Vulnerability of a Fractured Bedrock Aquifer to Emerging Sewage-Derived Contaminants and their use as Indicators of Virus Contamination

by

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A Thesis
presented to
The University of Guelph

In partial fulfillment of requirements for the degree of Master of Science in Environmental Science

Guelph, Ontario, Canada

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ABSTRACT

VULNERABILITY OF A FRACTURED BEDROCK AQUIFER TO EMERGING SEWAGE-DERIVED CONTAMINANTS AND THEIR USE AS INDICATORS OF VIRUS CONTAMINATION

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During an 8 month sampling campaign, 22 wells (11 private, 8 municipal, and 3 monitoring wells) completed in the fractured Silurian dolostone aquifers of southern Wellington County, Ontario, Canada were sampled for enteric viruses, fecal bacteria, artificial sweeteners, pharmaceuticals, and other common constituents of human and animal sewage. The vulnerability of fractured bedrock aquifers to sewage-derived contaminants was highlighted when 91% of the sampling wells exhibited at least one of the 49 sewage derived contaminants analyzed in this investigation. Low concentrations of viruses were found in 45% of the wells but each of these wells only exhibited viruses on one of the monthly sampling events. Current regulations in Canada and the United States require the monitoring of total coliforms and E. coli to determine the presence of a sewage influence in drinking water supplies, but statistical calculations of positive and negative predictive values, specificity, and sensitivity showed that the artificial sweetener acesulfame may act as a more effective tracer of sewage-derived contamination and the combination of ibuprofen and total coliforms may be able to indicate up to 70% of virus occurrences in southern Wellington County’s fractured bedrock aquifers. While the results may suggest that private well owners consuming untreated groundwater are at risk of acute gastrointestinal illness, the scope of the current study does not permit an assessment with regards to the risk of consuming water from municipal supplies.
PREFACE

The current document had been prepared in the form of three separate papers that are intended to be submitted as individual papers to scientific journals.

This thesis is the result of strongly collaborative work involving myself and others at the University of Guelph and researchers at several other institutions where the water analyses were conducted in laboratories with very advanced capabilities. This thesis is written as my contribution to science therefore below I explain my role in the work.

The first paper comprising Thesis Chapter 2, focuses on the vulnerability of a fractured bedrock aquifer to contamination with sewage-derived human enteric viruses. For this chapter, my responsibilities included collecting 118 virus samples throughout an 8 month sampling campaign of 22 wells across southern Wellington County, ON and analysing the data provided by Dr. Mark Borchardt’s lab. Dr. Mark Borchardt and his team from the USDA lab in Marshfield Wisconsin provided expertise with regards to virus sampling and analysis methods and strategy. The USDA lab also provided the project with the necessary equipment for the collection of virus samples and completed all virus analyses in their lab paid for by the University of Guelph (Dr. Parker research grants at reduced rates). Drs. Beth Parker and John Cherry provided expert input with regards to the hydrogeological aspects of the project and provided appropriate funding for the virus analyses. After I had compiled and analyzed the data and wrote it up in the form of Thesis Chapter 2, Drs. Mark Borchardt, Beth Parker, and John Cherry all reviewed and commented on this thesis chapter. A specific journal has yet to be determined for publication of this research chapter.

Thesis Chapter 3 focuses on the vulnerability of a fractured bedrock aquifer to emerging sewage-derived contaminants including artificial sweeteners, pharmaceuticals and various other anthropogenic
wastewater compounds. For this chapter, my responsibilities included collecting water samples from
the above mentioned 22 wells and shipping them to various labs where they were analyzed for artificial
sweeteners, pharmaceuticals, major ions, various water isotopes, tritium, and other anthropogenic
contaminants. Expert insight with regards to the transport, sample collection, and analysis of artificial
sweeteners was provided by Drs. William Robertson from the University of Waterloo and Dale Van
Stempvoort from the Canada Centre for Inland Waters located in Burlington, ON. Dr. Van Stempvoort’s
lab provided analysis of groundwater samples for 4 artificial sweeteners and perchlorate at no cost. Dr.
Chris Metcalfe and his lab crew at Trent University provided insight into the occurrence of
pharmaceuticals in groundwater and conducted all of the analyses of groundwater samples for
pharmaceutical compounds at research costs covered by Dr. Parker grants. After I compiled the analysis
results and summarized and discussed them in Thesis Chapter 3, Drs. Beth Parker and John Cherry
provided insight with regards to the hydrogeologic aspects of the project and provided editorial and
scientific feedback on the document presented here. A specific journal has yet to be determined for
publication of this research chapter.

Thesis Chapter 4 investigates the use of the above mentioned emerging sewage-derived contaminants
as novel indicators of virus contamination. As this paper draws from both of the papers comprising
Chapters 2 and 3, the collaborators include Drs. Mark Borchardt, Beth Parker, John Cherry, William
Robertson, Dale Van Stempvoort, and Chris Metcalfe. Each contributor offered services as mentioned
above. Dr. Kari Dunfield is the final collaborator on this final paper as she provided insight with regards
to traditional bacterial fecal indicators and the analyses used to assess their presence in groundwater.
Dr. Dunfield also provided lab space for these bacterial analyses to occur. Bacterial analyses were
conducted by myself and a wide range of field helpers, namely Loic Paquier and Amanda Malenica.

After I summarized and discussed the results in Chapter 4, comments on this final paper were provided
by Drs. Mark Borchardt, Beth Parker, and John Cherry. A specific journal has yet to be determined for publication of this research chapter.
ACKNOWLEDGEMENTS

As the current project examined the presence and significance of numerous contaminants in the fractured bedrock aquifers of the southern Wellington County, ON, it required a very large and diverse group of contributors without which this study would not have been complete. First off, the opportunity to undertake such a large project could only have been afforded to me by the grace of Dr. Beth Parker. I am therefore exceptionally grateful for this amazing opportunity she has provided me. Encouragement and support from Drs. Beth Parker, Gary Parkin, John Cherry, Kari Dunfield, William Robertson, and Mark Borchardt provided me with the stamina to see this project to its completion. Moral support and help during the sample collection process came from Loic Paquier, Carlos Maldaner, Amanda Malenica, Andrey Fomenko, Jonathan Munn, Thomas Coleman, and Adam Gilmore. I am extremely grateful for the support each of these people provided me. In addition, I am extremely grateful for the patience and support coming from the G360 Group staff including Andrea Harvie, Maria Gorecka, Deb Ruprecht, and Rashmi Jadeja. Without these employees my samples would never have arrived on time or been analyzed.

Apart from those that helped with the scientific side of the project, I must also extend additional gratitude to the well operators from Centre Wellington Water Services, the private well owners in Arkell, Eden Mills, and Aberfoyle and Len Yungblut and his operators at the University of Guelph for without these individuals I would never have had access to the diversity of wells that were investigated in this project.

My final thanks have to go to my family and friends that provided me with the support and encouragement that carried me through this project. The process was long and taxing, but because of you and your help through this project I am a stronger, better person today than I was 3 years ago.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW</td>
<td>All wells</td>
</tr>
<tr>
<td>BRW</td>
<td>Bedrock wells</td>
</tr>
<tr>
<td>CA</td>
<td>Carbonate</td>
</tr>
<tr>
<td>CH</td>
<td>Cabot Head Formation</td>
</tr>
<tr>
<td>CY</td>
<td>Crystalline</td>
</tr>
<tr>
<td>DNAPL</td>
<td>Dense non-aqueous phase liquid</td>
</tr>
<tr>
<td>E</td>
<td>Eramosa Formation</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
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<td>GA</td>
<td>Gasport Formation</td>
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<tr>
<td>GI</td>
<td>Goat Island Formation</td>
</tr>
<tr>
<td>GU</td>
<td>Guelph Formation</td>
</tr>
<tr>
<td>KCA</td>
<td>Karstic carbonate</td>
</tr>
<tr>
<td>LMWL</td>
<td>Local meteoric water line</td>
</tr>
<tr>
<td>LNAPL</td>
<td>Light non-aqueous phase liquid</td>
</tr>
<tr>
<td>MDL</td>
<td>Minimum detection limit</td>
</tr>
<tr>
<td>MSW</td>
<td>Municipal supply well</td>
</tr>
<tr>
<td>MW</td>
<td>Monitoring well</td>
</tr>
<tr>
<td>NA</td>
<td>Not applicable</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
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<tr>
<td>PPV</td>
<td>Positive predictive value</td>
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<tr>
<td>PQL</td>
<td>Practical quantitation limit</td>
</tr>
<tr>
<td>PW</td>
<td>Private well</td>
</tr>
<tr>
<td>q-PCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>Quantitative reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>SS</td>
<td>Sandstone</td>
</tr>
<tr>
<td>TNTC</td>
<td>Too numerous to count</td>
</tr>
<tr>
<td>UB</td>
<td>Undefined bedrock</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile organic compound</td>
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</table>
1.0 INTRODUCTION

1.1 Thesis introduction and literature review
Scientific investigations of virus contamination of aquifers used as sources for drinking water is vital as these pathogenic contaminants can quickly reduce groundwater quality and result in rapid and pervasive disease outbreaks (Summers, 1989; O’Connor, 2002). Virus adsorption and attenuation processes are limited during groundwater transport due to the small size of virus particles and the repulsive electrostatic forces developed between the negatively charged surfaces of both viruses and geologic materials at the neutral pH of groundwater (Gerba, 1983). Additionally, the cool temperatures of groundwater in comparison to surface water can prevent virus inactivation and ultimately promote virus longevity for over a year (Charles et al, 2009). Areas in temperate climates like those of Ontario, Canada are therefore at a relatively high vulnerability to virus contamination due to lower groundwater temperatures, between 9 and 12°C, relative to those in warmer climates such as the southern United States and Mexico. With limited adsorption and inactivation under the saturated conditions of groundwater flow, virus contamination of groundwater can therefore be exceptionally widespread and persistent relative to contamination with other pathogens or compounds in areas characterized by cooler climates.

In fractured bedrock aquifers, virus contamination can be even more widespread and rapid than in porous sediments on account of the even further reduced adsorption and diffusion and increased groundwater velocities associated with lower effective porosities governed by fractures. During groundwater transport, the transport of typical chemical contaminants of fractured bedrock aquifers such as LNAPLs and DNAPLs is significantly attenuated as these compounds are highly susceptible to matrix diffusion (Freeze and Cherry, 1979). In contrast, during groundwater transport, viruses behave like particles and are therefore less susceptible to diffusion into the rock matrix than classic solutes.
Attenuation of viruses is further reduced in fractured media as the surface area available for sorption to occur is significantly reduced relative to that of porous sediments. As fractured bedrock aquifers frequently exhibit low effective porosities on the order of 0.001 to 0.00001 (Freeze and Cherry, 1979), groundwater flow is isolated to fracture apertures and can reach velocities on the order of 1 to 10 m/day (Belan, 2010). With limited adsorption and inactivation during fracture flow, viruses can theoretically be transported several kilometers from their septic source before being inactivated.

While numerous lab-scale studies have established the predominant mechanisms governing colloid and virus attenuation during transport through fractured media (Mondal and Sleep, 2013; Rodrigues Dickson, and Qu, 2013), few field-scale studies have focused on the actual occurrence of human enteric viruses in fractured bedrock aquifers. Although studies by Davis and Witt (2000), Femmer (2000), Lindsey et al. (2002), and Johnson et al. (2011) assess the occurrence of viruses in fractured bedrock aquifers, low sampling frequencies make these studies inadequate representations of fractured bedrock vulnerability to virus contamination as a recent study by Bradbury et al. (2013) highlights the importance of quarterly or monthly sampling due to the high temporal variability of viruses in groundwater. Studies conducted by Lieberman et al. (2007), Borchardt et al. (2003 and 2007), Locas et al. (2007), and Bradbury et al. (2013) collected temporal samples for viruses but each of these studies investigated either a wide range of aquifer types and had only a limited focus on fractured bedrock aquifers in their assessment, or only sampled a small collection of wells representing a limited assortment of conditions influencing an aquifer’s or well’s susceptibility to virus contamination. To date, a single, large scale investigation focusing on the temporal susceptibility of various types of wells completed solely in fractured bedrock aquifers with appreciable spatial representation in a regional flow system context and the numerous contributing factors to their vulnerability has still not been conducted. Additionally, the relationship between the occurrence of viruses and other sewage-derived contaminants in varying hydrochemical settings has yet to be explored.
In addition to viruses, several contaminants including artificial sweeteners, pharmaceuticals, and various other sewage-derived contaminants have experienced very little attention by fractured bedrock hydrogeologists. Many of these contaminants have demonstrated elevated concentrations in both wastewater and surficial granular aquifers and have exhibited both rapid and conservative transport during groundwater flow. Studies by Drewes et al. (2003) and Godfrey et al. (2007) investigated the transport of various pharmaceuticals through several types of aquifers and highlighted the relatively persistent and conservative transport of compounds such as carbamazepine and sulfamethoxazole. Similarly Buerge et al. (2009) revealed the persistence of the artificial sweeteners acesulfame and sucralose when they didn’t exhibit any signs of degradation after being incubated in activated sludge for 7 hours. Large-scale investigation by Van Stempvoort et al. (2013), Barnes et al. (2008), and Loos et al. (2010) have revealed the widespread occurrence of several of these emerging contaminants in both the United States and Europe, but no studies have examined their presence or persistence during flow through fractured bedrock aquifers.

As artificial sweeteners and pharmaceuticals are released in high concentrations in urine and feces, the predominant source of these emerging contaminants in groundwater is human waste leaking from faulty sewers and septic systems. Similarly, many of the pathogens contaminating groundwater sources are enteric viruses which reside in mammalian intestinal tracts and are therefore shed in high concentrations in feces. As human enteric viruses, artificial sweeteners, and pharmaceuticals share a common source, these emerging contaminants could potentially act as novel indicators of virus contamination.

1.2 Thesis goals and hypotheses
The goals of the current study are therefore to assess the vulnerability of fractured bedrock aquifers to contamination with sewage-derived viruses and emerging contaminants and investigate whether any of these artificial sweeteners, pharmaceuticals, or other sewage-derived compounds can act as effective
indicators of groundwater vulnerability to human enteric viruses. The work will ultimately be divided into 3 separate chapters. In Chapter 2, an 8 month sampling campaign of 22 wells completed in the fractured Silurian dolostone aquifers of southern Wellington County, ON will be used to investigate the vulnerability of the fractured bedrock aquifers to contamination with both human and animal enteric viruses derived from leaking sewers and septic systems, agricultural runoff, and surface water bodies influenced by sewage sources. Focus will be laid on the factors contributing to virus contamination such as overburden type and thickness, well construction depth and year, well type, and the well’s proximity to potential sources.

The next chapter, Chapter 3, will use the same 22 sampling wells from the first chapter to examine the vulnerability of these fractured bedrock aquifers to sewage-derived contamination with 4 artificial sweeteners, 11 pharmaceuticals, and 23 other anthropogenic compounds and use hydrochemistry and isotopic analysis to assess the sources and relative timing of contamination. The occurrence of each of the emerging contaminants will be compared to detections previously reported in the literature in order to determine the relative susceptibility of fractured bedrock aquifers to contamination with these compounds.

The final research chapter of this thesis, Chapter 4, will statistically compare the effectiveness of total coliforms, *Escherichia coli* (*E. coli*), and each of the sewage-derived compounds detected in Chapter 3 as indicators of groundwater contamination with human enteric viruses through the calculation of indicator parameters including sensitivity, specificity, and positive and negative predictive values.

Results from the 8 month sampling campaign of 22 wells completed in the fractured Silurian dolostone aquifers within a 20 km radius of Guelph, Ontario, Canada are expected to reveal the high vulnerability of these types of aquifers to pervasive sewage-derived contamination with both viruses and various anthropogenic compounds on account of the highly variable thicknesses and permeabilities of the local
glacial overburden sediments and the cool groundwater temperatures and rapid flow velocities of the underlying fractured dolostone aquifers. As human enteric viruses, artificial sweeteners, pharmaceuticals, and other sewage-derived compounds share many of the same sources and exhibit rapid and conservative transport, it is hypothesized that some of these sewage-derived compounds will act as novel indicators of groundwater vulnerability to human enteric viruses.
Chapter 2: Vulnerability of a fractured dolostone aquifer to sewage-derived enteric viruses

ABSTRACT

Groundwater samples analyzed for human and animal viruses were collected from 22 wells, including 8 public water supply wells, 11 private domestic wells and 3 monitoring wells at intervals between 4 and 6 weeks over 8 months. The wells were situated in an important Silurian dolostone aquifer in southern Wellington County, Ontario where most of the population relies on this aquifer for its drinking water supply. Overall, only 10 (8%) of the 118 samples exhibited viruses but of the 22 wells sampled during this investigation, 10 (45%) were positive for human enteric viruses (polyomavirus, adenovirus A, and G2 norovirus). Of the 8 public supply wells, 5 (62.5%) showed detections of human enteric viruses while 5 out of the 11 private wells (45%) also exhibited human enteric viruses. However, each well showing a viral detection had only one virus occurrence during the 8 month sampling campaign and only one virus strain was detected in each well. The substantial percent of wells showing virus detections and the ephemeral occurrences are consistent with previous results reported in the literature for virus sampling in bedrock aquifers in populated regions. The finding of viruses is not unexpected because the hydrogeologic conditions are particularly favorable for sewage-derived viruses to enter and travel quickly in the network of interconnected fractures in this dolostone aquifer. The aquifer’s vulnerability to sewage-derived contaminants stems from many characteristics of the hydrogeologic system including the overburden thickness over much of the study area allowing rapid groundwater recharge, the shallow depth of well casings into the top of rock, typically fast groundwater velocities, and cold groundwater temperatures (~9°C). The unexpected aspects of the results are the lack of virus occurrences in the majority of the 22 sampling wells and the low detection frequency on a per sample basis. While
temporal virus sampling is required to monitor the ephemeral virus occurrences in groundwater, the most effective frequency for detecting viruses in wells completed in bedrock is still unknown. While some risk may be present for private well owners consuming untreated groundwater, for these virus occurrence results to be related to human health risk from consuming municipal water derived from groundwater it would be necessary to conduct a much different type of investigation.

2.0 INTRODUCTION

In a surveillance study conducted by the Centres for Disease Control and Prevention, 36 out of 48 waterborne disease outbreaks between 2007 and 2008 in the United States were attributed to contaminated drinking water (Brunkard, 2011). The tragedy in Walkerton, Ontario, Canada where 7 people died and more than 2,300 people became ill when Escherichia coli (E. coli) and Campylobacter jejuni contaminated the municipal water supply is an example of the potentially devastating effects of drinking water becoming contaminated by pathogens (O’Connor, 2002). While E. coli and Campylobacter jejuni are both common and important pathogens that can result in outbreaks, the case study of a norovirus outbreak in Wisconsin by Borchardt et al. (2011) highlights the uniqueness of virus contamination of groundwater as the virus exhibited rapid transport from a leaking septic system to a restaurant’s supply well in less than 1 week leading to over 200 individuals becoming infected with norovirus. In these cases mentioned above and many others, the pathogenic outbreaks resulted from sewage-derived contamination of ground-water sourced drinking water supplies. As enteric viruses reside in mammalian intestinal tracts and are shed in high concentrations in feces, the predominant sources of these viruses in groundwater are leaking sewers and septic systems, agricultural practices involving swine, cattle, and poultry, and surface water bodies influenced by agricultural runoff and human wastewater. When combining the results from 24 large scale studies investigating the occurrence of sewage-derived viruses and fecal indicators in public water systems, the United States 8
Environmental Protection Agency (EPA) projected that approximately 27% of all public water systems across the United States will be contaminated with enteric viruses similar at some point in time (EPA, 2006). As both history and research have illustrated the severe consequences of sewage-derived virus contamination of aquifers used as sources of drinking water, it is imperative that the factors contributing to this contamination are thoroughly examined.

Studies investigating the presence of viruses in groundwater have been conducted since the 1970s and have revealed that the major contributing factors to virus attenuation during groundwater transport are virus inactivation and adsorption. After reviewing data presented in 24 different reports examining the occurrence of viruses in groundwater, Keswick and Gerba (1980) determined that virus retention in sediments is predominantly dependent upon adsorption. Some of the first experiments investigating the role of adsorption in virus transport were conducted in the laboratory by Goyal and Gerba in 1979 when they used 14 different viruses in 9 different types of sediment to determine that both virus and sediment type dictate the amount of virus adsorption during flow through porous media. While several types of polio, coxackie, and echoviruses exhibited greater than 90% adsorption during transport through a sandy loam, echovirus types 1, 12, and 29 and simian rotavirus experienced only about 50% adsorption. In addition to virus type, soils promoting groundwater pH less than 5 exhibited between 65 and 99% adsorption whereas other sediments with higher pH values experienced little to no adsorption. While increased surface area of smaller grained sediments introduce more locations for adsorption to occur, recent studies have revealed that in addition to sediment surface area, the extent of adsorption is highly dependent upon electrostatic interactions and the surface charges expressed by both the virus and the geologic material at the near-neutral pH of groundwater (Gerba, 1983). Through the statistical analysis of the adsorption studies conducted in their 1979 investigation, Gerba and Goyal (1981) determined that the extent of these electrostatic interactions is predominantly controlled by the virus’s isoelectric point (the pH at which it exhibits a neutral charge), the ionic strength of the solution, the...
presence of organic and humic substances, the exchangeable iron content of the sediment, and the pH of the groundwater. For example, while both low pH and low organic matter levels promote increased adsorption by promoting increased charge differentials between the virus and geologic surfaces ($R = -0.9110$ and $-0.4920$, respectively), high levels of exchangeable iron in the sediment also lead to increased adsorption ($R = 0.5755$) on account of their introducing additional positively charged surfaces for negatively charged viruses to adsorb to.

In addition to adsorption, virus inactivation, or the process by which the virus loses its ability to infect a host, is a major contributing factor to virus persistence and therefore the mechanisms influencing virus inactivation are critical during virus transport. Several studies have suggested that virus inactivation is significantly influenced by microbial activity, groundwater temperature, and soil moisture content. An early study conducted by Hurst et al. (1988) monitored the infectivity of poliovirus in sterilized and unsterilized sandy loam and found that virus inactivation rates were 2 to 3 times lower in the sterile loam in comparison to the nonsterile loam. The study ultimately suggested that microbial antagonism was a major factor influencing the longevity of the viruses in water. Similar studies conducted more recently by Davies et al. (2006) found comparable results when sediments sterilized by irradiation exhibited much slower inactivation rates than nonsterilized sediments. The same study revealed that sediments maintained at temperatures of 4 and 20°C exhibited lower inactivation rates than sediments maintained at a temperature of 35°C. In an early study by Yates et al. (1985) the importance of temperature was made clear when groundwater samples of varying hydrochemistries were inoculated with 3 different viruses and stored at different temperatures. The study revealed that while properties such as pH, hardness, total dissolved solids, and turbidity varied between samples, temperature had the greatest influence on virus persistence in groundwater. In 2009, Charles et al. further investigated the influence of temperature on virus persistence in groundwater by storing enteroviruses, noroviruses, and adenoviruses in 3 types of groundwater at 12°C. After testing the samples for infectivity every month
for 2 years, Charles et al. revealed that viruses can remain viable for at least 1 year in groundwater at a temperature of 12°C. As groundwater temperatures mimic annual mean atmospheric temperatures and generally decrease with latitude, Canadian aquifers may be at a higher risk than aquifers in the southern United States and Mexico to persistent virus contamination on account of this increased longevity in cooler climates.

In addition to lower temperatures promoting virus longevity, Yeager and O’Brien (1979) also found that when poliovirus and coxsackievirus were monitored in sediments of varying moisture contents, more saturated sediments promoted increased virus persistence. While both moisture content and microbial antagonism have shown to significantly influence rates of virus inactivation, temperature is believed to have the greatest influence on virus inactivation. As groundwater is typically cooler than surface water, the conclusions by Yates et al. and Charles et al. would suggest that many types of aquifers are susceptible to prolonged virus contamination, but as these studies, as well as the early investigations of adsorption were limited to transport through porous media, investigations of virus transport through other types of aquifer material are necessary for furthering our understanding of virus transport.

In comparison to aquifers comprised of porous sediments, fractured bedrock aquifers exhibit several unique properties that put them at an exceptionally high risk of persistent and rapidly distributed virus contamination. In one of the first studies examining virus transport through fractured rock, Bales et al. (1989) showed that during transport through a core of fractured tuff, the bacteriophage MS-2 was eluted more than 3 times faster than a non-sorbing solute. McKay et al. (1993) also showed the rapid transport of pseudo-viruses (also known as bacteriophages) through fractured media during a field-scale tracer study in the upper 5.5 m of a weathered and fractured clay-rich till. MS-2 and PRD-1 bacteriophages were detected 4 m from the source between 1 and 2 days after injection while bromide required several months to travel the same distance. This rapid transport of the bacteriophages was
ultimately attributed to the fact that both bacteriophages and viruses act as colloidal particles and are therefore less susceptible to matrix diffusion than typical solutes. The arrival time for viruses ultimately reflects average linear groundwater velocities. In a recent lab-scale investigation by Weisbrod et al. (2013), during transport through a discrete fracture in a core of chalk, 3 bacteriophages were found to exhibit diffusion coefficients 2 to 3 orders of magnitude smaller than Li$^+$ and Br$^-$ and Peclet numbers between 4 and 7 orders of magnitude greater than Li$^+$ and Br$^-$. These results highlight the fact that adsorption, rather than diffusion, is the predominant source of attenuation during transport of colloids such as viruses through fractured rock. As summarized by Zhang et al. in their 2012 review of colloid transport in fractured rocks, however, relative to transport in porous media, virus transport through fractured rock is less prone to adsorption on account of the decreased surface area of fracture apertures in addition to the size and charge exclusion effects. Due to both the large size of virus particles relative to solutes and the repulsive forces generated from similar charges present of the surfaces of both viruses and geologic materials, viruses will remain primarily in the high-velocity central streamlines in a fracture therefore resulting in fewer solid-liquid interface interactions and limited chances for viruses to collide and adsorb to the fracture surface. With low affinity for both diffusion and adsorption, virus particles will ultimately exhibit transport speeds on the order of average groundwater velocity. As effective porosities on the order of 0.001 to 0.00001 in fractured rock promote large groundwater velocities (Freeze and Cherry, 1979) and colloids have shown Taylor dispersion coefficients up to 4 orders of magnitude greater than those of the solutes (Weisbrod, 2013), virus transport in fractured bedrock aquifers can be both rapid and widely distributed in a matter of days. While sediments overlying fractured bedrock aquifers can potentially offer some attenuation of viruses before they enter the aquifer, the predominant sources of enteric viruses are leaking sewers and septic systems which are frequently buried a few to several meters below ground surface and may lay on top of or into the top of rock. With cool temperatures promoting virus longevity up to and potentially longer than 1 year and
groundwater velocities on the order of 10 m/day typical of fractured dolostone aquifers (Belan, 2010), active viruses can potentially be transported several kilometers from a single source while maintaining their ability to infect a host.

To date, the number of studies investigating the presence of actual viruses in fractured bedrock aquifers is very limited (Table 2.1) and none of them have provided sufficient data to provide a reliable estimate of the vulnerability of wells completed in fractured bedrock to contamination with viruses on a regional basis. In the first study investigating the presence of viruses solely in a fractured bedrock aquifer, Borchardt et al. (2007) demonstrated the high vulnerability of these types of aquifers to virus contamination when monthly sampling of 3 public water supply wells in Madison, WI revealed the presence of human enteric viruses in 2 wells that were cased through a regionally extensive shale aquitard and open to the underlying confined sandstone aquifer. Later studies conducted by Bradbury et al. (2013) in the same wells as the Borchardt study, would reveal that while failed well casings could act as cross-connections from the upper unconfined aquifer to the lower confined aquifer, simultaneous detection of viruses in wells several kilometers apart would require several well casings to fail at the same time. As this scenario is unlikely, it was suggested that preferential flow paths from the upper aquifer through the aquitard were likely present in the form of naturally occurring fractures. While these studies did confirm the vulnerability of fractured bedrock aquifers to virus contamination, the number of wells and therefore type of wells investigated were relatively low.
Table 2.1. Summary of previous investigations of virus occurrence in fractured bedrock aquifers.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Location</th>
<th>Approximate area of study site (km²)</th>
<th>Type of bedrock</th>
<th>Number of wells</th>
<th>Sample Frequency</th>
<th>Total Number of samples</th>
<th>Virus Detection Rate Per Well (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>By RT-PCR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AW BRW AW BRW</td>
</tr>
<tr>
<td>Davis and Witt, 2000</td>
<td>Ozark Plateaus</td>
<td>122,000</td>
<td>Mix of CA and KCA</td>
<td>109</td>
<td>109</td>
<td>Twice</td>
<td>218</td>
</tr>
<tr>
<td>Femmer, 2000</td>
<td>Ozark Plateaus</td>
<td>122,000</td>
<td>Mix of CA and KCA</td>
<td>109</td>
<td>109</td>
<td>Once</td>
<td>106</td>
</tr>
<tr>
<td>Lindsey et al., 2002</td>
<td>Pennsylvania</td>
<td>119,283</td>
<td>25 CA; 25 CY</td>
<td>59</td>
<td>50</td>
<td>Once</td>
<td>50</td>
</tr>
<tr>
<td>Lieberman et al., 2002</td>
<td>USA</td>
<td>9,826,675</td>
<td>Mixed</td>
<td>30</td>
<td>13 (7 Karst)</td>
<td>Monthly for 1 year</td>
<td>360</td>
</tr>
<tr>
<td>Borchardt et al., 2003</td>
<td>Wisconsin</td>
<td>169,639</td>
<td>Mixed</td>
<td>50</td>
<td>30</td>
<td>Quarterly for 1 year</td>
<td>194</td>
</tr>
<tr>
<td>Borchardt et al., 2007</td>
<td>Madison, WI</td>
<td>243.5</td>
<td>SS</td>
<td>3</td>
<td>3</td>
<td>Monthly for 1 year</td>
<td>30</td>
</tr>
<tr>
<td>Locas et al., 2007</td>
<td>Quebec</td>
<td>1,542,056</td>
<td>UB, SS</td>
<td>12</td>
<td>2</td>
<td>Monthly for 1 year</td>
<td>113</td>
</tr>
<tr>
<td>(Bradbury et al., 2013)</td>
<td>Madison, WI</td>
<td>243.5</td>
<td>SS</td>
<td>6</td>
<td>6</td>
<td>Monthly</td>
<td>26</td>
</tr>
<tr>
<td>Johnson et al., (2011)</td>
<td>East Tennessee</td>
<td>~64,800</td>
<td>KCA (and 4 springs)</td>
<td>4</td>
<td>1-2 times</td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

AW = All Wells; BRW = Bedrock Wells; CA = Carbonate; CY = Crystalline; KCA = Karstic Carbonate; SS = Sandstone; UB = Undefined bedrock
*Results from RT-PCR analysis were suggested to be false negatives due to sample collection and concentration techniques. Viruses were positively detected using cell culture techniques but were not positive by RT-PCR. It was suggested that the possible factors contributing to these negative results were the sample concentration step, PCR inhibition, low virus concentrations, low sample volume, and/or the primers and probes.

-RT-PCR analysis not conducted.

In two studies funded by the USGS that were conducted by Davis and Witt (2000) and Femmer (2000), over 200 municipal supply wells completed in the carbonate and karstic carbonate aquifers of the Ozark Plateaus were sampled for human enteric viruses. While the large array of wells exhibited varying constructions, ages, and surrounding land use and therefore allowed for the evaluation of the various factors contributing to virus contamination, each of the wells was only sampled 1 or 2 times. In Bradbury et al.’s 2013 study, the importance of temporal sampling is highlighted as even wells highly prone to virus contamination did not always exhibit viruses during over a year of monthly sampling events. Other studies sampling a wide array of wells such as Lindsey et al. (2002) and Johnson et al. (2011) also completed a limited number of sampling events at each of their wells and so detection rates are not representative of the true vulnerability of the wells or aquifers. Studies including temporal sampling such as those conducted by Lieberman et al. (2002), Borchardt et al. (2003), and Locas et al.
(2007) therefore provide better insight with regard to the true vulnerability of a well completed in a fractured bedrock aquifer especially in the absence of other indicators of groundwater age and sewage-derived impacts. In the study by Lieberman et al. (2002) temporal sampling of 13 wells completed in karstic bedrock was conducted over an entire year and found that 43% of the wells exhibited culturable viruses. In the study by Locas et al. (2007), one of the only field scale studies of virus presence in Canadian groundwater, both of the bedrock wells that were sampled also exhibited viruses. While both of these studies produced temporal data, Lieberman et al. only sampled wells that had previously recorded contamination with microbial water quality indicators such as E. coli and Locas et al. sampled only a limited number of bedrock wells. In comparison, the investigation by Borchardt et al. conducted temporal sampling of a wide array of wells in a large variety of settings. Although Borchardt et al. temporally sampled 30 wells completed in bedrock and revealed that 10% of them were susceptible to virus contamination, all the wells were private wells and so no estimate for larger municipal supply wells was made. A 2006 estimate made by the EPA based on the results from 24 different studies across the United States would suggest that 27% of public supply wells will be contaminated with viruses at some point in time, but no distinction was made with regards to the susceptibility of wells in specific hydrogeologic settings such as fractured bedrock aquifers. Despite the fact that each of the studies summarized in Table 2.1 sampled wells completed in fractured rock, a single, large scale investigation focused on the temporal susceptibility of various types of wells completed solely in a regionally expansive fractured bedrock aquifer and the numerous well constructions and geologic factors contributing to their vulnerability has still not yet been conducted.

2.0.1 Hypotheses and Goals
Due to the low number of field-scale studies focusing on the vulnerability of fractured bedrock aquifers to virus contamination, the goal of the current study is to examine the various factors contributing to virus contamination including land use, wastewater management, overburden type and thickness, and
well type and determine the overall vulnerability of wells completed in a fractured dolostone aquifer used as the predominant source of drinking water in both urban and rural communities in southern Wellington County, Ontario, Canada. Over an 8 month period, an array of 22 private, municipal supply, and monitoring wells completed in the local Silurian dolostone aquifers were sampled monthly for the enteric viruses listed in Table 2.2. In order to provide the study with several types of scenarios that are typical to both urban and rural settings, each of the wells chosen to be sampled exhibited different characteristics with regards to well completion depths and pumping rates, overburden types and thicknesses, and proximity to different potential sources such as agriculture, surface water bodies, sanitary sewers, and septic systems. As the southern Wellington County is covered in glacially deposited overburden sediments with varying degrees of thickness and permeability in addition to the cool groundwater temperatures and rapid groundwater velocities on the order of 1-10 m/day (Belan, 2010), it is expected that all well types completed in the Wellington County’s fractured Silurian dolostone aquifers will be vulnerable to contamination with sewage-derived enteric viruses.
Table 2.2. Viruses investigated in this study included those derived from both human and animal waste.

<table>
<thead>
<tr>
<th>Virus Host</th>
<th>Virus Type</th>
<th>Analysis Method</th>
<th>Size (nm)</th>
<th>Symptoms attributed to infection in humans</th>
<th>Potential Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Enterovirus RNA</td>
<td>RT-qPCR</td>
<td>20-30</td>
<td>Fever, fatigue, sore throat, vomiting, rash, upper respiratory tract illnesses, acute gastroenteritis</td>
<td>Human waste (sewer or septic leachate)</td>
</tr>
<tr>
<td>Human</td>
<td>Hepatitis A virus RNA</td>
<td>RT-qPCR</td>
<td>27-32</td>
<td>Fever, fatigue, anorexia, nausea, abdominal discomfort, jaundice, liver damage</td>
<td>Human waste (sewer or septic leachate)</td>
</tr>
<tr>
<td>Human</td>
<td>Norovirus: genotypes I and II RNA</td>
<td>RT-qPCR</td>
<td>27-32</td>
<td>Nausea, vomiting, diarrhea, abdominal pain, and fever</td>
<td>Human waste (sewer or septic leachate)</td>
</tr>
<tr>
<td>Human</td>
<td>Polyomavirus DNA</td>
<td>qPCR</td>
<td>40-55</td>
<td>In immunocompromised individuals, persistent infection of kidneys and peripheral blood. Later spreading to central nervous system. May have tumorogenic potential.</td>
<td>Human waste (sewer or septic leachate)</td>
</tr>
<tr>
<td>Human</td>
<td>Adenovirus: 3 assays DNA</td>
<td>qPCR</td>
<td>70-100</td>
<td>Upper respiratory tract infection, gastroenteritis, diarrhoea, vomiting</td>
<td>Human waste (sewer or septic leachate)</td>
</tr>
<tr>
<td>Human</td>
<td>Polyomavirus DNA</td>
<td>qPCR</td>
<td>45</td>
<td>None</td>
<td>Human waste (sewer or septic leachate)</td>
</tr>
<tr>
<td>Avian</td>
<td>Avian influenza virus RNA</td>
<td>RT-qPCR</td>
<td>80-120</td>
<td>Fever, cough, acute respiratory distress, shortness of breath/difficulty breathing, abdominal pain, diarrhea</td>
<td>Avian waste (manure)</td>
</tr>
<tr>
<td>Various</td>
<td>Campylobacter jejuni* DNA</td>
<td>qPCR</td>
<td>2000</td>
<td>Abdominal pain, diarrhea, fever, and malaise</td>
<td>Animal waste</td>
</tr>
</tbody>
</table>

*Campylobacter jejuni* is not a virus, but a pathogenic bacterium frequently associated with waterborne disease outbreaks. While rarely found in groundwater, Campylobacter jejuni was included in the current analyses. It was not detected in any of the groundwater samples and therefore is excluded from discussion in the current manuscript.

2.1 SITE DESCRIPTION

2.1.1 Sampling Sites
The array of 22 sampling wells (Table 2.3) is located approximately 100 km west of Toronto, Ontario, Canada in several small communities within southern Wellington County including the City of Guelph, Fergus, Elora, Arkell, Eden Mills, and Aberfoyle (Figure 2.1A). While Guelph is a highly urbanized university town of approximately 127,000 people, the surrounding area is dominated by agricultural and rural communities. To the south of Guelph are Arkell and Aberfoyle, the 2 agricultural and residential areas where the nearly 7000 people of Puslinch Township reside. Eden Mills is a small population built along the banks of the Eramosa River northeast of Guelph while further northwest of...
Guelph are Fergus and Elora, the two largest communities within Centre Wellington Township with populations of 19,126 and 3,796 people, respectively.

Although the sampling area is entirely encompassed by the 6,800 km² of the Grand River watershed (Figure 2.1B), groundwater is the primary source of drinking water to each of the communities discussed above. The belt of Silurian dolostone that extends east from Lake Huron (Figure 2.1A) serves as an aquifer to the over 200,000 residents of Wellington County. All of the wells chosen for this investigation were completed in the Lower Silurian sequences as described by Brunton (2008) and consist of the Guelph, Eramosa, Goat Island, Gasport, Irondeqouit, Rockway, Merritton and Cabot Head Formations. Both the Guelph and Gasport Formations act as the predominant fractured bedrock aquifers in the region, supplying drinking water to much of southern Wellington’s population. The Guelph Formation is characterized by crinoidal grainstones and wackestones with a cream to light brown matrix. In the Guelph area, this formation is generally 15 to 22 m thick and exhibits a sharp basal contact with the black to dark grey shaly facies and crinoidal grainstones of the underlying Eramosa and Goat Island Formations, respectively. Although the Eramosa and Goat Island are water-bearing formations, their relatively low transmissivities suggest they are regional aquitards. When vertical fractures and preferential pathways in the form of cross-connecting boreholes are not present, the Eramosa and Goat Island Formations can limit the transport of surface contaminants present in the unconfined Guelph aquifer to the underlying Gasport aquifer. The thickness and presence of the Eramosa and Goat Island Formations, however, are highly variable in southern Wellington County and these formations are sometimes entirely absent. The 25 to 70 m of the blue-grey crinoidal grainstones of the Gasport Formation can therefore be in direct contact with the overlying Guelph Formation.
Figure 2.1. Sampling locations in southern Wellington County, Ontario, Canada and several surrounding communities within Wellington County. A. All of the sampling sites for this project were located in the Grand River watershed, the largest watershed in southern Ontario, covering a total area of 6800 km². The bedrock geology of Wellington County is dominated by a Silurian dolostone belt extending from Lake Huron to the Niagara River. The dolostone aquifers of this dolostone belt provide over 500 000 citizens with drinking water. All of the sampling wells are constructed into the Lower Silurian carbonate formations including the Guelph, Eramosa, Goat Island, and Gasport formations.
In masters theses by Belan (2010) and Munn (2012), the hydraulic properties of the Guelph and Gasport Formations were thoroughly examined using various combinations of hydraulic tests and core logging techniques. Belan used FLUTe tranmissivity profiling in an inactive, 12-inch diameter water supply well located in Guelph to obtain depth-discrete transmissivity values from the Guelph Formation down through the Gasport Formation. Temperature logging, packer tests, and a 13-day pumping test were also used to further define the flow within southern Wellington County’s aquifers. The tests yielded estimates of the transmissivity of the bulk aquifer (i.e. the Guelph and Gasport Formations combined) ranging from $2.5 \times 10^{-5}$ to $6 \times 10^{-4}$ m$^2$/s. Using these transmissivity values in the cubic law, Belan estimated that fracture apertures ranged from 9 to over 430 μm with a mean of 69 μm. Belan further estimated that hydraulic conductivity and groundwater velocities of the bulk aquifer ranged from $5.93 \times 10^{-6}$ m/s to $8.89 \times 10^{-6}$ m/s and from 0.002 to 28 m/day under natural gradients, respectively.

At the same study site, Munn (2012) completed a more detailed 3-D characterization of fracture orientation, spacing, and aperture using a suite of high-resolution, depth-discrete data collection methods including detailed core logging, borehole geophysics (acoustic televiewer), and hydraulic testing (FLUTe transmissivity profiling) in 2 inclined coreholes and several nearby vertical coreholes. Estimates of maximum transmissivity were on the same order as those by Belan as they ranged from $2.0 \times 10^{-4}$ to $4.2 \times 10^{-4}$ m$^2$/s. Using a similar rearrangement of the cubic law, Munn estimated fracture apertures ranging from 15 to 407 μm with a slightly larger geometric mean between 104 and 159 μm in comparison to the estimates made by Belan. While Belan estimated an effective fracture porosity of the bulk aquifer on the order of $5 \times 10^{-4}$, Munn suggested the mean effective fracture porosity of the Guelph Formation is approximately $9.7 \times 10^{-4}$ and between $3.1 \times 10^{-4}$ and $5.4 \times 10^{-4}$ for the Gasport Formation. Such low effective fracture porosities are rarely documented or estimated in the literature but are relevant to the estimation and occurrence of rapid groundwater velocities and contamination in fractured bedrock aquifers.
Overlying the fractured Silurian bedrock are various types of glacial deposits that potentially offer a source of attenuation of surface contaminants before they enter the rapid flow regime of the underlying fractured bedrock aquifers. The glacial spillways and drumlinized till plains that dominate southern Wellington County were deposited during the glacialiations of the Late Wisconsinan between 14.8 and 13.4 thousand years ago (Barnett, 1992). The study sites in this investigation are predominantly covered by the Port Stanley and Wentworth Tills (Figure 2.2). In southern Wellington County, the Port Stanley Till can range from 2 to 15 m thick and is frequently associated with glaciofluvial outwash sediments and both ground and end moraines. This till can range from a strongly calcareous and dolomitic silt to sandy silt till in the northern parts of southern Wellington County to a more clayey silt to silty clay till in the south. The Wentworth Till is typically present further south and is a strongly calcareous, sandy silt till associated with outwash gravel and sand and glaciolacustrine features. The presence and thickness of both of these tills is highly variable in southern Wellington County (Figure 2.3B) and studies investigating the vulnerability of the underlying fractured bedrock aquifers have established that the varying thicknesses and degrees of permeability of these sediments result in the aquifers being highly susceptible to surface contamination (Lake Erie Region Source Protection Committee, 2012). Although the highly urbanized centre of Guelph has shown to exhibit very low annual recharge on account of a large portion of the city being covered in impermeable pavement, areas surrounding the City have exhibited some of the highest recharge rates in the entire Grand River watershed with annual mean recharge rates greater than 400 mm/year (Wang, 2011). While the silty nature of the Port Stanley and Wentworth Tills may provide increased surface area for adsorption to occur, their variable thicknesses and presences can lead to some areas having an inherently higher vulnerability to surface contamination than others.

As Guelph, Fergus, and Elora are larger communities, they have both public water distribution systems and sanitary sewers serving the majority of their populations. Despite the availability of municipal
drinking water works, many residents of Fergus and Elora still rely on their own private wells and septic systems even within highly populated centres of the communities. While the sanitary systems of Guelph, Fergus, and Elora are expected to be the largest contributors of enteric viruses to the groundwater, private septic systems are also expected to be sources of contamination in Fergus and Elora, especially on the outskirts of these communities where the population is much more rural. As Fergus and Elora are both centred along the banks of the Grand River, it is also a likely source of contamination as it has the largest watershed in southern Ontario and therefore receives high volumes of sewage discharge from agricultural, municipal, and residential sources. Likewise, the Speed and Eramosa Rivers can convey these contaminants and be a source to groundwater in Guelph.

The populations of Arkell, Aberfoyle, and Eden Mills are entirely reliant upon private wells and septic systems. While the application of manure during farming in nearby agricultural areas may introduce viruses derived from swine, cattle, or poultry to the groundwater, the predominant source of human enteric viruses is likely to be septic leaching in each of these communities. As the Eramosa River runs through the centre of Eden Mills, it is also a potential source of viruses.
2.1.2 Sampling wells

The 22 wells sampled during this investigation (Table 2.3, Figure 2.2) consisted of existing private (PW), municipal supply (MSW), and monitoring wells (MW) completed in the local fractured dolostone aquifers within a 20 km radius of the City of Guelph. Each of the wells was chosen in order to represent the range of conditions influencing the vulnerability of typical bedrock wells to virus contamination and the variety of well constructions and pumping volumes. In order to promote diversity in sampling well characteristics, the final selections were based on 7 major criteria:

- well type, i.e. private, municipal supply, or monitoring well,
- well completion, including depth, length of open interval, and aquifer segment,
- the well’s proximity to potential sources such as sanitary sewers, septic systems, agriculture, or surface water bodies,
- the spatial distribution across the study area and flow system,
- surrounding land use, i.e. urban versus rural,
- overburden thickness, and
- accessibility/permission.
**Table 2.3** Details of the 8 large supply wells, 3 monitoring wells, and 11 private wells that were sampled in this project. The wells are located throughout southern Wellington County and are all completed to different depths allowing for the characterization of groundwater found in the Guelph, Eramosa, Goat Island, and Gasport formations. This array of wells also allowed for the examination of groundwater in close proximity to various land use and wastewater management practices. **Formation abbreviations**: Guelph (GU); Eramosa, (E); Goat Island, (GI); Gasport, (GA); Cabot Head, (CH). **Use abbreviations**: Municipal, (M); MW, Monitoring well, (MW); Research, (R); Domestic, (D). **Surrounding Land Use abbreviations**: R, Residential, (R); Agricultural, (A); Industrial, (I); Public parks, (P); Business, (B); University campus, (U). **Potential Sources abbreviations**: Sanitary sewer, (SS); Septic leaching, (SL); Surface water, (SW); Manure application, (M); Telescopic (in). **Land Use abbreviations**: R, Residential, (R); Agricultural, (A); Industrial, (I); Public parks, (P); Business, (B); University campus, (U).

<table>
<thead>
<tr>
<th>Location</th>
<th>Well ID</th>
<th>Total Depth (m)</th>
<th>Overburden Source</th>
<th>Overburden Type</th>
<th>Open Interval Formation</th>
<th>Well Diameter</th>
<th>Use</th>
<th>Surrounded Land Use</th>
<th>Potential Sources</th>
<th>Approximate Distance from Potential Sources (m)</th>
<th>Completion Year</th>
<th>Unique well characteristics improving diversity of well array</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elora</strong></td>
<td>MSW1</td>
<td>129.8</td>
<td>9.45 Gravel</td>
<td>9.4-13.0</td>
<td>GU, GA</td>
<td>3</td>
<td>M</td>
<td>R</td>
<td>SL, SL</td>
<td>30 m from sewer</td>
<td>1996</td>
<td>In close proximity to both urban severs and septic systems.</td>
</tr>
<tr>
<td></td>
<td>MSW2</td>
<td>121.9</td>
<td>13.1 Dismantle</td>
<td>15-115.9</td>
<td>GU, GA</td>
<td>10</td>
<td>M</td>
<td>A, I</td>
<td>SL, M</td>
<td>&lt; 30 m from agriculture; 100 m from septic</td>
<td>1991</td>
<td>Surrounded by agriculture but also in close proximity to automotive manufacturer with large and diverse staff contributing to sewer effluents.</td>
</tr>
<tr>
<td></td>
<td>MSW3</td>
<td>128</td>
<td>25 Dismantle</td>
<td>25-128</td>
<td>GU, GA</td>
<td>10</td>
<td>M</td>
<td>A, I</td>
<td>SL, M</td>
<td>&lt; 30 m from agriculture; 500 m from septic</td>
<td>Unknown</td>
<td>Exceptionally deep well surrounded by agriculture.</td>
</tr>
<tr>
<td></td>
<td>MSW4</td>
<td>110</td>
<td>4.88 Gravel</td>
<td>5.5-110</td>
<td>GU, E, GA</td>
<td>16</td>
<td>M</td>
<td>R, R</td>
<td>SL, SL SW</td>
<td>25 m from Grand River; &lt;50 m from septic</td>
<td>1966</td>
<td>In close proximity to both urban severs and septic systems and adjacent to the Grand River.</td>
</tr>
<tr>
<td></td>
<td>MSW5</td>
<td>125.1</td>
<td>23.5 Sand</td>
<td>80-129.1</td>
<td>E, GA</td>
<td>15-13-10 gravel</td>
<td>M</td>
<td>I, A</td>
<td>SL, SL</td>
<td>35 m from sewer; 300 m from agriculture, 100 m from industry</td>
<td>1972</td>
<td>Located within industrial area approximately 400 m east of residential area served primarily by severs.</td>
</tr>
<tr>
<td></td>
<td>MSW6</td>
<td>204</td>
<td>15.2 Gravel</td>
<td>24.1-134.4</td>
<td>GU, E, GA</td>
<td>15-10 gravel</td>
<td>M</td>
<td>A, R, P</td>
<td>SL, SL</td>
<td>100 m from park; 50 m from sewer; 50 m from agriculture</td>
<td>1975</td>
<td>In close proximity to residential area approximately 400 m east of residential area served primarily by severs.</td>
</tr>
<tr>
<td></td>
<td>PW1</td>
<td>8.4</td>
<td>1.8 Sand</td>
<td>6-36 Gravel</td>
<td>GU</td>
<td>2</td>
<td>M</td>
<td>R, P, R</td>
<td>SL, SW</td>
<td>&lt; 30 m from septic; &lt;50 m from Speed River</td>
<td>1969</td>
<td>Located through overburden and open to Guelph Formation only. In close proximity to main trunk of Guelph sewer system along Speed River and site with historic VOC contamination.</td>
</tr>
<tr>
<td></td>
<td>PW2</td>
<td>11.2</td>
<td>1.8 Sand</td>
<td>6-11.28</td>
<td>E</td>
<td>2</td>
<td>M</td>
<td>R, P, R</td>
<td>SL, SW</td>
<td>&lt; 30 m from septic; &lt;50 m from Speed River</td>
<td>1991</td>
<td>Located through overburden and Guelph Formation. Open only to Eramosa Formation. In close proximity to main trunk of Guelph sewer system along Speed River and site with historic VOC contamination.</td>
</tr>
<tr>
<td></td>
<td>PW3</td>
<td>25.4</td>
<td>1.8 Sand</td>
<td>18.5-15.4</td>
<td>GU, GA</td>
<td>2</td>
<td>M</td>
<td>R, P, R</td>
<td>SL, SW</td>
<td>&lt; 30 m from septic; &lt;50 m from Speed River</td>
<td>2000</td>
<td>Located through overburden, Guelph, and Eramosa Formations. Open only to Eramosa Formation. In close proximity to main trunk of Guelph sewer system along Speed River and site with historic VOC contamination.</td>
</tr>
<tr>
<td></td>
<td>PW4</td>
<td>18.6</td>
<td>12.8 Dismantle</td>
<td>12.8-58.8</td>
<td>GU, E, GA</td>
<td>10</td>
<td>R</td>
<td>U, R</td>
<td>SS</td>
<td>&lt; 10 m from septic</td>
<td>1958</td>
<td>Deep well with pumping rate of &lt;50 L/min on University of Guelph campus with dense and diverse population contributing to severs.</td>
</tr>
<tr>
<td></td>
<td>PW5</td>
<td>87.8</td>
<td>20.4 Gravel</td>
<td>14.3-87.8</td>
<td>GU, E, GA</td>
<td>10</td>
<td>R</td>
<td>R, R</td>
<td>SS</td>
<td>&lt; 10 m from septic</td>
<td>1953</td>
<td>Deep well with pumping rate of &lt;50 L/min located in university parking lot;~15 m from University of Guelph campus. Completed less than 10 m from sewers constructed less than 4 m above the top of bedrock and 12 m above the top of the well's open interval.</td>
</tr>
<tr>
<td></td>
<td>PW6</td>
<td>28</td>
<td>27.1 Gravel</td>
<td>27.4</td>
<td>GU</td>
<td>6</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 30 m from septic; &lt;100 m from agriculture</td>
<td>2006</td>
<td>Shallow private well cased through 27.1 m of gravel overburden and open to Guelph Formation. Surrounded by agriculture and residential septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW7</td>
<td>54.9</td>
<td>23.2 Gravel</td>
<td>26.6</td>
<td>GU, E, GA</td>
<td>5</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 15 m from septic</td>
<td>1991</td>
<td>Deep private well cased through 23.2 m of gravel overburden and open to Guelph, Eramosa, and Gasport Formations. Surrounded by agriculture and residential septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW8</td>
<td>24.4</td>
<td>21.3 Gravel</td>
<td>24.9</td>
<td>GU</td>
<td>6</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 15 m from septic</td>
<td>1992</td>
<td>Shallow private well cased through 21.3 m of gravel overburden and open to Guelph Formation. Surrounded by agriculture and small community relying on individual septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW9</td>
<td>15.4</td>
<td>25.8 Gravel</td>
<td>16.5-58.4</td>
<td>GU, E, GA</td>
<td>5</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 15 m from septic</td>
<td>1986</td>
<td>Deep private well cased through 15.8 m of gravel overburden and open to Guelph, Eramosa, and Gasport Formations. Surrounded by agriculture and residential septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW10</td>
<td>20.4</td>
<td>15.2 Gravel</td>
<td>15.2-26.4</td>
<td>GU</td>
<td>6</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 15 m from septic</td>
<td>1981</td>
<td>Shallow private well cased through 15.2 m of gravel overburden and open to Guelph Formation. Surrounded by agriculture and small community relying on individual septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW11</td>
<td>20.4</td>
<td>13.4 Gravel</td>
<td>13.8-20.45</td>
<td>GU</td>
<td>5</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 15 m from septic</td>
<td>1977</td>
<td>Shallow private well cased through 13.4 m of gravel overburden and open to only the Eramosa Formation. Surrounded by agriculture and small community relying in individual septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW12</td>
<td>74.7</td>
<td>11.3 Dismantle</td>
<td>12.19-74.67</td>
<td>GU, E, GA</td>
<td>6</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt;300 m from septic</td>
<td>1988</td>
<td>Deep private well cased through 11.3 m of diameter overburden and open from the Guelph to the Cabot Head Formation. Located on a golf course and surrounded primarily by agriculture and some septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW13</td>
<td>32</td>
<td>7.3 Gravel</td>
<td>7.6</td>
<td>GU, E, GA</td>
<td>6</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 15 m from septic</td>
<td>2006</td>
<td>Shallow, densely private well cased through 7.3 m of gravel overburden and open from the Guelph and Eramosa Formations. Surrounded primarily by agriculture and some septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW14</td>
<td>67.1</td>
<td>14.9 Gravel</td>
<td>13.8</td>
<td>GU, E, GA</td>
<td>6</td>
<td>D</td>
<td>A, R</td>
<td>SL</td>
<td>&lt; 15 m from septic</td>
<td>2007</td>
<td>Deep private well cased through 14.9 m of gravel overburden and open to the Guelph, Eramosa, Goat Island, and Gasport Formations. Surrounded by agriculture and small community relying on individual septic systems.</td>
</tr>
<tr>
<td></td>
<td>PW15</td>
<td>15.8</td>
<td>4.1 None</td>
<td>12-13.8</td>
<td>GU</td>
<td>6</td>
<td>D</td>
<td>A, R</td>
<td>SL, SW</td>
<td>&lt; 15 m from septic</td>
<td>2003</td>
<td>Shallow private well located in small community relying on septic systems along the Eramosa River where overburden is absent and fractured bedrock is exposed. Well is cased through 12 m of bedrock and open to the Gasport Formation.</td>
</tr>
<tr>
<td></td>
<td>PW16</td>
<td>11.1</td>
<td>0.3 None</td>
<td>15.2-31.1</td>
<td>GU</td>
<td>5</td>
<td>D</td>
<td>A, R</td>
<td>SL, SW</td>
<td>&lt; 15 m from septic</td>
<td>1985</td>
<td>Shallow private well deep located in small community relying on septic systems along the Eramosa River where overburden is absent and fractured bedrock is exposed. Well is cased through 15.2 m of bedrock and open to the Gasport Formation.</td>
</tr>
</tbody>
</table>
Figure 2.2. Location and surficial geology of study sites. A. In southern Wellington County, 11 private wells, 8 large supply wells, and 3 monitoring wells were sampled repeatedly over an 8 month period. B. Southern Wellington County is predominantly covered in the Port Stanley and Wentworth tills. C. The glacial deposits are primarily composed of diamicton, sand, and gravel. The Port Stanley Till is typified by a brownish-grey calcareous clayey silt till containing <5% clasts while the Wentworth Till can range from a sandy to very sandy till with between 7 and 10% clasts. D. Cross-section across southern Wellington County illustrating well completions and bedrock geology. Although several of wells are open in multiple formations, some are cased into a single hydrogeologic unit. MW1, PW1, PW3, and PW5 are solely open to the Guelph Formation, with casing being set into the top of rock. Wells MW2 and PW6 are open to the Eramosa formation while MW3, PW11, and PW10 are open only to the Gasport formation.
Using these criteria alongside maps illustrating the Ministry of the Environment’s Well Record Database (Appendix II Figure 1), 17 wells in Fergus, Elora, Arkell, Eden Mills, and Aberfoyle were chosen to be sampled. In addition to the above criteria and the Well Record Database, sewer invert elevations in the City of Guelph were plotted relative to the top of bedrock (Appendix II Figure 2) and used to select 5 wells in areas where sewers were constructed into or near the top of bedrock. In the end, a total of 22 wells including 11 private wells, 8 municipal supply wells, and 3 monitoring wells were chosen to be sampled. The characteristics of each well are summarized in Table 2.3 and so only a brief summary of the wells will be presented here.

In Centre Wellington, 6 municipal supply wells were sampled, including 3 wells in Elora and 3 wells in Fergus. Each well was cased through the overburden sediments into the upper few feet of the Guelph Formation. The wells were therefore open from the top of the Guelph Formation to varying depths within the Gasport Formation.

In the City of Guelph, no actual municipal supply wells were sampled, but the 5 wells chosen to be sampled in the City of Guelph consisted of 3 monitoring wells and 2 University of Guelph supply wells. The three 2-inch diameter monitoring wells with varying completion depths were located along the Speed River at a site of known industry-derived contamination with various volatile organic compounds and within 10 m of the main trunk line of Guelph’s sanitary sewer system that was constructed below the local water table and up to 4 m below the top of rock.

Two University of Guelph water supply wells located within the centre of the City of Guelph are both 10-inch-diameter wells and are pumped at an approximate rate of 280 L/min. for the purpose of this study, these wells are considered comparable to municipal supply wells and are labelled as such.

Four and five private wells of varying completion depths were sampled in the small Puslinch communities of Arkell and Aberfoyle, respectively. Along the Eramosa River in Eden Mills, 2 private...
wells of different total depths and completion years were the final wells chosen to be sampled in the current investigation.

2.2 METHODS

2.2.1 Sampling schedule
Sampling began in the second week of June 2012 and extended until January 2013. Each of the sampling wells was sampled once for general chemistry and several times for the enteric viruses listed in Table 2.2 within the 8-month sampling period. Most wells were sampled monthly for six months while other wells were sampled less frequently due to limited accessibility. Temporal sampling for viruses is required as previous studies have shown virus concentrations in groundwater to be extremely variable (Bradbury, 2013).

2.2.2 Virus Sampling
In accordance to the methods outlined in Millen et al. (2012), all sampling equipment coming in contact with raw well water was soaked in a 0.52% chlorine solution for 30 minutes prior to being rinsed with sodium thiosulfate for 5 minutes. All hoses and tubes were then flushed with 3 volumes of DI water and covered with parafilm.

Samples from municipal supply wells were taken directly from raw water taps within each of the well houses (Figure 2.3E). Private wells were sampled from external faucets bypassing water treatment such as filtration units or water softening systems (Figure 2.3F). Monitoring wells were sampled using a Grundfos Redi-Flo2 electrical submersible pump hooked up to a Honda EU2000KC2 generator. All sampling hoses were connected to a flow-through cell with a YSI 556 multiprobe system. Wells were purged until field parameters pH, temperature, and conductivity were constant.

During this investigation, all virus sampling followed the directions set forth in Millen et al. (2012). Glass wool filters provided by the United States Department of Agriculture, Agriculture Research Service
(USDA, ARS) in Marshfield, WI were attached to well taps and faucets by sterile ½” tubing (Figure 2.3). Then 500 to 1500 L of water was passed through each of the filters and discharge to ground surface. At one site known to have VOC contamination, filtered water was collected into 55 gal drums and treated at an onsite treatment centre.

Figure 2.3. Sampling materials and setup for virus sampling. Clockwise starting from top left. A. Filter housing made with Banjo dust caps. B. Glass wool that is packed into filter housing to act as virus filter. Filtration is based on charge differences between the glass wool and virus surfaces. To maximize charge difference, the pH of the water must be maintained between 6.5 and 7.5. If pH is above 7.5, it must be adjusted using HCl before the water reaches the glass wool filter. C. HCl injection setup with 0.5 M HCl and precision peristaltic pump connected to glass wool filter line via plastic barbed “T” connector between the filter and water faucet. D. Virus sampling setup for private well. Sampling tubes and glass wool filter attached directly to outdoor faucet bypassing any filtration or water treatment. E. Virus sampling setup for large supply well. Sampling tubes and glass wool filter attached directly to raw water sampling tap.

When initial pH levels were above 7.5, 0.5M HCl was injected before the filter using a Masterflex® precision peristaltic pump in order to establish a pH between 6.5 and 7.0. The pH adjustment maximizes the charge differences between the viruses and glass wool therefore increasing the likelihood for virus attachment to the filter. For the first 3 sampling events, the total volume of filtered water was
calculated by measuring the flow rate using a graduated cylinder and timing the entire filtration process. The remaining sampling events employed an Elster V100 volumetric meter to record more accurate flow volumes.

Once 500 to 1500 L of water had been sampled, the glass wool filters were packed on ice and shipped overnight to the USDA Agriculture Research Service in Marshfield, WI.

2.2.4 Virus analysis
Virus analysis was conducted at the USDA ARS laboratory in Marshfield, WI by the procedures outlined by Borchardt et al. (2012). Upon arriving at the USDA ARS laboratory, the glass wool filters were eluted using 3% beef extract containing 0.05 M glycine (pH 9.5). The pH of the eluent was adjusted to between 7.0 and 7.5 using 1 M HCl and flocculated with polyethylene glycol 8000 (8% [wt/vol]) and NaCl (final concentration, 0.2 M). This mixture was stirred for 1 hour, incubated over night, and centrifuged at 4200 x g for 45 minutes. The three preceding processes all occurred at 4°C, but once the pellet was resuspended in 2 mL of sterile 15 M Na2HPO4, the final concentrated sample was stored at -80°C.

Quantification of the viruses listed in Table 2.2 was completed using two-step quantitative reverse-transcription polymerase chain reaction (RT-qPCR) for the 5 RNA viruses while the DNA viruses were analyzed using only qPCR. The qPCR for all of the viruses was performed on a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) using PCR mixes prepared with the LightCycler DNA master hybridization probe kit (Roche Diagnostics) with fluorescence generated by TaqMan probes (TIB Molbio, Berlin, Germany). Detailed procedures including the specific primers, probes, and RT and PCR cocktails are discussed in more detail in Borchardt et al. (2012).

2.2.5 QA/QC
Virus sample collection followed a Standard Operating Procedure (SOP) developed in accordance with the procedures outlined in Millen et al (2012; Appendix I). Field staff for the current investigation were
trained by Mark Borchardt and Jordan Gonnering at the USDA, ARS to ensure adherence to previously established methods.

While cost of virus analyses impedes the collection of field duplicates, two 20 L matrix recovery controls were collected from two different sampling wells. Each of the samples was divided into two 10 L subsamples. The first 10 L sample was pumped through a sterile glass wool filter using a peristaltic pump and then eluted using a solution of 3% beef extract and glycine adjusted to a pH of 9.5. The pH of the final eluent is neutralized and a spike of known concentrations of Salmonella, Giardia, and enterovirus was added to the eluent, making this sample a seeded control. The second 10 L sample is spiked with known concentrations of Salmonella, Giardia, and enterovirus before it is pumped through a new glass wool filter. The filter is eluted and neutralized similar to above, to result in the seeded sample. Calculation of percent recovery follows Equation 1, below, and provides the recovery efficiency of the glass wool filter. The results from these recovery controls are presented in Appendix II Table 1. These methods also include equipment blanks, recovery controls, as well as various positive and negative controls for the qPCR analysis.

Equation 2.1

\[
\text{% Recovery} = \frac{\text{Concentration of Seeded Sample}}{\text{Concentration of Seeded Control}} \times 100\%
\]

2.2.6 Data analysis

Virus presence/absence as well as concentrations were plotted in ArcMap to illustrate the spatial distribution of viruses and their relationship to surficial geology. Virus detection rates were plotted against precipitation data acquired from the Guelph Turfgrass Institute and the Elora Research Station, both run by the University of Guelph. Correlation coefficients were then calculated for daily virus detection rates and precipitation data. Time lags were manually applied to the precipitation data so that virus detection rates were correlated with precipitation events occurring from 0 to 30 days prior to
the virus detection and 0 to 5 months prior to the virus detection. The correlation coefficients for each time lag were then compared to determine if virus detection rates were influenced by precipitation events within a given time frame as indicated by the highest correlation coefficient (Appendix II).

2.3 RESULTS
Average field pH values were between 6.5 and 7 for most wells, but the pH for groundwater taken from the MSW5, MSW6, PW11 and PW10 wells were consistently above 7.5. Glass wool filtration for these samples required in-line injection of 0.5 M HCl to lower the pH between 6.5 and 7 before the sample reached the filter.

The current study resulted in a 38.6% recovery of enterovirus from a seeded sample from MW1 and a recovery of 52.9% from MW1 which fall within the same range reported by Lambertini et al. in 2008 (glass wool recovery of enteroviruses from groundwater samples ranged from 56 to 12%). We therefore have confidence that the methods used in this study were consistent with methods established in previous works.

Of the 22 sampling wells in southern Wellington County that were chosen to be tested for viruses, 10 exhibited quantifiable concentrations of viruses (Table 2.4). Five of these wells were private wells while the other 5 were municipal supply wells. 5 out of 11 (45%) private wells that were sampled exhibited virus detections while 5 out of 8 (62%) municipal supply wells were also positive for viruses. The 3 viruses that were detected included human polyomavirus, adenovirus A, and G2 Norovirus (Figure 2.4). None of the wells that exhibited quantifiable virus concentrations tested positive on more than one occasion nor did they exhibit more than one type of virus.
Table 2.4. Summary of the 10 virus detections southern Wellington County, ON. 10 of the 22 sampling wells exhibited viruses during this investigation. Of the 10 wells that exhibited viruses, 5 were private wells and 5 were municipal supply wells. Of all the private wells sampled, 45% (5 out of 11) were positive for human enteric viruses. 62.5% of all the municipal supply wells that were sampled also exhibited viruses. G2 norovirus was consistently found in the highest concentrations followed by human polyomavirus then adenovirus A. GC/L = Genomic copies per litre.

<table>
<thead>
<tr>
<th>Date of Detection</th>
<th>Well ID</th>
<th>Well Type</th>
<th>Location</th>
<th>Virus</th>
<th>Concentration (GC/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/15/2012</td>
<td>PW4</td>
<td>Private</td>
<td>Aberfoyle</td>
<td>Human Polyomavirus</td>
<td>1.52</td>
</tr>
<tr>
<td>7/24/2012</td>
<td>MSW4</td>
<td>Municipal supply</td>
<td>Fergus</td>
<td>Adenovirus A</td>
<td>0.09</td>
</tr>
<tr>
<td>8/12/2012</td>
<td>PW1</td>
<td>Private</td>
<td>Aberfoyle</td>
<td>G2 Norovirus</td>
<td>3.34</td>
</tr>
<tr>
<td>8/12/2012</td>
<td>PW3</td>
<td>Private</td>
<td>Aberfoyle</td>
<td>G2 Norovirus</td>
<td>15.16</td>
</tr>
<tr>
<td>8/12/2012</td>
<td>PW2</td>
<td>Private</td>
<td>Aberfoyle</td>
<td>Human Polyoma Virus</td>
<td>1.16</td>
</tr>
<tr>
<td>8/14/2012</td>
<td>MSW7</td>
<td>Municipal supply</td>
<td>Guelph</td>
<td>Adenovirus A</td>
<td>1.23</td>
</tr>
<tr>
<td>8/20/2012</td>
<td>MSW6</td>
<td>Municipal supply</td>
<td>Fergus</td>
<td>Adenovirus A</td>
<td>0.54</td>
</tr>
<tr>
<td>9/11/2012</td>
<td>PW7</td>
<td>Private</td>
<td>Arkell</td>
<td>Human Polyoma Virus</td>
<td>1.99</td>
</tr>
<tr>
<td>11/7/2012</td>
<td>MSW1</td>
<td>Municipal supply</td>
<td>Elora</td>
<td>Human Polyoma Virus</td>
<td>1.01</td>
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<tr>
<td>11/7/2012</td>
<td>MSW2</td>
<td>Municipal supply</td>
<td>Elora</td>
<td>G2 Norovirus</td>
<td>15.63</td>
</tr>
</tbody>
</table>

Figure 2.4. Virus occurrence throughout southern Wellington County in 10 out of 22 wells. Each positive well only exhibited the presence of viruses once throughout the entire sampling schedule. While 3 wells were positive for adenovirus, 3 different wells were positive for G2 norovirus. Human polyomavirus was found in 4 wells.
Analyses of monthly precipitation and virus detection rates did not result in any observable relationships (Appendix II Figure 3) but did yield statistical correlations when 0 and 1 month lags were applied to Elora (R = -0.729) and Guelph (-0.396) sampling wells, respectively. Although the highest correlation coefficients during the monthly analyses were consistently negative, the high variability and low value of the correlation coefficients suggest that the experimental design of this investigation may not have been amenable to this kind of statistical analysis.

Correlations between overburden type and virus occurrence were also not obvious. Viruses occurred in wells surrounded by both gravel and diamict, and wells that one would expect to be at high risk of virus contamination due to a lack of overburden exhibited no viruses. While viruses occurred in wells with overburden thicknesses ranging from 4.88 to 27.1 m, a weak correlation (R = 0.6856) was found between virus concentrations and overburden thickness when two outliers were excluded from the statistical analysis (Appendix II Figure 4). Similarly, wells with longer open intervals seemed to correlate well with lower virus concentrations (R = -0.7587) (Appendix II Figure 5).

2.4 DISCUSSION & CONCLUSIONS
Previous assessments of the Grand River watershed have established that the dolostone aquifers of southern Wellington County are highly susceptible to contamination due to the combination of permeable overburden with spatially variably thicknesses and the highly fractured nature of the bedrock aquifers (Lake Erie Region Source Protection Committee, 2012). The detection of viruses in these aquifers, are therefore not surprising. In comparison to other studies, however, the detection of viruses in 10 out of the 22 wells sampled in this investigation is an exceptionally high detection rate of human enteric viruses in groundwater samples. On a per sample basis, however, the only 10 of 118 virus samples exhibited detectable concentrations of viruses. While the ephemeral nature of virus contamination has been well established in the literature, the low per sample detection frequency was
unexpected for the rapid flow regime and highly vulnerable setting of the fractured bedrock aquifers of southern Wellington County, ON.

2.4.1 Vulnerability of private wells
In the current investigation, 5 out of the 11, or 45% of private wells sampled, exhibited human enteric viruses including human polyomavirus, adenovirus A, and G2 norovirus. Compared to the only other study investigating virus concentrations in private wells completed in fractured bedrock aquifers, the current study would suggest that the private wells completed in the fractured Silurian dolostone aquifers of southern Wellington County, ON are several times more vulnerable to virus contamination than private wells in Wisconsin (Table 2.5)(Borchardt et al., 2003).

Table 2.5. Private well construction and virus sample summary statistics from the current study and Borchardt et al., 2003.

<table>
<thead>
<tr>
<th>Study</th>
<th>Statistic</th>
<th>Well Depth (m) (n=11)</th>
<th>Casing depth (m) (n=11)</th>
<th>Well age (yr) (n=11)</th>
<th>Sample volume (L) (n=66)</th>
<th>Sample pH (°C) (n=66)</th>
<th>Sample temp (°C) (n=64)</th>
<th>Detection Rate per sample</th>
<th>Detection Rate per well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen 2013</td>
<td>n</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>63</td>
<td>66</td>
<td>64</td>
<td>5/63 (8%)</td>
<td>5/11 (45%)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>39.0</td>
<td>16.7</td>
<td>20.1</td>
<td>917</td>
<td>7.31</td>
<td>11.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>31.1</td>
<td>15.8</td>
<td>22</td>
<td>897</td>
<td>7.28</td>
<td>10.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>15.8</td>
<td>7.62</td>
<td>6</td>
<td>623</td>
<td>6.92</td>
<td>8.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>74.7</td>
<td>27.4</td>
<td>36</td>
<td>1377</td>
<td>7.82</td>
<td>18.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borchardt 2003</td>
<td>n</td>
<td>44*</td>
<td>43</td>
<td>42</td>
<td>194</td>
<td>193</td>
<td>192</td>
<td>5/194 (3%)</td>
<td>4/50** (8%)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>36.6</td>
<td>25.3</td>
<td>7</td>
<td>1234</td>
<td>7.36</td>
<td>11.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>8.8</td>
<td>7.9</td>
<td>2</td>
<td>568</td>
<td>6.5</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>93.0</td>
<td>73.2</td>
<td>22</td>
<td>1605</td>
<td>8.54</td>
<td>18.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Of the 50 wells sampled in the Borchardt study, only 44 had well completion data.
** Of the 50 wells sampled in the Borchardt study, 30 were completed in fractured bedrock. The detection rate per well completed in fractured bedrock was 3/30, or 10%.

In the study by Borchardt et al. (2003), each private well was sampled 4 times throughout an entire calendar year while private wells in the current study were sampled 5 or 6 times between June and December of 2012. The lower number of samples per well in the Wisconsin study could have underestimated virus presence in comparison to the current investigation. Although the average age of
private wells sampled by Borchardt et al. was much younger than private wells sampled in the current study, the older wells in southern Wellington County did not express detectable viruses and so the age difference between the sampling wells is not likely to be responsible for the different detection rates. The volumes of water filtered by Borchardt et al. were relatively higher than the volumes filtered in the current study, but this would be expected to result in overestimations of virus occurrence in the Wisconsin study. While the types of fractured bedrock aquifers investigated by Borchardt et al. included igneous, sandstone, and dolostone aquifers, due to the similar average well depth, groundwater pH and temperature of the wells sampled in both studies, the two investigations are suitable for comparison as they are the only two investigations examining the vulnerability of private wells completed in fractured bedrock aquifers. It can therefore be concluded that private wells completed in the fractured bedrock aquifers of southern Wellington County are approximately 4.5 times more vulnerable to virus contamination than private wells in Wisconsin fractured bedrock aquifers. As relationships between virus occurrence and overburden or precipitation were absent, the elevated virus detection rates of the current study in comparison to the findings of Borchardt et al. are expected to be attributable to the higher sampling frequency of the current investigation. While both Borchardt et al. and the current investigation only found viruses in private wells in close proximity to septic systems, it is believed that they are the dominant source of the viruses in the wells. As private wells are generally located in rural areas removed from public infrastructure a septic system source is logical.

2.4.2 Vulnerability of municipal supply wells
Similar to above, the municipal supply wells of southern Wellington County also exhibited higher virus detection rates than previous investigations would suggest. In the US EPA’s 2006 Economic Analysis for the Final Ground Water Rule, the results of 15 groundwater investigations were culminated to arrive at an estimate that 27% of public water systems in the United States will be contaminated with viruses at some point. In the current study, 5 out of 8 (62%) large supply wells exhibited viruses during the 8-
month sampling period. As the EPA’s estimate was based on investigations of both porous media and fractured bedrock aquifers and relied on cell culture techniques for virus detections, it is not surprising to find a higher detection rate of viruses in municipal supply wells in the current investigation. Due to the individual grains of aquifers comprised of porous media, these aquifers will have an elevated surface area in comparison to fractured bedrock aquifers and will therefore have more sites available for virus adsorption and attenuation. Additionally, groundwater velocities within porous media are much slower and may result in virus inactivation before transport has been completed from the source to the vulnerable well. With elevated adsorption and inactivation in a porous media aquifer, the vulnerability of wells in these aquifers is decreased.

In comparison to the q-PCR technique used for virus detection in the current investigation, cell culture techniques have been shown to be limited in the type of viruses they can detect and the type of water they can detect viruses in. Cell culture techniques can also lead to significant underestimations of virus concentrations as the calculations are based on counting viral plaques as single virus particles when they can actually be formed from one or more virus particles. Although virus recovery from glass wool filters was between 38.6 and 52.9% for the current investigation (Appendix I Table 1), by using this detection method, this study is likely to have produced a better estimate of the actual concentrations of viral DNA in groundwater. By including investigations using cell culture detection methods to detect viruses in a number of different aquifer types, the EPA’s calculations may underestimate the true vulnerability of wells completed in fractured bedrock aquifers. As evidenced by the current investigation, municipal supply wells completed in fractured bedrock aquifers are highly vulnerable to contamination with sewage-derived viruses.

Other studies investigating the occurrence of viruses in municipal supply wells completed in fractured bedrock aquifers are summarized in Table 2.1. While the studies in the Ozark Plateaus (Davis and Witt,
2000; Femmer, 2000) and Pennsylvania (Lindsey et al., 2002) sampled a wide array of wells, the virus detection rates are suspected of being low due to insufficient sampling frequencies of each well. In contrast, studies by Lieberman et al. (2002), Borchardt et al. (2007), Locas et al. (2007), and Bradbury et al. (2013) each collected monthly samples in order to account for the temporal variability of viruses in groundwater. These studies, however, sampled a limited number of municipal supply wells with a limited assortment of surrounding land uses and potential sources. As the current study made an effort to sample several wells with varying constructions and surrounding land uses, the virus detection rate (62%) is expected to be more representative of all types of municipal supply wells constructed in fractured bedrock aquifers.

2.4.3 Influences on virus detection
In Bradbury et al.’s 2013 study, strong correlations were found between precipitation events and increased virus detection rates. In the current investigation, however, no observable relationship could be found between monthly precipitation and virus detections. The calculation of correlation coefficients for monthly virus detection rates and precipitation data suggested that contrary to the findings of Bradbury et al. (2013), an inverse relationship may be present between virus occurrence and precipitation events, and that during or up to one month following a heavy rain event, virus detection rates are expected to be low. While these relationships could be attributed to dilution after rain events resulting in lower detection rates or raised water tables leading to sewer infiltration rather than sewer exfiltration, the high variability and low values of the correlation coefficients suggest that the current investigation was not designed for such thorough statistical analysis and therefore the results are not conclusive evidence of the statistical relationship between virus occurrence and precipitation events.

As discussed in the introduction, certain properties of the overburden materials can provide a source of virus attenuation and can prevent viruses from reaching the underlying aquifer. In this investigation,
however, viruses were found in wells surrounded by sediments composed of both gravel and diamict. Additionally, wells in Eden Mills that did not have any overburden surrounding them to offer additional attenuation did not exhibit any viruses. The predominant source of contamination of the wells in Eden Mills is from the adjacent Eramosa River and while this river may be susceptible to sewer and septic effluents or agricultural runoff, the exposure to sunlight may limit virus concentrations due to UV denaturing of virus DNA. In any case, the combination of these results would suggest that overburden type has little influence over whether or not a given well will be susceptible to virus contamination. It is important to note, however, that transport through fractured rock is largely horizontal and so the overburden located at the wellhead may not have much influence on the transport of contaminants to the open interval of the well as this would require vertical transport. Due to rapid flow and therefore large capture zones in fractured bedrock aquifers, along with the high variability of overburden type and thickness in southern Wellington County, the type of sediments present at the wellhead become irrelevant. Although a well may be located in an area of tight diamict and therefore relatively low permeability, the sand and gravel deposits up gradient of the wellhead may provide a large portion of recharge to that area of the aquifer therefore increasing the well’s vulnerability to contamination. As the determination of well capture zones was beyond the scope of this investigation we cannot make a confident statement about the influence of overburden type on virus occurrence other than the fact that it seems the type of overburden located at the wellhead is irrelevant.

In contrast to overburden type, overburden thickness did show a weak correlation with virus concentrations. As this correlation was positive, however, the validity of this relationship is questionable. Due to the attenuating properties of unsaturated overburden sediments, one would expect that thicker sequences of overburden would result in decreased virus concentrations in the underlying fractured bedrock aquifer. In this investigation, the opposite relationship was found: thicker overburden lead to increased virus concentrations. Due to the weak correlation coefficient and lack of a
good explanation for these results, it cannot, in good confidence, be said that this relationship between overburden thickness and virus concentrations is valid.

The final relationship explored in this investigation was that between virus concentrations and the length of a well’s open interval. This relationship also exhibited a weak correlation (-0.7587), but with a more justifiable trend. This finding would suggest that wells with longer open intervals will exhibit lower virus concentrations. As most of the wells were only cased through the overburden and left open from the top of rock to the base of the well, deeper wells had greater open intervals. The deeper the open interval extended into the subsurface, the more material the viruses had to travel through. This would increase both the distance and time the virus had to travel to reach deeper sections of the open interval. Increased travel time and distance both increase the potential for virus inactivation and would result in lower virus concentrations at depth. Water from deeper sections of the open interval would then dilute any shallower water containing viruses and contribute to lowering virus concentrations in wells with greater open intervals. Although the correlation coefficient value is low, this relationship is present and important to report.

Another factor influencing virus detection could be the recovery from the glass wool filters (Appendix II, Table 1). While the current investigation yielded recoveries consistent with previous studies (38.6-52.9%), these recovery rates would suggest that the detection frequencies discovered in southern Wellington County could be artificially low due to low recovery and the actual detection frequency is somewhat higher. As the current investigation yielded both some of the highest recoveries of studies examining viruses in groundwater as well as some of the highest detection frequencies, the results are significant without adjusting for the glass wool recoveries less than 100%. It is still important to note that these rates are expected to be artificially low.
2.4.4 Risk associated with virus contamination of a fractured bedrock aquifer
While numerous wells within southern Wellington County exhibited positive virus detections throughout this study, the population is not necessarily at risk of contracting viral infections from drinking the local groundwater. First, the virus detection method used in this investigation (qPCR) quantifies both active and inactive viruses, but does not distinguish between the two. As infectivity analyses were not conducted in the current study, it is impossible to determine if the detections were of active or inactive viruses. The detections presented in the current study could therefore represent inactive, non-infectious viruses. Determining the likelihood of whether or not the viruses were or could be active or inactive based on travel times is not possible for the current study as capture zones and time of travel were not determined for the sampling wells. While virus infectivity such as that of adenovirus has been shown to degrade within 120 days, without accurate hydraulic information with regards to travel times from potential sources to each of the wells, it is impossible to determine if viruses could be transported from fecal sources to the wells in the finite amount of time that the viruses remain active in groundwater regimes. On the other hand, both Ogorzaly et al. (2010) and Charles et al. (2009) showed the adenovirus genome to exhibit degradation within less than 2 years in groundwater which would suggest that the wells exhibiting this virus in the current study must be influenced by water less than 2 years old. As Bofill-Mas at al. (2006) found human polyomavirus to exhibit similar persistence to adenovirus in sewage samples, southern Wellington County sampling wells exhibiting polyomavirus must also be under the influence of relatively young water. Regardless of whether or not the viruses were active, the wells containing these viral genomes are clearly susceptible to rapid sewage-derived contamination. As studies have yet to determine the maximum persistence of the GII norovirus genome in groundwater (Charles et al. 2009), determining the age of water influencing wells based on the occurrence of G2 norovirus DNA is not currently possible.
Although the infectious nature of the viruses detected in the current investigation was not determined, there may still be some risk associated with their detection. In a 2012 study by Borchardt et al. several qPCR detections of enterovirus and adenovirus were positively associated with acute gastrointestinal illnesses despite the fact that cell cultures did not exhibit any cytopathic effects or evidence of infectivity. As Borchardt et al. (2012) found that qPCR-positive results for waterborne viruses have value for predicting the risk of acute gastrointestinal illness without the presence of culturable viruses, the detections in southern Wellington County would suggest some risk to consumers of raw groundwater. As many private well owners drink untreated or minimally treated water, they may be at a risk of acute gastrointestinal illnesses derived from their drinking water. In Ontario, however, municipal water supplies must abide by the Safe Drinking Water Act (2002) and meet the Ontario Drinking Water Quality Standards. As these standards call for the treatment and chlorination of water distributed to the public as drinking water, low concentrations of viruses in some of the raw water from supply wells are likely to be inactivated prior to distribution. It is still important to note, however, that chlorine doses are determined through the detection of fecal indicators including total coliforms and E. coli which, in Chapter 4, are shown to be poor indicators of virus occurrence. The current treatment of municipal water may therefore not be adequate to eliminate all virus occurrences.

### 2.5 CONCLUSIONS

After an 8 month sampling campaign, human enteric viruses were found in a substantial number of municipal supply wells (5 out of 8) and private wells (5 out of 11) in the fractured bedrock aquifer of southern Wellington County, ON. Fractured bedrock aquifers are known for their relatively high degree of vulnerability to contamination and as previous investigations would suggest fractured bedrock aquifers to be susceptible to virus contamination, the high detection frequencies on a per well basis were expected. These results were anticipated because in the study area, the overburden has variable thickness and is thin in much of the area where groundwater recharge is expected to be large and rapid.
With only shallow casings generally extending only into the upper few feet of bedrock, wells completed in fractured bedrock aquifers are typically susceptible to surface contamination. Additionally, the shallow regional water table and large groundwater velocities in the fractures (1-10 m/day) are expected to promote rapid transport from near-surface fecal sources to the nearby wells and cool groundwater temperatures (~9°C) promote virus longevity. The hydrogeologic regime of fractured bedrock aquifers therefore creates an ideal setting for virus transport.

On a per well basis, virus detection rates were high relative to previous investigations, but on a per sample basis, the detection frequencies were much lower. Of the 118 samples, only 10 were positive for human enteric viruses. While the ephemeral nature of virus contamination of groundwater is well established, this absence of viruses in several of the wells and low detection frequency raise the question concerning the reason for this absence and low detection frequency; either the community contributing to the sewage source were not infected and therefore these wells have no virus presence, the frequency of sampling was inadequate for virus detection, or the local hydrogeological conditions prevent virus arrivals at these wells. As relationships between virus occurrences and well construction, overburden thickness and type, and precipitation were not observed in the current investigation, the reason for the low detection frequency on a per sample basis requires further investigation. Perhaps the wells not exhibiting any viruses are constructed in a way that future wells could be constructed in order to prevent virus occurrences. The current analyses, however, make the reason for the varying susceptibilities unclear.

Finally, while the current investigation failed to report on the actual infectivity of the viruses that were detected, the detection of their genomes is enough evidence to suggest that there may be some risk to those individuals consuming raw, untreated groundwater from southern Wellington County’s aquifers. As municipal water is heavily regulated and treated, any small concentrations of enteric viruses
contaminating the raw water are expected to be inactivated before water is distributed to the public. In contrast, private well owners rarely submit their well water to treatment systems and therefore these individuals are expected to be most at risk of contracting human enteric viruses from groundwater and experience acute gastrointestinal illnesses. For the current results of virus occurrences to be directly related to human health risk, however, it would be necessary to conduct epidemiological and infectivity experiments in the study area.
Chapter 3:
Artificial sweeteners, pharmaceutical compounds, and other sewage-derived contaminants in a fractured bedrock aquifer

ABSTRACT
While several investigations have explored the susceptibility of various types of wells and aquifers to sewage-derived contaminants, few studies have focused on the vulnerability of fractured bedrock aquifers to these organic wastewater contaminants. Pharmaceuticals, artificial sweeteners, and components of various cleaning products have been found in both surface and groundwater samples around the world, but the prevalence and transport properties of these contaminants in fractured bedrock aquifers are poorly understood. In the current investigation, groundwater samples from 22 wells completed in the fractured Silurian dolostone aquifers of southern Wellington County, ON, Canada were analyzed for 27 sewage-derived contaminants. Out of the 22 wells, 20 (91%) exhibited at least one of the wastewater contaminants. Although previous studies would suggest carbamazepine and sulfamethoxazole are the most prevalent pharmaceuticals in groundwater, each of them was only detected in 3 wells while ibuprofen was detected in 10 wells. Despite the high detection frequency of ibuprofen (45%), it was never detected above its practical quantitation limit of 2 ng/L. Perchlorate, PCE, and the artificial sweetener acesulfame were the most ubiquitous contaminants being detected in 19, 14, and 14, wells, respectively. Perchlorate and acesulfame were both found in concentrations several orders of magnitude above their respective minimum detection limits of 2 and 8 ng/L. While perchlorate and PCE have numerous potential sources, the predominant source of environmental acesulfame is sewer exfiltration and septic leaching. Its widespread occurrence is therefore strong
evidence suggesting that current wastewater management practices are ineffective at preventing anthropogenic waste streams from influencing local drinking water aquifers. Additionally, these findings would suggest that fractured bedrock aquifers are much more susceptible to sewage-derived contaminants than previous American and pan-European investigations would suggest (Barnes et al., 2008; Loos et al., 2010). The occurrence of acesulfame in shallow groundwater samples with tritium activities greater than 10 TU would also suggest that this artificial sweetener may be useful in determining relatively young water as this sweetener was only made available for consumption in 1988.

3.0 INTRODUCTION
As discussed in Chapter 2, sewer exfiltration and septic leaching are large potential sources of human wastewater contaminants in the environment. Other than viruses, this wastewater can also contain high levels of anthropogenic compounds such as artificial sweeteners, pharmaceuticals, and cleaning products, among others. Many of these compounds have shown to be exceptionally resistant to degradation even during wastewater treatment and can therefore persist for long durations in groundwater settings (e.g. van Stempvoort, 2011b). While some pharmaceuticals have exhibited toxicity to aquatic ecosystems, others, such as artificial sweeteners, are potentially more benign contaminants (Halling-Sorensen, 1988). In recent studies, the presence of many of these anthropogenic compounds in groundwater have suggested that they exhibit relatively conservative transport in porous media, but the literature on their transport in fractured bedrock regimes is extremely limited. The goal of the current investigation is to explore the presence of the wastewater contaminants listed in Tables 3.1 and 3.2 as well as other sewage-derived contaminants in the fractured Silurian dolostone aquifers of southern Wellington County in order to better understand the vulnerability of the aquifer to contamination with these types of contaminants and their persistence in the flow regimes of fracture rock aquifers.
Table 3.1. Characteristics of the artificial sweeteners and perchlorate examined in this investigation.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Hydrophobicity</th>
<th>Potential Sources</th>
<th>Persistence</th>
</tr>
</thead>
</table>
| Acesulfame                   | Anionic and strongly hydrophilic | • Human waste (sewer or septic leachate)  
• Food processing  
• Land fill leachate | • Low affinity for adsorption  
• Highly resistant to biodegradation  
• Exhibits highly conservative transport similar to chloride |
| Cylamate                     | Anionic and hydrophilic  | • Human waste (sewer or septic leachate)  
• Food processing  
• Land fill leachate | • Low affinity for adsorption  
• Rapid biodegradation |
| Saccharin                    | Anionic and hydrophilic  | • Human waste (sewer or septic leachate)  
• Food processing  
• Land fill leachate  
• Swine feed  
• Manure | • Low affinity for adsorption  
• Rapid biodegradation |
| Sucralose                    | Nonionic and hydrophilic  | • Human waste (sewer or septic leachate)  
• Food processing  
• Land fill leachate | • Exhibits less conservative transport than acesulfame due to increased susceptibility to biodegradation |
| Perchlorate (not a sweetener, but included in these analyses) | Anionic and hydrophilic  | • Rocket fuel  
• Fireworks  
• Road flares  
• Fertilizers  
• Munitions  
• Explosives | • Can persist in groundwater for several decades.  
• High solubility and little tendency to adsorb lead to high mobility. |
Sewer exfiltration and septic leaching are significant sources of human wastewater contaminants in the environment. In Chapter 2, an investigation of the vulnerability of wells completed in fractured bedrock aquifers to sewage-derived viruses revealed that the fractured Silurian dolostone aquifers of southern Wellington County, ON exhibit exceptionally high detection rates of human enteric viruses. As these viruses reside in mammalian intestinal tracts and are shed in high concentrations in feces, the predominant source of human enteric viruses are anthropogenic waste streams most likely originating in sewers and septic systems. A 2010 study by Hunt et al. linked the presence of viruses in groundwater to sewer exfiltration when they found both human enteric viruses and several unambiguous tracers of anthropogenic wastewater such as ionic detergents, flame retardants, and cholesterol in 33 municipal supply wells in close proximity to sanitary sewers. When sewer effluent and groundwater from nearby monitoring wells were analyzed by Wolf et al. (2004), simultaneous concentrations of X-ray contrast media and boron in both the sewers and groundwater revealed the direct influence of leaking sewers on groundwater quality. Additionally, in groundwater regimes such as fractured bedrock aquifers, sewage-derived contamination has been shown to follow preferential pathways and reach depths of 60 and 90 m in the unconfined and confined sandstone aquifers in the UK (Powell et al., 2003). Quantification of the leakage of sanitary and industrial sewage from sanitary sewers across the United States was conducted in 2000 by Amick et al. and revealed through continuous monitoring of flow within sanitary sewers that up to 56% of sewer flow is exfiltrated in some sewers in California, up to 49.1% in Maryland, and up to 34.5% in Kentucky. The study further summarized results from previous investigations in New Mexico, Hong Kong, and England where losses of wastewater from sanitary sewers were calculated using drinking water-wastewater balances and estimated that 11, 8, and 20 to 25% of sewer flow was exfiltrated, respectively. With up to 56% sewer leakage in some locations and numerous studies confirming the rapid release and penetration of anthropogenic contaminants from these leaking sewers to the surrounding aquifers, sewer exfiltration is a major source of sewage-derived contaminants.
In addition to sewer exfiltration, leaching septic systems are also a large contributor of emerging anthropogenic contaminants to groundwater. In 2007, Godfrey et al. observed the influence of a septic plume on groundwater quality when 3 of the 12 pharmaceutical compounds detected in a Montana high school’s septic tank were found to have infiltrated a 2-m-thick sand vadose zone and contaminated the underlying sand and gravel aquifer. Similarly, Carrara et al. (2008) revealed several pharmaceutical compounds including ibuprofen, gemfibrozil, and naproxen throughout almost the entire depth of a sand aquifer and at least 20 m down-gradient from a large septic system in Long Point, ON. In the same septic plume, Van Stempvoort et al. (2011) also detected 4 artificial sweeteners including acesulfame, sucralose, cyclamate, and saccharin. In a preliminary study for the current investigation, a small farming community outside the City of Guelph, ON that relies entirely on private septic systems also exhibited concentrations of artificial sweeteners several orders of magnitude above detection limits (Figure 3.1). As these sweeteners and pharmaceuticals are not naturally occurring in the environment, the predominant source of these anthropogenic contaminants in rural areas removed from landfill and food processing plants is septic leaching.

**Figure 3.1.** Results from a preliminary study illustrating the concentrations of acesulfame in a small farming community.
Widespread contamination of groundwater with these emerging compounds has been documented by several recent scientific investigations. The first study to find artificial sweeteners in the environment was a Swedish screening program conducted in 2008 in which sucralose was detected in natural aquatic environments in close proximity to sewage treatment plants (Brorstrom-Lunden et al., 2008). The first detection of artificial sweeteners in groundwater, however, was not until Buerge et al. analyzed several Swiss groundwater samples in 2009 for acesulfame, sucralose, saccharin and cyclamate. While Buerge et al. detected acesulfame in several of their groundwater samples, saccharin and cyclamate were not detected in groundwater until 2011 when Van Stempvoort et al. sampled groundwater influenced by a large septic plume in Long Point, Ontario, Canada which has since received much attention with regards to the presence, transport, and persistence of artificial sweeteners and pharmaceutical compounds (Robertson et al., 2013). Although a recent study by Van Stempvoort et al. (2013) detected these artificial sweeteners at two additional sites in Alberta and Ontario, the amount of literature examining the presence of artificial sweeteners in aquifers is very limited.

In contrast to artificial sweeteners, as summarized in Table 3.2, many studies have examined the occurrence of pharmaceutical compounds in groundwater, some of which have been at the national scale. Two smaller field-scale investigations of areas susceptible to wastewater contamination from septic or agricultural sources were conducted by Carrara et al. (2008) and Bartelt-Hunt et al. (2010), respectively, and discovered local contamination of groundwater with pharmaceuticals and hormones ranging from ibuprofen, gemfibrozil, naproxen, and triclosan, to androstenedione and estrone. In a larger study by Fram and Belitz (2011), 1231 groundwater samples from municipal supply wells across California were analyzed for 14 pharmaceutical compounds. In contrast to previous studies, the California study sampled from wells in a variety of hydrogeologic conditions with a range of surrounding
land uses. While 28 of the 1231 samples exhibited detectable pharmaceuticals, the detection frequencies and maximum concentrations were considerably lower than other field scale studies such as Barnes et al. (2008) and Loos et al. (2010) have reported.

In 2008, Barnes et al. conducted a national groundwater reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States in which 47 groundwater samples were collected from across the US and analyzed for 65 organic wastewater contaminants including various pharmaceutical compounds, cleaning products, and other common household products. The sampling sites provided analysis of varying land uses and hydrogeologic and climatic settings but were primarily located in areas in close proximity to potential sources and so the study ultimately exhibited positive detections at 81% of the sampling locations with 35 out of the 65 analytes being found at least once. The most frequently detected compounds were DEET, plasticizers, fire retardants, antibiotics, and detergent metabolites. A similar study conducted by Loos et al. in 2010, surveyed the occurrence of persistent groundwater pollutants across Europe by collecting 164 individual ground-water samples from 23 European countries and analyzing them for 59 organic compounds including pharmaceuticals, antibiotics, hormones, and other anthropogenic compounds. With no strict selection criteria for the sampling sites, results were representative of a wide array of hydrogeologic settings present across Europe. Every groundwater sample that was analyzed consisted of at least one of the organic compounds being analyzed for, with some samples exhibiting detections of up to 29 compounds. Similar to Barnes et al., compounds such as DEET were found in up to 84% of the groundwater samples where pharmaceuticals including carbamazepine and sulfamethoxazole were detected in 42 and 24% of samples, respectively. With widespread occurrence of these emerging organic compounds in both Europe and North America, studies investigating the properties governing their transport in groundwater are warranted in order to further understand the pathways of such ubiquitous sewage-derived contamination.
Table 3.2. Characteristics of the pharmaceutical compounds examined in this investigation. †Information from DrugBank.ca. *Information from chemicalize.org. <LR = Less than reporting limit.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CAS No.  †</th>
<th>Solubility (mg/L)</th>
<th>Charge at pH 7.4</th>
<th>Use*</th>
<th>Previous Study</th>
<th>Number of groundwater Samples</th>
<th>Detection Frequency: Max Concentration</th>
<th>Study Scale</th>
<th>Attenuation Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>103-90-2</td>
<td>1.4 × 10⁻⁴</td>
<td>Neutral</td>
<td>Analgesic and antipyretic</td>
<td>Barnes et al. 2008</td>
<td>47</td>
<td>6.4%; 0.38 μg/L</td>
<td>USA</td>
<td>High biodegradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fram and Bellitz, 2011</td>
<td>1231</td>
<td>0.32%; 1.89 μg/L</td>
<td>California</td>
<td>Moderate sorption</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>63-05-8</td>
<td>57.8</td>
<td>Neutral</td>
<td>Hormone</td>
<td>Bartelt-Hunt et al., 2010</td>
<td>51</td>
<td>0%; ND</td>
<td>4 livestock facilities in Nebraska</td>
<td>California</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>298-46-4</td>
<td>17.7</td>
<td>Neutral</td>
<td>Antiepileptic</td>
<td>Loos et al., 2010</td>
<td>164</td>
<td>42%; 390 ng/L</td>
<td>Europe</td>
<td>High persistence</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fram and Bellitz, 2011</td>
<td>1231</td>
<td>1.5%; 0.42 μg/L</td>
<td>California</td>
<td>Low adsorption Low biodegradability</td>
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<tr>
<td>Estrone</td>
<td>53-16-7</td>
<td>30</td>
<td>Neutral</td>
<td>Hormone</td>
<td>Loos et al., 2010</td>
<td>164</td>
<td>0.6%; 4 ng/L</td>
<td>Europe</td>
<td>Moderate biodegradation</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>25812-30-0</td>
<td>1.0 × 10⁻⁴</td>
<td>Negative</td>
<td>Lipid regulator</td>
<td>Barnes et al. 2008</td>
<td>47</td>
<td>0%; ND</td>
<td>USA</td>
<td>Moderate biodegradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Carrara et al., 2008</td>
<td>NA</td>
<td>NA; 1960 ng/L</td>
<td>Septic plume</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Loos et al., 2010</td>
<td>164</td>
<td>0%; ND</td>
<td>Europe</td>
<td>Moderate biodegradation</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>15687-27-1</td>
<td>21</td>
<td>Negative</td>
<td>NSAID</td>
<td>Barnes et al. 2008</td>
<td>47</td>
<td>2.1%; 3.11 μg/L</td>
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<td>Moderate biodegradation and sorption</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Carrara et al., 2008</td>
<td>NA</td>
<td>NA; 12, 000 ng/L</td>
<td>Septic plume</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Loos et al., 2010</td>
<td>164</td>
<td>6.7%; 395 ng/L</td>
<td>Europe</td>
<td></td>
</tr>
<tr>
<td>Meprobamate</td>
<td>57-53-4</td>
<td>4.7 × 10⁻³</td>
<td>Neutral</td>
<td>Anxiolytic</td>
<td>Hass et al., 2012</td>
<td>9</td>
<td>11%; 0.05 μg/L</td>
<td>Berlin, Germany</td>
<td>Extremely high persistence</td>
</tr>
<tr>
<td>Naproxen</td>
<td>22204-53-1</td>
<td>15.9</td>
<td>Negative</td>
<td>NSAID</td>
<td>Loos et al., 2010</td>
<td>164</td>
<td>0%; ND</td>
<td>Europe</td>
<td>High biodegradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Carrara et al., 2008</td>
<td>NA</td>
<td>NA; 5580 ng/L</td>
<td>Septic plume</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>723-46-6</td>
<td>6.1 × 10⁻³</td>
<td>Neutral</td>
<td>Antibiotic</td>
<td>Barnes et al. 2008</td>
<td>47</td>
<td>23.4%; 1.1 μg/L</td>
<td>USA</td>
<td>High persistence Low adsorption Low biodegradability</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fram and Bellitz, 2011</td>
<td>1231</td>
<td>0.41%; 0.17 μg/L</td>
<td>California</td>
<td></td>
</tr>
<tr>
<td>Triclosan</td>
<td>3380-34-5</td>
<td>6</td>
<td>Neutral</td>
<td>Antimicrobial</td>
<td>Barnes et al. 2008</td>
<td>47</td>
<td>14.9%; &lt;RL</td>
<td>USA</td>
<td>High photodegradation Moderate biodegradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Loos et al., 2010</td>
<td>164</td>
<td>1.8%; 9 ng/L</td>
<td>Europe</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>738-70-5</td>
<td>4.0 × 10⁻²</td>
<td>Neutral</td>
<td>Antibiotic</td>
<td>Barnes et al. 2008</td>
<td>47</td>
<td>0%; ND</td>
<td>USA</td>
<td>Moderate sorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fram and Bellitz, 2011</td>
<td>1231</td>
<td>0.08%; 0.018 μg/L</td>
<td>California</td>
<td></td>
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</tbody>
</table>
In recent studies, several of these emerging anthropogenic compounds were shown to be exceptionally resistant to degradation and exhibited persistent and conservative transport in groundwater. Laboratory studies by Buerge et al. (2009) revealed the persistence of artificial sweeteners when incubation experiments were conducted in activated sludge. In comparison to cyclamate and saccharin which exhibited elimination efficiencies of 99 and 78%, respectively, after a 3-hour incubation in the activated sludge, acesulfame and sucralose did not exhibit any signs of degradation within 7 hours of incubation. The conservative transport of both acesulfame and sucralose was shown by Van Stempvoort et al. (2011b) when they compared the sweeteners’ concentrations to concentrations of chloride throughout a septic plume in a sand aquifer in Long Point, ON. Acesulfame concentrations closely mimicked those of chloride, with only a slight indication of acesulfame degradation at increasing depth and distance from the source and little evidence of adsorption. While sucralose initially exhibited conservative transport similar to that of chloride and acesulfame, transport to increasing depths and distances from the septic source resulted in increasing sucralose degradation. A more recent study of acesulfame in the same septic plume used tritium/helium-3 dating techniques to confirm that acesulfame had persisted at high concentrations for at least 15 years (Robertson et al., 2013). While detections of saccharin, cyclamate, and sucralose in groundwater have been reported in several scientific investigations, their concentrations and persistence have shown to be significantly lower than that exhibited by acesulfame.

Several field studies investigating pharmaceuticals in groundwater, have demonstrated the attenuation mechanisms influencing pharmaceutical transport (Table 3.2) including carbamazepine’s high persistence and low affinity for adsorption during transport. During their assessment of a high school’s septic system, Godfrey et al. (2007) found that carbamazepine concentrations in the septic tank were reduced only 1.8 to 5 times during transport through 2-m-thick sand vadose zone to the underlying sand aquifer. This relatively conservative transport may be attributable to carbamazepine’s resistance to...
biodegradation. When Drewes et al. (2003) applied tertiary and secondary treated wastewater with measurable concentrations of various pharmaceuticals to the recharge basins of 2 alluvial aquifers, despite rapid biodegradation and removal of DOC during transport through vadose zone, carbamazepine showed very high persistence and was detected in a well with an estimated groundwater age of 8 years with no significant signs of reduction when exposed to both oxic and anoxic conditions. In a study by Clara et al. (2004), a slight reduction in carbamazepine concentrations was attributed to dilution, but as this reduction was small carbamazepine appeared to be uninfluenced by adsorption or degradation while being transported from a wastewater effluent injection well to a monitoring wells located down-gradient. In the study by Loos et al. (2010), carbamazepine was deemed the most relevant pharmaceutical compound as it was found in 42% of municipal supply wells across Europe with a maximum concentration of 390 ng/L. In that same study, sulfamethoxazole was found in the next highest percentage of wells (24%) with a maximum concentration of 38 ng/L. Similarly, in the study by Barnes et al. (2008), sulfamethoxazole was the 4th most frequently detected compound out of the 65 organic wastewater contaminants that were analyzed. While the concentrations of sulfamethoxazole were found to have decreased between 15 and 1200 times from the septic tank to the underlying aquifer in the Montana study by Godfrey et al. (2007), this antibiotic exhibited resistance to degradation and adsorption in a number of hydrogeological settings and is therefore characterized by high persistence relative to many other pharmaceuticals entering groundwater. The high mobility and persistence of each of these compounds is highly attributable to their neutral charges when exposed to the pH of groundwater.

After carbamazepine and sulfamethoxazole, the antimicrobial triclosan that is commonly found in personal care products has exhibited some of the highest detection frequencies in groundwater (Barnes et al., 2008). While Lindstrom et al. (2002) showed triclosan to be highly susceptible to photodegradation, the predominant factor contributing to attenuation of triclosan during groundwater
transport was suggested to be biodegradation when Xu et al. (2009) conducted column experiments involving sterilized and unsterilized soils which yielded triclosan half-lives almost two times greater in sterilized sediments than in unsterilized sediments.

Similar to triclosan, ibuprofen has also been shown to be susceptible to biodegradation, but is subject to sorption as well. During transport through a dolomitized limestone aquifer, ibuprofen exhibited a near 1000-fold decrease in concentration from wastewater effluent to a spring located down-gradient (Einsiedl et al., 2010). Using column experiments, Xu et al. (2009) examined the leaching potential of various pharmaceuticals in both sterilized and unsterilized sediments and found that half-lives of ibuprofen were 7.2 to 11.7 days in unsterilized soils and up to 21 days in sterilized soils, ultimately suggesting that biodegradation was a key contributing factor to ibuprofen attenuation. In 2004, Oppel et al. conducted column experiments with ibuprofen and when none of the pharmaceutical was recovered, it was suggested that although the net charge of ibuprofen at the near-neutral pH of groundwater is negative, ibuprofen’s protonated carboxyl group provided it with a positive charge that ultimately enhanced its sorption capacity. Ibuprofen was detected in only 2.1% of the wells sampled during Barnes et al.’s American study (2008) but exhibited a maximum concentration of 3.11 μg/L. Similarly, Loos et al. (2010) detected ibuprofen in 6.7% of their European wells and a maximum concentration of 395 ng/L. Although ibuprofen has not exhibited the highest detection frequencies, it is regularly found in some of the greatest concentrations of all pharmaceuticals in groundwater, such as the 12,000 ng/L detection in the Long Point, ON septic plume by Carrara et al. (2008). Although ibuprofen has exhibited susceptibility to both degradation and sorption, it is still an important groundwater contaminant to investigate due to its widespread use and high concentrations found in groundwater.
Gemfibrozil and naproxen have exhibited somewhat similar occurrences in groundwater to ibuprofen with low detection frequencies but high concentrations (Barnes et al., 2008; Carrara et al., 2008; Loos et al., 2010). The column experiments by Xu et al. (2009) have suggested that naproxen is highly susceptible to biodegradation, while a study by Fang et al. (2012) that investigated the occurrence, fate, and persistence of gemfibrozil in both water and soil also suggested that gemfibrozil is susceptible to biodegradation, but to a lesser degree. In a study by Loffler et al. (2005), acetaminophen exhibited complete transformation after being in a groundwater regime for 2 days therefore suggesting that acetaminophen is also highly susceptible to rapid biodegradation. While Barnes et al. (2008) and Fram and Belitz (2011) each found acetaminophen to have a detection frequency of 6.4 and 0.32%, respectively, the concentrations were significantly lower than those of other pharmaceuticals detected in the same samples. Due to its neutral charge at the typical pH of groundwater, acetaminophen may have a slightly elevated sorption capacity relative to negatively charged pharmaceuticals such as ibuprofen, naproxen, and gemfibrozil, but its high affinity for biodegradation will be one of the largest contributors to it having lower groundwater concentrations. Trimethoprim also exhibits a neutral charge at near-neutral pH, but it too has been found in only a small percentage of wells despite the fact that it is one of the most common pharmaceuticals found in wastewater effluents (Barnes et al., 2008; Richardson, 2009; Fram and Belitz, 2011). The absence of trimethoprim in the Montana aquifer underlying a septic system with detectable concentrations of the pharmaceutical suggested its susceptibility to physical and/or biological attenuating processes during transport through the vadose zone Godfrey et al. (2007).

In contrast to these highly biodegradable pharmaceutical compounds, meprobamate has been shown to persist for several decades, but as the use of this drug has severely declined in the past few years, it is not as common in groundwater as drugs such as carbamazepine and sulfamethoxazole. In a study investigating a landfill plume in a Florida limestone aquifer, Eckel et al. (1993), demonstrated the
persistence of meprobamate when it was detected in a groundwater sample 300 m from the landfill in water suggested to be 21 years old. In Canada, however, the use of meprobamate has severely declined in the past few decades (Allen, 2012) and, while it remains on the Veterinary New Drug List in Health Canada’s Drugs and Health Products, the previous 9 manufacturers of the drug have been reduced to a single producer. Although meprobamate has shown significant persistence in groundwater, its low use by the medical community suggests it is a rare contaminant of groundwater.

In addition to pharmaceuticals, hormones and by-products of cleaning solutions are important wastewater contaminants worth investigating as many of them have shown to severely influence drinking water quality and create health risks to both humans and animals alike (Farre et al., 2008). As Loos et al. (2010) only found estrone in one of the 164 European wells they sampled and Bartelt-Hunt et al. (2010) only found estrone in low concentrations in groundwater underlying an agricultural wastewater impoundment with positive detections of both estrone and adrostenedione, these hormones are rarely detected groundwater. The low detection frequency of each of these hormones has been attributed their high affinity for sorption and degradation as evidenced by the column experiments by Xu et al. (2009).

In contrast, compounds such as perchlorate and various volatile organic compounds and even boron derived from cleaning products have shown to enter groundwater from leaking sewers and have exhibited extremely long persistence and rapid transport in groundwater regimes. As boron can comprise between 5 and 15% of some detergents in the form of sodium perborate, it is a common component of anthropogenic wastewater and was detected in both sanitary sewer and groundwater in the immediate vicinity of the sewers in a study by Wolf et al. (2004) that investigated the influence of leaky sewers on groundwater quality. Similarly, volatile organic compounds (VOCs) such as chloroform, chloromethane, tetrachloroethene (PCE), and trichloroethene (TCE) are all solvents frequently use in
cleaning products as efficient degreasers and spot removers (EPA, 2013) and are also common constituents of wastewater. Chloroform can also form during the chlorination of drinking water, wastewater, and swimming pools, therefore further indicating anthropogenic sources. Perchlorate is another groundwater contaminant that can be derived from cleaning solutions as it can be spontaneously generated in both commercial strength (15%) hypochlorite solutions and in household bleach (Brown and Gu, 2006). Additionally, perchlorate was a common component of thyrostatic drugs given to livestock in efforts to increase body weight while reducing feed costs (Batjoens et al., 1993). While perchlorate is typically attributed to Chilean fertilizers and activities involving munitions, explosives, road flares, and pyrotechnics, the sources mentioned above are expected to be the predominant contributors of perchlorate to groundwater in areas removed from ammunitions factories and agricultural sites using Chilean fertilizers. While cleaning products are expected to only introduce very small amounts of perchlorate to the environment, the fact that perchlorate is kinetically inert with little affinity for adsorption or degradation (Brown and Gu, 2006) and can persist for several decades (California Department of Toxic Control, 2005; Brown and Gu, 2006) makes this an important contaminant to explore as even at low doses (>15 μg/L) it can disrupt the production of thyroid hormones which are critical to the regulation of metabolism in adults and the growth and development of the brain and central nervous systems in fetuses, infants, and young children even at low concentrations (Urbansky, 2002; EPA, 2008).

3.0.1 Hypotheses and goals
Excluding perchlorate, the low concentrations of most of these emerging contaminants in groundwater are not expected to impose health risks to individuals exploiting the contaminated aquifers as sources of drinking water, but investigation of the fate and transport of these compounds is still important as they may be ideal tracers of human and animal wastewater streams and other potentially more harmful sewage-derived contaminants such as enteric viruses. As mentioned above, several lab and field scale
studies have investigated the presence of these emerging contaminants in groundwater, but very few have examined the presence, transport, or persistence of these compounds in fractured bedrock aquifers. As Chapter 1 clearly showed the vulnerability of fractured bedrock aquifers to sewage-derived virus contamination, it is hypothesized that many of the mobile and persistent emerging contaminants such as acesulfame, carbamazepine, ibuprofen, and perchlorate will also be detectable in these aquifers. By collecting and analyzing groundwater samples from various types of wells completed at various depths in the aquifers of southern Wellington County, ON, the current investigation will illuminate the vulnerability of these aquifers to pharmaceuticals, artificial sweeteners, and several other wastewater contaminants in order to better understand the influence of anthropogenic waste streams on fractured bedrock aquifers.

3.1 SITE DESCRIPTION

The sampling wells and locations for this study were discussed in depth in Chapter 1 and will therefore only be summarized here and in Table 3.4.

Each of the sampling wells was completed in the fractured dolostone aquifers of southern Wellington County, Ontario, Canada and several small surrounding communities (Figure 3.2). These aquifers are part of a belt of Silurian-aged dolostone that spans from Lake Huron to the Niagara River. Low effective fracture porosities between $5 \times 10^{-4}$ and $9.7 \times 10^{-4}$ and average fracture apertures between 69 and 125 μm result in high transmissivities on the order of $10^{-4} \text{ m}^2/\text{s}$ therefore promoting very rapid groundwater transport in both the Guelph and Gasport formations (Belan 2010; Munn 2012). A variety of glacial deposits ranging from a silty clay till to gravel kame deposits are present in varying thicknesses across the entire county. Due to the high groundwater velocity within the fractured bedrock and the presence of permeable overburden of varying thicknesses, the aquifers of Wellington County have been found to be at an exceptionally high risk to contamination (Lake Erie Region Source Protection Committee 2012).
After an extensive selection process, 22 wells throughout southern Wellington County were chosen based on well type, completion depth, proximity to potential sewage sources, surrounding land use, overburden thickness and accessibility (Chapter 1). Wells were selected in close proximity to sanitary sewers and septic systems as well as in areas believed to be removed from these potential sources. The final selections provided an array of wells with varying characteristics and several different types of surrounding environments (Table 3.3).

A total of 11 private wells located in Arkell, Aberfoyle, and Eden Mills were selected for sampling due to their proximity to septic and agricultural sources, in addition to their having varying overburden thicknesses. In contrast, 3 monitoring wells located along the Speed River in downtown Guelph were selected for sampling as they would provide insight as to the susceptibility of these smaller wells in urban settings in close proximity to sanitary sewers.
Table 3.3. Completion details and average field parameters for each sampling well. *Overburden Type abbreviations: G, Gravel; D, Diamicton; S, Sand. †Surrounding Land Use abbreviations: A, Agricultural; R, Residential; I, Industrial; B, Business; P, Public Park; U, University campus.

<table>
<thead>
<tr>
<th>Well Completion Details</th>
<th>Aberfoyle</th>
<th>ARKELL</th>
<th>Eden Mills</th>
<th>Centre Wellington</th>
<th>Guelph</th>
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<tbody>
<tr>
<td>Total Depth (m)</td>
<td>PW1</td>
<td>PW2</td>
<td>PW3</td>
<td>PW4</td>
<td>PW5</td>
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<tr>
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<td>29</td>
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<td>PW7</td>
<td>PW8</td>
<td>PW9</td>
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<td>MSW7</td>
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<td>MSW7</td>
<td>MSW8</td>
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<td>0.31</td>
<td>0.38</td>
<td>0.42</td>
<td>0.89</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>PW11</td>
<td>MSW1</td>
<td>MSW2</td>
<td>MSW3</td>
<td>MSW4</td>
</tr>
<tr>
<td></td>
<td>10.14</td>
<td>9.41</td>
<td>11.19</td>
<td>12.51</td>
<td>11.77</td>
</tr>
<tr>
<td>Cond (µS/cm)</td>
<td>PW5</td>
<td>MSW5</td>
<td>MSW6</td>
<td>MSW7</td>
<td>MSW8</td>
</tr>
<tr>
<td></td>
<td>554</td>
<td>422</td>
<td>571</td>
<td>630</td>
<td>1307</td>
</tr>
</tbody>
</table>
Eight municipal supply wells located throughout southern Wellington County were sampled in order to investigate the vulnerability of wells with greater completion depths and pumping capacities than typical private and monitoring wells. While several of the municipal supply wells were located in the town centres, some were located near the city limits and were therefore more removed from urban life and sanitary sewers.

This final group of wells would allow for the investigation of fractured bedrock aquifer vulnerability to emerging contaminants in a wide variety of settings and conditions.

3.2 METHODS

3.2.1 Sample collection
Samples from private wells were collected directly from external faucets that by-passed any water treatment or filtration. Similarly, samples from municipal supply wells were collected from raw water faucets connected directly to the pumps. Samples from monitoring wells were collected using a Grundfos Redi-Flo2 electrical submersible pump connected to a Honda EU2000KC2 generator. Sample collection strictly adhered to the sampling protocol outlined in Appendix III. All sampling hoses were connected to a flow-through cell with a YSI 556 multiprobe system. Wells were purged until the pH, temperature, and conductivity were constant. Each well was sampled for general chemistry, isotopes, VOCs, and pharmaceuticals once, while samples for artificial sweeteners were collected twice, once in July and again in November 2012.

All general chemistry and isotope samples were collected into 500 mL HDPE bottles. Water samples for major ions were field filtered using a 0.45 μm filter and preserved using nitric acid. Samples were stored at 4°C. Within 2 days of sample collection, general chemistry samples were transported to the Maxxam Analytics laboratory located in London, ON. Isotope samples were shipped directly to the University of Waterloo’s Environmental Isotope Laboratory in Waterloo, ON.
Water samples analyzed for pharmaceuticals were collected in 500 mL amber Boston round bottles that had been pre-washed with acetone followed by hexane. Samples were transported on ice and stored at 4°C for no longer than 2 weeks.

Water being analyzed for artificial sweeteners was field filtered using an in-line 0.45 µm filter. Samples were then collected into 20 mL plastic scintillation vials allowing a head space of approximately 10%. Samples were immediately frozen and shipped to Dale van Stempvoort’s lab at the Canada Centre for Inland Waters in Burlington, Ontario.

Water samples for analysis of VOCs were collected into 40mL volatile organic analysis bottles of known masses. Bottles were filled to the point where no air bubbles remained when the closed bottle was inverted. Once the cap was on, Teflon tape was wrapped around the cap to ensure leaking did not occur. Samples were transported on ice and stored at 4°C.

3.2.2 Sample analysis
All general chemistry samples were analyzed by Maxxam Analytic’s laboratory located in London, ON.

All analytes were analyzed using methods approved by Environment Canada and are listed in Table 3.4.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Analysis Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metals</td>
<td>ICP-MS</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Colourimetry</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Cadmium Reduction Flow Injection</td>
</tr>
<tr>
<td>Sulphate and Chloride</td>
<td>Automated Colourimetry</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Titration</td>
</tr>
</tbody>
</table>

Analysis for ¹⁸O, ²H and ³H were conducted at the University of Waterloo’s Environmental Isotopes Laboratory. ¹⁸O was analyzed via automatic CO₂ equilibration-continuous flow-isotope ratio mass spectrometry while ³H was analyzed using chromium reduction of H₂O and continuous flow-isotope ratio
mass spectrometry (Drimmie et al., 2001a; Drimmie and Heemskerk, 2001b). $^3$H was first enriched 15 times by electrolysis before being quantified using a liquid scintillation counter (Taylor, 1977).

Each 500 ml sample collected for pharmaceutical analysis was separated into 3 aliquots before being extracted. Solid phase extraction of the pharmaceutical samples was conducted at the University of Guelph by following the standard operating procedure in Appendix III. The extracts were shipped on ice to Dr. Chris Metcalfe’s lab at Trent University in Peterborough, ON where the final analysis for pharmaceuticals emulated the methods described by Zhao and Metcalfe (2008; Appendix III). Samples were analyzed for ibuprofen, gemfibrozil, naproxen, and estrone in negative polarity mode by liquid chromatography and tandem mass spectrometry (LC-MS/MS) with a turbospray ionization source using a Q-Trap 5500 instrument purchased from AB Sciex (Concord, ON, Canada) and an Agilent 1100 series HPLC, including degasser, binary pump and autosampler (Agilent Technologies Canada, Mississauga, ON, Canada). Similarly, acetaminophen, carbamazepine, meprobamate, sulfamethoxazole, trimethoprim, and androstenedione were analyzed using LC-MS/MS but in positive polarity mode on an API 3000 also purchased from AB Sciex (Concord, ON, Canada).

Artificial sweeteners were analyzed by a Dionex 2500 ion chromatography system coupled with an AB Sciex QTRAP 5500 triple-quadrupole tandem mass spectrometrometer operated in negative electrospray ionization (ESI) mode (Van Stempvoort, 2011).

VOCs were analyzed at the University of Guelph using methods based on EPA method 8260B for analyzing VOCs by capillary column gas chromatography and mass spectrometry (GC/MS). This method requires that 5 mL of water sample containing VOCs is introduced into a purge-and-trap system (P&T) (Teledyne Tekmar, Model Atomx), separated using a gas chromatographic (GC) capillary column (Agilent, Model 7890A) and detected by a mass spectrometric detector (MSD) (Agilent, Model 5975C). The target VOCs are identified in the sample by analyzing the standards under the same conditions and comparing
the mass spectra and GC retention times. The target compounds were quantitated using the internal standard procedure. Certified standard solutions were obtained from Supelco (Bellefonte, Pennsylvania, USA). The method detection limits range from 0.08 to 0.20 µg/L.

3.2.3 QA/QC
The quality of general chemistry data can be attributed to the continuous and strict adherence to the standard operating procedure developed for this project based on Sections 9060A and 9060B of the 18th edition of *Standard Methods for the Examination of Water and Wastewater* (Appendix A). Sampling equipment including the YSI 556 multiprobe system was reliable and well maintained according to manufacturer guidelines by the rental company, Pine Environmental. Sample analysis was completed by the Maxxam Analytics laboratory in London, ON. This lab is accredited by MDDEP, SCC and/or CALA for specified tests, certified by PTI or licensed by the MOE. All shipments were tracked and monitored via chain of custody forms.

Analysis results were validated by the use of laboratory method blanks, blank spikes, matrix spike, duplicates, certified reference material, and surrogate recoveries. Field blind duplicates as well as equipment blanks were also included in the QA/QC process of general chemistry analyses. An ion balance was also calculated to ensure analytical quality.

Quality control of $^{18}$O and $^2$H samples was maintained by placing lab water standards (water kept in stock bottles and calibrated to the international reference materials VSMOW and VSLAP from the IAEA) at the beginning, middle and end of each sample batch. Duplicates were run approximately every tenth sample. Results were recorded based on standard corrections performed based on the calculated laboratory/VSMOW/VSLAP calibration. Quality of tritium analyses was controlled via duplicate analyses for every 10 samples analyzed. Isotope data was validated statistically by calculating percent difference of duplicates and ensuring no $^{18}$O or $^3$H duplicate exceeded a percent difference of 5% and no $^3$H
supplicate exceeded a percent difference of 10%. Sample shipment was recorded and tracked via chain of custody documentation.

The quality of the pharmaceutical results can be attributed to triplicate analysis of each sample as well as field duplicates and method blanks for each batch of samples. Triplicate analyses were averaged and reported alongside standard deviations. Calculation of percent differences was conducted for each field duplicate sample. Sample shipment was recorded and tracked via chain of custody documentation. The detection and quantitation limits for both pharmaceutical compounds and artificial sweeteners are presented in Table 3.5.

Table 3.5. Minimum detection limits and practical quantitation limits for pharmaceutical compounds, artificial sweeteners, and perchlorate.

<table>
<thead>
<tr>
<th>Analyte Type</th>
<th>Analyte</th>
<th>Minimum Detection Limit (MDL)</th>
<th>MDL unit</th>
<th>Practical Quantitation Limit (PQL)</th>
<th>PQL unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical</td>
<td>Acetaminophen</td>
<td>4</td>
<td>ng/L</td>
<td>10</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Androstenedione</td>
<td>0.3</td>
<td>ng/L</td>
<td>1</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Carbamazepine</td>
<td>0.3</td>
<td>ng/L</td>
<td>1</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Estrone</td>
<td>0.2</td>
<td>ng/L</td>
<td>0.7</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>2</td>
<td>ng/L</td>
<td>5</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>0.6</td>
<td>ng/L</td>
<td>2</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Meprobamate</td>
<td>2</td>
<td>ng/L</td>
<td>5</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Naproxen</td>
<td>2</td>
<td>ng/L</td>
<td>7</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxazole</td>
<td>0.3</td>
<td>ng/L</td>
<td>1</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Triclosan</td>
<td>3</td>
<td>ng/L</td>
<td>10</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
<td>0.6</td>
<td>ng/L</td>
<td>2</td>
<td>ng/L</td>
</tr>
<tr>
<td>Artificial</td>
<td>Acesulfame</td>
<td>8</td>
<td>ng/L</td>
<td>20</td>
<td>ng/L</td>
</tr>
<tr>
<td>Sweeteners</td>
<td>Cyclamte</td>
<td>3</td>
<td>ng/L</td>
<td>10</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Saccharin</td>
<td>21</td>
<td>ng/L</td>
<td>60</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Sucralose</td>
<td>5</td>
<td>ng/L</td>
<td>15</td>
<td>ng/L</td>
</tr>
<tr>
<td></td>
<td>Perchlorate</td>
<td>2</td>
<td>ng/L</td>
<td>5</td>
<td>ng/L</td>
</tr>
</tbody>
</table>

Sampling for artificial sweeteners included the collection of field duplicates and method blanks for every 10 samples collected. Sample shipment was recorded and tracked via chain of custody documentation. A standard curve was created for each analyte with a minimum of 5 points covering
the range from the minimum detection limit for each analyte up to 2 µg/L for acesulfame, saccharin and cyclamate and up to 50 µg/L for sucralose, matrix effects and instrument variations were corrected by internal standard response. All standard curves were linear regressions weighted 1/x² and the coefficients of determination (R²) of the regression equations were greater than 0.995. The area of the most sensitive MRM transition for each analyte was used for concentration calculations and the second transition was used to confirm compound identification. Samples with concentrations greater than the highest standard were diluted with Milli-Q water to be within the calibration curve range and re-run. In order for a positive identification the retention time match of the native and labeled analyte had to be within 2% and the relative abundance of the two MRM transitions had to be within a margin of 20%. With every set of 10 samples one continuing calibration check standard was run, as well as a duplicate injection of one of the samples and a blank. All check standards and duplicate injections were within 20% for levels greater than the practical quantitation level (pql) and within 50% for levels less than pql but greater than the minimum detection limit (MDL). The PQL and MDL VOC quality is assured by the collection of triplicate samples alongside trip, storage, and method blanks.

3.2.4 Data Analysis

General Chemistry Data Analysis

General chemistry data was imported into AquaChem software (Schlumberger, 2011) and used to create Stiff plots for each sampling well (Figure 3.3). Ratios of B/Cl were imported into ArcMap to illustrate their spatial distributions (Figure 3.4).

Isotope Data Analysis

To determine recharge conditions, 18O was plotted versus 2H with a local meteoric water line generated from data collected in Ottawa and reported in the Global Network of Isotopes in Precipitation
Historical tritium concentrations measured in Ottawa, ON were plotted with predictions of tritium concentrations in 2013 that were estimated using a simple decay formula and a half-life of 12.33 years (Figure 3.7). Measured ³H concentrations from each of the sampling wells were compared to this plot in order to estimate groundwater age. As the artificial sweetener acesulfame was made available for consumption in 1988, we plotted ³H data from our sampling wells versus well screen midpoint depth and concentrations of the acesulfame in an attempt to further deduce the age of the water in Wellington County’s aquifers (Figure 3.8).

**Pharmaceutical, Artificial Sweetener, and VOC Data Analysis**

All analytical results of pharmaceuticals, artificial sweeteners, perchlorate, and VOCs were summarized in Table 3.5 while concentrations of pharmaceuticals and artificial sweeteners were plotted in ArcMap to determine the special variability of each compound (Figure 3.6). Concentrations of pharmaceuticals and artificial sweeteners were plotted versus one another to determine if a relationship was present between their relative concentrations. Concentrations of each analyte were also plotted against overburden thickness and depth of the well screen midpoint to determine if geology and/or well completion influence the occurrence of these compounds. Detections on a per well basis can be found in Appendix IV Table 1.

### 3.3 RESULTS

#### 3.3.1 Water chemistry

Stiff plots illustrating the concentrations of major ions in each of the sampling wells reveal that almost all of the private wells exhibit similar water chemistries with Ca and HCO₃ being the dominant ions in solution (Figure 3.3). Water collected from private wells in Arkell contained the highest concentrations of nitrate with an average of 4.5 mg/L. In addition, PW9 exhibited elevated concentrations of Na, Cl, Ca,
and Mg. In Aberfoyle, both PW4 and PW5 showed elevated concentrations of nitrate with PW5 also containing higher concentrations of Na and Cl than other wells in the area. MW1 and MW2, the shallow monitoring wells along the Speed River, showed very similar groundwater chemistry to the previously mentioned private wells. MW3, however, exhibited drastically higher concentrations of all ions.

Figure 3.3. Stiff plots from each of the sampling wells indicating spatial variations in water chemistry. All groundwater samples were predominantly comprised of Ca and HCO₃, which is consistent with groundwater coming from a carbonate aquifer. Some wells exhibited elevated levels of Mg, Cl, and Na which were mostly attributable to the application of road salt. Large supply wells showed elevated SO₄ which was attributed to the influence of the evaporate deposits in the Salina formation located to the west of the sampling sites.
All of the municipal supply wells in southern Wellington County that were sampled in this investigation showed distinctly different water chemistry from the private wells. In Guelph, MSW7 and MSW8 on the University of Guelph campus contained slightly elevated Cl and Na as well as higher concentrations of Mg and SO₄ relative to other wells in southern Wellington County. In Centre Wellington, municipal supply wells were characterized by slightly elevated concentrations of Ca, low concentrations of Cl, and significantly high levels of SO₄.

While both of the large supply wells located on the University of Guelph campus exhibited relatively low ratios of B/Cl, only one large supply well in Centre Wellington had detectable boron (Figure 3.4). MSW5, located in Fergus, exhibited the highest B/Cl ratio of the entire investigation. The monitoring wells along the Speed River also exhibited elevated B/Cl ratios as did PW6 and PW7.

Figure 3.4. Spatial distributions of B/Cl ratio suggesting areas under the influence of human wastewater streams. Colours in the adjacent table correspond with the coloring coding of symbols in the figure indicating samples with the highest B/Cl ratios in red and non-detects in white.

<table>
<thead>
<tr>
<th>Well</th>
<th>B/Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSW1</td>
<td>ND</td>
</tr>
<tr>
<td>MSW2</td>
<td>ND</td>
</tr>
<tr>
<td>MSW3</td>
<td>ND</td>
</tr>
<tr>
<td>MSW4</td>
<td>ND</td>
</tr>
<tr>
<td>MSW6</td>
<td>ND</td>
</tr>
<tr>
<td>PW5</td>
<td>ND</td>
</tr>
<tr>
<td>PW5</td>
<td>0.15</td>
</tr>
<tr>
<td>PW11</td>
<td>0.23</td>
</tr>
<tr>
<td>PW4</td>
<td>0.26</td>
</tr>
<tr>
<td>PW9</td>
<td>0.31</td>
</tr>
<tr>
<td>PW8</td>
<td>0.33</td>
</tr>
<tr>
<td>MSW7</td>
<td>0.39</td>
</tr>
<tr>
<td>PW11</td>
<td>0.41</td>
</tr>
<tr>
<td>PW10</td>
<td>0.43</td>
</tr>
<tr>
<td>MSW8</td>
<td>0.44</td>
</tr>
<tr>
<td>PW7</td>
<td>0.54</td>
</tr>
<tr>
<td>PW3</td>
<td>0.60</td>
</tr>
<tr>
<td>PW2</td>
<td>0.65</td>
</tr>
<tr>
<td>PW4</td>
<td>0.95</td>
</tr>
<tr>
<td>PW6</td>
<td>1.13</td>
</tr>
<tr>
<td>MSW5</td>
<td>3.13</td>
</tr>
</tbody>
</table>

On average, the pH was between 6.95 and 7.5 except for MSW5 and MSW6 in Centre Wellington and PW10 and PW11 in Eden Mills where the pH was consistently above 7.5 (Table 3.3). While most wells exhibited dissolved oxygen concentration below 1 mg/L, PW6, PW7, PW8, and PW9 located in Arkell had DO concentrations between 2.01 and 6.89 mg/L. These wells were also accompanied by the greatest oxidation-reduction potential (ORP) values while their salinity, temperature, and conductivity all remained within the same range as the rest of the sampling wells.

The single surface water sample taken during this study from the Speed River showed very similar chemistry to the private wells, with Ca and HCO₃ being the dominant ions (Figure 3.3). Isotope analysis of the Speed River sample plotted below the local meteoric water line (LMWL) for ²H and ¹⁸O (Figure 3.5). Similarly, samples from PW7 and PW1 showed isotopic enrichment as they too plotted below the LMWL. Groundwater taken from LSW8, PW6, PW4, PW5, and PW11 plotted directly on the LMWL. All of the other groundwater samples collected in this study plotted above and to the left of the LMWL for ²H and ¹⁸O. Samples from the municipal supply wells in Centre Wellington and LSW7 on the University of Guelph campus all plotted the farthest from the LMWL, therefore suggesting the greatest amount of isotopic depletion.

Tritium analysis revealed a range of concentrations in the groundwater of southern Wellington County (Figure 3.8). Deeper wells, including all of the municipal supply wells, as well as MW3 along the Speed River, exhibited the lowest concentrations of both tritium and the artificial sweetener acesulfame in comparison to the concentrations found in the private wells and Speed River. Deeper wells tended to exhibit tritium activities between 1 and 10 TU while the shallower private wells had activities between 10 and 25 TU.
3.3.2 Artificial sweeteners, perchlorate, and pharmaceuticals

Results from the analysis for artificial sweeteners, perchlorate, and pharmaceuticals are all summarized in Table 3.6 and Figure 3.8. After several sampling events, the current study revealed that the artificial sweeteners acesulfame and saccharin can be found in quantifiable concentrations in various well types across southern Wellington County. While only one large supply well exhibited detectable concentrations of acesulfame, this artificial sweetener was found in 10 out of 11 (91%) private wells. All 3 of the monitoring wells exhibited acesulfame. The concentrations of acesulfame reached levels up to 3 orders of magnitude above the detection limit. The artificial sweetener saccharin was detected in one private well and one large supply well. Sucralose and cyclamate were not detected in any wells.
Table 3.6. Summary of analytical results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Analyte</th>
<th>Number of Wells with Detections</th>
<th>Final Detection Frequency, (%)</th>
<th>Maximum Concentration units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Private Wells</td>
<td>Large Supply Wells</td>
<td>Monitoring Wells</td>
</tr>
<tr>
<td>Artificial Sweeteners</td>
<td>Acesulfame</td>
<td>14</td>
<td>10/11, (91)</td>
<td>1/8, (13)</td>
</tr>
<tr>
<td></td>
<td>Sucralose</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Saccharin</td>
<td>2</td>
<td>1/11, (9)</td>
<td>1/8, (13)</td>
</tr>
<tr>
<td></td>
<td>Cyclamate</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>Ibuprofen</td>
<td>10</td>
<td>6/11, (55)</td>
<td>4/8, (50)</td>
</tr>
<tr>
<td></td>
<td>Triclosan</td>
<td>3</td>
<td>3/11, (27)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Carbamazepine</td>
<td>3</td>
<td>3/11, (27)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxazole</td>
<td>3</td>
<td>2/11, (18)</td>
<td>1/8, (13)</td>
</tr>
<tr>
<td></td>
<td>Naproxen</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Meprobamate</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Acetaminophen</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Androstenedione</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>Estrogen</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Vinyl chloride</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1,1-DCE</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>c-DCE</td>
<td>3</td>
<td>-</td>
<td>1/8, (13)</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>14</td>
<td>6/11, (55)</td>
<td>5/8, (63)</td>
</tr>
<tr>
<td></td>
<td>1,1,1-TCA</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>3</td>
<td>1/11, (9)</td>
<td>1/8, (13)</td>
</tr>
<tr>
<td></td>
<td>Chloromethane</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>t-DCE</td>
<td>2</td>
<td>-</td>
<td>1/8, (13)</td>
</tr>
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<td></td>
<td>1,2-DCA</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PCE</td>
<td>14</td>
<td>6/11, (55)</td>
<td>6/8, (75)</td>
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<tr>
<td>Volatile Organic Compounds</td>
<td>1,1,-TCA</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1,2-Dichloropropane</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Carbon tetrachloride</td>
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Results for all sewage-derived contaminants other than VOCs detected in the current investigation. Acesulfame and perchlorate were the most prevalent contaminant throughout the entire aquifer with concentrations several orders of magnitude above their detection limits.
Perchlorate was detected in 19 out of all 22 wells sampled in this investigation. All of the monitoring wells exhibited perchlorate as did 10 out of 11 private wells and 6 out of 8 large supply wells.

The only pharmaceutical compounds detected in this investigation were ibuprofen, triclosan, carbamazepine, and sulfamethoxazole. While ibuprofen and triclosan were found in more wells than carbamazepine and sulfamethoxazole, none of the concentrations were above their respective practical quantitation limits (PQL). The results for ibuprofen and triclosan can therefore only be reported as present or absent. All detections of carbamazepine and sulfamethoxazole, however, were found in concentrations above the PQL, but these concentrations were still several orders of magnitude less than concentrations of acesulfame. Of all the pharmaceuticals, carbamazepine exhibited the greatest concentration at 23.05 ng/L in PW9.

Attempts to correlate acesulfame concentrations to pharmaceutical concentrations were unsuccessful due to few pharmaceutical detections above their respective PQLs. This investigation therefore cannot report on the statistical relationship between the concentrations of artificial sweeteners and pharmaceuticals.

Comparing acesulfame and perchlorate concentrations to both boron and a B/Cl ratio did not yield any significant correlations. Relationships between pharmaceutical and artificial sweetener occurrences were also independent of well construction and depth as well as overburden thickness and type.

### 3.4 DISCUSSION

As summarized in Table 3.6 and Figure 3.6 the fractured Silurian dolostone aquifer in southern Wellington County, ON is at risk of contamination with sewage-derived contaminants. Several artificial sweeteners, pharmaceuticals, and other wastewater contaminants were detected in private, large supply, and monitoring wells of various constructions and ages. Out of the 22 wells sampled, only one
large supply well in Fergus, ON did not exhibit any of the analytes included in this investigation. As most of these compounds are not naturally occurring, their presence in groundwater is irrefutable evidence that anthropogenic waste streams are influencing the local aquifers. While most of these contaminants are not a health risk to humans at such low concentrations, their detection may provide insight as to the aquifer’s vulnerability to more threatening contaminants such as viruses and pathogens. It is therefore important that we acknowledge the presence of each of these analytes in these fractured bedrock aquifers and attempt to discern its significance.

3.4.1 Hydrochemistry

While detection of the artificial sweeteners and pharmaceutical compounds detected in this investigation is interesting, it is important to understand the basic chemistry of the aquifers in order to appreciate the occurrence of each of the sewage-derived contaminants. All of the wells sampled in this investigation exhibited large contributions of Ca and HCO₃ in their ionic compositions. Both of these ions are consistent with carbonate rock aquifers and are derived from the dissolution of the calcite and dolomite comprising the surrounding rock matrix. Some wells also exhibited elevated concentrations of Na, Cl, and Mg. While the Mg can also be attributed to dolomite dissolution, it, as well as Na and Cl, can be found in high concentrations where road salt applications influence an aquifer. The wells that showed elevated Na included PW4, PW5, PW8, PW9, MSW4, MSW7, MSW8, MW2, and MW3. Excluding PW8, each of these wells is located in a high traffic area relative to other wells in the area and would therefore be more likely to experience elevated road salt applications in the winter months. The elevated concentrations found in PW8 may be attributed to the influence of a septic plume impacted by water softened with NaCl.

While most of the wells exhibited Na:Cl ratios near 1, PW9 had a ratio of 0.5 suggesting an additional source of Cl. As this well also showed elevated concentrations of K, the high Cl content can be
attributed to the agricultural use of KCl found in many commercially available fertilizers. As PW9 also exhibited some of the highest concentrations of nitrate, an agricultural influence on this well is almost certain.

Sulphate was another ion occurring in high concentrations in some of the wells. All of the large supply wells in both Centre Wellington and the University of Guelph campus as well as MW3 exhibited elevated sulphate levels. The common trend for these wells is that they are all constructed with long open intervals extending deep into the Gasport Formation. While other wells included in this study are open to the Gasport Formation, the wells exhibiting elevated sulphate concentrations are located farther west in southern Wellington County and are in much closer proximity to the Salina Formation of the Upper Silurian dolostone belt crossing southern Ontario (Figure 3.2). The Salina Formation is comprised of evaporitic carbonates and shales with interbeds of anhydrite and gypsum up to 2 m thick as well as beds 30 to 60 m thick of halite (Barnett, 1992). Due to the proximity of the municipal supply wells to the Upper Silurian dolostone belt, it is expected that the gypsum and anhydrite of the Salina Formation are large contributing factors to the elevated sulphate concentrations in each of these wells.

The final ion of interest in this investigation is boron. As mentioned in the introduction, boron has been used as an indicator of anthropogenic waste streams due to its ubiquitous presence in industrial and domestic detergents. More specifically, high B/Cl ratios have been used to distinguish anthropogenic boron from naturally occurring boron accumulations. In this investigation, boron concentrations ranged from non-detectable concentrations to concentrations over 120 μg/L. While the elevated B/Cl ratios in the monitoring wells and PW6 and PW7 can be attributed to anthropogenic waste streams due to the presence of the other analytes included in this investigation, the exceptionally high ratio found in MSW5 does not fit this trend. MSW5 was the only large supply well of this entire investigation not to exhibit any of the analytes attributed to sewage effluents. This finding would lead one to believe that MSW5 is
the least susceptible well to sewage-derived contaminants. The presence of such high boron concentrations, however, would suggest otherwise.

While the dominant source of anthropogenic boron is commercial and industrial detergents, boron’s presence has also been attributed to use in several industries including wire drawing (Vengosh et al., 1994). Wire drawing is a process used to reduce the cross-section of a wire. In Fergus, directly adjacent to MSW5, is a manufacturing plant that produces wire and electrical cables. While boron is the sole sewage-derived analyte detected in MSW5, it is suspected that its source may be the manufacturing of wires rather than a sewage source. As this manufacturing plant began productions in 1965, this boron data can be used to further constrain estimated groundwater ages as will be discussed below.

3.4.2 Isotopic signatures and groundwater age

Most of the groundwater samples collected during this study exhibited $^{18}$O and $^2$H concentrations above and to the left of the local meteoric water line (LMWL) suggesting these waters were recharged at a time when the isotopic ratios of precipitation were slightly different from modern ratios (Figure 3.5). As isotopes condense preferentially with mass, clouds moving inland will have heavier isotopes precipitate out much sooner within warm climates leaving only the lighter isotopes to be precipitated in colder climates. This means that higher temperature climates receive precipitation enriched in $^{18}$O and $^2$H while cooler climates have more depleted isotopic ratios. This could indicate that groundwater with depleted isotopic ratios was recharged during a time of colder temperatures relative to current climatic trends and would therefore suggest that the water of depleted isotopic ratios is older than water with more enriched ratios. Samples from the municipal supply wells in Centre Wellington exhibited some of the greatest amount of isotopic depletion suggesting the smallest influence of modern precipitation and potentially some of the oldest water of this entire investigation. Samples such as those from MSW8,
PW4, PW5, PW6, and PW11 that lie directly on the LMWL, however, are more consistent with modern precipitation and were probably recharged more recently.

The single surface water sample collected from the Speed River exhibited an isotopic ratio that plotted below the LMWL suggesting isotopic enrichment. This tends to happen when water is exposed to evaporative processes and is therefore expected for surface water bodies. The proximity of the Speed River sample to the LMWL is consistent with the fact that this is a gaining stream and so should therefore exhibit similar water chemistry to the local groundwater in addition to limited isotopic enrichment. The Stiff plots of the Speed River sample, as well as a sample from MW1, exhibit nearly identical ionic compositions. As their isotopic ratios also both plot below the LMWL, it is clear these waters were both recently recharged with relatively young and slightly enriched water. The isotopic ratio of PW7 also plotted below the LMWL suggesting that this well too was recharged more recently than some of the other wells included in this study.

In the past, tritium analyses have been used to elaborate on the approximate groundwater ages suggested by the $^{18}$O and $^2$H data but as modern atmospheric tritium levels seem to be reaching a plateau (Figure 3.7) and levels from the nuclear bomb testing era have decayed to around the same activity as this plateau, determining groundwater age from tritium data is becoming increasingly difficult. Plotting tritium concentrations versus depth did show a general trend with deeper wells showing lower tritium activities than shallower wells, but the greatest indicator of groundwater age came when tritium activities, well screen midpoints and acesulfame concentrations were plotted simultaneously (Figure 3.8).
Figure 3.7. Measured tritium concentrations in precipitation from Ottawa, ON and predicted decay concentrations of tritium between 1953 and 2007.
Figure 3.8. Tritium and acesulfame concentrations versus well screen midpoint depth. Suggests acesulfame found only in groundwater influenced by modern water.

In order to estimate the approximate groundwater ages of samples collected in this investigation, 3 main concepts were considered.

1. As Figure 3.7 shows that modern precipitation in southern Ontario exhibits tritium concentrations between 10 and 30 TU, it is expected that more recently recharged groundwater will exhibit tritium concentrations within this range.

2. As acesulfame was not approved for use in food and beverages until 1988 any water containing this artificial sweetener must be under the influence of water recharged less than 25 years ago.

3. As groundwater is generally older with increasing depth, one can expect that shallower wells will contain much younger water than deeper wells.

With all of these concepts considered, one can expect that shallow groundwater with tritium concentrations between 10 and 30 TU and measurable concentrations of acesulfame will have been recharged within the last 25 years.
When plotting tritium and acesulfame concentrations versus the depth of the well screen’s midpoint, it becomes apparent that all of the wells exhibiting tritium concentrations greater than 10 TU and with a well screen midpoint shallower than 60 m are under the influence of relatively modern water (Figure 3.8). While plotting acesulfame concentrations alone versus well screen mid-points would suggest that perhaps acesulfame is being attenuated and therefore restricted to the upper few meters of an aquifer, decreasing tritium activities would suggest that deeper water is older and therefore may have been recharged at a time when acesulfame was not available. Analysing acesulfame concentrations with depth may therefore be a new way of suggesting relative groundwater ages.

Although groundwater samples collected from greater depths did exhibit measurable concentrations of acesulfame, the low sweetener concentrations occurring with low tritium activities would suggest that this groundwater from most of the large supply wells and MW3 may be a blend of modern (<25 years) and submodern (<60 years) water. The deepest groundwater samples from MSW3 and MSW5 were characterized by detectable tritium concentrations below 1 TU associated with undetectable acesulfame. The absence of acesulfame is not enough evidence to suggest that these samples are older than 25 years, but the low tritium activities in addition to the great depths from which the water is coming would support this idea. Furthermore, MSW5 had the highest B/Cl ratio of the entire study that may be attributable to wire manufacturing plant across the street. As this plant was constructed in 1965, the boron in the adjacent well must not have been recharged any earlier than 1965, ultimately constraining the time of recharge of groundwater from these older wells to the mid to late 1960s.

In the California investigation of pharmaceutical occurrence in California, pharmaceutical concentrations were found to correlate with younger water exhibiting tritium activities less than 0.2 TU (Fram and Belitz, 2011). As all of the groundwater samples collected in the current investigation expressed tritium activities greater than 0.2 TU, pharmaceutical detections in these samples is expected.
3.4.3 Trends in Sewage-Derived Contamination of Bedrock Wells

Despite the current investigation analysing for fewer sewage-derived contaminants than the US reconnaissance study by Barnes et al. (2008), wastewater contaminants were detected in 95% of the wells sampled in southern Wellington County in comparison to the 81% of wells exhibiting these contaminants in the US. As the well selection process of this investigation was less biased towards wells in close proximity to potential sources, a lower detection rate was expected. But as the US study sampled wells in several types of hydrogeological settings, the high susceptibility of fractured bedrock aquifers in comparison to porous media aquifers is suspected of being responsible for the higher detection rates in the current investigation.

In comparison to Loos et al.’s European study, the wells in southern Wellington County were exceptionally more susceptible to contamination with pharmaceuticals as 11 out of 22 wells, or 50% of wells sampled in this study exhibited pharmaceuticals and only 2.3% of wells had detections of pharmaceuticals in Europe. On average, the wells in southern Wellington County exhibited 4 sewage-derived contaminants. MSW4 and MW1 exhibited the greatest number of contaminants, with 8 and 13 compounds, respectively. As MSW4 is adjacent to the Grand River and MW1 to the Speed River, the reason for these two wells to express such a diverse group of compounds may be a result of the wells being influenced by these large rivers. While MSW4 is an established GUDI well, the hydraulic gradients located at MW1 would suggest that the Speed River is a gaining stream and therefore not likely to influence groundwater quality. MW1 is located on a site historically contaminated with VOCs and as the majority of contaminants detected in MW1 are VOCs they are likely a result of the historical contamination and not sewer exfiltration (ARCADIS, 2013). In the end, wells in close proximity to both sewers and septic systems were vulnerable to sewage-derived contaminants with 78 and 92% of wells in close proximity to sewers and septic systems, respectively, exhibiting wastewater contaminants.
3.4.4 Pharmaceuticals, artificial sweeteners, and other sewage-derived contaminants

Of all the compounds examined in this investigation, perchlorate occurred in the highest number of wells. Despite the vast distribution of detections, the presence of perchlorate should not cause alarm as the concentrations were all several orders of magnitude below the EPA’s interim health advisory level of 15 μg/L. The presence of perchlorate, however, still remains intriguing as there are no known industries working with munitions or explosives in the area. While 2 gun ranges are present in Eden Mills, the concentrations of perchlorate extend all the way across the county making these gun ranges unlikely sources, at least for wells outside of Eden Mills. The use of flares in road construction and police operations are common in southern Ontario as are celebratory fireworks displays on holidays. Environmental perchlorate can also be attributed to the use of detergents and so can potentially be an indicator of human wastewater. As mentioned earlier, high concentrations of boron in the environment are frequently attributed to the influence of common household bleach products and so the weak positive correlation ($R^2 = 0.4637$) between boron and perchlorate could suggest that some of the perchlorate in southern Wellington County is from a similar source (Figure 3.9). Additionally, while thyrostatic drugs are currently banned for use in livestock, the persistence of perchlorate and heavy use of these drugs in the 1970s could still be a contributing factor to the overall presence of perchlorate in Wellington County. In the end, the diversity of perchlorate sources makes it very difficult to determine origins of the perchlorate in groundwater. The most likely cause for perchlorate’s occurrence is a combination of the sources mentioned above, each contributing a small portion to the total concentrations. Although the presence of perchlorate is interesting, the non-uniqueness of potential sources ultimately makes perchlorate a poor tracer for sewage-derived contaminants.
Figure 3.9. Plot of boron versus perchlorate resulted in a weak ($R^2 = 0.4637$) positive correlation suggesting that the two contaminants may have a common source.

The second most common contaminant in this investigation was PCE. While the majority of detections were below the PQL they are not of health concern, but their presence is still important to note. The majority of wells included in this investigation are not in close proximity to large industries and therefore the source of PCE must be low levels of commercially available solvents leaching into the groundwater potentially from sewer and septic systems. As with perchlorate, PCE is a very non-unique contaminant as it is used in many different products. While PCE is indicative of anthropogenic influences, distinguishing between septic and non-septic PCE is beyond the scope of this project.

In contrast to perchlorate and PCE, acesulfame has much fewer potential sources and can generally be attributed to sewer and septic effluents. In this investigation, acesulfame was found in 13 wells. In addition, the concentrations of acesulfame were consistently several orders of magnitude above the MDL. As only one municipal supply well had detectable concentrations of acesulfame, it would seem as though shallower private wells are more vulnerable to contamination with this artificial sweetener. As discussed above, however, the low acesulfame concentrations at depth may not necessarily be due to a
lack of sewage influences but rather a lack of acesulfame usage when the deeper portions of the aquifer were being recharged. Additionally, deeper wells are generally associated with longer open intervals and therefore may result in increased dilution to the point where analyte concentrations may be lowered to non-detectable levels.

Saccharin only occurred in measurable levels in 2 wells. Due to the high degradability of this sweetener, its presence in groundwater suggests rapid recharge and transport to the wells. While saccharin was found alongside acesulfame in PW1 and can therefore be attributed to a septic influence, its presence in MSW3 was independent of any other sewage-derived contaminant, even boron. As Buerge et al. (2011) pointed out that saccharin is frequently added to piglet feed to aid in the weaning process, the sweetener’s environmental presence may be attributable to the application of swine manure during agricultural practices. As MSW3 lacks evidence of other sewage-derived contaminants and is located in a relatively rural, low-population area, the saccharin in this well is expected to be a result of swine manure being used in an agricultural setting. While this sweetener was only detected in 2 out of 22 wells, it can still convey a well’s susceptibility to different contaminant sources and highlight areas of exceptionally rapid recharge and transport.

As investigations by Xu et al. (2010) and Oppel et al. (2004) would suggest that ibuprofen is susceptible to both biodegradation and adsorption, it is not surprising that ibuprofen was detected in fewer wells than acesulfame. In previous studies by Barnes et al. (2008), Carrara et al. (2008), and Loos et al. (2010), ibuprofen was detected at concentrations up 3.11 μg/L, 12,000 ng/L, and 395 ng/L, respectively. Although ibuprofen was never detected above its 2 ng/L pql, it was detected in a much larger percentage of wells than in both the American investigation by Barnes et al. (2008) and the pan-European study by Loos et al. (2010). In this investigation, ibuprofen was detected in 45% of the groundwater samples, while the European and US studies found ibuprofen in 6.7 and 2.1% of their
sampling wells, respectively. While 8 out of 10 ibuprofen detections occurred alongside acesulfame, the unique ibuprofen detections in MSW1 and MSW2 suggest that this pharmaceutical may be able to indicate sewage influences in locations where acesulfame cannot. In Figure 3.8, MSW1 and MSW2 exhibit 2 of the lowest tritium activities suggesting that groundwater from these wells is older than groundwater from the other wells. The positive ibuprofen detection could potentially suggest that the groundwater in these two wells was under the influence of sewer or septic wastewater when it was recharged, but at a time when acesulfame was not heavily used. If this is the case, it would suggest a persistence of ibuprofen for more than 25 years. While the low temperatures and DO of these two wells could potentially reduce microbial activity, a persistence of 25 years would be remarkable, but as no previous studies have reported on the persistence of ibuprofen in fractured bedrock aquifers, this may be possible.

Triclosan, carbamazepine, and sulfamethoxazole were also detected, but in a very limited number of wells. Like ibuprofen, triclosan was never detected in concentrations above the PQL. Additionally, triclosan was only detected in 3 wells, each of which had positive detections of both ibuprofen and acesulfame. As Halden and Paull (2005) would suggest that the aromatic structure and high chlorine content of triclosan are expected to impart a resistance to biodegradation, the low detection frequencies of triclosan despite high detection frequencies of other sewage-derived contaminants would suggest that triclosan was influenced by some attenuating process. While sorption is generally a limited process in fractured bedrock aquifers due to a reduced surface area of the fracture surface in comparison to porous media, sorption and subsequent sedimentation of triclosan in septic tanks and leach fields may account for the low detection frequencies of triclosan in southern Wellington County’s groundwater. In both the European and US studies, triclosan was also found in a low percentage of wells and in exceptionally low concentrations (Loos, 2010; Barnes, 2008).
Carbamazepine and sulfamethoxazole had few detections and only one was unique; both of these pharmaceuticals were always detected alongside ibuprofen and acesulfame except for one detection of carbamazepine in PW5. Contrary to ibuprofen and triclosan, however, carbamazepine and sulfamethoxazole were always detected in quantifiable concentrations. In fact, out of all the pharmaceutical compounds, carbamazepine was found in the greatest concentrations. The low detection frequency of carbamazepine and sulfamethoxazole is, however, contrary to the results from previous investigations as the pharmaceuticals were consistently considered to be two of the more relevant pharmaceuticals found in the environment due to their longevity and general lack of attenuation and removal (Clara et al., 2004; Drewes et al., 2003). In the European investigation by Loos et al. (2010), carbamazepine and sulfamethoxazole were detected in 42 and 23% of wells, respectively. With only 3 detections of both carbamazepine and sulfamethoxazole in this investigation, these pharmaceuticals seem to be much less prevalent in the fractured bedrock aquifers of southern Wellington County than both the European and US studies would have suggested.

The absence of the artificial sweeteners sucralose and cyclamate as well as the pharmaceuticals acetaminophen, androstenedione, estrone and gemfibrozil, is likely attributable to their affinities for biodegradation and sorption. In the investigations by Loos et al. (2010) and Barnes et al. (2008), these compounds consistently exhibited the lowest detection frequencies. Equally rare in groundwater are trimethoprim and naproxen. While the column experiments by Xu et al. (2009) showed naproxen to have one of the greatest susceptibilities to biodegradation, Godfrey et. al. (2007)showed trimethoprim's high susceptibility to significant attenuation during transport from a septic system to an underlying sand aquifer. As the reported detection rates of each of these compounds was low in previous investigations, their lack of detection in the current investigation is not a unique result.
3.5 CONCLUSIONS

Although one private well did not exhibit any sewage-derived contaminants, the overall conclusion from this study is that all well types completed in the fractured bedrock aquifers of southern Wellington County are susceptible to contamination with at least one type of organic wastewater contaminant regardless of the well’s construction, depth, surrounding land use, or overburden thickness. The wide
array of wells sampled in this investigation revealed that areas served by sewers and septic systems are both vulnerable to human waste streams. As 95% of the wells exhibited wastewater contaminants, fractured bedrock seems to be more vulnerable to these contaminants than previous American (Barnes et al., 2008) and pan-European (Loos et al., 2010) studies would suggest. In the European investigation, 2.3% of wells were susceptible to pharmaceuticals while 45% of the wells sampled in southern Wellington County contained at least one pharmaceutical. While previous investigations would suggest that ibuprofen is found in some of the highest concentrations out of all the pharmaceuticals in the environment, this was not the case in the current study. Here, while ibuprofen was the most prevalent pharmaceutical compound, it was never detected above its practical quantitation limit.

Similarly, low detection frequencies of carbamazepine and sulfamethoxazole in the fractured bedrock aquifers of southern Wellington County are contrary to previous studies investigating organic wastewater contaminants. In the previous investigations by Barnes et al. (2008) and Loos et al. (2010), carbamazepine and sulfamethoxazole exhibited the highest detection rates out of all the pharmaceutical compounds analyzed. In the fractured dolostone aquifers of southern Wellington County, however, carbamazepine and sulfamethoxazole exhibited two of the lowest detection frequencies of all the sewage-derived contaminants that were analyzed.

In the end, perchlorate, PCE, and the artificial sweetener acesulfame were the most prevalent of all the contaminants analyzed in the 22 groundwater samples. While perchlorate and PCE have numerous potential sources, the high detection frequency of acesulfame (14/22 wells) provides strong evidence that leaking sanitary sewers and leaching septic systems are influencing the drinking water aquifers of southern Wellington County as this compound is not naturally occurring and, when consumed, is shed in high concentrations in human waste. Although this artificial sweetener has not exhibited any health
risks to humans, its presence could indicate the potential for more serious sewage-derived contaminants such as viruses to be in the aquifer.

Apart from the evident vulnerability of the local aquifers to sewage-derived contaminants, the current study also suggested the use of a unique technique for further distinguishing groundwater ages using acesulfame. Despite a few large peaks in tritium activities from the mid 1960s, most tritium activities from the last 60 years have decayed to approximately the same range. Using only tritium for dating groundwater is therefore becoming increasingly difficult. As every shallow groundwater sample collected in this investigation that expressed tritium activities above 10 TU also exhibited quantifiable concentrations of acesulfame, it can be said with very high confidence that these samples were recharged in the last 25 years. Combining the analysis of tritium and acesulfame can then be used to distinguish old groundwater samples from samples recharged in the last 25 years. While the ages may be approximate, determining young versus old water is relevant in the study of hydrogeology as it can identify an era during which contamination occurred.
Chapter 4:
Artificial sweeteners and pharmaceutical compounds as indicators of sewage-derived waste in a fractured dolostone aquifer

ABSTRACT
After 2 previous investigations revealed that the fractured bedrock aquifers of southern Wellington County, Ontario were highly susceptible to contamination with human enteric viruses, pharmaceuticals, artificial sweeteners, and perchlorate, it was suggested that perhaps one of these emerging contaminants could be an efficient and accurate indicator of sewage-derived contamination of a fractured bedrock aquifer. As the primary concern with fecal contamination of an aquifer is the transport of potentially dangerous pathogens to water supplies, the concentrations of pharmaceuticals, artificial sweeteners, traditional bacterial indicators, and compounds associated with anthropogenic waste streams were compared to the occurrence of viruses. Efficiency of each of the potential indicators was examined through the calculation of positive and negative predictive values (PPV and NPV, respectively) as well as true positive and specificities (TPR and TNR, respectively). Ibuprofen exhibited the highest of all 4 of these values suggesting that it may be the best indicator of potential virus occurrences in a fractured bedrock aquifer (PPV = 60%; NPV = 67%; sensitivity = 60%; specificity = 67%). Although ibuprofen occurrences exhibited the highest statistical correlation with virus occurrences, this pharmaceutical was never detected in concentrations above the practical quantitation limit and so its concentrations could not be compared to the concentrations of viruses. On the other hand, concentrations of the artificial sweetener acesulfame exhibited the best correlation with concentrations of viruses ($R^2 = 0.7729$). Total coliforms exhibited some of the highest indicator statistics (PPV = 45%; NPV = 55%; Sensitivity = 50%; Specificity = 50%), but due to the highly unspecific nature of
total coliform sources, it is not as specific of an indicator as pharmaceuticals and artificial sweeteners whose predominant environmental sources are human waste. *E. coli* exhibited the indicator statistics (PPV = 17; NPV = 44; Sensitivity = 10%; Specificity = 58%) of all the indicator parameters suggesting that this traditional fecal indicator is not as efficient at predicting virus occurrences as some of these new emerging contaminants. While triclosan, carbamazepine, sulfamethoxazole, and saccharin were detected, the number of detections was low, suggesting that their commercial use is not as widespread in southern Wellington County as ibuprofen and acesulfame and they may not be the best compounds to use as tracers of wastewater influences in groundwater. In the end, the combination of ibuprofen and acesulfame was suggested as being the best tool for indicating the potential for wastewater and viral influences on the fractured Silurian dolostone aquifers of southern Wellington County, ON.

4.0 INTRODUCTION

As discussed in Chapters 1 and 2, septic leaching and sewer exfiltration can release various types of potentially dangerous contaminants to the subsurface environment. In particular, Chapter 1 revealed that the fractured bedrock aquifers of southern Wellington County, Ontario, Canada are more vulnerable to virus contamination than previous investigations would suggest. As 45% of the private wells and 62.5% of the municipal supply wells sampled in southern Wellington County exhibited human enteric viruses during an 8 month sampling regime, it becomes clear that these fractured dolostone aquifers will require vigilant monitoring in order to prevent viral outbreaks.

Monitoring groundwater for viruses and various pathogenic bacteria and protozoa is an extremely time-consuming and expensive process and therefore warrants the development of a simpler, less expensive technique to indicate potential pathogenic contamination of an aquifer. Quantitative-polymerase chain reaction (qPCR) is a real time molecular technique used to amplify DNA in order to quantify virus concentrations present in a groundwater sample. While this technique is fast and sensitive, it requires
specific primers for each virus desired to be analyzed and a sterile laboratory fully equipped with PCR instruments, mixes, probes, and probe kits. The components for this type of analysis can therefore drastically increase the cost of a virus monitoring program.

Cell culture methods are frequently used as the simpler, less expensive technique for virus detection. These techniques require that viruses present in the groundwater sample infect a standard host cell line that will exhibit cytopathic effects once it has been infected. The infected cells or plaques are then counted as singular virus particles and used to estimate the concentration of infectious viruses present in the groundwater sample. While these methods may be less complicated than molecular techniques, they are limited as only a few of the viruses that can occur in groundwater can be detected by this method (EPA, 2006 economic analysis). When using this technique, varying hydrochemistries can significantly influence virus recovery. While molecular techniques also cannot produce perfect virus recoveries, cell culture methods can result in significant underestimates of virus concentrations as plaques are counted as singular viruses whereas they frequently consist of several viral particles and strains. Additionally, while q-PCR can detect both active and inactive viruses, cell culture methods can only detect active viruses.

While qPCR is arguably the best technique for quantifying virus concentrations in groundwater, the technique is highly cost restrictive. In addition, a recent study by Bradbury et al. (2013) showed that virus occurrence in groundwater can be extremely temporally variable and that successive samples must therefore be collected. The expensive qPCR technique must therefore be repeated quarterly, or more preferably, monthly in order to adequately assess the vulnerability of an aquifer to contamination with human enteric viruses. As these costs are extremely restrictive, the use of a chemical or biological indicator with less expensive and time-consuming analyses have become the standard method for monitoring drinking water for fecal contamination.
In both Canada and the US, public water systems are required to sample for fecal indicator bacteria in order to monitor and control the potential distribution of fecally-derived viruses to the public. In the United States, the Ground Water Rule (EPA, 2006 Groundwater Rule) relies heavily on the requirements of the Total Coliform Rule (EPA, 1989) which ultimately calls for the monitoring of the distribution systems of all public water systems for total coliforms at a frequency proportional to the number of people served by that water system. Similarly in Ontario, the Safe Drinking Water Act (Government of Ontario, 2002) requires that samples be taken from entry points into the drinking water system’s distribution system at a frequency proportional to the size of the population being served by the system. These samples must all be analyzed for \textit{E. coli}, total coliforms, and heterotrophs.

As these requirements are based on population size, the distribution system that supplies the 2.6 million residents of Toronto, ON would ultimately be required to sample its system approximately 360 times per month, which averages out to roughly 1 sample every two hours. In smaller cities, such as Guelph, ON, with a population of 127 000, the distribution system would need to be sampled about 102 times throughout a month, equating to about 3 samples a day. With such frequent sampling events it is assumed that any drastic changes to the water quality will be detected early enough so that the appropriate measures can be taken to treat the water before it is distributed to the public. But these monitoring strategies are based entirely on the analysis for total coliforms and \textit{E. coli} and therefore rely on the assumption that these microbes are reliable indicators of fecal and pathogenic contamination.

An effective fecal indicator will exhibit all of the characteristics listed in Table 4.1. These pillars of a fecal indicator ultimately constrain an indicator to a fecal-source specific compound or microbe that shares environmental transport and persistence characteristics with enteric viruses. Additionally, the methods used for the analysis of the indicator must be cheap, simple, applicable to a wide array of hydrochemistries, and characterized by low detection limits. Simple and inexpensive detection methods
have been developed for total coliforms, but as 4 of the 5 genera included in this group are either not unique to fecal sources or can multiply and occur naturally in the environment (Edberg et al., 2000), total coliforms do not fulfill all the pillars listed in Table 4.1. While *E. coli* also has several simple and cost-effective detection methods and is present in high concentrations in feces (approximately $10^9 \text{g}^{-1}$), it can be up to 100 times larger than human enteric viruses and can therefore experience elevated levels of physical straining during groundwater transport. Although *E. coli* has long been thought to be the sole genera within the total coliforms group to be predominantly derived from feces, recent investigations would suggest that several strains of *E. coli* exist and persist naturally in the environment and are therefore autochthonous members of the soil microbial community (Ishii et al., 2007). In other words, several strains of *E. coli* that are not fecally derived are naturally occurring in the environment and contributing to high counts of *E. coli* in water. Additionally, these naturalized *E. coli* strains have shown the ability to persist and replicate to high cell densities when incubated at temperatures ranging from 4°C to 37°C in unsterilized sediments (Ishii et al., 2006). In comparison to strains derived from human feces, the adapted strains have exhibited enhanced growth at cool temperatures (Gordon et al., 2002) and so the detection of *E. coli* in cool groundwater may very well be naturally occurring *E. coli* and not a true indication of a fecal source. As Ishii et al. (2006) isolated identical *E. coli* strains from the same sites over multiple seasons, they suggested that these bacteria can persist in soil over long durations. With natural background concentrations of *E. coli* that are indistinguishable from fecally-derived strains when standard membrane filtration methods are used, *E. coli* becomes a much less specific and less valuable fecal indicator than previously thought. As neither total coliforms nor *E. coli* meet all of the pillars outlined in Table 4.1, the current standards for monitoring a water supply for potential virus contamination are insufficient.
Table 4.1. The pillars of an effective virus indicator as summarized from Gasser et al., 2010 and Lin and Ganesh, 2013.

<table>
<thead>
<tr>
<th>The indicator must:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Share a common predominant source with viruses</td>
</tr>
<tr>
<td>2 Be universally present in high concentrations in human wastewater</td>
</tr>
<tr>
<td>3 Have low background levels and not exhibit appreciable amounts of replication in the environment</td>
</tr>
<tr>
<td>4 Exhibit conservative and persistent behaviour similar to viruses</td>
</tr>
<tr>
<td>5 Exhibit high mobility in groundwater regimes</td>
</tr>
<tr>
<td>6 Have inexpensive and accessible analytical methods</td>
</tr>
<tr>
<td>7 Have low detection limits</td>
</tr>
<tr>
<td>8 Be capable of being detected in groundwater with varying hydrochemistries</td>
</tr>
<tr>
<td>9 Exhibit a similar degree of removal to viruses during septic leaching or sewer exfiltration</td>
</tr>
</tbody>
</table>

To date, numerous studies have reported on the inability of these traditional fecal indicators to predict and indicate fecally-derived virus contamination of water supplies. In 1980, Keswick and Gerba reviewed 24 studies investigating the presence of viruses in groundwater and of the 6 studies that reported on the co-occurrence of viruses with indicator bacteria, 4 of the studies reported the simultaneous detection of viruses with either fecal coliforms, fecal streptococcus, total coliforms, or E. coli while the remaining 2 studies reported virus contamination without any detections of bacterial contamination. Several more recent field scale studies investigating both the occurrence of viruses and their co-occurrence with various indicators have produced a wide range of results. Lindsey et al. (2002) sampled 59 noncommunity water supply wells across the commonwealth of Pennsylvania for pathogens, including viruses, and several microbiological indicator organisms and reported that 60% (3 out of 5) of virus detections had simultaneous detections of total coliforms while only 40% (2 out of 5) of the virus detections were accompanied by E. coli. When Borchardt et al. (2003) sampled 50 private wells across Wisconsin for human enteric viruses, total coliforms, E. coli, fecal enterococci, F-specific RNA coliphages, nitrate, and chloride, they found that total coliforms exhibited a 25% sensitivity
meaning that 25% of wells exhibiting viruses also had detections of total coliforms at some point during
the study. *E. coli*, however, exhibited a sensitivity of 0% when none of the wells with positive detections
of viruses ever exhibited *E. coli*. In contrast, Locas et al. (2007) reported total coliforms as having 100% sensitivity when over one year twelve municipal wells in Quebec were sampled monthly for bacterial indicators, viral indicators, total culturable human enteric viruses, and noroviruses. While total coliforms were always present with human enteric viruses, 1 of the four wells to exhibit viruses never exhibited *E. coli* throughout the entire study therefore resulting in *E. coli* having a sensitivity of 75%.

With total coliform sensitivities ranging from 25 to 100% and *E. coli* sensitivities from 0 to 75%, the efficiency of these bacterial indicators to suggest the presence of fecal and pathogenic contamination is highly variable and therefore unreliable.

Several pharmaceutical compounds and artificial sweeteners can be characterized by a number of the pillars presented in Table 4.1 and are therefore expected to be better indicators of human waste streams. Studies by Van Stempvoort et al. (2011a and b; 2013) and Buerge et al. (2009 and 2011) have highlighted the ubiquitous presence of high concentrations of artificial sweeteners in wastewater and have demonstrated the relatively conservative and persistent transport of the artificial sweetener acesulfame in groundwater. In studies investigating the presence of pharmaceuticals across the United States and Europe, Barnes et al. (2008) and Loos et al. (2010) have respectively revealed the persistence and widespread occurrence of several pharmaceutical compounds including ibuprofen, carbamazepine, and sulfamethoxazole. In Chapter 2, the occurrence of carbamazepine, sulfamethoxazole, triclosan, ibuprofen and acesulfame in the fractured bedrock aquifers of southern Wellington County, Ontario, Canada was attributed to septic leaching and sewer exfiltration as each of these synthetic compounds was detected in remote areas removed from landfills and food processing plants. As human enteric viruses are shed in high concentrations in feces, these pathogens share a common source with the pharmaceuticals and artificial sweeteners found in southern Wellington County’s aquifers. Additionally,
while carbamazepine, sulfamethoxazole, and triclosan exhibit neutral charges at the near-neutral pH of groundwater, ibuprofen, acesulfame, and viruses all exhibit negative charges in natural groundwaters and are therefore expected to experience similar levels of sorption during transport. While the current cost of the analysis of each of these compounds is not comparable to the inexpensive analyses for total coliforms and *E. coli*, the fact that ibuprofen and acesulfame fulfill 8 out of the 9 pillars presented in Table 4.1 suggests that they may be novel fecal indicators worth pursuing.

Other sewage-derived contaminants were detected in southern Wellington County’s groundwater and may also hold potential for being novel fecal indicators. In Chapter 2, low levels of perchlorate, boron, and chloroform were reported in a number of the study’s sampling wells. As these compounds are associated with cleaning products and treated drinking water, their environmental occurrence has occasionally been attributed to human wastewater, but these contaminants also have a myriad of additional sources that are not related to sewage inputs. For example, the environmental occurrence of perchlorate is most frequently attributed to products containing explosives such as munitions or fireworks (California Department of Toxic Control, 2005) and boron can be found in high concentrations due to the manufacturing of wires and cables (Vengosh, 1994). Although these contaminants are known for their environmental persistence and some of their environmental occurrences may be attributed to septic leaching and sewer exfiltration, the fact that they are not unique to septic sources reduces their potential of being efficient fecal indicators. Nonetheless, their widespread occurrence throughout the fractured bedrock aquifers of southern Wellington County, ON begs for their use as fecal indicators to be investigated.

4.0.1 Hypotheses and goals

While several studies have investigated and established the presence of artificial sweeteners, pharmaceuticals, and other sewage-derived compounds in groundwater, to our knowledge, no studies
have simultaneously collected samples for these compounds alongside virus samples in order to assess the effectiveness of these emerging contaminants as fecal or virus indicators. In the current investigation, concentrations and detection frequencies of traditional fecal indicators including total coliforms and *E. coli* are compared to the concentrations and detection frequencies of artificial sweeteners, pharmaceuticals, viruses, and other sewage-derived compounds reported in Chapters 1 and 2 in order to examine the relative reliability of each of these contaminants to act as indicators of fecal and virus contamination in fractured bedrock aquifers. As stated earlier, total coliforms and *E. coli* have exhibited varying levels of reliability in predicting virus occurrences. The inconsistency of these bacterial indicators has been attributed to their short half lives and larger size relative to viruses. Using compounds that have shown prolonged environmental persistence and conservative transport is therefore expected to result in more reliable indicators. As viruses, artificial sweeteners, and pharmaceutical compounds can exhibit prolonged environmental persistence and can all be introduced to the environment by septic leaching and sewer exfiltration, it is hypothesized that these emerging compounds will act as valuable tracers of human waste streams. Due to the high detection frequency of ibuprofen and acesulfame noted in Chapter 2, these 2 compounds are expected to exhibit the highest indicator parameter values and be the best indicators of fecal and virus contamination. Therefore, the goal of this chapter is to determine statistically, using sensitivity, specificity and positive and negative predictive values (PPV and NPV, respectively), if these compounds can be used as indicators of fecal and virus contamination.

### 4.1 SITE DESCRIPTION

As described in Chapter 1, the sites that were investigated in this study consisted of 22 locations within a 20-km radius of the City of Guelph, Ontario, Canada (Figure 4.1; Table 4.2). The sampling wells were located in Centre Wellington, the City of Guelph, Arkell, Eden Mills, and Aberfoyle and included 11 private wells (PW), 8 municipal supply wells (MSW), and 3 monitoring wells (MW) located along the
Speed River in downtown Guelph. Each well was chosen to highlight different aspects of the local aquifers and therefore resulted in a diverse array of wells allowing for the examination of aquifer vulnerability in a wide range of settings. Surrounding land use ranged from predominantly agricultural uses in the Arkell area to dense urban centres like that of Guelph. The wells also exhibited a range of completions from simple completions into the upper few meters of the Guelph Formation to very deep completions into the lower reaches of the Gasport Formation.

Both the Guelph and Gasport Formations are comprised of crinoidal grainstones and wackestones characterized by transmissivities on the order of $10^{-4}$ m$^2$/s and effective fracture porosities around $10^{-4}$ (Munn, 2012). The combination of these transmissivities and low effective fracture porosities promote extremely rapid groundwater velocities. The Guelph Formation is separated from the underlying Gasport Formation by the Eramosa Formation which is characterized by lower transmissivities and is frequently considered a regional aquitard (Brunton, 2008). Overlying sediments are composed of glacial deposits exhibiting varying thicknesses and permeabilities across the county.
Figure 4.1. Sampling locations in southern Wellington County consisted of 8 municipal supply wells, 3 monitoring wells, and 11 private wells.
Table 4.2. Details for 22 sampling wells including 11 private wells, 3 monitoring wells and 8 municipal supply wells in southern Wellington County, ON.

<table>
<thead>
<tr>
<th>Location</th>
<th>Well ID</th>
<th>Total Depth (m)</th>
<th>Overburden Thickness (m)</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre Wellington</td>
<td>MSW1</td>
<td>129.8</td>
<td>9.45</td>
<td>Municipal</td>
</tr>
<tr>
<td></td>
<td>MSW2</td>
<td>121.9</td>
<td>13.1</td>
<td>Municipal</td>
</tr>
<tr>
<td></td>
<td>MSW3</td>
<td>128</td>
<td>25</td>
<td>Municipal</td>
</tr>
<tr>
<td></td>
<td>MSW4</td>
<td>110</td>
<td>4.88</td>
<td>Municipal</td>
</tr>
<tr>
<td></td>
<td>MSW5</td>
<td>129.5</td>
<td>78.9</td>
<td>Municipal</td>
</tr>
<tr>
<td></td>
<td>MSW6</td>
<td>124.4</td>
<td>15.2</td>
<td>Municipal</td>
</tr>
<tr>
<td>Fergus</td>
<td>MW1</td>
<td>8.4</td>
<td>1.8</td>
<td>Monitoring</td>
</tr>
<tr>
<td></td>
<td>MW2</td>
<td>11.3</td>
<td>1.8</td>
<td>Monitoring</td>
</tr>
<tr>
<td></td>
<td>MW3</td>
<td>25.4</td>
<td>1.8</td>
<td>Monitoring</td>
</tr>
<tr>
<td>City of Guelph</td>
<td>MSW7</td>
<td>58.8</td>
<td>12.8</td>
<td>Research</td>
</tr>
<tr>
<td></td>
<td>MSW8</td>
<td>87.8</td>
<td>20.4</td>
<td>Research</td>
</tr>
<tr>
<td>Speed River</td>
<td>PW1</td>
<td>29</td>
<td>27.1</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW2</td>
<td>54.9</td>
<td>23.2</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW3</td>
<td>24.4</td>
<td>21.3</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW4</td>
<td>59.4</td>
<td>15.8</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW5</td>
<td>20.4</td>
<td>15.2</td>
<td>Domestic</td>
</tr>
<tr>
<td>Aberfoyle</td>
<td>PW6</td>
<td>20.4</td>
<td>13.4</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW7</td>
<td>74.7</td>
<td>11.3</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW8</td>
<td>32</td>
<td>7.3</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW9</td>
<td>67.1</td>
<td>14.9</td>
<td>Domestic</td>
</tr>
<tr>
<td>Arkell</td>
<td>PW10</td>
<td>15.8</td>
<td>0.1</td>
<td>Domestic</td>
</tr>
<tr>
<td></td>
<td>PW11</td>
<td>31.1</td>
<td>0.3</td>
<td>Domestic</td>
</tr>
</tbody>
</table>

4.2 METHODS

While Chapters 1 and 2 fully cover the detailed methods used to sample and analyze the various sewage-derived viruses and compounds included in this investigation, the list of these analytes and their analysis methods are summarized in Table 4.3 and are briefly mentioned below. Details pertaining to the sampling and analysis methods for total coliforms and *E. coli* are discussed in more detail below and in Appendix V.

4.2.1 Sample collection

Each of the wells was sampled for viruses on a monthly basis up to a maximum of six times between June 2012 and January 2013. Some wells were sampled less frequently due to limited accessibility. During virus sampling events in July and November 2012, samples for both artificial sweeteners and
bacterial fecal indicators were also collected. Pharmaceutical samples were collected once during the virus sampling event in September 2012 while general chemistry samples were collected once alongside the virus samples in November 2012.

Pathogens were collected using the glass wool filtration methods described by Millen et al. (2012) and analyzed at the USDA lab in Wisconsin using quantitative polymerase chain reaction (Borchardt et al., 2012). Groundwater samples for pharmaceutical analyses were collected into pre-washed 500 mL amber bottles and shipped to Dr. Chris Metcalfe’s lab at Trent University in Peterborough, ON for analysis via the liquid chromatography and tandem mass spectrometry methods developed by Zhao and Metcalfe (2008). Artificial sweeteners and perchlorate were analyzed at the Canada Centre for Inland Waters in Burlington, ON by ion chromatography coupled with triple-quadrupole tandem mass spectrometry (van Stempvoort, 2011). VOCs were analyzed at the University of Guelph using Method 8260B for analyzing VOCs by capillary column gas chromatography and mass spectrometry (EPA, 1996).

Groundwater samples collected from private wells were drawn directly from external faucets that bypassed any treatment or filtration. Similarly, samples from municipal supply wells were taken from faucets connected to the pump and were therefore raw water. Monitoring wells were sampled using a sterilized Grundfos Redi-Flo2 electrical submersible pump hooked up to a Honda EU2000KC2 generator. Field parameters including pH, temperature, conductivity, and dissolved oxygen were monitored using a YSI556 multiprobe. Samples were not collected until field parameters had stabilized.

The specific sampling methods for pathogens, pharmaceuticals, artificial sweeteners, and other sewage-derived contaminants were described in depth in Chapters 1 and 2. Collection of groundwater samples for *E. coli* and total coliforms closely followed the guidelines set forth by EPA Method 1604 (2002). As groundwater concentrations of *E. coli* and total coliforms were expected to be low, sample volumes were increased from the suggested 100 mL to 1000 mL. Groundwater samples for bacterial analyses
were collected in triplicate into autoclaved 1 L HDPE bottles and transported on ice to the University of Guelph.

4.2.3 Bacterial analysis

Samples were analysed within 24 hours of their collection using vacuum filtration following the standard operating procedure outlined in Appendix V that was based on the EPA Method 1604 (2002). Prior to sample collection, sterile Petri dishes were prepared with Oxoid CM 1038 Differential Coliform (DC) agar. Each groundwater samples was filtered onto pre-sterilized 47-mm diameter, grid-marked, cellulose ester membranes and placed on Petri dish filled with DC agar. After 24 hours of incubation at 35°C, the plates were removed and checked for bacterial growth. Pink colonies were counted as total coliforms and blue colonies were counted as *E. coli* (Figure 4.2). The plates were incubated for an additional 24 hours followed by another round of colony counting. Quality of these techniques was maintained by strictly adhering to the protocol presented in Appendix 1 and monitoring method blanks between each sample filtration.

![Figure 4.2. Membrane filter on differential coliform agar. White colonies indicate heterotrophs while pink colonies indicate total coliforms and blue colonies indicate *E. coli.*](image-url)
4.2.4 Data analysis

Bacterial concentrations from triplicate analyses for *E. coli* and total coliforms were averaged and plotted with standard deviations for each well. Filters exhibiting greater than 200 colony forming units (CFU) were deemed “too numerous to count” and plotted as such. Average concentrations of *E. coli* and total coliforms were compared to the maximum contaminant levels set forth by the EPA.

Sensitivity and specificity were calculated for each of the potential virus indicators using the equations presented below. The sensitivity is the percent of virus detections that are actually indicated by a given tracer while the specificity is the percent of wells with non-detectable concentrations of pathogens that also exhibited non-detectable concentrations of a given tracer. Positive and negative predictive values were also calculated for each potential indicator of virus contamination. The positive predictive value will indicate the percentage of indicator occurrences that actually indicated virus occurrences while the negative predictive value will indicate the percentage of wells that did not exhibit an indicator that also did not exhibit pathogens.

Equations (Sox et al., 1988):

\[
\text{Sensitivity} = \frac{\text{Number of wells positive for both viruses and indicator}}{\text{Total number of wells positive for viruses}} \times 100\%
\]

\[
\text{Specificity} = \frac{\text{Number of wells negative for both viruses and indicator}}{\text{Total number of wells negative for viruses}} \times 100\%
\]

\[
\text{Positive Predictive Value} = \frac{\text{Number of wells positive for both viruses and indicator}}{\text{Total number of wells positive for indicator}} \times 100\%
\]

\[
\text{Negative Predictive Value} = \frac{\text{Number of wells negative for both viruses and indicator}}{\text{Total number of wells negative for indicator}} \times 100\%
\]
In the end, all four of these statistical parameters were used to determine the overall best indicator of virus occurrences.

Measureable concentrations of indicators were plotted versus virus concentrations in order to determine the ability of the indicators to predict the extent of virus contamination that may be occurring.

4.3 RESULTS
Detection frequencies of all the analytes included in the 3 chapters of this investigation are summarized in Table 4.3 and will be further discussed below.
Table 4.3. Summary of analytes, analysis methods and detection frequencies on a per well basis. ND = Non-detect. PPM = Positive polarity mode. NPM = Negative polarity mode.

<table>
<thead>
<tr>
<th>Analyte Type</th>
<th>Analyte</th>
<th>Detection Frequency</th>
<th>Analysis Method*</th>
<th>Analytical Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Virus</td>
<td>Enterovirus</td>
<td>ND</td>
<td>RT-qPCR</td>
<td>USDA Marshfield, WI</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A virus</td>
<td>ND</td>
<td>RT-qPCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norovirus: geotypes I and II</td>
<td>3/22 (14%)</td>
<td>RT-qPCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polymavirus</td>
<td>4/22 (18%)</td>
<td>qPCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenoviruses</td>
<td>3/22 (14%)</td>
<td>qPCR</td>
<td></td>
</tr>
<tr>
<td>Bovine Virus</td>
<td>Bovine Polyomavirus</td>
<td>ND</td>
<td>qPCR</td>
<td></td>
</tr>
<tr>
<td>Swine Virus</td>
<td>Hepatitis E virus</td>
<td>ND</td>
<td>RT-qPCR</td>
<td></td>
</tr>
<tr>
<td>Avian Virus</td>
<td>Avian influenza virus</td>
<td>ND</td>
<td>RT-qPCR</td>
<td></td>
</tr>
<tr>
<td>Protozoan</td>
<td>Cryptosporidium</td>
<td>ND</td>
<td>qPCR</td>
<td></td>
</tr>
<tr>
<td>Bovine fecal indicator</td>
<td>Bovine M3 Bacteroides-like</td>
<td>1/22 (5%)</td>
<td>qPCR</td>
<td></td>
</tr>
<tr>
<td>Pharmaceutical Compound</td>
<td>Acetaminophen</td>
<td>ND</td>
<td>LC-MS/MS in PPM</td>
<td>Water Quality Centre, Trent University, Peterborough, ON</td>
</tr>
<tr>
<td></td>
<td>Androstenedione</td>
<td>ND</td>
<td>LC-MS/MS in PPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbamazepine</td>
<td>3/22 (14%)</td>
<td>LC-MS/MS in PPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Estrone</td>
<td>ND</td>
<td>LC-MS/MS in NPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>ND</td>
<td>LC-MS/MS in NPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>10/22 (45%)</td>
<td>LC-MS/MS in PPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meprobamate</td>
<td>ND</td>
<td>LC-MS/MS in PPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Naproxen</td>
<td>ND</td>
<td>LC-MS/MS in PPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxazole</td>
<td>3/22 (14%)</td>
<td>LC-MS/MS in PPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triclosan</td>
<td>3/22 (14%)</td>
<td>LC-MS/MS in NPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
<td>ND</td>
<td>LC-MS/MS in PPM</td>
<td></td>
</tr>
<tr>
<td>Artificial Sweetener</td>
<td>Acesulfame</td>
<td>14/22 (67%)</td>
<td>IC-MS in NPM</td>
<td>Canada Centre for Inland Waters, Burlington, ON</td>
</tr>
<tr>
<td></td>
<td>Sucralose</td>
<td>ND</td>
<td>IC-MS in NPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saccharin</td>
<td>2/22 (9%)</td>
<td>IC-MS in NPM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclamate</td>
<td>ND</td>
<td>IC-MS in NPM</td>
<td></td>
</tr>
<tr>
<td>Perchlorate</td>
<td>Perchlorate</td>
<td>19 (86%)</td>
<td>IC-MS in NPM</td>
<td></td>
</tr>
<tr>
<td>Bacterial fecal indicator</td>
<td>Escherichia coli</td>
<td>6/22 (27%)</td>
<td>Membrane filtration</td>
<td>University of Guelph</td>
</tr>
<tr>
<td></td>
<td>Total coliforms</td>
<td>11/22 (50%)</td>
<td>Membrane filtration</td>
<td></td>
</tr>
<tr>
<td>Volatile Organic Compounds</td>
<td>Vinyl chloride</td>
<td>2/22 (9%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,1-DCE</td>
<td>1/22 (5%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,1-DCA</td>
<td>2/22 (9%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c-DCE</td>
<td>3/22 (14%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>14/22 (67%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,1,1-TCA</td>
<td>1/22 (5%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>3/22 (14%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloromethane</td>
<td>3/22 (14%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t-DCE</td>
<td>2/22 (9%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2-DCA</td>
<td>1/22 (5%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PCE</td>
<td>14/22 (67%)</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,1,-TCA</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2-Dichloropropane</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloroethane</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbon tetrachloride</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dichloromethane</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethylenbenzene</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m+p Xylene</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o-Xylene</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Styrene</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>ND</td>
<td>GC-MS</td>
<td></td>
</tr>
</tbody>
</table>
4.3.1 Bacterial indicator results

*E. coli* was detected in 6 out of the 22 wells (27%) sampled in this investigation including 1 municipal supply well, 2 monitoring wells and 3 private wells (Figure 4.3A). In Chapter 2, low tritium levels and the absence of acesulfame suggested that the water in MSW1 was much older than the water in the majority of the other wells. Despite the seemingly old water in MSW1, *E. coli* was detected. Only PW11 exhibited *E. coli* in concentrations above the EPA’s maximum allowed contaminant level of 1 CFU/100 mL. While both PW10 and PW11 exhibited *E. coli* during both sampling events, MSW1, MW2, MW3, and PW9 only exhibited *E. coli* once. The detection of human polyomavirus in MSW1 was the only virus detection to coincide with the detection of *E. coli*. *E. coli* ultimately had the second lowest positive predictive value (17%) and sensitivity (10%) of all the potential virus indicators examined in this investigation (Table 4.4). Additionally, PW10 and PW11, the wells exhibiting the highest concentrations of *E. coli*, did not exhibit any viruses.

None of the membrane filtration method blanks exhibited *E. coli* colonies.

Total coliforms were detected in 11 out of 22 wells (50%) including 3 municipal supply wells, 2 monitoring wells, and 6 private wells (Figure 4.3B). Total coliform concentrations were significantly higher than *E. coli* with PW7 and PW10 both exhibiting too many colony forming groups to count. MW3, PW7, PW10, and PW11 each produced concentrations of total coliforms significantly above the EPA’s maximum allowable contaminant level of 1 CFU/100 mL. Of the 11 wells to exhibit positive detections of total coliforms, 5 were also positive for viruses resulting in total coliforms having some of the highest values for each of the indicator parameters (Table 4.4).

None of the membrane filtration method blanks exhibited total coliform colonies.
Figure 4.3. A. Average E. Coli concentrations from sampling events in July (Event #1) and November (Event #2) 2012. B. Average total coliform concentrations from sampling events in July and November 2012. Error bars indicate the standard deviation of the triplicate samples. The maximum contaminant level for both E. Coli and total coliforms is 1 CFU/100 mL and is indicated by the yellow line. Some plates exhibited total coliform colonies that were too numerous to count (TNTC). MSW5, MW1, MW2, and MW3 were not sampled during Event #1 due to limited site access.
Table 4.4. Summary of indicator detection frequencies and statistics.

<table>
<thead>
<tr>
<th></th>
<th>All Wells (n=22)</th>
<th>Private Wells (n=11)</th>
<th>Municipal Supply Wells (n=8)</th>
<th>Monitoring Wells (n=3)</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Coli</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>17</td>
<td>44</td>
<td>10</td>
<td>58</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>45</td>
<td>55</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>60</td>
<td>67</td>
<td>60</td>
<td>67</td>
</tr>
<tr>
<td>Triclosan</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>53</td>
<td>10</td>
<td>83</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>33</td>
<td>53</td>
<td>10</td>
<td>83</td>
</tr>
<tr>
<td>Acesulfame</td>
<td>14</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>36</td>
<td>38</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Saccharin</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>50</td>
<td>45</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>19</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>42</td>
<td>33</td>
<td>80</td>
<td>8</td>
</tr>
<tr>
<td>Chloroform</td>
<td>14</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>33</td>
<td>20</td>
<td>40</td>
<td>17</td>
</tr>
</tbody>
</table>

4.3.2 Other indicator parameter results

The remaining results of the indicator parameter statistics are summarized in Table 4.4. Ibuprofen exhibited the greatest positive and negative predictive values, second greatest sensitivity and third greatest specificity of all the proposed indicators. As ibuprofen was never detected in concentrations above its practical quantitation limit, concentrations could not be correlated with virus concentrations.

Perchlorate had the highest sensitivity at 80% but also the lowest specificity. In contrast, triclosan, carbamazepine, sulfamethoxazole, and saccharin exhibited the highest specificity values and the lowest positive predictive and sensitivity values. None of the concentrations of these compounds exhibited a correlation with virus concentrations.

The indicator parameters calculated for acesulfame were generally in the middle of the pack, with no value exceptionally low or exceptionally high. B/Cl and ^3^H activities showed weak relationships with virus concentrations but acesulfame was the only potential indicator included in this study that exhibited a strong positive correlation with virus concentrations (R^2^ = 0.7729) (Figure 4.4). One exceptionally high virus concentration from PW3 was excluded from this analysis.
4.4 DISCUSSION

4.4.1 E. Coli and total coliforms as fecal indicators in a fractured bedrock aquifer

*E. coli* provided little insight as to the vulnerability of a well to virus contamination. As only 6 of the 22 wells sampled in this investigation exhibited *E. coli* and only one of these *E. coli* detections was accompanied by a virus detection, *E. coli* exhibited the second lowest positive predictive and sensitivity values of all the potential virus indicators examined in this project. *E. coli* was second only to chloroform for the lowest indicator parameters.

When *E. coli* was detected in the highest concentrations in PW10 and PW11 alongside detections of acesulfame, perchlorate, triclosan, and ibuprofen, it was expected that these wells would contain some...
of the highest concentrations of viruses out of all the wells examined in this investigation. Although these wells exhibited some of the highest concentrations of a diverse group of sewage-derived contaminants, PW10 and PW11 did not exhibit any signs of virus contamination. As both of these wells are within several meters of the Eramosa River, they are expected to be under the influence of surface water which may very well be the source of the above mentioned sewage-derived contaminants. If this is the case, it is important to note that there are several additional environmental factors that can influence the persistence of sewage-derived contaminants in surface waterbodies.

Contaminants of surface waterbodies are exposed to numerous environmental factors that are not present in the subsurface. While oxygen from the atmosphere can promote increased biodegradation, ultraviolet (UV) light from the sun can result in varying degrees of the inactivation and death of virus particles and bacteria, respectively. When DNA is exposed to UV light, dimerization of thymine bases can distort the conformation of the DNA’s double helix therefore leading to mutations, death, or inactivation of the microbe (Harris et al., 1986). While *E. coli* and viruses are both susceptible to this UV damage, *E. coli* has been found to be capable of enzymatic DNA repair after exposure to UV light (Novick and Szilard, 1949). A process known as photoreactivation is initiated when some bacteria are exposed to visible light after exposure to UV light. Photoreactivation ultimately repairs the cell’s DNA and promotes its reactivation. This process ultimately decreases the death toll UV light would have inflicted on *E. coli* communities. As this process is completed through enzymatic activity, only cells with functioning photoreactivating enzymes are capable of repairing their DNA after experiencing UV damage. As viruses tend to lack these repair enzymes, photoreactivation is more commonly associated with bacteria (Harris et al., 1986).

In one investigation, viruses were shown to require between 6 and 10 times the UV light dose required to inactivate 99.99% of *E. coli* (Harris et al., 1986). While *E. coli* may be more susceptible to initial UV
damage, its propensity for photoreactivation may play a role in its persistence in the samples from PW10 and PW11. Additionally, even if the \textit{E. coli} was from a fecal source, if the population contributing to the fecal input was not infected with viruses at the time of exfiltration or leakage, viruses will not be present in the sewage-influenced groundwater. While viruses were not detected in these wells during the sampling events of this investigation, they may still occur on a later date as fecal influences on the groundwater of Eden Mills are certain as indicated by the high concentrations of other sewage-derived contaminants in PW10 and PW11.

In contrast to \textit{E. coli}, total coliforms exhibited the second highest indicator parameters of all the potential indicators examined in this investigation. Out of the 11 total coliform detections, 5 occurred in wells with virus detections. The efficiency of total coliforms at predicting virus contamination was somewhat surprising as 4 of the 5 genera included in this group can be found naturally in the environment and are therefore not solely attributed to fecal sources. The one genus of total coliforms that is predominantly attributed to fecal sources is \textit{E. coli}, but as discussed above, it was not widely detected in this investigation. Despite the fact that total coliforms are associated with sources other than feces, they seem to be good indicators of virus occurrence.

Compared to other studies investigating the effectiveness of total coliforms and \textit{E. coli} as virus indicators at the field scale, the current study would suggest that these bacterial indicators are much less effective in fractured bedrock aquifers than aquifers comprised of different materials. For both total coliforms and \textit{E. coli}, the current study produced the second lowest sensitivity, lowest specificity, second highest PPV, and lowest NPV when compared to the statistics published by Lindsey et al. (2002), Borchardt et al. (2003), and Locas et al. (2007) (Table 4.5). The low NPV would suggest that the absence of total coliforms and \textit{E. coli} does not effectively predict the absence of viruses as almost half of the wells negative for total coliforms and \textit{E. coli} exhibited viruses. In contrast, Locas et al. (2007) claimed:
“the absence of viruses can be presumed in the absence of total coliforms...as illustrated by a negative predictive value of 100%.”

Comparing the experimental designs of the current study to those by both Borchardt et al. (2003) and Locas et al. (2007), the discrepancies in the indicator statistic may be attributable to the aquifer types examined by each investigation. While Locas et al. and Borchardt et al. each collected samples from aquifers with compositions ranging from clay or sand to rock or sandstone, the current investigation collected samples solely from fractured dolostone aquifers. As shown in Chapter 1, fractured bedrock aquifers are much more susceptible to virus contamination as these aquifers can promote rapid and extensive transport of viruses and colloids on account of the low effective porosity and reduced sorption associated with colloidal transport through fractured media. These colloidal particles, including both viruses and bacteria such as total coliforms and E. coli, can therefore be transported exceptional distances from the source. While viruses have been shown to persist in the cool temperatures of groundwater for over a year, bacterial indicators will typically only survive 4 to 12 weeks (Edberg et al., 2000). While a year of transport through a fractured bedrock aquifer could carry both the viruses and bacterial indicators several kilometers from a fecal source, the indicators will have died of long before virus inactivation occurs. The absence of total coliforms or E. coli in a fractured bedrock aquifer therefore does not aptly predict virus absence as these indicators exhibit significantly decreased longevity and persistence in comparison to viruses.
Table 4.5. Indicator parameters of total coliforms and *E. coli* from previous studies in comparison to the statistics generated in the current investigation.

<table>
<thead>
<tr>
<th>Study Aquifer Type</th>
<th>Total coliforms</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Porous and</td>
<td>Porous</td>
</tr>
<tr>
<td></td>
<td>Fractured media</td>
<td>Fractured media</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Specificity</td>
<td>71.7</td>
<td>50</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>7.1</td>
<td>50</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>91.7</td>
<td>100</td>
</tr>
</tbody>
</table>
Contrary to Locas et al. (2007) and Borchardt et al. (2003), Lindsey et al. collected virus and bacterial indicator samples solely from fractured bedrock aquifers. Interestingly, the sensitivity and specificity of total coliforms deduced from Lindsey et al. and the current investigation were within 10% of each other, respectively. However, the statistical gap between the two study’s PPVs and NPVs were significantly greater. This difference is likely attributable to the different virus detection methods used by Lindsey et al. and the current investigation. While the current study relied on quantitative polymerase chain reaction (qPCR) for virus quantification, Lindsey et al. used cell culture methods. These methods are notorious for resulting in underestimates of virus occurrence as they can only detect active viruses while qPCR can detect both active and inactive viruses. The low number of positive virus detections could therefore result in the low PPV and high NPV produced by Lindsey et al. (2002). As inactive viruses are no longer infectious, they do not pose a pathogenic threat to individuals consuming water contaminated with inactive viruses. Quantifying concentrations of both active and inactive viruses could therefore result in higher risk estimates, but it is still important to understand the vulnerability of an aquifer to colloidal transport whether the viruses are active or not as potentially more persistent viral strains or pathogens could contaminate the aquifer in the future.

4.4.2 Significance of sampling frequency

As discussed in the introduction, the EPA’s Ground Water Rule and the Safe Drinking Water Act in Ontario both require frequent monitoring of total coliforms in public water systems drawing from groundwater sources (EPA, 2006; Government of Ontario, 2002). While Ontario requires that any detection of *E. coli* or total coliforms is followed by corrective action, the Ground Water Rule only requires corrective action if fecal contamination is confirmed by the presence of *E. coli*, fecal enterococci, or coliphages. As this investigation has shown that 50% of virus occurrences were not accompanied by total coliforms and 90% of virus occurrences were not accompanied by *E. coli*, perhaps the indicators currently being used to indicate public health risks are not entirely sufficient. It is
important to note, however, that the sampling frequency in this investigation for total coliforms and *E. coli* was drastically lower than the frequency required for large public water systems. This could have resulted in artificially low detection frequencies of these indicator species. Virus samples were collected up to 6 times from some of the sampling wells while samples for bacterial indicators were only collected twice. Of the 10 virus occurrences, 4 occurred during sampling events when bacterial samples were also being collected. Of these 4 occurrences, only one of the virus detections was accompanied by a bacterial indicator.

The detection of Adenovirus A in MSW4 was accompanied by the detection of total coliforms at an average concentration of 0.043 CFU/100 mL. The goal of this part of the investigation was to determine the co-occurrence of viruses and traditional fecal indicators in a fractured bedrock aquifer. As the concentrations of the fecal indicators were expected to be low, sample volumes were increased to approximately 1000 mL in comparison to the 100 mL suggested by Method 1604 (EPA, 2002). The maximum concentration of the triplicate samples collected at MSW4 was 0.147 CFU/100 mL (1 colony/680 mL). As the minimum detection for 100 mL samples would be 1 CFU/100 mL, the detection in MSW4 is likely to have been missed if only the suggested 100 mL sample had been collected. With no fecal indicators present, the virus detection in MSW4 would have gone undetected. Therefore, even with the simultaneous collection of samples for viruses and bacterial fecal indicators, *E. coli* and total coliforms failed to indicate the presence of sewage-derived viruses. This would suggest that even with high frequency sampling of large public water systems, fecally-derived viruses could go undetected.

<table>
<thead>
<tr>
<th>Well ID</th>
<th>Virus</th>
<th>Date of Detection</th>
<th>Total coliforms</th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>MSW4</td>
<td>Adenovirus A</td>
<td>7/24/2012</td>
<td>7/24/2012</td>
<td>ND</td>
</tr>
<tr>
<td>PW2</td>
<td>Human Polyoma Virus</td>
<td>8/12/2012</td>
<td>7/15/2012</td>
<td>ND</td>
</tr>
<tr>
<td>MSW7</td>
<td>Adenovirus A</td>
<td>8/14/2012</td>
<td>7/17/2012</td>
<td>ND</td>
</tr>
<tr>
<td>PW7</td>
<td>Human Polyoma Virus</td>
<td>9/11/2012</td>
<td>7/18/2012</td>
<td>ND</td>
</tr>
<tr>
<td>MSW1</td>
<td>Human Polyoma Virus</td>
<td>11/7/2012</td>
<td>7/16/2012</td>
<td>7/16/2012</td>
</tr>
</tbody>
</table>

*Table 4.6. Virus detections as indicated by total coliforms and *E. coli*.***
The remaining 6 virus detections could only have been indicated by the detection of total coliforms or *E. coli* during one of the 2 separate sampling events for bacterial fecal indicators. As Public Health Ontario (2013) suggests that private well owners sample their water 3 times a year, the results for the fecal indicators that are presented here are more representative of sampling frequencies of private wells. Of the 6 virus detections that occurred without simultaneous sampling for fecal indicators, 4 of the wells that exhibited viruses had positive detections of total coliforms 1 to 4 months prior to the virus detection (Table 4.6). If these samples that exhibited bacterial indicators were representative of the samples collected by private well owners, they may have suggested that the wells were under the influence of a septic source and the well owners could take preventative actions to treat their water in case the fecal contamination lead to a virus contamination in the future. As 3 out of the 4 detections were of total coliforms and not *E. coli*, however, fecal contamination is not certain. Additionally, as discussed above, the sample volumes were 10 times greater than typical methods would suggest and so many of these detections may have actually presented as negative if the lower sample volume had been used. In the end, sampling twice within 6 months for bacterial fecal indicators resulted in only 1 positive indication by *E. coli* that virus contamination may occur. Of the remaining 9 detections of viruses, 4 were indicated by total coliform detections during sampling events prior to the virus detections, while only 1 virus detection occurred alongside a total coliform detection on the same date. This still left 5 of the 10 virus detections occurring without any indication that a fecal source may be impacting the well. Of these 5 unpredicted virus detections, 2 were from municipal supply wells and may have been indicated by bacterial indicators if more frequent bacterial sampling had been conducted. As most private well owners only have their water sampled 1 to 3 times a year, however, the results shown here are realistic. This would mean that 3 out of 5 (60%) virus detections in private wells will go undetected if only bacterial fecal indicators are used. A single sample from a private well only acts as a snapshot of the water influencing that well and so a sample testing negative for total coliforms and *E. coli* could
provide the well owner with a false sense of security in their water quality. Although 2 samples collected within 4 months of each other do not exhibit fecal indicators, this investigation reveals that viruses are capable of impacting the water between those sampling events.

In comparison to the variability of the bacterial results from one sampling event to the next, the artificial sweetener acesulfame showed much more consistent detections throughout this investigation. Although the concentrations of the artificial sweetener acesulfame showed some variability between sampling events, if a well was positive for the sweetener during one sampling event, it was also positive for the sweetener in the following sampling event. This would suggest that perhaps acesulfame is more persistent in groundwater or more present in human waste streams than bacterial indicators and may be a more reliable indicator of fecal contamination in private wells experiencing a low sampling frequency. Finding this artificial sweetener in a well will be irrefutable evidence that the well is under the influence of a septic source and so precautionary measures may be taken in order to protect the well owner and their family from any potential virus contamination events that may occur in the future. Upon discovering their well is under the influence of septic waste streams, the well owner can install at-home treatment such as a reverse osmosis system that will reduce any future virus concentrations to safe levels.

4.4.3 Sewage-derived contamination and groundwater age

As discussed in Chapter 3, the majority of groundwater samples from southern Wellington County’s fractured bedrock aquifers indicated that the sampling wells of these investigations are under the influence of modern water and are therefore vulnerable to contamination with sewage-derived contaminants. While the low tritium levels in some of the municipal supply wells would suggest that the groundwater was old, the very presence of tritium suggested that these waters were recharged after nuclear bomb testing began in the 1950s. MSW1 exhibited one of the lowest tritium activities at 1.9 TU and is therefore expected to produce some of the oldest water of this investigation. As reported in
Chapter 3, acesulfame was not detected in this well which was originally suggested to be due to the fact that this artificial sweetener was not made available to the public until 1988 and so would not be expected to be found in water with a low tritium activity and age greater than 25 years. The presence of human enteric viruses in this well, however, would suggest an influence of modern water as the persistence of virus genomes in groundwater is not expected to be longer than a few years (Charles et al., 2009; Ogorzaly et al., 2010). The absence of acesulfame in MSW1 must therefore not be due to a lack of modern water influencing the well.

From the remaining wells, viruses were detected in groundwater samples with tritium activities ranging from 13.1 to 1.6 TU therefore suggesting that viruses are occurring in groundwater with various ages. As mentioned above, virus genomes are not expected to persist for several decades and so it becomes even clearer that despite the low tritium activities suggesting somewhat older water in some of these wells, modern water is influencing their supply. As these wells have long open intervals starting less than a meter from the top of rock and only a few meters below ground surface, the influence of modern water on these wells is expected. The low tritium activities therefore indicate a large contribution of relatively old water potentially from the deeper regions of the aquifer, but as the water in these wells is a blend of both old and modern water from both the Guelph and Gasport Formations, the low tritium activities do not adequately reflect the well’s susceptibility to modern contaminants such as viruses.

4.4.4 Artificial sweeteners, pharmaceuticals, and other sewage-derived compounds as novel fecal indicators

As mentioned above, acesulfame detections were very consistent throughout the investigation. Although acesulfame was detected in high concentrations in a large number of wells, it did not exhibit the highest indicator parameter statistics. Despite these low indicator statistics, acesulfame did exhibit a strong correlation between its concentrations and the corresponding concentrations of viruses and may therefore be able to indicate the extent of virus or fecal contamination (Figure 4.4). Similarly, B/Cl
and tritium activities also exhibited positive correlations with virus concentrations. These relationships are all expected. Environmental B is generally associated with commercial bleach and cleaning products and higher tritium is consistent with modern water. Additionally, the only source of the artificial sweetener acesulfame in groundwater apart from landfills will be human wastewater. Higher values for each of these parameters would therefore indicate a higher sewage impact as well as the potential for higher virus concentrations should the wastewater be contaminated with viruses. As acesulfame had the greatest correlation with virus concentrations it would suggest that it may be the best indicator to estimate the magnitude of virus contamination.

Another interesting observation of acesulfame occurrences was that the 4 wells exhibiting the highest acesulfame concentrations, PW5, PW6, PW8, and PW9, did not exhibit measurable concentrations of viruses. The presence of other sewage-derived contaminants in PW6, PW8, and PW9 would support the fact that these wells are under the influence of septic waste streams and so the absence of viruses may be attributed to the fact that perhaps the population contributing to the septic plume was not infected with viruses at the time the waste was released. Another reason that acesulfame was present in high concentrations while viruses are not detected could be that acesulfame is exhibiting prolonged persistence in comparison to viruses. As little is known about the persistence of acesulfame in fractured bedrock aquifers, this could be a potential explanation for the sweetener being detected without viruses. As each of these wells is relatively shallow and exhibited relatively high tritium concentration suggesting extremely modern groundwater, the former explanation attributing the absence of viruses to the lack of infection is expected to be more accurate.

In the end, ibuprofen exhibited the greatest positive and negative predictive values, second greatest sensitivity, and third greatest specificity. In Figure 4.5 the true positive and false negative rates for each of the indicators are plotted against one another to illustrate the effectiveness of each of the potential
indicators to act as virus indicators. Indicators exhibiting the highest true positive rate and lowest false negative rate will plot closest to the upper left corner of the plot and will be the best virus indicator. Figure 4.5 would therefore suggest that ibuprofen and total coliforms are the best indicators of virus occurrences in the fractured bedrock aquifers of southern Wellington County, ON. It is important to note, however, that correlations between ibuprofen and virus concentrations could not be determined as the concentrations of ibuprofen were all below the method’s practical quantitation limit. This investigation could therefore not report on how ibuprofen concentrations relate to virus concentrations.

Figure 4.5. True positive rate versus false positive rate for each of the potential virus indicators. Ibuprofen and total coliforms exhibited the greatest true positive rate and lowest false positive rate and acted as the best virus indicators.

Triclosan, carbamazepine, and sulfamethoxazole were only detected in 3 wells each and therefore have exceptionally high negative predictive and specificity values. As viruses were also only detected in a few wells, the high number of non-detects of both the indicators and the viruses resulted in simultaneous non-detects. Each of these indicators was only detected once alongside a virus, but the low number of detections of each of the indicators resulted in greater statistical values. As the number of positive
detections for each of these indicators was low, triclosan, carbamazepine, and sulfamethoxazole are expected to be poor human enteric virus indicators in the fractured bedrock aquifers of southern Wellington County, ON.

Perchlorate was detected in 19 out of 22 sampling wells and exhibited the highest sensitivity (80%) despite its having numerous potential sources other than human wastewater. As discussed in Chapter 2, environmental perchlorate concentrations have been attributed to activities ranging from bomb and munitions manufacturing to household cleaning products. As the source of this contaminant is not solely sewer or septic wastewater as it is with enteric viruses, suggesting potential virus contamination based on the presence of perchlorate would be unreliable. Additionally, perchlorate has shown to persist for several decades in groundwater settings. With no attenuation processes like those influencing virus transport and persistence, water contaminated with perchlorate could be several decades old and any viruses that were once present will have been inactivated several years prior to the groundwater sample being collected. Perchlorate was ultimately detected in 11 wells where no viruses were detected, resulting in perchlorate having the lowest specificity of all the potential indicators included in this investigation. Due to its widespread nature, numerous potential sources, and long environmental persistence, perchlorate is not an ideal virus indicator as it is frequently present when there is no foreseeable threat of virus contamination.

4.5 CONCLUSIONS

In the end, *E. coli* proved to be one of the worst indicators of the presence of the 8 pathogens analyzed in Chapter 1. Additionally, the use of total coliforms and *E. coli* as viral indicators proved to be less efficient in the fractured bedrock aquifers of the current investigation than in aquifers comprised of different materials that were investigated by Borchardt et al. (2003) and Locas et al. (2007). The sensitivity and specificity of total coliforms (50% and 50%, respectively) were comparable to statistics
generated by Lindsey et al. (2002) in the fractured bedrock aquifers of Pennsylvania, and we are therefore confident in the results presented here.

Ibuprofen ultimately exhibited the best statistical relationships with virus detections suggesting that it may be the best indicator for potential virus contamination in fractured bedrock aquifers. As ibuprofen was not detected above the analysis method’s practical quantitation limit, this pharmaceutical could not be used to indicate the extent of virus contamination. Acesulfame concentrations, on the other hand, showed relatively strong correlations with virus concentrations but the presence of statistical outliers in Figure 4.4 force one to question the true value of this relationship between acesulfame and virus concentrations. As acesulfame is not a naturally occurring compound, its presence in groundwater is irrefutable evidence of human wastewater, but using acesulfame to indicate the potential presence of viruses is not expected to be strong based on its low parameter statistics. The current investigation would ultimately suggest that the tandem use of total coliforms and ibuprofen could provide the best indication of an aquifer’s susceptibility to contamination with sewage-derived viruses. Alone, total coliforms indicated 50% of the virus occurrences in the current investigation, while ibuprofen indicated 60%. If the monitoring of these two compounds were to be combined, 70% of the virus occurrences in this investigation would be indicated.

It is important to note, however, that each of the three suggested indicators above do not meet all of the pillars described in Table 4.1 and exhibited some low values when their indicator parameters were calculated. Total coliforms can be derived from several sources other than leaking sewers and septic systems and may therefore lead to the false identification of a fecal source. The persistence and transport properties of both ibuprofen and acesulfame are poorly understood for fractured bedrock aquifers and their similarity to the characteristics of virus transport and persistence in groundwater can only be speculated. Additionally, the analysis for pharmaceuticals and artificial sweeteners are not
widely available and are therefore not cost-effective. The combination of these 3 new indicators could, however, overcome their individual shortcomings and provide well operators and owners with the best indication of a well’s susceptibility to contamination with viruses.

For ibuprofen and acesulfame to become leading indicators of virus and fecal contamination, future investigations will need to establish each of these compounds’ transport and persistence properties in various types of aquifers and compare them to those properties of a wide range of viruses and pathogens. Additionally, the efficiency of these compounds at indicating virus detections should be compared to abilities of traditional fecal indicators monitored at the higher sampling frequencies required for large public water systems. Future investigations should therefore collect monthly samples for viruses along with the proposed indicators to determine their simultaneous co-occurrence and indicating abilities.

Despite the high sampling frequency for bacterial fecal indicators in large public water systems, the sampling schedule of the current investigation mimics sampling of many private wells. As only 2 of the 5 wells that exhibited viruses exhibited total coliforms prior to the virus detection, the other 3 detections would have gone unnoticed. As acesulfame showed relatively consistent concentrations between sampling events, an annual sample for this artificial sweetener in private wells could indicate the potential for virus contamination throughout the rest of the year. Despite its current high cost, a single acesulfame analysis of private well water could lead to the prevention of the infection of individuals consuming water from private wells with waterborne viruses and pathogens.

5.0 THESIS DISCUSSION AND CONCLUSIONS

5.1 Vulnerability of fractured bedrock aquifers to sewage-derived virus contamination
In the end, fractured bedrock aquifers were found to exhibit an expected high vulnerability to contamination with sewage-derived human enteric viruses. While previous studies by Borchardt et al
(2003) and the EPA (2006) would suggest 10 and 27% of private and municipal wells, respectively, will be susceptible to virus contamination, temporal virus samples from each of the 22 wells resulted in 10 wells (45%) exhibiting human enteric viruses including human polyomavirus, adenovirus A, and G2 norovirus. Of the 11 private wells sampled, 5 (45%) exhibited viruses while 5 (62.5%) of the municipal supply wells exhibited viruses.

Although porous sediment were present in the form of glacially deposited sediments, the underlying fractured bedrock aquifers of southern Wellington County were still highly susceptible to contamination with human enteric viruses. The type of overburden present at the wellhead seemed not to influence virus detection rates, but overburden thickness, the length of a well’s open interval, and precipitation exhibited weak correlations with virus occurrences (R = 0.6856, R = -0.7587, and R = -0.729 to -0.396, respectively). While downward gradients are frequently present in southern Wellington County, much of the flow through the fracture network is horizontal. This therefore makes the type and thickness of overburden located directly at the wellhead less significant. Additionally, as the predominant source of human enteric viruses are buried sewers and septic systems, the presence of any overburden sediments that may be able to provide some source of attenuation of contaminants before reaching the horizontal flow regime of the underlying aquifer also becomes less significant as the amount of material separating the contaminants from the aquifer is significantly reduced when the source is buried several feet into them.

A long open interval of a well can result in reduced virus detections and concentrations as these wells are typically much deeper and require the viruses to travel through much more geological material before reaching the well at depth. This will result in a greater time of travel and will promote virus inactivation before the virus reaches the open interval. Low concentrations at the deeper portions of
the well can result in overall dilution of higher concentrations that may be present at shallower depths and therefore result in lower total virus concentrations in blended water drawn from the well.

The relatively inconclusive and variable results from the analysis of precipitation’s influence on virus detection rates suggested that perhaps low precipitation can result in higher virus concentrations within one month, but as these results are contrary to results published by Bradbury et al. (2013) more stringent investigations need to be conducted to thoroughly understand the influence of precipitation on virus occurrences in groundwater.

In comparison to previous large-scale investigations of virus contamination of groundwater, on a per well basis the detection rates of the current study were much higher. Part of this is attributable to the fact that the current investigation collected temporal samples and could therefore capture virus occurrences despite their temporal and ephemeral occurrence in groundwater. Another reason this study demonstrated high detection rates on a per well basis could be due to the fact that samples were taken from a fractured bedrock aquifer where cool groundwater temperatures can promote virus longevity and rapid groundwater velocities can increase virus transport from sewage sources. Additionally, the qPCR method that was used for virus detection has been shown to exhibit enhanced sensitivity in comparison to cell culture techniques as it can detect both active and inactive viruses. For this reason, detection rates determined from qPCR results can significantly overestimate the risk associated with virus contamination of an aquifer as many of the detected viruses may not actually be infectious. Due to the finite amount of time that a virus can remain active in groundwater, detections of active viruses in a groundwater sample can provide irrefutable evidence of rapid contamination of an aquifer by a septic source. Although the current study did not include infectivity analyses, the genomes of human adenovirus and polyomavirus have exhibited degradation in groundwater in less than 2 years and therefore their detection can still suggest rapid contamination of a supply aquifer. As it is still
unclear as to the persistence of the GII norovirus in groundwater its detections can only be used to suggest the potential risk of gastrointestinal illness to individuals consuming impacted water and cannot be used to comment on time of travel.

5.2 Vulnerability of fractured bedrock aquifers to emerging sewage-derived contaminants
After 2 rounds of sampling for artificial sweeteners and single sampling events for pharmaceuticals and volatile organic compounds (VOCs), well construction and depth as well as overburden thickness and type showed to have little influence on the occurrence of these compounds as 20 out of the 22 wells exhibited at least one of the sewage-derived contaminants. While 18 of the 38 sewage-derived compounds were detected at least once, the most prevalent contaminants were the artificial sweetener acesulfame (number of wells: 14; maximum concentration: 4366 ng/L), perchlorate (19; 625 ng/L), ibuprofen (10; <PQL), chloroform (14; 2.46 µg/L), and PCE (14; 0.40 µg/L). As investigations conducted by Van Stempvoort et al. (2013) and Buerge et al. (2009) have documented the widespread occurrence and persistence of acesulfame in groundwater, its presence in the aquifers of southern Wellington County are not surprising. In contrast, ibuprofen has consistently been shown to exhibit susceptibility to biodegradation, but in the current study it was found in the highest number of wells of all the pharmaceutical compounds analyzed. The low concentrations are therefore suspected to be due to ibuprofen’s affinity for biodegradation, but the pervasive occurrence is most likely attributable to the widespread use of this anti-inflammatory pharmaceutical. In contrast, previous large-scale studies by Barnes et al. (2008) and Loos et al. (2010) would suggest carbamazepine, sulfamethoxazole, and triclosan to be the most persistent and prevalent pharmaceuticals in groundwater, but each of these compounds was detected in only 3 wells with maximum concentrations of 23.05 ng/L, 4.17 ng/L, and <PQL, respectively. While both Barnes et al. (2008) and Loos et al. (2010) found these pharmaceutical compounds in a high number of groundwater samples, each of the drugs has been shown to be susceptible to reductions in their concentrations during transport (Godfrey et al., 2007; Xu et al., 2009).
and so their low detection rates in the current study could be due to various attenuating mechanisms acting on these compounds.

5.3 Using emerging sewage-derived compounds as indicators of groundwater age and contamination
Isotopic analyses revealed a range of groundwater ages in the 22 sampling wells. Deep municipal supply wells exhibited the greatest depletion of $^{18}$O and $^2$H while shallower private wells with lower pumping rates exhibited isotopic concentrations consistent with more modern precipitation. The use of the artificial sweetener acesulfame alongside tritium activities suggested that higher concentrations of acesulfame in shallower wells are consistent with lower tritium concentrations and recently recharged groundwater. The results suggest that analyzing acesulfame concentrations could be a new method of indicating relative groundwater ages.

When the detections of human enteric viruses were statistically compared to the occurrences of the above wastewater contaminants, ibuprofen exhibited the highest indicator parameters (Positive predictive value (PPV) = 60%; Negative predictive value (NPV) = 67%; Sensitivity = 60%; Specificity = 67%). While acesulfame did not exhibit indicator parameters as high as ibuprofen (PPV = 36%; NPV = 38%; Sensitivity = 50%; Specificity = 25%), concentrations of this artificial sweetener exhibited the best correlation with virus concentrations ($R^2 = 0.7729$).

Collection and analysis of both total coliforms and E. coli resulted in comparatively lower indicator parameters (total coliforms: PPV = 45%; NPV = 55%; Sensitivity = 50%; Specificity = 50%; E. coli: PPV = 17%; NPV = 44%; Sensitivity = 10%; Specificity = 58%) than previous studies investigating the effectiveness of these traditional fecal indicators would suggest. Of the 5 privates wells to exhibit human enteric viruses, only 2 (40%) exhibited signs of total coliforms and none showed signs of E. coli. In contrast, ibuprofen and/or acesulfame was detected in 4 out of the 5 private wells exhibiting viruses. If total coliforms, ibuprofen, and acesulfame were used in tandem, 100% of the virus detections would
have been indicated. Similarly, in the municipal supply wells, 4 out of the 5 wells to exhibit viruses would have been revealed if ibuprofen had been used as the fecal indicator.

While the sampling for artificial sweeteners twice and pharmaceuticals once is similar to sampling frequencies of total coliforms and *E. coli* in private wells, the current standards outlined by the Groundwater Rule in the US and the Safe Drinking Water Act in Ontario require municipal drinking water supplies to experience much more frequent sampling due to much higher pumping rates and volumes. For the true effectiveness of ibuprofen and acesulfame to act as fecal and virus indicators to be compared to total coliforms and *E. coli*, future investigations will need to collect several rounds of simultaneous samples for viruses and the various potential indicators in order to determine the temporal variability of each of the potential indicator’s relationship with virus occurrences.

In the end, fractured bedrock aquifers were found to be at a high risk to contamination with sewage-derived human enteric viruses and several anthropogenic wastewater compounds. While acesulfame was suggested to be a very strong indicator of sewage-derived contamination, the combination of ibuprofen and total coliforms are suggested to be the best indicators of virus contamination of a fractured bedrock aquifer.
6.0 LITERATURE CITED

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California Department of Toxic Control. 2005. Section 3: Sources of perchlorate in the environment.


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Appendix I

Research Chapter A: Virus sampling protocol

0.0 Preparations

0.1 Prepare 0.52% chlorine
   1. In 5 L carboy, add 500 mL of household bleach to 4250 mL of DI H₂O.
   2. Adjust the pH of the solution to 6-7 with 1 M HCl.
   3. Bring to 5 L with DI H₂O.

0.2 Prepare sodium thiosulfate
   1. Add 20 g sodium thiosulfate to 20 L of DI H₂O and mix well.

0.3 Decontaminate sampling equipment
   1. Place the inlet and outlet hoses in the 0.52% bleach solution and turn on pumps, recirculating the chlorine solution in the container until bleach is contacting all surfaces. Turn off the pump and let soak for 30 min. Pump the hoses dry.
   2. Place the inlet and outlet in the sodium thiosulfate solution and pump until the solution is contacting all surfaces. Turn off the pump and let soak for 5 minutes. Pump the hoses dry.
   4. Rinse equipment thoroughly with DI H₂O and store for next use. Reduce exposure to air by storing tubing in plastic bag or sealing ends with foil or paraffin seal.

1.0 Sample Collection:

1.1 To obtain a water sample, use an outdoor faucet or the faucet near the pressure tank that bypasses any softener or filtering devices.

1.2 While wearing sterile gloves, wipe the faucet with 70% EtOH.

1.3 Connect sample tubing in accordance with Figure 1, with discharged water directed to ditch, swale, or lawn.
1.4 Purge water until the field parameters (pH, conductivity, temperature, oxidation-reduction potential, and dissolved oxygen) are stable.

1.5 Measure and record field parameters.

1.6 Connect glass wool filter and flowmeter in accordance with Figure 2.

Figure 6. Preliminary sampling set-up for glass-wool filtration.

Figure 7. Sampling setup for glass-wool filtration,
1.7 Record the initial flowmeter reading before turning on the tap and achieving a flow rate no greater than 4 L/minute.

1.8 If the pH is above 7.5, open the acid valve and turn on the peristaltic pump to establish a constant pH between 6.5 and 7.0.

1.9 Filter 200-1500 L total.

1.10 Turn off the acid pump, close the tap and record the date, time of day, final flow rate, and total volume.

Disconnect the glass wool filter and drain excess water. Label the filter and place it into a sterile plastic bag before putting it into a cooler with ice packs.
### Table 3. Results from matrix recovery control. FCSV = final concentrated sample volume.

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<th>Giardia</th>
<th>Enterovirus</th>
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<td>% Recovery</td>
<td>Cal. Conc.</td>
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<td>1.70E+01</td>
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</table>
Appendix II Figure 1. Figures illustrating well selections methods. Elevations of sanitary sewer inverts were plotted in relation to the top of bedrock. Overburden thickness was also plotted to further identify areas of high risk to contamination.

Appendix II Figure 2. Figures illustrating well selections methods. MOE well records were plotted in Wellington County in order to identify at-risk wells.
Figure 3. Plots of precipitation and pathogen occurrence in A) Guelph and B) Elora show no observable correlation with one another.
Figure 4. Correlation plot of overburden thickness versus pathogen concentrations weakly suggesting that thicker overburden will lead to higher pathogen concentrations. Two outliers and non-detects were excluded from correlation and trendline calculations.

Figure 5. Correlation plot of length of open interval versus pathogen concentrations weakly suggesting that longer open intervals will lead to lower pathogen concentrations. Two outliers and non-detects were excluded from correlation and trendline calculations.
Appendix III

Research Chapter B: Standard Operating Procedures

Standard Operating Procedure for Pharmaceutical, VOC, Isotope, General Chemistry, and Artificial Sweetener Sample Collection

0.0 Preparations

0.4 Prepare **0.52% chlorine**
   1. In 5 L carboy, add 500 mL of household bleach to 4250 mL of DI H₂O.
   2. Adjust the pH of the solution to 6-7 with 1 M HCl.
   3. Bring to 5 L with DI H₂O.

0.5 Prepare **sodium thiosulfate**
   2. Add 20 g sodium thiosulfate to 20 L of DI H₂O and mix well.

0.6 **Decontaminate sampling equipment**
   1. Soak all sampling hoses and attachments in 0.52% chlorine solution for 30 minutes. After 30 minutes, remove hoses and attachments from solution.
   2. Soak all sampling hoses and attachments in the sodium thiosulfate solution for 5 minutes. After 5 minutes, remove hoses and attachments from solution.
   3. Rinse equipment thoroughly with DI H₂O and store for next use. Reduce exposure to air by sealing hoses and attachments with Parafilm.

0.7 **Wash 500 mL amber Boston round bottles:**
   1. Wash bottles with acetone.
   2. Rinse bottles with hexane and allow to evaporate.

1.0 Sample Collection:

1.11 To obtain a water sample from private wells, use an outdoor faucet or the faucet near the pressure tank that bypasses any softener or filtering devices. For large supply wells, collect sample from a raw water faucet. For collection from monitoring wells, use Grundfos Redi-Flo2 electrical submersible pump connected to a Honda EU2000KC2 generator, ensuring that all hoses and pump heads have been decontaminated using the instructions above.

1.12 While wearing sterile gloves, wipe the faucet with 70% EtOH.
1.13 Connect sample tubing in accordance with Figure 1, with discharged water directed to ditch, swale, or lawn.

Figure 8. Preliminary sampling set-up for glass-wool filtration.

1.14 Purge water until the field parameters (pH, conductivity, and temperature) are stable.

1.15 Measure and record field parameters.

1.16 Disconnect flow-through cell and begin collecting samples.

**Pharmaceutical sample collection**

1. Fill one 500 mL amber Boston round bottle with a water sample for pharmaceutical analyses.
2. Transport on ice and store at 4°C for no longer than 2 weeks.
3. Collect 1 duplicate for every 10 samples collected.

**VOC sample collection**

1. Fill 3 40 mL VOA bottles with water allowing meniscus to form at bottle neck to ensure that no air is trapped when the cap is put on.
2. Invert the bottle to ensure no air bubbles are present and seal the cap with Teflon tape.
3. Transport samples on ice and store at 4°C.
4. Accompany samples with trip and storage blanks.

**Isotope sample collection**
1. Fill one 500 mL HDPE bottle with water sample ensuring no air bubbles are present.
2. Store at 4°C.

**General chemistry sample collection**

1. Fill one 500 mL HDPE bottle with water sample for general water parameter analysis.
2. Attach 0.45 μm filter in line.
3. Fill one acidified 250 mL HDPE bottle provided by MAXXAM Laboratories to the designated fill line for metals analysis.
4. Collect field duplicates for each batch of samples.

**Artificial sweetener sample collection**

1. With 0.45 μm filter still attached, collect water sample into 20 mL plastic scintillation vial, leaving approximately 10% head space.
2. Transport samples on ice and freeze upon returning to lab.
3. Collect one field duplicate for every 10 samples collected.
Standard Operating Procedures for Multi-Residue Extraction of Acidic, Phenolic and Neutral Compounds

Sample Preparation

1. Vacuum filter an appropriate volume of aqueous sample (e.g. wastewater, surface water) through a 1.5µm glass-fiber filter.
2. Pour filtered sample into glass container. If there are 3 replicates, prepare 3 separate aliquots.
3. Adjust the pH of the samples to pH=8.0 by adding NH₄OH (1.0 % for large amount, 0.125% for small amount).
4. Spike the samples with surrogate standards and mix.
5. The water samples are now ready to be extracted by SPE, but the SPE cartridges must first be pre-conditioned.
6. Clean all equipment before extracting a different sample.

SPE Cartridge Pre-conditioning

1. Place the cartridges (OASIS MAX SPE cartridges, 6 mL, 500 mg) on top of the SPE manifold and begin to condition each of them sequentially with 6 ml MeOH, 6 ml 0.1 M NaOH in water, and 6 ml distilled water. DO NOT LET THE CARTRIDGES GO DRY.
2. Once conditioned, the samples can be passed through the cartridges.

Extraction

1. The Teflon tubes that connect the samples to the SPE manifold must first be cleaned with methanol, and then rinsed with HPLC water.
2. Attach one clean tube to each of the cartridges, and then place the other end in one of the samples. Clearly label all of the cartridges.
3. The water samples should be passed through the SPE cartridges at a rate of < 5 mL/min (drop by drop will be good).
4. Rinse each of the sample bottles with approx. 10 mL of pH=8.0 distilled water and pass the rinsings through the cartridge.
5. At this point, ensure the solid phase material is kept wet by trapping the last rinse in the cartridge. Once all samples have passed through, let the cartridges go dry for a one minute in order to remove unwanted water.

6. The Teflon tubing should be quickly removed. Wash the cartridge with 2 mL of pH=8.0 water. Let the cartridge aspirate to dryness for about 20 min with vacuum.

7. A 15 mL collection tube should be placed under the sample in order to collect the eluate. Small Teflon “Guides” must be placed under the top of the manifold, so the samples are properly guided into the 15 mL collection tube.

8. Elute the cartridge as follows:
   a) Elute the cartridge with 2 ml MeOH at a flow rate of about 1 ml/min (drop by drop).
   b) Then, elute the cartridge with 3 × 3 mL 2% formic acid in MeOH a flow rate of about 1 ml/min (drop by drop). After elution, aspirate the cartridge to dryness.

   AT THIS POINT, SEND THE EXTRACTS TO TRENT

9. Evaporate to almost dryness, solvent exchange to methanol (add 1-2 ml methanol), evaporate to near dryness again. Reconstitute the sample with 0.4 mL methanol for analysis.

10. Analyse by LC-MS/MS.
Methods for liquid chromatography and tandem mass spectrometry for pharmaceutical analysis

The pharmaceuticals were analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) with an electrospray ionization (ESI) source using either an AB Sciex API 3000 instrument for compounds detected in positive ion mode or an AB Sciex QTrap 5500 instrument for compounds detected in negative ion mode (Table 1). All analyses were completed at the Water Quality Centre at Trent University under the supervision of Prof. Chris Metcalfe.

The API 3000 system was equipped with a Series 200 autosampler from Perkin Elmer (Waltham, MA, USA), and pumps (LC-10AD), degasser (DGU-14A) and system controller (SCL-10A) from Shimadzu (Columbia, MD, USA). Analytes were separated chromatographically using a Genesis C18 column (150 mm x 2.1 mm ID; 4 µm particle size) and a guard column (Genesis C18, 10 mm x 2.1 mm ID; 4 µm); both purchased from Chromatography Specialties (Brockville, ON, Canada). The LC mobile phases in gradient elution were (A) water (100%) with 0.1% acetic acid and (B) acetonitrile (100%) with 0.1% acetic acid. The precursor and product ion transitions for multiple reaction monitoring (MRM) of the analytes detected in positive ion mode and their corresponding labelled surrogates are listed in Table 1. For quantification, an external standard method with a five-point calibration curve was used, and the data were adjusted according to the response for the surrogate internal standards in order to compensate for matrix effects.

For determination of analytes in negative ion mode, the Q-Trap 5500 instrument was equipped with an Agilent 1100 series (Mississauga, ON, Canada) separation system, including degasser, binary pump and auto sampler. The analytes were separated chromatographically using a Genesis C-18 column and a guard column of the same stationary phase (Chromatographic Specialties). The LC mobile phases for gradient elution were the same as described above. MRM detection was performed using the precursor and product ion transitions and their corresponding labelled surrogates listed in Table 1. An external standard method with a five-point calibration curve was used for quantification, and the data were adjusted according to response for the surrogate internal standards.
Quantification was performed using an internal standard method, and a stable isotope-labelled compound was used as an internal standard for each analyte. The curve was plotted by the peak area ratios of the target analyte and the internal standard against the concentration ratios of the analyte and the internal standard. The analyte concentration ranged from 2 to 1000 ng/ml, and the internal standard concentration was constant (50 ng/ml) in the calibration solutions. The stable isotope-labelled internal standards were spiked into the samples prior to extraction in order to compensate for the mass loss during the sample preparation and the matrix effects during LC-MS/MS analysis (Zhao and Metcalfe, 2008).

Three replicate samples from each site were prepared for analysis to evaluate the reproducibility of the analytical method. Laboratory procedural blanks spiked with labelled surrogates were processed and analysed exactly as described for the field samples. The Lab Blanks were run at a rate of every 8 field samples. No target analytes was detected in the laboratory procedural blanks at concentrations higher than the limits of detection. Good Laboratory Practice was used in the preparation and analysis of all samples. The Limits of Detection (LODs) and Limits of Quantitation (LOQs) for each analyte were determined by spiking standards into dechlorinated tap water at concentrations that produced a signal <10x the baseline signal. The LODs and LOQs in the water matrix (Table 1) were calculated as, respectively, 3x and 10x the standard deviation (n=3) of the signal monitored at the retention time for each analyte.

Reference:
### Appendix IV

#### Research Chapter B: Excluded Tables and Results

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Appendix IV. Table 1. Summary of analysis results per well. x > PQL. MDL < o < PQL. Pharmaceuticals found in 10 out of 22 wells (45%); 4/8 MSWs (50%); 6/11 PWs (54%); 0/3 MWs. Acesulfame in 14/22 wells (64%); 1/8 MSWs (13%); 10/11 PWs (91%); 3/3 MWs. 7/10 (70) pharmaceutical detections co-occuring with VOCs. 10/14 (71%) Acesulfame detections co-occuring with VOCs.
Preparations:

0.1 Prepare agar and plates for *E. coli* and total coliform analyses according to label directions

1. Add Oxoid CM 1038 Differential Coliform Agar to water.
2. Dissolve agar by heating the mixture to 100°C while stirring constantly.
3. Add 12 mg Cefsulodin.
4. In a sterile hood, pour enough agar into each plate so that the entire area of the plate is covered by agar.
5. Store in refrigerator.

0.2 Prepare 10x Phosphate Buffered Saline (PBS)

1. Dissolve the following in 800 mL of distilled water:
   - 80 g NaCl
   - 2.0 g KCl
   - 14.4 g Na$_2$HPO$_4$
   - 2.4 g KH$_2$PO$_4$
2. Adjust pH to 7.4.
3. Adjust volume to 1 L with additional distilled water.
4. Sterilize by autoclaving.
5. Dilute to 1xPBS with sterile distilled water.

0.3 Autoclave *E. coli* and total coliform sample bottles and filtration apparatuses.

Filtration and Plating:

1. Sterilize work station and gloved hands by spraying down with 70% EtOH.
2. Label agar plates according to Figures 1 and 2.
3. Shake sample bottle 25+ times.
4. Measure volume of water in bottle #1 with sterilized graduated cylinder and record volume.
5. Using flamed forceps, place sterile filter membrane on filtration apparatus and filter above volume of water using vacuum pump.
6. Follow sample with filtration of 30 mL of sterile PBS buffer x2, making sure that buffer touches all surfaces of upper filtration unit. Using a sterile beaker is useful in this step.
7. Using flamed forceps, remove filter from filtration apparatus and place on appropriately labeled DC agar plate.
8. Empty filtration apparatus flask into sink.
9. Rinse graduated cylinders with PBS buffer x1 followed by sterile DI water x3.

   **For proper rinse, place approximately 10 mL of rinse solution into graduated cylinder and cover the top of the graduated cylinder with a sterilized, gloved hand and rock the cylinder back and forth while spinning it to make sure that the solution has touched every interior surface of the graduated cylinder.

10. Rinse filtration apparatus with 30 mL of sterile PBS buffer, making sure that buffer touches all surfaces of upper filtration unit. Using a sterile beaker is useful in this step.

11. Place sterile filter membrane on filtration apparatus and filter 100 mL of sterile DI water using vacuum pump.

12. Remove filter from filtration apparatus and plate on DC agar plate as “Control #1”.

13. Empty filtration apparatus flask into sink.

14. Repeat Steps 1-12 for Bottle #2, ending with “Control #2” on separate plate.

15. Repeat Steps 1-7 for Bottle #3.

16. Rinse graduated cylinders with 70% EtOH x1 followed by sterile DI water x3.

17. Sterilize work station and gloved hands by spraying down with 70% EtOH.

18. Assemble new autoclaved filtration apparatus for next sampling site.

19. Repeat Steps 1-15 for all sampling sites.

20. Incubate plates at 35°C ± 0.5°C, with approximately 90% humidity for 24 hours.

21. Count pink colonies and record as total coliforms.

22. Take picture of plate with scale and label.

23. Incubate for additional 24 hours.

24. Count pink colonies and record as total coliforms.

25. Count blue colonies and record as E. coli.
Figure 1. Summary of method.

1. Filter 1000 mL
2. Rinse with 30 mL PBS
3. Plate filter
4. Empty filtration apparatus flask.
5. Rinse graduated cylinders with sterile PBS buffer
6. Rinse graduated cylinders with sterile DI water x3
7. Rinse filtration apparatus with sterile PBS buffer
8. Filter 100 mL of sterile DI water and plate as C_l #1
9. Empty filtration apparatus flask.
10. Repeat for Bottle #2, ending with
11. Repeat for Bottle #3 (no control).
12. Rinse graduated cylinders with 70% EtOH
13. Rinse graduated cylinders with sterile DI water x3
Figure 2. Plate labeling system (bottom view).
### APPENDIX VI

#### Research Chapter C: Excluded Table

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Appendix VI. Table 1. Summary of analysis results per well. \( x > \text{PQL. MDL} < \text{MDL} \). Viruses detected in 10/22 (45%) wells; 5/8 (62.5%) MSWs; 5/11 (45%) PWs. *E. coli* detected in 6/22 (27.3%) wells; 1/8 (12.5%) MSWs; 3/11 (27.3%) PWs. Total coliforms detected in 11/22 (50%) wells; 3/11 (27.3%) MSWs; 6/11 (54.5%) PWs; 2/3 (66.7%) MWs. Pharmaceuticals found in 10 out of 22 wells (45%); 4/8 MSWs (50%); 6/11 PWs (54%); 0/3 MWs. Acesulfame in 14/22 wells (64%); 1/8 MSWs (13%); 7/10 (70%) PWs (91%); 3/3 MWs. 7/10 (70) pharmaceutical detections co-occurant with VOCs. 10/14 (71%) Acesulfame detections co-occurant with VOCs.