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Investigating non-targeted screening and accurate quantitation of trace surface water contaminants using high-resolution mass spectrometry

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Abstract

The human impact on surface water is a growing concern and the chemical surveying of contaminants including pharmaceuticals and pesticides is currently lacking. Neonicotinoids in particular, are among the most widely used insecticides that have prompted environmental concern and require monitoring. Chemical contaminants in environmental water samples are commonly analyzed by targeted tandem mass spectrometry. However, this requires a prior knowledge of the contaminants in the area of interest. Here, surface water samples were screened by utilizing optimized data-independent acquisition (DIA) methods and the spectra were databased for future retrospective analysis. This circumvents the requirement for target analytes prior to analysis and allows for improved method development.

Methods were produced for the improved screening of contaminants using DIA, for the quantitation of targeted compounds, and to allow for the high-throughput analysis of neonicotinoids. Quantitation was completed for the detected compounds at various surface water sites and wastewater treatment plants.

Keywords

Analytical Chemistry, Environmental Analysis, Solid Phase Extraction, Lyophilisation, Mass Spectrometry, Non-Targeted Screening, Data-Independent Acquisition, Trace Residue Quantitation.

Co-Authorship Statement

Chapter 2: Morrison LM, Lapen D, Topp E, Manoli K, Renaud J, Yeung K and Sumarah M

This chapter was written in its entirety by LMM. LMM and KM carried out all experiments with the help of apprentices. LMM designed and carried out all sample extractions and analyses. KM completed oxidation reactions on samples in section 2.6. Samples for section 2.4, 2.7 and 2.8 were provided by DL. Samples for section 2.5 and 2.6 were provided by ET and KM respectively. JR, KY, and MS provided expert advice on experimental design and analysis.

Chapter 3: Morrison LM, Renaud J, Lapen D, Sabourin L, Yeung K and Sumarah M

This paper was written by LMM. All experiments were executed by LMM. Samples were provided by DL. LMM and JR developed extraction protocol. LMM produced and carried out all LCMS analyses. LS, KY, and MS provided expert advice and authors read and provided edits for approval of the final manuscript.

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List of Abbreviations

AAFC	Agriculture and Agri-Food Canada
ac	Alternating current
ACN	Acetonitrile
ACS	American Chemical Society
AGC	Automatic gain control
AIF	All ion fragmentation
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
C18	18 carbon alkyl chain stationary phase
CEC	Contaminants of emerging concern
CEM	Chain ejection model
ChE	Cholinesterase
CID	Collision-induced dissociation
CRM	Chain residue model
Da	Daltons
DAI	Direct aqueous injection
dc	Direct current
DDA	Data dependent acquisition

DIA	Data independent acquisition
EAWAG	Eigenössische Anstalt für Wasserversorgung, Abwasserreinigung und Gewässerschutz (Swiss Federal Institute for Environmental Science and Technology / ETH)
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
ESI	Electrospray ionization
FA	Formic acid
Fe(VI)	Ferrate
FFT	Fast Fourier transform
FMOC	9-fluorenylmethyl chloroformate
FWHM	Full width half maximum
GC-MS	Gas chromatography mass spectrometry
H_2O	Water
HCD	Higher collision energy dissociation
HCl	Hydrochloric acid
HESI	Heated electrospray ionization
HILIC	Hydrophilic interaction liquid chromatography
HLB	Hydrophilic-Lipophilic-Balanced
HM	High mass
HPLC	High performance liquid chromatography
HRMS	High-resolution mass spectrometry

IEM	Ion evaporation model
INST	Internal standard
IT	Injection time
IUPAC	International Union of Pure and Applied Chemistry
kV	Kilovolts (103 volts)
LC	Liquid chromatography
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LFB	Laboratory fortified blank
LFSM	Laboratory fortified sample matrix
LM	Low mass
LOD	Limit of detection
LOQ	Limit of quantitation
m/z	Mass-to-charge ratio
MAC	Maximum acceptable concentration
MDL	Method detection limit
MQL	Method quantitation limit
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NCE	Normalized collision energy

NTU	Nephelometric turbidity units
PCA	Principle component analysis
PFAS	Polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PICT	Pollution-induced community tolerance
PPCP	Pharmaceutical and personal care product
ppm	Parts-per-million
ppt	Parts-per-trillion
PRM	Parallel reaction monitoring
PRMA	Pest management regulatory agency
QqQ	Triple quadrupole
Qual	Qualifier fragment ion
Quan	Quantifier fragment ion
R_E	Recovery efficiencies
rf	Radio frequency
RT	Retention time
sp.	Species (singular)
SPE	Solid phase extraction
spp.	Species (plural)

SRM	Selected reaction monitoring
TCP	3,5,6-trichloro-2-pyridinol
TDS	Total dissolved solids
TIC	Total ion current
TiO2	Titanium dioxide
TSS	Total suspended solids
USEPA	United States Environmental Protection Agency
WWT	Wastewater treatment
WWTP	Wastewater treatment plant

Chapter 1

1 Introduction

1.1 Environmental Contamination

The human impact on water systems is a constant concern as we continue to add foreign compounds to the environment to meet cultural and societal demands. Contaminants of emerging concern (CEC) can occur naturally, from synthetic engineering, as by-products from manufacturing processes and from degradation of other compounds. The extent of their presence in water is dependent on the amount entering the environment, physicochemical properties, environmental conditions and preferential distribution to different environmental compartments (1). Some of these factors that can influence the fate of chemicals in the environment include temperature, humidity, solar irradiation, volatility, polarity and sorption to soil and water. The persistence and accumulation of CECs is particularly concerning, as they can cause unexpected adverse effects from long-term exposure and synergistic effects (2).

Many studies have monitored pollutants in surface waters such as rivers and lakes, as well as effluent contamination in point sources of urban regions (3-8). Agricultural run-off from fertilizer and pesticide application is a common source of water pollution. Fortunately, pesticides are applied increasingly through seed treatment rather than spraying, to improve safety of both farmers and nearby ecosystems by preventing wind transfer (9). Industrial and wastewater treatment plants (WWTP) are common urban point sources studied for acute toxicity to the environment. However, these examples represent only a fraction of the sources of pharmaceuticals, pesticides, sweeteners, personal care products, heavy metals and other compounds in biological communities (3, 4).

Currently, there are many regulations for chemicals in the environment based on reported toxicity and environmental fate. Chlorpyrifos, for example, is a regulated organophosphorus pesticide with its primary toxicity through inhibition of cholinesterase (ChE), which causes continual nerve stimulation (10). The most recent evaluation in 2008 determined the short- and long-term guidelines for chlorpyrifos level in water to be 20 and 2 ng L^{-1} respectively (11). This study admits that there was limited data for long-term exposure and that this cut-off is an approximation. Some pesticides are banned completely in Ontario including glyphosate for cosmetic home use, while other classes of pesticides require special permits such as neonicotinoids, which are applied to nearly

100% of maize (*Zea mays*) kernels and 60% of soybean (*Glycine max*) seeds sold in the province (12).

1.1.1 Pesticides

Pesticides used by the agricultural sector as well as on turf grass in urban areas can enter the environment through a myriad of transport and fate pathways. For instance, pesticides can be transferred by wind during spray application to crops, transported through volatilization into the atmosphere or by run-off erosion and leaching from the treated land. Focus for chemical pollution is often on pesticides with acute toxicity or carcinogenic properties (7). However, the long half-lives of many compounds need to be taken into account when monitoring water contamination. Chronic exposure to low levels of micro pollutant residues is an emphasized issue in the World Health Organization's 2008 report (13). Drift during spray application is dependent on droplet size, such as 100 µm droplets take 11 s to fall 3 m and can travel 20 m in 8 km/h wind (14). Even areas completely out of range from agricultural sectors can be affected due to volatilization; Rose *et al*. (2018) found that 10% of all metolachlor applied is taken up into the atmosphere (15).

Degradation products of chemical pollutants are often overlooked by water assessment studies, though they are included in drinking water regulations (16). Chloroacetanilide and triazine are common herbicides found in surface and drinking water and their respective degradants are often responsible for a substantial portion of the pesticide load (17). Pesticides that enter the environment in their intact form degrade through several biological and chemical degradation mechanisms (18). Metolachlor, a chloroacetanilide herbicide extensively used for maize (*Zea mays*), soybean (*Glycine max*) and cotton (*Gossypium hirustum*) weed control, rapidly degrades to form oxanilic and ethanesulfonic acids (17). The degradation pathway for metolachlor to oxanilic acid occurs through oxidation of the acetyl group, while ethanesulfonic acid is formed through glutathione conjugation (19). Similarly, atrazine, a triazine herbicide that is widely used to prevent broadleaf weeds on corn (*Zea mays*), sugarcane (*Saccharum officinarum*) and on turf (*Poaceae*) is commonly found in surface waters and has persistent degradants (e.g. desethylatrazine) (20). Persistent degradation products can be hazardous themselves or

they can serve as biomarkers for nonpoint source pollution and thus should be included in environmental screening.

Neonicotinoid pesticides in particular are a current global public health issue because of their widespread use and persistence in both soil and water resulting in accumulation in drinking water (21, 22). They are the most commonly used insecticide worldwide, accounting for roughly one third of the global market (23). Neonicotinoids are applied approximately 60% of the time through seed treatment to reduce loss from spraying, but they are highly water soluble ($\log K_{ow} < 2$) and can easily be washed out unintentionally into the surrounding environment (24). Many studies have found potentially devastating unintended effects on non-target insects such as honeybees (*Apis mellifera*) (25, 26). The contamination of neonicotinoids in surface waters has recently also become concerning to the invertebrates living in aquatic environments (26-28). Their mode of action is through selective binding of electronegative groups to the nicotinic acetylcholine receptors in the central nervous system of invertebrates (reviewed in (23, 29)). In doing so the neurons are constantly being stimulated and this can lead to death.

Low volatility and high sorption to soil are characteristics that are desired in pesticides. Neonicotinoids have advantageously low volatility with vapor pressures between 2.8×10^{-8} and 2.0×10^{-3} mPa at room temperature (24). They also have acceptable sorption in soil containing high levels of organic matter due to hydrophilic bonding interactions between phenolic hydroxyl and carboxyl acidic groups in soil and cyano and nitro groups in neonicotinoids (23, 24, 29). The persistence of pesticides can also be a sought-after design, though if left unchecked this can result in accumulation and increased chance of chronic toxicity. Neonicotinoids have notably long half-lives, up to 1000 days at a neutral pH, with increased alkalinity and acidity decreasing the half-life (DT_{50}) (23, 24). These factors lead to the requirement of a long-term monitoring method (30).

1.1.2 Pharmaceuticals

Pharmaceuticals can enter the environment as part of the manufacturing process, through human or animal excretion as well as from improper disposal (5). Wastewater treatment plants are currently not capable of removing all organic compounds and the effluent

regularly contains high concentrations of pharmaceuticals (6). Many treatment plants collect the sediment following sanitization and process it for a fertilizer called sludge (7). This often heavily contaminated product is used by the agricultural sector to treat fields, potentially resulting in human medicine contaminating soil and surface water environments. Veterinary medicine and additives used in feeding operations are also commonly present in manure used as fertilizer. Treated crops contaminate surface waters through runoff and wash-out in heavy rain (8).

Chemical pollutant research has historically focused on acutely toxic compounds and carcinogens such as pesticides and industrial intermediates. Pharmaceuticals and their bioactive metabolites are becoming a larger concern as they are continually introduced into aquatic environments. New and innovative drugs are constantly being produced to increase potency and prevent degradation. This leads to the potential for persistent pollutants entering the environment and their subsequent accumulation. The concentration of pharmaceutical residues in the environment is often below acute toxicity guidelines but it is unknown if other receptors in non-targeted organisms are affected or if there are synergistic effects from drugs with similar mechanisms (31). Even at low parts-per-trillion concentrations (ng L^{-1}) these aquatic pollutants can lead to bacterial adaption and the development of resistance (7).

The unprecedented rate of antibiotic resistance in patients suffering from pathogens from *Enterobacteriaceae* and *Pseudomonadaceae* families, among others, has become a public-health crisis (32). Antibiotics are the largest category of therapeutics and growth promoters in human and veterinary medicine worldwide (33). Antibiotic resistance is becoming increasingly problematic; as more bacteria evolve and require stronger medications there is alarm that this will outpace our ability to produce the drugs required to treat diseases. Virtually all older antibiotics have become inadequate for many contagions such as cutaneous infections from *Streptococcus pneumoniae, Streptococcus pyogenes* and *Staphylococcus* spp. (32). There has also been evidence of resistance to macrolides such as azithromycin in *Neisseria gonorrhoeae*(34) and tylosin, an antibiotic used in veterinary medicine as a bacteriostatic feed additive (35).

1.1.3 Artificial Sweeteners

In the last decade, studies have found that the presence of artificial sweeteners in the environment is widespread (36-39). The extensive consumption of diet beverages and other low calorie food products has introduced sweeteners into the environment through excretion. This is particularly true of acesulfame, saccharin and cyclamate, as humans have little ability to metabolize the large amounts of sweetener in each product (40). Artificial sweeteners are also poorly degraded by WWTPs and have been detected in effluent, surface water and potable water at ug L^{-1} concentrations (37-39).

The recalcitrance to treatment and their low absorption to soils make acesulfame, sucrose and other sweeteners ideal candidates for waste water contamination markers (37, 39). One study on Singapore surface water found that even without direct discharge from WWTP, sweeteners including acesulfame, sucralose, saccharin and cyclamate were ubiquitous (36). The use of a urinary marker can allow improved control over water quality. One example of this technique has been completed using acesulfame in pool and hot tub water (41). Using the known average level of acesulfame in human urine (4 ug mL^{-1}), concentrations found in bodies of water can be used to approximate the volume of urine (42). This is particularly useful in stagnant bodies of water such as small lakes, which will be investigated in this thesis.

1.1.4 Industrial Pollutants

Industrial surfactants are commonly found as contaminants, such as 2-naphthalenesulfonic acid and the polyfluoroalkyl substances (PFAS). PFAS in particular are used for a multitude of applications due to their resistance to heat, water and oil. Examples include fire-fighting foams, apparels, upholstery, food paper wrappings and metal plating. Their popularized use has resulted in PFAS compounds being abundant in the environment and even in blood samples of the U.S. population (18). Their persistence and resistance to degradation has led to bioaccumulation in the environment as well as organs and blood (18). Additionally, it has been shown that traditional wastewater treatment has little ability to diminish these compounds (19).

Due to societal uproar and government restrictions on the traditionally used PFAS compounds including perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) for their reproductive toxicity and environmental persistence, industries have transitioned to the less studied PFAS compounds (20). One major substitute is a chemical known as GenX, a perfluoroalkyl ether carboxylic acid. A recent study near a manufacturing site in the Netherlands found concentrations as much as 13 times greater than the sum of many PFAS compounds (21). Due to their widespread presence, environmental monitoring efforts need to include these extremely persistent compounds.

1.1.5 Wastewater Treatment Plants

The growing concern toward clean water shortages has led to increased innovations in wastewater treatment. Primary treatment generally includes screening, grit removal and the addition of disinfectants, such as chlorine (43). Secondary treatment involves the addition of inorganic coagulants such as alum [$Al_2(SO_4)_3$] or organic coagulants known as polyelectrolytes to induce sedimentation by flocculation, thereby decreasing turbidity (44). Polyelectrolytes are polymers containing anions or cations which dissociate in aqueous solutions and destabilize colloidal materials, thereby causing agglomeration of small particles into larger flocs that settle out of solution into sludge (45). Sludge is then collected, processed and treated for use in agriculture as a fertilizer substitute.

Waste water and drinking water treatment plants currently have little ability to treat CEC contamination. The use of photolysis is becoming more common and other possible methods for degrading pollutants are being investigated by the engineering community (46-48). Oxidation methods including ozonation are useful for the removal of pharmaceuticals, but can also result in the production of bromate from bromide, which is classified as a probable human carcinogen (49, 50). Further strategies have been proposed including using photo catalysis of metal oxides, such as titanium dioxide (TiO_2), to create hydroxyl radicals that oxidize pollutants (51, 52). These new techniques have the potential to treat water for the removal of harmful organic compounds with limited risk (53).

1.2 Sample Preparation

The majority of water samples are simple matrices that do not require a large amount of clean-up. This is ideal for screening experiments as clean-up steps can lead to the loss of important compounds along with the matrix intended for removal by washing with clean solvent. The major biological components that interfere with water analysis are simply removed by filtering. The samples can then be processed by multiple methods including direct aqueous injection (DAI), solid phase extraction (SPE), or lyophilisation. The two latter options are advantageous in efforts to enrich the compound concentrations, which are often at trace levels in the surface water samples provided. There is evidence that many compounds including the neonicotinoid imidacloprid are toxic at low parts-per-trillion (ppt) levels with long-term exposure (26). These extraction methods can produce significant sample enrichment, therefore allowing for detection of these important low-abundance compounds.

1.2.1 Solid Phase Extraction (SPE)

Techniques for extracting analytes of interest from a matrix depend on the questions being asked and the instrumentation available. The most common technique for water analysis involves SPE at a particular pH depending on the analytes being targeted, such as acidifying to promote protonation (54-58). Developing a protocol to screen for a large number of compounds can be very difficult with multiple classes having a variety of chemical properties. The SPE cartridge determines what type of compounds will be collected and what conditions are needed for optimal extraction. The four main types of cartridges include nonpolar, polar, ion exchange and mixed mode. Nonpolar cartridges are used to extract nonpolar compounds out of polar solvents, whereas polar cartridges work vice versa for polar compound extraction. Ion exchange binds charged compounds to the oppositely charged (cation to anion) sorbent and mixed mode has a solid phase that allows both types of interactions.

Hydrophilic-Lipophilic-Balanced (HLB) nonpolar reversed-phase cartridges are ideal for binding the nonpolar compounds of interest out of polar water. HLB is stable at pH extremes, which allows analyte retention to be optimized for a large range of compounds.

The Van der Waals interactions are ideal when the compounds are not charged. Thus, the recommendation is for the pH to be 2 units above or below the pK_a of bases or acids, respectively, to limit ionization. This allows for the attractions between compounds and sorbent to be broken with the use of a polar solution such as methanol. When screening a large number of compounds, the pH is adjusted to low (2), neutral (6.5) and high (10) values in order to collect the majority of organic compounds, similar to the Environmental Protection Agency (EPA) method (59). A downfall of this technique, however, is that it is difficult to wash the column without analyte loss, as some compounds may be weakly interacting.

Large volumes are required for SPE of water samples in order to collect a detectable level of contaminants, as all compounds have some level of polarity and their collection on the stationary phase of the cartridge is in equilibrium with their mobile phase interactions with the water. These large volumes lead to high expenses for transporting and storing samples. The SPE technique is also very time consuming due to equipment limitations for large sample volumes and requires intensive man hours with the large volume.

1.2.2 Lyophilisation

Lyophilisation is a simple concentration method requiring much smaller volumes compared to SPE, which first involves freezing a sample so that the material only exists in the solid state. Ideally the material is spread over a large surface area and is frozen slowly to allow for larger crystal formation for rapid sublimation. The frozen samples are then put under vacuum to ensure sublimation directly to the gas phase without the intermediate melting step to maintain analyte stability. The residue remaining from the lyophilized sample can be rather complex depending on the sample matrix, causing difficulties in recovery as the major tool for extraction is solvent polarity differences. Methods for groundwater and surface water analysis have used metal chelators such as Ethylenediaminetetraacetic acid (EDTA) and citric acid to overcome this issue (60, 61). Ultimately, this can lead to an increase in sample matrix complexity and prove to limit analyte ionization.

Using small reconstitution volumes, analyte concentration can be immensely increased. The most convenient technique for sample processing, DAI, by definition offers no opportunity for enrichment or sample clean-up, resulting in limited detection and decreased screening abilities. Similarly, lyophilisation does not function as a clean-up technique but does allow fortification by concentrating the analytes present in the sample. Lyophilisation is capable of both high-throughput analyses by using smaller volumes and analyte enrichment comparable to SPE. Nonetheless, lyophilisation is likely not ideal for large scale screening, though it allows for inexpensive and effective analysis of a narrow range of compounds measured over time. A project involving the development of a method capable for analyzing hundreds of samples for neonicotinoids with limited user input using this technique is discussed in this thesis.

1.2.3 Chromatographic Separation

Traditionally, pesticides as well as many other compounds were analyzed using gas chromatography coupled to mass spectrometry (GC-MS) (62, 63). However, due to increasing safety regulations, emerging compounds have become less volatile to prevent loss at high temperatures (24). Pharmaceuticals are also often non-volatile and require a different mode of separation or derivatization. This has lead researchers to use liquid chromatography coupled to mass spectrometry (LC-MS) to cast the largest net with little manipulation of samples (3, 64, 65).

1.2.3.1 Reverse Phase Chromatography

Environmental contaminants have a variety of structures and characteristics that make it difficult to produce a simple all-encompassing method of analysis. Organic pollutants are often relatively nonpolar and can be separated using reverse phase liquid chromatography with nonpolar stationary phase (e.g. alkyl chain). The gradient change in solvent polarity from an aqueous mobile phase to an organic phase allows analytes with different polarities to be separated. The more polar a compound is, the less affinity it will have for the nonpolar stationary phase and therefore earlier it will elute by chromatography. Additionally, the mobile phase can be supplemented with an acid or base to improve chromatographic peak shape and improve ionization.

1.2.3.2 Normal Phase Chromatography

Some highly polar compounds, like glyphosate, are also found in the environment, requiring normal phase separation; for example hydrophilic interaction liquid chromatography (HILIC) comprising polar stationary phase. The gradient will change from an organic solvent to aqueous mobile phase over the course of separation. Glyphosate and its main metabolite are also difficult to collect by the solid phase extraction protocol previously discussed as they have been shown to have an affinity for glass adsorption (66). Some studies have employed derivatization with 9-fluorenylmethyl chloroformate (FMOC) in an effort to decrease the structure's polarity and improve chromatographic retention (66, 67). However, in order to capture and separate these compounds, there is a risk that other compounds will be altered or missed.

1.2.3.3 Stationary Phase Dimensions

The particle pore size of the column controls the efficiency or band broadening as well as the pressure. The relatively small particle size used for the separation column (1.2 µm) allows for idyllic resolving power between analytes. The small sample volume (2 µL) can be rapidly separated compared to larger particle sizes, which result in greater mass transfer. The 18 length alkyl chain stationary phase (C18) is useful here, again because small molecules have limited hydrophobicity when compared to larger compounds such as proteins. The limited opportunities for interactions between the compounds and the stationary phase therefore necessitate the larger surface area to interact with and improve the likelihood of capturing analytes.

1.3 Analytical Instrumentation

Mass spectrometry requires vacuum and the sample to be analyzed needs to be within a gas phase system. Many compounds are volatile enough to transition to gas state simply by heating. For these compounds, including pesticides, GC-MS would be an ideal choice for analysis. However, many compounds are not volatile and so an alternative method for ionization is required. Atmospheric pressure ionization (API) probes have allowed for the major conversion from popular use of GC-MS to LC-MS because of the ability to ionize a wider variety of compounds (68). API is often chosen to allow for soft ionization by

preferential protonation or deprotonation (69). The most commonly used API sources include electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) (68).

1.3.1 Electrospray Ionization

ESI uses a strong electric field and often the assistance of heat to convert liquid droplets into a fine aerosol (70). The dispersion of analytes from these drops is shown in **Figure 1** for positive ionization mode. Negative mode works by switching the direction of electrons at the power supply in the kV range, resulting in excess negative charge at the capillary. The formation of a charged droplet at the tip of a capillary is known as a Taylor cone, after Sir Geoffrey Ingram Taylor theorized the formation of a stable liquid cone in 1964 (71). There is an accumulation of charge at the source capillary repelling the droplet and the counter electrode capillary leading to mass analyzer is attracting the droplet. The Taylor cone is a consequence of this charge difference in competition with the droplet's surface tension.

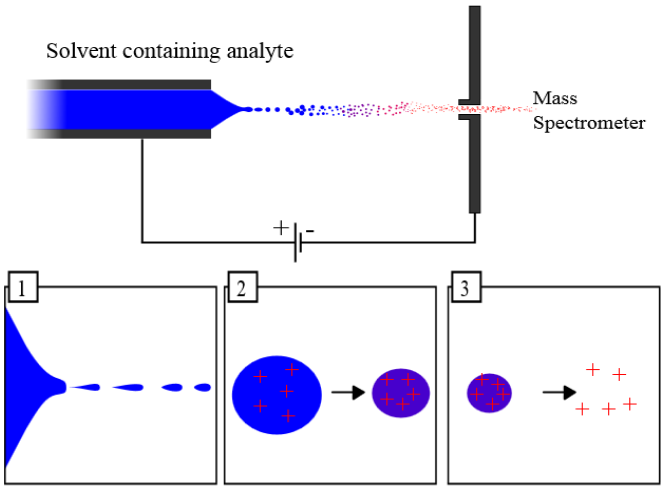


Figure 1: Schematic of positive mode electrospray ionization
The Taylor cone shown in 1 is produced from a charge differential between the capillary and mass spectrometer and rapid desolvation in 2 decreases droplet size until the analytes are converted into gas phase in 3 by coulomb fission and evaporation (72)

The constant jet of liquid ejected from the Taylor cone breaks down spontaneously into smaller droplets as it travels toward the analyzer (73). Each drop will undergo multiple consecutive coulomb fissions. This occurs from solvent evaporation when the point which the surface tension is exceeded by the coulomb force of repulsion from like charges on the surface, also known as the Rayleigh limit (**Equation 1**).

$$q = 8\pi\sqrt{\varepsilon_o\ \gamma\ R^3} \qquad \textbf{Equation 1}$$

q is the charge on the droplet
ε_o is the electric permittivity
γ is the surface tension of the droplet
R is the radius of the droplet

Solvent evaporation and coulomb fissions proceed until the solvent is completely evaporated. If there are large compounds present, they will receive the remaining surface charges, leading to the formation of multiply charged species as per the charged residue model (CRM) shown in **Figure 2** (74, 75). The ion evaporation model (IEM) depicted would occur for the majority of low mass compounds, such as pharmaceuticals and pesticides (76-78). Additionally, Konermann *et al*. have proposed a new chain ejection model (CEM) for the exploration of disordered polymers, such as unfolded proteins (75). As the Rayleigh limit is surpassed in the IEM each released droplet is likely to contain an analyte of interest.

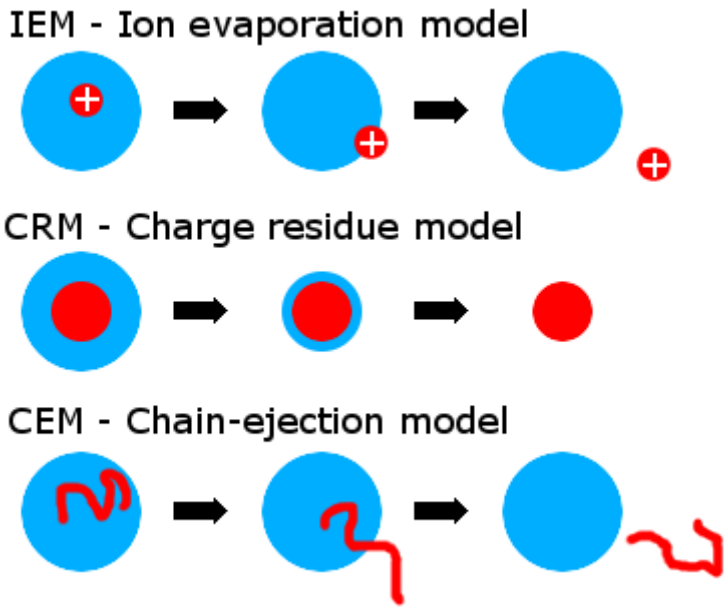


Figure 2: Electrospray ionization mechanism for the generation of intact gas-phase ions
Analytes shown in red are evaporated out of the solution shown in blue through IEM, CRM and CEM (79)

1.3.2 Atmospheric Pressure Chemical Ionization

APCI is nearly identical to ESI, with the main difference being the positioning of the electric field. First, the sample and mobile phase are nebulized and desolvated using applied heat and a gas flowing around the capillary, as shown in **Figure 3**. **C**onsequently, this ionization technique is particularly useful with thermally stable, low mass compounds. Next, a corona discharge needle ionizes the gas and solvent mixture producing ions such as hydronium H_3O^+ and N_4^+ (80). This occurs from the high positive current on the needle flowing into the air creating plasma around the electrode. The generated ions will pass charge to nearby areas of lower potential through collisions.

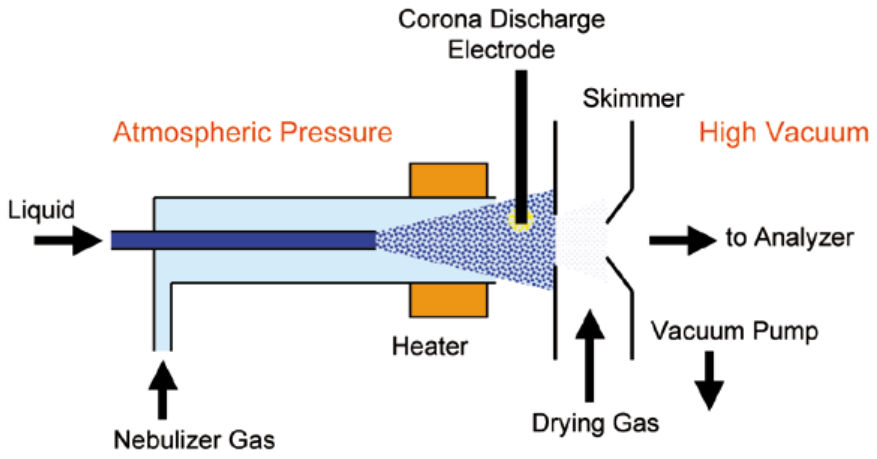


Figure 3: Atmospheric pressure chemical ionization schematic
The liquid containing the mobile phase and sample is nebulized and desolvated prior to ionization by the corona discharge (81)

APCI is suited for analysis of a narrower range of compounds than ESI, though it is generally accepted as having decreased susceptibility to matrix effects (69, 82, 83). The potential for improved ionization of analytes improves compound signal by limiting charge competition. This is a direct result of ESI applying current and heat simultaneously resulting in charged droplets rather than the initial desolvation prior to charging as with APCI. With the analysis of complex environmental matrices, these droplets contain a significant amount of impurities, resulting in greater variances (82). The potential for an analyte to be ionized is based on the highest charge affinity of the different eluting species (83). APCI employs desolvation prior to ionization allowing increased target analyte responses by decreasing the competition for charge. This competition is generally accepted as the primary mechanism of signal suppression or enhancement (SSE), which inhibits reproducibility and hinders quantitation (69, 82, 83).

1.3.3 Mass Spectrometry

Mass spectrometry is the gold standard for qualitative and quantitative compound detection, based on separation by mass-to-charge ratios (*m/z*). Mass measurements are in a standardized, unified atomic mass unit also known as Daltons (Da). A single Dalton is

equal to $1/12^{th}$ the mass of a free carbon 12 atom, absent of excitation, approximately $1.66x10^{-27}$ kg (84). Small compounds are often singly charged, whereas due to instrument limitations larger compounds often require multiple charges to reduce the *m/z* to be within the analyzer range. Mass spectrometers are versatile and can be tailored for particular experimental goals. They range from a simple quadrupole with an electron multiplier for single *m/z* detection to a high-resolution mass spectrometer (HRMS) capable of collecting and distinguishing compounds across a large *m/z* range. While electron multipliers amplify and count the analytes transmitted by the quadrupole, HRMS is able to fully resolve analytes including isotopes with a difference of 1 Da.

Resolution is a term used to describe an instrument's power to resolve between two peaks. The International Union of Pure and Applied Chemistry (IUPAC) describes resolution as the experimental *m* of an analyte peak with singly charged ions divided by the *m* change across the peak at full width and at half its maximum height (FWHM) as seen in **Equation 2** and **Figure 4**. Similarly, resolving power R_p can be described as the *m* divided by the difference between the *m* of the two separate peaks as seen in **Equation 3** and **Figure 4**. HRMS instruments including time-of-flight and Orbitrap mass analyzers are capable of resolution near 50,000 and over 100,000 (FWHM) respectively. Triple quadrupole are considered low resolution instruments with a maximum of 1,000 (FWHM). This improved resolution is particularly important for experiments where it is impossible to select all analytes of interest prior to analysis.

$$Resolution = \frac{m}{\Delta m} \qquad \textbf{Equation 2}$$

$$Resolution = \frac{m}{m_1 - m_2} \qquad \textbf{Equation 3}$$

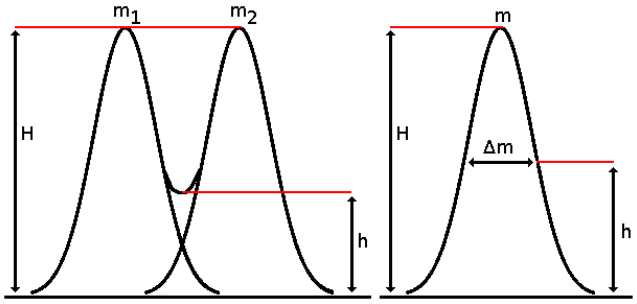


Figure 4: Mass spectrometry resolving power and resolution
The depiction shows resolving power on the left between two peaks and resolution on the right of a single peak using FWHM (85)

The Q-Exactive™ Orbitrap HRMS (**Figure 5**) desorbs and ionizes analytes with atmospheric pressure ion sources. Ions are captured using an S-lens as they enter the vacuum, which focuses the ions to increase sensitivity. A bent flatapole reduces noise by preventing neutrals from entering the quadrupole, thereby improving robustness. An initial quadrupole ion filter transmits the analytes that are within an acceptable range of the selected mass. Optionally selected fragmentation occurs in a separate higher-energy collisional dissociation cell (HCD) and a collection trap accumulates the analytes (C-trap). Ion cooling occurs before and after entering the HCD cell throughout the run. Finally the ions are injected into the Orbitrap mass analyzer which measures the signal over time and converts to *m/z* using the calculated frequency from a Fourier transform (86).

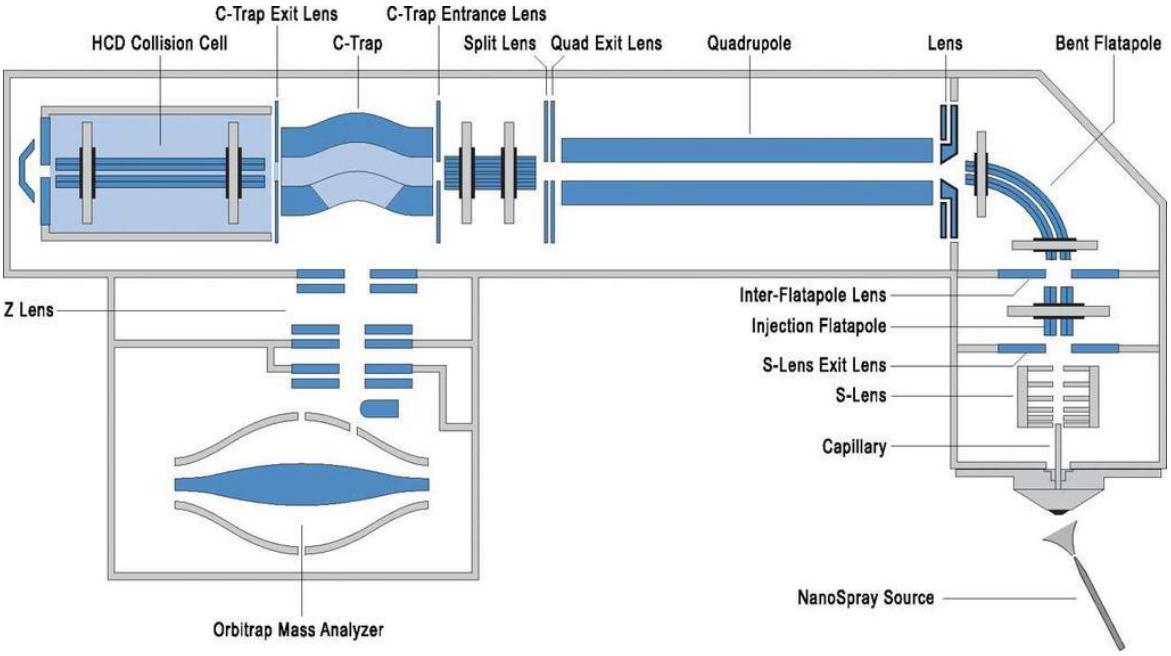


Figure 5: Thermo Fisher Scientific Q-Exactive™ Orbitrap mass spectrometer schematic

1.3.3.1 Quadrupole Mass Filter

Traditional bioanalysis of environmental samples incorporates the use of a triple quadrupole (QqQ) mass analyzer for targeted quantitation. Quadrupoles can function as a mass filter, ion trap and collision cell. Ions are controlled by applying time independent direct current (dc) and time-dependent alternating current (ac) by radio frequency (rf) voltages across four parallel rods (87). The rods can be visualized using a three dimensional XYZ axis (Error! Reference source not found.), where ions travel the Z plane in parallel to the length of the rods. The rods in the X and Y plane are separate pairs where, each pair has either a positive or negative charge that are of equal magnitude and rf voltages 180^{o} out of phase with the other pair (87, 88). The high and low mass limits are set by the X and Y-axis quadrupole pairs, respectively, for negative ions (**Figure 6**). Therefore, the X-axis rods will have an oscillating rf voltage to stabilize low *m/z* ions being attracted by the dc voltage. The Y-axis rods with the same charge will repel the low *m/z* ions and the rf will induce destabilization, forcing ions to collide with a rod and become neutralized. Importantly, the rf is proficient at affecting small *m/z* ions, while

large ions with low charge will be directed mostly by the dc field. High *m/z* ions are stabilized by Y-axis rods with the same charge and destabilized by the X-axis rods with opposite charge.

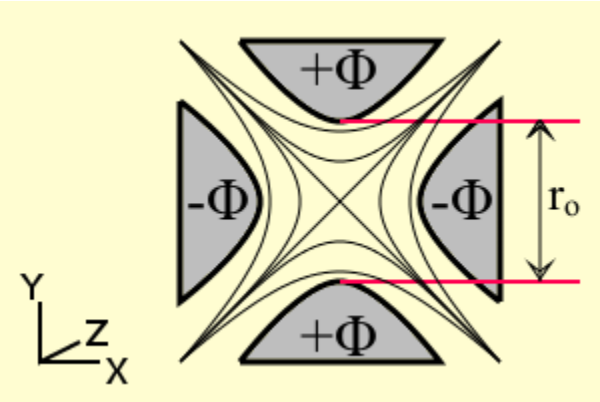


Figure 6: Quadrupole mass filter schematic
The pairs of electrodes are used for transmission of charged analytes through the channel with a small r_o (89)

The four rods select ions by setting high and low mass filters that determine the *m/z* range of ions stable enough to pass through. By adjusting electrical parameters, a range of *m/z* window selections can be scanned across a range to acquire full mass spectra of a sample. In QqQ, the second quadrupole can be used to transmit ions or as a collision cell for quantitation experiments using tandem MS. This role is often replaced by a hexapole to allow for improved transmission at higher collision energies (90). The third quadrupole in tandem MS is used to select a daughter/fragment ion from the previously transmitted parent/precursor ions in a technique known as selected reaction monitoring (SRM). Quantitative analysis requires a quantifier (Quan) and qualifier (Qual) fragment ion that came from the previously selected parent/precursor ion to be acquired for confident identification known as multiple reaction monitoring (MRM).

When using HRMS for analyte quantitation, the main difference is that the final quadrupole is replaced with an Orbitrap. With this change, all daughter ions created in the HCD cell are acquired instead of selecting Quan/Qual ions prior to analysis. This is known as parallel reaction monitoring (PRM), where the Quan and Qual ions used for

quantitation are selected and analyzed post acquisition. This allows for a change of selection due to interference without re-running samples. PRM is more specific than SRM due to additional product ions and it has shown similar linearity, precision and repeatability for quantitation, though there is a loss of sensitivity from the QqQ setup due to interfering ions (91-93).

1.3.3.2 C-trap and HCD Cell

The C-trap, as shown in **Figure 5**, is positioned parallel to the quadrupole and HCD cell and perpendicular to the Orbitrap. The ions transmitted by the quadrupole are held in the C-trap using rf voltage until the duty cycle is complete, where they are cooled by a gentle stream of inert nitrogen to prevent internal collisions. The maximum number of ions that can be stored in the C-trap prior to each scan is set as the automatic gain control (AGC) and an injection time is also set for when the AGC is not reached. The resolving power chosen is directly proportional to the scan time and the injection time is set by interpreting how long the analyzer will require to complete the duty cycle.

Once the duty cycle is complete and the C-trap is sufficiently filled, all the collected ions are sent for analysis following optional fragmentation in the HCD cell. This allows for multiplexing capabilities where multiple ions are selected then simultaneously fragmented and analyzed. HCD is a type of collision induced dissociation (CID) specific to the Thermo Scientific Orbitrap instrument. Ions are accelerated out of the C-trap into the HCD cell, where an inert gas such as nitrogen is used to convert the kinetic energy of the ions into internal energy via collisions (94). The amount of energy imparted on the ions set prior to experimentation is known as normalized collision energy (NCE), which is altered internally so that larger *m/z* ions receive higher energy. Following fragmentation, ions are transferred through the C-trap to the Orbitrap.

1.3.3.3 Orbitrap Mass Analyzer

Based on ion trap technology developed in the early twentieth century by Kingdon (95), the Orbitrap operates by trapping ions between a central spindle electrode and an outer barrel-like electrode (96). Ions travel along the central electrode while spiraling around it, becoming packaged based on *m/z* (97). The *m/z* values are measured based on frequency

of the oscillations. The time-domain current transients of the ions are converted to *m/z* using fast Fourier transforms (FFT), producing a mass spectra. The Orbitrap has a large dynamic range; it is capable of measuring large mass compounds up to 6000 *m/z* and can achieve significant mass accuracy due to ion frequency being independent of energy and spatial spread (98).

The high mass accuracy of the Orbitrap in the parts-per-million (ppm) range allows improved non-target screening. As described in **Equation 4**, mass accuracy is defined as the error of the experimental mass to the theoretical exact mass. Attempting to elucidate the structure of an unknown compound requires exceptional mass accuracy to resolve from other highly similar compounds. In order to obtain a reasonable molecular formula, the minimum mass accuracy required is 5 ppm (99). The Orbitrap can achieve low ppm mass accuracy, whereas a QqQ can only resolve a difference of about 0.1 da (100 ppm). With high mass accuracy, molecular formulae can be deciphered by interpreting isotopic ratios, adducts and mass defects. Mass defect is the difference between experimental mass and nominal mass and can reveal the presence of atoms with a high mass defect such as sulfur (100).

$$mass\ accuracy = \frac{experimental\ mass - exact\ mass}{exact\ mass} \qquad \textbf{Equation 4}$$

1.3.3.4 Non-Targeted Screening

Techniques for non-targeted analysis using fragmentation of all or most of the ions in a sample are becoming popularized on HRMS instruments. This trend began late in the twentieth century to push limits of discovery in ‘omics’ fields such as, proteomics and metabolomics (101-103). The goal in non-targeted screening is to get a full view of what is in a sample. With environmental analysis, the use of a non-targeted screen allows for a chemical profile of the area of interest to be built and the spectra to be stored for retrospective analysis.

All ion fragmentation (AIF) is a method used to simultaneously fragment all precursor ions across a large *m/z* range. This acquisition method is arguably the most broadly encompassing, as half of the spectrometer's duty cycle is spent detecting ions, while the other half is spent detecting their fragments. However, AIF is limited as it produces a surplus of data with a high difficulty of compound elucidation. Data dependent acquisition (DDA), on the other hand, is limited to fragmenting individual precursor ions above the selected threshold abundance. It is particularly useful if the main compounds of interest are expected to be at a high concentration, as it allows for simple detection and quantitation. DDA and AIF however are not capable of analyzing trace compounds.

Data independent acquisition (DIA) is similar to AIF in that it allows nearly all ions to enter the HCD for fragmentation, but it is designed with the capabilities of the C-trap in mind. The quadrupole transmits smaller ranges of precursor ions for co-fragmentation; this is repeated to acquire the entire mass range throughout the run. DIA is the only option for environmental screening of trace compounds because the low levels would be missed by DDA and impossible to link precursor and fragment using AIF. Using peptides, this technique is demonstrated in **Figure 7** (104). In this example the *m/z* range is 500-900 and fragmentation is completed on 16 windows in 25 *m/z* sections.

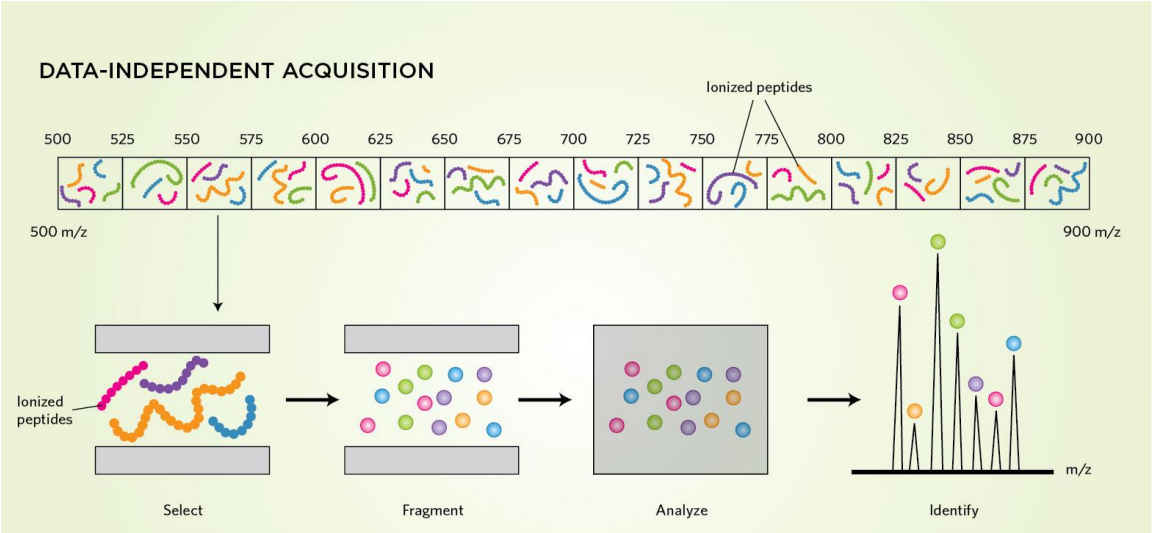


Figure 7: Data-independent acquisition
Using peptides the large mass range of *m/z* 500 – 900 is broken into windows of *m/z* 25 that are separately transmitted to the collision cell for fragmentation (104).

1.4 Rationale and Aim

As the field of analytical chemistry is advanced by improved instrumentation we need to continue to adjust our approach for bioanalytical investigations. Developing non-targeted screening methods for the analysis of surface water allows for an increased data acquirement from an initial investment. The aim of this work was to utilize the DIA methods discussed and tailor it toward improving environmental monitoring. Similarly, targeted methods traditionally used for quantitation were optimized for contaminants of particular concern to produce a survey of a previously unstudied Canadian watershed. Through this work we will show the power of retrospective analysis of databased spectra and its ability to allow for confident detection and identification of compounds present without the requirement of analytical standards. This technique will provide improved method development by targeting pharmaceuticals and pesticides detected in the environment of interest.

Chapter 2

2 Comprehensive Screening of Environmental Contaminants in Surface Waters by Non-Targeted LC-MS and Quantitation on a Q-Exactive Orbitrap

Parts of this chapter will be published in:

Kyriakos Manoli, **Lucas M. Morrison**, Mark W. Sumarah, George Nakhla, Virender K. Sharma, Ajay K. Ray. *Pharmaceuticals and pesticides in secondary effluent wastewater: Identification and enhanced removal by acid-activated ferrate(VI)*. Water Research. Submitted

Morrison, L. M., Renaud, J. B., Yeung, K. K-C., Sumarah, M. W. *Negative Mode Non-Targeted Screening and Retrospective Analysis of Trace Surface Water Contaminants*. Journal to be determined. In progress.

2.1 Abstract

Chemical contaminants in environmental samples are often detected by targeted LC-MS/MS screening techniques. This requires prior knowledge of the contaminants of concern in the area of interest. Recent developments in high-resolution mass spectrometry has allowed for the advancement of non-targeted scanning techniques and retrospective analysis. Using positive mode data-independent acquisition (DIA), samples are analyzed by three independent 6 minute runs for high, medium and low mass ranges. Micro pollutants can then be identified by retention time, accurate mass, isotope pattern and product ions. The datasets are than archived to allow for emerging contaminants of interest to be detected retrospectively.

Using this DIA approach, samples are screened against an in-house library produced from hundreds of analytical standards. Detected compounds are then targeted using parallel reaction monitoring (PRM) for quantitation; DIA can be difficult to achieve enough scans for trace analysis, particularly in complex matrices. Accurate quantitation requires method recovery, limitation and linearity to be determined. Recoveries can be optimized by altering extraction and analysis techniques for particular compounds. These experiments are costly and time consuming, which again points to the need for a screening protocol prior to implementation of a targeted analysis to ensure the compounds are indeed present in the subject area.

Even with these combined methods, compounds will be missed if they are absent from the in-house library. In order to achieve comprehensive screening, an open source data analysis tool, XCMS can be used to detect constituents that stand out in a group of samples (105). Using this technique a previously unidentified compound was detected at high intensities in samples from a particular site. The compound is a metabolite of the chlorpyrifos insecticide known as 3,5,6-trichloro-2-pyridinol, which requires negative mode ionization and was therefore undetected by the current DIA method. This prompted the development of a negative screening mode which analyzes samples by two 6 minute runs which encompasses low and high mass ranges.

2.2 Introduction

Considerable resources go toward environmental monitoring and water protection projects in Canada yearly as there is growing concern of possible health risks (106-112). The public attention on drinking water quality has become amplified since recent waterborne infection outbreaks in Walkerton and North Battleford (113). The dialogue has since moved from concentrating solely on microbiology to including chemical contamination (114, 115). In August 2014, the town of Toledo Ohio had undrinkable tap water for more than 2 days due to an unmonitored chemical (microcystins) naturally produced by algae blooms (116). The South Nation watershed has previously been extensively studied for pathogens (106-109). Nevertheless, the work presented in this thesis was the first chemical survey of the South Nation watershed.

Water quality protection is a constant concern that requires accurate detection of contaminants of emerging concern (CEC). Health Canada has guidelines for many CEC, including pesticides; however, the United States Environmental Protection Agency (USEPA) has a more extensive list of CEC guidelines (117, 118). The analysis of environmental samples for the presence of contaminants typically relies upon targeted analyses produced from reference standards (119-125). This requires prior knowledge of the pollutants in the area that are concerning to the ecosystem and nearby populations. High-resolution mass spectrometry (HRMS) instruments such as time-of-flight and Orbitrap detectors allow for accurate-mass measurements that improve screening techniques for trace CEC (119, 126).

Full scan mode is often used as the main technique for screening a sample and accurate-mass measurements of HRMS improves compound detection using this method. Fragmentation has been shown to be required for many sample analyses, such as compound identification; particularly, when there is high potential for analyte interference from background ions with nearly identical *m/z* (127-129). Methods have been investigated using HRMS for non-targeted and semi-targeted screening with fragmentation, including all-ion fragmentation (AIF), data-independent acquisition (DIA) and data-dependent acquisition (DDA). These screening techniques are advantageous to

analyze samples by multiple ionization and acquisition modes, without modifying the instrument.

The initial screening technique using DIA is a published method that runs each sample three times based on the c-traps ability for ion collection (64). The runs are sectioned into low, medium and high mass ions with the *m/z* windows set at 128-351, 349-651 and 649-1051 respectively. The mass ranges for the three runs overlap so that any ions at the upper and lower ends of the range can be sufficiently scanned. The limits were chosen based on the size range of compounds present in environment (64). Resolution is set at 17500 to allow more scans as there is limited time for analysis in each of the runs. The low mass range is the tightest since more ions are expected in this section. Throughout each of the three runs the quadrupole is set to transmit even smaller ranges of *m/z* to the c-trap known as windows. All the ions collected from the window in the c-trap are then sent to the HCD cell for fragmentation prior to analysis (**Figure 8**).

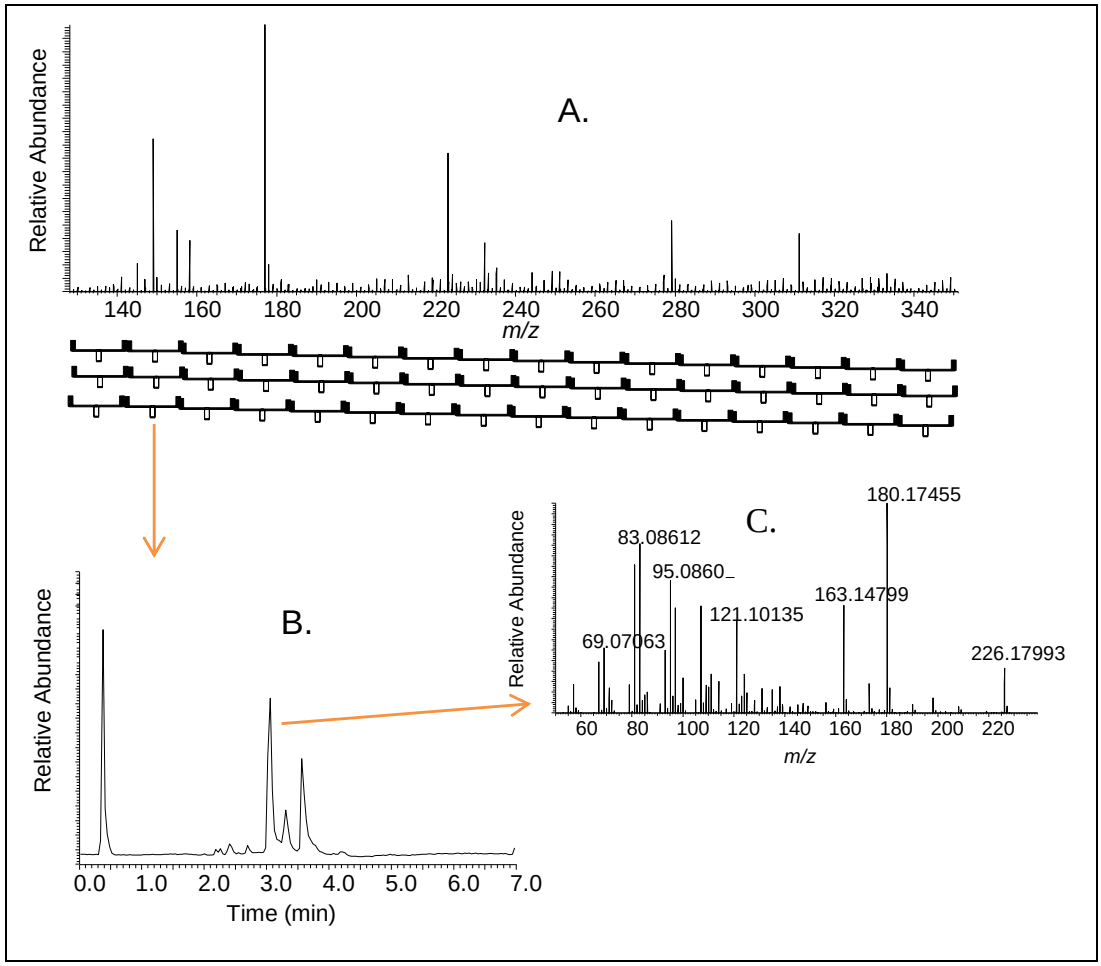


Figure 8 Diagram of the DIA workflow
A. the mass windows selected for the quadrupole are sequentially scanned throughout the run giving B. the chromatogram of peaks that contained precursor masses in said window which are C. sent to the HCD cell for fragmentation before the Orbitrap analyzer

Quantitation of analytes can be difficult in biological matrices due to variations between samples. Studies have shown that in order to determine true recovery of analytes it is important to assess multiple sample sources by spiking the analytes of interest (130). There are many techniques for improving recovery, including altering the extraction procedure, clean up steps to remove matrix interferences, changing chromatographic conditions, using internal standards (INST) and changing the ionization source interface between the LC and MS. In this study the goal is to extract the majority of contaminants

and store data for retrospective analysis and comparisons. This means altering extraction protocols is difficult since it could comprise any ability to compare between sites across years. Optimizing chromatography was employed to improve quantitation and parallel reaction monitoring (PRM) allowed for improved scanning across the separation.

Using these developed methods, two additional studies were conducted on secluded Lac Hughes near Montreal and on local wastewater treatment plants (WWTP) in London. Lac Hughes is a unique site of analysis, as it represents a virtually agriculture free pool of water and the interpretation value is increased for this reason. Additionally, the human influence is limited to septic tanks as there is no municipal treatment. Conversely the WWTP study was hypothesized to have many sources of CEC that were compared between the two sites of study (Adelaide and Greenway). This work was in collaboration with a chemical engineering group to not only survey and quantify the chemical composition of the sites but also accurately measure the degree of a novel oxidation process to remove organic pollutants.

Finally, this chapter involved the use of the above DIA screening for retrospective analysis and extending the method to more completely survey analytes in a sample. This involved the analysis of multiple samples for the presence of compounds using accurate precursor mass and fragments from an online spectral database. Additionally, a metabolomics approach was taken to study a variety of samples for contaminants which were missed in the screen. This prompted the development of a negative mode DIA method to encompass these missed compounds in future analyses.

2.3 Experimental methods

Quantitation using INST is the most accurate method of quantitation and provides assurance that the method continues to be reproducible. This involves spiking samples with labelled analytes prior to extraction. Compounds are often labelled by deuterium in place of hydrogen or by C_{13} in place of native C_{12} atoms. These labelled standards can be quite expensive and it is excessive to use one for each compound in a large scale

environmental screening study. Alternatively, external calibration curves can be used by spiking a range of relative levels for the compounds of interest into a matrix matched solution. The majority of analyses in this chapter were completed using external calibrations for quantitation with the addition of a few INST related to the particular study.

2.3.1 Solid Phase Extraction (SPE)

Each SPE cartridge requires conditioning using methanol to fill (5 mL) the cartridge twice, as it can penetrate the bonded alkyl groups and is water miscible. The second step is to equilibrate the cartridges in the same manner using water with the correct pH for the sample being extracted. At this point the 200 mL sample can be loaded using the vacuum set up depicted in **Figure 9**, which constantly pulls sample from the flask reloading the SPE cartridge as the sample passes through the solid phase.

Extraction was completed on two 200 mL aliquots of each sample using Waters Oasis HLB 6 cc 200 mg solid phase extraction cartridges (Milford, MA, USA). The first aliquot was adjusted to a neutral pH of 6.5 ± 0.02 using formic acid, ACS reagent (Sigma-Aldrich, St. Louis, MO) and the second was acidified to a pH of 2.0 ± 0.02 using hydrochloric acid. Ammonium hydroxide reagent was used when the pH was below the target. Finally, the sample is connected and the vacuum system initiated and monitored for a rate of 1 drop per second (**Figure 9**).

The loaded compounds are then eluted into 15 mL polypropylene conical tubes (Corning Science, Mexico, S.A.) using three fractions of 1.5 mL of methanol. The final combined volume of 4.5 mL is then dried under a gentle flow of nitrogen in a water bath set at 45^{o}C (Meyer N-Evap, Organomation Associates Inc., Berlin, MA, USA). Reconstitution is completed using 300 µL of methanol followed by 100 µL of LC-MS grade water. The combination is mixed using a Vortex-Genie 2 model G-560 (Scientific Industries, Bohemia, NY) and transferred to amber HPLC grade vials (Agilent Technologies, Santa Clara, CA) for storage at -20^{o}C. Prior to analysis, 75 µL of the combined fractions is transferred to an amber HPLC grade vial with 250 µl glass inserts and diluted to 150uL

using an 80:20 water to methanol solution to produce a half and half mixture, resulting in a 250 fold enrichment.

Figure 9: SPE setup for three samples
Volumes measured in volumetric flasks on the left and using vacuum pressure to allow a controlled interaction with the stationary phase (1 drop/sec)

2.3.2 Chromatography Conditions

All chromatographic separation was achieved on an Agilent 1290 infinity high-performance liquid chromatography (HPLC) system with a Zorbax Eclipse Plus RRHD C18 rapid resolution HD column (2.1 × 50 mm, 1.8 µm particle size; Agilent Technologies) and an Eclipse Plus C18 guard column (2.1 × 5 mm, 1.8 µm particle size; Agilent Technologies) maintained at 35 °C. The mobile phase was comprised of 0.1% formic acid (>99% purity, Thermo Scientific, Waltham, MA) in water (A) (Optima grade, Fisher Scientific, Lawn, NJ, USA) and 0.1% formic acid in acetonitrile (B) (Optima grade, Fisher Scientific, Lawn, NJ, USA). Chromatography for the DIA screening (**Figure 10**) was: 100% A held from 0–0.5 min, 100%-0% A from 0.5-3.5 min, held at 0% A from 3.5-5.5 min and 0%-100% A from 5.5-6 min. The injection volume was 5 µL and the flow rate was 0.3 mL/min.

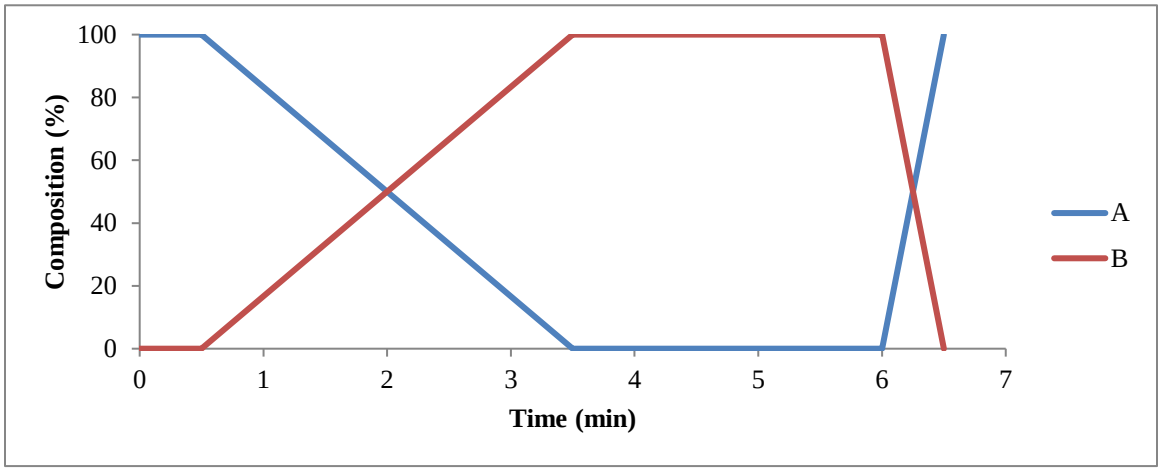


Figure 10: Chromatographic separation for the DIA screening method
A is water with 0.01% formic acid (FA) and B is acetonitrile with 0.01% FA

The screening method discussed previously must remain unaltered to allow for comparisons across samples and to the library. The 6 minute run consists of a simple 3 minute gradient from 0-100% acetonitrile. The library used for comparison was prepared by running analytical standards by the same method for ‘fingerprint’ retention times and

fragmentation. This database has been produced and maintained by many contributors at the Agriculture and Agri-Food Canada London Research facility as new compounds have been purchased and studied.

Following the identification of compounds in a wide variety of samples studied, a targeted PRM method was developed to allow for quantitation of the identified compounds while keeping in mind that more compounds may need to be added. Standards stored at the facility were accurately weighed and two master mixes were prepared; one mix with the 34 pharmaceuticals and the other with the 40 pesticides. The chromatographic separation then needed to be optimized to allow for sufficient scanning of each compound.

The finalized targeted method involved a 13 minute separation with a gradient from water to acetonitrile over 10.5 minutes involving sections of nearly isocratic elution between 0.7 – 3 minutes where a large number of compounds elute (**Figure 11**). The mobile phase was comprised of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The full chromatography was: 100% A held from 0–0.5 min, 100%-70% A from 0.5-0.7 min, 70%-65% A from 0.7-3 min, 65%-0% A from 3-10 min, held at 0% A from 10-12 min and 0%-100% A from 12-13 min.

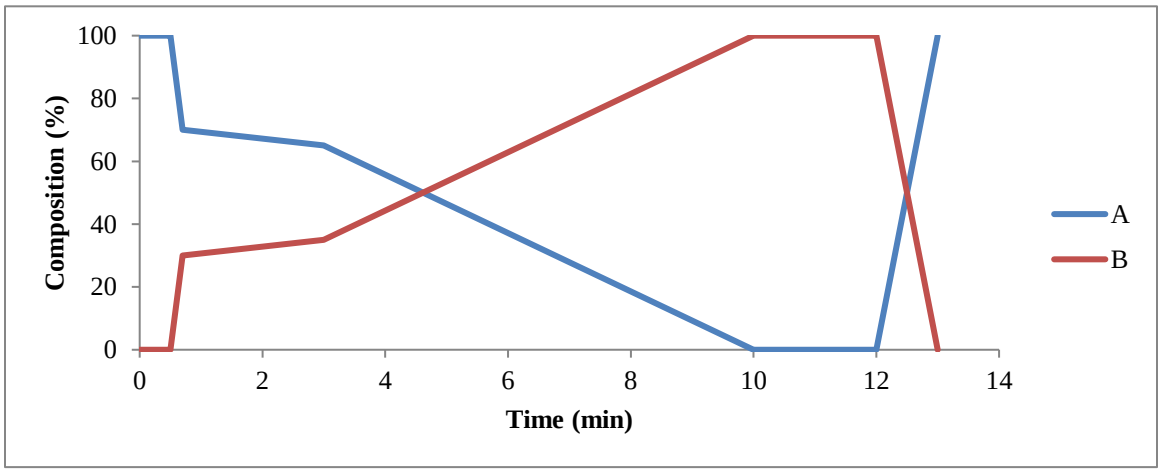


Figure 11: Chromatographic separation for the targeted method Used to quantify both pharmaceutical and pesticide contamination in surface water samples, where A is water with 0.01% formic acid (FA) and B is acetonitrile with 0.01% FA

2.3.3 Mass Spectrometry Conditions

All MS data in this chapter was obtained using a Q-Exactive™ Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA), coupled to a heated electrospray ionization (HESI). HESI settings were: capillary temperature, 400 °C; sheath gas flow rate, 19 arbitrary units; auxiliary gas flow rate, 8 arbitrary units; probe heater temperature, 450 °C; S-lens RF level, 45%; and capillary voltage, 3.9 kV.

The resolution was kept at 17500 and injection time was set to 64 seconds with an automatic gain control (AGC) of 3 X 10^6 maximum ion population in the c-trap and 1.2 m/z isolation windows. Peak integration was completed using Xcalibur® software Genesis peak detection algorithm.

2.3.4 Detection

The screening technique using positive mode DIA is based on a published method that runs each sample three times based on the c-traps ability for ion collection (128, 131). The NCE was set to 35 in order to achieve sufficient dissociation of some very stable compounds with the caveat that some more fragile molecules will knowingly become obliterated and likely result in less than ideal fragmentation patterns. The low range has been shown to contain a large number of compounds resulting in a smaller range and tight *m/z* windows of 11.3 whereas the other two ranges are larger and *m/z* windows are set to 15.3 for mid and 20.3 for high (64). Each window overlaps by a *m/z* of 0.3, based on a mass defect study of common pharmaceuticals, to limit ions at the edge of the quadrupole range (64). Each sample scanned by DIA is compared to a database of approximately 300 analytical standards (**Appendix 1**) for identification by accurate mass, retention time and fragment masses.

An optimized method for quantitation using PRM was developed based on an initial screening from a variety of sites in the South Nation watershed. The quadrupole was set to select 34 pharmaceuticals or 40 pesticides. These specific precursor ions are then sent to the HCD cell for fragmentation. The limitation on the ions entering the c-trap improves the ability to achieve sufficient scanning on each analyte. The monitored ions, retention times and specific collision energies for each compound are listed in **Table 1**.

Table 1: Liquid chromatography-mass spectrometry parameters for parallel reaction monitoring
Used for quantitation of pharmaceuticals and pesticides identified in the South Nation watershed and the labelled internal standards used in routine analysis

Pharmaceutical	Formula	RT	Ion	*m/z*	NCE	Quan	Qual
albuterol	$C_{13}H_{21}NO_3$	1.72	$[M+H]^+$	240.1594	29	148.0758	166.0864
amitriptyline	$C_{20}H_{23}N$	3.36	$[M+H]^+$	278.1905	48	233.1322	91.0547
amitriptyline-D_6	$D_6C_{20}H_{17}N$	3.36	$[M+H]^+$	284.2300	48	233.1326	91.0548
atenolol	$C_{14}H_{22}N_2O_3$	1.74	$[M+H]^+$	267.1703	46	145.0649	190.0862
azithromycin	$C_{38}H_{72}N_2O_{12}$	2.04	$[M+2H]^{2+}$	375.2615	15	83.0498	591.4214
bacitracin	$C_{66}H_{103}N_{17}O_{16}S$	2.19	$[M+3H]^{3+}$	474.9235	16	669.3391	227.0852
celecoxib	$C_{17}H_{14}F_3N_3O_2S$	6.87	$[M+H]^+$	382.0831	56	282.0958	362.0762
celecoxib-D_7	$D_7C_{17}H_7F_3N_3O_2S$	6.87	$[M+H]^+$	388.1193	56	369.1206	289.1387
clarithromycin	$C_{38}H_{69}NO_{13}$	3.64	$[M+H]^+$	748.4841	17	158.1174	590.3898
clarithromycin N-metyl-^{13}C-D_2	$^{13}C\ D_2C_{37}H_{67}NO_{13}$	3.64	$[M+H]^+$	752.4998	17	162.1396	594.4117
cycloheximide	$C_{15}H_{23}NO_4$	2.84	$[M+H]^+$	282.1699	31	246.1489	229.1224
diclofenac	$C_{14}H_{11}Cl_2NO_2$	6.48	$[M+H]^+$	296.0240	24	250.0184	215.0494
diphenhydramine	$C_{17}H_{21}NO$	2.62	$[M+H]^+$	256.1695	19	167.0856	152.0619
doxycycline	$C_{22}H_{24}N_2O_8$	2.27	$[M+H]^+$	445.1605	33	428.1342	321.0741
enrofloxacin	$C_{19}H_{22}FN_3O_3$	1.99	$[M+H]^+$	360.1718	42	245.1083	316.1819
erythromycin a	$C_{37}H_{67}NO_{13}$	2.70	$[M+H]^+$	734.4685	16	576.3740	158.1175
gliclazide	$C_{15}H_{21}N_3O_3S$	5.68	$[M+H]^+$	324.1376	39	110.0966	127.1229
gliclazide-D_4	$D_4C_{15}H_{17}N_3O_3S$	5.68	$[M+H]^+$	327.1549	39	110.0968	127.1232
lincomycin	$C_{18}H_{34}N_2O_6S$	1.82	$[M+H]^+$	407.2210	25	126.1280	359.2177
melengestrol	$C_{23}H_{30}O_3$	7.18	$[M+H]^+$	397.2373	26	279.1746	337.2164
metsulfuron	$C_{13}H_{13}N_5O_6S$	3.99	$[M+H]^+$	382.0815	16	167.0562	141.0771
miconazole	$C_{18}H_{14}Cl_4N_2O$	5.30	$[M+H]^+$	414.9933	37	158.9764	69.0455
monensin	$C_{36}H_{62}O_{11}$	11.1	$[M+Na]^+$	693.4184	56	461.2881	501.3196
nortriptyline	$C_{19}H_{21}N$	3.25	$[M+H]^+$	264.1746	40	233.1328	91.05501
oxolinic acid	$C_{13}H_{11}NO_5$	2.79	$[M+H]^+$	262.0710	70	234.0400	244.0607
oxytetracycline	$C_{22}H_{24}N_2O_9$	1.96	$[M+H]^+$	461.1554	18	426.1183	444.1290
ractopamine	$C_{18}H_{23}NO_3$	1.96	$[M+H]^+$	302.1750	28	164.1070	121.0650
ranitidine	$C_{13}H_{22}N_4O_3S$	1.76	$[M+H]^+$	315.1482	27	176.0490	224.0981
salinomycin	$C_{42}H_{70}O_{11}$	10.92	$[M+Na]^+$	773.4810	37	431.2403	531.3291
sarafloxacin	$C_{20}H_{17}F_2N_3O_3$	2.08	$[M+H]^+$	386.1310	49	299.0991	342.1414
sertraline	$C_{17}H_{17}Cl_2N$	3.71	$[M+H]^+$	306.0810	41	158.9765	129.0702
spiramycin	$C_{43}H_{74}N_2O_{14}$	2.02	$[M+2H]^{2+}$	422.2642	22	174.1126	142.1228
sulfacetamid	$C_8H_{10}N_2O_3S$	1.87	$[M+H]^+$	215.0484	9	156.0114	108.0449
sulfamethazine	$C_{12}H_{14}N_4O_2S$	2.23	$[M+H]^+$	279.0910	44	204.0439	124.0873
sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	2.61	$[M+H]^+$	254.0593	35	156.0114	108.0448
thiabendazole	$C_{10}H_7N_3S$	1.90	$[M+H]^+$	202.0433	52	175.0326	131.0604
tylosin	$C_{46}H_{77}NO_{17}$	2.97	$[M+H]^+$	916.5264	24	174.1123	772.4470

warfarin	$C_{19}H_{16}O_4$	5.83	$[M+H]^+$	309.1121	27	163.0387	251.0698
Pesticide							
3-hydroxycarbofuran	$C_{12}H_{15}NO_4$	2.34	$[M+H]^+$	238.1073	10	181.0859	163.0753
acetamiprid	$C_{10}H_{11}ClN_4$	2.56	$[M+H]^+$	223.0742	47	126.0105	56.0503
aldicarb sulfone	$C_7H_{14}N_2O_4S$	2.00	$[M+Na]^+$	245.0566	54	109.0503	166.0714
ametryn	$C_9H_{17}N_5S$	3.14	$[M+H]^+$	228.1277	56	186.0799	96.0556
atraton	$C_9H_{17}N_5O$	2.23	$[M+H]^+$	212.1505	58	170.1028	100.0505
atrazine	$C_8H_{14}ClN_5$	4.56	$[M+H]^+$	216.1010	57	174.0532	96.0556
azoxystrobin	$C_{22}H_{17}N_3O_5$	6.29	$[M+H]^+$	404.1241	13	372.0973	344.1018
buprofezin	$C_{16}H_{23}N_3OS$	6.91	$[M+H]^+$	306.1634	24	201.1051	116.0529
butachlor	$C_{17}H_{26}ClNO_2$	8.63	$[M+H]^+$	312.1724	22	238.0988	162.1274
carbofuran	$C_{12}H_{15}NO_3$	4.14	$[M+H]^+$	222.1124	26	165.0910	123.0442
clothianidin	$C_6H_8ClN_5O_2S$	2.34	$[M+H]^+$	250.0154	33	169.0543	131.9671
clothianidin-D_3	$D_3C_6H_5ClN_5O_2S$	2.34	$[M+H]^+$	253.0348	33	172.0730	131.9671
cyanazine	$C_9H_{13}ClN_6$	3.59	$[M+H]^+$	241.0963	53	214.0842	132.0317
cyproconazole	$C_{15}H_{18}ClN_3O$	5.85	$[M+H]^+$	292.1211	36	70.0406	125.0152
cyprodinil	$C_{14}H_{15}N_3$	5.08	$[M+H]^+$	226.1338	80	93.0576	210.1024
cyromazine	$C_6H_{10}N_6$	1.66	$[M+H]^+$	167.1034	59	80.0512	125.0819
diethatyl-ethyl	$C_{16}H_{22}ClNO_3$	7.19	$[M+H]^+$	312.1361	17	238.0992	266.0942
dinotefuran	$C_7H_{14}N_4O_4$	1.88	$[M+H]^+$	203.1136	29	129.0897	114.1028
diphenamid	$C_{16}H_{17}NO$	5.44	$[M+H]^+$	240.1382	47	134.0959	167.0847
fenbuconazole	$C_{17}H_{17}ClN_4$	6.65	$[M+H]^+$	337.1214	42	125.0153	70.0406
fenfuram	$C_{12}H_{11}NO_2$	4.87	$[M+H]^+$	202.0862	50	109.0286	120.0445
fenpropimorph	$C_{20}H_{33}NO$	4.36	$[M+H]^+$	304.2634	59	147.1163	116.107
imazalil	$C_{14}H_{14}Cl_2N_2O$	3.12	$[M+H]^+$	297.0556	48	158.9762	69.0455
imidacloprid	$C_9H_{10}ClN_5O_2$	2.45	$[M+H]^+$	256.0590	31	209.0589	175.0979
imidacloprid-D_4	$D_4C_9H_6ClN_5O_2$	2.45	$[M+H]^+$	260.0847	31	213.0839	179.1229
metolachlor	$C_{15}H_{22}ClNO_2$	6.79	$[M+H]^+$	284.1411	22	252.1137	176.1425
metribuzin	$C_8H_{14}N_4OS$	3.74	$[M+H]^+$	215.0961	59	187.1003	131.0380
napropamide	$C_{17}H_{21}NO_2$	6.51	$[M+H]^+$	272.1645	31	129.1142	171.0794
octhilinone	$C_{11}H_{19}NOS$	6.02	$[M+H]^+$	214.1260	50	102.0011	57.0707
pirimiphos-ethyl	$C_{13}H_{24}N_3O_3PS$	8.44	$[M+H]^+$	334.1348	36	198.1060	182.1288
promecarb	$C_{12}H_{17}NO_2$	6.07	$[M+H]^+$	208.1332	12	109.0651	151.1117
propachlor	$C_{11}H_{14}ClNO$	5.22	$[M+H]^+$	212.0836	45	170.0365	94.0655
propazine	$C_9H_{16}ClN_5$	5.55	$[M+H]^+$	230.1167	58	146.0221	188.0688
pyracarbolid	$C_{13}H_{15}NO_2$	4.33	$[M+H]^+$	218.1175	46	125.0597	97.0288
schradan	$C_8H_{24}N_4O_3P_2$	2.17	$[M+H]^+$	287.1396	23	242.0816	135.0681
simazine	$C_7H_{12}ClN_5$	3.40	$[M+H]^+$	202.0854	65	132.0324	124.0871
tebuconazole	$C_{16}H_{22}ClN_3O$	6.49	$[M+H]^+$	308.1524	41	70.0407	125.0153
tebuthiuron	$C_9H_{16}N_4OS$	2.98	$[M+H]^+$	229.1117	52	172.0895	116.0275
thiacloprid	$C_{10}H_9ClN_4S$	2.99	$[M+H]^+$	253.0304	41	126.0105	90.0343
thiamethoxam	$C_8H_{10}ClN_5O_3S$	2.15	$[M+H]^+$	292.0260	17	211.0649	131.9670

thiamethoxam-D_3	$D_3C_8H_7ClN_5O_3S$	2.15	$[M+H]^+$	295.0454	17	214.0836	131.9667
trifloxystrobin	$C_{20}H_{19}F_3N_2O_4$	8.07	$[M+H]^+$	409.1369	19	186.0525	206.0812
triflumizole	$C_{15}H_{15}ClF_3N_3O$	6.55	$[M+H]^+$	346.0928	8	278.0555	73.0655

RT – Retention time
NCE – Normalized collision energy
Quan – Quantifier fragment ion
Qual – Qualifier fragment ion

2.3.5 Chemicals

Reference standards – 3,5,6-trichloro-2-pyridinol, acetamiprid, albuterol, amitriptyline, atenolol, azithromycin, bacitracin, celecoxib, clarithromycin, clothianidin, cycloheximide, diclofenac, dinotefuran, diphenhydramine, doxycycline, enrofloxacin, erythromycin a, gliclazide, imidacloprid, lincomycin, melengestrol, metsulfuron, miconazole, monensin, nortriptyline, oxolinic acid, oxytetracycline, ractopamine, ranitidine, salinomycin, sarafloxacin, sertraline, spiramycin, sulfacetamide, sulfamethazine, sulfamethoxazole, thiabendazole, thiacloprid, thiamethoxam, tylosin and warfarin were purchased from Sigma Aldrich (St. Louis, MO). NSI pesticide standard mixes 5, 8, 9 and 10 containing 3-hydroxy carbofuran, aldicarb, ametryn, atraton, atrazine, azoxystrobin, buprofezin, butachlor, carbaryl 9, carbofuran, chlorpyrifos oxon, cyanazine, cyproconazole, cyprodinil, cyromazine, diethatyl-ethyl, diphenamid, fenbuconazole, fenfuram, fenpropimorph, imazalil, isopropalin, metolachlor, metribuzin, napropamide, octhilinone, pirimiphos-ethyl, promecarb, propachlor, propazine, pyracarbolid, schradan, simazine, tebuconazole, tebuthiuron, trifloxystrobin, triflumizole were bought from NSI Lab Solutions (Raleigh, NC, USA). All standards were ≥ 98% pure.

Internal standards – amitriptyline-D_6, celecoxib-D_4, clarithromycin N-metyl-^{13}C-D_2 and gliclazide-D_4 were purchased from ALSACHIM (Illkirch-Graffenstaden, France); clothianidin-D_3, imidacloprid-D_4 and thiamethoxam-D_3 were obtained from Sigma Aldrich (St. Louis, MO). All INST standards were ≥ 98% pure.

Reagents and solvents – Hydrochloric acid (36.5%-38.0%) was purchased from Caledon Laboratory Chemicals (Georgetown, ON, Canada) and ammonium hydroxide ACS

reagent (28%–30 %) was acquired from Thermo Scientific (Waltham, MA, USA). Optima grade methanol and acetonitrile were bought from Fisher Scientific (Pittsburgh, PA, USA). Formic acid, ACS reagent (>96% purity) was purchased from Sigma-Aldrich, St. Louis, MO and Formic acid (>99% purity) was purchased from Thermo Scientific (Waltham, MA, USA). Whatman glass microfiber filters (GF/C; 1.2 µm pore size) were purchased from GE Healthcare Life Sciences (Buckinghamshire, UK). Reverse osmosis deionized water was produced by Thermo Scientific 18 MΩ-cm Barnstead Nanopure Water Purification System and used for SPE extraction preparation.

2.3.6 Standard spiking solutions

Each individual analytical standard was accurately weighed on a Mettler analytical balance AL54 (Mettler-Toledo Columbus, OH) and dissolved in methanol or water to a stock concentration of 1 mg mL^{-1}. Working solutions of unlabeled standards were prepared at 10 µg mL^{-1} with methanol for pharmaceuticals and pesticides separately. Daily mixes were prepared by combining the individual working solutions and diluting them with methanol to a final concentration of 100 ng mL^{-1}. These solutions were used for development of external calibration curves daily prior to analysis. Working solutions of labeled standards were prepared at 1 µg mL^{-1} with methanol and spiked at 50 ng L^{-1} in 200 mL water samples prior to extraction.

2.3.7 Quantitation

Peak area was integrated from PRM analysis on Xcalibur software using Genesis peak detection algorithm with 5 point smoothing and 50 baseline set for integration. External calibration was completed with calibration curves at the following concentrations: 0.005, 0.025, 0.1, 0.25, 0.625, 2.5, 25 and 50 ng mL^{-1}. INSTs were added to the standard curve at 12.5 ng mL^{-1} per level. Compounds with isotopically labeled standards were calculated using **Equation 5.** Whereas, the externally calibrated compounds are calculated the same way only without the INST, so it is just the equation for a straight line.

$$\frac{peak\ area_{\text{unlabelled}}}{peak\ area_{internal\ standard}} = slope\,\frac{[unlabelled]}{[internal\ standard]} + intercept \qquad \textbf{Equation 5}$$

In order for accurate quantitation the method must first be validated for each compound. Laboratory fortified sample matrix (LFSM) was prepared by spiking 200 mL of environmental water at quantifiable levels in triplicate prior to extraction. The same water sample was similarly analyzed for native levels of the compounds of interest in triplicate. Each sample was then processed as described in section **2.3**. Laboratory fortified blanks (LFB) were also analyzed by spiking an equal amount to LFSM into empty conical tubes in triplicate prior to nitrogen evaporation and processed by the remainder of the method described in **2.3.1.** Recovery efficiencies (R_E) were calculated using **Equation 6.**

$$R_E = \frac{[LFSM-Native]}{[LFB]} \qquad \textbf{Equation 6}$$

Instrumental limits were determined by extending the calibration curve to determine the lowest detectable and quantifiable level for each compound. The limit of detection (LOD) was determined as the lowest level that the compound produced a detectable peak from 5 consecutive injections by the Xcalibur® peak detection software in Genesis mode. The limit of quantitation (LOQ) was determined as the level at which the standard deviation between 5 injections was below 25%. Method detection limits shown in Table 2 were determined by applying the R_E for each compound to their respective LOD and LOQ. Method detection (MDL) and quantitation (MQL) were selected for the particular pH extraction which produced the best R_E for the individual analytes.

Table 2: Preferential extraction pH for each pharmaceutical and pesticide analyzed by the targeted quantitation and their respective method limitations

Pharmaceutical		MDL (ng L^{-1})	MQL (ng L^{-1})	Pesticide	pH	MDL (ng L^{-1})	MQL (ng L^{-1})
albuterol	pH6	0.05	0.23	3-hydroxycarbofuran	pH2	0.92	9.17
amitriptyline	pH2	0.16	0.82	acetamiprid	pH6	0.10	0.39
atenolol	pH6	0.88	0.88	aldicarb sulfone	pH6	2.08	2.08
azithromycin	pH2	1.13	5.66	ametryn	pH6	0.01	0.02
bacitracin	pH2	150.54	301.07	atraton	pH2	0.01	0.01
celecoxib	pH6	4.33	43.30	atrazine	pH6	0.02	0.10
clarithromycin	pH6	0.05	0.26	azoxystrobin	pH2	0.01	0.02
cycloheximide	pH2	48.82	48.82	buprofezin	pH2	0.13	0.13
diclofenac	pH2	1.12	4.48	butachlor	pH6	0.08	0.34
diphenhydramine	pH6	0.04	0.18	carbofuran	pH6	0.09	0.36
doxycycline	pH2	9.74	97.40	clothianidin	pH6	0.93	9.26
enrofloxacin	pH2	4.75	19.00	cyanazine	pH6	0.09	0.09
erythromycin a	pH2	1.18	4.73	cyproconazole	pH6	0.10	0.38
gliclazide	pH2	0.25	1.27	cyprodinil	pH2	0.12	0.49
lincomycin	pH6	1.04	1.04	cyromazine	pH6	0.64	1.61
melengestrol	pH2	1.15	1.15	diethatyl-ethyl	pH6	0.09	0.36
metsulfuron	pH6	0.04	1.09	dinotefuran	pH6	1.63	4.07
miconazole	pH2	0.08	0.40	diphenamid	pH2	0.01	0.01
monensin	pH6	0.27	54.85	fenbuconazole	pH2	0.42	0.42
nortriptyline	pH2	0.01	0.20	fenfuram	pH6	0.08	0.08
oxolinic acid	pH6	1.58	6.34	fenpropimorph	pH6	0.10	0.10
oxytetracycline	pH2	92.27	230.68	imazalil	pH6	0.14	0.14
ractopamine	pH2	0.38	0.38	imidacloprid	pH2	0.94	0.94
ranitidine	pH6	0.49	0.49	metolachlor	pH6	0.01	0.02
salinomycin	pH6	0.05	1.26	metribuzin	pH2	0.11	0.42
sarafloxacin	pH2	3.01	30.13	napropamide	pH2	0.01	0.02
sertraline	pH2	0.85	0.85	octhilinone	pH6	0.08	0.32
spiramycin	pH6	0.40	2.01	pirimiphos-ethyl	pH2	0.01	0.03
sulfacetamid	pH6	90.49	90.49	promecarb	pH6	0.81	2.01
sulfamethazine	pH6	1.08	1.08	propachlor	pH6	0.01	0.04
sulfamethoxazole	pH6	1.04	1.04	propazine	pH6	0.01	0.02
thiabendazole	pH2	0.36	1.80	pyracarbolid	pH6	0.10	0.10
tylosin	pH6	1.08	4.30	schradan	pH2	0.01	0.11
warfarin	pH6	0.04	0.21	simazine	pH6	0.11	0.11
				tebuconazole	pH6	0.01	0.10
				tebuthiuron	pH2	0.01	0.01
				thiacloprid	pH6	0.09	0.36
				thiamethoxam	pH6	1.13	1.13

	trifloxystrobin	pH6	0.02	0.11
	triflumizole	pH6	0.60	1.50

MDL – Method detection limit
MQL – Method quantitation limit

2.4 South Nation Watershed Survey

2.4.1 Sample Characteristics and Handling

The region of interest in this chapter is the South Nation watershed area located just north of the St. Lawrence River near Ottawa, ON. Its total area is 3,900 km^2, consisting of mostly flat land with tile drainage and groundwater allowing flow (109). Land use in this watershed is mixed-use, but primarily (60%) agricultural (i.e., cash crops, livestock, corn, soybean and forage cropping practices). This includes a series of rivers that have connection to both agriculture and urban influence, as well as putatively uncontaminated surface water, which is located upstream from farming. Additionally, a sampling site included, borders a reported organic, pesticide-free farming operation.

Previous studies in this area have concentrated on pathogens that pose a threat to human health and found that the pathogenic *L. monocytogenes* in this region showed resistance to many antibiotics, including lincomycin, erythromycin and penicillin (109). This has been hypothesized to be a result of antibiotics being overused by physicians and ineffective removal during WWT (32). The reduced effectiveness of current medicines will require improved treatment strategies to continuously be developed if environmental contamination of these compounds continues to be a concern.

Surface water samples were collected biweekly at six sites in 2016 between June and November and from 9 sites in 2017 between May and November. Samples were collected in sterile containers and shipped overnight on ice to Agriculture and Agri-Food Canada's Research and Development Centre in London, Ontario and immediately frozen at -20 °C. The samples were thawed at room temperature on the day of use and filtered through Whatman glass microfiber filters (GF/C), 47 mm diameter and 1.2 µm pore size (GE

Healthcare Life Sciences, Buckinghamshire, UK) to remove solid particulate prior to extraction.

2.4.2 Results and Discussion

The non-targeted screening method provided an overview of compounds that are at detectable levels in the area of interest by comparing to an in-house library of nearly 300 compounds (**Appendix 1**) from which 72 provisional identifications were made. The analysis found a large variety of contaminants in the South Nation watershed including 34 pharmaceuticals (**Table 3**) and 38 pesticides (**Table 4**). These trace CEC found in surface waters are often below drinking water guidelines. However, there is potential for long-term exposure to affect human health and it has been shown that the effects of antidepressants, pesticides and other CEC can negatively influence aquatic communities (7, 132-134).

Pharmaceuticals in surface water have become a topic of increasing concern as the prevalence of antibiotic resistance across multiple pathogens has emerged. Antibiotics are used immensely worldwide as the largest category of therapeutics and growth promoters in human and veterinary medicine (33). Focus for pesticide pollution commonly concentrates on compounds with acute toxicity or carcinogenic properties (7). However, the long half-lives of many compounds need to be taken into account when monitoring water contamination. Here, we have identified many compounds and produced a targeted PRM method in order to quantify many of the detected compounds as well as some other CEC of particular interest.

Table 3: Pharmaceuticals identified by positive mode data-independent acquisition Samples collected from multiple sites in 2016 and analyzed on four separate days to provide a qualitative analysis on the South Nations watershed.

Date Analyzed	Oct 12			Nov 18				Nov 25				Dec 9			
Samples →	July 18 Site C	July 18 Site A	July 18 Site B	Aug 30 Site D	Sept 12 Site D	June 20 Site B	Sept 26 Site A	Oct 11 Site A	Sept 12 Site E	July 18 Site D	Aug 30 Site E	June 20 Site F	Sept 12 Site A	Oct 11 Site B	Nov 7 Site A
Compound															
albuterol									+						+

amitriptyline			+			+	+							+		
atenolol										+				+		+
azithromycin				+		+	+		+	+		+	+			
bacitracin	+															
carbenicillin														+		
clarithromycin	+	+	+			+	+	+	+	+	+	+	+	+	+	+
cycloheximide	+								+		+			+		
diphenhydramine		+					+		+					+		
doxycycline															+	
enrofloxacin		+		+										+		
erythromycin a	+	+					+							+	+	
lincomycin				+	+											
melengestrol									+							+
meropenem	+	+	+		+					+						
metsulfuron	+		+			+										
miconazole							+	+								
monensin	+		+	+	+	+	+	+	+	+	+					
nortriptyline						+										
oxolinic acid						+									+	
oxytetracycline	+															
ractopamine	+		+			+	+			+	+			+	+	+
ranitidine												+	+			
salinomycin				+	+											
sarafloxacin									+			+				
sertraline		+	+		+	+		+							+	+
spectinomycin	+		+							+						+
spiramycin						+										
sulfacetamide	+															
sulfamethazine									+							
sulfamethoxazole											+					
thiabendazole				+		+	+									
tylosin	+										+					
warfarin				+												+

+ designates a detection

Table 4: Pesticides identified by positive mode data-independent acquisition Samples collected from multiple sites in 2016 analyzed on four separate days to provide a qualitative analysis on the South Nations watershed.

Date Analyzed	Oct 12			Nov 18				Nov 25				Dec 9			
Samples →	July 18 Site C	July 18 Site A	July 18 Site B	Aug 30 Site D	Sept 12 Site D	June 20 Site B	Sept 26 Site A	Oct 11 Site A	Sept 12 Site E	July 18 Site D	Aug 30 Site E	June 20 Site F	Sept 12 Site A	Oct 11 Site B	Nov 7 Site A
Compound															

3-hydroxycarbofuran				+		+	+		+	+	+	+	+	+	+
aldicarb				+											
ametryn	+	+	+	+	+		+	+		+		+	+		
atraton	+	+	+			+						+			+
atrazine	+	+	+	+	+	+	+	+		+		+		+	+
azoxystrobin		+								+		+			
buprofezin				+			+								
butachlor				+											
carbaryl 9	+	+		+		+		+	+						
carbofuran			+			+		+				+			
clothianidin	+		+	+		+									
cyanazine														+	+
cyproconazole	+														
cyprodinil				+		+	+								
cyromazine				+	+										
diethatyl-ethyl			+												
diphenamid	+														
fenbuconazole		+	+												
fenfuram				+	+									+	+
fenpropimorph				+		+	+								
imazalil							+	+							
isopropalin				+											
metolachlor	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
metribuzin	+		+			+									
napropamide								+							
octhilinone								+							
pirimiphos-ethyl		+		+	+	+	+		+				+		
promecarb		+	+												
propachlor												+			
propazine	+		+			+				+					
pyracarbolid								+							+
schradan	+	+		+	+	+	+						+		
simazine	+		+			+				+					
tebuconazole	+	+			+	+				+	+	+			
tebuthiuron	+														
thiamethoxam	+		+			+									
trifloxystrobin						+	+			+					
triflumizole						+									

+ designates a detection

The pharmaceutical presence across the South Nation watershed is depicted in **Figure 12** for compounds detected over their MQL. The analytes that could not be quantitated due

to high MQLs include bacitracin, cycloheximide, doxycycline, monensin, oxytetracycline, sarafloxacin, and sulfacetamid. Extraction recovery could be improved for these compounds by altering the pH of the sample prior to extraction or the cartridge used for SPE. For example, bacitracin has been shown to transform to 1-epibacitracin in the presence of low pH which can occur by molecular rearrangement over the long SPE process (135). Using a higher pH aliquot may improve the recovery of bacitracin with the addition of significant extraction time or by affecting other recoveries. Therefore, non-targeted screening can detect many compounds but quantitating all may not be feasible and a selection of the most appropriate candidates is required. Thus, most studies concentrate on a few classes of compounds, such as coccidiostats (136) like monensin, or fluoroquinolones (137) like sarafloxacin. Here, we quantify a wide variety of classes including anticoagulants, antidepressants, coccidiostats and antibiotics, such as penicillins, macrolides and fluoroquinolones.

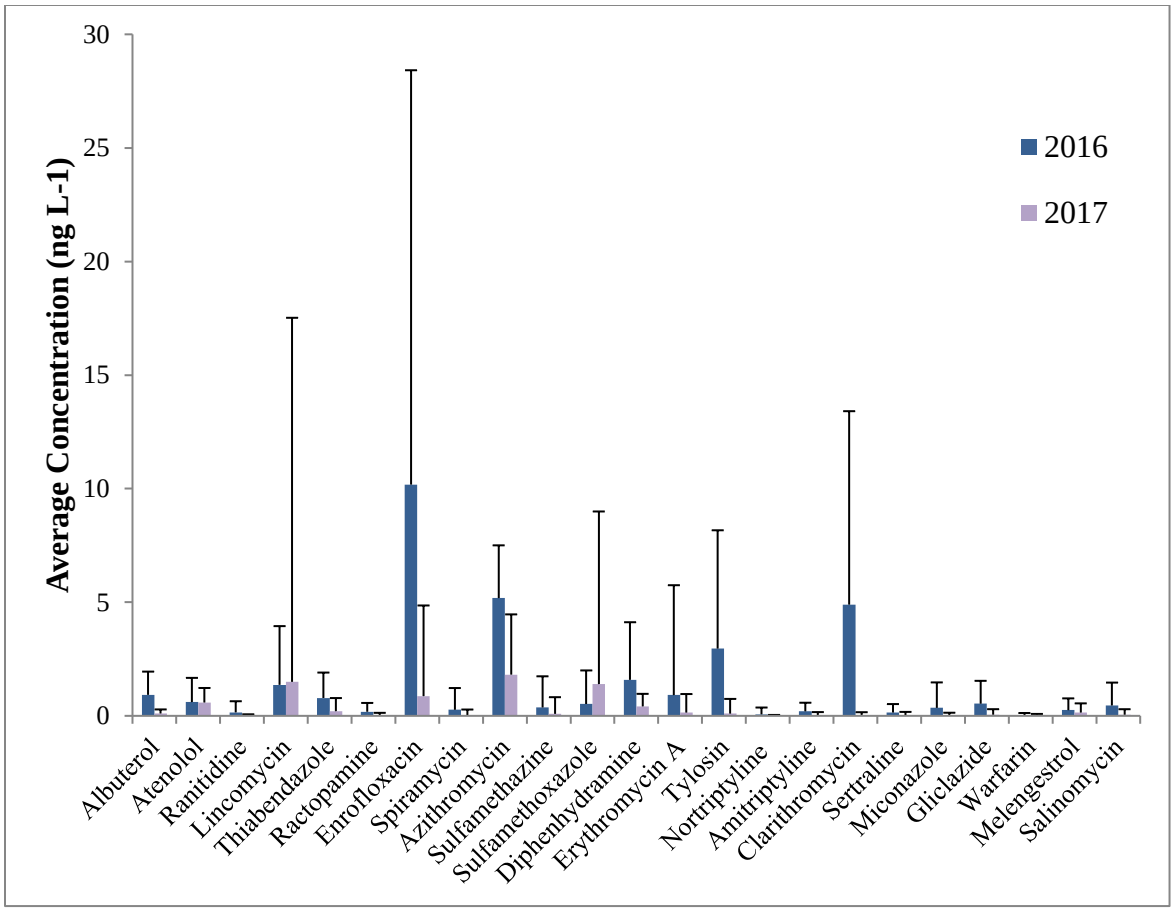


Figure 12: Average concentration of quantifiable pharmaceuticals in the South Nation watershed
66 samples from 6 sites collected biweekly in 2016 and 2017 between May and September with standard error bars depicting the high variance across seasons

Pharmaceuticals derived from human influence on the watershed were found at detectable levels in Error! Reference source not found.. Diphenhydramine was found here at an average of 1.55 ng L^{-1} and 0.36 ng L^{-1} in 2016 and 2017 respectively. It is the active ingredient found in antihistamine allergy medicine, such as Benadryl®. It could be used as a human effluent marker. The other five compounds responsible for the main presence of pharmaceutical contamination are all used as antibiotics. Clarithromycin was only quantifiable in 2016, with an average of 4.87 ng L^{-1}. This again points to urban influence as clarithromycin is a macrolide antibiotic usually sold as Biaxin. Lincomycin was found at 1.21 ng L^{-1} and 2.70 ng L^{-1} in 2016 and 2017 respectively. It is a lincosamide antibiotic

usually only used when the patient has an allergy to other antibiotics such as penicillin, as it has shown adverse effects such as the development of colitis (138).

Veterinary pharmaceuticals in the watershed were found at detectable levels in Error! Reference source not found.. Tylosin is a macrolide antibiotic used as a feed additive to stop bacterial growth. It was only found at quantifiable levels in 2016 with an average concentration of 1.55 ng L^{-1}. Incidentally, research has shown no evidence toward pollution-induced community tolerance (PICT) from tylosin (139). PICT is the strengthening of a species through evolution by the compound eliminating those with higher sensitivity (140). Azithromycin is a macrolide antibiotic commonly prescribed for infections including strep throat and chlamydia. It was also found at high levels in the surface water samples from the majority of the sites as it is also used in veterinary medicine to treat *Rhodococcus equi* and other infections. Enrofloxacin was found to have an extremely high presence in 2016, particularly at Site A with levels up to 102.85 ng L^{-1}. This led to the high average seen in Error! Reference source not found. at 3.64 ng L^{-1}, as the remaining sites had low to undetectable levels. It is used as an antibiotic to treat bacterial infections in humans and animals. The majority of these antibiotics have wide spectrum uses for gram positive organisms, allowing for simple medication. There are no guidelines for these compounds in drinking water. However, their presence in the environment at such high levels presents the question of whether PICT and antibiotic resistance will occur.

The majority of sites have direct contributions from rural and urban influences including cattle operations near Site A and B, a golf course and urban development at Site C, Site D is fed by rural tile drainage ditches and further details about the site descriptions are given by Ruecker *et al*. (141) and Lyautey *et al*. (109). Comparing the chemical composition across sites in **Figure 13** shows that the sampling from 2016 found a higher concentration of pharmaceuticals than 2017. However, Site C is the irregularity from this trend showing a large presence in 2017. This could point to the limitations of grab sampling for monitoring surface water as there is a high potential for pulse spikes from a recent fertilizer application or wash out from recent weather events.

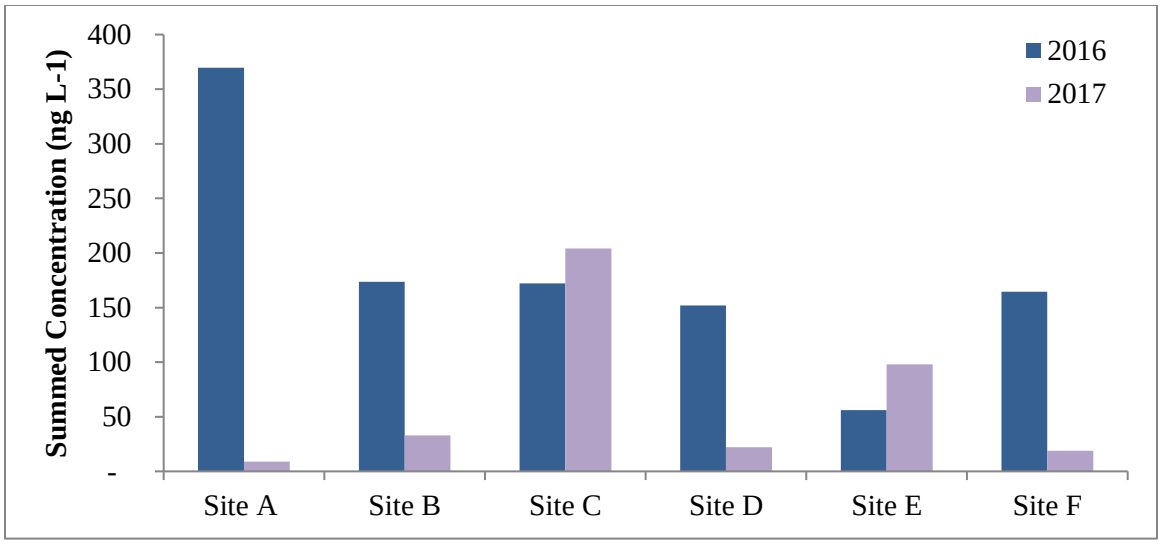


Figure 13: Summation of pharmaceutical concentrations for 2 year study Pharmaceuticals above their respective limit of quantitation in each of the 6 sites studied in both 2016 and 2017 between May and September sampled biweekly from South Nation watershed

Analysis of 66 samples resulted in only 12 pesticides being detected above their MQL. Their average concentration across six sites for 2016 and 2017 is shown in Error! Reference source not found.. Commonly used pesticides atrazine and metolachlor are often found at high levels, especially in areas prominently growing maize (142-144). The vast presence of these compounds is therefore not surprising in this area. Metolachlor and atrazine are used widely for broadleaf weeds in corn (Zea mays) and soybeans (Glycine max) to prevent weeds from overgrowing crops. Metolachlor at 20^{o}C is known to have high water solubility (530 mg L^{-1}), vapor pressure of (1.7×10^{-3} Pa) and a log octanol-water partition coefficient (log K_{ow}) of 2.9 allowing it to easily be transferred by both water and atmosphere pathways (15, 17). Atrazine has similar physiochemical properties at 20^{o}C with a solubility of 30 mg L^{-1}, a vapor pressure of 4.0×10^{-6} Pa and a log K_{ow} of 2.3 (16).

Metolachlor had an average of 14.40 ng L^{-1} and 11.98 ng L^{-1} in 2016 and 2017 respectively. It has displayed toxicity to aquatic organisms through growth inhibition as well as possible synergistic effects with other CEC including atrazine (142, 145). There are currently no maximum environmental concentrations of metolachlor or atrazine as the

research has shown no adverse effects or PICT when used as the sole methane oxidation inhibitor (146). The average concentration of atrazine was 12.67 ng L^{-1} and 13.28 ng L^{-1} for 2016 and 2017 respectively. It has been banned in Europe due to the high levels that exceed benchmark limits of toxicity. The EPA's controversial position remains that there is insufficient evidence that the pollutant could lead to reproductive issues in human or amphibian sexual development (147, 148). Currently the maximum acceptable concentration (MAC) in Canadian drinking water for atrazine and its bioactive N-dealkylated metabolites is 5 ppb (µg L^{-1}) and the MAC for metolachlor is 50 ppb. These guidelines are based on studies done between 1971 and 1987 (Health Canada, 1989).

The remaining pesticide pollution in the area was largely due to the presence of the neonicotinoid insecticides as shown in Error! Reference source not found.. Imidacloprid is often found in high concentrations as it was the first neonicotinoid to be introduced (21, 115, 149). However, the data from this project currently shows higher levels of clothianidin and thiamethoxam. The average concentration of clothianidin was 3.86 ng L^{-1} and 2.80 ng L^{-1} for 2016 and 2017 respectively. The EPA guideline for acute exposure of clothianidin is 11 ppb and 1.1 ppb for chronic exposure, whereas for thiamethoxam only acute exposure is set (17.5 ppb). The average concentration of thiamethoxam was 1.47 ng L^{-1} and 0.51 ng L^{-1} for 2016 and 2017 respectively. The levels found here were far below limits again though, as previously mentioned long-term monitoring could allow for further interpretations to be drawn.

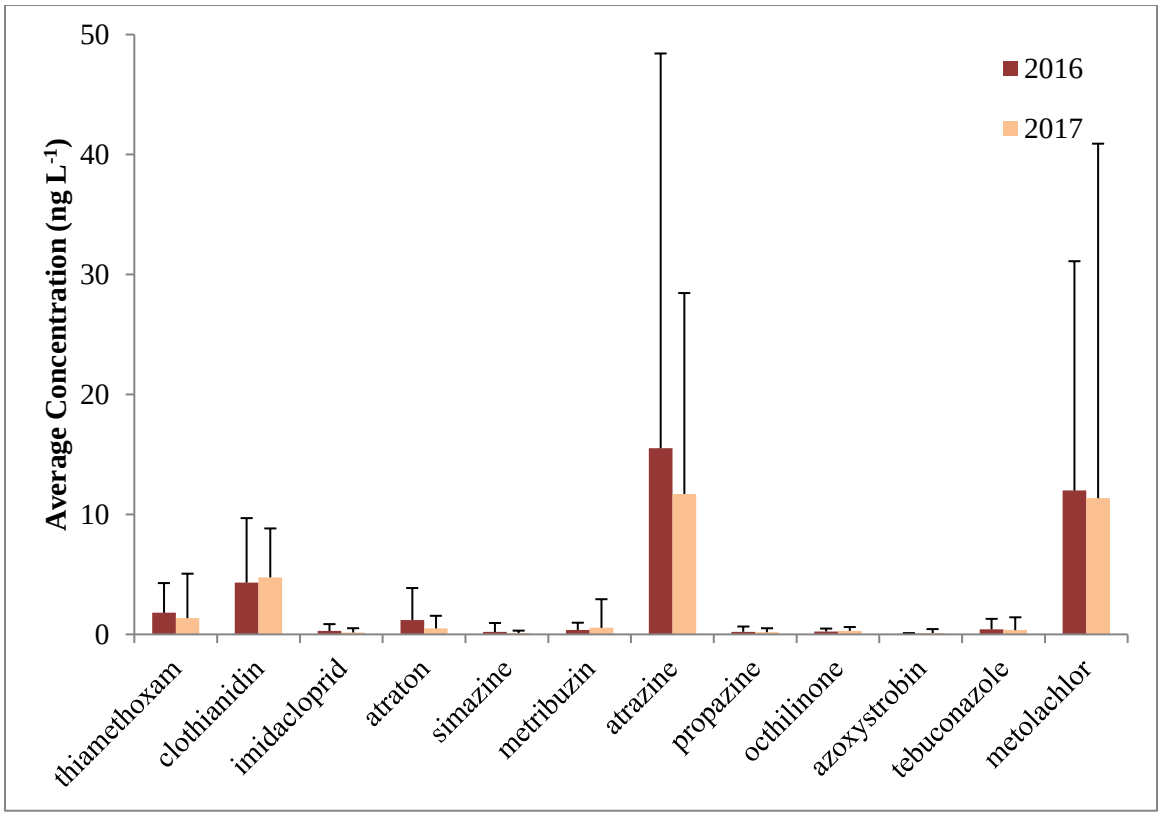


Figure 14: Average concentration of quantifiable pesticides in the South Nation watershed
66 samples from 6 sites collected biweekly in 2016 and 2017 between May and September with standard error bars depicting the high variance across seasons

An in-depth evaluation of atrazine and metolachlor concentrations analyzing changes throughout the 2017 year can be seen in **Figure 15**. Through this comparison, during periods when farmers commonly spray their crops, the presence of the herbicides in the watershed is increased; spraying often occurs in spring following the crop sprouting leaves and at the end of the year as preventative maintenance. Though the exact spraying schedule of the farmers in the area is not known, the trend clearly points to the physiochemical properties of the compounds to easily travel into ground water and streams following application. This phenomenon appears to occur over time as there is not a single spike in the spring but rather an increasing concentration to an apex where it slowly dilutes and moves through the water system.

The highest level of metolachlor and atrazine found across the survey was 559 ng L^{-1} and 360 ng L^{-1} respectively **Figure 15**. These levels are drawing near the MAC guidelines

and this is in the absence of the degradation products included in the guidelines. The MAC for drinking water is not directly related to surface water but is important to monitor changes in the levels found in the environment. With increased monitoring, one could draw conclusions to the possibility of accumulation in the watershed and make comparisons to the amount sold each year. However, this would require the inclusion of metabolites, which have been shown to prefer negative mode ionization (150). Hence, there is a need for a negative mode monitoring method that this thesis discusses later in further detail.

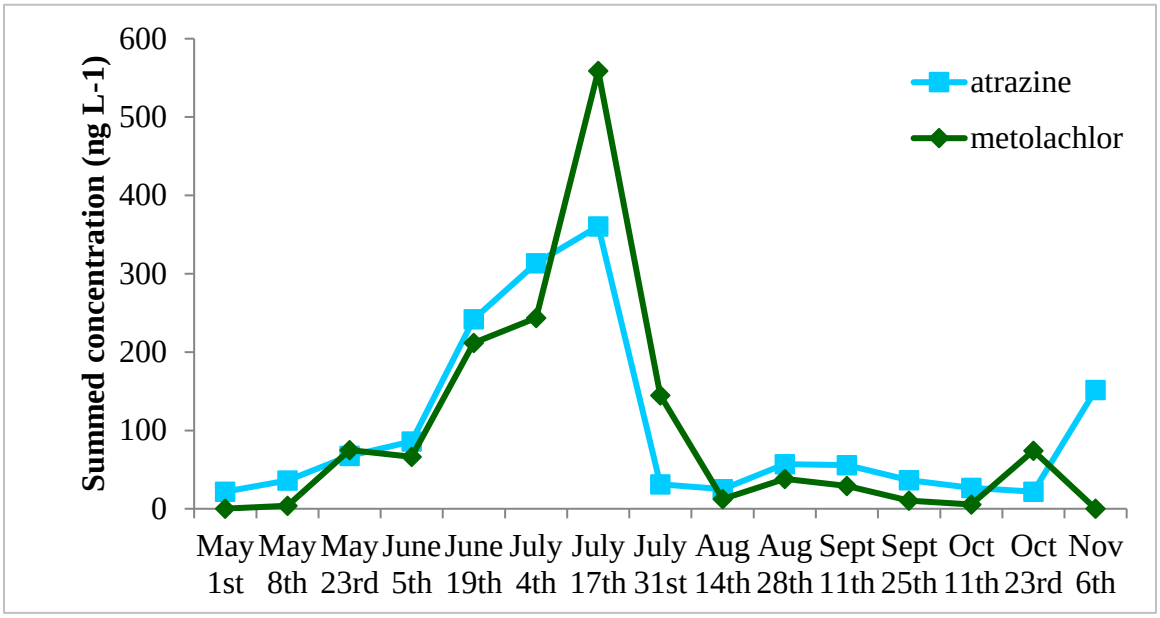


Figure 15: Summed concentration of metolachlor and atrazine in 2017
Summed atrazine, in blue, and metolachlor, in green, concentrations from all 9 sites collected biweekly in 2017 from May to November

Interestingly, the comparisons across the six sites can allow for some interpretations to be made. As previously discussed the locations studied are well known as primarily agricultural and it can be expected that each will have a relatively high degree of pesticide presence. However, Site E is located near organic pesticide free farming operations. As shown in **Figure 16** the Site E samples showed very little pesticide

presence. This serves as both confirmation that the site is indeed pesticide free and that the analysis is accurate.

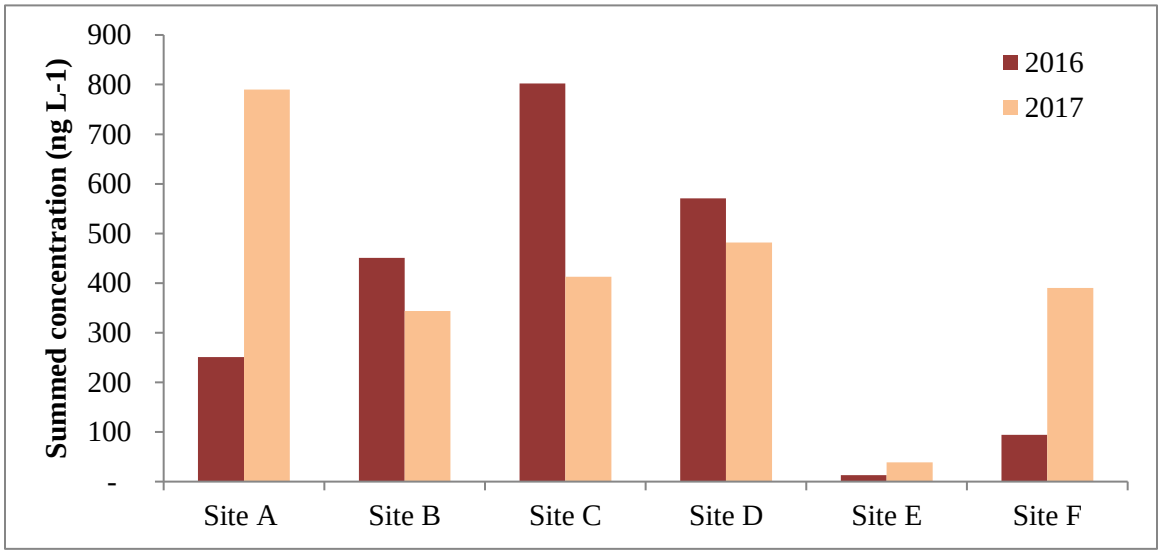


Figure 16: Summation of pesticide concentrations from 2 year study
Pesticides above their respective limit of quantitation in each of the 6 sites studied in both 2016 and 2017 between May and September sampled biweekly from South Nation watershed

The South Nation watershed has been extensively monitored and studied over the past few decades, though the presented data is the first chemical survey. The compounds quantitated were all below Canadian and USEPA guidelines. Trends across the two year study found comparable levels between years with a slightly higher concentration in 2016. Herbicides, insecticides and antibiotics were all commonly detected throughout the study. The two year study has allowed for interpretation of water contamination which correlates to land use knowledge and with further data comparison from the upcoming years may allow for improved understanding of the fate of these chemicals in the environment.

2.5 Lac Hughes

Similar to the previous study of the South Nation watershed, this project involved the survey of possible contaminants in a previously unstudied secluded lake about 90 km North of Montreal. This study was completed in a similar manner by first screening the samples to provide an overview of the CEC presence. The PRM method discussed in **2.3.2** and **2.3.3** was then used for quantitation of the identified compounds. With this study additional investigation and interpretation was attempted on the direct human contribution. Using full scan mass spectra and the DIA spectra the samples were analyzed retrospectively for illicit drugs and screening chemicals as the area is primarily surrounded by cottages for vacationers. Additionally, using the known average level of acesulfame in human urine (4 ug mL^{-1}), concentrations found in bodies of water can be used to approximate the volume of urine input (41, 42). This is applicable with Lac Hughes as it is a relatively stagnant source.

2.5.1 Sample Characteristics and Handling

This survey included 12 samples collected from multiple locations around the lake and from a ground water well. The area is free from nearby crop pesticide application and there is no municipal sewage plant in the region. The main toxicity is expected to be a result of turf pesticide application as well as septic tanks from the local residents. The area has only been inhabited for approximately 100 years, the last 40 of which have been completely free of agriculture presence. Any agriculture pesticides present can then be assumed to be a result of persistence or volatile transport from the atmosphere.

2.5.2 Results and discussion

Lac Hughes represents a well conserved lake free from rural and urban input for at least 40 years. It is surrounded by cottages containing individual septic systems. If there is a large source of human contamination it could be due to cracked or damaged septic tanks. The DIA method was again employed to survey the lake prior to selecting compounds for targeted analysis. This produced a much smaller list than the South Nation watershed as expected. The targeted compounds are quantified in **Table 5**, where sites 1–10 are lake samples and 11–12 are well sites. Notably, the largest CEC detected is the insect repellent

DEET that is commonly used by campers. It was found at levels ranging from 63.2 ng L^{-1} to 2396 ng L^{-1} in the lake with an average concentration of about 699.1 ng L^{-1}.

Table 5: Summary of quantified compounds at Lac Hughes
Samples include the laboratory blank tap water (TW) and 12 samples provided and concentrations are in ng L^{-1} units

Pesticides	MQL	TW	1	2	3	4	5	6	7	8	9	10	11	12
azoxystrobin	0.02	—	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	—	—
atrazine	0.1	—	0.31	0.35	0.26	0.13	0.33	0.14	0.14	0.19	0.17	0.37	—	—
diphenamid	0.01	—	—	—	<LOQ	<LOQ	<LOQ	<LOQ	—	—	—	—	—	—
metolachlor	0.02	—	0.06	0.08	0.16	0.27	0.16	0.16	0.21	0.22	0.33	0.37	0.03	0.03
octhilinone	0.33	—	<LOQ	<LOQ	—	—	—	—	<LOQ	<LOQ	—	—	—	—
tebuconazole	0.1	—	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	—	—
DEET	0.1	18.7	719.9	1652	648.7	912	523.5	479.6	334.6	2396	289.4	300.1	69.8	63.2
Pharmaceuticals														
albuterol	0.23	—	<LOQ	—	<LOQ	—	—	—	—	—	—	—	—	—
atenolol	0.88	—	<LOQ	<LOQ	<LOQ	—	—	—	<LOQ	—	—	—	—	—
azithromycin	5.56	—		<LOQ	—	—	<LOQ	—	—	—	—	—	—	—
diphenhydramine	0.22	—	7.8	3.3	<LOQ	3.8	2.6	1.8	1.4	2.5	<LOQ	10.7	3.3	3.1
thiabendazole	1.82	—	—	—	—	—	—	—	<LOQ	—	—	—	—	—
Artificial Sweeteners														
acesulfame	0.28	—	7.78	1.73	0.90	0.67	8.19	5.54	2.06	1.87	1.13	0.89	4.75	5.50

— = Not detected
<LOQ = confirmed detection below limit

Two compounds detected ubiquitously across the lake, diphenhydramine and acesulfame, are direct indicators of human impact. Diphenhydramine is an antihistamine and though it is commonly found, only 1 in 13 Canadians have allergies (151). However, acesulfame is found to have an estimated concentration of 4 ug mL^{-1} in urine and is a more functional biomarker for volume entering the lake (41, 42). The lake has an approximate area volume of 4.59×10^9 L (152). The estimated volume of urine in the lake at the time of sampling was calculated as 3530 L using the **Equation 7** average concentration of 3.08 ng L^{-1} of acesulfame across site 1-10.

$$C_1V_1 = C_2V_2 \qquad \textbf{Equation 7}$$

Additional compounds found nearly universally across the samples are atrazine and metolachlor. The herbicides are not registered for turf maintenance and they could be a result of atmospheric transportation or legacy contaminants from the historical farming in the area over 40 years prior to this study. Alternatively, this could be the result of an unlawful application by property owners on the lake. Using sites 1–10 in Table 5 the levels of atrazine and metolachlor had an average of 0.24 ng L^{-1} and 0.20 ng L^{-1} in the lake respectively. What is primarily important is the large decrease in concentration compared to the South Nation watershed study, which had average concentrations above 10 ng L^{-1}.

Additional retrospective analysis was completed on the samples to further target possible human contributions to the lake. This additional screening shown in **Table 6** targeted primarily sunscreens and illicit drugs. Additionally, compounds missing from the DIA library of particular concern were analyzed, including glyphosate and a pharmaceutical and personal care products (PPCP) commonly used as a birth control and hormone regulator 17a-ethylnylestradiol. Glyphosate, 17a-ethylnylestradiol, and octyl-methoxycinnamate were analyzed by comparing to analytical standards purchased.

The remaining compounds were scanned using their accurate mass and by implementing a cross laboratory comparison technique using a mass spectra database known as MassBank (153). This database has a wide variety of compounds that have been analyzed using multiple collision energies on multiple mass spectrometers. Selecting a similar MS instrument scan that also uses Fourier transform technology allows for similar fragmentation to be expected. The contributor with the most adequate comparison is EAWAG, who often also uses a collision energy of 35, which allows ideal comparison to our DIA spectra for the samples. The fragments come with predicted formulas which can then be scanned for in our samples to improve detections based on precursor mass. Unfortunately, the database does not include comparable retention times. An ideal inter-laboratory comparison system would require a retention index for the compounds rather than retention time as proposed by Quilliam *et al* (154).

Retrospective analysis determined that illicit drugs were not detected in the lake by the current extraction method. Similarly, the majority of sunscreen chemicals were not present. The detections made include octisalate, also known as octyl salicylate, and octocrylene. Both are organic compounds with extended conjugation to absorb ultraviolet rays from 280 nm – 320 nm. Their presence in the lake is not unexpected but could not be confirmed without the use of analytical standards for more accurate detection.

Table 6: Summary of the non-targeted retrospective analysis
Samples include the laboratory blank tap water (TW) and 12 samples and compound detection is based on MassBank spectra fragmentation patterns

Sunscreen Chemicals	TW	1	2	3	4	5	6	7	8	9	10	11	12
● benzophenone-3	-	-	-	-	-	-	-	-	-	-	-	-	-
● homosalate	-	-	-	-	-	-	-	-	-	-	-	-	-
● 4-methyl-benzylidene camphor	-	-	-	-	-	-	-	-	-	-	-	-	-
● octyl-methoxycinnamate	--	--	--	--	--	--	--	--	--	--	--	--	--
● avobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-
● octisalate	-	+	-	+	-	+	+	-	-	+	+	-	-
● octinoxate	-	-	-	-	-	-	-	-	-	-	-	-	-
● octocrylene	-	+	-	-	-	-	-	-	-	-	-	-	-
Illicit Drugs													
● nicotine	-	-	-	-	-	-	-	-	-	-	-	-	-
● Δ^9-tetrahydrocannabinol	-	-	-	-	-	-	-	-	-	-	-	-	-
● fentanyl	-	-	-	-	-	-	-	-	-	-	-	-	-
● methamphetamine	-	-	-	-	-	-	-	-	-	-	-	-	-
● cocaine	-	-	-	-	-	-	-	-	-	-	-	-	-
Pesticides and PPCP													
● Glyphosate	--	--	--	--	--	--	--	--	--	--	--	--	--
● 17a-ethylnylestradiol	--	--	--	--	--	--	--	--	--	--	--	--	--

- Putatively not detected with MassBank database
-- Confirmed not detected with analytical standards
+ Putative detection with MassBank database

2.6 Wastewater Effluent

This study first sought to identify and quantify the pharmaceutical and pesticide presence in two WWTP effluents using the previously described DIA and PRM methods (**2.3.2** & **2.3.3**). The removal of these organic contaminants was then studied using ferrate (VI) (Fe(VI), FeO_4^{2-}) for oxidation with and without first catalyzing the reaction with different levels of hydrochloric acid (HCl). This oxidation technique was developed by Manoli *et al.* and has previously been shown to have enhanced oxidation of caffeine with acid activation in laboratory water (155, 156). This study compared two WWTPs and how their different water characteristics affect the novel treatment technique.

2.6.1 Sample Characteristics and Handling

This WWTP study analyzed effluent samples collected from the Adelaide and Greenway sewage treatment plants located in London, Ontario, Canada. Sewage effluent (10 L) from Adelaide (plant A) and Greenway (plant B) was collected on July 17, 2017 and July 20, 2017 respectively. Both plants employ primary treatment and activated-sludge based secondary treatment. Plant A employs phosphorus removal by chemical addition in addition to the primary and secondary treatment. Each sample was separated into 2 L aliquots for a variety of treatments including untreated, 5 and 10 mg L^{-1} ferrate-treated, and acid-catalyzed treatments of both concentrations. A certain amount of the solid Fe(VI) was added to the sample and in the case of acid activation the desired amount of HCl was added dropwise in equal parts with the ferrate.

Plant B was found to have a higher complexity, with a turbidity of 5.7 ± 1.0 nephelometric turbidity units (NTU), measured using a Thermo Orion AQUAfast II AQ2010 Turbidity Meter, compared to plant A's turbidity of 4.3 ± 1.0 NTU. Many other factors led to the same conclusion including total suspended (TSS) and dissolved solids (TDS) measured according to standard methods (157). TSS of plant A and B were 8 ± 3 mg L^{-1} and 12 ± 3 mg L^{-1} respectively. TDS of plant A and B were 622 ± 33 mg L^{-1} and 1098 ± 58 mg L^{-1} respectively.

2.6.2 Results and Discussion

Hundreds of compounds were scanned by the DIA method and those detected were quantified in **Table 7** for 22 pharmaceuticals and pesticides. As expected the main contaminants found from sewage effluent are pharmaceuticals commonly prescribed as medication, including a variety of antibiotics. The only pesticide detected at quantifiable levels was imazalil. The majority of the compounds were found at similar levels for both WWTPs. Analysis of degradation is only achievable on compounds over their quantitation limits.

Notably, sulfamethoxazole was found at high levels of 348.94 ng L^{-1} and 413.03 ng L^{-1} in plant A and B respectively. It is a common medicine for urinary tract infection diagnosis as well as bronchitis and prostatitis. Evidence points to the development of pathogenic resistance toward sulfamethoxazole and it's ubiquitously found in WWTP effluent showing a high potential to persist in the environment, with less than 1% degrading to its metabolites naturally (158, 159). Previous studies have used photocatalytic degradation and adsorptive removal techniques for the treatment of sulfamethoxazole (160, 161). Similarly, many other pharmaceuticals were detected above MQL and their degradation was studied.

It is clear that the presence of these CECs in WWTP effluent points to the inability of conventional biological treatment processes to remove the pollutants. Previous studies on the Fe(VI) treatment have shown potential to disinfect and oxidize organic contaminants (162-164). As with most innovative remediation techniques the major concern is potentially harmful byproducts of the treatment, which was shown to be of limited concern for this procedure (155, 156). Importantly, it has also been observed that the Fe(VI) without acid activation can have limited degradation capabilities of many compounds (165).

Table 7: Pharmaceutical and pesticide concentrations in untreated WWTP effluent from both plant A and plant B

Compound	Plant A (ng L^{-1})	Plant B (ng L^{-1})	MDL[b] (ng L^{-1})	MQL[c] (ng L^{-1})
albuterol	4.69	4.50	0.05	0.23
ranitidine	155.40	129.13	0.49	0.49

lincomycin	12.57	21.42	1.04	1.04
thiabendazole	6.97	18.08	0.36	1.80
ractopamine	ND[a]	<MQL	0.38	0.38
spiramycin	ND	<MQL	0.40	2.01
sulfamethazine	ND	2.41	1.08	1.08
sulfamethoxazole	348.94	413.03	1.04	1.04
tylosin	<MQL	<MQL	1.08	4.30
nortriptyline	1.83	1.04	0.01	0.20
amitriptyline	23.50	20.00	0.16	0.82
clarithromycin	60.18	16.74	0.05	0.26
sertraline	12.55	5.30	0.85	0.85
miconazole	ND	<MQL	0.08	0.40
warfarin	0.88	7.50	0.04	0.21
diclofenac	<MQL	<MQL	0.98	3.93
cyromazine	ND[a]	<MQL	0.64	1.61
schradan	ND	<MQL	0.01	0.11
thiacloprid	ND	<MQL	0.09	0.36
imazalil	0.34	0.40	0.14	0.14
cyprodinil	<MQL	<MQL	0.12	0.49
trifloxystrobin	ND	<MQL	0.02	0.11

ND - Not Detected
MDL - Method Detection Limit
MQL - Method Quantitation Limit

The Adelaide WWTP had high levels of many CEC including sulfamethoxazole, ranitidine, clarithromycin, and lincomycin. **Figure 17** shows the degradation of the compounds detected above their MQL. Many pollutants were easily oxidized simply using Fe(VI) including albuterol, ranitidine, lincomycin, and imazalil. The remaining compounds are clearly shown to have improved degradation when catalyzed with the acid. The majority of these CECs have an improved degradation from adding more Fe(VI) as well as from adding HCl. Thiabendazole is an example where the amount of Fe(VI) added does not improve degradation in the absence of acid catalysis.

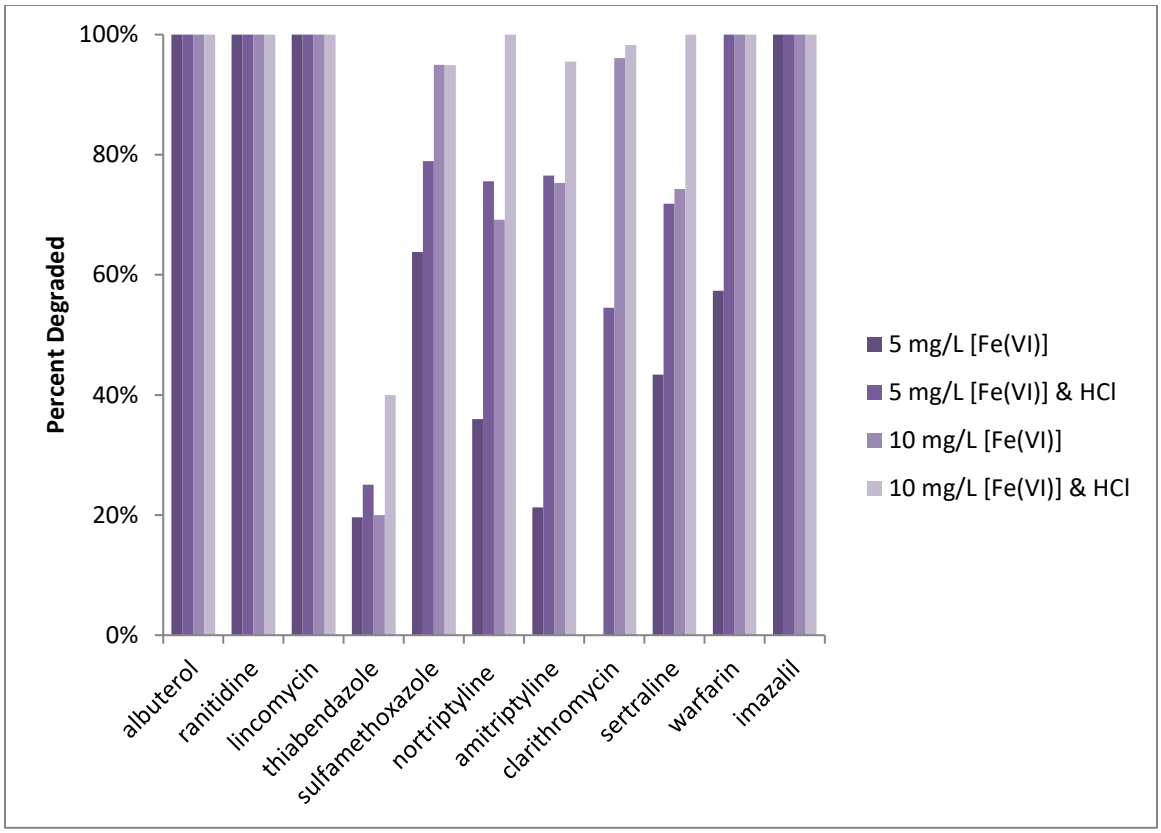


Figure 17: Degradation of quantifiable contaminants detected in Adelaide WWTP effluent.
Where hydrochloric acid (HCl) was added at 1.5 mol per Fe(VI) mol

The comparison between two WWTPs makes it clear that there is a requirement of acid activation for improved degradation. **Figure 18** shows the degradation of effluent from plant B, which was considerably more complex than plant A. Some of the compounds that easily degraded at plant A also degraded in plant B by all treatments, including albuterol and ranitidine. However, problematic compounds such as thiabendazole had decreased degradation efficiency, requiring 10 mg L^{-1} of Fe(VI) with acid activation to even achieve 6% degradation. Similarly, imazalil requires much more treatment to be removed from the difficult matrix. At plant A all treatments were successful at removing imazalil, whereas the plant B sample required extra ferrate and HCl.

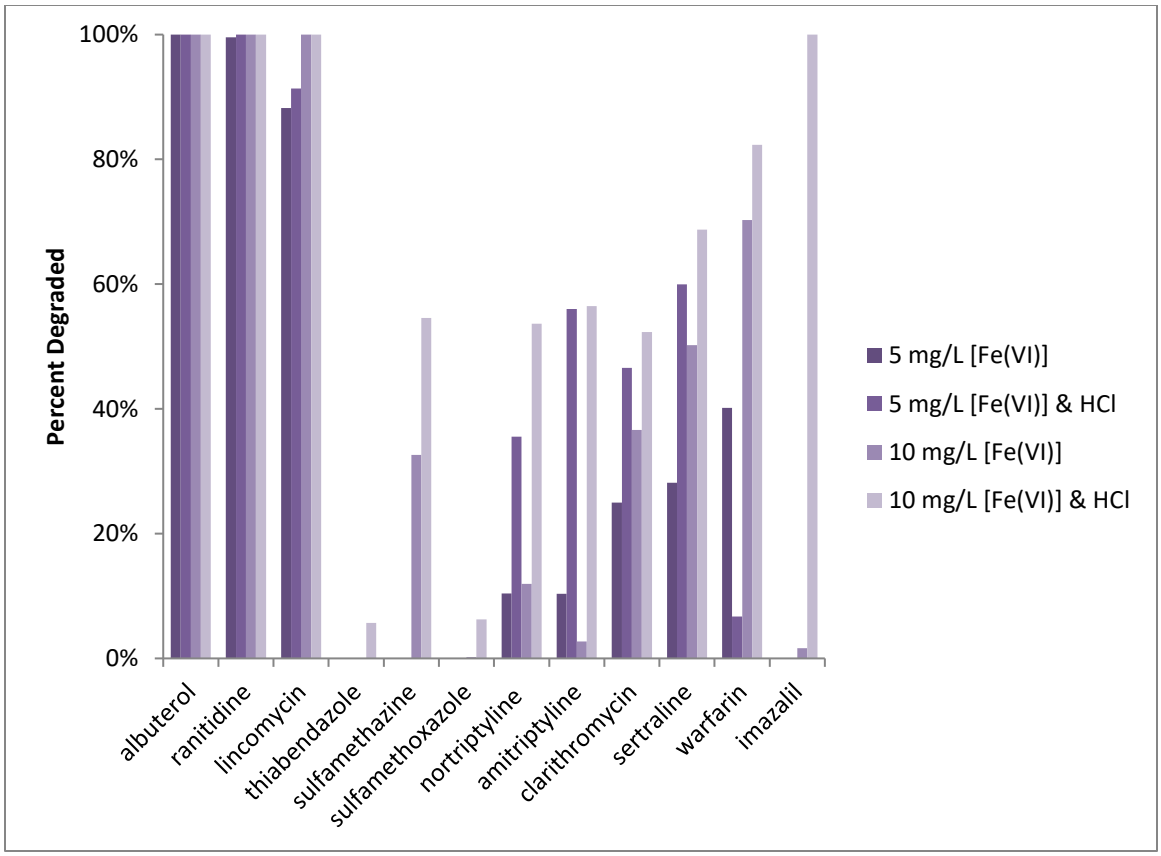


Figure 18: Degradation of quantifiable contaminants detected in Greenway WWTP effluent.
Where hydrochloric acid (HCl) was added at 1.5 mol per Fe(VI) mol

This work has unique results that answer questions in the field of water treatment. There is clear need for removal or pharmaceuticals and pesticides from both wastewater and drinking water. The acute and chronic health risks of water contaminants for humans and has been noted here repeatedly. Here, it is shown that methods for oxidation of these compounds could be useful. Additionally, by comparing two WWTPs it is clear that treatment needs to be proven in multiple matrices as degradation is particularly affected by the water characteristics. The developed methods were efficient at detecting and quantitating a wide variety of compounds. Furthermore, they allowed small differences between concentrations before and after treatment to be determined.

2.7 The Power of Databased DIA

Two emerging pesticides, flupyradifurone and sulfoxaflor, with the potential to be replaced; the currently controversial neonicotinoids have recently been approved for use by Health Canada and the EPA (118, 166). Studies have shown their structural similarities to the neonicotinoids give them comparable activity profiles by binding agonistically to the nicotinic acetylcholine receptors (167). It can be hypothesized that they will also have analogous fates in the environment pertaining to water solubility and persistence in soil, water, atmosphere, etc. Over 200 extracted samples that were collected from the South Nation watershed between June 2016 and November 2017 and analyzed using the above described DIA method, sections **2.3.2 & 2.3.3,** were then compared retrospectively to the accurate mass of the new insecticides. This allowed for the confident determination that the pesticides were not currently being detected in the area.

The new neonicotinoids, flupyradifurone and sulfoxaflor are shown in **Figure 19** with their fingerprint product ions at the collision energy used for DIA (35 NCE). The retention times of flupyradifurone and sulfoxaflor are 2.95 minutes and 3.19 minutes respectively. The fragments shown, along with their accurate precursor masses (in red) and retention time, were used to screen the sample retrospectively to confirm the compounds were not present in the watershed between 2016 and 2017. This result is as expected as the insecticides have only recently become approved for use.

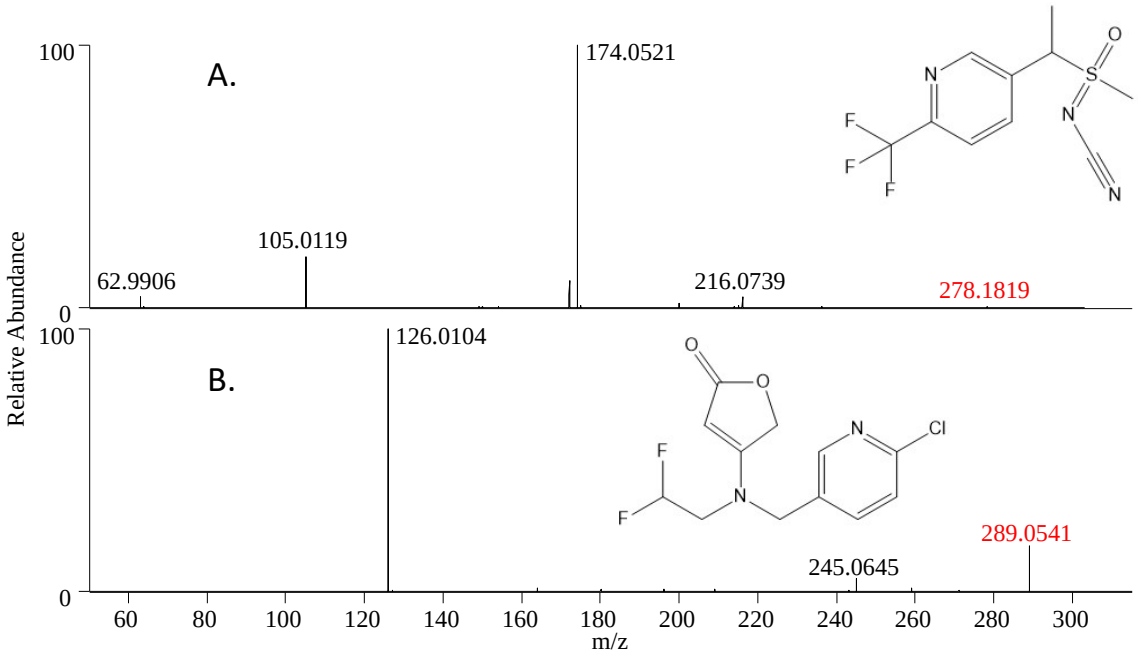


Figure 19: Targeted tandem mass spectra used for screening against samples A. sulfoxaflor and B. flupyradifurone were fragmented at normalized collision energy of 35. The structure of each is shown and its precursor *m/z* is in red

The databased DIA data allows for endless post-acquisition analysis. This technique was used many times throughout my work. The example of sulfoxaflor and flupyradifurone are particularly interesting as Section **2.4** depicted the common occurrence of neonicotinoids. Additionally, **Chapter 3** is concentrated on the development of simple and accurate analysis of the original 7 neonicotinoids. This retrospective analysis was completed after **Chapter 3** was published and it provided an argument for their absence from the method.

2.8 Contaminants missed by the DIA screening

Though the DIA screening technique has been shown to detect a large variety of compounds, it is not all encompassing. Full scan HRMS was used to compare all positive and negative ions across samples for any CEC missing from the original analyses. The spectra were converted to MZML format and using the R program: the differences

between all features in a variety of samples were compared using principle component analysis (PCA). Though there was little noticeable difference among the samples in positive mode, **Figure 20** depicts the negatively ionized compounds from 23 variable samples. Circled in red is group A, containing only samples from Site D and circled in blue is group B, which contains the majority of the remaining samples. Group A contains significant analytes which are not present in group B or vice versa. In order to determine the ions responsible for the large difference, statistical manipulations were completed on the data exported from R using excel.

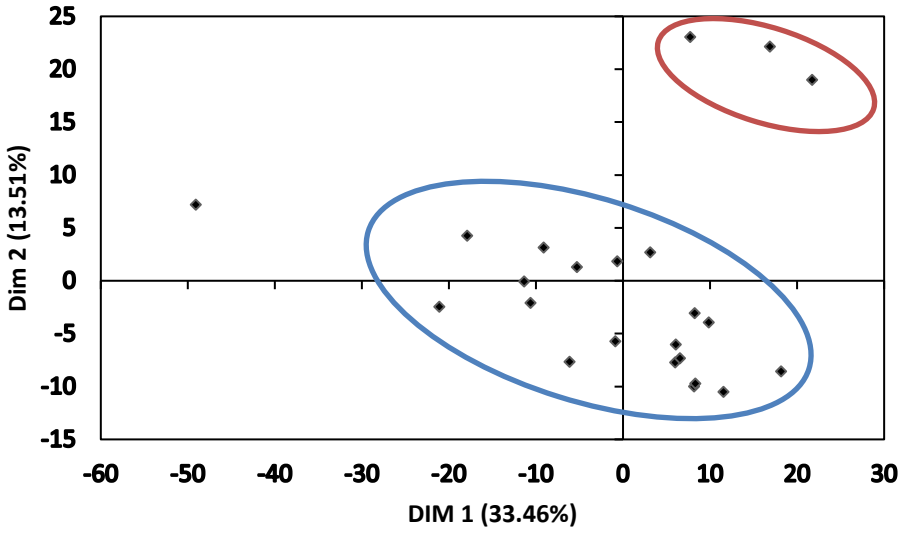


Figure 20: PCA plot of 23 samples from a variety of sites from the South Nation watershed analyzed in negative full mass spectrometry mode.
Group A in red are from site D and clearly have components causing them to be separated from the main group B in blue

Further investigation was completed by comparing group A to three samples from group B to determine the difference in their chemical composition (**Figure 20**). The three samples chosen were from site E as they had large variance across Dim 2 but little across Dim 1, allowing an individual determination of what caused the Dim 2 variance. Additionally, site E is downstream of an organic pesticide-free farm. **Figure 21** depicts

the difference between the average features of each sample group known as the fold change (X-axis) against the T-test for significant difference (Y-axis). Again it was clear that some features stood out from the majority (red data points) and they all related to a signal peak at 3.43 min with an *m/z* of 195.9119.

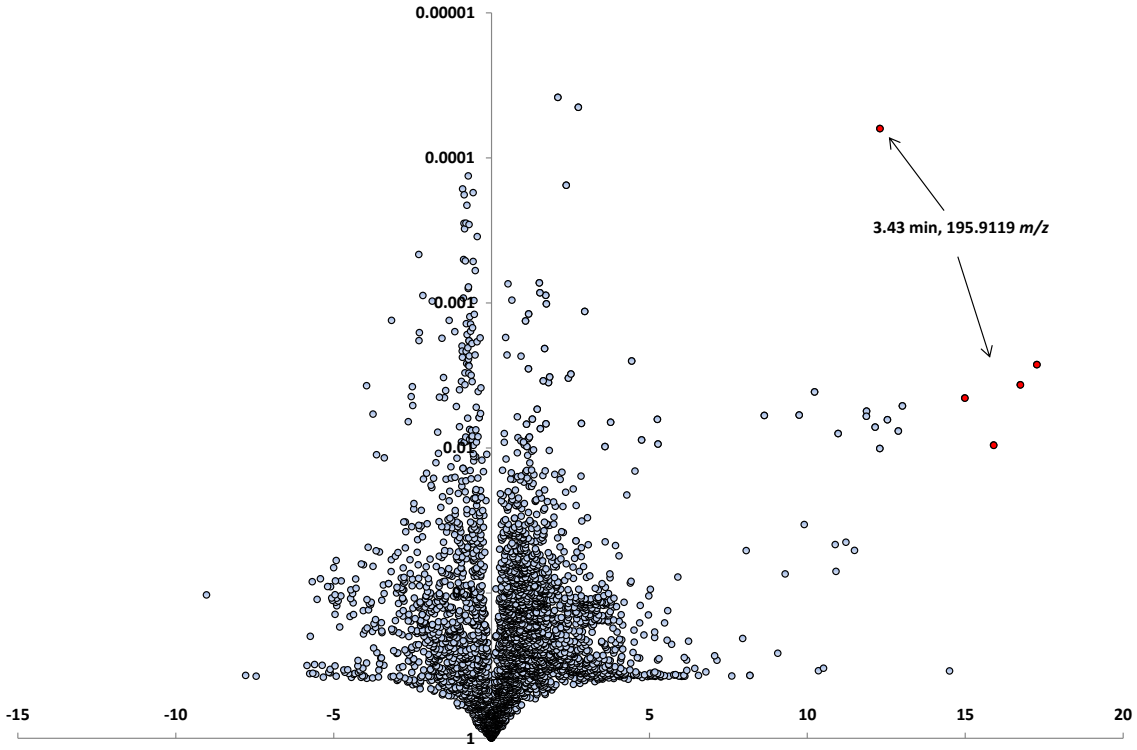


Figure 21 Volcano plot features comparing Site D from Site E
Red data points describe features relating to a particular peak with high significance (Y-axis) and a large fold change (X-axis) between the groups

The groups were then compared by manually inspecting their respective chromatograms in negative mode and an intense peak at 3.43 min was found among the Site D samples with a base peak *m/z* of 195.9119 that was absent in the Site E samples (**Figure 22**). The DIA screen previously discussed only scans positively ionized analytes, which presented some limitations for confirming the identification, since no fragmentation data was available.

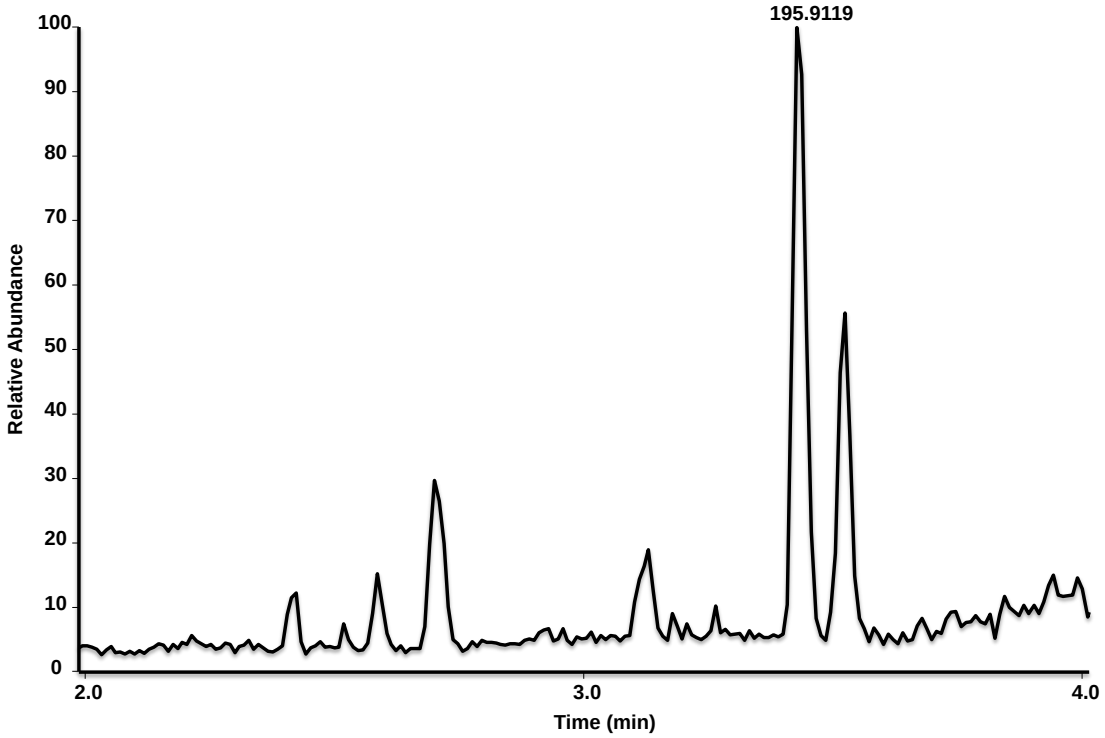


Figure 22: Site D sample chromatogram (3 – 4 min)
An intense peak (6.25x107) at the respective time (3.43 min) and with the same bass peak (*m/z* =195.9119) identified as the main discriminator between Site D and Site E

Using the Full Mass Spectrum depicted in **Figure 23,** identification was made using the isotopic ratio. The isotopes chlorine-35 (75.77%) and chlorine-37 (24.23%) are separated by 2 *m/z* units, with a 1:3 probability of the heavier atom (168). The 195 and 201 mass would represent 100% of the chlorine atoms being isotope 35 and 37 respectively. The 197 mass is a 2:1 split favoring the lighter atoms and the 199 is a 2:1 split favoring the heavier. The compound was identified as the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCP). Confirmation was then completed upon acquisition of an analytical standard.

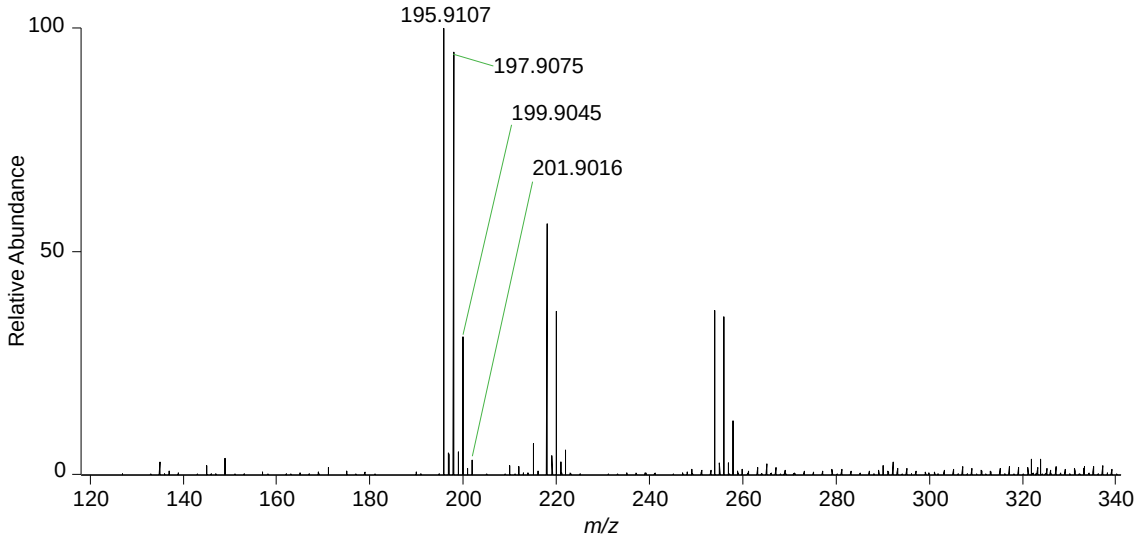


Figure 23: Full mass spectrum of the peak at 3.43 min from a site D sample Collected on August 30, 2016 depicting the characteristic isotopic pattern of a compound containing 3 chlorine atoms

Chlorpyrifos rapidly metabolizes to TCP in water by hydrolytic cleavage of the phosphate ester bond (**Figure 24**), leading to the detection of TCP rather than the parent compound in surface water analysis (169). TCP has been reported to have links to low testosterone levels in humans (170). Initial confirmation attempts of TCP using a standard compound revealed difficulty fragmenting the stable ring structure. Further analysis on this compound required targeted selected ion monitoring (T-SIM) rather than the traditional parallel reaction monitoring. The 197 and 199 isotopes can be used as accurate qualifier and quantifier ions in this case.

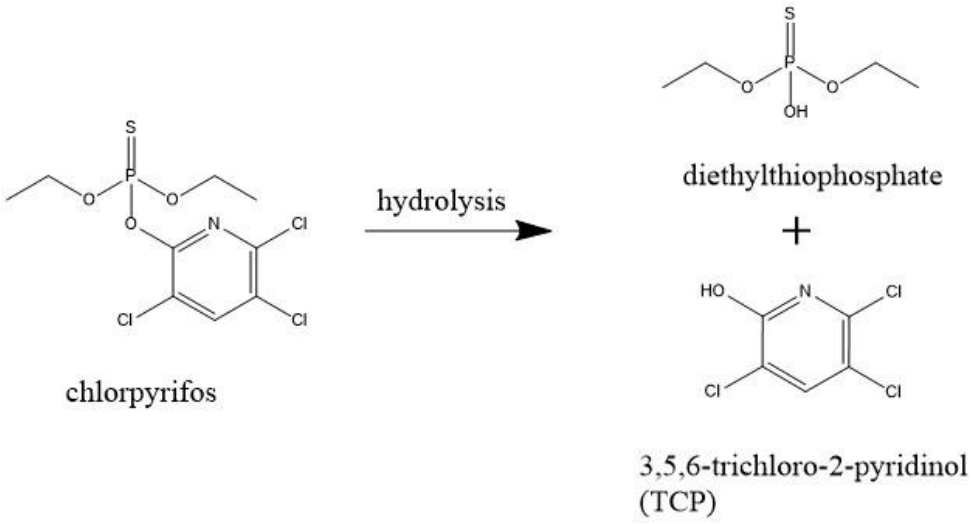


Figure 24: Chlorpyrifos hydrolysis to the TCP molecule that was identified at substantial levels in Site D

2.8.1 Development of Negative DIA

It is clear with the identification of a new contaminant (TCP), which has shown adverse effects from exposure in the literature, that an improved screening method is required if the goal is to collect and analyze all contaminants of possible interest. The development of a negative ionization DIA method will allow for improved detection, as well as provide insights into the current database. Some of the compounds which are difficult to ionize and fragment by the positive method could have improved spectrometry through the negative ionization mode.

Current environmental screening strategies concentrate primarily on the analysis of positively ionized CECs (123, 124, 129, 131, 171). This is due to the improved ionization of many compounds in positive mode. Solely monitoring in positive mode misses many compounds with the potential to have detrimental effects in the environment (e.g. polyfluoroalkyl substances). Due to the increased detections in positive mode, there is also a larger number of background ions ionized (172). The analytes detected by positive mode therefore have a higher likelihood of being suppressed by other signals when compared to those in negative mode.

Recent work has delved into the determination of signal suppression from spectral interference and how this can be quantified as an individual selectivity value for each respective ion (131). The method concentrated on positively ionized pharmaceuticals in surface water and it was shown that the majority of contaminants were small molecules. This can be explained by the degradation of compounds, ionization conditions (e.g. in-source fragmentation) and compounds that are natively small (131). A similar trend can be expected with the development of a method for screening negative contaminants.

2.8.2 Negative Mode Ionization

In order for the negative screening method to be compatible with the previous positive DIA technique it needs to maintain the same mobile phase at the identical pH to allow simultaneous analysis moving forward. It has previously been shown that using acidic mobile phase can improve ionization efficiency, giving stronger signals in negative mode by deprotonating anions and giving a highly delocalized charge (171). Ionization efficiency in negative mode has also been studied by comparing different polar protic and aprotic solvents, which showed good response and the best separation with water/acetonitrile (173). Though it is clearly not ideal, it is hypothesized that these imperfect ionization conditions will further reduce the signal suppression from background ions.

2.8.3 Precursor Ion Selectivity

Four surface water samples and two sewage effluent samples were analyzed in negative ionization in full scan mode. The *m/z* of all collected ions resulted in over 7 million signals. The frequency of each nominal mass is shown in **Figure 25** between 100 *m/z* and 1200 *m/z*, which was particularly dense in the low mass region. It is unsurprising that there is a low likelihood of large molecular weight compounds to be detected in negative mode. Noticeably, the compounds with an even nominal mass have a lower chance of experiencing spectral interference in comparison to odd mass ions. The analytes with an even nominal mass will then have better selectivity compared to odd mass ions. This can be explained by the nitrogen rule where compounds with an odd number of nitrogen atoms will give a positive *m/z*. It is common among small molecules to have a structure

containing zero to one nitrogen atom. However, nitrogen groups such as amines prefer to accept a proton for ideal ionization in positive mode.

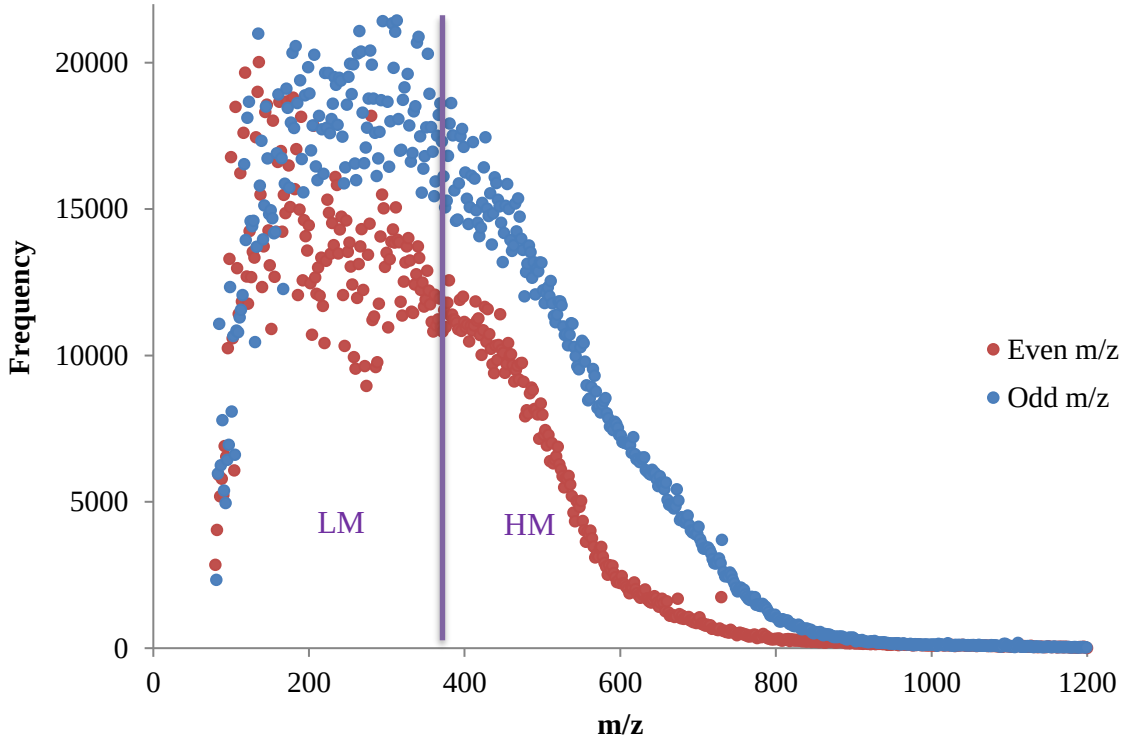


Figure 25: Histogram of nominal mass ions in negative mode
The even (blue) analytes being more abundant than the odd (red) mass compounds. The highly dense lower mass (LM) region at the lower molecular weight trends down to the less populated high mass (HM) region

The high density of low mass ions requires a smaller range and tighter windows, whereas the limited ions in the high range can have expanded windows. The full scan range for negative mode was set between 120 *m/z* and 800 *m/z* due to the potential for compounds outside this range being limited. The full range was separated into two smaller scanning ranges compared to the three required in positive mode. The low mass (LM) range is small due to the high ion population and only scans between 120 *m/z* and 340 *m/z*. The high mass (HM) range scans between 340 *m/z* and 800 *m/z*.

2.8.4 Mass Defect Assessment

The same six samples discussed above were manipulated using a python script to develop a dataset containing the precursor *m/z* and its mass defect. Using R, a scatterplot was produced shown in **Figure 26** comparing *m/z* and mass defect. There is a clear trend within all samples that there are far fewer ions producing signals with a mass defect between 0.5 – 0.8 *m/z*. The method was then produced using these defects as the edge of each ion collection window to limit the possibility of the quadrupole not transmitting important analytes. Due to the high frequency of low mass ions, the low mass range employs tight windows of 11.3 *m/z*, whereas the high mass range windows are wider at 23.3 *m/z*. Each window has an overlap of 0.3 *m/z* between them to allow for the highest possibility of collecting all ions and to prevent missing an analyte between the LM and HM scanning methods.

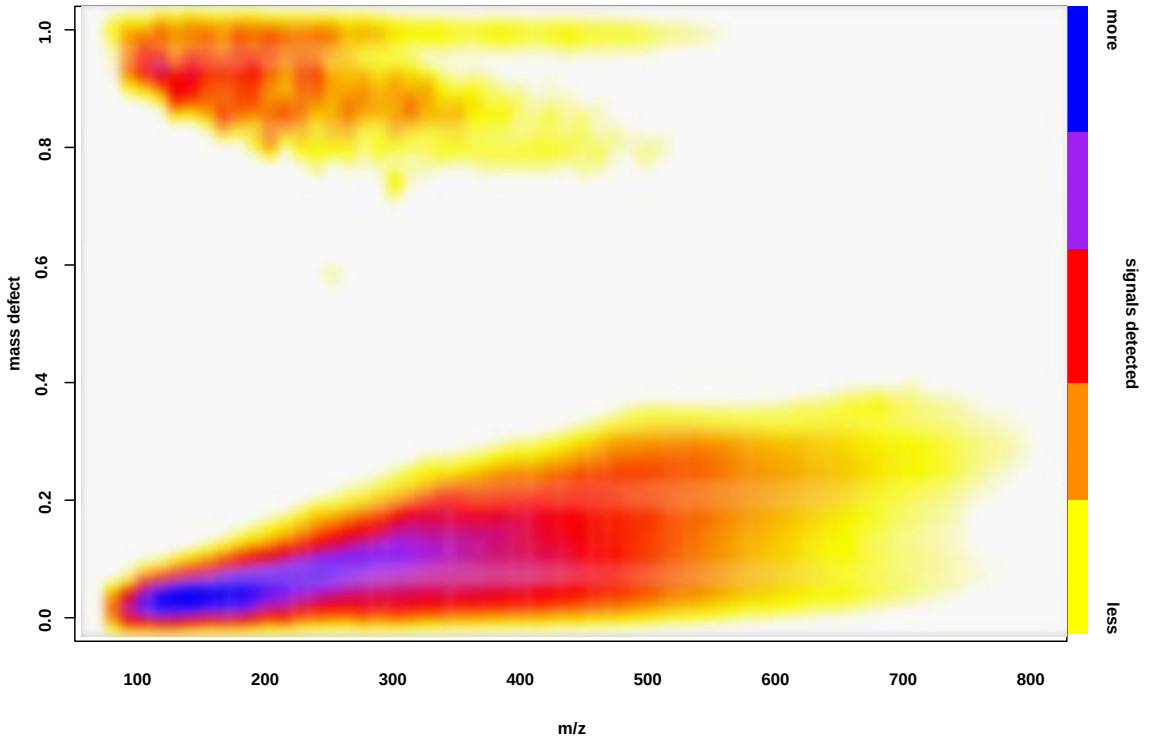


Figure 26: Smooth scatter plot of mass defect compared with the *m/z* of each ion There is clearly a high frequency of low mass defects and a limited spectral occurrence between 0.5 and 0.8 m/z

2.8.5 Chlorpyrifos metabolite

The newly developed DIA method was used to analyze the sample collected on August 30th, 2016 from Site D and is depicted in **Figure 27** for the peak of interest at 3.4 minutes that was analyzed under two filters, denoted by the green dotted line. There are few fragment peaks of value for structure elucidation. The isotopic ratio characteristic of the compound containing three chlorine atoms is the only identification method. This isotope pattern will allow for confident identification with the 195 peak being detected in the first filter (185.5-196.8*m/z*) and the 197 and 199 peaks selected in the second filter (196.5-207.8*m/z*).

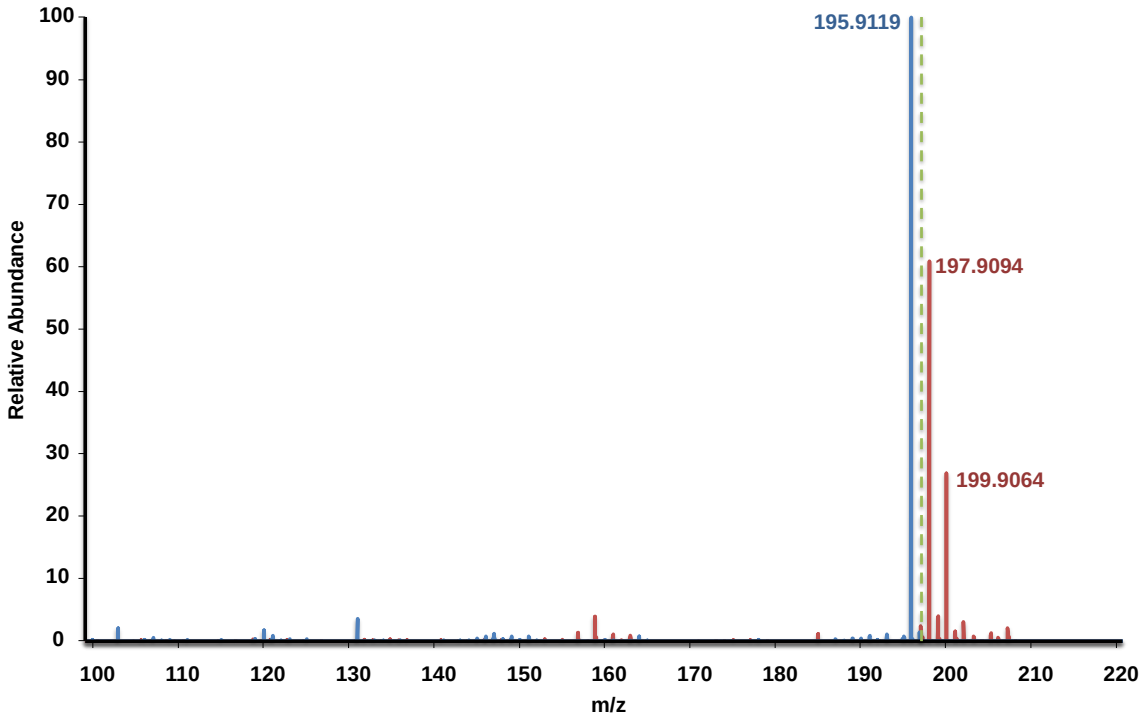


Figure 27: DIA spectra of TCP molecule
MS/MS fragmentation of the peak at 3.43 minutes required two separate window filters (segregated by green dotted line). The first filter transmits ions between 185 *m/z* and 196 *m/z* while the second filter transmits ions from 196 *m/z* -207 *m/z*

The extracted ion chromatogram of the three isotope peaks can be seen from the analysis of an analytical standard below in **Figure 28**. The isotopic ratio of 1:3 heavy to light chlorine atoms can clearly be seen in the relative abundance of the analyte. Separation of the peaks into two windows increases the selectivity of the compound and limits the chance of false identification using the DIA method.

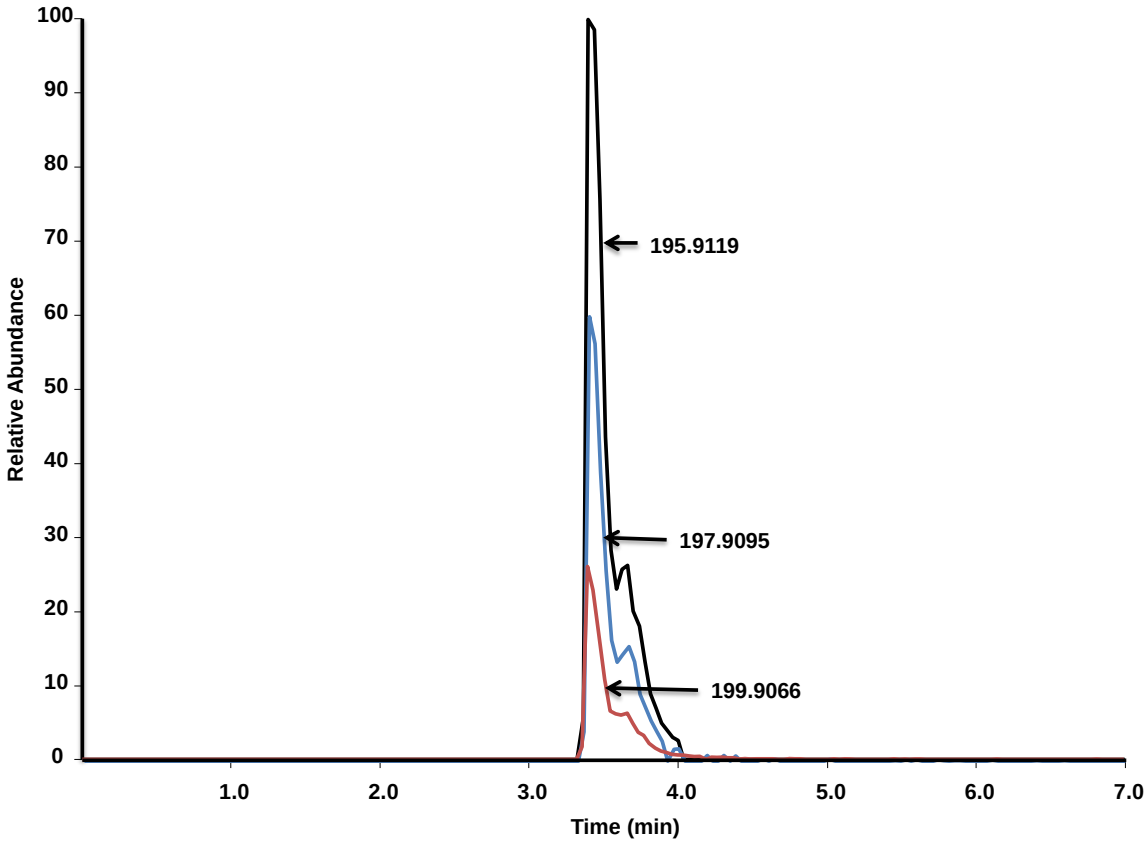


Figure 28: Extracted ion chromatogram of chlorpyrifos metabolite TCP depicting the relative abundance comparison of the isotopes

2.8.6 Comparing DIA to Databased Spectra

The survey on the original 15 samples scanned in positive mode (**2.4.2**) was expanded using the newly developed negative mode DIA. The samples screened in negative mode were compared against many in-house standards similar to the positive mode survey but

to increase the opportunity for identifications the online MassBank dataset was utilized. This expansion of possible compounds is clearly justified by the aforementioned decrease in compounds readily ionized in negative mode. The difficulty with comparing LC-MS/MS spectra comes with the different collision energy setting, mobile phases, gradients and mass analyzers. Importantly, EAWAG has a large database of compounds that have been scanned by many collision energies using similar instrumentation to our laboratory (**Appendix 1**).

EAWAG similarly uses a Fourier transform mass analyzer using collision energies comparable to our method (35 NCE). The main caveat for attempting to compare to their database is the difference in retention times. With the absence of retention index data we instead compared a wide range of 27 compounds run by our chromatography of 7 minutes to theirs of 30 minutes. Using a quadratic equation of the compared data we were able to estimate the retention time of unknown compounds to within a minute (**Figure 29**).

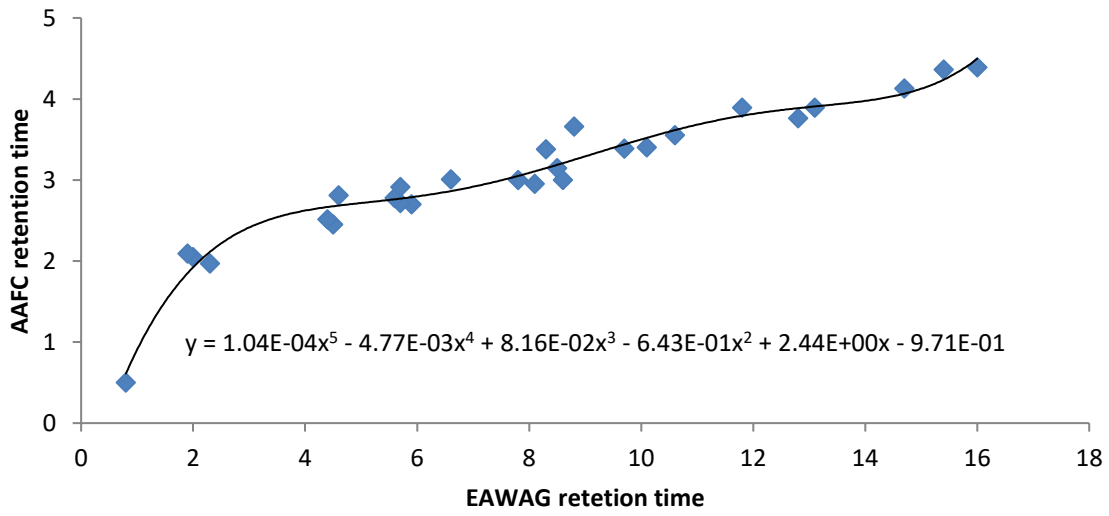


Figure 29: Relationship between Agriculture and Agri-Food Canada (AAFC) and EAWAG's retention times
Using a fifth degree polynomial trend for 26 analytical standards

2.8.7 Negative mode screen of South Nation Watershed

The developed negative mode DIA method was employed to scan 23 variable samples that were previously analyzed in positive mode. The 14 confident identifications in **Table 8** include a variety of CECs including pharmaceuticals, pesticides, artificial sweeteners, industrial contaminants and degradation products of pesticides. It has been shown in this thesis that artificial sweeteners are particularly useful as human biomarkers (**2.5.2**), particularly acesulfame, detected here in 10 of the 15 sites.

Table 8: Data-independent acquisition of negative ions
Samples collected from multiple sites in 2016 analyzed on four separate days to provide a qualitative analysis on the South Nations watershed.

Date Analyzed	Oct 12			Nov 18				Nov 25				Dec 9			
Compound	July 18 Site C	July 18 Site A	July 18 Site B	Aug 30 Site D	Sept 12 Site D	June 20 Site B	Sept 26 Site A	Oct 11 Site A	Sept 12 Site E	July 18 Site D	Aug 30 Site E	June 20 Site F	Sept 12 Site A	Oct 11 Site B	Nov 7 Site A
acesulfame	+	+	+	+		+	+	+					+	+	+
sucralose								+			+		+		+
3,5,6-trichloro-2-pyridinol	+	+		+	+	+	+			+		+	+	+	+
metolachlor OXA	+	+	+	+	+	+	+	+		+		+	+	+	+
metolachlor ESA	+	+	+	+	+		+	+		+		+	+		
ranitidine				+											
furosemide									+						
genistein		+	+	+	+	+		+	+		+	+	+	+	+
mycophenolic Acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
dinoseb										+					
dinoterb					+					+				+	
2-naphthalenesulfonic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
perfluorooctanesulfonic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
perfluorooctanoic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ designates confident identification

Current wastewater treatment (WWT) has been shown to have limited ability to degrade these contaminants. Sweeteners, for example, are poorly degraded and have been detected in effluent, surface water and potable water at ug L-1 concentrations (37-39). This recalcitrance to treatment and their low absorption to soils make acesulfame, sucrose and other sweeteners ideal candidates for waste water contamination markers (37, 39). One study on Singapore surface water found that even without direct discharge of WWT sweeteners including acesulfame, sucralose, saccharin and cyclamate were ubiquitous (36). This again points to the need of the newly developed screening method to analyze these compounds as they are all preferentially ionized in negative mode as shown in **Figure 30**.

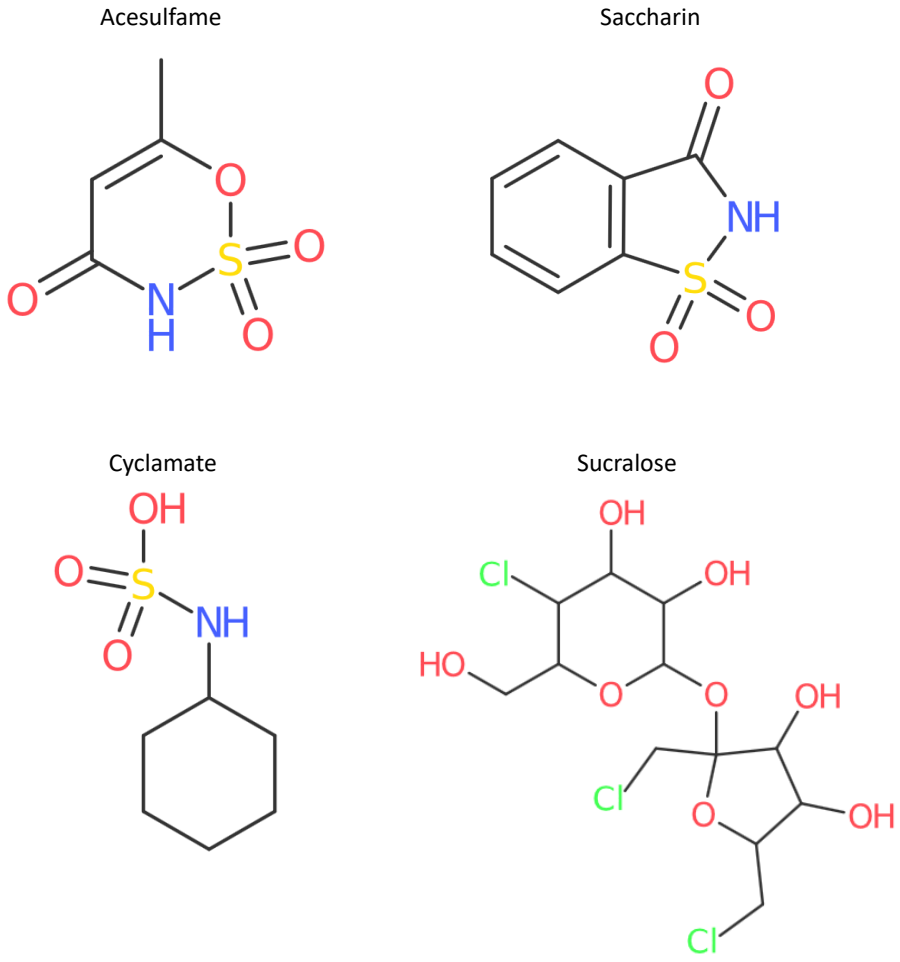


Figure 30: Structure of artificial sweeteners acesulfame, saccharin, cyclamate and sucralose

Metolachlor is a chloroacetanilide, which was commonly found in the above survey of the South Nation watershed (**2.4.2**). Chloroacetanilide degradates are also often commonly found in water analyses though they are commonly overlooked in water regulations (13). The degradation pathway for metolachlor oxanilic acid (MOXA) occurs through the oxidation of the acetyl group and metolachlor ethanesulfonic acid (MESA) is formed through glutathione conjugation (15). Here we found both metabolites and the TCP molecule across the majority of sites. Importantly, the pesticide degradates were not detected in the samples from site E downstream from the pesticide-free farming operation. The structures of metolachlor and both degradation products can be seen in **Figure 31**, where it is clear all compounds are capable of positive mode ionization but the degradates would prefer negative mode.

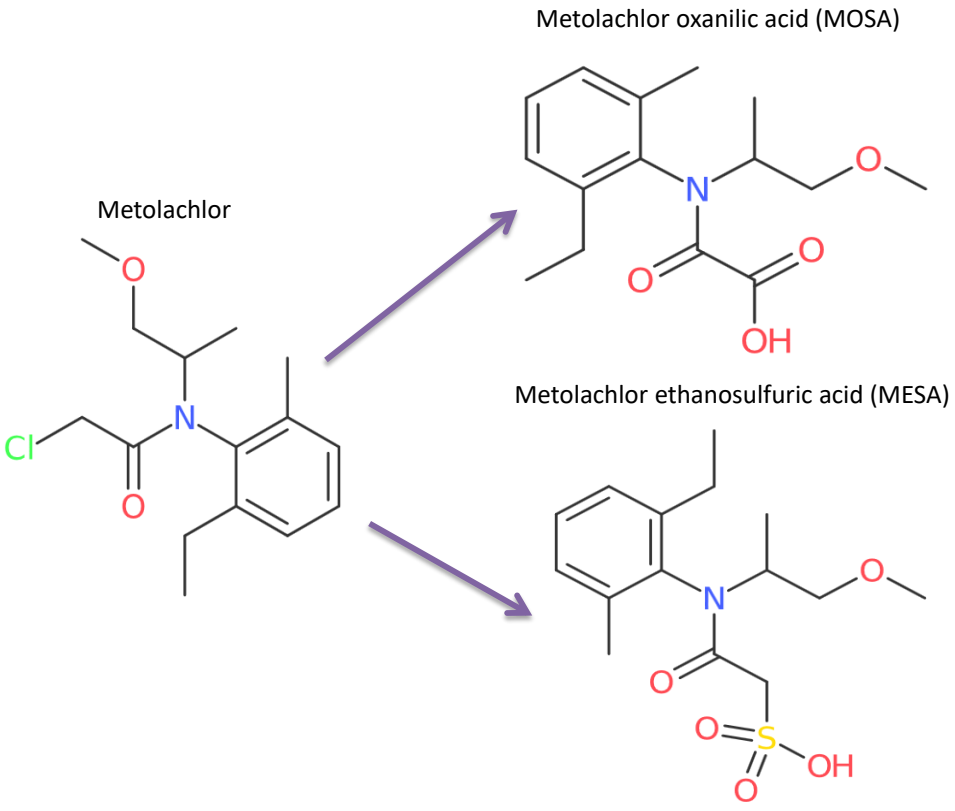


Figure 31: Structures of metolachlor and its degradation products MOXA and MESA

Industrial surfactants such as 2-naphthalenesulfonic acid and the per- and polyfluoroalkyl substances (PFAS) were detected from the negative screen in **Table 8**. The PFAS compounds screened were perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). PFAS in particular have a multitude of purposes due to their resistance to heat, water and oil. Examples of their uses include fire-fighting foams, apparels, upholstery, food paper wrappings and metal plating. Their popularized use has resulted in PFAS presence being abundant in the environment and even in blood samples of the general U.S. population (174). Their persistence and resistance to degradation has led to bioaccumulation in the environment as well as organs and blood (174). Additionally, it has been shown that traditional wastewater treatment has little ability to diminish these compounds (175).

Due to societal uproar and government restrictions on the traditional PFAS compounds (PFOS and PFOA) for their reproductive toxicity and environmental persistence, the industry has developed new compounds (176). One major substitute is a chemical known as GenX, which is a perfluoroalkyl ether carboxylic acid. A recent study near a manufacturing site in the Netherlands found concentrations as much as 13 times greater than the sum of other PFAS compounds (177). **Figure 32** depicts the structures of PFOA, PFOS and the newly developed GenX compound. It is clear that these compounds require negative mode to be detected. The manufacturers are clearly attempting to influence degradation by decreasing size and introducing central bridging oxygen into their chemicals. This however could result in even higher persistence and the potential for increased volatilization that could result in the transport of the pollutants to previously uncontaminated environments.

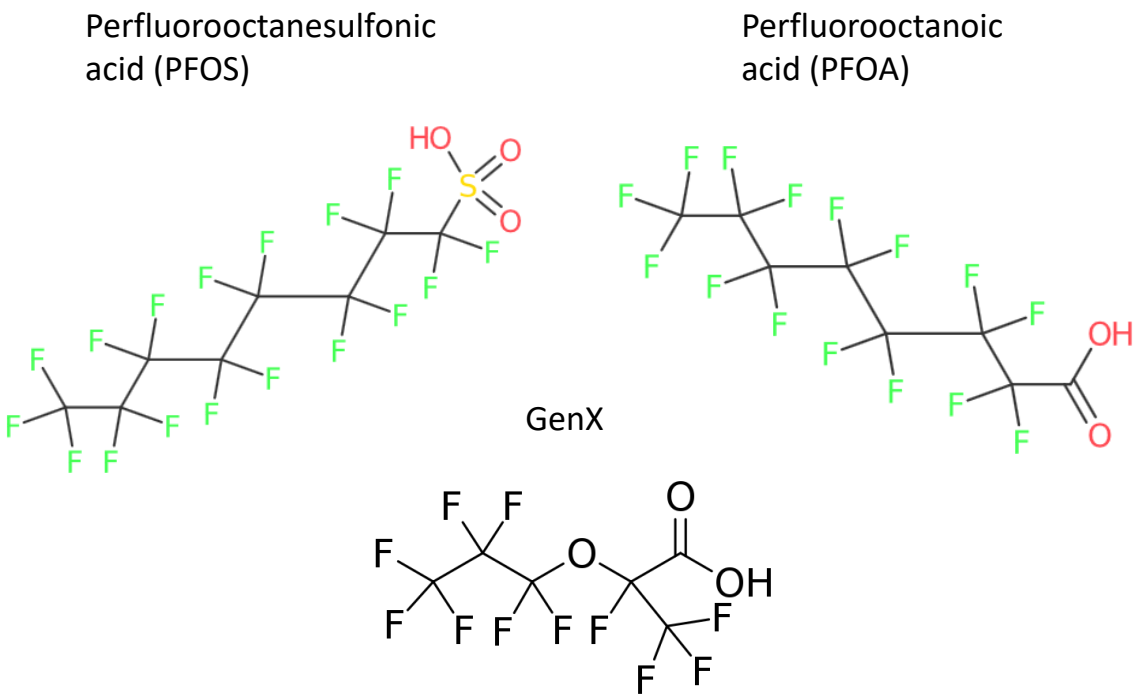


Figure 32: Structures of the PFOS, PFOA and GenX chemicals

The development of a negative mode screening technique improves the capabilities of environmental analyses. It increases the possible detections that can be made by including compounds requiring negative ionization. PFAS, artificial sweeteners, pesticide degradation products and other compounds with environmental importance have been shown to prefer or require negative rather than positive ionization. These compounds would be missed by the originally developed methods discussed in section **2.3.4**. With this negative DIA method these compounds will no longer be missed and the databased scans for each sample will have more value as new CEC are manufactured and enter the environment. Furthermore, compounds able to be ionized in either mode will have improved selectivity by the negative DIA method due to limited spectral interference.

Chapter 3

3 High-Throughput Quantitation of Neonicotinoids Using Small Sample Volumes by Lyophilisation

A version of this chapter has been published in:

Morrison LM, Renaud JB, Sabourin L, Sumarah MW, Yeung KK, Lapen DR. “High-Throughput Quantitation of Neonicotinoids in Lyophilized Surface Water by LC-APCI-MS/MS.” *Journal of AOAC International*. 2018 May. (Permission in **Appendix 2**)

3.1 Abstract

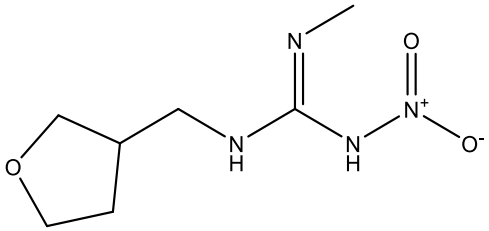
Neonicotinoids are among the most widely used insecticides. Recently, there has been concern associated with unintended adverse effects on honeybees and aquatic invertebrates at low parts-per-trillion levels. There is a need for LC-MS/MS methods that are capable of high-throughput measurements of the most widely used neonicotinoids at environmentally relevant concentrations in surface water. This method allows for quantitation of acetamiprid, clothianidin, imidacloprid, dinotefuran, nitenpyram, thiacloprid and thiamethoxam in surface water. Deuterated internal standards are added to 20 mL environmental samples, which are concentrated by lyophilisation and reconstituted with methanol followed by acetonitrile. A large variation of mean recovery efficiencies across five different surface water sampling sites within this study was observed, ranging from 45 to 74%. This demonstrated the need for labelled internal standards to compensate for these differences. Atmospheric pressure chemical ionization (APCI) performed better than electrospray ionization (ESI) with limited matrix suppression, achieving 71–110% of the laboratory fortified blank signal. Neonicotinoids were resolved on a C18 column using a 5 min LC method, in which MQL ranged between 0.93 and 4.88 ng L^{-1}. This method enables cost effective, accurate and reproducible monitoring of these pesticides in the aquatic environment.

Highlights: Lyophilisation is used for high throughput concentration of neonicotinoids in surface water. Variations in matrix effects between samples were greatly reduced by using APCI compared with ESI. Clothianidin and thiamethoxam were detected in all samples with levels ranging from below method quantitation limit up to 65 ng L^{-1}.

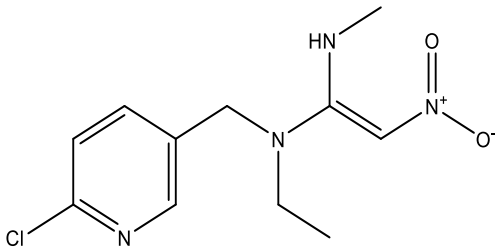
3.2 Introduction

Neonicotinoids are among the most commonly used insecticides worldwide (**Figure 33**); for example, imidacloprid is registered for use on over 140 crops in more than 120 countries (23). Since its introduction, additional neonicotinoids have been commercialized including: acetamiprid, clothianidin, dinotefuran, nitenpyram, thiacloprid and thiamethoxam. Neonicotinoids possess either an electronegative nitro- or cyano-functional group that selectively binds to nicotinic acetylcholine receptors, affecting the

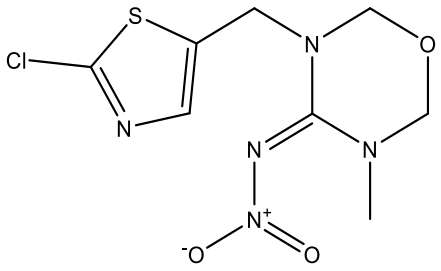
central nervous system of insects (29, 178). Thiamethoxam and clothianidin are among the most widely used, particularly through seed treatment for improved application (179). The Pest Management Regulatory Agency (PMRA) report on pesticides (2014) listed clothianidin as one of the top ten commercial insecticides in Canada, with annual sales exceeding 100,000 kg of the active ingredient (180). Sales of clothianidin were followed by thiamethoxam (>50,000 kg), imidacloprid (>50,000 kg) and acetamiprid (<50,000 kg) (180). Due to their widespread use and persistence, neonicotinoids can accumulate in soils and waterways (21) and have been detected in drinking water (181).



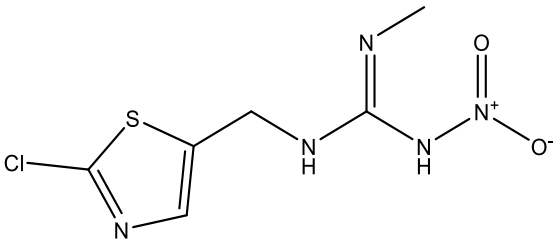
dinotefuran



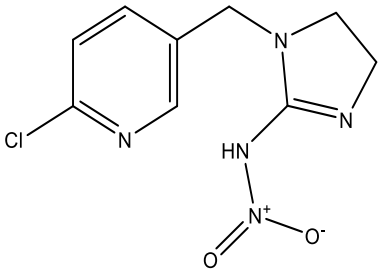
nitenpyram



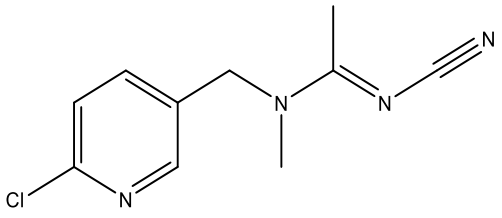
thiamethoxam



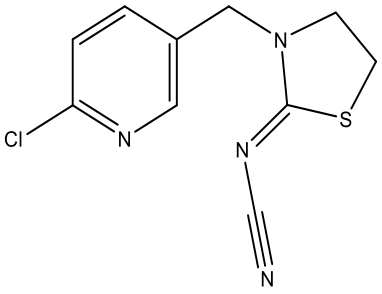
clothianidin



imidacloprid



acetamiprid



thiacloprid

Figure 33: Neonicotinoid structures

The use of neonicotinoids by the agricultural sector has been scrutinized due to potential unintended adverse effects on non-target insects such as honeybees (*Apis mellifera*) (25, 26). There is also increasing concern regarding the concentrations of neonicotinoids found in aquatic environments and their effects on invertebrates (26-28, 115). A recent study established a direct link between imidacloprid exposure and invertebrate community impairment (182). One particular study pointed to a significant response by Baetis mayflies to imidacloprid treatment (183). The 2017 U.S. benchmarks for neonicotinoid acute and chronic toxicity in water invertebrates are listed in **Table 9** (184). The only Canadian benchmark toxicity that has been defined for fresh water quality guidelines is for imidacloprid long term exposure at 230 ng L^{-1} (185). However, lower short- and long-term ecological thresholds of 200 ng L^{-1} and 35 ng L^{-1} have been proposed based on species sensitivity distributions (26, 186). Imidacloprid concentrations as high as 10.2 µg L^{-1} were detected in surface waters from Southwestern Ontario (115). A similar study from Sweden reported levels of 15 µg L^{-1} in some surface waters (28). Although use of clothianidin and thiamethoxam have surpassed imidacloprid in Canada, there is currently no guideline for these neonicotinoids due to limited data on environmental fate, exposure and biological effect. Increased public concern demands an efficient method to help regulatory jurisdictions determine the fate and persistence of all major neonicotinoids in surface water.

Table 9: Environmental benchmarks of neonicotinoids toxicity in water invertebrates from USEPA for 2017 in ug L^{-1} concentrations (184)

compound	Acute	Chronic
dinotefuran	>484,150	>95,300
nitenpyram	—	—
thiamethoxam	17.5	—
clothianidin	11	1.1
imidacloprid	0.385	0.01
acetamiprid	10.5	2.1
thiacloprid	18.9	0.97

— = data not published

Conceptually, direct aqueous injection (DAI) offers the highest throughput and ease of application of all available LC-MS/MS methods. Using a modern triple quadrupole system and injecting 50-100 µL of each sample, Hao *et al*. (2015) reported neonicotinoid method detection limits (MDL) between 2 and 8 ng L^{-1} (187). The increased sensitivity of modern mass spectrometers has allowed DAI to be more applicable for trace analysis; however, this technique may not be suitable for labs without state-of-the-art instrumentation and for samples with strong matrix effects.

The key feature of DAI is the absence of a sample enrichment or cleanup step; a necessary procedure for many existing LC-MS/MS systems. Common approaches for improving sensitivity by concentrating analytes include solid phase extraction (SPE) and lyophilisation. SPE is a popular enrichment technique that has previously been used for parts-per-trillion (ppt) detection of neonicotinoids in surface water samples (54-56, 188, 189). Hladki and Calhoun (2012) concentrated neonicotinoids from a 1 L surface water sample using Waters Oasis® hydrophobic-lipophilic balance SPE cartridges to achieve method detection limits between 3.6 and 6.2 ng L^{-1} (54). The disadvantages of SPE include the cost of cartridges and additional time requirements for sample processing, particularly with large volumes and sample numbers. Therefore a sample concentration method that is inexpensive, accurate, sensitive and allows for high-throughput processing, is desirable for monitoring neonicotinoids from environmental samples.

Lyophilisation offers a compromise between the enrichment by SPE and the high-throughput and reduced costs of DAI. LC-MS/MS methods have been developed to analyze pharmaceuticals and pesticides by simple and effective methods using lyophilisation (60, 61, 190, 191). The majority of these compounds have been detected in the ppt (ng L^{-1}) range.

We report a LC-MS/MS method specific for neonicotinoids that is capable of achieving low ppt limits of quantitation using lyophilisation for sample enrichment. We also demonstrated that atmospheric pressure chemical ionization (APCI) drastically reduced matrix effects. Furthermore, internal standards were required to correct for variation in recovery efficiencies between samples. The method was optimized and validated using environmental surface water samples collected from a long-term water quality surveillance initiative.

3.3 Experimental

3.3.1 Reagents and Supplies

(a) *Water* – reverse osmosis deionized water, 18 MΩ-cm, Barnstead Nanopure Water Purification System (Thermo Scientific, Waltham, MA)

(b) Methanol, acetonitrile and water – Optima Grade (Fisher Scientific, Pittsburgh, PA)

(c) Formic acid – highly purified grade (Thermo Scientific, Waltham, MA)

(d) *Reference standards* – acetamiprid, clothianidin, imidacloprid, dinotefuran, nitenpyram, thiacloprid, thiamethoxam, thiamethoxam-d_3, imidacloprid-d_4 and clothianidin-d_3 purchased from Sigma Aldrich (St. Louis, MO). Standards were ≥ 98% pure

(e) *Filters* – Whatman glass microfiber filters (GF/C), 47 mm diameter and 1.2 µm pore size (GE Healthcare Life Sciences, Buckinghamshire, UK)

(f) *Scintillation vials* – unattached urea cap with conical liner (VWR, Radnor, PA)

(g) *Filter vials* – SINGLE StEP™ nano filter vial 0.45 µm PTFE, with non-slit blue cap (Thomson Instrument Company, Oceanside, CA)

(h) HPLC vials – amber glass (Agilent Technologies, Santa Clara, CA)

3.3.2 Apparatus

(a) *Analytical balance* – Mettler AL54 (Mettler-Toledo Columbus, OH)

(b) *Lyophilizer* – FreeZone Plus 12L Cascade Console Freeze Dryer (Labconco, Kansas City, MO)

(c) *Mixer* – Vortex-Genie 2 model G-560 (Scientific Industries, Bohemia, NY)

(d) *Vacuum concentrator* – centrivap concentrator and cold trap (Labconco, Kansas City, MO)

(e) *Shaker* – Eppendorf™ ThermoMixer F1.5 (Fisher Scientific)

(f) *Chromatography* – Agilent 1290 infinity HPLC, binary pump, autosampler and column compartment (Agilent Technologies)

(g) *Mass spectrometer* – Q-Exactive Orbitrap (Thermo Scientific)

3.3.3 Sample Characteristics and Handling

Surface water samples were collected at five sites in the South Nation River watershed near Ottawa Ontario, between May and June 2017. Land use in this watershed is mixed-use, but primarily agricultural (i.e., cash crops, livestock, corn, soybean and forage cropping practices) (192). Water samples were collected in sterile containers and shipped overnight on ice to Agriculture and Agri-Food Canada's Research and Development Centre in London, Ontario and immediately frozen at -20 °C. The samples were thawed at room temperature on the day of use and filtered through 1.2 µm glass microfiber filters to remove solid particulate prior to analysis.

3.3.4 Standard Solutions

Individual standards of clothianidin, imidacloprid, nitenpyram, thiacloprid, thiamethoxam, thiamethoxam-d_3, imidacloprid-$d_4$and clothianidin-d_3 were accurately weighed and dissolved in methanol to a stock concentration of 1 mg mL^{-1}. Acetamiprid and dinotefuran were dissolved in water to a stock concentration of 1 mg mL^{-1}. Working solutions (10 µg mL^{-1}) of labelled and unlabeled standards were prepared for each compound. Labelled and unlabeled mixes were prepared by combining the individual working solutions and diluting them with methanol to a final concentration of 100 ng mL^{-1}.

3.3.5 Lyophilisation

Laboratory fortified sample matrices (LFSM) were prepared by transferring 20 mL of filtered surface water samples into a polypropylene scintillation vial. Five µL of labelled standard spiking solution (100 ng mL^{-1}) was added, resulting in a final concentration of 25 ng L^{-1}. Samples were vortexed for 30 seconds before being placed at -80 °C. After freezing, the scintillation vial caps were loosened to allow air flow and the frozen samples were placed immediately inside a lyophilizer for 24 hours or until completely sublimated.

Following lyophilisation, the analytes were recovered with the addition of 500 µL of methanol, vortexed for 30 seconds and transferred into a PTFE SINGLE StEP™ nano filter vials. The recovery process was repeated by adding 500 µL of acetonitrile and combining it with the methanol fraction prior to drying using a centrivap. The dried residue was reconstituted with 80 µL of 7:2:1 water:methanol:acetonitrile and samples were mixed (1400 rpm) on a thermomixer for one hour at room temperature. Nano filter vials were used for a final filtering to remove all solid particulate matter prior to LC-MS/MS analysis.

3.3.6 Chromatography Conditions

(a) *Analytical column* – Zorbax Eclipse Plus C18 rapid resolution HD, 2.1 x 50 mm, 1.8 µm particle size (part No. 959757-902; Agilent Technologies)

(b) *Guard column* – Eclipse Plus C18 2.1 x 5 mm, 1.8 µm particle size (part No. 821725-902; Agilent Technologies)

(c) *HPLC mobile phase* – 0.1% formic acid in water (mobile phase A), 0.1% formic acid in acetonitrile (mobile phase B)

(d) *Flow rate* – 0.3 mL min^{-1}

(e) *Gradient* (**Figure 34)**– 100% A held from 0–0.5 min, 100%-70% A from 0.5-0.7 min, 70%-65% A from 0.7-3.12 min, 65%-0% A from 3.12-3.5 min, held at 0% A from 3.5-4.5 min, 0%- 100% A from 4.5-5.0 min

(f) *Injection volume* – 5 µL

(g) *Column temperature* – 35 °C

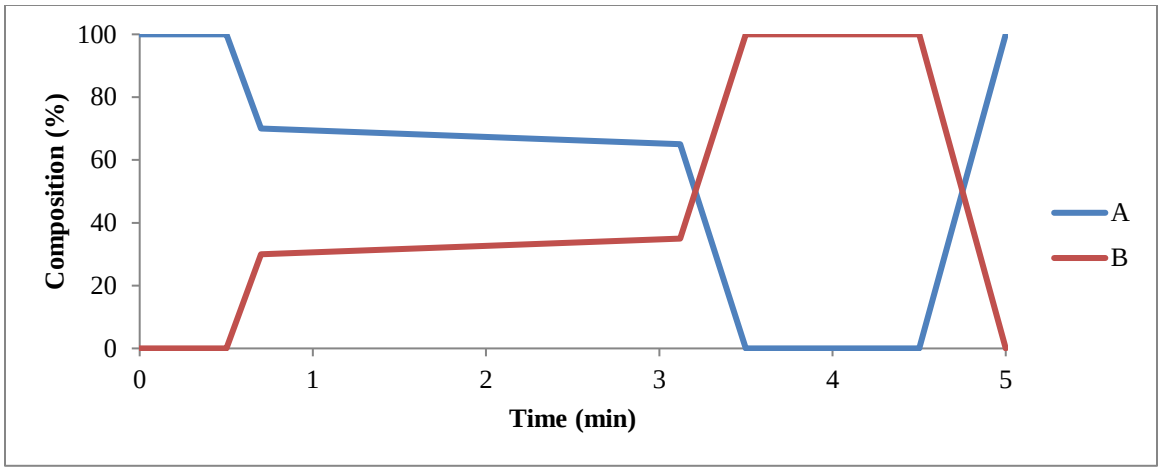


Figure 34: Chromatographic separation for the targeted neonicotinoid method Used for quantitation of seven neonicotinoids, where A is water with 0.01% formic acid (FA) and B is acetonitrile with 0.01% FA

3.3.7 Mass Spectrometry Conditions

APCI settings were: capillary temperature, 260 °C; sheath gas flow rate, 25 arbitrary units; auxiliary gas flow rate, 15 units; probe heater temperature, 425 °C; S-lens RF level, 50%; and corona discharge voltage, 4.3 kV.

Heated electrospray ionization (HESI) settings were: capillary temperature, 400 °C; sheath gas flow rate, 19 arbitrary units; auxiliary gas flow rate, 8 arbitrary units; probe heater temperature, 450 °C; S-lens RF level, 45%; and capillary voltage, 3.9 kV.

Samples were analyzed in parallel reaction monitoring mode (PRM) at 17,500 resolution, automatic gain control (AGC) 3×10^6, maximum injection time 64 ms and isolation window of 1.2 *m/z*.

3.3.8 Quantitation

(a) A calibration curve containing all seven unlabelled neonicotinoids was made at the following concentrations: 0.1, 0.25, 0.625, 2.5, 25, and 50 ng mL^{-1}. Isotopically labelled compounds were added to each calibration solution at a concentration of 12.5 ng mL^{-1}.

(b) Xcalibur software using ICIS peak detection algorithm, 5 point smoothing and 50 baseline was used to integrate peak areas.

(c) A linear calibration curve was obtained by plotting using 1/x weighting factor:

$$\frac{peak\ area_{\text{unlabelled}}}{peak\ area_{internal\ standard}} = slope\frac{[unlabelled]}{[internal\ standard]} + intercept$$

3.3.9 Method validation

(a) *Recovery efficiency* – Each LFSM was spiked either before or after lyophilisation with unlabelled and labelled compounds to final concentrations of 25 ng L^{-1} and 250 ng L^{-1} to evaluate recovery efficiencies (R_E). Concentrations were corrected from baseline using sample blanks.

$$R_E = \frac{[pre\ lyophilization\ spike]}{[post\ lyophilization\ spike]}$$

(b) *Signal suppression/enhancement (SSE)* – Laboratory fortified blanks (LFB) were prepared by spiking empty filter vials with 25 ng L^{-1} and 250 ng L^{-1} of unlabelled and labelled neonicotinoids before the final drying and reconstitution steps and SSE was evaluated as:

$$SSE\% = \frac{[post\ lyophilization\ spike]}{[LFB]}$$

(c) Accuracy and precision – The accuracy between five replicates from each of the five study sites was evaluated using the ratio of experimentally determined concentrations and the amount spiked. Accuracy and precision (RSD) was determined using five replicates of LFSM spiked at 25 ng L^{-1}.

$$accuracy = \frac{[measured] - [blank]}{[level\ spiked]}$$

(d) *Limits and linearity* – The method detection limit (MDL) was defined as the lowest concentration where five consecutive injections produced a detectable signal, which was corrected for percent recoveries of each compound. The method quantitation limit (MQL) was defined as the lowest concentration where the peak area RSD of five consecutive injections was below 25%, when corrected for percent recoveries. Linearity of the calibration curve for each compound was determined in a range from the lowest quantifiable levels to 50 ng L^{-1} with a 1/x weighting factor.

3.4 Results and Discussion

3.4.1 Sample pre-concentration

Following lyophilisation, all freeze-dried surface water samples yielded a solid residue. Initial method optimization found significant discrepancies in analyte recoveries between surface water samples and deionized water (which did not yield any solid residue following lyophilisation). Therefore, environmental water samples were used for method optimization and evaluation. In this method, the process starts with a 20 mL environmental surface water sample and results in a final volume of 80 µL, a 250 fold increase in concentration. The combination of both methanol and acetonitrile was found to be optimal for recovery. The addition of 0.1% formic acid decreased recovery and similarly the addition of EDTA or citric acid had little effect on recovery and increased signal suppression.

When using a high resolution mass spectrometer, the 'noise' level that is commonly present for low resolution instruments may be absent. Therefore, in place of a signal to noise based definition, the MDL for each compound was defined as the lowest concentration at which five injections were consecutively detected (**Table 10**). The MQL was the lowest concentration where the RSD of the peak area was less than 25%. The MQL ranged between 0.67 - 8.7 ng L^{-1}, which is comparable to the literature reports that used much greater sample volumes (54-58).

Table 10: Linearity and Instrument Limitations

compound	calibration curve equation[a, b]	correlation coefficient, r	MDL[c] (RSD)[d] ng L^{-1}	MQL[c] (RSD)[d] ng L^{-1}	SUR RR [e]
dinotefuran	y = 0.7674x - 0.0061	0.9990	2.52 (9)	2.52 (9)	90%
nitenpyram	y = 0.1935x - 0.0036	0.9989	4.88 (21)	4.88 (21)	88%
thiamethoxam	y = 1.3466x - 0.0203	0.9990	2.42 (11)	2.42 (11)	-
clothianidin	y = 0.9819x - 0.0154	0.9995	1.73 (16)	1.73 (16)	-
imidacloprid	y = 1.2329x - 0.0309	0.9998	2.22 (20)	2.22 (20)	-
acetamiprid	y = 2.7631x - 0.0083	0.9996	0.89 (26)	1.78 (10)	124%
thiacloprid	y = 2.5104x + 0.0045	0.9997	0.47 (30)	0.93 (16)	121%

[a] The calibration curve was prepared between 0.01 – 50 ng mL^{-1}.

[b] 1/x weighting factor of peak area ratio unlabeled/labelled.

[c] MDL and MQL were determine at the levels where n = 5 injections were detected and those with a RSD less than 25% respectively.

[d] RSD from n = 5 injections.

[e] The relative response (RR) correction was determined for acetamiprid, dinotefuran, nitenpyram and thiacloprid, using thiamethoxam-d_3 as the labelled surrogate (SUR).

3.4.2 Chromatography conditions

A major objective of this work was to enable high throughput sample analysis. This entails decreasing the LC-MS method duration, while still achieving chromatographic resolution of the analytes. Using the gradient described above, the seven neonicotinoids were resolved within 5 minutes (**Table 11**). Ideal separation of the analytes shown in **Figure 35** allowed for a maximum number of scans to be acquired from each chromatographic peak.

Table 11: LC-MS parameters for compound identification

compound	formula	ion	retention time (min)	precursor (*m/z*)	quantifier/ qualifier (*m/z*)	normalized collision energy (NCE)
dinotefuran	$C_7H_{14}N_4O_4$	$[M+H]^+$	1.91	203.1136	129.0897/ 114.1027	29
nitenpyram	$C_{11}H_{15}ClN_4O_2$	$[M+H]^+$	1.96	271.0954	225.1022/ 99.0922	27
thiamethoxam	$C_8H_{10}ClN_5O_3S$	$[M+H]^+$	2.20	292.0260	211.0649/ 131.9670	17
thiamethoxam-d_3	$D_3C_8H_7ClN_5O_3S$	$[M+H]^+$	2.20	295.0454	214.0836/ 131.9667	17
clothianidin	$C_6H_8ClN_5O_2S$	$[M+H]^+$	2.39	250.0155	169.0539/ 131.9668	33
clothianidin-d_3	$D_3C_6H_5ClN_5O_2S$	$[M+H]^+$	2.39	253.0348	172.0730/ 131.9670	33
imidacloprid	$C_9H_{10}ClN_5O_2$	$[M+H]^+$	2.50	256.0590	209.0584/ 175.0975	31
imidacloprid-d_4	$D_4C_9H_6ClN_5O_2$	$[M+H]^+$	2.50	260.0847	213.0839/ 179.1229	31
acetamiprid	$C_{10}H_{11}ClN_4$	$[M+H]^+$	2.62	223.0742	126.0105/ 56.0503	47
thiacloprid	$C_{10}H_9ClN_4S$	$[M+H]^+$	3.05	253.0305	126.0105/ 69.0341	41

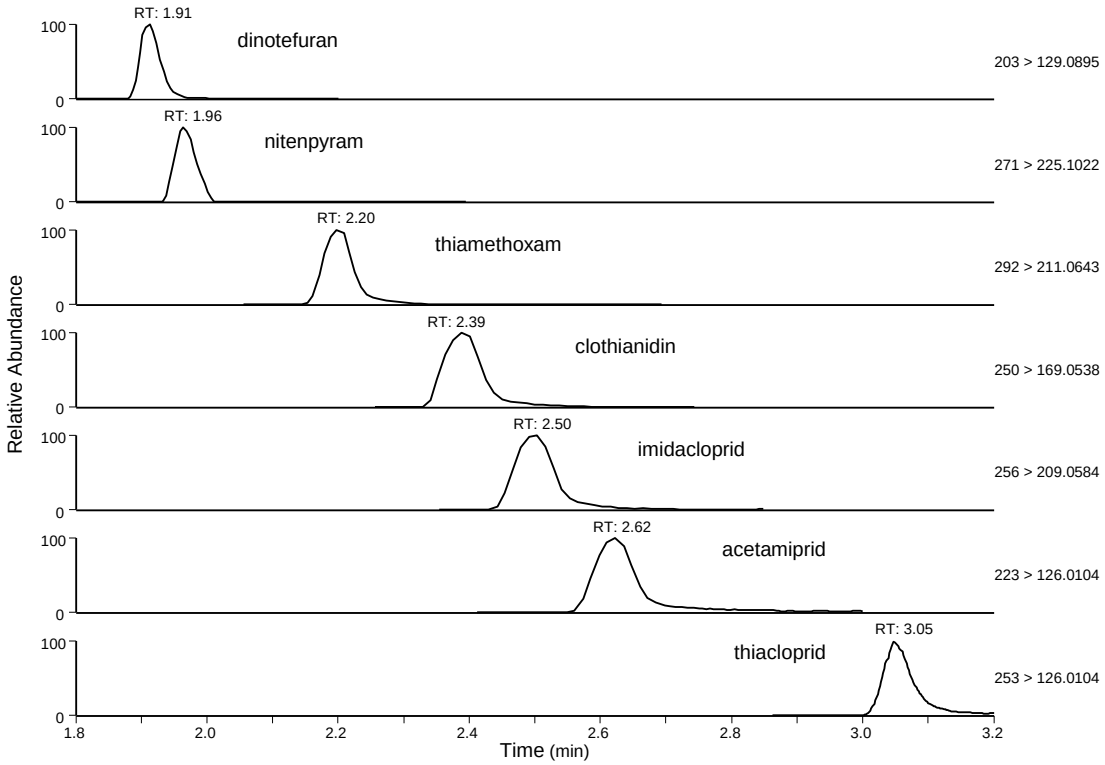


Figure 35: Extracted Ion Chromatogram of 0.25 ppb standard solution

3.4.3 Evaluation of Ionization Source

Current methods for the analysis of neonicotinoids have relied predominately on electrospray ionization (193, 194). Using our sample preparation and extraction conditions, signal suppression was significant when using our HESI source (**Table 12**). Preliminary analyses with the HESI source demonstrated that the signal intensity of the compounds recovered from spiked samples were reduced to between 23–65% compared to LFB signals. However, neonicotinoids were found to have decreased susceptibility to matrix effects when using an APCI source. This observation is in agreement with Wang and Gardinali (2012), who found APCI to have improved ionization over ESI for some pharmaceuticals and personal care products in water (83). APCI resulted in a large diminution of overall signal suppression as well as inter-analyte differences between the neonicotinoids within this study; the SSE of the analytes ranged between 73–92%. Based on these results, APCI was selected as the optimal ionization source for subsequent analyses.

Table 12: Ionization evaluation of 250 ng L^{-1} spike in a sample comparing mean signal suppression (SSE) from five replicates when using APCI and HESI source probes

compound	APCI SSE (%)	HESI SSE (%)
dinotefuran	71	23
nitenpyram	90	33
thiamethoxam	74	39
clothianidin	80	43
imidacloprid	86	61
acetamiprid	110	57
thiacloprid	103	65

APCI is suited for analysis of a narrower range of compounds than HESI; however, it is generally accepted as having decreased susceptibility to matrix effects (69, 82, 83). With APCI, the injected sample and mobile phase are heated prior to charging, enabling gas phase ionization, whereas HESI heats and charges the solution simultaneously (69, 82). Ionization in the liquid phase generates charged droplets, which contain a significant

amount of impurities, resulting in greater variances due to different matrix compositions (82). These impurities can decrease target analyte responses caused by competition for ionization based on the highest charge affinity of the different eluting species (83). This competition is generally accepted as the primary mechanism of signal suppression or enhancement (SSE), which inhibits reproducibility and hinders quantitation (69, 82, 83). In comparing matrix effects across five different sites, we found that APCI also performed well (**Table 13**), where the average SSE for all analytes was 81%, with RSD values below <15%.

Table 13: Recovery and signal suppression of 25 ng L^{-1} spike in five different sample matrices using APCI (n=5)

average recovery efficiency (%)	site A	site B	site C	site D	site E	mean (%)	RSD (%)
dinotefuran	72	48	37	30	37	45	33
nitenpyram	98	36	34	38	25	46	57
thiamethoxam	90	63	48	48	58	61	25
clothianidin	106	84	59	53	68	74	26
imidacloprid	102	61	41	41	53	60	38
acetamiprid	100	62	41	41	49	59	38
thiacloprid	113	68	51	48	58	68	35
average SSE (%)							
dinotefuran	90	90	82	79	94	87	6
nitenpyram	84	81	78	87	109	88	13
thiamethoxam	86	62	61	58	69	67	15
clothianidin	92	75	58	77	85	77	15
imidacloprid	74	67	69	77	84	74	8
acetamiprid	99	85	86	92	107	94	9
thiacloprid	75	72	70	83	92	78	10

3.4.4 Sample recovery

Lyophilisation allows for the concentration of all components present in a sample with little manipulation. In addition to the analytes of interest, all other compounds present in the residue remaining after lyophilisation are concentrated, including inorganic salts. Organic solvents, rather than aqueous, were added in order to reconstitute the analytes and minimize salt reconstitution. Sample recovery was evaluated by comparing the

measured concentrations in samples spiked with analytes prior to lyophilisation against the identical spike following reconstitution, in order to negate any differences in SSE. As depicted in **Table 13**, recovery showed greater variance across samples than between analytes. For example, the average recovery efficiency in Site D ranged from 72 – 113%, whereas the range for acetamiprid across samples was 41 – 100% (mean 50%, RSD 38%). This demonstrated the need for the internal standards that we used in the experimentation in order to compensate for sample-to-sample variation.

3.4.5 Method Validation

Good linearity ($r^2 > 0.9989$) was obtained from the calibration curve (0.1 – 50 ng/mL) as shown in **Table 10**. The accuracy of the method was determined by spiking 25 ng L^{-1} of each compound into the five LFSM. The calculated results were compared to the expected concentration of 25 ng L^{-1} (**Table 14**). The compounds had a mean percent accuracy ranging from 94%-110% across the five samples, validating the accuracy of this method at ppt concentrations from 20 mL sample volumes. Additionally, the precision was acceptable with RSD values below 10%.

Table 14: Precision and accuracy as a percent of the 25 ng L^{-1} spike pre lyophilisation (n=5)

	site A		site B		site C		site D		site E		mean	
compound	mean (%)	RSD (%)	mean (%)	RSD (%)	mean (%)	RSD (%)	mean (%)	RSD (%)	mean (%)	RSD (%)	mean (%)	RSD (%)
dinotefuran	99	8	106	9	117	3	85	3	98	3	101	5
nitenpyram	120	3	100	2	109	5	96	3	91	2	103	7
thiamethoxam	104	4	92	4	93	9	94	8	93	9	95	5
clothianidin	107	7	109	8	89	6	92	4	95	9	98	9
imidacloprid	91	9	93	8	95	8	96	4	96	7	94	2
acetamiprid	109	7	113	9	108	4	116	3	105	7	110	4
thiacloprid	86	7	91	8	92	4	109	8	101	4	96	9

3.4.6 Quantitation of neonicotinoids in South Nation Watershed

Five surface water samples collected from the South Nation watershed were spiked with labelled standards and screened using the described method. Thiamethoxam and clothianidin were detected in all sites; all other neonicotinoids screened were not detected above the MDL. Imidacloprid was not detected in any samples, despite its current regulatory focus. Clothianidin, which was found at levels ranging from 18 - 65 ng L^{-1}, was present in all sites at higher amounts than thiamethoxam, which ranged from 2.4 - 7.1 ng L^{-1} (**Table 15**). These concentrations are in agreement with neonicotinoid sales data for Canada (195).

Table 15: Average concentration and RSD of neonicotinoids in five replicates from each site in ng L^{-1}

compound	site A	site B	site C	site D	site E
dinotefuran	—	—	—	—	—
nitenpyram	—	—	—	—	—
thiamethoxam	7.11 ± 0.04	3.98 ± 0.09	<MQL	3.2 ± 0.4	5.0 ± 0.4
clothianidin	38.0 ± 0.2	65.2 ± 0.1	60.7 ± 0.2	18.6 ± 0.2	18.91 ± 0.08
imidacloprid	—	—	—	—	—
acetamiprid	—	—	—	—	—
thiacloprid	—	—	—	—	—

— = not detected <MDL
<MQL = A detection below the quantifiable level

This method was shown to be effective for quantitative neonicotinoid monitoring, particularly for large-scale surveillance-style studies that typically require hundreds or thousands of samples. The method decreases manual labor, reduces costs, and requires only a small sample volume. The lyophilization step allows this method to be applied across modern and existing LC-MS/MS platforms. All seven neonicotinoids are detectable at low ppt levels, which is comparable with currently available SPE and DAI methods. Finally, the combination of deuterated internal standards and APCI produces highly reproducible results that are ideal for minimizing sample-to-sample differences in recovery efficiencies and SSE.

Chapter 4

4 General Discussion and Conclusions

4.1 Discussion

The traditional method of analysis for contaminants in the environment is limited, as it requires the selection of the compounds to be studied prior to method development. This is a result of the exclusive implementation of tandem mass spectrometry. Including an initial non-targeted screen using data-independent acquisition (DIA) allows for a more encompassing analysis of each sample. Compounds detected in the screening can then be targeted, providing a more relative selection for quantitation. Additionally, the DIA spectra can be retrospectively analyzed for emerging contaminants that weren't selected as original targets.

This work has produced a series of analytical methods for the identification and quantitation of environmental contaminants. Pharmaceuticals and pesticides have been studied in particular, with the two year study of a watershed, the analysis of a secluded lake and the examination of a wastewater treatment process. The development of a negative DIA method led to improved detections of compounds previously missed. In particular, neonicotinoids required an improved method that would allow for high-throughput of a large number of samples to get a true picture of their presence in the environment.

4.1.1 SNC Survey

The South Nation watershed has been extensively monitored and studied over the past few decades (106, 108, 192). The data presented here, is the first chemical survey. The overall contamination levels between the two years of study appeared to be generally analogous in comparison. Significantly, it was seen that there was clear changes in concentration across the months, particularly for pesticides that have a tendency to be heightened during months when application takes place. The continuation of this research will allow for further interpretational value, including possible persistence of compounds, accumulation and disappearance as they become banned or restricted for use.

The variety of contaminants detected includes herbicides, insecticides, antibiotics and antidepressants. The largest contaminations came from the herbicides atrazine and metolachlor. This is theorized to be due to their widespread use and ease of transport into

water. No pollutants were found above environmental or drinking water guidelines. This could be due to the analyses missing degradation products, which are often also bioactive compounds. Atrazine, for instance, has been banned in the European Union following the excessive detection of the native compound and degradants in water (196).

The major pharmaceutical presence consisted of antibiotics including erythromycin, clarithromycin, enrofloxacin and azithromycin. This is particularly concerning as the mutation of pathogens to be resistant against our medicine has become a crisis (32, 34, 197). Currently, there are no environmental guidelines for these compounds. Whether or not the contaminants are at dangerous levels, it is important to monitor the changing concentrations to increase awareness of their presence and the possible implications.

4.1.2 Secluded Lake Analysis

Lac Hughes was found to have very little contamination, as expected. However, some herbicides were found that could be considered surprising, based on land use information. This could be construed as a result of legacy contamination from historic rural activity, misuse by nearby residents, or transportation by volatilization. Considerably, the ubiquitous level of acesulfame, an artificial sweetener, was interpreted to determine the input volume of human urine in the lake. The main contamination found at immense levels in the lake was the insect repellent DEET. This was foreseeable as the lake is prominently used by cottagers. This study in particular is a great example of human influence on a secluded lake with no industrial, rural or urban influence. The only contamination can be expected to come from local septic tanks of the surrounding cottages and their residents.

4.1.3 Wastewater Treatment

Wastewater treatment plants (WWTP) are currently limited in their ability to remove chemical pollutants, such as pharmaceuticals and pesticides. Pharmaceuticals are commonly found in WWTP effluent due to medications only being partially metabolized or from improper disposal (123). Antibiotics entering the environment are particularly concerning with the emergence of resistant bacteria (32). Treatment techniques including oxidation of these contaminants have shown some ability for remediation. Unfortunately,

the majority of experiments have been completed in laboratory samples and many compounds have shown recalcitrance (163). Here, we have employed a previously developed method of oxidation for the remediation of contaminants in real WWTP samples (155, 156).

Implementation of the developed methods of analysis for screening and targeted analysis allowed for evaluation of the oxidation treatment. Here, we found a variety of pharmaceuticals and pesticides. Pharmaceuticals were the major source of contamination, including antibiotics, antidepressants and anticoagulants. The oxidation showed potential for treatment of many chemicals and with the supplementation of acid-catalysis even recalcitrant chemicals were removed. Additionally, by comparing two WWTPs remediation techniques clearly need to be proven in multiple matrices, as it was particularly affected by water characteristics. Further implementation of this treatment could lead to improved WWTP techniques.

4.1.4 Negative mode data independent acquisition

Non-targeted analysis of environmental contamination is ideal for surveying the majority of chemicals in a sample. Unfortunately, it is impossible to collect and analyze every analyte present. A new non-targeted DIA method was developed for negatively ionized compounds that were previously being missed and have been shown to have potential human and environmental impact. These chemicals include polyfluoroalkyl substances (PFAS), pesticide metabolites and artificial sweeteners.

The development of the method required determining potential ion m/z distribution. A multitude of samples were scanned to determine the mass range required for the new DIA method. It was determined that the range was much smaller than the previously developed screening method, as there are far fewer analytes ionized in negative mode compared to positive mode. This is particularly important as the compounds detected will have improved selectivity due to decreased spectral interference. Additionally, this allowed the new screening technique to require less analysis time compared to the previous method. Using mass defect distribution the DIA windows were optimized to prevent ions at the edge of each window.

The finalized method was used to scan a wide variety of samples for analysis. The samples were screened against in-house analytical standards as previously completed with positive DIA. An online open source mass spectra database was also screened against to expand the potential for identifications. The PFAS chemicals identified are particularly concerning due to their persistence in the environment. Additionally, the metolachlor degradation products that were identified are required for an accurate risk assessment of the herbicide. Furthermore, the detection of chlorpyrifos is difficult, due to it readily degrading in water. The primary hydrolysis metabolite, 3,5,6-trichloro-2-pyridinol (TCP), is the optimal marker for tracing chlorpyrifos presence in the aquatic environment (169). TCP has also been sown to have toxic effects and notably, it requires negative ionization (198).

4.1.5 High-throughput method for small sample volumes using lyophilisation

The optimization of extraction and analysis is completed for a single class of compounds in an effort to provide improved monitoring. Neonicotinoids are among the most widely used insecticides and they have recently become controversial for their effect on unintentional species. There is particular concern for their presence in aquatic environments and their effects on invertebrates (26-28, 115). This method implemented simplification of the current extraction technique using SPE on 200 mL of surface water by instead lyophilizing 20 mL water samples. This provides similar analyte concentration without the laborious and expensive isolation. Furthermore, this allows for the simultaneous analysis of a multitude of samples with little user input. This method is not ideal for a variety of compounds and DIA would have little value, but it points instead to the importance of simple and directed analysis for compounds of particular concern.

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Appendices

Appendix 1: Compounds compared to non-targeted DIA spectra using accurate precursor mass, retention time and characteristic fragment ions

Analytical standards	EAWAG negatively ionized compounds
17a-ethylnylestradiol	10phiC10SPC
3-Hydroxycarbofuran	1-Chlorobenzotriazole
4`,6` Diamidino - 2 - Phenylindole	1H-1-2-3-triazole-5-OH
4-aminophenyl sulfone	1H-Benzotriazole
Acetamiprid	1H-Benzotriazole- 4(or 5)-methyl-
Acetominophen	1H-Benzotriazole-5-carboxylic acid
Acibenzolar-S-methyl	1-Hydroxybenzotriazole
Alachlor	2-(3-Hydroxycyclohexyl)-5-(2-methyl-2-octanyl)phenol
Albuterol	2'-2'-Difluoro-2'-deoxyuridine
Aldicarb sulfone	2-Aminobenzimidazole
Ametryn	2-Hydroxybenzothiazole
Amikacin	2-Mercaptobenzothiazole
Amitriptyline	2-Naphthalenesulfonic acid
Amoxicillin	2-Naphthoxyacetic acid
Ampicillin	2-Toluenesulfonamide
Apramycin sulfate	3-[(4-chlorobenzoyl)amino]propanoic acid
Atenolol	3-Phenoxybenzoic acid
Atraton	4`-Hydroxy Diclofenac
Atrazine	4-Amino-6-chloro-1-3-benzenedisulfonamide
Azithromycin	4-Chlorophenol
Azoxystrobin	4-Hydroxybenzotriazole
Bacitracin	4-Toluenesulfonamide
Benzyldimethyldodecylammonium chloride	5-Fluorouracil
Biphenyl	5-Hydroxy Diclofenac
Bromacil	5-Methyl-1H-benzotriazole
Buprofezine	8phiC8SPC
Butachlor	Acamprosate
Butylate	Acemetacin
Caffeine	Acetamiprid
Capecitabine	Acetazolamide
Carbadox	Acifluorfen
Carbamazepine	Adenosine
Carbaryl	Albendazole
Carbenicillin	Albuterol
Carbofuran	Alfuzosin
Cefotaxime	Aliskiren

Cefsulodin
Ceftazidime
Ceftiofur
Celecoxib
Cephalexin
Chlorhexidine
Chlorpropham
Cimetidine
Ciprofloxacin
Clarithromycin
Clinafloxacin
Clothianidin
Cruformate
Cyanazine
Cycloate
Cycloheximide
Cyproconazole
Cyprodinil
Cyromazine
Decoquinate
Dichlobutrazole
Diclofenac
Diethatyl-ethyl
Diltiazem HCl
Dinitramine
Dinotefuran
Diphenamid
Diphenhydramine
Diphenhydramine N-Oxide
Doxycycline
Enrofloxacin
Eprinomectin
EPTC
Erythromycin A
Erythromycin B
Erythromycin C
Esfenvalerate
Etridiazole
Febuconazole
Fenamiphos sulfone
Fenamiphos sulfoxide
Fenarimol
Allopurinol
Amidosulfuron
Amisulpride
Amisulpride N-Oxide
Amoxicillin
Ampicillin
Aspartame
Aspirin
Asulam
Atazanavir
Atenolol acid
Atorvastatin
Atrazine-2-hydroxy
Atrazine-desethyl-2-hydroxy
Azoxystrobin
Benserazide
Bentazone
Benthiavalicarb-isopropyl
Benzenesulfonamide
Benzisothiazolone
Betamethasone
Bexarotene
Bezafibrate
Bicalutamide
Boscalid
Bromacil
Bromazepam
Bufexamac
Candesartan
Capecitabine
Carbaryl
Carbetamide
Cefaclor
Cefadroxil
Cefalexin
Cefazolin
Ceftazidime
Celiprolol
Cetirizine
Cetirizine N-Oxide
Chloramphenicol
Chlordiazepoxide
Chloridazon

Fenfuram
Fenpropimorph
Flucythrinate
Fludioxonil
Flumequine
Fluridone
Flusilazole
Gliclazide
Glybenclamide
Halofuginone
Heptachlor epoxide
Heptenofos
Hexaconazole
Hexazinone
Iamda-Cyhalothrin
Imazalil
Imidacloprid
Iprobenfos
Isazophos
Isopropalin
Isoprothiolane
Ivermectin
Kanamycin
Ketoconazole
Kresoxim-methyl
Leptophos
Levamisole Hydrochloride
Linezolid
Linuron
maduramicin
Mecarbam
Melengestrol
Meropenem
mesasulfuron
Metabromuron
Metazachlor
Metformin
Methiocarb
Methiocarb sulfone
Methiocarb sulfoxide
Methomyl
Methoprotryne
Chloridazone-desphenyl
Chloridazone-methyl-desphenyl
Chlorthalidone
Chlorthiazide
Cilastatin
Cimetidine
Climbazol
Clofibric acid
Clopidogrel carboxylic acid
Clothianidin
Cortisone
Coumachlor
Coumafuryl
Cyclamate
Cycloxydim
Darunavir
Deferasirox
Dexamethasone acetate
Dicamba
Dichlorvos
Diclofenac
Dicloxacillin
Didanosine
Dienogest
Diethyl-phthalate
Dimefuron
Dimethachlor ESA
Dimethachlor OXA
Dimethenamid OXA
Dimethenamide ESA
Di-n-butyl phthalate
Dinotefuran
Diuron
Diuron-desdimethyl
Diuron-desmethyl
Dopamine
Doxazosin
Doxycycline
Efavirenz
Emtricitabine
Epinephrine

Methoxycinnimate
Methyl pentachlorophenyl sulfide
Metolachlor
Metribuzin
MGK-264
Miconazole
Minocycline
Molinate
Monensin
Monolinuron
Myclobutanil
N,N-Diethyl-3-methylbenzamide
Nalidixic acid
Napropamide
Nitenpyram
Nitralin
Nitrapyrin
Nitrofurantoin
Nitrothal isopropyl
Norflurazon
Norgestimate
Nortriptyline
Nuarimol
Octhilinone
Ofloxacin
o-Phenylphenol
Oxacillin
Oxadiazon
Oxadixyl
Oxamyl
Oxolinic Acid
Oxycarboxin
Oxychlordane
Oxyfluorofen
Oxytetracycline
Paraoxon
Pebulate/Vernolate
Penconazole
Pendimethalin
Penicillin G
Pentachloroaniline
Phenthoate
Piperonyl butoxide
Eprosartan
Etodolac
Fenhexamid
Fenofibric acid
Fexofenadine
Fipronil
Fipronil-sulfide
Flecainide
Flonicamid
Florfenicol
Fluazifop
Flubendazole
Fluconazole
Flucytosine
Fludrocortisone
Flufenacet ESA
Flufenacet OXA
Flufenamic acid
Flunixine
Fluometuron
Fluroxypyr
Flurtamone
Fluvastatin
Foramsulfuron
Forchlorfenuron
Fosinopril
Furosemide
Gabapentin
Gemcitabine
Gemfibrozil
Genistein
Glycyrrhetinic Acid
Haloxyfop
Heptenophos
Hydrochlorothiazide
Ibuprofen
Imatinib
Imazamox
Imazaquin
Imidacloprid
Indapamide
Indomethacin
Iodosulfuron-methyl

Pirimicarb
Pirimiphos-ethyl
Pirimiphos-methyl
Polymyxin B sulphate
Prochloraz
Procymidone
Profenofos
Profluralin
Promecarb
Prometon
Prometryne
Pronamide
Propachlor
Propanil
Propargite
Propazine
Propetamphos
Propham
Propiconazole
Propyzamide
Prothiofos
Pyracarbolid
Pyrazophos
Pyridaben
Quinalphos
Quinomethionate
Quintozene
Ractopamine
Ranitidine
Roxarsone
Roxithromycin
Salinomycin
Sarafloxacin
Schradan
Secbumeton
Sertraline
Simazine
Simetryn
Spectinomycin
Spiramycin
Streptomycin
sulfacetaminde
Sulfachloropyridazine
Irbesartan
Isoproturon-monodemethyl
Isoxaben
Isoxaflutole
Ketoprofen
Kresoxim-methyl acid
Lacosamide
Lansoprazole
Letrozole
Lincomycin
Linoleic acid
Lorazepam
Losartan
Mandipropamid
Mebendazole
Meclofenamic Acid
Mefenamic acid
Mesosulfuron-methyl
Mesotrione
Metamitron
Metamitron-desamino
Metaxalone
Metazachlor OXA
Methoxyfenozide
Metolachlor ESA
Metolachlor OXA
Metosulam
Metoxuron
Metribuzin-desamino
Metribuzin-diketo
Metsulfuron-methyl
Mianserin-N-Oxide
Microcystin-LA
Microcystin-LF
Microcystin-LY
Microcystin-RR
Microcystin-YR
Minocycline
Monuron
Mycophenolic acid
N4-Acetylsulfadiazine
N4-Acetylsulfadimethoxine
N4-Acetylsulfamethazine

Sulfadiazine
Sulfadimethoxine
Sulfaguanidine
Sulfallate
Sulfamerazine
Sulfamethazine
Sulfamethoxazole
Sulfamethoxypyridazine
sulfanilamide
Sulfaquinoxaline
Sulfathiazole
Sulfisoxazole
Sulfotep
Sulprophos
tau-Flauvalinate
TCMTB
Tebuconazole
Tebuthiuron
Tecnazene
Terbacil
Terbufos
Terbumeton
Terbuthylazine
Terbutryn
Terbutryne
Tetrachlorvinphos
Tetradifon
Tetramethrin
Tetrasul
Thiabendazole
Thiacloprid
Thiamethoxam
Thiobencarb
Ticarcillin
Tolclofos-methyl
Tolylfluanid
Triadimefon
Triadimenol
Triallate
Triazophos
Tribufos
Tricyclazole
Trifloxystrobin
N4-Acetylsulfamethoxazole
N4-Acetylsulfathiazole
Nafcillin
Naproxen
Naptalam
Nateglinide
Neotame
Niclosamide
Nicosulfuron
Niflumic acid
Nilotinib
Nitrazepam
N-N-Dimethyl-N'-phenylsulfamide
N-N-Dimethyl-N'-p-tolylsulphamide
Nodularin
Nordiazepam
Nystatin
Olopatadine
Oryzalin
Oseltamivir carboxylate
Oxacillin
Oxazepam
Oxytetracycline
Pantoprazole
Paracetamol
Penciclovir
Pencycuron
Perfluorooctyl phosphate
Perindopril
Phenobarbital
Phenylbutazone
Phenytoin
Pioglitazone
Prednisone
Pregabalin
Prolinamide
Propachlor ESA
Propanil
Propazine-2-hydroxy
Propyzamide
Prothioconazole-desethio
Ranitidine
Ranitidine N-oxide

Triflumizole
Trifluralin
Trimethoprim
Tylosin
Vancomycin
Vinclozolin
Virginiamycin
Warfarin

Ranitidine-S-oxide
Repaglinide
Ribavirin
Rimsulfuron
Ritalinic acid
Rosuvastatin
Salicylic acid
Simazine-2-hydroxy
Sotalol
Sulcotrione
Sulfadimethoxine
Sulfamethoxazole
Sulfanilic acid
Sulfathiazole
Sulfentrazon
Sulpiride
Tebufenozide
Teflubenzuron
Telmisartan
Tembotrione
Tenofovir
Tepraloxydim
Terbutylazine-2-hydroxy
Terbutylazine-desethyl-2-hydroxy
Tetracycline
Theophyline
Thiamphenicol
Thifensulfuron-methyl
Tiapride
Ticlopidine
Topiramate
Torsemide
Triclabendazole
Triclocarban
Triflumuron
Triflusulfuron-methyl
Trinexapac
Tritosulfuron
Valsartan
Valsartan acid
Warfarin
Zidovudine
Zonisamide

July 17, 2018

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"High-Throughput Quantitation of Neonicotinoids in Lyophilized Surface Water by LC-APCI-MS/MS" by Morrison, Lucas M. ; Renaud, Justin B. ; Sabourin, Lyne ; Sumarah, Mark W. ; Yeung, Ken K.C. ; Lapen, David R.
DOI: https://doi.org/10.5740/jaoacint.17-0486. Published online ahead of print May 21, 2018

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Curriculum Vitae

Lucas Morrison, BSc. MSc. (Candidate)

PART A – PROFESSIONAL HISTORY:

Education:

Western University, London, ON.	2016 – 2018
Analytical Chemistry	MSc. Candidate
Laurentian University, Sudbury, ON.	2012 – 2016
Forensic Science and Chemistry	BSc. Double Major
4th Year Honors Thesis	Published in JAT

Awards:

Academic Excellence Scholarship	2012, 2013, 2015
Chemistry Award	2012
Leadership Award	2012

Teaching Experience:

Teaching Assistantship	2016 – 2018
Organic Chemistry 2213, 2223, 2273	Western University
Teaching Assistant Training Program	2016
Improved presentation and instruction skills	Western University

Community Involvement:

Teaching Assistantship 2016 – 2018

Organic Chemistry 2213, 2223, 2273 Western University

PART B – CONTRIBUTIONS:

Publications:

Morrison, L.M., Renaud, J. B., Sabourin, L., Lapen, D. R., Yeung, K. K-C., Sumarah, M. W. *High-Throughput Quantitation of Neonicotinoids in Lyophilized Surface Water by LC-APCI-MS/MS.* Journal of AOAC International Vol 101, May 2018

- I contributed 80% including the development of extraction and analysis procedure and implementation on real samples. I drafted the manuscript and took part in the editing and reviewing process.

Morrison, L. M., Unger, K. A., & Watterson, J. H. *Analysis of Dextromethorphan and Dextrorphan in Skeletal Remains Following Differential Microclimate Exposure: Comparison of Acute vs. Repeated Drug Exposure*. Journal of Analytical Toxicology, 41.6 (2017), 566-572.

- I contributed 70% including the harvesting, extracting and analysis of the specimen. I also contributed to the drafting and editing of the manuscript.
- This work was presented at Canadian Society of Forensic Science conference (2016)

Morrison, L. M., Renaud, J. B., Yeung, K. K-C., Sumarah, M. W. *Negative Mode Non-Targeted Screening and Retrospective Analysis of Trace Surface Water Contaminants*. In Progress

- I contributed 80% completing solid phase extraction on hundreds of samples and analyzed the mass spectra from non-targeted screening positive and negative ions. Produced a method for quantitation of 73 pharmaceuticals and pesticides detected in the screened samples.
- This work was presented at Canadian Society of Chemistry conference (2017) and Western University Envirocon (2016).

Kyriakos Manoli, **Lucas M. Morrison**, Mark W. Sumarah, George Nakhla, Virender K. Sharma, Ajay K. Ray. *Pharmaceuticals and pesticides in secondary effluent wastewater: Identification and enhanced removal by acid-activated ferrate(VI)*. In Progress

- I contributed 50% by producing a method of analysis for pharmaceuticals and pesticides of concern in wastewater effluent. Extracted approximately 50 samples and analyzed the mass spectra for the quantitation of 57 pharmaceuticals and pesticides. I wrote introduction, experimental and results sections involving the analytical data.

Renaud, J. B. Ph.D., Sabourin, L., Lapen, D. L. PhD., Gottschall, N., Wilkes, G., **Morrison, L. M**., Risha, Y., Top, B., Sumarah, M. W Ph.D., Topp, E. Ph.D. *Human and Veterinary Pharmaceuticals in the Canadian Environment.* MOU between the New Substances Assessment and Control Bureau (Health Canada) and the London Research and Development Centre (Agriculture & Agri-Food Canada). 2016 – 2017

- I contributed 25% to this work including method development and the extraction of real samples.

Summary of Course Work:

CHEM 9544A – Mass Spectrometry – 88

CHEM 9703R – Chemical Biology – 80

CHEM 9507Q – Advanced Chemical Communications – 86