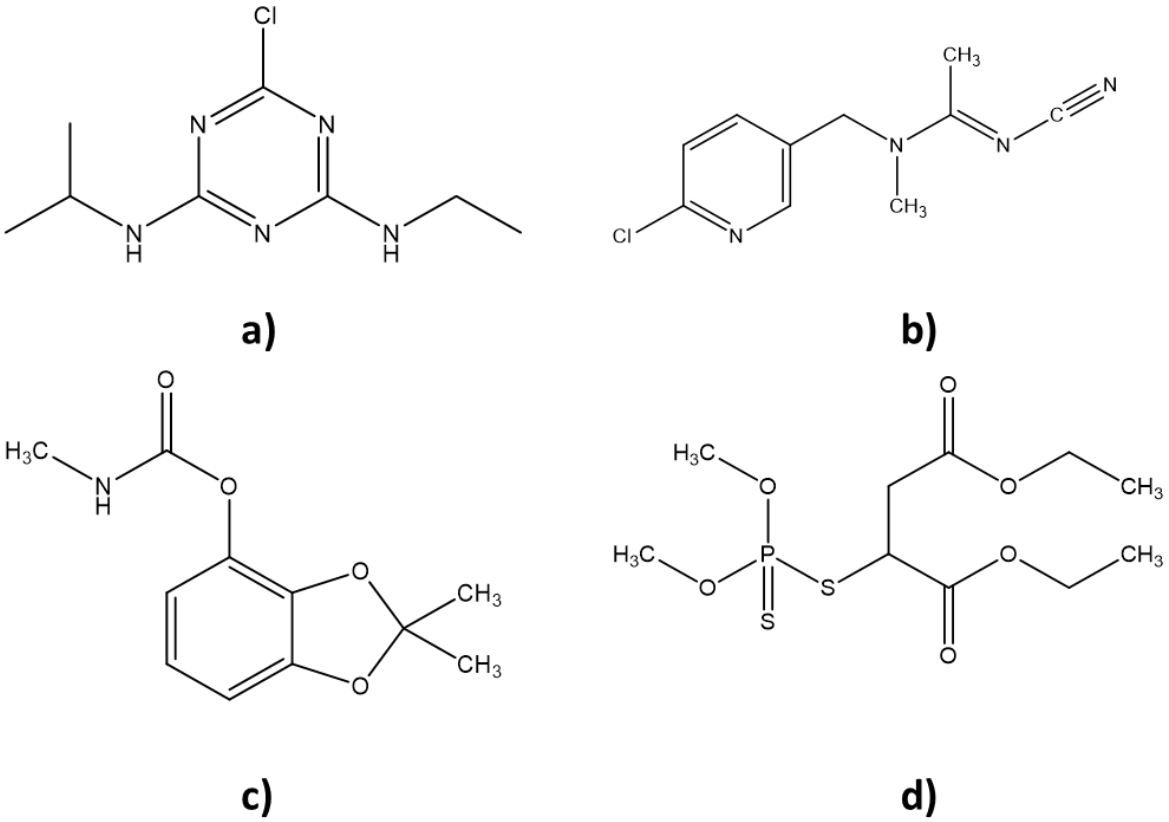


Figure 2: Examples of classes of pesticides. a) Triazine herbicide atrazine b) neonicotinoid insecticide acetamiprid c) carbamate insecticide bendiocarb and d) organophosphate insecticide malathion

Though many pesticides have toxic effects, their usage in agriculture reduces the impact of pests on growth of crops, making their use a practical necessity to maximize overall yield.[29] The proliferation of these compounds in the environment can occur in a variety of ways. Wind during aerosol application of the pesticides can cause the spray to spread beyond the target, contaminating surrounding areas.[30] Additionally, heavy rainfall can cause the pesticides to leach into the groundwater and diffuse about the watershed in the surface water.[24,31] Different sampling sites in the same region may contain different compounds due to the variability in human activity and inputs. For more agriculturally influenced areas, it is expected that the concentration of pesticides would therefore be higher than in a more urban area.[32] Urban areas are not immune to pesticide

contamination however, as residential use on lawns and in gardens may also lead to groundwater contamination after heavy rainfall.[33]

1.2 Environmental Matrices

The compounds identified in environmental samples depend on the matrix that is being investigated. Certain compounds remain soluble in surface water, some may be retained by the sediment, while others may be taken up by organisms in the region.[34] This means that different matrices sampled from the same location may give different results, both in compounds identified and in their concentrations. Due to the different characteristics of environmental contaminants, it is important to investigate multiple matrices in an area to discern the extent of the contamination. Each matrix and target compound requires a separate optimized method to ensure accurate identification and quantification of the contaminants. The matrices to be investigated in this work are surface water, sediment, and biosolids.

1.2.1 Surface water

Environmental contaminants often end up in surface water.[18,35–37] Rivers, lakes, and streams amass a wide variety of contaminants from the surrounding areas, and even the most isolated of places are known to contain small amounts of pharmaceuticals or pesticides that propagate from populated areas.[1] For the purposes of this research, six watersheds from Ontario and Quebec were examined based on existing sampling infrastructure and differences in human activity (**Figure 3)**. Conservation authorities at each watershed took one sample from each site every month from May to October or November. Monthly sampling allows for a comprehensive assessment of the contamination at each site, and insight into how contamination changes throughout the agriculture season. Freezing water samples allows for retrospective analysis of the samples for any target analyte that is desired. For the purposes of this study, frozen samples from 2017, 2018 and 2019 were investigated alongside 2020 samples to study the long-term trends at each site. The methods used to examine these water samples can

be adjusted to change the target compounds, and in this work, several different targets are examined using different methods of extraction and analysis.

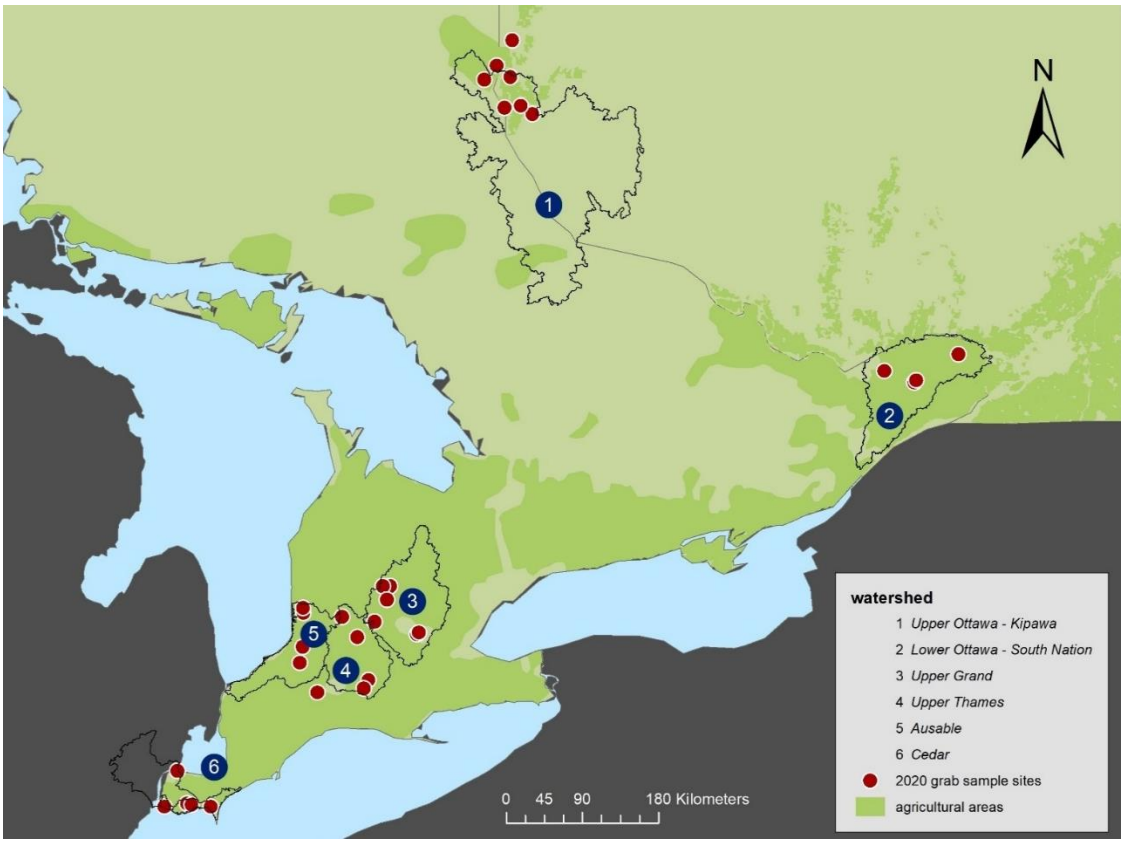


Figure 3: Six Target watersheds around Ontario and Quebec for 2020. Each sample site remained constant through the year, and most were sampled through all years of sampling (2018-2020). Grab samples are small water samples taken to represent one specific time point at a location.

The first investigation in this work on surface water was to research prevalence of the pharmaceutical metformin and its degradation product guanylurea in surface water. Identification and quantification of the compounds in Ontario and Quebec waterways will be elaborated on in Chapter 2. Each of the watersheds exhibit distinct characteristics due to their surrounding populations and the degree of agricultural activity occurring nearby, so this examination is to identify the primary sources of the compounds in the

environment, and how much is present at each site. Because metformin is a pharmaceutical, its usage and occurrence are similar to other PPCPs, and it should therefore be most prevalent near populated areas and WWTPs.[21] Consequently, because guanylurea is the degradation product of metformin, guanylurea should be in higher concentrations at sites where more metformin is found, and it should also be present in the same watersheds.

The second investigation on surface water was to retrospectively analyze the general trends of concentration for a large target list of ESOCs in water samples taken from 2017-2020. This will use a large target database to detect as many compounds as possible from the list to understand the extent of contamination of many ESOCs at the sampling sites. The samples taken vary both temporally and geographically, so trends can be identified based on the region, influence at the site, or time. The data resulting from this study can be saved and retrospectively analyzed based on which compounds are becoming of interest in environmental studies.

1.2.3 Sediment

Surface water data alone may not give the total representation of environmental contamination in a region. Sediment underlying water sampling sites in rivers, lakes, and streams is necessary to investigate as different compounds may have varying mobility through the matrices.[34] Some compounds that end up in surface water may preferentially bind to sediment, meaning they can accumulate rather than remaining soluble in surface water, while others may not be absorbed or adsorbed at all.[38,39] Depending on the conditions of the water, some compounds bound to sediment may resolubilize, causing contamination.[40] Sampling of sediment in this work occurs at the same timepoints and locations as surface water, providing the best possible insight into overall contamination at each site.

In this work, sediment samples were examined to support the occurrence of metformin and guanylurea. The extraction method was adapted from a method optimized specifically for metformin quantification in soil, which was modified to include guanylurea.[41] Because metformin and guanylurea may exhibit different mobility in soil

and surface water,[6] it is important to investigate both matrices to ensure that the compounds have been identified everywhere they appear. In general, sediment analysis allows for a deeper understanding of an analyte's mobility through matrices and is an invaluable tool in characterization of environmental contaminants.

1.2.4 Biosolids

Biosolids are the solid waste remaining once WWTP treatment has been completed, and the liquid fraction has been released back into the environment in WWTP effluent. As biosolids are primarily human waste, they contain many of the same compounds present in wastewater effluent.[42] These biosolids cannot be released back into the environment in the same manner as wastewater due to micropollutants and bacteria present in the sludge. Millions of tons of biosolid waste are produced every year, so it is of interest in conservation efforts to find some use for these solids.[43] Several companies have developed techniques in removing contaminants from biosolids such that they can be recycled into fertilizer.[44] Because these techniques have been developed primarily to remove bacteria, it is unknown if ESOCs are present, or if the application of these fertilizers is a point source for their introduction to the environment. There is evidence to suggest that uptake of some complex pharmaceuticals into crops is possible,[7,9] so it is important to discern if there are ESOCs present in the treated biosolid fertilizer, and if they are present in hazardous concentrations.

The goal of this study is to determine if the same process that removes bacteria and micropollutants is also able to remove or reduce concentrations of ESOCs in the treated fertilizer. Instead of only quantification of the contaminants in the treated fertilizer, what is being investigated in this case is the difference in concentration before and after treatment. Treatment of the untreated biosolids (colloquially known as "cake") utilizes a thermal hydrolysis process (THP), which exposes the cake to high heat and base while shearing the solids against a high speed blade to grind the solid material, removing bacteria and micropollutants. The cake and the treated fertilizer can be subjected to the same extraction method to quantify the target ESOCs in each. Previous work done by this lab targeted 5 specific PPCPs in biosolids and demonstrated that the treatment was able to

remove 100% of the targeted compounds in water, and around 40% when spiked into biosolids.[45] This may not, however, provide the full picture, as spiked compounds may not be able to completely absorb into the organic material, and there may be some degree of protection from degradation by the complex organic matter in the untreated cake. This can be seen by the drastic decrease in degradation efficiency from water to spiked cake in the same study.[45] This work seeks to further investigate this effect in biosolids using a general method for ESOC extraction, and to determine conclusively if targeted ESOCs are removed in the treated fertilizer. Extraction of compounds in biosolids and their identification will be elaborated on further in Chapter 3.

1.3 Extraction

Extractions from environmental matrices are used to transfer a compound from its original matrix to another utilizing a molecule's varying affinity for different environments. One method of extraction used in this work is accomplished through thorough mixing of the sample with a second solvent in which it is preferentially soluble. When the phases intermix, and the analyte comes into contact with the second phase, it solubilizes into the new solvent, where it remains. The initial solution is then removed to prepare the targets for analysis. Often the solvents used in extraction are not suitable for analysis via mass spectrometry as they may be too viscous, may not be miscible with the mobile phase, or may corrode the tubing or column, among many other possible issues. To limit these effects, a drying step is used to evaporate the solution and leave the dried target analytes to be reconstituted in a suitable solvent, usually methanol (MeOH), acetonitrile (ACN), or water. For environmental samples, specialized methods are developed based on the matrix and the targets to effectively isolate them from the complex mixture so they can be analyzed.

Water is a simple matrix for extraction, as it usually requires few steps to clean the solution, and filtration of the sample is often adequate to remove major biological contaminants such as algae or silt. Many of the target analytes can be extracted with a simple extraction method, and concentration of samples from low concentrations is possible for trace analysis. Sediment and biosolids are more complex matrices that

require extensive methods to extract the desired compounds and may require multiple different methods applied to a single sample to accurately quantify all contaminants.

1.3.1 Solid Phase extraction

One valuable method for extraction of environmental contaminants from surface water is solid phase extraction (SPE).[46] This work uses SPE with Oasis Hydrophilic-Lipophilic Balance (HLB) cartridges to extract a wide variety of ESOCs from surface water. By slowly passing the surface water under vacuum through the HLB cartridge, the compounds of interest are loosely bound to the sorbent through electrostatic interactions. These HLB cartridges contain a copolymer of one hydrophilic monomer (N-vinylpyrrolidone) and one lipophilic monomer (divinylbenzene) **(Figure 4)**.[47] These monomers allow for adequate retention of both polar and nonpolar target analytes, as well as retention of impurities which can be removed by a washing step prior to elution.

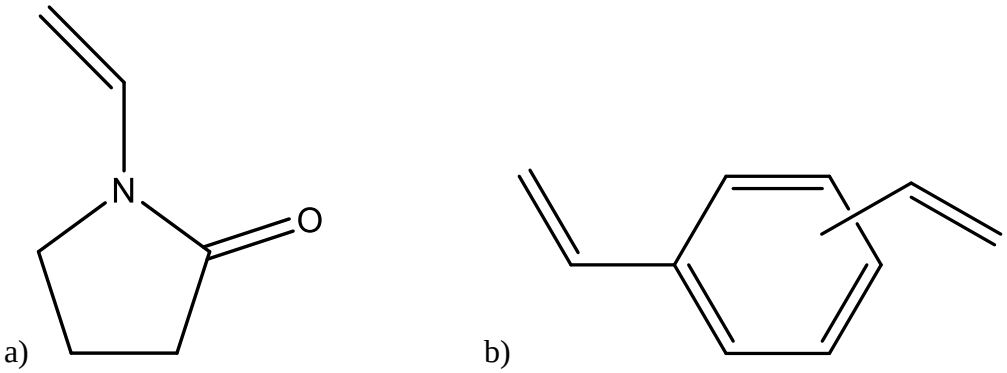


Figure 4: a) Hydrophilic monomer N-vinylpyrrolidone and b) Lipophilic monomer divinylbenzene used in the copolymer of HLB cartridges. The second vinyl group on monomer b) can be meta or para substituted.

Compounds of interest can be chemically changed by altering the pH such that they are uncharged and nonpolar. The weak interactions between the nonpolar analytes and the cartridge allow the analytes to preferentially adsorb to the cartridge and they can therefore be removed from the water sample. These weak electrostatic interactions retain the target compounds which can easily be eluted with a small amount of polar solvent, such as

MeOH or ACN. This method concentrates the compounds from a larger volume of sample onto a much smaller cartridge, allowing us to increase the concentration of compounds that are present in original concentrations of ng/L or lower. As many sampling sites are located far from WWTP influences, compounds resulting from WWTP effluent should be quite dilute, so this concentration step is necessary for detection. Additionally, pesticides in surface water stemming from agriculture are also generally found in low amounts, which may still be environmentally relevant. Chronic exposure to some compounds at low levels can still exhibit adverse effects,[48] so trace analysis is necessary to detect and quantify them in these trace amounts. SPE is effective in amplification of signals for trace analysis of a wide variety of compounds, however each extraction can be lengthy, which limits the utility of the method.[49]

1.4 Liquid Chromatography

Although different methods are used depending on the target compounds, one constant in the environmental analytical chemistry performed here is liquid chromatography (LC). Chromatography is the separation of a mixture into its components, which is especially useful when isolating target compounds in a complex mixture, as in an environmental sample. Separation in LC is derived from the varying affinity of target analytes for the phases in a column. This separation occurs in columns which can be an open tube or packed with beads consisting of the same material as the solid stationary phase. The solid stationary phase is an adsorbent that remains fixed to the inside of the column, and a liquid mobile phase can be eluted through it. The varying affinity of a compound for the stationary or mobile phase is what guides separation. The phases used in the most common types of chromatography are of opposite polarity, so the order of elution for the analytes in a solution depends on how polar they are. There are exceptions to this however, such as hydrophilic interaction liquid chromatography (HILIC) which uses mobile and stationary phases of the same polarity and separates with a more intricate mechanism.

There exist several methods of liquid chromatography: the overarching types used are normal phase and reverse phase. Normal phase chromatography employs a nonpolar

mobile phase and a polar stationary phase. Highly nonpolar compounds are pulled through the column by the mobile phase rapidly with minimal retention to the stationary phase. Reverse phase chromatography is similar in concept, but the mobile phase and stationary phase are of opposite polarity from normal phase chromatography, with a polar mobile phase and nonpolar stationary phase. Reverse phase columns typically consist of silica functionalized with a long chain alkane, such as a C8 or C18 alkane. Mobile phase solvents are typically polar, and include MeOH, ACN, and water. Order of elution in reverse phase is different from normal phase, though generally the most polar compounds elute first, and least polar elute last. The composition of the mobile phase can also be adjusted over time through gradient elution to optimize separation and improve the resolution and separation of the peaks. In many cases, the gradient must be adjusted to ensure that compounds with high affinity for the stationary phase can elute, and analytes with low affinity can still be retained in the column such that they all elute in the same method. The mobile phase usually involves two solvents of opposite polarity, and by starting with a high concentration of one solvent then slowly increasing the concentration of the other solvent the polarity can be altered. This allows analytes that were strongly interacting with the stationary phase to leave in favour of the mobile phase so they can then be eluted. This lowers the retention time of the slow eluting analytes and improves resolution, increasing the quality of the resulting spectra. Reverse phase liquid chromatography is employed for much of this work as many of the target ESOCs are non-polar and can be separated effectively in a reverse phase method.

1.4.1 HILIC

Hydrophilic interaction chromatography (HILIC) is a technique used in this work to separate highly polar compounds. HILIC columns use a polar stationary phase and a polar mobile phase to separate analytes that would elute early in reverse phase conditions and may not be soluble in the nonpolar mobile phases used in normal phase chromatography. The term was first used to describe a polar stationary phase with an aqueous-organic mobile phase, and in that study, it was used in the separation of peptides, amino acids, and other polar molecules.[50] HILIC employs the varying hydrophilicity of polar

molecules, as well as other electrostatic interactions with the stationary phase, to separate these polar molecules from each other. The mobile phase in HILIC typically consists of a mixture of ACN and water. In this mobile phase, water is more polar than ACN and therefore adsorbs more effectively to the polar stationary phase **(Figure 5)**.[51] This effectively turns water into a new stationary phase on the surface of the column, and ACN acts more like a traditional mobile phase. When the analytes enter the column, the more hydrophilic molecules enter the water layer and are retained, while the less hydrophilic compounds continue to move through the mobile phase. Also, water and the column stationary phase exhibit other electrostatic effects that differently retain analytes, including hydrogen bonding and electrostatic effects on ionized functional groups. This means that retention times for compounds in a HILIC column do not increase linearly with hydrophilicity.[52]

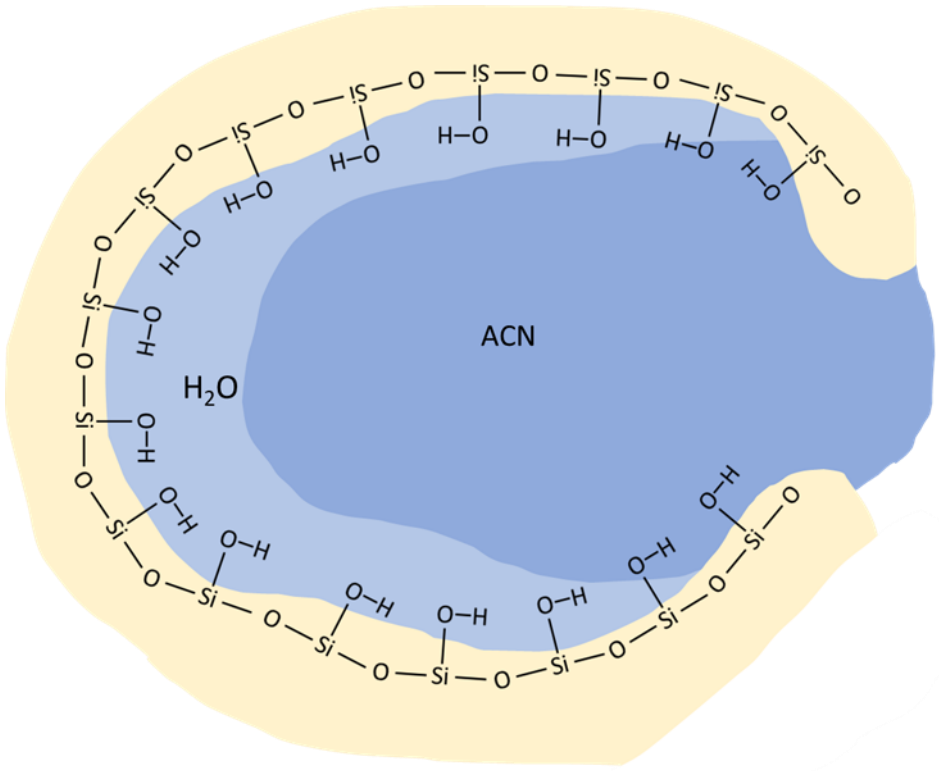


Figure 5: Separation of layers in a particle in a packed HILIC column. Bare silica is given as the stationary phase.[53]

1.5 Mass Spectrometry

Following separation of target analytes in a liquid chromatography system, LC can be coupled with mass spectrometry (MS) to then identify and quantify the compounds that had been separated in the column. Tandem mass spectrometry is a valuable tool in the identification and characterization of analytes in environmental samples as it can aid in determining the identity of contaminants, and how much is in the sample. The separation of target analytes in mass spectrometry is based on the mass-to-charge ratio (*m/z)* of an ion, where units of mass are in daltons (Da), which are equivalent to 1/12 the mass of an unbound carbon-12 atom.[54] Analytes are ionized, then are broken into charged fragments, where each compound typically has at least one product ion specific to the precursor ion that is created by this fragmentation. The product ion varies based on the collision energy (CE) specified by the method, and the optimal collision energy for fragmentation varies for each compound. Most analytes are singly charged, giving a *m/z* value based on the fragment's atomic mass, though some larger analytes can be multiply charged, which causes their *m/z* value to be a fraction of their atomic mass. Target analytes vary in both their chemical structures and their properties, so different methods must be used to best separate them such that detection of the compounds is as effective as possible.

1.5.1 Ionization

Ionization in a mass spectrometer generally occurs after liquid chromatography, and before the target analytes are guided into the mass spectrometer. Heated electrospray ionization (HESI) is the method of choice in this work, though many other forms of ionization can be used depending on the purpose of the experiment. Solvents for HESI typically include a mixture of water and a volatile organic solvent, like MeOH or ACN, and often a weak acid or base to aid in ionization of the analytes, such as 0.1% formic acid.[55] HESI functions by aerosolizing droplets of solvent using heat and a strong electric field (**Figure 6)**.[56] The solvent eluting from the column is injected into the ionization chamber through a charged spray needle where it begins to form a stable liquid cone,

called the Taylor cone (first described by Sir Geoffrey Ingram Taylor).[57] In positive mode, the source capillary is positively charged and the capillary leading to the mass analyzer is negatively charged. Positively charged solvated analytes are expelled from the source and are drawn towards the capillary outflow. Surface tension competes with electrostatic forces, causing the characteristic shape of the Taylor cone with an excess of positive charges on its surface. The Taylor cone begins to spray jets of liquid when the surface tension of the cone is exceeded by the coulombic repulsion of the surface charge. This phenomenon was first theorized by Lord Rayleigh in 1882 when estimating the maximum charge a droplet could hold before throwing out fine jets of emission, and the maximum surface tension is called the Rayleigh limit.[58]

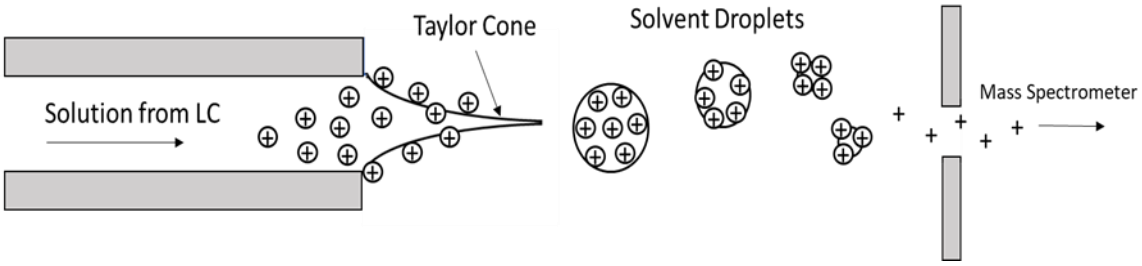


Figure 6: General mechanism for electrospray ionization in positive mode. Positively charged ions are selected and guided through to the mass spectrometer. The ionization chamber is filled with nitrogen gas to aid in formation of droplets.

The droplets resulting from the electrospray evaporate spontaneously as they travel towards the spectrometer. In positive mode, the droplets form with an excess of positive charge, and are then guided towards the mass spectrometer. These larger droplets evaporate as they move through the chamber, and once the repulsive charge in the droplet exceeds the surface tension, the droplet breaks down further into smaller droplets until they consist of only single ions. There exist three theories to explain the final production of gas-phase ions in electrospray ionization. These are the ion evaporation model (IEM) thought to be the method in which small molecules evaporate,[59,60] the charged residue model (CRM), which is used to explain evaporation of larger molecules,[61] and the chain

ejection model (CEM)[62] which explains evaporation of unfolded proteins. Most of the target analytes examined here are pesticides and pharmaceuticals, which are small molecules, meaning CEM is not relevant in this case. It is generally accepted that both IEM and CRM occur in tandem in evaporation, so both are applicable in this work **(Figure 7)**. In IEM, the electric field experienced by the droplet is sufficient to cause the small molecules to eject from the surface of the solvent droplet as gas phase ions. In CRM, the large molecule remains while the solvent droplets evaporate from the surface to dryness. These methods explain how a target analyte adopts charge from the solvent droplets resulting from jet emission and are valuable in this work to understand how ionization is occurring in a mass spectrometer.

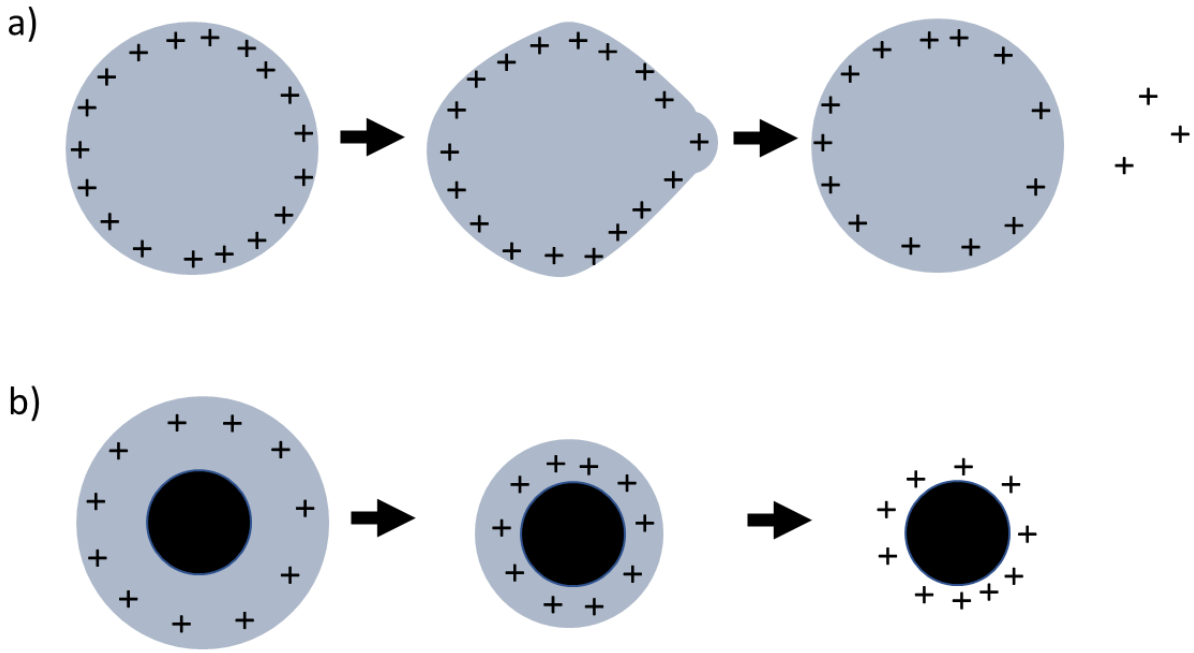


Figure 7: a) Ion evaporation model and b) Charged residue model used to explain evaporation of solvent droplets to gas-phase ions in electrospray ionization.

1.5.3 Quadrupole Mass Filter

Once compounds have been isolated from their original complex mixtures and ionized, it is now necessary to filter out just the analytes that are of interest. The quadrupole mass

filter is a very important tool in spectrometry for selection of target analytes using their mass-to-charge ratio. The quadrupole consists of four parallel rods, where rods on opposite sides are paired. All four of the rods receive the same voltage, but the pairs have opposite signs. Direct current remains consistent, while alternating current is applied to the rods at radiofrequency (rf) voltages. These alternating currents form an electric field that is able to guide molecules through the quadrupole. This oscillating field can be modified to select ions of one specific *m/z* or a range of *m/z* values that will maintain a stable trajectory. Ions that are smaller than this *m/z* are destabilized too much by the field and are ejected out of the quadrupole, ions larger than this *m/z* are not affected enough, and are similarly ejected upon collision with the rods **(Figure 8)**.

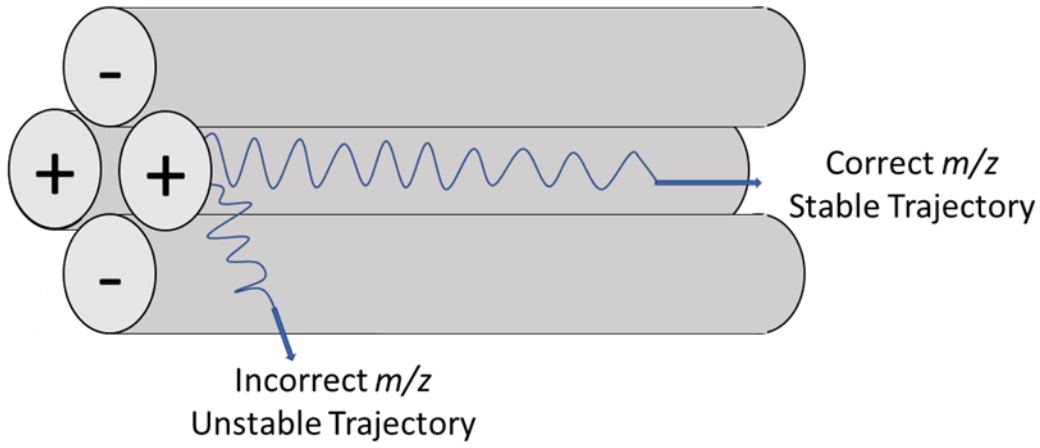


Figure 8: Example trajectories of ions entering a quadrupole mass filter. The front pole is omitted to observe the ion pathway at the centre of the quadrupole.

1.5.3 Fragmentation

Following selection of the target ions, fragmentation is performed to break the ion into fragments such that a unique pattern of fragmentation occurs. Putative identification can occur when the resulting fragmentation pattern is compared to literature or previous scans. When this is paired with the retention time (RT) from LC, we can confidently determine the identity of the original ion. Fragmentation in mass spectrometry involves

collision with an inert gas, such as argon, helium, or nitrogen gas. The two mass spectrometry methods used in this work use two different types of collision for fragmentation of the target analytes. In an Orbitrap mass spectrometer, higher-energy collisional dissociation (HCD) is used in an HCD collision cell. This cell is aligned perpendicular to the Orbitrap mass analyzer, and the ions are guided in by a radiofrequency-only quadrupole. HCD is a variation of collision-induced dissociation (CID), where the ions collide with an inert gas to break into pieces, and in HCD this occurs outside of the ion trap. The collision causes fragmentation by converting kinetic energy to internal energy to break bonds between atoms. Upon fragmentation, the ions accumulate in the C-trap until either the maximum number of ions set by the method has accumulated, or until a specified time has elapsed, whereupon all ions are injected into the Orbitrap mass analyzer simultaneously for analysis. In triple quadrupole mass spectrometry, CID is used, and it occurs in the second quadrupole which employs only radiofrequency to guide the analytes through the collision cell. Collision energy in both cases can be modified based on the analyte, as the energy at which the analytes collide with the inert gas can alter the fragmentation of the target. Collision must be optimized based on the target, as too little energy will not cause sufficient fragmentation, and too much energy will cause the analyte to fragment into too many pieces, and the product ion will not be characteristic of the precursor.

1.5.4 Orbitrap Mass Spectrometry

Detection of analytes in a complex mixture is often difficult, and there is an ever-increasing list of compounds to detect. The Orbitrap mass spectrometer is a powerful HRMS instrument used for a variety of environmental analyses due to its ability to analyze a wide range of analytes at the same time.[63] A simplified overview of a Q-exactive Orbitrap mass spectrometer is shown in **(Figure 9)**.[64] When paired with liquid chromatography, the compounds that are eluted from the column are ionized as previously described via HESI and are directed into the spectrometer. The instrument is under vacuum, and the ions are pulled in by vacuum electronic-field forces, then the S-lens uses radiofrequency to focus them into a beam and direct them into the instrument.

Upon entering the instrument, the analytes are guided through the bent flatapole, where the electric field in the flatapole sends charged ions into the quadrupole mass filter. Due to the bend in the flatapole, neutral atoms and solvent droplets are not guided by the electronic field and are removed at the bend. Ions that make it through the bent flatapole are then focused into the quadrupole mass filter which employs both rf and direct currents to guide ions through. Ions are then selected in the quadrupole mass filter as described above.

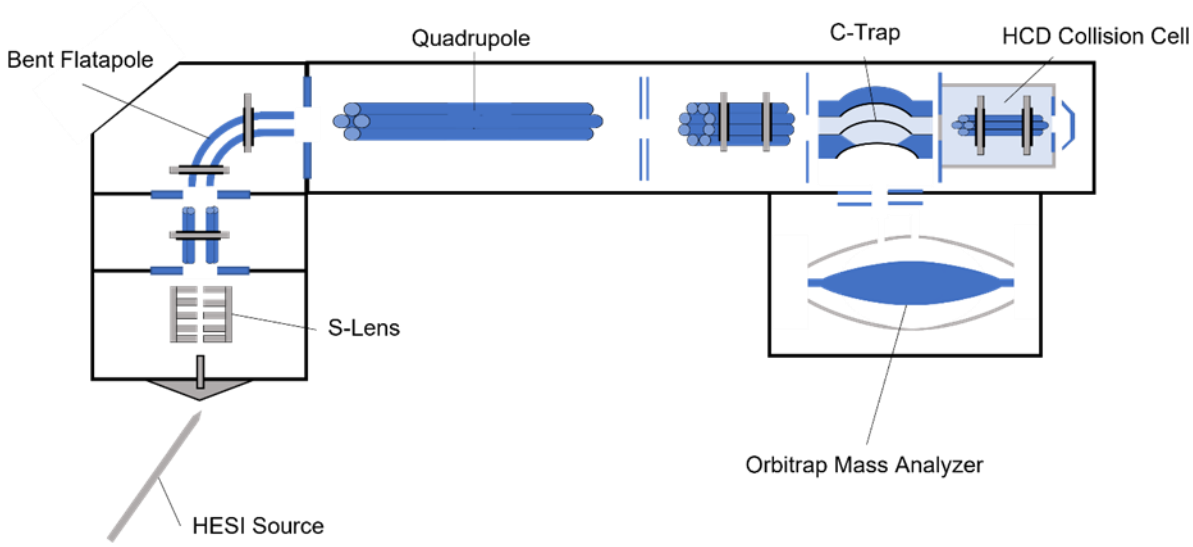


Figure 9: Simplified schematics of a Q-Exactive™ Orbitrap mass spectrometer.

Upon exiting the quadrupole mass filter, the ions are guided through into a curved linear trap (C-trap). In this C-trap, the ions begin to accumulate until they are either injected into the HCD cell or the Orbitrap mass analyzer. Analytes can be injected into the Orbitrap mass analyzer intact, or can be injected into the HCD cell for fragmentation. Ions that enter the HCD cell are fragmented as described above against inert gas. Once fragmented, the product ions accumulate in the C-trap before being injected simultaneously into the Orbitrap mass analyzer. The Orbitrap mass analyzer consists of two outer electrodes surrounding a spindle-shaped central electrode.[64,65] In the Orbitrap, ions separate by *m/z* based on their trajectory in the radial and axial directions **(Figure 10)**. Ions in the

Orbitrap oscillate and begin to form stable trajectories in bands characteristic of their *m/z*. The frequency at which these ions oscillate around the electrode depends solely on their *m/z*, so a mixture of ions will separate into different trajectories, and these frequencies can be detected by the outer electrodes. Through a Fourier transform of the detected signal, the *m/z* of all ions in the mass analyzer can be detected.

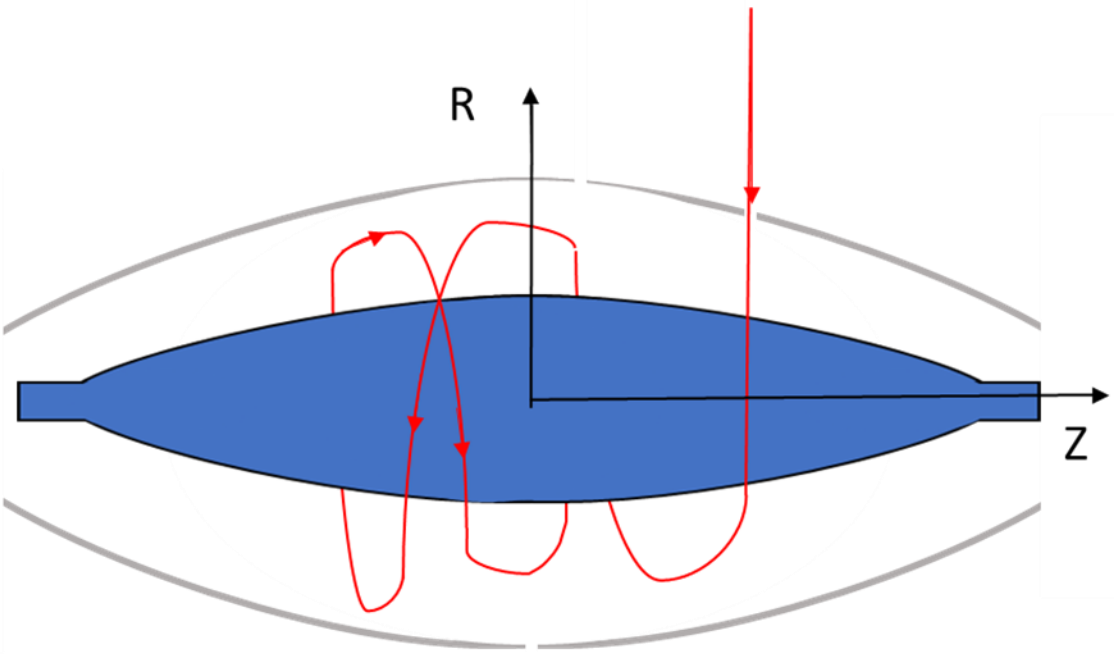


Figure 10: Simplified diagram of an Orbitrap mass analyzer. Trajectory of an example ion is expressed as a red line. The radial (R) and axial (Z) directions are labelled.[65]

The utility of an Orbitrap mass spectrometer is in its ability to be used for nontargeted analysis methods on a complex mixture. These methods vary based on how they collect data from an injected sample, and can be chosen based on the intended result. For example, all ion fragmentation (AIF) is a method in which all the available precursor ions are fragmented and injected into the Orbitrap mass analyzer simultaneously. AIF produces complicated spectra, especially when analyzing a mixture of compounds, which leads to difficulty in identification of each individual peak. A more specific method of detection that produces comparable results with less noise is data dependant acquisition

(DDA). DDA limits fragmentation of ions to those that appear above a selected level of abundance. This is useful in detecting the most prominent compounds in a mixture or sample, as no compounds below the set abundance will be injected into the mass analyzer.[66] One important drawback of AIF and DDA for this work is that trace analysis is not possible. Because DDA would not select trace contaminants, and AIF would be too difficult to deconvolute and determine the precursor and product ions, neither method is able to detect many compounds that are present in low concentrations.

For nontargeted environmental analysis one valuable method is data independent acquisition (DIA), a method designed around the capabilities of the Orbitrap and C-trap to perform trace analysis of a complex mixture. This method involves separating the scans into mass ranges, then fragmenting and analyzing all ions in those windows. This differs from AIF however, in that smaller ranges of the precursor ions are transmitted at one time, allowing for much easier identification of the resulting fragment.[67] The precursor ions in DIA are divided into low mass, medium mass, and high mass windows of 128-351 *m/z*, 349-651 *m/z*, and 649-1051 *m/z*. The slight overlap in the *m/z* window ensures that product ion data are collected for every ionizable compound in the mixture. The spectra produced from this method can also be stored and retrospectively analyzed to identify any new analytes of interest in the future.

1.5.5 Triple Quadrupole Mass Spectrometry

Much environmental analysis employs a triple quadrupole mass spectrometer (QqQ) paired with high-performance liquid chromatography (HPLC) to examine contaminants. By employing three quadrupoles in succession[68], a QqQ instrument can accurately separate and quantify these contaminants from an environmental sample containing many different analytes **(Figure 11)**. QqQ methods can vary based on the type of analysis to be performed. There are several scan modes used in QqQ: precursor ion scan, neutral loss scan, product ion scan, selected reaction monitoring (SRM) and multiple reaction monitoring (MRM).[69] For the purposes of the environmental analysis in this work, MRM is the method used. In MRM, the first and third quadrupole are used as mass analyzers set to select for the *m/z* of many different parent and product ions. By rapidly switching

between these pairs of parent and product ions, multiple fragmentation patterns can be detected. This enhanced selectivity of the instrument is possible with minimal loss in sensitivity, making it especially effective in trace analysis of environmental contaminants, where concentrations are typically low. As described above, the second quadrupole is where fragmentation of the precursor occurs.

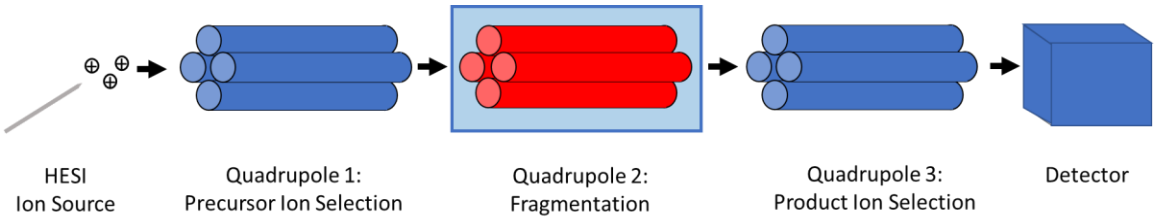


Figure 11: Triple quadrupole mass spectrometry setup overview for multiple reaction monitoring.[68]

QqQ is especially effective in determination of environmental samples, as many of the matrices in this work are complex and contain a wide variety of organic compounds. When multiple compounds co-elute, their interactions can cause the retention time of an analyte to shift away from its expected value. These matrix effects can cause either a suppression or an enhancement of the ionization, which are a constant struggle in identification and quantification of compounds in mass spectrometry. The selectivity of MRM enables a QqQ instrument to overcome matrix effects, making it especially utile for biosolids analysis. Much of the work in this thesis uses a targeted analysis method with a QqQ instrument with an extensive target list. Due to the excellent selectivity of the instrument and the large target list of important environmental contaminants, QqQ is the best instrument to use for the purpose of this research.

1.6 Thesis Objectives

The overall objective of this thesis is use mass spectrometry to investigate environmental contamination, its extent, and the avenues in which it occurs. Chapter 2 elaborates on the

compounds of interest metformin and guanylurea and their occurrence in Ontario and Quebec surface water and sediment. Chapter 3 discusses treatment of biosolids with a thermal hydrolysis chemical treatment to create fertilizer, and the effects of this treatment on ESOC concentration. Chapter 4 discusses the use of methods for extraction of various ESOCs from surface water. Solid phase extraction is used to analyze water for a wide variety of environmental contaminants using one method. The resulting data from this method should provide insight into what compounds are most common contaminants in the environment. The data can be stored and retrospectively analyzed to determine long term temporal trends for a wide variety of ESOCs across Ontario and Quebec.

Chapter 2

Investigation of the Diabetes Medication Metformin and its Degradation Product Guanylurea in Ontario and Quebec watersheds using Mass Spectrometry

2.1 Chapter 2 Objectives

Metformin and guanylurea are two common environmental contaminants found around the world. Metformin has been in use for decades as a diabetes medication, and it has recently been proven that guanylurea is created when metformin is bacterially degraded. The goal of this study is to identify and quantify both compounds in the environment using multiple years of stored surface water and sediment samples. Using this information, it can aid in elucidating the origins of the compounds in the environment, and how prevalent they are across Canada.

2.2 Introduction

2.2.1 Metformin

One particular pharmaceutical of interest in this work is metformin. Metformin is a small-molecule drug used as a first-line medication in treatment of type 2 diabetes. First discovered in 1929[70], metformin and other similar biguanide derivatives were not investigated for their hypoglycemic effects in humans until the late 1950s[71]. This was due to the ground-breaking discovery of insulin, for which Sir Frederick Banting won the Nobel prize in 1923, and insulin became one of the first and most well-known compounds used in treatment of diabetes. Due to the increasing availability of insulin, hypoglycemic drugs such as metformin were generally forgotten about until the 1940s where metformin was rediscovered in a search for antimalarial drugs[72,73]. The antimalarial biguanide drug proguanil was modified to metformin, which was then used in the Philippines to treat influenza. Metformin hydrochloride was prescribed under the name fluamine, where its ability to lower blood glucose was also noted.[74] Following the rediscovery of the hypoglycemic effects of biguanides, the 1950s saw three clinical trials performed on three separate biguanides by different laboratories. The compounds tested in these trials were metformin under the name Glucophage (“glucose eater”) by Jean Sterne in France[75], buformin by Mehnert et al. in Germany[76], and phenformin by Ungar et al. in the United States[77] **(Figure 12)**.

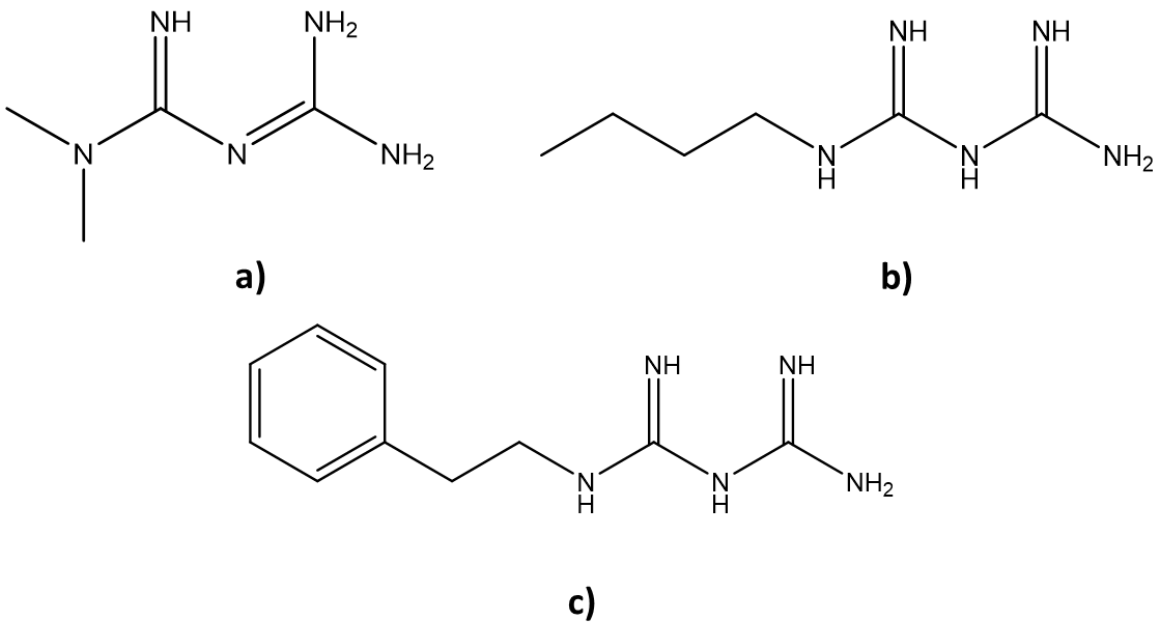


Figure 12: Structures of the initially investigated biguanide drugs a) Metformin, b) buformin and c) phenformin

All three of the investigated biguanides exhibited similar effects. Though the exact mechanism was not known at the time, it has since been discovered that activation of AMP-activated protein kinase is required to exhibit the glucose lowering effects[78,79]. Through this interaction, each of the drugs inhibit gluconeogenesis in the liver and promote uptake of insulin through the cell membranes. One important drawback to buformin and phenformin is that their longer alkyl chains make them more lipophilic and therefore less prone to metabolism. This characteristic means that patients receive prolonged exposure to the compounds when compared to metformin, and the clinical trials were ended due to an increase in the side effect of lactic acidosis, causing phenformin and buformin to be withdrawn from most countries in the 1970s.[80,81] Lactic acidosis is a buildup of lactic acid in the blood and is a possible side effect of all biguanides as they inhibit the formation of new glucose from lactate through gluconeogenesis. Metformin is known to cause lactic acidosis, but it is much less common and is mostly an issue in patients with existing kidney impairment, as roughly 80% of metformin is excreted by the kidneys.[82,83] When the kidneys are impaired, they

are unable to efficiently excrete metformin and prolonged action of the drug then causes this buildup of lactic acid, which can be fatal.

Metformin has been available in the UK since 1958 and Canada since 1972 but was not approved by the Food and Drug Administration (FDA) until 1994 due to lingering concerns around toxicity of phenformin, which had been used widely before its withdrawal. Many studies were performed to investigate the impact of clinical use of metformin, and following its approval, a study was published in the New England Journal of Medicine confirming the efficacy of metformin in treatment of diabetes mellitus.[84] Since then, it has become the most common oral drug prescribed for treatment of diabetes in the world. Further investigation of metformin has also revealed that it is useful in treatment of several other maladies, including polycystic ovary syndrome (PCOS),[85] cancer,[86,87] and COVID-19.[88] Prescriptions of metformin increase annually both with the increasing human population, and the new illnesses in which it is finding use. The total prescriptions of metformin surpassed 120 million worldwide in 2012[89], and have been increasing ever since.[90] Metformin is a pharmaceutical that is excreted mostly unchanged from the body, and many WWTPs do not sufficiently degrade it[15,91], so this widespread usage may cause significant contamination.

2.2.2 Guanylurea

One important subsection of ESOCs that has been less extensively researched is their degradation products or metabolites. Both pesticides and pharmaceuticals can be degraded environmentally. Whether this is instigated by bacteria, UV, heat, pH, or some other pathway, the original compound is broken down into a different form. Due to the constant replenishment of ESOCs in the environment from WWTPs and agriculture, it is possible that their degradation products are accumulating in surface water or sediment. This could be hazardous if these degradation products exhibit any notable toxicity. This is of increasing importance for metformin as its usage has been widespread for decades and its degradation product guanylurea was only discovered relatively recently.[92,93] Guanylurea arises from the degradation of metformin when exposed to certain bacteria, at least one of which belonging to the species *Aminobacter* has been isolated.[94] The exact

mechanism of degradation is not known, though spectral data indicate two dealkylations and an oxidative deamination[93] occur to form guanylurea from metformin **(Figure 13)**.

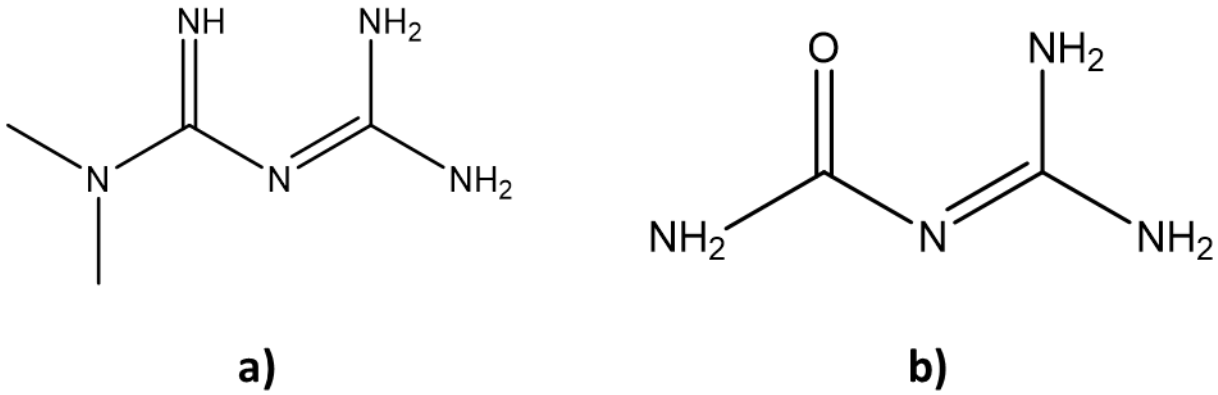


Figure 13: Chemical structures of a) the pharmaceutical metformin and b) its main metabolite guanylurea

2.2.3 Contamination and Toxicity of Metformin and Guanylurea

The prominence of metformin and guanylurea in the environment is due to the inability of WWTPs to fully degrade complex pharmaceuticals. As metformin is an important chemical in diabetes treatment and is commonly prescribed around the world, it has been identified in waterways in many countries, including Canada,[2,95] the United States,[2,96,97] Greece,[98] the Netherlands,[99,100] Germany,[21,93,101] and China.[102] In areas with a higher number of metformin prescriptions, higher concentrations of metformin in the surrounding surface water have also been noted. This may indicate that as the world population increases, metformin prescriptions and therefore environmental concentrations may increase as well.

Studies have examined the change in concentration from WWTP treatment and noted that in some cases the concentration of guanylurea increases from influent to effluent corresponding to a decrease in metformin concentration. Some other cases, however, did not note any change in metformin concentration. This is evidence that degradation of metformin results mainly in the formation of guanylurea, and that only certain bacterial

species that are not always present in WWTPs are able to perform this degradation.[92–94] After the discovery of guanylurea, retroactive studies of water samples containing metformin found guanylurea in the same samples where it had not been previously detected.[92]

In March of 2022, a study determined that metformin may be responsible for genital birth defects in human males when the father was prescribed metformin[103]. Metformin exposed children were more likely than the control group to exhibit birth defects, and further research is being conducted to determine the severity of these findings. One different study noted increases in childhood obesity for children whose mothers had been prescribed metformin to treat PCOS.[104] These toxic effects were only discovered recently despite the long-term use of the drug, so there may be as of yet undiscovered toxic effects occurring in the environment as well.

One important set of toxicological studies performed by Environment and Climate Change Canada (ECCC) identified toxic effects on the Japanese medaka (*Oryzias latipes)*.[105–107] When exposed to concentrations of guanylurea lower than 0.25 µg/L for their whole life cycle, Ussery et al. noted intersexuality in juvenile male fish, as well as negative effects on growth and total body length. The study is especially relevant to this work as environmental concentrations we have observed can reach and exceed the concentrations at which these effects occur.[98,99] Typical toxic concentrations of metformin or guanylurea for many organisms are not seen to be as close to the toxic concentrations below 0.25 µg/L of guanylurea on Japanese medaka as identified by Ussery *et al.***.** It is important, however, to know toxic concentrations for as many other organisms as possible to attempt to extrapolate that information to similar organisms and more easily eliminate sources of contamination in the environment. Several aquatic organisms and the toxic concentrations of metformin and guanylurea at which effects begin to occur are listed in **Table 1**.

Table 1: Literature concentrations of metformin and guanylurea that exhibit effects on aquatic organisms

Organism	Concentration	Effects
Fathead minnows (Pimephales promelas)[108]	40 µg/L Metformin	Intersexuality and Weight loss
Japanese Rice Fish (*Oryzias latipes)*[48]	10 µg/L Metformin	Weight and length decrease
Japanese Rice Fish (*Oryzias latipes)*[105]	>0.25 µg/L Guanylurea	Weight and length decrease
Freshwater Plankton *(Daphnia magna)*[91]	EC_{50} 40 mg/L Guanylurea	Immobilization
Duckweed[109] (*Lemna minor)*	EC_{50} 24.2 mg/L Metformin	Growth Inhibition
Water fleas[109] (*Daphnia similis)*	EC_{50} 14.3 mg/L Metformin	Immobility
Siamese Fighting Fish[110] (*Betta splendens)*	40 µg/L Metformin	Decreased Aggression

Although ecotoxicological data are minimal for both compounds, it is advantageous for scientists and regulatory agencies to know typical environmental concentrations of these contaminants. This allows for investigation into the effects of environmentally relevant concentrations on aquatic organisms that may be exposed to them. We hypothesized that the concentrations of compounds depend primarily on the location of the sampling, with the highest concentrations of both metformin and guanylurea occurring near the influence of sewage/wastewater treatment effluents, with lower concentrations in agriculturally dominated landscapes where fertilizers are applied to fields. We believe that the data presented here will help us to better understand how both compounds behave in impacted water resources and sediment, help us untangle pollution sources, and determine where and when concentrations could be of concern from a toxicological perspective.

2.3 Methods

2.3.1 Surface Water

Water samples were taken in monthly intervals from May to November from 2018 to 2020 by the conservation authorities at each of the watersheds studied. One-litre polyethylene terephthalate (PET) bottles (SystemsPlus, Baden, ON, CA) were filled at the sampling site using a pole sampler. Filled bottles were then stored on ice in insulated coolers and shipped to Agriculture and Agri-Food Canada (AAFC) at the London Research and Development Centre (LRDC). Samples were logged and stored at -20°C until ready for analysis. Prior to the monitoring program, unpublished stability studies determined metformin and guanylurea are stable under these conditions and will not degrade before simultaneous analysis. Monthly sampling over three years allows for long-term analysis of each site and is more accurate in assessing risk of the compounds by determining an average concentration that organisms at each site are exposed to over a prolonged period.

Surface water samples were analyzed by a Thermo Vanquish™ Duo tandem UHPLC system coupled to a TSQ Altis™ triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Sample vials were stored in an autosampler at 10 °C and 10 µL were injected onto one of two Agilent Zorbax Eclipse Plus columns (2.1 x 50 mm, 1.8 µm, Agilent Technologies, Santa Clara, CA, USA) maintained at 35 °C with a flow rate of 300 µL/minute. Mobile phase A (H_2O + 0.1% FA; Optima LC-MS Grade) (Fisher Scientific, Lawn, NJ, USA) was held at 2% for 0.750 minutes. Mobile phase B (acetonitrile + 0.1% FA; Optima LC-MS Grade) (Fisher Scientific, Lawn, NJ, USA) was then increased to 15% over 1.05 minutes and to 24% over 5.6 minutes. Finally, mobile phase B was increased to 98% over 15.1 minutes and held for 2.4 minutes. While analytes were being resolved on one column, the second column was re-equilibrated for 3.5 minutes at 300 µL/minute mobile phase A in preparation for the subsequent injection. The OptaMax NG HESI source was operated with capillary voltages of 3.5 kV in both positive and negative mode, ion transfer tube temperature of 325 °C and vaporizer temperature of 350 °C. The sheath, auxiliary and sweep gases were set to 35, 10, and 1

arbitrary units, respectively. Quantification was performed in Thermo TraceFinder 5.0 (Thermo Scientific, Waltham, MA, USA).

2.3.2 Sediment

Sediment samples were collected in 2020 at the same sampling sites the surface water was collected. A stainless-steel scoop was cleaned with MeOH and used to take sediment samples which were then placed in 500-mL PET bottles (SystemsPlus, Baden, ON, CA). Samples were stored in insulated coolers on ice and shipped to the LRDC, then were frozen alongside surface water samples at -20°C. Previously performed unpublished stability tests confirmed that metformin does not degrade to guanylurea under these conditions, so concentrations of guanylurea calculated in the samples are due only to its original occurrence in the sample. For the sediment extractions, roughly 3-4 g of wet sediment from each site was weighed into a 15-mL conical polypropylene (PPE) Falcon tube. Each tube was centrifuged for 10 minutes at 4000 rpm in an Eppendorf 5810R benchtop centrifuge (Eppendorf, Mississauga, ON, CA) before carefully decanting water from the sediment. Each tube was then weighed to determine the mass of the remaining sediment. The sediment samples were air-dried for 90 minutes prior to extraction. A 10 µg/mL metformin- $^{13}C_4$ $^{15}N_5$ and guanylurea-$^{15}N_4$ (Sigma Aldrich, St Louis, MO, USA) internal standard spiking solution was added to the sediment at a ratio of 10 µL/2.5 grams to simulate 40 ng/g of IS in sediment. Based on the protocol of Ostensvik et. al,[41] the spiked sediments were extracted using 6 mL of extraction buffer consisting of 1:9 formic acid:0.5 M ammonium acetate (Sigma-Aldrich, St Louis, MO, USA) for every 2.5 g of sediment. The solutions were vortexed for 30 seconds on a Vortex-Genie 2 model G-560 (Scientific Industries, Bohemia, NY) to ensure the sediment material was completely dislodged from the base of the Falcon tube. The sediments were sonicated at room temperature in a Cole-Parmer ultrasonic cleaner (Cole-Parmer, Vernon Hills, IL, USA) for 20 minutes then vortexed for 30 seconds. Samples were then placed in a tube rack on a VWR DS-500 orbital shaker (Avantor, Radnor, PA, USA) operating at 400 rpm for 30 minutes at a 45° angle to assist with mixing. Following this, the tubes were centrifuged at 4000 rpm for 10 minutes to separate the solid and liquid layers. The top liquid layers were then carefully decanted into clean 15-mL tubes and spiked with an IS of 10 µg/mL

metformin-D_6 at the same volume as the original metformin- $^{13}C_4$ $^{15}N_5$ and guanylurea-$^{15}N_4$ IS spike to simulate the same 40 ng/g concentration. From these extracts, 500 µL of each was removed and placed into an amber HPLC vial (Agilent Technologies, Santa Clara, CA). The solutions were dried with a gentle stream of nitrogen and reconstituted in 250 µL H_2O, then 250 µL of acetonitrile. This dry down step is important due to the high concentration of formic acid used in the extraction buffer which leads to poor separation in the chromatogram. The 500-µL reconstituted extracts were vortexed for 30 seconds to ensure dissolution of the solids, then placed into the HPLC autosampler. The initially spiked IS mixture of metformin- $^{13}C_4$ $^{15}N_5$ and guanylurea-$^{15}N_4$ underwent the extraction method and as extraction efficiency is not 100%, the peak height was diminished. The post-spike metformin D_6 was not diminished because it was added to the final extract, therefore recovery efficiency (R_E%) can be calculated by comparing the peak heights of the two analytes.

All samples were analyzed by a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled to an Agilent 1290 HPLC. A 2-µL sample was injected onto a Waters BEH amide column; (2.1 x 50mm, 1.7 µm) maintained at 35 °C with a flow rate of 400 µL min^{-1}. Mobile phase A (10 mM ammonium formate with 0.1% formic acid in water: acetonitrile, 1:9) was held at 100% for 1 minutes. Mobile phase B (10 mM ammonium formate with 0.1% formic acid in water) was then increased to 5% over 1 minutes and to 50% over 0.6 minutes. Mobile phase B was held at 50% for 1 minute before returning to 0%. The column was re-equilibrated with mobile phase A for 2 minutes prior to the subsequent injection. The HESI-II source was operated in positive ionization mode with capillary voltages of 3.5 kV, ion transfer tube temperature of 400 °C and vaporizer temperature of 300 °C. The sheath and auxiliary gases were set to 25 and 8 units, respectively. Target analytes and their corresponding internal standards were monitored using the settings listed in **Table 2**. Quantification was performed in Thermo Xcalibur platform version 4.3 with a mass accuracy of 5 ppm and RT window of ± 0.05 minutes relative to the internal standard.

2.3.3 Quantification

Quantification using internal standards (IS) is an accurate method for determining quantities of metformin and guanylurea in surface water and sediment. Labelled standards use stable isotopes in place of several atoms on the analyte such that the same fragmentation patterns and characteristics are the same, but the *m/z* is slightly different. In the case of the metformin and guanylurea IS, ^{15}N, ^{13}C, and deuterium (^{2}H or D) are all used. The internal standards are spiked into the solution at a known concentration which enables a simple comparison of the responses from the known concentration of IS and the unknown concentration of metformin and guanylurea already present in the sample.

In the sediment extraction method, samples were spiked with two different IS mixtures. The first mixture, added before the extraction, contained metformin- $^{13}C_4$ $^{15}N_5$ and guanylurea-$^{15}N_4$. This mixture allows for quantification of the unknown amount of metformin and guanylurea by calculating the ratio of the peaks of our unknown concentration to our known concentration of the internal standard. The second internal standard of metformin-d_6 was spiked after the extraction method at the same concentration of the initial mixture. The recovery can then be determined by comparing the peak height of metformin-d_6 to metformin-$^{13}C_4$ ^{15}N as the only loss of signal would be due to the recovery efficiency, and signal suppression or enhancement in the method. Internal standards used are metformin- $^{13}C_4$ $^{15}N_5$, guanylurea-$^{15}N_4$, and Metformin-D_6 which were detected using the mass spectrometer settings outlined in **(Table 2).**

Table 2: Mass Spectrometer settings for Metformin, Guanylurea, and related Internal Standards

Analyte	*Ion Type*	*RT (min)*	*Precursor m/z*	Collision Energy (NCE)	*Quantifier ion m/z (CE)*	*Qualifier ion 1 m/z (CE)*
Metformin	$[M+H]^+$	2.24	130.1	42	60.0564	71.0610
Metformin ($^{13}C_4$,$^{15}N_5$)	*$[M+H]^+$*	*2.24*	*139.1*	*42*	*64.0508*	-
Metformin (D_6)	*$[M+H]^+$*	*2.24*	*136.1*	*42*	*60.0564*	-
Guanylurea	$[M+H]^+$	2.12	103.1	21	60.0563	86.0355
Guanylurea ($^{15}N_4$)	*$[M+H]^+$*	*2.15*	*107.1*	*21*	*63.0474*	

2.4 Results and Discussion

2.4.1 Surface Water and Sediment

Quantification of metformin and guanylurea at each sampling site revealed the widespread occurrence of both compounds. In surface water, each of the six individual watersheds registered guanylurea at every site at least once, and metformin in all but two of the 40 total sites. Metformin was identified above the limit of detection (10 ng/L) in 51.0% of samples, and guanylurea above the limit of detection (LOD) (100 ng/L) in 50.7% of the 673 total samples. Despite the LOD being a magnitude higher for guanylurea, it was only detected in two fewer surface water samples, meaning guanylurea would likely be present in many more samples if the LODs were lower. Metformin was found in 343 different samples, and guanylurea was found in 71.4% of them. In water samples in which both compounds appeared, guanylurea was higher in 89.0%, or 218 out of 245. Guanylurea was also found above the LOD in 104 samples where metformin was not found. These factors are both indicative of degradation of metformin leading to increasing concentrations of guanylurea in surface water. In 70 of the 98 samples where guanylurea was not found, metformin was found at concentrations below 100 ng/L, meaning the initial presence of metformin may not have been sufficient for detectable concentrations of guanylurea to appear.

For sediment analysis, samples were taken only in 2020 from sites in which it was safe to do so. From the 40 surface water sampling sites, sediment was taken from 29 of them for a total of 155 samples. In these samples, metformin was extracted and detected above the limit of quantification (0.05 ng/g metformin and 0.6 ng/g guanylurea) in 98 of them. Of the 155 samples, there were 56 samples which registered neither compound. Metformin and guanylurea appeared in the same sample in 26 of the 30 samples that contained guanylurea. Conversely to the trends observed in surface water, guanylurea was found both in fewer samples, and in much lower concentrations than metformin. Previous studies have analyzed the sorption of metformin and guanylurea to sediment and have determined that their solubilities are high, and binding to sediment is weak.[6] As few sites did measure metformin and guanylurea through the sampling period, this is reinforced by

the data. Average concentrations of metformin and guanylurea calculated in surface water and sediment for all sites are listed in **Table A1** and **Table A2,** respectively.

To best compare the concentrations of metformin and guanylurea in surface water and sediment between sites in the same watershed, ratios of metformin and guanylurea are plotted on maps of the sampling site locations **(Figures 14-20)**. The average concentrations of metformin and guanylurea were calculated in samples in which they were detected, and samples below the LOD are omitted in calculations. Average concentrations during the sampling period are expressed as a pie chart, and the size of the pie chart increases with the total concentration of the two compounds at the sampling site.

2.4.2 Ausable Bayfield Results

The Ausable Bayfield Conservation Authority (ABCA) samples the Ausable Bayfield watershed, which lies along Lake Huron and includes cities such as Grand Bend and Goderich. Ausable Bayfield is one of the less populated watersheds investigated in this work with a population of roughly 45000.[111] Its area is 2440 km^2, most of which is agricultural areas. The sampling sites investigated are therefore influenced mostly by agriculture, and the human input in the region should be minimal compared to more populous watersheds **(Table 3)**.

Table 3: Activity and Input at ABCA sampling sites

Sampling Site	Primary Activity
A1	Human input
A2	Agriculture
A3	Agriculture
A4	WWTP

The primary expected sources of both metformin and guanylurea in the environment are urban influences and WWTPs. As there were limited urban influences at this watershed, and the only WWTP was from a small human population, overall concentrations of metformin and guanylurea were quite low **(Figure 14)**. In surface water, the highest

average concentration of metformin at any of the sites was 0.040 ± 0.03 µg/L at site A4, and the highest concentration of guanylurea was 0.313 ± 0.5 µg/L at site A3. These concentrations are much lower than those known to cause toxic effects, even with chronic exposure. In sediment, concentrations of metformin and guanylurea were low as well. Site A1 only registered metformin on one occasion through the sampling period, and it, as well as A4, did not contain guanylurea in sediment even once. Guanylurea was not more prevalent in the other sites either, as both A2 and A3 registered it only once through the six-month period. The highest concentration recorded in the watershed was 133.05 ng/g in June 2020, while no other samples taken in the following months from the same site were over 5 ng/g. This may indicate that metformin in this region is not persistent, and degrades over time to guanylurea, or resolubilizes and dilutes around the watershed.

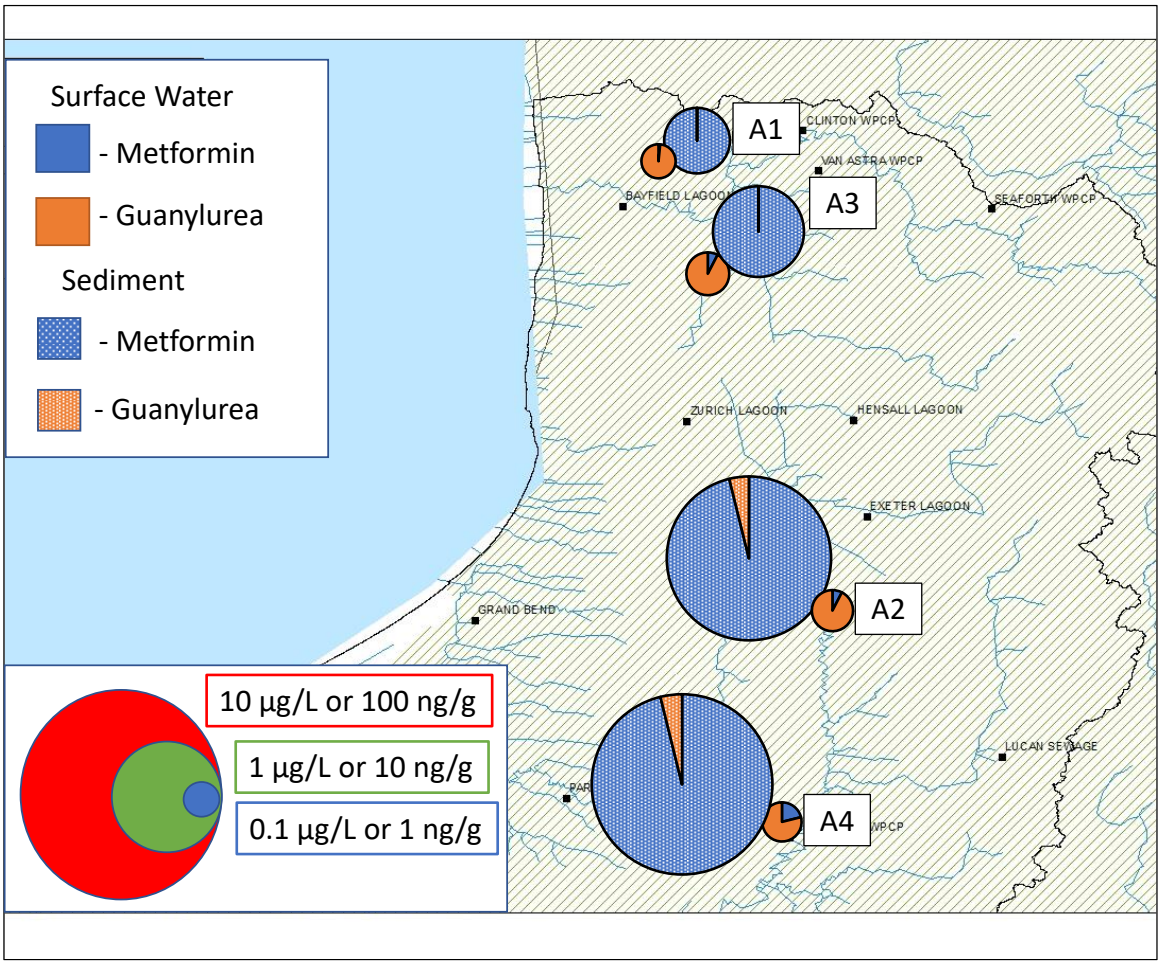


Figure 14: Site map for the Ausable Bayfield watershed. Ratios of metformin to guanylurea at each site are provided as pie charts, the size of the pie chart increases with total concentrations of both compounds.

2.4.3 Cedar Results

The Essex Region Conservation Authority (ERCA) samples from the Cedar watershed which encompasses the southwestern most tip of Ontario and includes sites along Lake Erie and Lake St. Clair near the Detroit River. Both Detroit and Windsor are cities near these sampling sites with a heavy manufacturing presence. The population in this region is relatively high at over 420 000 people,[112] so human input in the area is a significant factor. Four distinct types of inputs are examined in this watershed, one WWTP, one urban, one greenhouse, and two agriculture influences **(Table 4).**

Table 4: Activity and Input at ERCA sampling sites

Sampling Site	Primary Activity
C1	WWTP
C2	Greenhouse
C3	Agriculture
C4	Agriculture
C5	Urban

The highest combined concentration identified in this watershed was 4.55 µg/L at C1, where an average of 4.20 ± 7 µg/L of guanylurea was measured in 2018-2020. C1 is sampled near a WWTP, so it is expected to exhibit higher concentrations of pharmaceuticals, and it is the only site to average over 1 µg/L guanylurea. Every sampled site in this watershed measured an average concentration of guanylurea in surface water higher than the benchmark for chronic toxicity of 0.25 µg/L as highlighted by Ussery et al. in 2019. Variation in concentration between timepoints is large however, as all 2020 samples at C1 registered over 0.25 µg/L, while only one sample in 2018 contained guanylurea at all. This may indicate increased use of the compound and therefore

increasing risk of accumulation of guanylurea in the environment. C1 was also the only site to not only have an average over 1 µg/L, but to register above that level in any of the samples taken through all three sampled years.

Fewer sites in this watershed were able to be safely sampled for sediments. Similarly to the Ausable Bayfield watershed, guanylurea was found much less frequently in sediment than guanylurea, appearing only once in B3 and nowhere else at any point. The concentration of guanylurea found at this site was also much lower than the average concentrations of metformin, at 2.93 ng/g guanylurea. Of the 10 samples in which metformin was found through the sampling period, only one sample was lower than 2 ng/g. All three of the sites had low concentrations of guanylurea but had an average concentration of over 20 ng/g metformin **(Figure 15)**. This indicates that guanylurea does not adsorb as effectively to soil as metformin, and that accumulation in soil is not occurring extensively at this watershed.

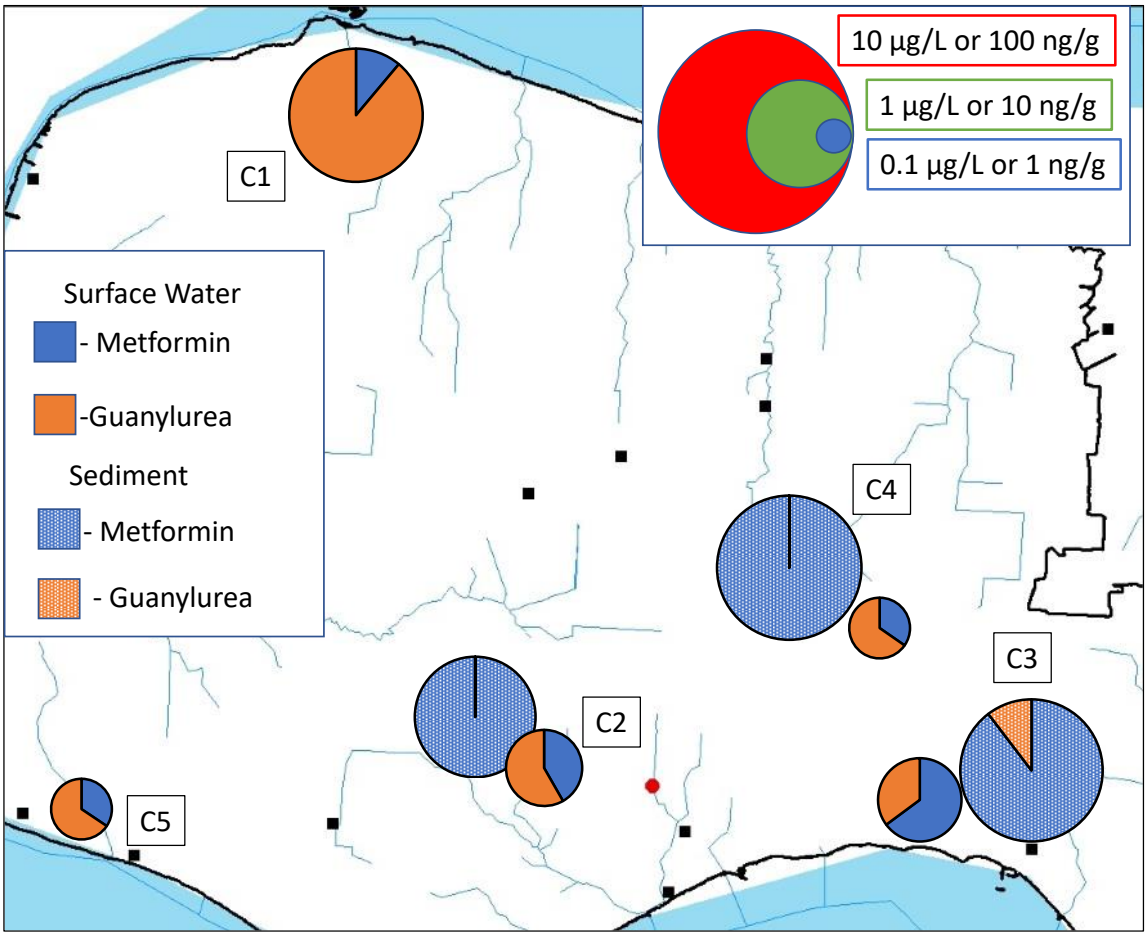


Figure 15: Site map for the Cedar watershed. Ratios of metformin to guanylurea at each site are provided as pie charts, the size of the pie chart increases with total concentrations of both compounds.

2.4.4 Upper Grand Results

The Grand River Conservation Authority (GRCA) samples the Grand River watershed containing the cities of Kitchener, Waterloo, Guelph, Brantford, and Cambridge, as well as several other smaller communities. The Grand River watershed is the largest watershed in Ontario at 6800 km^2, with a total population of over 1 million people, most of whom live in the larger cities.[113] Two sampling sites are sampled near Kitchener and Waterloo and are influenced primarily by WWTPs in the region, while the rest are further from the larger cities and receive input mostly from agriculture **(Table 5)**. The more populous

regions are expected to exhibit the highest concentrations of metformin and guanylurea in surface water especially due to the nearby WWTP effluents that receive input from the surrounding population.

Table 5: Activity and Input at GRCA sampling sites

Sampling Site	Primary Activity
UG1	WWTP
UG2	Rural
UG3	Agriculture
UG4	WWTP
UG5	Agriculture
UG6	Agriculture

Of the six sites with different activities, the highest recorded concentrations of both metformin and guanylurea occurred at the WWTP sites near Kitchener and Waterloo: UG1 and UG4. This is due to the higher populations leading to increased use of the compounds in the area, and the main source in the watershed being WWTP effluent. Both UG1 and UG4 contained average concentrations of guanylurea higher than 2 µg/L, at 2.82 ± 2 µg/L and 2.01 ± 1 µg/L, respectively. Out of 16 samples taken from each of UG1 and UG4 through the three-year sampling period, 14 contained over 0.7 µg/L of guanylurea in both. For both sites, the samples that contained below 0.7 µg/L were all from 2018. These concentrations are well above concentrations that can cause toxic effects in Japanese medaka, so there may be toxic effects already occurring to organisms in the region.

Sediment data for the Upper Grand region follow similar trends to those observed in the previous watersheds. Guanylurea was found twice at UG6, and was found only once at each other site. The highest single occurrence of 1.99 ng/g guanylurea was at UG1 in May 2020, where the concentration of metformin at the same timepoint was found to be 34.857 ng/g. Repeating the previous trends, metformin was more common in sediment samples than guanylurea, and was in higher concentrations **(Figure 16)**.

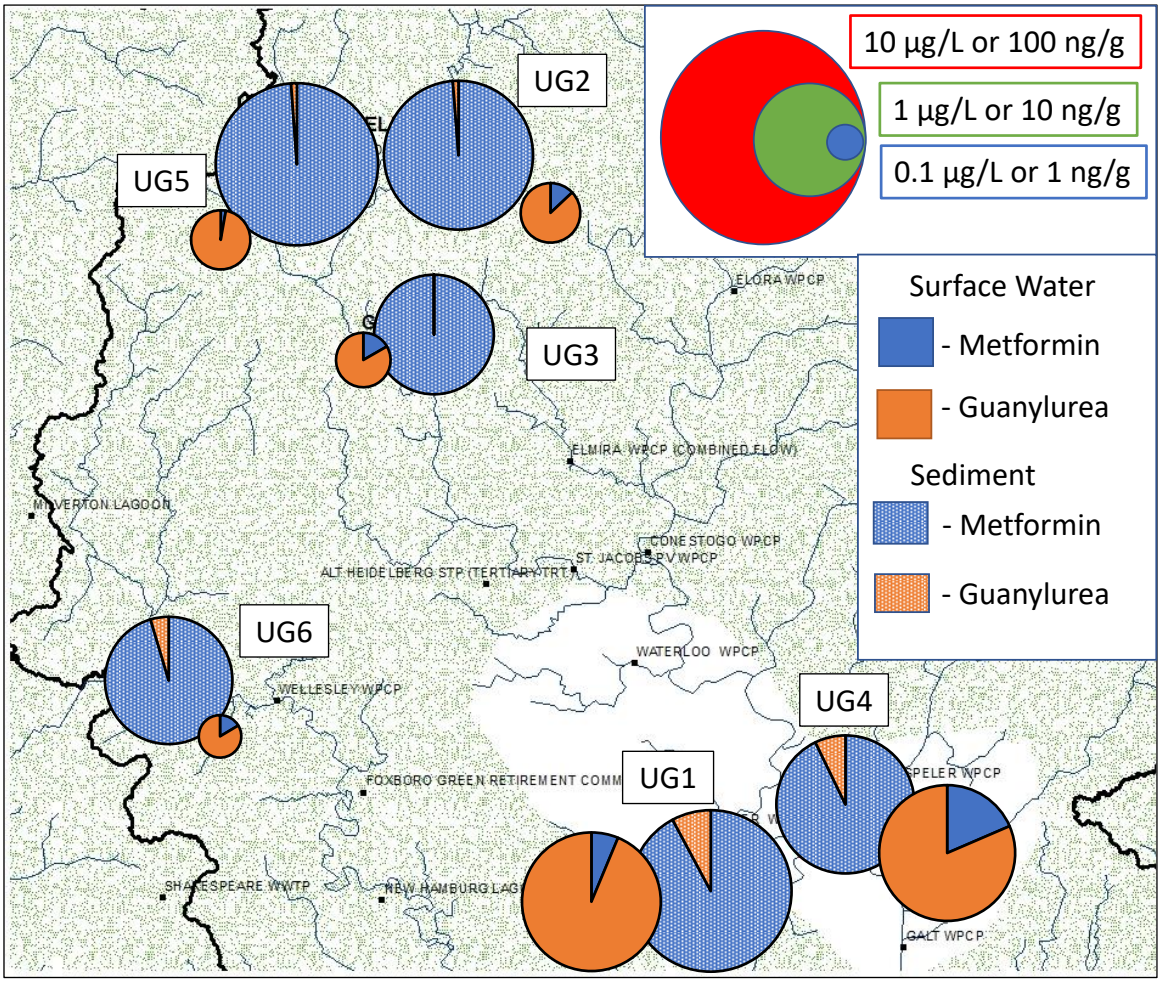


Figure 16: Site map for the Upper Grand watershed. Ratios of metformin to guanylurea at each site are provided as pie charts, the size of the pie chart increases with total concentrations of both compounds.

2.4.5 Upper Ottawa – Kipawa Results

The Organisme de Bassin Versant du Témiscamingue (OBVT) is at the remote region of Upper Ottawa- Kipawa, located along the border of Northern Ontario and Quebec. The watershed is quite small, with a total population of roughly 59 000 and a population density of only 1.7 inhabitants/km^2.[114] One important site in this watershed is site 6, which was the only site in the dataset that was sampled directly from the effluent pipe of a WWTP **(Table 6)**. As these water samples have not had time to dilute and diffuse about the watershed, UOK site 6 should contain the highest concentration of all chemicals in the watershed that result from WWTP contamination. Sampling in this watershed only occurred in 2020, so long term data are not available.

Table 6: Activity and Input at OBVT sampling sites

Sampling Site	Primary Activity
UOK1	Agriculture
UOK2	Agriculture
UOK3	Agriculture
UOK4	Agriculture
UOK5	Agriculture
UOK6	WWTP
UOK7	Agriculture

UOK6 offers the greatest insight of any of the sampling sites as to how WWTPs introduce contamination of metformin and guanylurea in the aquatic environment. Despite being one of the smallest watersheds investigated in the course of this work, UOK6 registered the highest average concentration of both metformin and guanylurea in surface water of 3.63 ± 3 µg/L metformin and 14.6 ± 4 µg/L guanylurea, much higher than any other site. All other sites in the region are agriculturally influenced and exhibited trends similar to the previous watersheds, where no sites but the WWTP-influenced UOK6 contained above 0.25 µg/L guanylurea. The UOK1 site measured metformin only once in the sampling period, and UOK2 did not contain metformin at all. The surface

water data in this watershed emphasize the link between population size and widespread contamination of metformin and guanylurea, as well as the link between WWTP effluent and their presence in the environment.

For sediment sampling in the watershed, five of the six sites only contained guanylurea once, while UOK2 did not register guanylurea above the LOD at all. Three sites (UOK1, UOK2, and UOK5) only contained metformin once, at concentrations below 10 ng/g. Interestingly, the agriculture site UOK3 which contained an average of 0.106 ± 0.1 µg/L metformin in surface water had 237.683 ± 100 ng/g metformin in sediment. This may indicate that the type of soil at this site can more easily adsorb metformin, or that the bacterial species that degrades metformin to guanylurea is not present at this location. All concentrations of both metformin and guanylurea in sediment at this watershed are among the lowest of all sites examined in this study **(Figure 17)**.

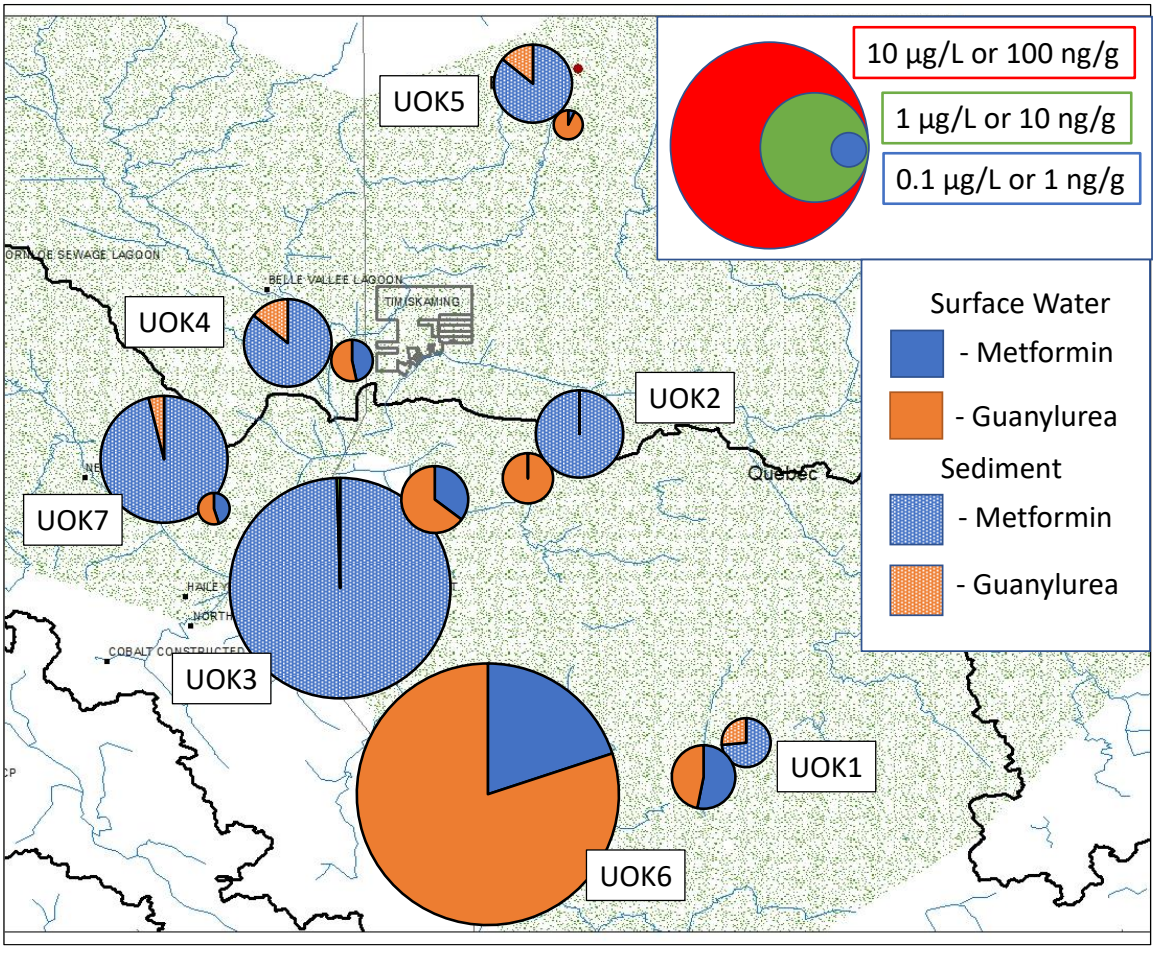


Figure 17: Site map for the Upper Ottawa – Kipawa watershed. Ratios of metformin to guanylurea at each site are provided as pie charts, the size of the pie chart increases with total concentrations of both compounds.

2.4.6 Lower Ottawa – South Nation Results

The Lower Ottawa – South Nation watershed is sampled by South Nation Conservation Authority (SNCA). The region lies just south of Ottawa and is 4441 km^2, containing 16 different municipalities.[115] The Lower Ottawa – South Nation watershed has extensive sampling infrastructure, and contains the highest number of sampling sites of any watershed with 13 total sites. The region is primarily agricultural, and that is reflected by the 9 sampling sites with agriculture activity nearby **(Table 7)**. There are, however, four sampling sites taken from two lagoons and their outflows. A lagoon is a type of WWTP which is treated over time using bacteria, UV and sometimes added chemicals. Samples taken from this location are more analogous to samples taken directly from a WWTP mid-treatment. This means that if degradation is occurring actively at these lagoon sites, then samples taken mid-treatment should contain higher concentrations of metformin than guanylurea. Each lagoon site was also paired with a sampling site at the lagoon outflows where the treated effluent is then released. If concentrations of guanylurea are higher than concentrations of metformin in the outflow, then this further reinforces that bacterial degradation is occurring in the lagoon WWTP sites. If total concentrations are diminished, this also indicates that the lagoon sites can completely degrade guanylurea as well.

Table 7: Activity and Input at SNCA sampling sites

Sampling Site	Primary Activity
1	Agriculture
2	Agriculture
3	Agriculture
4	Agriculture
5	Agriculture
6	Agriculture

7	Agriculture
8	Agriculture
9	Agriculture
10	Lagoon #1
11	Lagoon #1 Outflow
12	Lagoon #2
13	Lagoon #2 Outflow

The highest observed concentrations in the watershed were, as expected, the samples from the lagoon sites. In surface water, lagoon sites 10 and 12 registered 2.409 ± 4 µg/L and 1.602 ± 2 µg/L of metformin, and 0.932 ± 0.8 µg/L and 1.941 ± 1 µg/L guanylurea, respectively. These lagoon sites contained a much higher ratio of metformin to guanylurea with LOSN10 containing more metformin than guanylurea, a trend not seen in any of the agriculture sites sampled in the watershed. Most notably from these results is that the ratio does favour metformin, indicating that bacterial degradation is occurring in the lagoons. Concentrations at the lagoon outflow sites 11 and 13 however, detected only 0.014 ± 0.007 µg/L and 0.141 ± 0.2 µg/L of metformin and 0.430 ± 0.010 µg/L and 0.406 ± 0.2 µg/L of guanylurea, respectively, much lower than those concentrations seen in the lagoons themselves. This is indicative of further bacterial degradation of guanylurea occurring in the lagoon prior to release of the treated effluent back into the environment. Other sites at the watershed even further reinforce those trends as seen in other watersheds through the sampling, where agriculturally influenced sites were not prominent sources of either compound. Not including the lagoon-influenced sites, only 1 site measured over 0.25 µg/L guanylurea at LOSN site 9, with a concentration of 0.301 ± 0.2 µg/L.

For the sediment sampling, trends proved similar to surface water. Guanylurea was only detected once through the sampling period, at site 3. Metformin, however, was detected at all the sampled sites, with the highest average concentration of metformin of 4047 ± 6111 ng/g occurring at the lagoon site LOSN12. This exceeds all other sampled sites by 10-fold, and the next highest concentration of metformin was 351.09 ng/g at LOSN13, the outflow of the same site. Despite the extremely high concentration of metformin seen

in sediment in site 12, concentrations of metformin in surface water for the site were lower than the lagoon at site 10 and were comparable to several other non-lagoon sites. This means that metformin is likely not persistent in lagoon outflow and the surrounding surface water and can still resolubilize and degrade into guanylurea **(Figure 18)**.

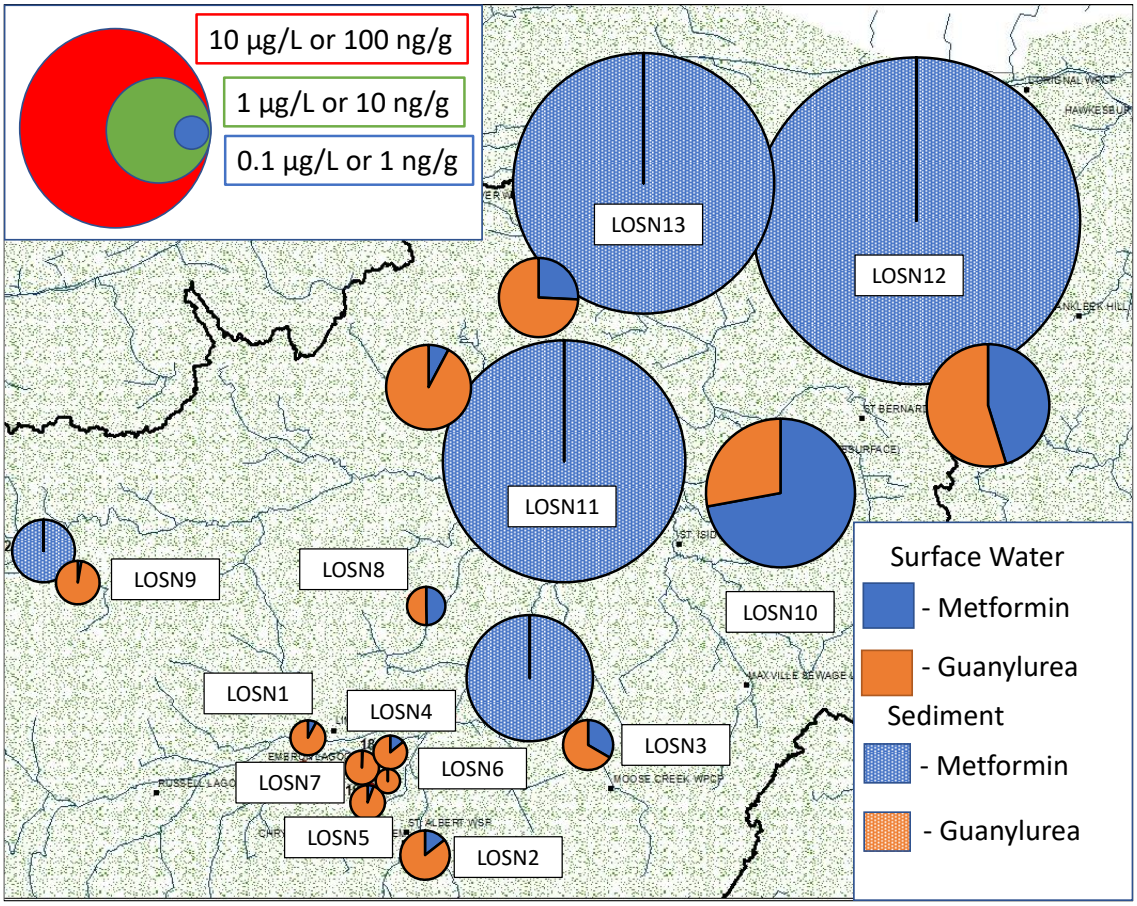


Figure 18: Site map for the Lower Ottawa - South Nation watershed. Ratios of metformin to guanylurea at each site are provided as pie charts, the size of the pie chart increases with total concentrations of both compounds.

2.4.7 Upper Thames Results

The final watershed examined in this study is the Upper Thames region, which is sampled by the Upper Thames River Conservation Authority (UTRCA). The Upper Thames watershed contains the large urban centres of London, Woodstock, and Stratford, and the

rest is agriculture.[116] With a total population of nearly 540 000, this region is the second-highest population watershed investigated in this work. Three of the sampled sites are near high-population areas, and the other two are more rural to give a complete picture of contamination in the region **[Table 8]**.

Table 8: Activity and Input at UTRCA sampling sites

Sampling Site	Primary Activity
1	Agriculture/Urban
2	WWTP
3	Urban
4	Agriculture
5	Agriculture

This watershed offered the interesting insight into higher populations and the general effects those have on concentration of metformin and guanylurea. The highest concentration of guanylurea measured at this watershed was at site 1, at 9.015 ± 7 µg/L, which was the highest average concentration of guanylurea other than the WWTP effluent pipe at UOK6. The influence at this site was urban and agriculture, not a WWTP, which exhibits the effects that high populations can have on overall concentration of the compounds. Total concentrations at sites 2 and 3 were also relatively high, at 3.31 and 4.65 µg/L, respectively. These sites had WWTP and urban activities nearby, while the two agriculture-influenced sites at UT4 and UT5 measured average total concentrations of 0.627 and 0.266 µg/L. Concentrations of guanylurea at all sites in this watershed were above 0.2 µg/L and were higher than most other watersheds.

Sediment data for this watershed contained three of the highest concentrations of metformin as detected through the sampling period that were not from lagoon or lagoon outflow sites. Of the three sites with urban or WWTP influences, the lowest concentration of metformin at these sites was 174.878 ± 60 ng/g. Of the total 30 samples that had

guanylurea in quantifiable amounts, 13 of them were at this watershed. This may indicate that higher population watersheds are constantly replenishing these compounds in the environment through WWTP effluent or urban runoff as the compounds are known to be very soluble and do not adsorb to sediment for long.[6] This watershed further reinforces the data seen previously at all other watersheds, but adds insight into the role population and sampling sites have on average concentrations of the compounds. The sampling sites containing greater than 1 µg/L total concentration in surface water and greater than 100 ng/g total concentration in sediment were all urban or WWTP influenced sites sampled from close to highly populated cities **(Figure 19)**.

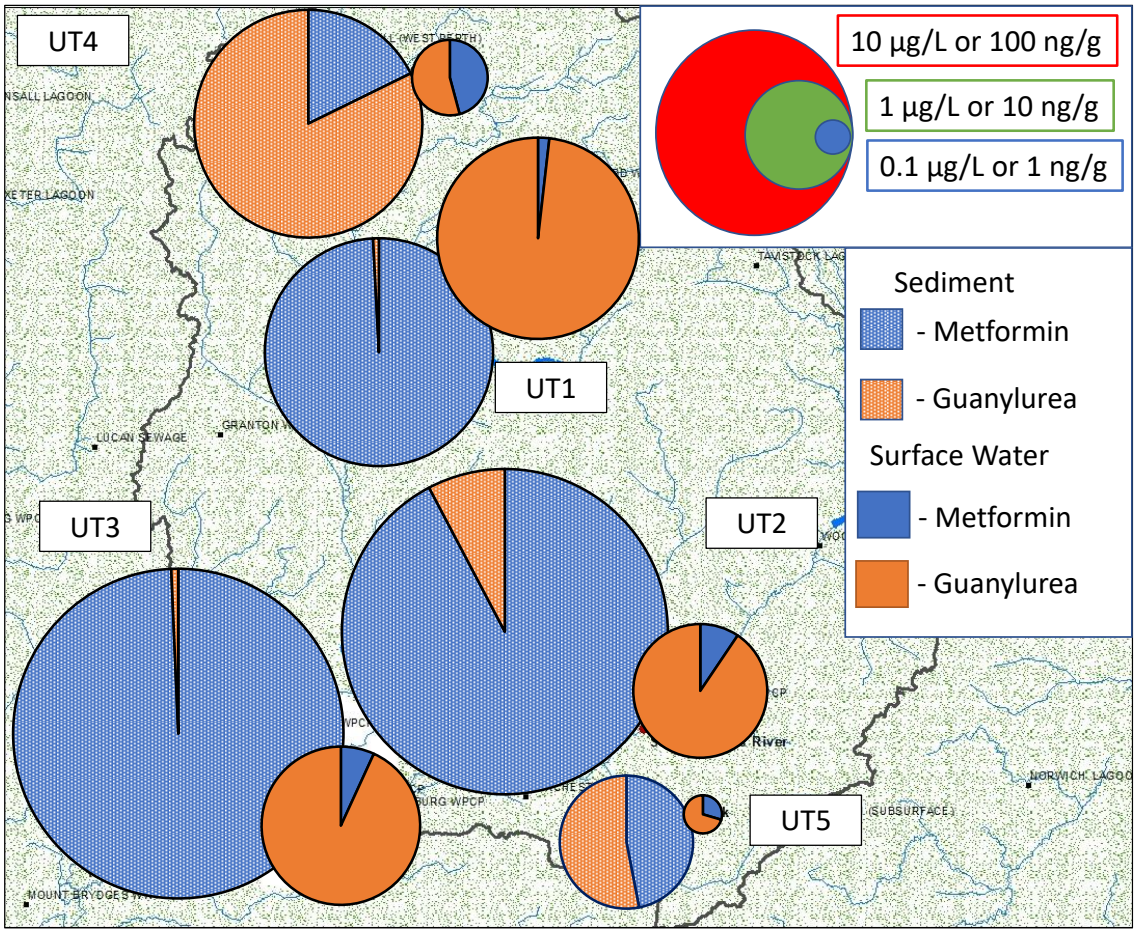


Figure 19: Site map for the Upper Thames watershed. Ratios of metformin to guanylurea at each site are provided as pie charts, the size of the pie chart increases with total concentrations of both compounds.

2.5 Conclusions

This work has clearly exhibited the prevalence of both metformin and guanylurea in Ontario and Quebec waterways, and the utility of the method in detecting both compounds. With LODs of 10 ng/L and 0.05 ng/g metformin and 100 ng/L and 0.6 ng/g guanylurea in surface water and sediment respectively, levels of the compounds well below toxic concentrations can be measured. Metformin and guanylurea were each seen to be present in slightly over half of all water samples taken from 2018-2020, indicating that they are common in the environment, and likely have been for some time. Guanylurea was in higher concentration in most water samples than metformin, and the opposite was true for sediment, indicating metformin is much more easily bound to sediment where it can be more sufficiently retained. High concentrations at sampling sites were not consistently high throughout the sampling period, further reinforcing previous studies that indicate low sorption to sediment and high solubility in water. Higher concentrations of guanylurea in most samples that contained both compounds as well as the common occurrence of guanylurea in samples that did not contain metformin are indicative of degradation of metformin in the environment to form its metabolite. The sites that contained concentrations of guanylurea registering above the highlighted potentially chronically toxic concentration of 0.25 µg/L were primarily WWTP, urban, or lagoon influenced sites. This suggests that agriculture is not a prominent source of contamination for either compound, and that insufficient degradation of metformin and guanylurea in WWTPs is where most of its environmental contamination arises.

Chapter 3

Investigating Effects of the Thermal Chemical Hydrolysis Treatment on ESOCs in Biosolids

3.1 Chapter 3 Objectives

The solid waste remaining after WWTP treatment of sewage can be used as fertilizer provided it has been sufficiently treated. This treatment is only required to remove bacteria and micropollutants, but it is unknown if it can remove or degrade other compounds. As WWTP treatment cannot sufficiently degrade many complex pharmaceuticals, these biosolids may contain environmental contaminants that are then reintroduced into the environment. This study seeks to assess methods for extracting ESOCs that may be present in the biosolids, and to determine if any of the compounds change in concentration after treatment.

3.2 Introduction

Pharmaceuticals and personal care products (PPCPs) are an ever-increasing class of chemicals used in everyday life by millions of people worldwide. This class includes a wide array of common chemicals used in over-the-counter medications, cosmetics, and veterinary drugs. Among them are some of the most widely used compounds in the world, including caffeine, ibuprofen, and acetaminophen. While some of these compounds are seen to decrease from influent to effluent in wastewater treatment plants (WWTPs) such as caffeine,[117] others are known to be insufficiently degraded. As such, many PPCPs that enter a WWTP remain in the treated effluent and are released back into the surface water.[16,19,118,119] Human use and consumption of PPCPs in a region are therefore linked to occurrence of those same PPCPs in the surrounding aquatic environment.[120] As new PPCPs are in constant development, their environmental impacts are often unknown. Clinical trials on safety of newly developed drugs focus on their efficacy in treating an illness and often overlook environmental effects upon their excretion. This means that toxicity of environmental contaminants is an ever-present issue that must be investigated for every new PPCP that is introduced to the aquatic environment.

Municipal wastewater treatment plants receive millions of tons of solid waste per year.[43] This solid waste, known as biosolid, remains behind with little productive use. The

organic material that remains can be used as fertilizer in agriculture, provided it has been processed to remove harmful micropollutants and bacteria. The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) regulates the land use of biosolids to ensure they do not contain trace amounts of harmful elements like arsenic, lead, and mercury.[121] One method to process the solid and remove chemical contaminants is the Thermal Hydrolysis process (THP), which uses basic pH conditions, heat and shearing of the waste.[122] The treatment of biosolids creates what is labelled by the Environmental Protection Agency (EPA) as Class A fertilizer. Class A fertilizer are treated to be determined pathogen-free and therefore safe to be distributed in public land without restrictions.[123] It is known that the THP method removes bacteria and does in fact create Class A fertilizer, however it is unknown if ESOCs remain recalcitrant in these biosolids and are therefore spread back into the environment when applied in agriculture.[124] This is a concern as many WWTPs are unable to fully degrade compounds like complex pharmaceuticals, and many ESOCs are therefore present in the treated effluent wastewater that is released into the environment every day.[16,19,20,36] As the solid waste is subjected to a similar process to wastewater, it is likely that many of the same contaminants remain in the solid and should be investigated. It is of importance to ensure that no environmental contaminants are present in high concentrations in the fertilizer before it is applied such that biosolid application does not become a point source for ESOCs in the environment. Biosolids before and after THP treatment will be analyzed to allow for a direct observation of the effects of treatment on ESOCs present in organic solids treated in WWTPs.

Two time points were examined for each WWTP to determine if there are temporal effects that may change which compounds are detected in each sample, and whether they increase or decrease in concentration. May and December samples from 2021 were collected then extracted to have two data points in different seasons. This allowed for comparison of the same compounds at different time points, and therefore the seasonal distributions of compounds that may only be detectable in one point but not the other.

Mass spectrometry will be used to identify and quantify ESOCs in the biosolid samples before and after treatment. The first method to be used is a nontargeted data independent

analysis (DIA) method using the Orbitrap mass spectrometer. The purpose of this analysis is to assess a nontargeted method for detecting ESOCs in biosolids. This DIA method will be used to determine if it can effectively separate a wide variety of compounds from the complex organic matrix.

A QqQ method has also been developed that will be used for targeted analysis of several compounds of interest in environmental toxicology. Triple quadrupole mass spectrometry is especially useful in examining complex organic solutions as the specificity of the instrument for its target analytes allows it to overcome matrix effects which are so prevalent. By individually analyzing target analytes and determining their fragmentation, the compounds can be added to a target list with the parameters, such that subsequent analyses will detect every compound on the list. This allows for a wide range of environmentally relevant target compounds to be detected and analyzed, which can be used to discern the severity of their contamination in the aquatic environment.

3.3 Materials and Methods

Solid waste samples were taken by a collaborator from three WWTPs at distinct locations in May and December of 2021. Samples were treated with shearing in a benchtop reactor at 75°C in 6M KOH for 90 minutes. Plastic containers were filled with roughly 500 mL of sample and transported on ice before being extracted immediately then stored at 4°C. Each of the three cake and fertilizer samples were extracted and analyzed in triplicate.

3.3.1 Nontargeted analysis method

To begin extraction of aqueous biosolid samples, 250 mg of the solid samples were transferred into 15-mL conical PPE Falcon tubes, followed by 5 mL of 60:40 acetonitrile:pH 2 water. The pH of the mixture was adjusted to 2 with HCl. The mixture was vortexed on a Vortex-Genie 2 model G-560 (Scientific Industries, Bohemia, NY) to suspend the solid, then shaken for at 300 rpm on a VWR DS-500 orbital shaker (Avantor, Radnor, PA, USA) for 30 minutes. The tubes were then centrifuged at 3000 rpm for 10 minutes in an Eppendorf 5810R benchtop centrifuge (Eppendorf, Mississauga, ON, CA),

and the liquid was decanted into a clean 50-mL tube. The solid was then extracted again with 5 mL of 50:50 acetonitrile:acetone, which was shaken and centrifuged in the same conditions as the first extraction. The liquid extract was decanted into the same tube as the first extract. HLB SPE cartridges were activated with 6 mL of methanol, followed by 6 mL acetonitrile, 6 mL methanol, then conditioned twice with 6 mL of pH 2 water each time. The liquid extract was drawn under vacuum through the SPE cartridges at a rate of roughly 1 drop per second. Once all liquid had been drawn through the cartridge, the cartridges were washed with 3 mL of hexanes, then were allowed to air dry for 5 minutes. The dried cartridges were then eluted into new 15-mL tubes with 3 additions of 1 mL 50:50 acetonitrile:methanol. The extracts were dried under a gentle stream of air, then reconstituted in 1 mL methanol. The final extract was then filtered into 2-mL amber HPLC vials (Agilent Technologies, Santa Clara, CA) through a 0.22 µm filter prior to LC-MS analysis.

The extracts were analyzed using a Thermo Q-Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) coupled to an Agilent 1290 HPLC (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was performed using an Agilent Zorbax EclipsePlus RRHD C18 column with an injection volume of 5 µL for each sample at 35℃. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) using a flow rate at 0.3 mL min^{-1}. The ion trap mass spectrometer was used with a heated electro-spray ionization source (HESI), with capillary temperature of 400°C; sheath gas, 17 arbitrary units; auxiliary gas, 8 units; probe heater temperature, 450°C; S-Lens rf level, 45%; and capillary voltage, 3.9 kV.

Identification of contaminants using the DIA method was performed in MSDial software version 4.90. To do so, .raw spectra files were converted into .mzml files using Proteowizard msconvert version 3, then analyzed against the Swiss Federal Institute of Aquatic Science and Technology (EAWAG, in original German Eidgenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz) pesticide and pharmaceutical database. The software identifies correlation of the resulting spectra with stored spectra for the target compounds and gives results based on their similarities.

3.3.2 Targeted Triple Quadrupole method

Prior to weighing out the biosolids, roughly 5 g of each sample was spread onto aluminum foil and left in a fume hood for 3 days to air dry at room temperature. Dried biosolids were homogenized with a mortar and pestle then transferred for storage into a 50-mL conical PPE Falcon tube. Moisture content for each was measured to calculate concentration of each ESOC in dry weight. This was done using a Sartorius MA37-1 Infrared Moisture Balance (Sartorius, Oakville, CA), which heats the sample and determines the moisture percentage based on mass lost to evaporation. A phosphate buffer was made with 5.99 g of KH_2PO_4, 5.40 g of K_2HPO_4 in 500 mL of NanoPure water adjusted to pH 2 with 6M HCl. To begin extraction, 250 mg of each dried biosolid sample was weighed and placed into a clean and dry 15-mL conical PPE Falcon tube. To each tube, 5.0 mL of 60:40 acetonitrile:phosphate buffer (pH 2) was added. The mixture was vortexed vigorously then shaken on a rotary shaker at 300 rpm for 30 minutes. The solution was centrifuged for 10 minutes at 4000 rpm to separate the solid from the liquid extract. The top liquid layer was decanted into a clean 15-mL tube. The solid was extracted again with 5.0 mL of 50:50 acetonitrile:acetone, which was then vortexed, shaken and centrifuged as described previously. The extract was decanted and combined in the same 15-mL tube as the previous extract. Approximately 100 mg of ethylenediaminetetraacetic acid (EDTA) iron (III) sodium salt (Sigma Aldrich, St Louis, MO) was added to each extract, then the samples were shaken for 8 minutes prior to spinning down by centrifugation at 4000 rpm. Finally, 2 mL of the extract was removed and dried under a steady stream of nitrogen. The dried extracts were reconstituted in 1 mL of methanol, then diluted 1:1 in water.

All extracts were analyzed using a Thermo Vanquish Duo HPLC system coupled to a Thermo Altis triple quadrupole mass spectrometer ((Thermo Fisher Scientific, Waltham, MA, USA). The HPLC performed online preconcentration onto a Thermo hypersil GOLD aQ (20x2.1mm, 12µm, Thermo Fisher Scientific, Waltham, MA, USA) precolumn and backeluted onto an Agilent Zorbax C-18 column (50 x 2.1 mm; 1.8 µm, Agilent Technologies, Santa Clara, CA) held at 35°C. A flow rate of 300 µL/min aqueous mobile phase (A) of H_2O + 0.1% Formic Acid (Optima™ LC/MS Grade, Fisher scientific, Waltham, MA, USA) and organic mobile phase (B) of Acetonitrile + 0.1% Formic acid

(Optima™ LC/MS Grade, Fisher Scientific, Waltham, MA, USA) was used throughout. The gradient began with 2% B for 0.75 minutes before increasing to 15% over 1.05 minutes. B was increased to 24% over 5.6 minutes and to 98% over 15.1 minutes. B was held at 98% for 2.4 minutes before returning to 2% in 0.1 minutes.

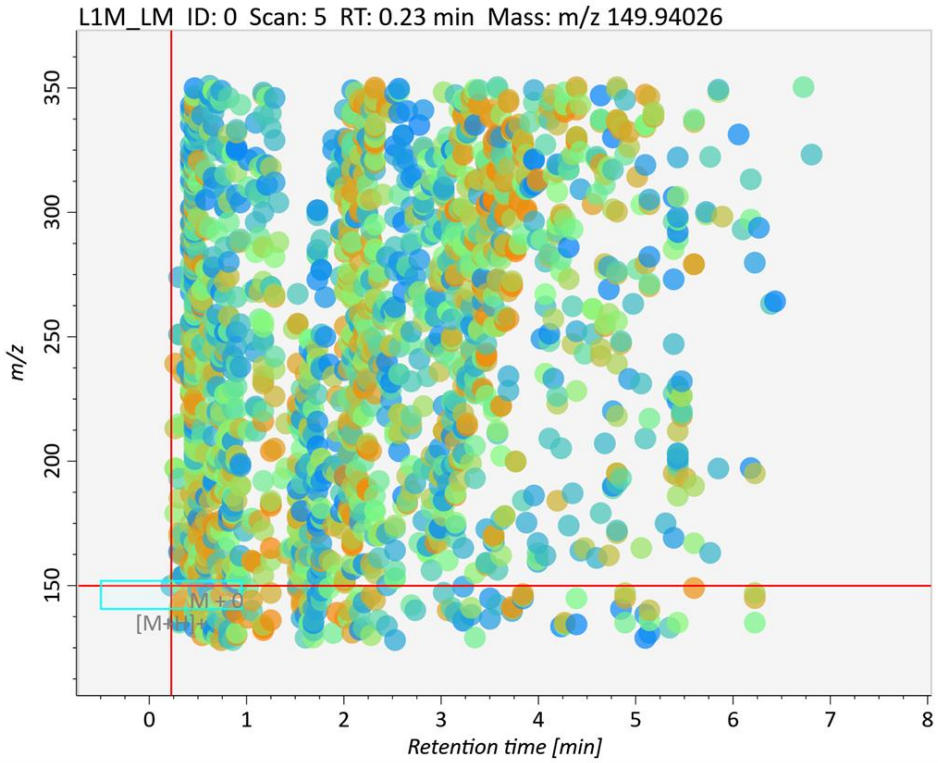
The heated electrospray ionization (HESI) source was set at the following conditions: capillary voltage, 3.5kV; Sheath Gas, 35; Aux Gas, 10; Sweep gas, 1; Ion transfer tube temperature, 325 °C; vaporizer temperature, 350 °C. A 0.7 Da and 1.2 Da resolution was used for the first and third quadrupole, respectively. The collision gas was maintained at a pressure of 1.5 mTorr. The multiple reaction monitoring (MRM) settings for 405 target environmental contaminants are listed in **Appendix 2**. The bound residues were quantified using Thermo TraceFinder software version 5.0 (Thermo Scientific, Waltham, MA, USA). Final concentrations in the extracts were back calculated using volumes of solvent and moisture percentage to enable comparison of dry weight concentration of each contaminant.

3.4 Results and Discussion

3.4.1 Nontargeted Analysis of Biosolids

The nontargeted method resulted in a wide array of spectra. Each peak recorded in the MS spectrum was fragmented to result in a paired MS2 spectrum. MSDial software then analyzed every MS2 spectrum against the EAWAG database and returned a list of hits and the level of confidence in its identification **(Figure 20).**

A)



B)

RT [min]	m/z	Type	Metabolite name	Chromatogram
2.70	278.2112	[M+H]+	Venlafaxine	
2.94	294.2058	[M+H]+	Pramoxine; LC-ESI-QFT; MS2; CE	
2.09	152.0712	[M+H]+	Paracetamol; LC-ESI-ITFT; MS2; CE	
2.09	152.0711	[M+H]+	Paracetamol; LC-ESI-ITFT; MS2; CE	
2.86	256.1689	[M+H]+	Diphenhydramine; LC-ESI-QFT; MS2; CE	
2.82	272.2007	[M+H]+	Dextromethorphan; LC-ESI-ITFT; MS2; CE	
2.86	325.1718	[M+H]+	Citalopram; LC-ESI-ITFT; MS2; CE	
2.86	325.1717	[M+H]+	Citalopram; LC-ESI-ITFT; MS2; CE	
3.18	237.1026	[M+H]+	Carbamazepine	
2.78	134.0603	[M+H]+	5-Methyl-1H-benzotriazole; LC-ESI-ITFT; MS2;	

Figure 20: Example of a) a low mass scan using DIA and b) resulting identification of targets against the EAWAG pharmaceutical and pesticide database in MSDial software.

Despite the expectation of a large number of compounds to be present in the biosolid sample, Orbitrap mass spectrometry with a nontargeted method proved difficult. Of the several thousand chromatograms generated with distinct peaks, only 10 compounds were identified by the software with low confidence. This indicates that matrix effects experienced by the coeluting compounds are severe enough that the analytes are not eluting where they are expected to or are supressed by other compounds. To conclusively determine what compounds are present, a more selective targeted method should be used with the QqQ.

3.4.2 Targeted Analysis of ESOCs in Biosolids

Quantification of the 405 target compounds in both cake and the treated fertilizer revealed what occurred as a result of THP chemical treatment. **(Figures 21-23).** Data are expressed as a comparison of concentration in the initial cake against the concentration in the treated fertilizer on a logarithmic scale. This allows for a linear expression of each data point and its concentration in both matrices. Compounds that appear along the y-axis are present in only the treated fertilizer, meaning they were not extracted by the method in the cake samples. This suggests some level of shielding or inhibition of extraction by the complex organic matter in the cake. As the THP chemical treatment does not introduce any new compounds and simply alkalizes and heats the cake, the compounds must be present but are not available to extract. Compounds that are along the x-axis are present only in the initial cake, and not in the treated fertilizer. This may in fact be indicative of degradation of the compound, and the compounds along the x-axis may be removed by THP treatment. Samples were analyzed in triplicate, and compounds were omitted from the charts if they did not appear in at least two different samples between the 12 points at each WWTP. This is done to ensure that the identification of a compound is not a false positive, such that trends can accurately be assessed.

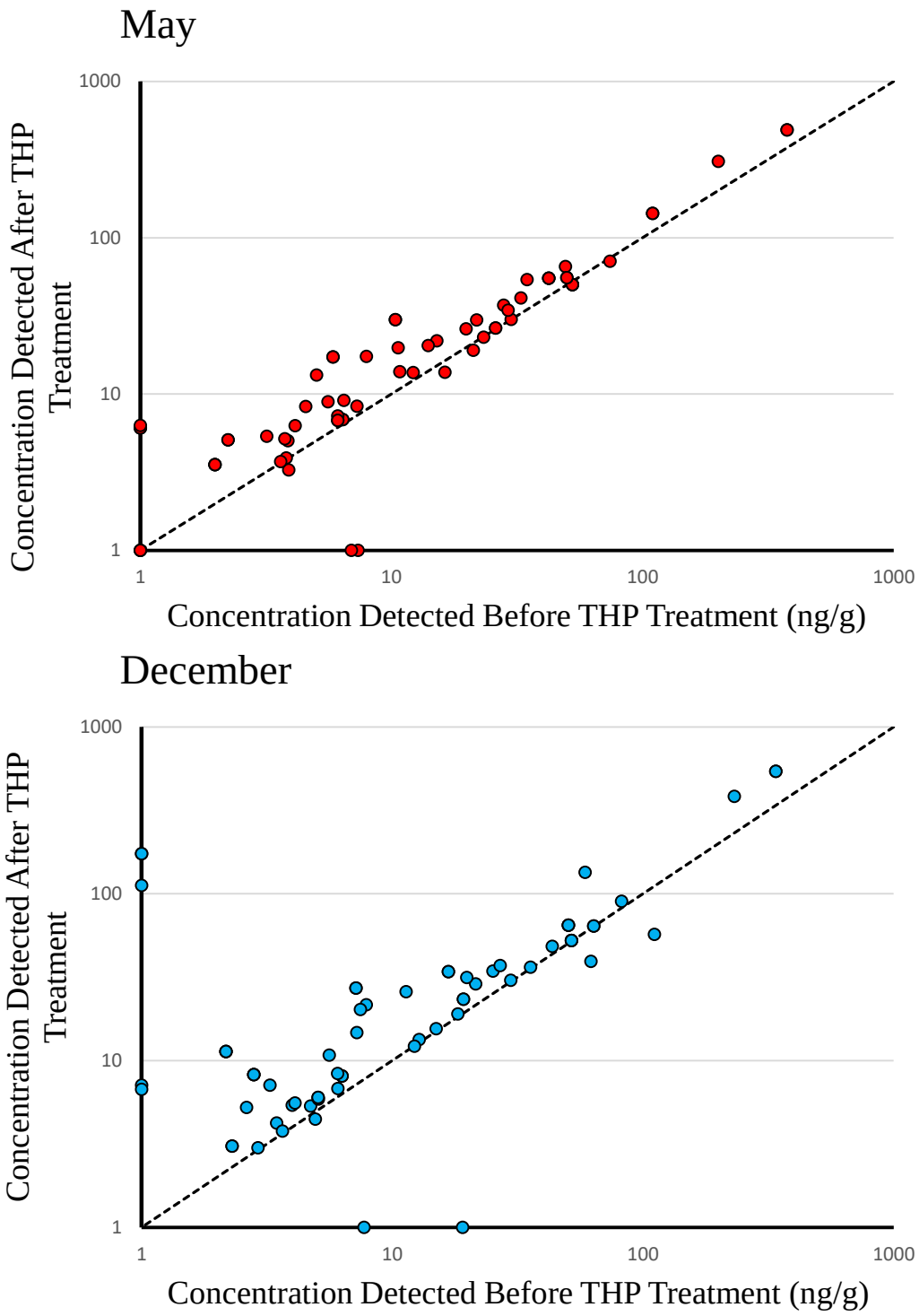


Figure 21: Concentration of Target Compounds in Biosolid Cake from WWTP-A before and after THP treatment

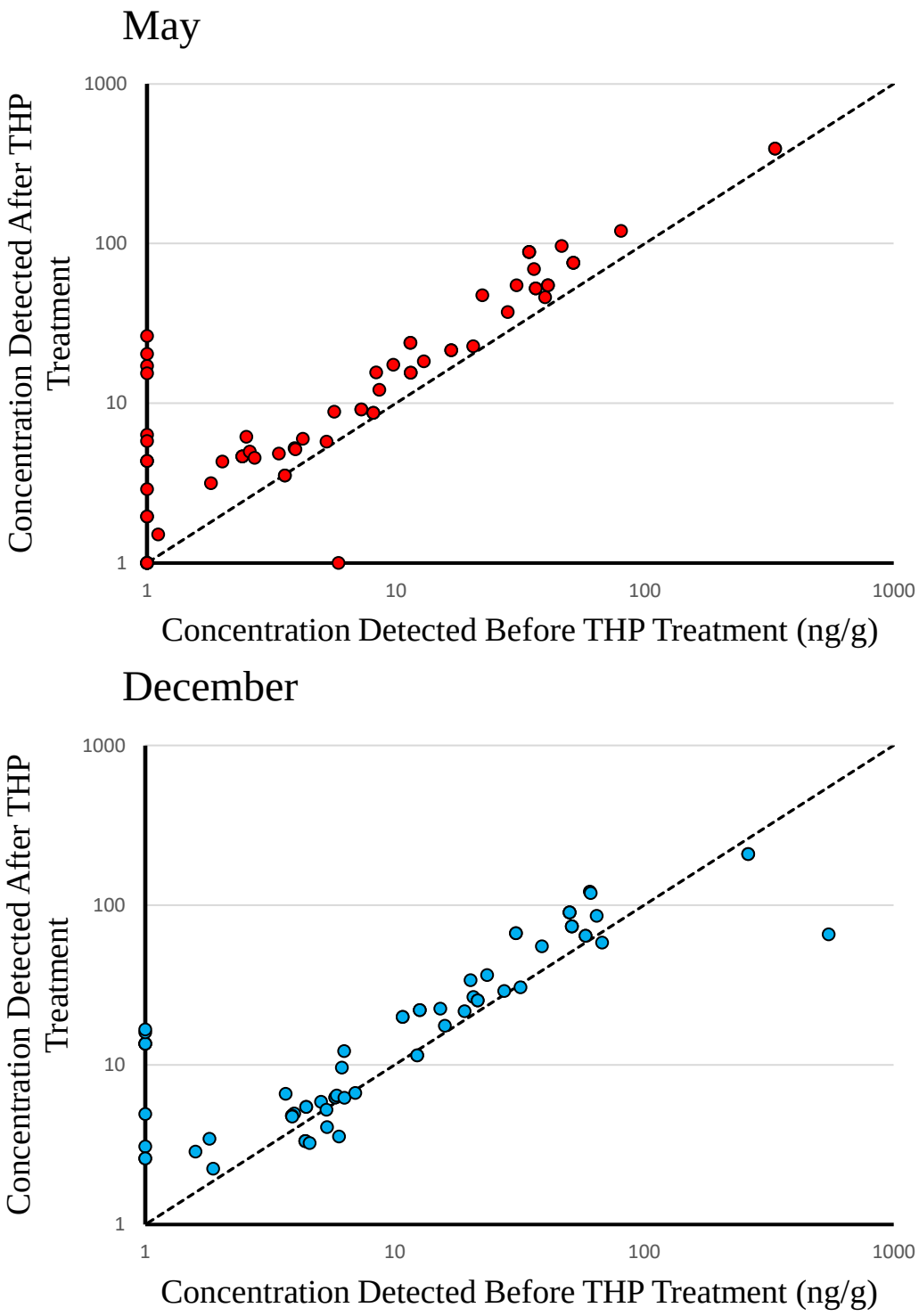


Figure 22: Concentration of Target Compounds in Biosolid Cake from WWTP-B before and after THP treatment

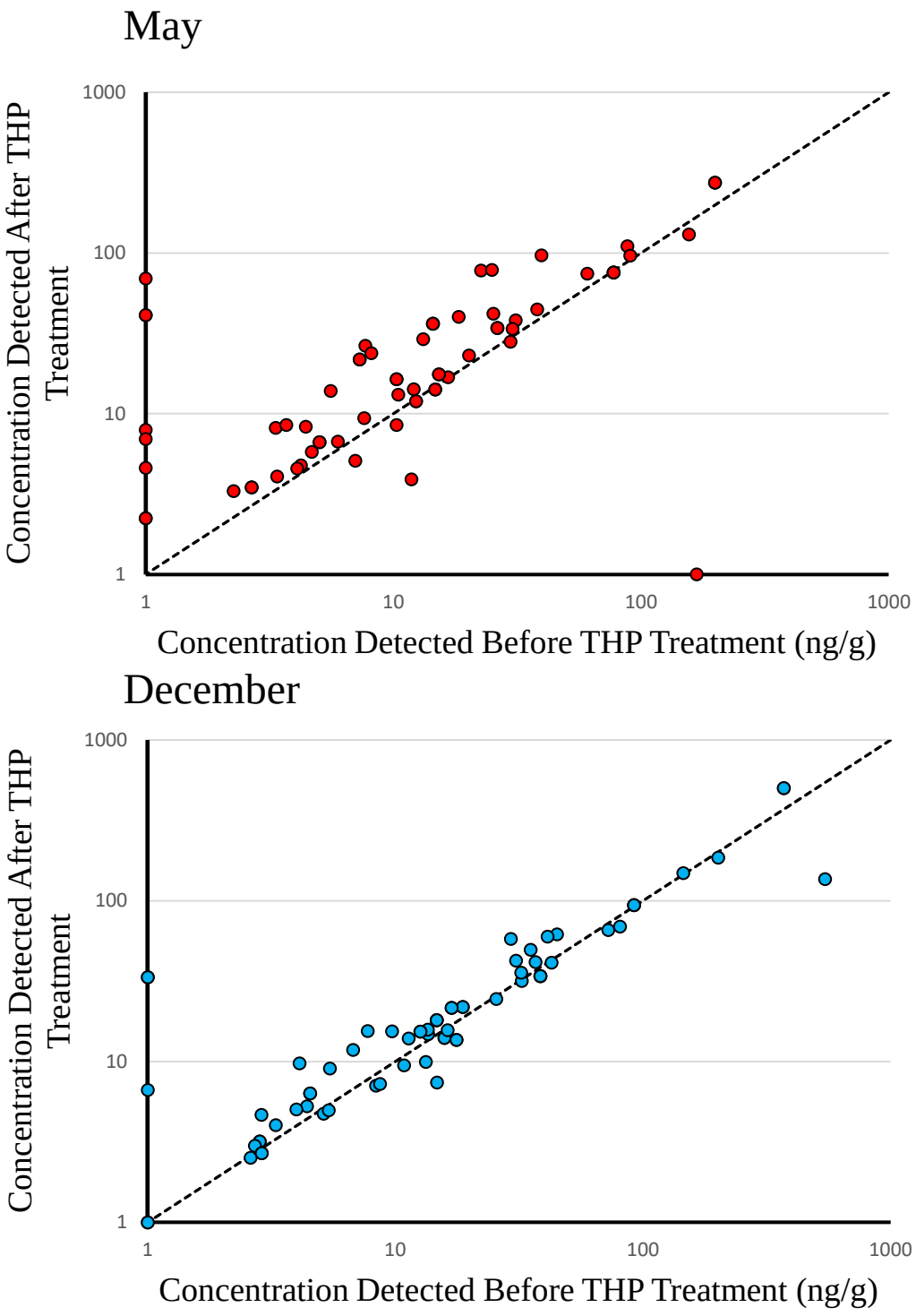


Figure 23: Concentration of Target Compounds in Biosolid Cake from WWTP-C before and after THP treatment

Compounds above the trendline in each graph are seen to increase in concentration after THP treatment. In general, many compounds did appear to increase, but most detected compounds remained along the trendline and therefore did not change due to the treatment. The significance of the concentration changes due to THP treatment was examined using a Kruskal-Wallis test for non-parametric data, with false discovery rate adjustment (FDR) and did not identify any of the changes as significant. This means that the slight changes in concentration from cake to fertilizer can be attributed to random variance between the samples. Therefore, THP treatment did not have any discernible significant effect on ESOC concentration in biosolid samples. This does not account for the presence of many compounds in the treated samples that were not present in the original cake. We hypothesize that this is due to the complex organic matter in the cake shielding ESOCs from extraction. Then, once exposed to the base, heat, and shearing that are characteristic of THP, the matrix has been broken open to expose the target compounds. This makes them extractible in fertilizer where they were not in cake. There may be other factors that cause this increase in concentration, but it is not possible that THP itself is introducing these chemicals as nothing beyond 6M KOH is added to the untreated solids. Despite the limited effects of THP treatment, the QqQ method did prove to be effective at detecting many different compounds of various classes **(Appendix 2).**

The general method enabled for extraction of ESOCs was able to extract 71 different compounds in quantifiable levels. Some of these compounds detected are pesticides, like the herbicide metolachlor, the fungicide imazalil, or the insecticide piperonyl butoxide, among others. However, the most common compounds as detected in biosolid samples were pharmaceuticals and other personal care products. This is expected as the primary input to WWTPs is human waste from sewage systems. These pesticides may be derived from agricultural runoff that find their way into surface water and then into water treatment services, but the vast majority of these compounds should be from human waste.

Despite the wide range of compounds detected in the samples, they were all in generally low concentrations. Most of the compounds detected were in concentrations of 10 ng/g or lower, while very few approached 100 ng/g. One compound, however, that was

consistently present in all samples and was not affected by THP treatment was telmisartan. Telmisartan was the highest concentration compound in all the time points and samples tested, measuring over 100 ng/g in every sample. Telmisartan is an oral drug used in treatment of hypertension which is left unchanged by the body. Roughly 98% of telmisartan is excreted in its original form in feces, and is also stable from degradation in basic conditions.[125,126] Compounds with these characteristics are ones most expected to be detected in biosolid samples.

3.5 Conclusions

Despite the expected effects of THP treatment, little change was observed from untreated cake to class A biosolid fertilizer. None of the changes in concentration as a result of the treatment were seen to be significant when tested in a Kruskal-Wallis significance test. Though the changes were not significant, many of the concentrations were seen to increase slightly after the treatment which may suggest that degradation of complex polymers in the untreated cake in some way releases the bound contaminants. Of the compounds detected in cake, many were insufficiently degraded in WWTPs and were stable from degradation in basic conditions.

Chapter 4

Solid Phase Extraction of ESOCs in Surface Water

4.1 Chapter 4 Objectives

Contamination with ESOCs in Canadian waterways is an issue that must be constantly monitored. These compounds vary greatly in their chemical structures and characteristics, so a method that is able to extract and identify as many as possible would be valuable. This study evaluates the use of solid phase extraction in isolating ESOCs from surface water. Triple quadrupole mass spectrometry in conjunction with a large list of target spectra created by our group enables these methods to detect many compounds with one method of extraction.

4.2 Introduction

Due to the prevalence of many ESOCs in surface water around the world, it is important to develop a method to isolate and identify as many compounds as possible. This would simplify analysis for many compounds in surface water and increase our ability to process a large number of samples rather than subjecting a single sample to multiple different methods. Our group has created a target list for many common environmental contaminants by injecting standards of the target at a known concentration and storing spectral data, increasing our ability to detect each compound in an unknown mixture. This target list can be employed to test for every compound on that list with a selective targeted method and accurately identify if it is in the environmental sample. Compounds can then be quantified to determine the severity of their contamination, and if they are approaching toxic levels.

This study uses the extensive sampling from watersheds around Ontario and Quebec as outlined in Chapter 2 to examine the extent of environmental contamination and how it arises. This work seeks to further expand our understanding of contamination by observing contamination of target compounds at various sites of different activity. By categorizing the target analytes and at which sites they are most common, we can discern which compounds are most relevant in environmental toxicology. The utility of this dataset is in its ease of analysis and reliability of results from the targeted method, so it can be applied to a multitude of investigations with collaborators.

4.3 Materials and Methods

Water samples were taken in monthly intervals from May to November from 2018 to 2020 using the same sampling method and geolocations as outlined in Section 2.3.1. One-litre polyethylene terephthalate (PET) bottles (SystemsPlus, Baden, ON, CA) were filled at the sampling site and shipped to AAFC at LRDC. Samples were stored at -20°C until ready for analysis.

All glassware used in extraction was cleaned sequentially three times each with hot tap water, MeOH, then acetone. The glassware was then dried in a 100 °C oven for at least one hour, and up to 24 h until visible liquid had evaporated. This process removes all trace contaminants that would be amplified by SPE and therefore detectable in the resulting extracts. Water samples in PET bottles were placed into a sink filled with warm water to thaw before being filtered under vacuum through a 1.6 µm inert glass microfiber filter (Whatman, GF/C) to remove large particles like algae and silt. From each 1-L bottle, two 200-mL aliquots were taken and decanted into separate clean and dry 500-mL beakers. The aliquots were adjusted to pH 6.50 ± 0.02 and pH 2.00 ± 0.02 using hydrochloric acid and sodium hydroxide.

Waters Oasis® HLB 200-mg solid phase extraction cartridges (Waters, Milford, MA) were then used to extract the ESOCs from the surface water samples. Each 200-mL aliquot taken from the original 1-L sample was extracted through one HLB cartridge, so two solid phase extractions were performed for each original water sample. To begin SPE, the cartridges were conditioned twice with 5 mL methanol to penetrate the bonded alkyl groups on the cartridge. They were then equilibrated with water of pH 6.5 or pH 2, to match the pH of the 200-mL aliquot being extracted. The 200-mL water samples were then drawn at a rate of 1-2 drops/second through Teflon tubing under vacuum onto the cartridges 12 aliquots at a time **(Figure 24)**. The constant replenishment of water over the course of the extraction caused the cartridges to remain wet throughout. This is important as the pores in the sorbent must be wet to retain analytes. Once the entire water sample had been drawn onto the cartridge, the tubing was removed, and the cartridges were allowed to air dry for 5 minutes with the vacuum turned off to ensure no solvent remained

in the cartridge. This leaves just the target analytes bound loosely to the monomer in the SPE cartridge.

Figure 24: Typical setup for a set of SPE extractions. Two of these setups were utilized in one round of extractions for a total of 12 extractions simultaneously.

Following extraction from the water samples onto the HLB cartridges, the bound analytes were eluted into 15-mL conical Falcon PPE tubes **(Figure 25)**. This was done with 1 mL of methanol three times, then 1 mL of acetonitrile three times for a total of 6 mL. These polar organic solvents were used one after the other to ensure that all bound analytes are eluted from the cartridges. The extracts were then dried at 30 °C under a gentle stream of

air in a gentle flow evaporator. The tubes were removed, and the remaining solids were reconstituted in a solvent of 80:20 methanol:water. Prior to analysis, 100 µL of each fraction was combined into a single sample to detect all analytes with a single LC-MS/MS experiment. As different compounds are eluted in the pH 6.5 and pH 2 fractions, they are combined to allow for a full analysis of the compounds present in the original water sample.

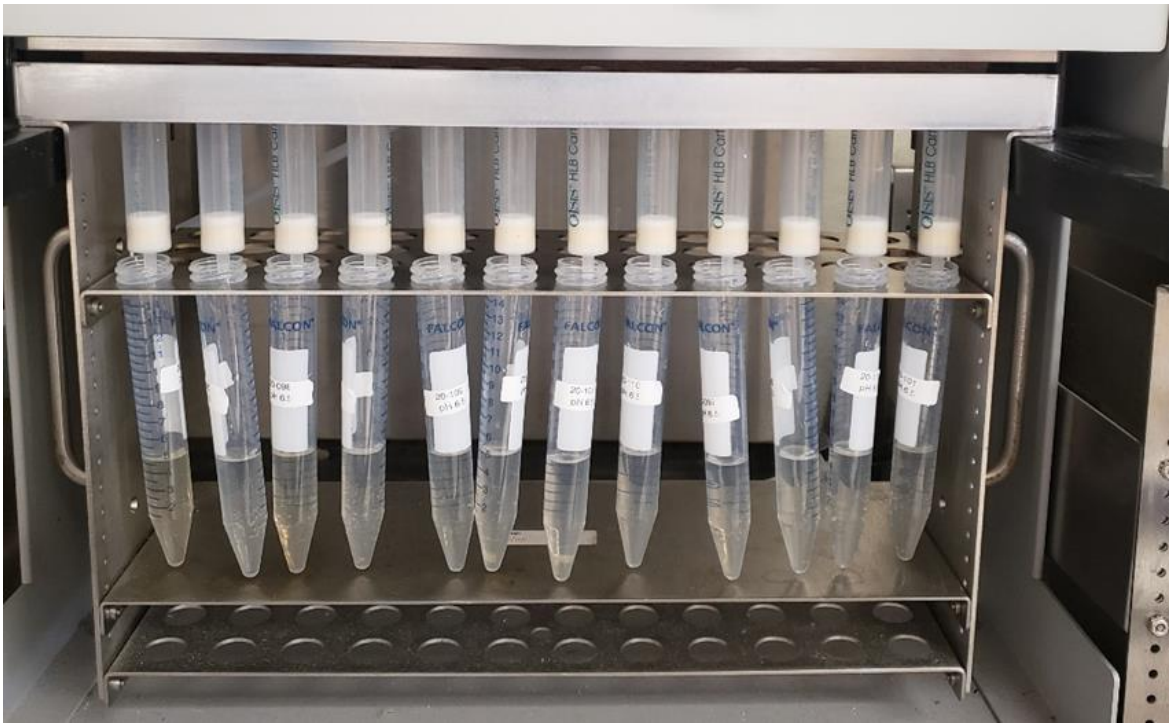


Figure 25: Setup for elution of one set of 12 HLB cartridges into 15-mL conical tubes.

4.4 Results and Discussion

The targeted analysis of surface water was very effective at detecting a wide array of ESOCs in surface water. A total of 833 samples were examined through four years at 40 different sites, in six different watersheds. Of the common environmental contaminants on the target list, 257 different compounds were detected at least once in surface water samples taken from 2017-2020 in Ontario and Quebec waterways **(Table A5)**. Most

compounds were detected at several different time points at different sampling sites. Influences and activities at each site are consistent with Chapter 2. The compounds that were found most often were the insect repellant N,N-diethyl-meta-toluamide (DEET), and caffeine. DEET was found in every single water sample, and caffeine was found in all but three of the 833 total samples. These two compounds are the most indicative of human presence due to their extensive use and occurrence in surface water. Beyond these two compounds, 110 of the 257 different identified compounds were PPCPs, and 111 were pesticides. The compounds from outside of these classes include chemicals such as the industrial surfactant perfluorooctanoic acid, and the artificial sweetener acesulfame. Of the 257 target compounds, 100 of them appeared in more than 10% of samples. These compounds are those that would be most relevant in ecotoxicology as their occurrences through the years of sampling can be more easily analyzed for trends.

Many compounds were found in fewer than 100 of the samples, among these was the selective serotonin reuptake inhibitor (SSRI) antidepressant sertraline. Despite high usage of the drug, even a study on hospital effluent in the Netherlands only found a maximum concentration of 19.9 ng/L.[127] This indicates that degradation of sertraline is likely occurring in WWTPs, then it is further diluted through the watershed to remain present in low concentrations. This is supported in this data as sertraline was only detected more than once in 8 of the 40 total sites, all of which were WWTP influenced. In fact, every single sample take from the WWTP effluent site UOK6 and the WWTP influenced site UT1 contained sertraline while only 2 of its 73 total occurrences were in agriculturally influenced sites. Even the agriculturally influenced sites in the same watershed as these sites did not record sertraline, further indicating its low persistence and likely degradation.

The corrosion inhibitor 4-methyl-1H-benzotriazole was one compound found frequently through the surface water data, appearing in 819 off the 833 total samples. The compound is also used as aircraft de-icing fluid, and has been seen to leech into surface water downstream from airports.[128] This extensive occurrence of the compound indicates that it may be persistent in the environment, as it was seen in every sampling site multiple times through each year, including the agricultural sites. Other compounds seen in over 90% of

samples were the herbicides atrazine and metolachlor, as well as the common NSAID acetylsalicylic acid, appearing in 814, 806, and 787 samples, respectively. Each of these drugs sees extensive use in Canada, and the herbicides have been investigated previously for their common occurrences.[129,130] Acetylsalicylic acid is a common over the counter drug used in Canada to treat inflammation, and has been previously detected in water systems in Canada,[131] as well as India,[132] Korea,[133] and many others. The common occurrence of these compounds in this data further reinforces the validity and exhibits the efficacy of the method in detecting a wide range of compounds. The data set resulting from this work is extremely large but can be separated by average concentration of the target ESOC in each site **[Table 9]**. This table is one small portion of the dataset, showing only the 32 most abundant compounds as they appear in one of the six watersheds. Total occurrences through all samples are noted to demonstrate the prevalence of certain compounds, and they are classified by category to show the widespread contamination from a variety of classes.

Table 9: The Most Abundant ESOCs in Sampled Sites and their Average Concentrations in the Ausable Bayfield Watershed

Compound Name	Category	Site Total # of Occurrences	A1 Average Concentration (ng/L)	A2 Average Concentration (ng/L)	A3 Average Concentration (ng/L)	A4 Average Concentration (ng/L)
Deet	Insect repellant; human	833	230.40 ± 670	90.50 ± 189	113.15 ± 262	240.50 ± 466
Caffeine	Human	830	6.50 ± 9	22.71 ± 24	32.83 ± 81	26.93 ± 57
4-Methyl-1H-benzotriazole	industrial; corrosion inhibitor	819	33.65 ± 100	20.07 ± 12	52.72 ± 166	126.93 ± 370
Metolachlor	herbicide	814	71.97 ± 138	525.35 ± 1174	314.36 ± 554	627.41 ± 785
Atrazine	Herbicide; triazine	806	49.55 ± 138	70.16 ± 108	122.22 ± 293	145.46 ± 181
acetylsalicylic acid	Pharmaceutical; human	787	216.42 ± 432	163.02 ± 325	154.87 ± 187	121.00 ± 134
Metalaxyl	fungicide	719	10.91 ± 23	215.80 ± 801	11.13 ± 16	64.10 ± 94
Paraxanthine	Human; metabolite; caffeine	715	4.22 ± 4	5.57 ± 4	13.45 ± 32	6.24 ± 7
Chlorantraniliprole	insecticide	694	16.02 ± 20	23.31 ± 54	18.86 ± 32	50.93 ± 41
24D	Herbicide	648	15.04 ± 24	176.95 ± 304	37.93 ± 86	42.86 ± 53
Sebacic acid	Industrial; Natural	643	114.40 ± 74	78.10 ± 24	99.19 ± 52	84.39 ± 37
Imazethapyr	herbicide	636	10.79 ± 21	59.18 ± 99	27.65 ± 76	186.77 ± 320
Fenuron	Herbicide	601	2.11 ± 2	3.24 ± 3	3.55 ± 8	1.82 ± 2
Acesulfame	Artificial Sweetener	596	24.06 ± 13	75.60 ± 128	31.28 ± 63	38.58 ± 48
Tebuconazole	fungicide	594	5.45 ± 4	9.04 ± 9	6.78 ± 8	20.95 ± 52
Mirtazapine	pharmaceutical; antidepressant	559	16774.04 ± 25376	7903.58 ± 8276	9727.59 ± 8210	4835.17 ± 4336
Pyraclostrobin	fungicide	551	11.53 ± 1	38.03 ± 87	11.33 ± 2	31.74 ± 62
Ethiofencarb	insecticide; carbamate	530	21.59 ± 16	21.64 ± 16	113.36 ± 350	23.67 ± 27
Bentazon	Herbicide	528	361.46 ± 1298	122.11 ± 300	141.38 ± 534	44.20 ± 78
Clothianidin	insecticide; neonicotinoid	508	22.06 ± 40	39.70 ± 29	14.75 ± 18	16.48 ± 14
Trifloxystrobin	fungicide	499	3.29 ± 1	3.95 ± 2	4.36 ± 3	12.37 ± 34
Propiconazole	fungicide	458	4.34 ± 4	9.42 ± 14	7.44 ± 12	4.95 ± 9
Difenoconazole	fungicide	435	2.64 ± 1	649.31 ± 2482	3.56 ± 3	5.29 ± 4
Azoxystrobin	fungicide	424	2.18 ± 3	8.26 ± 12	6.52 ± 18	7.59 ± 21
Methamidophos	Insecticide	419	16.62 ± 20	17.00 ± 31	34.03 ± 36	8.94 ± 8
Carbamazepine	pharmaceutical; anticonvulsant	398	1.95 ± 3	6.19 ± 7	3.65 ± 3	2.67 ± 6
Gabapentin	pharmaceutical	359	23.21 ± 6	199.26 ± 400	38.06 ± 36	35.43 ± 58
Venlafaxine	pharmaceutical; human; antidepressant	317	2.03 ± 4	2.25 ± 2	5.37 ± 4	1.60 ± 2
Carbendazim	Fungicide	314	0.21 ± 0.3	0.52 ± 0.3	0.33 ± 0.3	0.14 ± 0.2
Thiamethoxam	insecticide; neonicotinoid	301	13.09 ± 11	311.64 ± 821	21.76 ± 38	25.49 ± 33
Cetirizine	pharmaceutical; human; antihistamine	298	2.91 ± 4	1.95 ± 3	2.57 ± 2	1.31 ± 1
Tebuthiuron	Herbicide	294	1.17 ± 1	1.83 ± 1	0.61 ± 0.4	0.69 ± 1

The trends observed in the dataset are to be further analyzed while examining specific classes and types of compounds. One collaboration with ECCC is ongoing to analyze pesticide concentrations throughout these waterways, as well as temporal trends associated with those compounds. Due to the large amount of data points over three years, distinct trends can be tracked for each compound or type of compound detected by the method. One further ongoing work with this dataset is on long-term temporal trends of pharmaceuticals and related compounds as they change through the course of the 2020 COVID-19 pandemic. This work will focus primarily on pharmaceuticals, as use of medication through the pandemic may change in response to health initiatives and treatment strategies.

4.5 Conclusions

These data are valuable in environmental analyses as any of the detected compounds can be further investigated if they are identified as targets of interest. For example, with the discovery of guanylurea, metformin became increasingly of interest in environmental toxicology studies in 2014, though it had seen frequent usage since the 1950s. As bacterial degradation of ESOCs can create any number of metabolites or degradation products with varying toxicological effects, any of the ESOCs detected by this method could become a future target. These data are also a useful tool in collaborations as any study into compounds that were detected in these watersheds can be further supported by this dataset. QqQ proved to effectively detect over 250 compounds from surface water samples, and expansion of the target list would allow for even further identification.

Chapter 5

General Discussion and Conclusions

5.1 Metformin and Guanylurea in Ontario and Quebec Waterways

The pharmaceutical metformin was found to be quite common throughout the surface water at the watersheds examined, while its degradation product guanylurea was even more so. In sediment, metformin was found in much higher amounts, and in far more samples than guanylurea. This allowed for another view into contamination of metformin and where it may accumulate. As sorption to soil is weak for both compounds but slightly higher for metformin, these results were expected. The variety in activity at each sampling site emphasized the primary route in which metformin and guanylurea enter the environment. As agriculturally influenced sites measured the lowest concentrations of both compounds in all watersheds tested, it can be concluded that agriculture is not a point source for either compound in the environment. WWTPs, lagoons, and urban influences were all much more common sources of the compounds and sites with those activities nearby should be closely monitored to limit accumulation and linked toxic effects.

5.2 ESOC Contamination of Biosolid Fertilizers

The method utilized in these extractions and quantifications of ESOCs in biosolids may remain an invaluable tool in quantifying environmental contaminants that may be applied in agriculture. Though the samples tested in this work were low in hazardous compounds, it may remain an ongoing issue as populations increase and environmental PPCP concentrations increase correspondingly. The work done in this section demonstrated the issues with nontargeted analysis of complex matrices such as biosolids, and the need for selective instrumentation such as with a QqQ. The ability for the QqQ to overcome the matrix effects seen in the DIA method reinforces the utility of the instrument.

5.3 Mass Spectrometry methods for Environmental Analyses

Mass spectrometry and its versatility is displayed throughout Chapter 4. A wide variety of methods exist to extract and analyze ESOCs and the utility of several of them were exhibited. Each of these methods will be used in the future as the sampling program mentioned throughout this work has continued through 2021 and 2022 and should continue in future years. Alternatives to SPE are being examined to eliminate the slow turnover between runs and should allow for rapid evaluation of environmental samples. The dataset resulting from targeted analysis of SPE samples if being evaluated for several purposes and may yet be used in more ongoing investigations of compounds detected in the surface water.

5.4 General Conclusions

In general, this work utilizes a variety of methods to identify and quantify emerging substances of concern from several different matrices and demonstrates their utility. All data gathered during this research can be used to further our understanding of environmental contamination, and where it occurs. Mass spectrometry remains the gold standard in environmental analyses due to its plethora of uses and selectivity of targets.

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Appendices

Appendix 1: Metformin and Guanylurea in Ontario and Quebec Waterways

Table A1: Average Metformin and Guanylurea Concentrations in water samples taken from 2018-2020 with standard deviation

Watershed	Site	Metformin Concentration (µg/L)	Std. Dev.	Guanylurea Concentration (µg/L)	Std. Dev.
1) Ausable Bayfield	**1**	0.016	0.007	0.176	0.08
	2	0.019	0.03	0.234	0.1
	3	0.023	0.02	0.313	0.5
	4	0.040	0.03	0.226	0.07
2) Cedar	**1**	0.350	0.4	4.201	7
	2	0.190	0.1	0.265	0.1
	3	0.413	0.3	0.308	0.1
	4	0.100	0.1	0.315	0.2
	5	0.131	0.07	0.393	0.1
3) Upper Grand	**1**	0.189	0.1	2.825	2
	2	0.035	0.06	0.212	0.1
	3	0.027	0.02	0.219	0.1
	4	0.429	0.4	2.012	1
	5	0.010	0.008	0.246	0.1
	6	0.028	0.04	0.193	0.07
4) Upper Ottawa-Kipawa*	**1**	0.144		0.127	0.07
	2	LOQ		0.243	0.1
	3	0.106	0.1	0.243	0.06
	4	0.058	0.04	0.067	0.005
	5	0.008	0.004	0.069	0.01
	6	3.630	3	14.568	4
	7	0.051	0.02	0.083	0.03

5) Lower Ottawa-South Nation	**1**	0.027	0.03	0.195	0.1
	2	0.035	0.04	0.208	0.09
	3	0.110	0.2	0.219	0.1
	4	0.046	0.03	0.180	0.1
	5	0.020	0.03	0.188	0.08
	6	LOQ		0.079	0.01
	7	0.005	0.000	0.141	0.09
	8	0.110	0.000	0.111	0.05
	9	0.008	0.003	0.301	0.2
	10	2.409	4	0.932	0.8
	11	0.014	0.007	0.430	0.01
	12	1.602	2	1.941	1
	13	0.141	0.2	0.406	0.2
6) Upper Thames	**1**	0.166	0.1	9.015	7
	2	0.271	0.2	3.039	2
	3	0.298	0.3	4.350	3
	4	0.186	0.2	0.441	0.3
	5	0.056	0.06	0.209	0.1

Table A2: Average concentrations of Metformin and Guanylurea in 2020 Sediment Samples

	Site	Average Metformin Concentration (ng/g)	Std. Dev.	Average Guanylurea Concentration (ng/g)	Std. Dev.
1) Ausable Bayfield	**A1**	3.1		<LOQ	
	A2	46.1	75.3	2.64	0.376

	A3	8.68	10.4	<LOQ	
	A4	37.3	26.2	1.13	0.382
2) Cedar	**C2**	21.2	34.0	<LOQ	
	C3	25.4	27.1	2.93672	
	C4	28.2	16.9	<LOQ	
3) Upper Grand	**UG1**	34.9	32.1	1.99	
	UG2	44.3	57.2	0.544	
	UG3	16.3	14.2	<LOQ	
	UG4	25.8	34.4	1.05	
	UG5	47.1	64.5	0.973	
	UG6	20.2	11.5	0.672	0.173
4) Upper Ottawa-Kipawa	**UOK1**	1.96		1.155	
	UOK2	7.43		<LOQ	
	UOK3	238	104	0.703	
	UOK4	5.28	2.90	0.870	
	UOK5	4.26		0.704	
	UOK7	21.2	1.31	1.25	
5) Lower Ottawa-South Nation	**LOSN3**	17.5	11.7	<LOQ	
	LOSN9	4.04	0.267	<LOQ	
	LOSN10	222	270	<LOQ	
	LOSN12	4047	6111	<LOQ	
	LOSN13	351		<LOQ	
6) Upper Thames	**UT1**	175	61.4	1.44	
	UT2	276	216	23.0	22.7
	UT3	251	300	1.71	1.75
	UT4	30.329	30	141	128
	UT5	17.1	16	19.3	19.0

Appendix 2: Target ESOCs for Biosolid Analyses

Table A3: All ESOCs detected in biosolid samples

10-hydroxyamitriptyline	Fluxapyroxad
6PPD	Imazalil
Abiraterone Acetate	Irbesartan
Alectinib	Ketoconazole
Ametryn	Labetalol
Amitryptaline	Lamotrigine
Amlodipine	Lidocaine
Aripiprazole	Losartan
Atorvastatin	Metolachlor
Azithromycin	Metoprolol
Carbamazepine	Miconazole
Carvedilol	Mirtazapine
Celecoxib	Montelukast
Cetirizine	Moxifloxacin
Chlorhexidine	Norfluoxetine
Citalopram	O-desmethylvenlafaxine
Clarithromycin	Ofloxacin
Clethodim	Paroxetine
Clindamycin	Piperonyl butoxide
Clotrimazole	Propranolol
Clozapine	Quetiapine
Cyclobenzaprine	Sertraline
Deet	Sitagliptin
DesHEE-quetiapine	Spirodiclofen
Desmethylcitalopram	Sulisobenzone
Dextromethorphan	Tamsulosin
Diclofenac	Telmisartan
Didemethyl-citalopram	Testosterone enanthate

Diltiazem	Tolnaftate
Diphenhydramine	Tolvaptan
Duloxetine	Trazodone
Fenhexamid	Triclocarban
Fexofenadine	Venlafaxine
Fludioxonil	Vilazodone
Fluoxetine	Ziprasidone
Flutriafol	

Table A4: MRM settings for biosolid target analytes

Compound	CAS	Category	RT (min)	Ion Type	Precursor m/z	Quantifier m/z (CE)	Qualifier m/z (CE)	instrumental LOD (ng/L)
10-hydroxyamitriptyline	1159-82-6	pharmaceutical	6.15	[M+H]+	294.2	216 (28)	231 (21)	50
24D	94-75-7	Herbicide	11.39	[M-H]-	219	161 (17)	125 (28)	50
3-hydroxycarbofuran	16655-82-6	pesticide	4.94	[M+H]+	238.1	181 (10)	163 (15)	5
4-Methyl-1H-benzotriazole	29878-31-7	industrial	5.08	[M+H]+	134.1	77 (27)	79 (20)	10
abacavir	136470-78-5	pharmaceutical	3.13	[M+H]+	287.2	134 (42)	79 (21)	5
Abiraterone Acetate	154229-18-2	pharmaceutical	15.31	[M+H]+	392.3	332 (34)	316 (48)	5000
acesulfame	55589-62-3	Artificial	1.44	[M-H]-	162	82 (14)	78 (31)	50
acetamiprid	135410-20-7	insecticide	5.78	[M+H]+	223.1	126 (21)	90 (33)	5
acetominophen	103-90-2	Pharmaceutical	2.69	[M+H]+	152.1	110 (16)	93 (25)	100
acetylsalicyclic acid	50-78-2	Pharmaceutical	6.09	[M-C2H2O-H]-	137.1	93 (17)	65 (29)	5000
acibenzolar-s-methyl	135158-54-2	Fungicide	13.03	[M+H]+	211	136 (31)	140 (25)	500
advantame	245650-17-3	Artificial	9.03	[M+H]+	459.2	252 (20)	102 (28)	50
albuterol	18559-94-9	pharmaceutical	5.99	[M+H]+	240.2	166 (14)	-	50
aldicarb	116-06-3	insecticide	7.28	[M+NH4]+	208.1	116 (10)	89 (16)	100
ametryn	834-12-8	Herbicide	8.27	[M+H]+	228.1	186 (18)	96 (25)	5
aminocarb	2032-59-9	insecticide	2.63	[M+H]+	209.1	152 (14)	137 (24)	1
amitryptaline	50-48-6	pharmaceutical	10.73	[M+H]+	278.2	105 (25)	117 (24)	500
Amlodipine	88150-42-9	pharmaceutical	10.58	[M+H]+	409.2	238 (10)	294 (11)	100
amoxacillin	26787-78-0	antibiotic	2.59	[M+H]+	366.1	114 (21)	208 (13)	100
anhydroerythromycin A	23893-13-2	antibiotic	10.66	[M+H]+	716.5	558 (17)	158 (30)	1000
aspartame	22839-47-0	Artificial	3.89	[M+H]+	295.1	120 (25)	235 (14)	500
atenolol	29122-68-7	pharmaceutical	2.7	[M+H]+	267.2	145 (27)	190 (20)	5
Atorvastatin	134523-00-5	pharmaceutical	14.34	[M+H]+	559.3	440 (23)	250 (42)	50
atrazine	1912-24-9	Herbicide	10.71	[M+H]+	216.1	174 (17)	132 (23)	1
azithromycin	83905-01-5	antibiotic	5.75	[M+2H]2+	375.3	591 (16)	434 (22)	100
azoxystrobin	131860-33-8	fungicide	13.98	[M+H]+	404.1	372 (15)	329 (31)	5
benalaxyl	71626-11-4	fungicide	15.59	[M+H]+	326.2	148 (22)	266 (14)	10
bendiocarb	22781-23-3	insecticide	10.11	[M+H]+	224.1	167 (10)	109 (17)	100

bentazon	25057-89-0	Herbicide	10.74	[M-H]-	239.1	132 (26)	197 (20)	100
benzoximate	29104-30-1	insecticide	16.99	[M+H]+	364.1	199 (10)	184 (34)	1000
bezafibrate	41859-67-0	pharmaceutical	12.61	[M+H]+	362.1	316 (14)	139 (27)	100
bifenazate	149877-41-8	insecticide	14.53	[M+H]+	301.2	198 (10)	170 (19)	1000
boscalid	188425-85-6	fungicide	14.09	[M+H]+	343	307 (20)	271 (33)	500
Bromoxynil	1689-84-5	herbicide	10.97	[M+H]+	275.9	81 (35)	79 (50)	500
bromuconazole	116255-48-2	fungicide	13.51	[M+H]+	376	70 (21)	159 (28)	500
bupirimate	41483-43-6	fungicide	12.49	[M+H]+	317.2	166 (25)	237 (20)	1
buprofezin	69327-76-0	insecticide	14.54	[M+H]+	306.2	201 (13)	116 (17)	100
Bupropion	34911-55-2	pharmaceutical	5.95	[M+H]+	240.1	184 (13)	131 (29)	5
caffeine	1958-08-02	Human	3.29	[M+H]+	195.1	138 (19)	110 (22)	50
capecitabine	154361-50-9	pharmaceutical	8.79	[M+H]+	360.2	244 (11)	174 (21)	50
Carbadox	6804-07-05	veterinary	3.76	[M+H]+	263.1	231 (14)	129 (31)	50
Carbamazepine	298-46-4	pharmaceutical	9.97	[M+H]+	237.1	194 (21)	179 (34)	10
carbamazepine-10-11-epoxide	36507-30-9	pharmaceutical	7.44	[M+H]+	253.1	180 (30)	210 (15)	50
carbaryl	63-25-2	insecticide	10.81	[M+H]+	202.1	145 (10)	127 (29)	500
carbendazim	10605-21-7	Fungicide	3.02	[M+H]+	192.1	160 (18)	132 (31)	5
carbenicillin	4697-36-3	antibiotic	9.67	[M+H]+	379.1	204 (20)	238 (19)	5000
carbetamide	16118-45-9	insecticide	7.87	[M+H]+	237.1	192 (10)	118 (13)	100
carbofuran	1563-66-2	insecticide	10.05	[M+H]+	222.1	123 (22)	91 (35)	10
carvedilol	72956-09-3	pharmaceutical	10.26	[M+H]+	407.2	224 (22)	222 (24)	5000
Cefotaxime	63527-52-6	pharmaceutical	3.83	[M+H]+	456.1	396 (10)	324 (14)	500
Ceftiofur	80370-57-6	antibiotic	7.82	[M+H]+	524	241 (18)	210 (22)	100
ceftriaxone	73384-59-5	antibiotic	3.48	[M+H]+	555.1	396 (13)	324 (17)	100
Celecoxib	169590-42-5	pharmaceutical	15.01	[M+H]+	382.1	362 (29)	300 (30)	500
cetirizine	83881-51-0	pharmaceutical	10.85	[M+H]+	389.2	201 (19)	165 (55)	50
chloramphenicol	56-75-7	antibiotic	6.84	[M-H]-	321	152 (17)	257 (12)	100
chlorantraniliprole	500008-45-7	insecticide	12.81	[M+H]+	482	284 (13)	451 (17)	100
chlorbromuron	13360-45-7	herbicide	13.13	[M+H]+	293	125 (35)	182 (17)	500
chloroxuron	1982-47-4	herbicide	13.56	[M+H]+	291.1	72 (21)	164 (17)	100
Cilastatin	82009-34-5	pharmaceutical	3.98	[M+H]+	359.2	202 (15)	97 (23)	50
ciprofloxacin	85721-33-1	antibiotic	3.82	[M+H]+	332.1	288 (18)	245 (23)	50
citalopram	59729-33-8	pharmaceutical	9.2	[M+H]+	325.2	109 (29)	262 (20)	5
Clarithromycin	81103-11-9	antibiotic	11.15	[M+H]+	748.5	158 (29)	590 (18)	500
Clindamycin	18323-44-9	antibiotic	6.5	[M+H]+	425.2	126 (30)	377 (19)	10
Clindamycin phosphate	24729-96-2	pharmaceutical	6.2	[M+H]+	505.2	126 (32)	457 (21)	50
clofentezine	74115-24-5	insecticide	16.42	[M+H]+	303	138 (15)	102 (34)	5000
clopidogrel	113665-84-2	pharmaceutical	12.99	[M+H]+	322.1	212 (16)	184 (22)	1
clopidol	2971-90-6	antibiotic	3.11	[M+H]+	192	101 (27)	87 (32)	10
clopyralid	1702-17-6	Herbicide	3.12	[M-H]-	190	146 (9)	-	10000
clothianidin	210880-92-5	insecticide	4.72	[M+H]+	250	169 (13)	132 (17)	50
Clotrimazole	23593-75-1	pharmaceutical	11.28	[M-C3H4N2+H]+	277.1	165 (24)	241 (26)	1000
Clozapine	5786-21-0	pharmaceutical	6.3	[M+H]+	327.1	270 (25)	192 (46)	100
colchicine	64-86-8	pharmaceutical	8.18	[M+H]+	400.2	358 (22)	310 (26)	100

cotinine	486-56-6	Human	0.92	[M+H]+	177.1	80 (24)	98 (25)	50
cyazofamid	120116-88-3	fungicide	15.62	[M+H]+	325.1	108 (14)	261 (10)	500
Cyclobenzaprine	303-53-7	pharmaceutical	10.45	[M+H]+	276.2	215 (41)	216 (24)	100
Cycluron	2163-69-1	Herbicide	10.83	[M+H]+	199.2	89 (15)	72 (22)	50
cyproconazole	94361-06-5	fungicide	13.17	[M+H]+	292.1	125 (30)	89 (53)	100
cyprodinil	121552-61-2	fungicide	11.66	[M+H]+	226.1	93 (35)	118 (31)	100
cyproterone acetate	427-51-0	pharmaceutical	15.05	[M+H]+	417.2	357 (16)	279 (25)	5000
cyromazine	66215-27-8	Insecticide	1.27	[M+H]+	167.1	68 (33)	125 (18)	100
Decoquinate	18507-89-6	pharmaceutical	17.5	[M+H]+	418.3	372 (25)	204 (44)	50
deet	134-62-3	Insect	11.18	[M+H]+	192.1	119 (18)	91 (30)	1
desHEE-quetiapine	5747-48-8	pharmaceutical	6.57	[M+H]+	296.1	210 (30)	253 (21)	50
Desmetryn	1014-69-3	Herbicide	6.16	[M+H]+	214.1	172 (18)	82 (29)	500
Desmycosin	11032-98-7	antibiotic	9.44	[M+H]+	772.4	174 (30)	407 (25)	5000
dextromethorphan	125-71-3	pharmaceutical	8.05	[M+H]+	272.2	215 (24)	171 (43)	5
dicamba	1918-00-9	Herbicide	8.13	[M-H]-	219	175 (7)	-	5000
dichlorprop	120-36-5	Herbicide	12.63	[M-H]-	233	161 (28)	125 (28)	500
Diclazuril	101831-37-2	antibiotic	14.7	[M-H]-	405	334 (19)	299 (28)	1000
diclobutrazol	66345-62-8	fungicide	14.23	[M+H]+	328.1	159 (39)	123 (55)	1000
Dicrotophos	141-66-2	insecticide	3.9	[M+H]+	238.1	127 (19)	72 (26)	5
diethofencarb	87130-20-9	fungicide	13.5	[M+H]+	268.2	226 (10)	180 (18)	500
difenoconazole	119446-68-3	fungicide	15.65	[M+H]+	406.1	251 (26)	337 (18)	500
Diltiazem	42399-41-7	pharmaceutical	9.75	[M+H]+	415.2	178 (26)	150 (45)	50
dimefuron	34205-21-5	herbicide	12.65	[M+H]+	339.1	167 (22)	140 (40)	500
dimethoate	60-51-5	insecticide	5.35	[M+H]+	230	199 (10)	125 (22)	50
dimethomorph	110488-70-5	fungicide	13.06	[M+H]+	388.1	301 (21)	165 (32)	100
Dimoxystrobin	149961-52-4	fungicide	15.03	[M+H]+	327.2	205 (10)	116 (23)	10
diniconazole	76714-16-4	fungicide	14.9	[M+H]+	326.1	70 (25)	159 (30)	50
Dinitolmide	148-01-6	Coccidiostat	5.24	[M-H]-	224	181 (11)	42 (14)	500
dinotefuran	165252-70-0	insecticide	2.94	[M+H]+	203.1	129 (12)	113 (10)	50
diphenhydramine	58-73-1	Pharmaceutical	4.56	[M+H]+	256.2	167 (18)	152 (42)	50
Diuron	330-54-1	herbicide	11.37	[M+H]+	233	72 (18)	46 (17)	100
epoxiconazole	135319-73-2	fungicide	13.99	[M+H]+	330.1	121 (21)	123 (18)	50
Erythromycin A	114-07-8	antibiotic	9.71	[M+H]+	734.5	158 (30)	576 (20)	1000
Esomeprazole	119141-88-7	pharmaceutical	5.55	[M+H]+	346.1	198 (11)	136 (32)	5
Etaconazol	60207-93-4	fungicide	13.72	[M+H]+	328.1	159 (29)	205 (17)	100
Ethiofencarb	29973-13-5	insecticide	11.17	[M+H]+	226.1	107 (10)	169 (10)	500
ethiprole	181587-01-9	insecticide	13.43	[M-H]-	395	331 (10)	262 (29)	500
ethirimol	23947-60-6	Fungicide	4.43	[M+H]+	210.2	140 (23)	98 (27)	5
Etoxazole	153233-91-1	insecticide	18.66	[M+H]+	360.2	141 (31)	304 (18)	5000
fenamidone	161326-34-7	fungicide	14.03	[M+H]+	312.1	236 (15)	92 (25)	50
Fenarimol	60168-88-9	fungicide	13.52	[M+H]+	331	268 (23)	189 (50)	100
fenbuconazole	114369-43-6	fungicide	14.61	[M+H]+	337.1	194 (17)	91 (32)	10000
Fenhexamid	126833-17-8	fungicide	14.04	[M+H]+	302.1	97 (25)	55 (35)	500
fenobucarb	3766-81-2	insecticide	13.04	[M+H]+	208.1	95 (15)	152 (10)	100

fenoxycarb	72490-01-8	insecticide	14.9	[M+H]+	302.1	88 (19)	116 (11)	5000
fenpropimorph	67564-91-4	fungicide	11.65	[M+H]+	304.3	147 (29)	130 (25)	500
Fenuron	101-42-8	Herbicide	4.7	[M+H]+	165.1	72 (16)	46 (14)	5
fexofenadine	83799-24-0	pharmaceutical	10.9	[M+H]+	502.3	466 (27)	171 (38)	10
Finasteride	98319-26-7	pharmaceutical	12.7	[M+H]+	373.3	305 (32)	317 (24)	500
fluazinam	79622-59-6	Fungicide	17.97	[M-H]-	463	416 (20)	398 (16)	5000
Flubendiamide	272451-65-7	insecticide	15.76	[M+H]+	683	408 (10)	274 (29)	10000
fludioxonil	131341-86-1	Fungicide	13.52	[M-H]-	247	180 (29)	152 (39)	1000
flufenacet	142459-58-3	herbicide	15.02	[M+H]+	364.1	152 (19)	194 (10)	500
Flunixin	38677-85-9	pharmaceutical	11.77	[M+H]+	297.1	264 (35)	259 (29)	50
fluometuron	2164-17-2	herbicide	10.88	[M+H]+	233.1	72 (19)	160 (27)	100
fluoxastrobin	193740-76-0	fungicide	15.15	[M+H]+	459.1	427 (0)	383 (22)	500
Fluoxetine	54910-89-3	pharmaceutical	11.12	[M+H]+	310.1	44 (14)	148 (9)	500
flupyradifurone	951659-40-8	insecticide	6.8	[M+H]+	289.1	126 (20)	90 (40)	10
Fluquinconazole	136426-54-5	fungicide	14.17	[M+H]+	376	349 (20)	307 (28)	500
fluridone	59756-60-4	herbicide	13.17	[M+H]+	330.1	309 (36)	310 (29)	10
Flusilazole	85509-19-9	fungicide	14.43	[M+H]+	316.1	247 (18)	165 (28)	50
flutolanil	66332-96-5	fungicide	14.89	[M+H]+	324.1	262 (19)	242 (26)	10
flutriafol	76674-21-0	fungicide	11.25	[M+H]+	302.1	123 (28)	95 (48)	100
Fluvoxamine	54739-18-3	pharmaceutical	10.55	[M+H]+	319.2	71 (17)	258 (12)	1000
Forchlorfenuron	68157-60-8	plant	11.05	[M+H]+	248.1	129 (18)	93 (32)	50
Fuberidazole	3878-19-1	Fungicide	3.41	[M+H]+	185.1	157 (22)	65 (42)	5
Furosemide	54-31-9	pharmaceutical	9.98	[M-H]-	329	285 (16)	205 (16)	500
gliclazide	21187-98-4	pharmaceutical	12.9	[M+H]+	324.1	91 (33)	127 (19)	500
Glipizide	29094-61-9	pharmaceutical	11.8	[M+H]+	446.2	321 (13)	286 (25)	100
GS-441524	1191237-69-0	pharmaceutical	2.53	[M+H]+	292.1	147 (32)	202 (11)	1000
guanylurea	141-83-3	Pharmaceutical	0.5	[M+H]+	103.1	60 (12)	43 (25)	500
halofuginone	55837-20-2	pharmaceutical	6.51	[M+H]+	414	120 (23)	138 (19)	50
hexaconazole	79983-71-4	fungicide	14.5	[M+H]+	314.1	70 (21)	159 (31)	100
hexaflumuron	86479-06-3	insecticide	15.15	[M+H]+	461	429 (10)	141 (38)	1000
hexazinone	51235-04-2	herbicide	8.15	[M+H]+	253.2	171 (16)	71 (30)	10
hexythiazox	78587-05-0	insecticide	18.26	[M+H]+	353.1	228 (15)	168 (24)	10000
Hydrochlorothiazide	58-93-5	pharmaceutical	3.24	[M-H]-	296	269 (20)	205 (24)	100
hydroxybupropion	357399-43-0	pharmaceutical	4.56	[M+H]+	256.1	130 (46)	139 (27)	50
imazamox	114311-32-9	herbicide	5.26	[M+H]+	306.1	261 (20)	246 (25)	50
imazethapyr	81335-77-5	herbicide	7.13	[M+H]+	290.1	245 (22)	177 (28)	10
imidacloprid	138261-41-3	insecticide	5.12	[M+H]+	256.1	209 (16)	175 (18)	50
ipconazole	125225-28-7	fungicide	15.69	[M+H]+	334.2	70 (23)	125 (39)	1000
Iprovalicarb	140923-17-7	fungicide	13.71	[M+H]+	321.2	119 (20)	203 (10)	50
Irbesartan	138402-11-6	pharmaceutical	11.01	[M+H]+	429.3	195 (22)	180 (41)	50
isoprocarb	2631-40-5	Insecticide	11.69	[M+H]+	194.1	95 (15)	137 (10)	500
Isoproturon	34123-59-6	Herbicide	11.34	[M+H]+	207.1	72 (18)	165 (14)	10
labetalol	36894-69-6	pharmaceutical	6.75	[M+H]+	329.2	162 (26)	294 (19)	100
lamotrigine	84057-84-1	pharmaceutical	4.28	[M+H]+	256	211 (25)	166 (28)	100

lidocaine	137-58-6	pharmaceutical	3.79	[M+H]+	235.2	86 (18)	58 (32)	5
lincomycin	154-21-2	antibiotic	3.14	[M+H]+	407.2	126 (28)	359 (19)	5
linezolid	165800-03-3	antibiotic	5.76	[M+H]+	338.2	296 (17)	235 (21)	50
linuron	330-55-2	herbicide	13.24	[M+H]+	249	160 (18)	182 (15)	500
Lisinopril	76547-98-3	pharmaceutical	3.18	[M+H]+	406.2	84 (28)	246 (23)	500
Losartan	114798-26-4	pharmaceutical	11.24	[M+H]+	423.2	207 (22)	377 (15)	50
maduramicin	84878-61-5	antibiotic	23.98	[M+Na]+	939.5	877 (37)	895 (50)	1000
malathion	121-75-5	insecticide	14.77	[M+H]+	331	127 (12)	285 (10)	1000
Mandipropamid	374726-62-2	fungicide	14.28	[M+H]+	412.1	328 (15)	356 (10)	500
mecoprop	93-65-2	Herbicide	12.64	[M-H]-	213	141 (17)	-	500
mefenacet	73250-68-7	herbicide	13.94	[M+H]+	299.1	148 (15)	120 (25)	50
melengestrol acetate	2919-66-6	Feed	15.4	[M+H]+	397.2	337 (14)	279 (20)	1000
Meloxicam	71125-38-7	pharmaceutical	12.49	[M+H]+	352	115 (19)	141 (20)	50
mepanipyrim	110235-47-7	fungicide	14.18	[M+H]+	224.1	77 (38)	106 (26)	100
Mepronil	55814-41-0	fungicide	14.6	[M+H]+	270.1	119 (24)	228 (15)	50
meropenem	119478-56-7	antibiotic	3.02	[M+H]+	384.2	141 (16)	254 (16)	500
metalaxyl	57837-19-1	fungicide	11.49	[M+H]+	280.2	220 (14)	248 (10)	50
metconazole	125116-23-6	fungicide	14.72	[M+H]+	320.2	70 (24)	125 (39)	500
metformin	657-24-9	Pharmaceutical	0.5	[M+H]+	130.1	60 (22)	85 (16)	100
methabenzthiazuron	18691-97-9	Herbicide	10.31	[M+H]+	222.1	165 (17)	150 (33)	50
Methamidophos	10265-92-6	Insecticide	1.36	[M+H]+	142	94 (14)	125 (14)	50
methiocarb	2032-65-7	insecticide	13.18	[M+H]+	226.1	169 (10)	121 (19)	500
methomyl	16752-77-5	Insecticide	3.48	[M+H]+	163.1	106 (10)	88 (10)	50
Methoprotryne	841-06-5	herbicide	8.78	[M+H]+	272.2	240 (19)	198 (23)	10
methoxyfenozide	161050-58-4	insecticide	14.59	[M+H]+	369.2	149 (17)	313 (10)	50
Metobromuron	3060-89-7	herbicide	11.71	[M+H]+	259	148 (15)	170 (19)	100
Metolachlor	51218-45-2	herbicide	14.68	[M+H]+	284.1	252 (15)	176 (26)	10
metoprolol	51384-51-1	pharmaceutical	4.59	[M+H]+	268.2	116 (18)	159 (21)	5
metribuzin	21087-64-9	Herbicide	8.83	[M+H]+	215.1	187 (18)	84 (22)	50
Mexacarbate	315-18-4	insecticide	3.38	[M+H]+	223.1	151 (24)	166 (15)	5
mirtazapine	85650-52-8	pharmaceutical	4.04	[M+H]+	266.2	195 (28)	72 (20)	500
monensin	17090-79-8	antibiotic	22.57	[M+Na]+	693.4	461 (55)	479 (55)	1000
monocrotophos	6923-22-4	insecticide	3.51	[M+H]+	224.1	127 (16)	193 (10)	10
monolinuron	1746-81-2	Herbicide	11.15	[M+H]+	215.1	126 (18)	148 (15)	500
morpholino diethyl ether	6425-39-4	Industrial	0.6	[M+H]+	245.2	114 (31)	158 (18)	50
moxifloxacin	151096-09-2	antibiotic	5.79	[M+H]+	402.2	358 (19)	261 (26)	500
myclobutanil	88671-89-0	fungicide	13.77	[M+H]+	289.1	125 (33)	89 (55)	50
mycophenolate mofetil	128794-94-5	pharmaceutical	9.5	[M+H]+	434.2	114 (28)	285 (25)	100
Narasin	55134-13-9	antibiotic	23.37	[M+Na]+	787.5	431 (53)	531 (45)	10000
neotame	165450-17-9	Artificial	10.13	[M+H]+	379.2	172 (24)	319 (18)	5
nitenpyram	150824-47-8	insecticide	3.42	[M+H]+	271.1	225 (12)	99 (15)	50
nitrofurantoin	67-20-9	Pharmaceutical	4.09	[M-H]-	237	152 (12)	194 (9)	500
norfluoxetine	126924-38-7	pharmaceutical	10.84	[M+H]+	296.1	134 (7)	-	1000
novobiocin	303-81-1	antibiotic	15.45	[M+H]+	613.2	189 (27)	218 (12)	500

Nuarimol	63284-71-9	fungicide	12.42	[M+H]+	315.1	252 (22)	207 (47)	50
O-desmethylvenlafaxine	93413-62-8	pharmaceutical	3.99	[M+H]+	264.2	58 (20)	133 (27)	10
ofloxacin	82419-36-1	antibiotic	3.73	[M+H]+	362.2	318 (20)	261 (28)	10
olanzapine	132539-06-1	pharmaceutical	3.03	[M+H]+	313.1	256 (24)	198 (44)	100
omethoate	1113-02-6	insecticide	2.69	[M+H]+	214	183 (11)	125 (22)	10
oxadixyl	77732-09-3	fungicide	9.09	[M+H]+	279.1	219 (10)	132 (31)	500
oxolinic acid	14698-29-4	pharmaceutical	6.95	[M+H]+	262.1	216 (30)	160 (39)	100
oxytetracycline	79-57-2	antibiotic	3.71	[M+H]+	461.2	426 (21)	337 (30)	100
Paclobutrazol	76738-62-0	fungicide	12.9	[M+H]+	294.1	70 (21)	125 (38)	50
paliperidone	144598-75-4	pharmaceutical	5.76	[M+H]+	427.2	207 (29)	110 (41)	50
Pantoprazole	102625-70-7	pharmaceutical	7.19	[M+H]+	384.1	200 (15)	138 (31)	10
Paroxetine	61869-08-7	pharmaceutical	10.2	[M+H]+	330.2	192 (22)	151 (22)	1000
penconazole	66246-88-6	fungicide	14.49	[M+H]+	284.1	123 (49)	173 (30)	100
pencycuron	66063-05-6	fungicide	16.53	[M+H]+	329.1	125 (26)	218 (16)	50
Picoxystrobin	117428-22-5	fungicide	15.86	[M+H]+	368.1	145 (22)	205 (10)	10
piperacillin	61477-96-1	antibiotic	9.55	[M+H]+	518.2	143 (21)	160 (11)	100
pirimicarb	23103-98-2	insecticide	4.35	[M+H]+	239.2	182 (16)	72 (21)	5
Pravastatin	81093-37-0	pharmaceutical	10.34	[M-H]-	423.2	321 (14)	303 (13)	500
Prednisone	1953-03-02	pharmaceutical	9.05	[M+H]+	359.2	267 (16)	265 (17)	500
prochloraz	67747-09-5	fungicide	13.01	[M+H]+	376	308 (12)	266 (17)	500
promecarb	2631-37-0	insecticide	13.47	[M+H]+	208.1	151 (10)	109 (16)	500
Prometon	1610-18-0	Herbicide	6.8	[M+H]+	226.2	142 (23)	184 (19)	10
Prometryn	7287-19-6	Herbicide	10.4	[M+H]+	242.1	158 (24)	200 (19)	10
propamocarb	24579-73-5	Fungicide	2.75	[M+H]+	189.2	102 (18)	144 (13)	10
Propazine	139-40-2	Herbicide	12.49	[M+H]+	230.1	146 (23)	188 (18)	50
propham	122-42-9	Herbicide	12.03	[M+H]+	180.1	138 (10)	120 (17)	5000
Propiconazole	60207-90-1	fungicide	14.91	[M+H]+	342.1	159 (30)	123 (54)	100
propoxur	114-26-1	insecticide	9.95	[M+H]+	210.1	111 (14)	168 (10)	100
propranolol	525-66-6	pharmaceutical	7.5	[M+H]+	260.2	116 (18)	183 (19)	50
prothioconazole	178928-70-6	fungicide	14.81	[M+H]+	344	326 (10)	154 (28)	5000
pymetrozine	123312-89-0	Insecticide	2.41	[M+H]+	218.1	105 (20)	78 (38)	10
pyraclostrobin	175013-18-0	fungicide	16.35	[M+H]+	388.1	194 (13)	163 (24)	5000
pyrimethanil	53112-28-0	Fungicide	8.48	[M+H]+	200.1	107 (24)	183 (24)	100
Quetiapine	111974-69-7	pharmaceutical	7.47	[M+H]+	384.2	253 (21)	221 (42)	50
Quinoxyfen	124495-18-7	fungicide	16.72	[M+H]+	308	197 (33)	162 (45)	5000
Ractopamine	97825-25-7	Feed	3.96	[M+H]+	302.2	164 (18)	121 (24)	50
Ramipril	87333-19-5	pharmaceutical	10.03	[M+H]+	417.2	234 (20)	117 (40)	50
Ranitidine	66357-35-5	pharmaceutical	2.76	[M+H]+	315.2	176 (19)	130 (26)	50
remdesivir	1809249-37-3	pharmaceutical	12.09	[M+H]+	603.2	200 (39)	402 (15)	5000
rimsulfuron	122931-48-0	herbicide	11.15	[M+H]+	432.1	182 (22)	325 (15)	100
risperidone	106266-06-2	pharmaceutical	5.9	[M+H]+	411.2	191 (29)	110 (49)	500
rosuvastatin	287714-41-4	pharmaceutical	12.02	[M+H]+	482.2	258 (33)	272 (34)	100
Saccharin	1981-07-02	Artificial	2.75	[M-H]-	182	42 (26)	106 (23)	1000
salinomycin	53003-10-4	antibiotic	22.41	[M+Na]+	773.5	431 (55)	531 (44)	500

Salmeterol	89365-50-4	pharmaceutical	11.18	[M+H]+	416.3	380 (19)	232 (22)	10000
sebacic acid	111-20-6	Industrial	8.58	[M-H]-	201.1	139 (15)	137 (21)	500
sebuthylazine	7286-69-3	Herbicide	12.52	[M+H]+	230.1	68 (36)	174 (18)	500
sertraline	79617-96-2	pharmaceutical	11.15	[M+H]+	306.1	159 (30)	275 (13)	1000
simazine	122-34-9	Herbicide	8.1	[M+H]+	202.1	132 (19)	124 (18)	50
Simetryn	1014-70-6	Herbicide	6.09	[M+H]+	214.1	124 (20)	96 (24)	10
sitagliptin	486460-32-6	pharmaceutical	5.65	[M+H]+	408.1	235 (18)	193 (25)	50
sotalol	3930-20-9	pharmaceutical	2.68	[M+H]+	273.1	133 (27)	213 (18)	10
spiroxamine	118134-30-8	fungicide	11.8	[M+H]+	298.3	144 (21)	100 (31)	500
stevioside	57817-89-7	Natural	10.15	[M-C6H10O5-H]-	641.3	479 (42)	317 (47)	10000
sucralose	56038-13-2	Artificial	3.88	[M+FA-H]-	441	395 (11)	359 (15)	5000
sulfadoxine	2447-57-6	antibiotic	5.59	[M+H]+	311.1	156 (20)	108 (25)	50
sulfamethazine	57-68-1	pharmaceutical	4	[M+H]+	279.1	186 (17)	124 (26)	50
sulfentrazone	122836-35-5	Herbicide	11.21	[M-H]-	385	307 (23)	240 (31)	500
sulisobenzone	4065-45-6	Sunscreen	5.92	[M-H]-	307	211 (40)	227 (24)	100
Tadalafil	171596-29-5	pharmaceutical	11.51	[M+H]+	390.1	268 (13)	204 (55)	500
Tamsulosin	106133-20-4	pharmaceutical	6.9	[M+H]+	409.2	228 (23)	271 (19)	100
Tazobactam	89786-04-9	antibiotic	2.64	[M+H]+	301.1	207 (16)	168 (15)	500
tebuconazole	107534-96-3	fungicide	14.26	[M+H]+	308.2	70 (23)	125 (37)	50
tebufenozide	112410-23-8	insecticide	15.42	[M+H]+	353.2	133 (19)	297 (10)	100
tebufenpyrad	119168-77-3	insecticide	17.2	[M+H]+	334.2	117 (36)	147 (26)	10000
Tebuthiuron	34014-18-1	Herbicide	7.81	[M+H]+	229.1	172 (18)	116 (27)	10
telmisartan	144701-48-4	pharmaceutical	10.96	[M+H]+	515.2	276 (39)	289 (50)	5000
terbumeton	33693-04-8	Herbicide	7.1	[M+H]+	226.2	170 (18)	142 (25)	10
Terbuthylazine	5915-41-3	Herbicide		[M+H]+	230.1	174 (17)	132 (25)	5
Terbutryn	886-50-0	Herbicide	10.51	[M+H]+	242.2	186 (19)	91 (28)	5
tetraconazole	67915-31-5	fungicide	14.26	[M+H]+	372	159 (30)	70 (22)	100
tetracycline	60-54-8	antibiotic	4.03	[M+H]+	445.2	410 (18)	241 (35)	100
thiabendazole	148-79-8	Fungicide	3.23	[M+H]+	202	175 (26)	131 (33)	10
thiacloprid	111988-49-9	insecticide	7.52	[M+H]+	253	126 (21)	90 (36)	50
Thiamethoxam	153719-23-4	insecticide	4.05	[M+H]+	292	211 (10)	132 (23)	50
Thidiazuron	51707-55-2	Herbicide	8.81	[M+H]+	221	102 (16)	128 (16)	500
thiobencarb	28249-77-6	herbicide	16.38	[M+H]+	258.1	125 (19)	89 (47)	500
thiophanate-methyl	23564-05-8	fungicide	9.98	[M+H]+	343.1	151 (20)	311 (10)	500
tolnaftate	2398-96-1	fungicide	17.7	[M+H]+	308.1	148 (15)	120 (29)	10000
Tolvaptan	150683-30-0	pharmaceutical	13.77	[M+H]+	449.2	252 (20)	119 (38)	5000
Trazodone	19794-93-5	pharmaceutical	6.8	[M+H]+	372.2	176 (26)	148 (34)	50
triadimefon	43121-43-3	fungicide	13.98	[M+H]+	294.1	197 (15)	225 (13)	100
triadimenol	55219-65-3	fungicide	12.9	[M+H]+	296	70 (10)	99 (15)	100
tricyclazole	41814-78-2	Fungicide	6.05	[M+H]+	190	163 (23)	136 (29)	50
trifloxystrobin	141517-21-7	fungicide	17.21	[M+H]+	409.1	186 (17)	145 (43)	1000
triflumizole	68694-11-1	fungicide	14.37	[M+H]+	346.1	278 (10)	43 (22)	500
Trimethoprim	738-70-5	antibiotic	3.49	[M+H]+	291.1	230 (24)	261 (25)	5
tylosin	1401-69-0	antibiotic	10.38	[M+H]+	916.5	174 (37)	772 (29)	500

vamidothion	2275-23-2	insecticide	4.66	[M+H]+	288	146 (12)	118 (23)	5
Vancomycin	1404-90-6	antibiotic	2.84	[M+2H]2+	724.7	1305 (14)	1143 (17)	1000
venlafaxine	93413-69-5	pharmaceutical	6.35	[M+H]+	278.2	58 (18)	121 (31)	10
Vilazodone	163521-12-8	pharmaceutical	8.06	[M+H]+	442.2	155 (38)	197 (31)	10000
Virginiamycin M1	21411-53-0	antibiotic	11.78	[M+H]+	526.3	355 (17)	337 (21)	5000
warfarin	81-81-2	pharmaceutical	13.18	[M+H]+	309.1	163 (15)	251 (19)	100
zoxamide	156052-68-5	fungicide	16.12	[M+H]+	336	187 (22)	159 (39)	100

Appendix 3: Widespread ESOC Analysis in Surface Water

Table A5: Analytes detected in Surface water samples from 2018-2020

10-hydroxyamitriptyline	imazethapyr
24D	imidacloprid
3-hydroxycarbofuran	iminostilbene
4-Methyl-1H-benzotriazole	ipconazole
abacavir	Iprovalicarb
acephate	Irbesartan
acesulfame	isoprocarb
acetamiprid	labetalol
acetominophen	lamotrigine
acetylsalicyclic acid	lidocaine
acibenzolar-s-methyl	lincomycin
advantame	linuron
albuterol	Lisinopril
aldicarb	malathion
amitryptaline	Mandipropamid
Amlodipine	mecoprop
amoxacillin	mefenacet
aripiprazole	Meloxicam
aspartame	mepanipyrim
atenolol	Mepronil
Atorvastatin	metalaxyl
atrazine	methabenzthiazuron

azoxystrobin
bendiocarb
bentazon
benzoximate
bezafibrate
bifenazate
boscalid
Bromoxynil
bromuconazole
bupirimate
buprofezin
Bupropion
caffeine
capecitabine
Carbadox
Carbamazepine
carbamazepine-10-11-epoxide
carbaryl
carbendazim
carbenicillin
carbofuran
Cefotaxime
Ceftiofur
ceftriaxone
cetirizine
chloramphenicol
chlorantraniliprole
chlorbromuron
chloroxuron
Cilastatin
ciprofloxacin
Methamidophos
methiocarb
methomyl
Methoprotryne
methoxyfenozide
Metobromuron
Metolachlor
metoprolol
metribuzin
Mexacarbate
mirtazapine
monocrotophos
morpholino diethyl ether
moxifloxacin
myclobutanil
mycophenolate mofetil
Narasin
neotame
nicarbazin
nitenpyram
nitrofurantoin
norfluoxetine
novobiocin
Nuarimol
O-desmethylvenlafaxine
ofloxacin
olanzapine
omethoate
oxadixyl
oxolinic acid
oxytetracycline

citalopram
Clindamycin
Clindamycin phosphate
clopidogrel
clopidol
clothianidin
Clozapine
colchicine
cotinine
cyazofamid
cyclamate
Cyclobenzaprine
cyproconazole
cyprodinil
cyromazine
Decoquinate
deet
desHEE-quetiapine
dextromethorphan
dicamba
dichlorprop
Diclazuril
diclobutrazol
Dicrotophos
diethofencarb
difenoconazole
Diltiazem
dimethoate
dimethomorph
diniconazole
Dinitolmide
Paclobutrazol
paliperidone
Pantoprazole
paraxanthine
Paroxetine
pencycuron
penicillin V
Perfluorooctanoic acid
Picoxystrobin
piperacillin
pirimicarb
Pravastatin
Prednisone
prochloraz
Prometon
Prometryn
propamocarb
Propazine
propham
Propiconazole
propoxur
propranolol
pymetrozine
pyraclostrobin
pyrimethanil
Quetiapine
Quinoxyfen
Ractopamine
Ramipril
Ranitidine
remdesivir

dinotefuran
diphenhydramine
Diuron
epoxiconazole
Esomeprazole
Etaconazol
Ethiofencarb
Ethiofencarb sulfoxide
Etoxazole
fenamidone
Fenarimol
fenbuconazole
Fenhexamid
fenobucarb
fenpropimorph
Fenuron
fexofenadine
Finasteride
fluazinam
fludioxonil
Flunixin
fluometuron
fluoxastrobin
Fluoxetine
flupyradifurone
Fluquinconazole
fluridone
Flusilazole
flutolanil
flutriafol
Fluvoxamine
risperidone
rosuvastatin
Saccharin
sebacic acid
sebuthylazine
sertraline
simazine
Simetryn
sitagliptin
sotalol
spiroxamine
sucralose
sulfadoxine
sulfamethazine
sulfentrazone
sulisobenzone
Tadalafil
Tamsulosin
Tazobactam
tebuconazole
tebufenozide
Tebuthiuron
terbumeton
Terbutryn
tetraconazole
tetracycline
thiabendazole
thiacloprid
Thiamethoxam
Thidiazuron
thiobencarb

Forchlorfenuron	Tolvaptan
Furosemide	Trazodone
Gabapentin	triadimefon
gliclazide	triadimenol
Glipizide	tricyclazole
GS-441524	trifloxystrobin
guanylurea	triflumizole
Halofuginone	Trimethoprim
hexaconazole	Vancomycin
hexaflumuron	venlafaxine
hexazinone	Virginiamycin M1
Hydrochlorothiazide	warfarin
hydroxybupropion	Ziprasidone
imazamox	

CURRICULUM VITAE

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CHEM 9713R – Advanced Medicinal Chemistry - 88

CHEM 9522B - Analytical Separations – 90

CHEM 9657 Seminar - Completed