INVESTIGATION OF MOLECULAR MARKERS TO IDENTIFY SOURCES OF NITRATE CONTAMINATION IN GROUNDWATER

by

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ABSTRACT

Molecular markers were investigated as potential tools for differentiating between the sources of elevated nitrate-N in the Hopington AB Aquifer. Residential use (septic systems) and agriculture (livestock) have been identified as key land use activities, which overlay the Hopington AB Aquifer, and thus possible contributors of nitrate-N to the groundwater. Harmful levels of nitrate-N concentrations above the drinking water limit of 10 mg/L have been detected in the well of a private resident (14 mg/L) and spring water (17 mg/L), which were located on the aquifer.

DAS 1 (a diaminostilbene) and DSBP (a distyrylbiphenyl) are fluorescent whitening agents (FWAs), which in the Fraser Valley are present in 3 out of 4 popular laundry detergents, and have been detected in domestic wastewater at concentrations of 7.84 and 2.36 µg/L respectively; thus they are suitable markers for septic systems in Langley. Sulfamethazine, which is an antimicrobial approved solely for veterinary use in Canada, is widely used in the livestock industry.

Good maximum recoveries for DAS 1 (60%), DSBP (125%) and sulfamethazine (125%), coupled with low method detection limits ranging from of 0.01 – 0.04 µg/L implied that solid phase extraction (SPE) and high-performance liquid chromatography (HPLC) with an ultra violet (UV) detector were adequate for the determination of the molecular markers.

The detection of DAS 1 (3.14 µg/L) and DSBP (0.05 µg/L) in the final effluent at a BNR (biological nutrient removal) pilot plant suggested that the FWAs were not completely removed by wastewater treatment processes including primary clarification, biological (aerobic and anaerobic), and membrane filtration; thus, once released, these FWAs may persists in the environment. In this study, DAS 1 (0.01 – 0.13 µg/L) was detected in 4 wells belonging to private residences, which were located on the Hopington Aquifer. DAS 1 (0.05 µg/L) and DSBP (0.02 µg/L) were also detected in spring water, which were located down gradient of septic systems. These results suggested that septic tank systems have contributed to the overall nitrate in the aquifers. The non-detection of the FWAs at the two control sites (Hopington C and Abbotsford) confirmed the specificity of DAS 1 and DSBP in relation to source.
Overall, the FWAs exhibited fairly conservative behaviours due to their abilities to be source specific and persistent in the environment. As a result, they are useful tools for the identification of septic system sources of contamination in the environment. Sulfamethazine was not detected in any of the Hopington AB wells; however, further research is needed in order to determine if this antimicrobial was an appropriate molecular marker for livestock activities.
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In memory of my loving nephew

Nicholas Anthony Mitchell
1 INTRODUCTION

Worldwide, groundwater is a vital resource for many communities for both economic and domestic purposes, and often it is the same users of the aquifers who are responsible for the degradation of the water quality (Close et al., 1989). In British Columbia (B.C.), groundwater is an important source of potable water for many residents. Up to 23% of the population depends upon groundwater as a source of drinking water. In smaller semi-rural communities such as the Township of Langley, B.C, an estimated 27% (23,000) of the residents depend upon groundwater from private wells as their sole water resource for domestic purposes; currently there are about 5000 private wells located in the Township (Stjepovic, 2004).

According to Close et al., (1989), aquifers particularly unconfined and in semi-rural areas are frequently subjected to pollution from various sources, including septic tanks and agricultural activities; therefore, well water abstracted from these aquifers particularly for domestic use may pose serious health problems. One of the many contaminants in elevated concentrations, which can adversely affect groundwater quality and ultimately human health, is nitrate.

Knobeloch et al., (2000) reported that nitrate contaminated water is the most common environmental pathway associated with a fatal health condition in infants called methemoglobinemia or “blue baby syndrome”. Blue baby syndrome occurs when ingested nitrate is reduced to nitrite, which binds to the hemoglobin (Fe$^{2+}$) (Ward et al., 2005). Subsequently, hemoglobin (Fe$^{2+}$) in the red blood cells is oxidized to methemoglobin (Fe$^{3+}$) (Knobeloch et al., 2000). The health risk to infants is that methemoglobin levels above 10% interferes with the oxygen-carrying capacity of the blood (Ward et al., 2005), which can result in cyanosis symptoms, such as bluish colored skin, irritability and lethargy. In addition, methemoglobin levels > 50% in untreated infants can quickly result in coma and death (Knobeloch et al., 2000). As a result, the recommended health-based guideline for nitrate in drinking water is 50 mg/L, which is equivalent to 11 mg/L as nitrate-N (WHO, 2004). In Canada and the USA, the Maximum Acceptable Concentration (MAC) and the Maximum Contaminant Level (MCL) for nitrate-N in drinking water is 10 mg/L respectively.
The nitrate-N concentration in most ambient groundwater in British Columbia is less than 1 mg/L, therefore, concentrations above 3 mg/L usually signifies anthropogenic impacts (BCMoE, 2007). The results of water quality studies on the Hopington Aquifer suggested that there was an ongoing anthropogenic impact on the groundwater, which in some cases at elevated levels, which can potentially harm young infants. For example, based on historic nitrate-N data for the periods 1994-2004, out of a total of 473 sampling events for the Hopington Aquifer, 38% of the data were ≥ 3 mg/L; whereas, nitrate-N concentrations greater than the drinking water quality limit of 10 mg/L were observed in about 5% of the data. The maximum nitrate-N concentration for the above sampling period was 48 mg/L, which was observed in the well belonging to a private resident in 1995 (Schreier et al., 1996; Gartner Lee Limited, 1998; TOL, 2004).

Several studies on the Hopington Aquifer have implicated agriculture and residential use (septic) systems as the main contributors of nitrate-N to the groundwater (Schreier et al., 1996; Gartner Lee Limited, 1998). In fact, Golder Associates, (1999) reported that there are some unresolved issues, which are associated with nitrogen contamination in the Hopington Aquifers from non point sources. One of the issues is an argument over the nitrogen input from septic systems versus agricultural sources.

Often, the identification of a contaminant from a non point source in groundwater is difficult due to a mixture of overlying land use activities. As a result, successful management of an aquifer may be hindered, resulting in the continuous degradation of water quality, as observed with the Hopington Aquifer. In fact, according to the current Township of Langley Water Management Plan (WMP) Draft Report, May, 2008, one of the requirements to effectively manage groundwater in Langley is the need to obtain basic and reliable data including land use contamination effects. For example, one of the recommendations of the WMP is to implement a comprehensive monitoring program and study, which will include a Township-Wide Contaminated Inventory Study / Nitrate Loading Analysis. In the WMP, some of the research questions, which would need addressing, include “What sources of potential contaminants are present in municipal well capture zones”, “What land use activities and practices are likely adversely affecting groundwater quality”, and “What are the most damaging sources (septic vs. manure) of nutrient loadings” (IAPT, 2008).
The above WMP research questions could be answered with the aid of molecular markers. Eganhouse (1997), proposed the use of molecular markers to identify the specific source of a pollutant. The rationale for using a molecular marker is that since the marker is linked to a specific origin, then if one of the markers is found in the environment, it is possible to identify the specific source of the material. Ideally, a successful molecular marker should exhibit "source specificity" and "conservative behavior". In addition, Takada et al., (1997) stated that a marker should be used and released in sufficient quantities so that detection is possible after dilution in the environment.

Traditionally, molecular markers used to identify groundwater pollution from sources such as septic tank systems, included conductivity, chloride, nitrate, ammonia, coliform bacteria and faecal coliform bacteria. Other researchers used stable isotopes ($^{15}$N) to differentiate between nitrate from animal and human wastes, and inorganic fertilizers (Kaplan and Magaritz, 1986; Wassenaar, 1995; Batt et al., 2006). Hydrophobic markers including coprostanol ($5\beta$), which is a sterol that is produced in the digestive tract of humans had been used as a marker to detect sewage pollution in marine waters and sediments (Leeming et al., 1997) and rivers (Dachs et al., 1999).

Hydrophilic markers have become increasingly useful for tracking pollutants, which are water soluble in aquatic systems (Hayashi et al., 2002). For tracking sewage in the environment, Takada et al., (1997) propose the use of the natural products urobilin and caffeine, and synthetic compounds such as linear alkylbenzene sulphonates (LAS) and 4, 4'-bis (2-sulfoctyl) biphenyl (DSBP). Other researchers combined DAS 1 (4, 4'-bis-[4-anilino-6-morpholino-1,3,5-triazin-2-yl]amino] together with DSBP to detect human wastewater effluents in marine environments (Hayashi et al., 2002), rivers (Poiger et al., 1996; Hayashi et al, 2002), lakes (Stoll et al., 1997, 1998) and sediments (Stoll et al., 1997). DAS 1 and DSBP, which are optical brighteners or fluorescent whitening agents (FWAs), are minor components of laundry detergent. The main purpose of FWAs is to adsorb to the fabric to improve and return its whiteness during washing (Siegrist et al., 2002). Several researchers (Stoll and Giger, 1998; Hayashi et al., 2002) have reported that DSBP and DAS 1 are useful molecular markers to track domestic or municipal wastewater in the environment, because they are water soluble due to their ionic sulfonyl group (Hayashi et al., 2002), they do not degrade by hydrolysis or biodegradation (Stoll et al., 1998;
Poiger et al., 1998), they may sorb to sediments but can be removed by flushing (Stoll et al., 1998) and mostly exists in the dissolve phase in secondary effluent (Poiger et al., 1998). One limitation of FWAs in the environment is that they can photodegrade (Stoll et al., 1998). However, this photochemical process is not expected to affect the FWAs from the laundering process to the septic system, due to the lack of sunlight.

A potential marker for the impact of agricultural waste, notably livestock, in groundwater is an antimicrobial called sulfamethazine, which has been approved by Health Canada as a drug in the feed solely for livestock, including cattle, swine, horses and poultry (Health Canada, 2007). In general, sulfamethazine belongs to a group of sulfonamides, which are broad-spectrum synthetic antimicrobials (Batt et al., 2006). Some of the treatment claims for sulfamethazine include the maintenance of growth rate and feed efficiency in periods of stress, and in the prevention of diseases such as swine pneumonia (Health Canada, 2007). Overall, sulfonamides have low octanol-water partition coefficients, thus these compounds have the potential to be highly mobile in soil and leach into groundwater (Batt et al., 2006).

1.1 Research Objectives

The continuous input of harmful levels of nitrate-N into the Hopington Aquifer from non point sources is a major concern for the residences of the Township of Langley. As a result, the primary objectives of this research will be

1. Using land use information, determine the possible sources of nitrate-N in the Hopington aquifer, and identify appropriate molecular markers, which can differentiate between these sources.

2. Obtain or adapt suitable analytical methods for the determination of these molecular markers.

3. Investigate the efficiency of the molecular markers for the determination or separation of the sources of nitrate-N in groundwater from this aquifer.
2 LITERATURE REVIEW

2.1 Introduction

In British Columbia, more than 750,000 residents rely on groundwater for their drinking water supply (BCMoE, 2008). Between 1977 and 1993, over 12,000 samples of groundwater were analysed for nitrate-nitrogen concentration in the province by the Ministry of Environment (MoE). The Maximum Acceptable Concentration (MAC) for nitrate in drinking water in British Columbia is 45 milligrams per litre (mg/L), however, for nitrate-nitrogen (NO₃⁻N) reported in laboratory tests, the MAC is 10 mg/L. Nitrate-nitrogen levels above the Canadian drinking water guideline of 10 mg/L were detected in 186 or 1.5% of the samples analysed; whereas, there were nitrate-nitrogen concentrations greater than or equal to 3 mg/L in 7% of the samples analysed, which may indicate human impact (BCMoE, 2007).

High nitrate concentrations in drinking water can cause methemoglobinemia commonly called “blue baby syndrome” particularly in babies under six months old. “Blue baby syndrome” results from oxygen deficiency in the body due to the inability of the blood to transport oxygen; in extreme cases, death can occur (BCMoE, 2007). A case of nitrate-induced methemoglobinemia was observed in an infant in Wisconsin, USA. In 1999, a 3 week old infant with symptoms of irritability, blue skin and breathing difficulties was found to have a methemoglobin concentration of 91.2%. The cause of the illness was reported to be ingestion of nitrate contaminated well water. Nitrate-N concentrations in the well samples were reported to be 27.4 mg/L; in addition, the water was also tested positive for E. coli bacteria. Barnyard runoff, septic tank effluent, and agricultural fertilizers were implicated as potential sources of the nitrate contamination in the well water (Knobeloch et al., 2000).

Nitrate in groundwater can originate from non-point sources such as leaching of chemical fertilizers, leaching of animal manure and septic and sewage discharges. However, the problem is that non-point sources may be widespread or diffuse, and as a result, detection of a specific source may be difficult (BCMoE, 2007). According to Takada et al., (1997), anthropogenic markers are useful tools for identifying the source of pollutants in the environment, particularly
from areas where there are multiple sources. In addition, knowledge about the specific source of the pollutant can ultimately assists management to decide where to focus their efforts for controlling the sources of the inputs.

2.1.1 Hopington aquifers

In the above groundwater study conducted by the MOE in British Columbia, nitrate-nitrogen concentrations above the drinking water guidelines were observed in some rural wells in the vicinity of Langley, Abbotsford, Armstrong, Grand Forks, Kamloops, Oliver, Osoyoos, Salmon Arm, Vernon and Williams Lake (Figure 2.1). These areas are in predominately intensive agricultural or septic tank locations (BCMoE, 2007).

Based on new maps, 18 major aquifers have been identified in Langley with most of the aquifers being contained within deposits by the Fraser River or by glaciers during the last ice age. One of the major aquifers is the Hopington (Hopington AB and Hopington C), which underlies the Salmon River Uplands, and is comprised of glacial outwash sediments up to 50 metres in thickness. The upper unconfined portion of the aquifer (Hopington AB) covers about 30 square kilometres, and consists of thick permeable sand and gravel deposits. The unconfined Hopington AB Aquifers, which is somewhat close to the surface, is an important groundwater recharge area. The confined aquifer (Hopington C), which underlies the Hopington AB, extends further south and west covering an area of 50 square kilometres. The Hopington C Aquifer is characterised by low- permeability surface sediments (TOL, 2001; TOL, 2008).
An estimated 6600 residents on 1,951 properties were reported to obtain groundwater from the Hopington Aquifer through private wells, private community systems or are connected to the Township’s east water system. Overall, the largest groundwater users on the Hopington Aquifer were reported to include private residential wells (940,000 m³), hobby farms (102,000 m³/y), commercial farms and businesses (867,000 m³/y) and golf courses and fish farms (368,000 m³/y). The estimated total volume of water extracted is approximately $2.74 \times 10^6$ m³/y (TOL, 2001).

Figure 2.1  Locations of some wells in British Columbia with high nitrate concentrations. Source: BCMoE (2007). Copyright © Province of British Columbia. All rights reserved. Reprinted with permission of the Province of British Columbia.
2.2 Land use activities and sources of nitrate-N contamination

According to Katz and Choquette (1991), in a typical sand and gravel aquifer unaffected by anthropogenic activities, naturally occurring chemicals such as nitrate may exist in the groundwater, as a result of physical, chemical and biological processes. These complex processes occur as water from precipitation percolates through the soil, the unsaturated zone, and the saturated zone. In addition, the background water chemistry throughout the aquifer may be affected by residence times, soil type, vegetation, lithology, mineralogy and groundwater flow patterns. Freeze and Cherry (1979) reported that in an aquifer overlain with anthropogenic activities, dissolved nitrogen in the form of nitrate ($\text{NO}_3^-$) is the most common contaminant found in groundwater. To a lesser degree dissolved nitrogen also occurs in the form of ammonium ($\text{NH}_4^+$), ammonia ($\text{NH}_3$), nitrite ($\text{NO}_2^-$), nitrogen ($\text{N}_2$), nitrous oxide ($\text{N}_2\text{O}$) and organic nitrogen.

Fukada et al., (2004) reported that there are two main but separate sources of nitrate in urban groundwater. The first source is nitrifying $\text{NH}_4^+$ derived from the conversion of organic nitrogen in soil, inorganic fertilizer, sewage and urea in sewage. The second source is from the direct input of $\text{NO}_3^-$ from inorganic fertilizer and sewage. In fact, Freeze and Cherry (1979) stated that the widespread occurrence of nitrate in groundwater is increasingly due to agricultural practices and disposal of sewage on or beneath the land surface. In agreement, Aelion and Conte (2004) also reported that some common sources of nitrate in groundwater due to anthropogenic input include residential and agricultural used fertilizers, animal waste and broken septic tanks; as a result, aquifers systems around the world are being threatened by nitrate contaminations. For the Hopington Aquifer, nitrate-N reaching a maximum concentration of 48.4 mg/L had been detected in the well water demonstrating severe contamination in areas of this local aquifer.

2.3 Septic tank systems

Within the Township of Langley, private septic systems are used in the areas that are not generally serviced by the Township sanitary system (Golder Associates, 1999). Wernick et al., (1998) reported that from the 1930s to 1994, there were an estimated 3,250 septic systems
installed in the Salmon River watershed (which contains the Hopington AB Aquifer). However, in the 1970s, the highest rate of septic system installation occurred, resulting in the addition of 1500 new septic systems. Overall, there are some 5000 to 13,000 septic tanks in the Township of Langley, and an estimated 200 to 600 new septic permits are issued each year. In addition, septic systems, which are required in secondary buildings on agricultural properties, are also permitted in the Township of Langley (Golder Associates, 1999).

Golder Associates (1999) reported that in general, areas where there are high densities of septic systems together with rapidly permeable soils, do not facilitate adequate remediation, which can result in contamination of the underlying aquifer systems. According to Canter and Knox (1985), the general concern with septic tank system is the quality of the effluent from the septic tank itself, and the efficiency of the underlying soil to remove the constituents, as it passes through the unsaturated soil to the groundwater. In general, an estimated 610,000 cubic meters is recharged back into the Hopington Aquifers via septic fields (TOL, 2001), creating the potential for groundwater contamination.

2.3.1 Nitrate-N

The components of domestic sewage may include organic matter (proteins, amino acids, volatile acids, hormones, vitamins and pesticides), inorganic constituents including chloride, boron and phosphate, and micro organisms including bacteria, viruses, fungi and protozoan. However, to a greater extent, human excreta in domestic sewage are the main contributor of nitrate contamination in groundwater, with smaller contributions from food preparation and laundry effluent (Misstear and Bishop, 1997). In British Columbia, nitrate can be considered the principal contaminant of groundwater, due to various sources including septic tank effluent, where nitrogen is one the main components of concern from this system (Freeze et al., 1994).

In a septic tank, there is separation of heavier sludge and lighter solids from the influent, with the sludge forming a blanket at the bottom of the tank, and the lighter solids including fats and grease floating to the surface to form scum. Much of the sludge and scum are liquefied through decomposition and anaerobic digestion with gas being released from these processes and additional solids settling to the bottom of the tank. Between the sludge and the scum, the
partially clarified liquid flows through an outlet to the soil absorption system, where essentially the ammonium ion from the effluent is converted to nitrates ($\text{NO}_3^-$) through nitrification (Canter and Knox, 1985).

The attenuation of septic tank effluent is expected to occur in an engineered subsurface environment called a drainfield. A typical septic system for a household may consist of a holding tank and a weeping tile bed 100 $\text{m}^2$ in area. The tiles which are usually perforated PVC (polyvinylchloride) pipe are situated in gravel lined trenches 2 metres apart and at a depth of 0.6 m (Robertson et al., 1991).

In general, much of the decomposition of the organics from septic tank effluent is expected to occur within the drainfield and the soil absorption system by aerobic microbial (biological) activities, with the treated water percolating through the soil. Aeration of the absorption field is provided naturally through the backfill, which covers the trenches (Viessman and Hammer, 1993; Hammer and Hammer, 1996).

Within the drainfield system, nitrogen readily changes form (organic $\text{N} \rightarrow$ ammonia $\rightarrow$ nitrate), in the presence of oxygen (Freeze and Cherry, 1979). For example, Harman et al., (1996) reported that active oxidation and nitrification processes were also implicated for the removal of ammonia from septic tank effluent and the high concentration of nitrate below tile beds. Based on septic tank effluent concentrations of 128 $\text{mg/L}$ and 0 $\text{mg/L}$ for ammonia and nitrate respectively, the authors observed a reduction of ammonia and an increase in nitrate with concentrations of 0.2 $\text{mg/L}$ and 112 $\text{mg/L}$ respectively. In addition to nitrification, ammonia may also be removed from the effluent in the drainfield through adsorption to soil colloids or the filtering of solid-phase particles (Walker et al., 1973a, b; Harrison et al., 2000).

The concern with nitrogen in the unsaturated zone is that after decomposition of the organic material from the septic tank effluent, the resulting nitrogen predominantly in the form of nitrate may leave this zone and reach groundwater through leaching by percolating water (Davis and De Wiest, 1966). Gardner (1965) stated that an important factor, which can determine the mobility of nitrogen through soil is based on the type of interactions which exists between the nitrogen in solution and the surface area of the soil particles. For example, the author reported
that nitrate ion is weakly adsorbed by few soils and not at all by most soils, therefore, it can readily move through the soil profile. In contrasts, the ammonia ion which is strongly adsorbing will move less readily than the nitrate ion as it percolates through the soil profile. Thus, the only process that could reduce nitrate as it percolates downward is denitrification (Walker et al., 1973a, b).

One of the limiting factors, which may inhibit denitrification processes in the subsurface environment of a septic tank system particularly situated in sand aquifer, is the lack of, or the unavailability of carbon for the denitrifying bacteria. Aquifer soils, which have a predominantly sand aggregate such as the Hopington Aquifers, may contribute very little organic matter as a source of carbon necessary for denitrification processes, and the available carbon itself may not biodegrade, thus facilitating the export of nitrate to groundwater (Bedessem et al., 2005). An example of the contribution of organic matter towards denitrification in sandy soils lacking carbon was determined by Bedessem et al., (2005). Based on laboratory model leach fields, synthetic septic tank effluent and native fine Wyoming soils (sand) containing 0.4% organic matter (dry weight), the authors observed that with the addition of an organic layer (30% pine saw dust) to the sand (for denitrification), there was an increase in the removal of total nitrogen by up to 67% from the effluent, based on a sawdust-sand mixture of 5.5% total organic matter (dry weight). The authors also added that adsorption to the organic matter may also have contributed to the overall reduction of total nitrogen from the effluent.

In addition to carbon deficient groundwater, a well aerated sandy subsoil may not facilitate any considerable amount of denitrification (Walker et al., 1973b), this is because effluent from septic systems situated in sands may undergo primarily aerobic activities with nitrate as the end product (Bouma, 1979) in possibly high concentrations in groundwater beneath drainfields. Walker et al., (1973b) proposed that the only major mechanism for reducing nitrate concentration in groundwater below drainfields, which are situated above aerobic soils is dilution using uncontaminated groundwater.
2.4 Agriculture

In addition to septic tank systems, nitrogen compounds may also enter soils via mineralization of plant material (organic), atmospheric deposition, and the spreading of organic and inorganic fertilizers. After the application of animal manures and commercial fertilizers, nitrogen compounds in the soils are oxidized to form mainly nitrates, which are taken up by crops or lost to the atmosphere through denitrification. However, if crop uptake or atmospheric losses do not occur, then it is possible that the excess nitrate may accumulate in the soil when these fertilizers exceed the nutrient requirements of the crops. As a result, nitrate in the soil may leach into the water table through the unsaturated zone (Hii et al., 1999; Mitchell et al., 2003).

Agriculture is possibly one of the most important anthropogenic activities that influence the quality of groundwater. Fertilizers, pesticides, livestock or fowl wastes are some of the main agricultural activities that can contribute or cause the degradation of groundwater quality. Fertilizers, which can be manufactured chemically (inorganic) or derived from animal or human waste (organic) may have the most widespread impact on groundwater quality especially if the aquifer is unconfined (Freeze and Cherry, 1979).

Some of the key agricultural activities within the Township of Langley include dairy, poultry, beef, swine and sheep farming (TOL, 2008). Wernick et al., (1998) also added that due to an increase in the number of livestock such as horses and sheep, hobby farms were increasing in numbers, and thus becoming significant agricultural activities. In addition, the authors examined the animal density of the Salmon River Watershed (includes the Hopington AB Aquifers) to evaluate the potential for pollution to the watershed, due to livestock and poultry operations. An average animal stocking density of 2.5 animal units per hectare (AU.ha\(^{-1}\)) was used as an index to determine the pollution potential to the watershed. Within the watershed, the overall animal unit density was 1.5 animal units per hectare (AU.ha\(^{-1}\)), which was below the pollution potential index. However, in some regions of the watershed, notably the Hopington AB aquifer, the authors reported an animal unit density of 3.0 AU.ha\(^{-1}\) which indicated the possibility of groundwater contamination due to the overlying livestock activities.
2.4.1 Livestock and nitrate-N

Agricultural activities including the storage or disposal of livestock or poultry wastes on land, have been implicated as potential causes of groundwater quality degradation. For example, nitrate from small source areas such as farm manure piles, poultry waste lagoons, and feedlots, have contributed to the degradation of water quality in many agricultural areas (Freeze and Cherry, 1979). For the most part, manure is generally defined as the feces and urine from animals, which are deposited primarily in buildings or collection yards, and stored for spreading on land (Chadwick and Chen, 2002).

In fresh animal excreta, the main form of nitrogen is organic, such as proteins, urea, or uric acid. However, due to enzymes and microorganisms in the animal excrement, nitrogen transformation occurs resulting in ammonia (Porter, 1975). For crops, the ammonium fraction in the manure is readily available as inorganic nitrogen; however, the rest of the nitrogen in the manure is not immediately available for crop uptake, as it is held within the organic matter (BCMAL, 2005b). Under aerobic conditions, nitrification of ammonia (NH₃) and ammonium (NH₄⁺) in the soil or unsaturated zone results in the formation of nitrates (Zebarth et al., 1998). According to Hatch et al., (2002), ammonia levels are usually low in agricultural soils, typically 1-3 mg N/L due to the non limiting rate of nitrate production. Thus, the negligible concentrations of ammonia and the high concentrations of nitrate in groundwater, particularly for the agricultural regions, may be due to complete nitrification processes of the residual nitrogen in the soil and unsaturated zone (Wassenaar, 1995).

Overall, in the Lower Mainland, there is a nitrogen surplus from manure, which is projected to grow by about 37% by 2010 (Timmenga and Associates, 2003). In Langley, it was reported that manure was the most significant source of nitrogen in the region, and one major source of nitrogen surplus is possibly as a result of horse operations (TOL, 2004). Within the Township of Langley, 506 farms have raised over 4,724 horses and ponies. In fact, Langley has been reported to be the horse capital of the British Columbia, due to an annual industry of over $50 million (TOL, 2007a). Typical horse operations may vary from only one or two horses (hobby farms) to larger farms where there are over 11 horses (BCMAFF, 2002). Larger horse farms may consist of commercial breeding and training facilities (TOL, 2007a).
The main pathways for nitrate contamination of the Hopington AB aquifers from livestock such as horse operations, would probably be through poor manure management including, storage, handling and spreading on crop fields. In general the nitrogen content in the manure for a single 455 kg horse for one year may total 45 kg (TOL, 2006), or 2.4 kgN/tonne for manure with shavings (BCMAFF, 2005). Thus, mismanaged piles of horse manure may have detrimental impacts to the environment including pollution of groundwater (LEPS, 2007).

In terms of storage and handling, according to BCMAL (2006), one 455 kilogram horse may produce 26 liters of waste (urine and feces) per day, and need a suggested storage capacity for 56 liters per day, with bedding. For an operation with 5 horses, in 6 months the manure produced may fill a two car garage, and thus require a storage area of approximately 6 m x 6 m stacked 1.4 m high. However, according to LEPS (2007), most horses now live in “condo style” accommodations, which means that a great number of horses are housed in confined areas, resulting in a large amount of manure produced and not enough land to spread it on. Other horse manure management problems reported included manure being left in piles uncovered, some large piles have been left sitting on the property for 10 years, exceeding the 9 month limitation. Manure piles were also found to be located within close proximity to watercourses and wells. BCMAL (2006) reported that manure stored in short term facilities, should be located 30 meters from a watercourse and a well.

In British Columbia, the use of horse manure as a fertilizer is encouraged for pasture or forage crops. In general, horse manure/bedding mixture from only 3-4 horses can be spread on each acre of productive pasture (BCMAL, 2006). Essentially, forage is an excellent horse feed, and a pasture which is well managed and productive can provide a cheap source of feed for one mare and a foal for 4 to 5 months of the year on 1.5 to 2 acres of land (BCMAL, 2005a). As mentioned earlier, in B.C., manure spreading is not permitted outside the growing seasons (April to September) or late fall and winter. In addition, manure must be stored to avoid rain from washing out the nutrients, which can cause pollution. However, according to LEPS (2007), raw manure spread on fields during the months of October to April is a common mismanagement practice.
According to BCMAL (2006), the spreading of composted manure rather than raw manure on fields is encouraged, as it has a slightly higher nutrient content than fresh manure; in addition, there is the potential for reduced leaching to the environment. However, farmers may be discouraged to use the composted manure due to a higher stability of the nutrients in the manure as they are bonded to organic matter, also, composted manure may be expensive for the spreading on fields.

As mentioned earlier, beef cattle is a key agricultural activity in the Township of Langley. Typical nitrogen content in the manure (solid) of beef cattle is 4.2 kg/tonne, and thus can be used as a fertilizer for crops such as in the berry production (BCMAFF, 2005). Berry and grapes production, particularly destined for the wine industry, has been thriving in Langley. In 2006, it was reported that the there were 163 berry and grape farms in the Township. Furthermore, there are five wineries currently located in Langley (TOL, 2008).

In BC, similar to horses, beef cattle may also be pastured during the forage growing season, and thus have access to pasture 24 hours a day (BCMAFF, 2004). Other key agricultural activities within the Township of Langley were poultry, dairy, swine and sheep (TOL, 2007b). Nutrient balances for livestock such as one dairy cow weighing 640 kg and producing 7760 kg of milk, and 100 layers (1.8 kg per bird) producing 25,000 eggs per year, may result in excreted nitrogen quantities of 116 kg/year and 80 kg/year respectively. These figures are based on a nitrogen dietary ingestion of 164 kg/year minus 48 kg/year of nitrogen in animal product for a dairy cow, and nitrogen dietary ingestion of 107 kg/year minus 27 kg/year of nitrogen in the product for 100 layers (BCMAFF, 1982). Typical nitrogen contents in manure for dairy (solid), dairy (liquid), swine (covered pits) and swine (covered pits) are 3.9, 2.9, 6.3 and 3.5 kg/tonne respectively (BCMAFF, 2005).

For the Hopington AB aquifer, animal manure including poultry and horse is commonly used as a main fertilizer for crops and pastures. However, after the application of animal manure (also commercial fertilizers), nitrogen compounds in the soils are oxidized to form mainly nitrates, which are taken up by crops or loss to the atmosphere through denitrification. However, if crop uptake or atmospheric losses do not occur, then it is possible that the excess nitrate may accumulate in the soil when these fertilizers exceed the nutrient requirements of the crops. As a
result, nitrate in the soil may leach into the water table through the unsaturated zone (Hii et al., 1999; Mitchell et al., 2003); in addition, the continuous use of manure increases the amount of nitrogen released from soil organic matter (BCMAFF, 1999). In general during the growing season, nitrogen from manure may be taken up by the forage grass, which has high nitrogen requirement (BCMAFF, 1994).

An increase in nitrate contamination in streamwater, and animal unit density was observed by Wernick et al., (1998) in the region of the Hopington AB Aquifers. The authors reported that downstream of an area where the animal unit density was 3.0 AU.ha⁻¹, which exceeded the animal pollution index of 2.5 AU.ha⁻¹, nitrate concentration of about 5 mg N/L was observed. However, it should be noted that the authors also observed a high septic system density in this region, thus the possibility of nitrate contamination of the aquifers from both sources. In addition, contamination of the streamwater was predominantly from groundwater discharged from the Hopngton AB aquifers. During the summer when the flow of the stream is generally low, groundwater makes up the bulk of the water; as a result, elevated nitrate is observed in streamwater.

In British Columbia, as a result of contamination of watercourses, the spreading of manure in late fall and winter is not permitted due to sensitive fisheries stages and periods of high rainfall (BCMAFF, 2004). Essentially, the majority of nitrogen, which has been applied to crop fields by fall will leach during fall and winter (BCMAFF, 1999), possibly contaminating the underlying aquifer. In some situations, livestock farmers spread manure on fields during the no spread period, when soil, crop and weather conditions are not appropriate. For example, the no spread period is about 210 days or seven months, however, about 60% of the dairy and hog farms reported liquid manure storage capacity of 150 days or less. Also, over 60% of the manure from the farms with insufficient storage was reported to be stored in open tanks (Bertrand, 2006). In addition, manure bound for raspberry farms for spreading and incorporation, may remain uncovered in stockpiles, or spread on land during the winter period. The end result is that selected nitrogen compounds in the manure may move downwards into the aquifers with percolating rain and snowmelt (Liebscher et al., 1992).
2.5 Land use activities and molecular markers

Molecular markers (chemical markers), under suitable conditions can provide information about sources of pollution in the environment, because their structures are linked to a specific origin. Therefore, if one of these markers is found in the environment, then it is possible to identify the specific source of the contaminating material. Additionally, markers can be used to estimate and identify contributions of contaminants made by different sources (Eganhouse, 1997).

In general, “source specificity” and “conservative behaviour” are the two main criteria for an ideal molecular marker. The link between a marker and a given source is called source specificity, and ideally, this link should be “direct” and “unique” (Eganhouse, 1997). In addition to its source, a marker should be used and released in sufficient quantities so that detection is possible after dilution in the environment (Takada et al., 1997).

The conservative behaviour of a marker is mainly governed by its fate from its source through the environment. Essentially, for a marker to have a conservative behaviour, it should be persistent and resist environmental processes that cause deteriorations over time, which may result in loss of the marker (Takada et al., 1997). Some examples of environmental processes, which may affect or alter a potential marker include physical processes (particle transport, advection and sedimentation), phase transfer (volatilization, dissolution, adsorption and desorption) photolytic, microbial degradation (Eganhouse, 1997) and chemical degradation.

Close et al., (1989) reported that some of the usual markers of pollutants in groundwater studies include conductivity, chloride, nitrate, ammonia, coliform bacteria and faecal coliform bacteria. Chloride and conductivity were found to be conservative markers of septic effluent because the concentrations were found to be relatively unchanged as the effluent passed through a septic tank and boulder pit to a lysimeter. However, all the above mentioned usual markers may not uniquely implicate septic tank effluent as a contamination source. Thus, markers such as chemicals and micro-organisms are used as specific associations with human sewage.
2.5.1 Isotopes

Stable isotopes have also been used as markers to evaluate the origin of nitrate in groundwater (Batt et al., 2006; Kaplan and Magaritz, 1986; Wassenaar, 1995). According to Wassenaar (1995), $\delta^{15}$N values can be used to differentiate between nitrate derived from synthetic fertilizer ($\delta^{15}$N $\approx$ -1 to +2\%o) and animal waste sources or sewage ($\delta^{15}$N $\approx$ +8 to +16\%o). For example, the authors conducted isotopic analysis to determine the origin of NO$_3^-$ in the Abbotsford aquifer system, which ranged from 0 to 151 mg/L NO$_3^-$. Stable isotopes including $\delta^{15}$N in NO$_3^-$ from environmental samples such as groundwater, and contamination source materials (manure, synthetic fertilizers) were analysed for this study. According to the results, the organic $\delta^{15}$N composition in the poultry manure samples (organic N plus NO$_3^-$ - N) generally could be differentiated from the three nitrogen based synthetic inorganic fertilizers (urea, ammonium sulphate and ammonium phosphate) used on the raspberry fields. The $\delta^{15}$N in the manure and the fertilizers ranged from +7.9 to +8.6 \%, and -1.5 to -0.6 \% respectively. In the groundwater samples, the $\delta^{15}$N of NO$_3^-$ ranged from +2 to 23\%, this indicated that poultry manure ($\delta^{15}$N $>$ +8) was the primary source of NO$_3^-$ in the aquifer.

Wassenaar (1995) reported that there are some disadvantages of using isotopes in groundwater studies. Firstly, NH$_3$ volatilization in the source material, such as manure, can result in a significant enrichment of the $^{15}$N. In addition, the enrichment can lead to a wide range in $\delta^{15}$N values of nitrate as a result of nitrification of the manure. As a result, the authors suggested the use of O ($\delta^{18}$O) isotope in conjunction with the N ($\delta^{15}$N) isotope, which would offer more reliable source identification. Secondly, nitrate originating from human wastes is isotopically indistinguishable from animal waste. Based on primarily the latter information, then perhaps isotopes may not be appropriate markers to distinguish between nitrate from human wastes (septic systems) and livestock (manure) in Langley.

2.5.2 Hydrophobic markers

Molecular markers have also been selected based on their polarity. For example, Hayashi et al., (2002) reported that the most commonly used markers for tracing anthropogenic sources of contamination in the environment have been hydrophobic. These markers are useful for
tracking sewage particles and particle bound pollutants. Some examples of hydrophobic markers from sewage sources include coprostanol and α- Tocopheryl acetate (natural products) and linear alkylbenzenes (synthetic detergent) (Takada et al., 1997).

In the field, hydrophobic markers such as coprostanol have been used to detect and distinguish the sources of fecal pollution in the environment including marine waters and sediments (Leeming et al., 1997) and rivers (Dachs et al., 1999). Coprostanol (5β-cholestan-3β-ol) is a sterol, which is produced in the digestive tract of humans, and may be used as marker for sewage pollution. Traditionally, microbiological indicators have been used as markers for sewage pollution in the environment; however, due to limitations such as die-off and lack of correlation with sewage pathogens, coprostanol has been used as an alternative marker (Leeming et al., 1997). In addition to humans, animals also produce fecal sterols, thus care should be taken with source identification. For example, Chin-Cheng and Yu-Pin (2004) observed a similarity between coprostanol fractions of pigs and humans in the receiving waters of Taiwan’s rivers. Out of a total of nine different sterols, the concentrations of coprostanol, which were represented as percentages of total sterols in human (n=4) and pigs (n=9) were 35.8% and 29.5% respectively, compared with 11.5% and 2.3% for cows and ducks respectively.

2.5.3 Hydrophilic markers

In comparison to hydrophobic markers, hydrophilic markers have become increasingly useful for the tracking of pollutants, which are water soluble in aquatic systems (Hayashi et al., 2002). According to Takada et al., (1997), hydrophilic or water soluble markers in sewage may include the natural products urobilin and caffeine, and synthetic compounds such as linear alkylbenzene-sulfonates (LAS) and 4, 4′-bis (2-sulfostyryl) biphenyl (DSBP).

2.6 Fluorescent whitening agents

Fluorescent whitening agents (FWAs) are minor components of laundry detergents, accounting for about 0.1 to 0.6% of the total mass (Poiger et al., 1996; Eckhardt and von Rütte, 1975). However, they are important to residential and commercial laundering processes, due to its
ability to restore the “perceived” loss of whiteness in white fabrics (Eckhardt and von Rütte, 1975).

The most commonly used FWAs are anionic derivatives of the diaminostilbene type (DAS 1) or distyrylbiphenyl type (DSBP) (Kramer, 1992). The stilbene class of “optical brightening agents” or “fluorescent whitening agents” (Figure 2.2) are derived from synthetically produced organic compounds called diaminostilbene or distyrylbiphenyl (Anliker, 1975; Kramer, 1992). DAS 1 (4,4′-bis-[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2′-disulfonate) and DSBP, which are trade names, have small white pellet and yellow-green powder forms respectively.

FWAs are generally hydrophobic, however, they are anionic in nature and the sulfonate groups increases their water solubility. There is also an affinity for cellulose due to the FWAs large carbon structures (Kramer, 1992; Stoll and Giger, 1997, 1998). These colourless to weakly coloured compounds absorb ultraviolet light from daylight ~300-430 nm, and reemit most of the absorbed energy as blue fluorescent light between ~ 400 and 500 nm (Siegrist et al., 2002). Natural organic fabrics generally have an absorption band extending from the short UV-region partly into the blue visible region, causing a yellowish shade (Kramer, 1992). As a result, in daylight aesthetically undesirable yellowish discolorations found in white industrial materials, such as textiles, papers, or plastics can be masked by the blue fluorescence from optical brighteners to give a dazzling white appearance. In laundry detergents only the DAS1 and DSBP types of FWAs are used (Stoll and Giger, 1998), and these FWAs adsorbs to the fabric improving and returning its whiteness during washing (Siegrist et al., 2002).
FWAs are generally in the parent E (DAS 1) and E,E (DSBP) isomeric forms, because only the E and the E,E isomers are the active whitening agents, which gives them their fluorescence (Kramer et al., 1996). These E and E,E isomeric forms can be altered when exposed to light to produce the Z and EZ photoisomers respectively. As a result, some laundry detergents contain the E and E,E isomeric forms to replace the FWAs on fabric, which can photochemically degrade during wear (Stoll and Giger, 1998).

2.6.1 Occurrence and use

World-wide, DAS 1 and DSBP, the two most used FWAs, which are only found in laundry detergents (Stoll and Giger, 1998) had consumptions of an estimated 3000 and 14000 t/yr respectively in 1990 (Poiger et al., 1996). Hayashi et al., (2002) reported that in Japan both
DAS 1 and DSBP are major FWAIs presently found in laundry detergents. In a study by Poiger et al., (1998), out of an annual consumption of FWAIs by householders in Zürich, an estimated 38 and 97% of DAS 1 and DSBP respectively were reported to be discharged with household wastewater. The authors suggested that a large proportion of the remaining fraction of DAS 1 and DSBP from householders' wastewater were perhaps adsorbed to the laundry during washing, where they replace the FWAIs lost during wear.

In contrast, Zinkernagel, (1975) reported that around 10 to 15% of FWAIs used by consumers in the household product is ultimately disposed of in the waste water. Overall, Stoll and Giger (1998) reported that a fraction of FWAIs remain in the washing liquor when textile is laundered. The liquor containing the FWAIs is discharged to sewers for treatment in municipal waste water treatment plants. At a municipal sewage treatment plant in Zürich, Switzerland, FWAIs have been found in raw sewage at concentrations of 18 and 23 µg/L for DSBP and DAS1 respectively (Poiger et al., 1996).

2.6.2 Fate of FWAIs

(i) Laundry process

Poiger et al., (1998) reported that of the annual consumption of FWAIs, the total amount of DAS 1 and DSBP discharged with household effluents is 38 and 97% respectively; the remaining fraction of FWAIs would be adsorbed to fabric. In the household effluent (toilet, sink, garbage disposal, bath, shower, dishwasher, washing machine (Bennett et al., 1974) particularly the laundry effluent, it is anticipated that the FWAIs would adsorb to suspended solids, since adsorption is the dominant removal mechanism for FWAIs from wastewater (Poiger et al., 1998). Typical concentrations of total solids and suspended solids in a washing machine wastewater based on three washing cycles for a family of 2-5 persons were reported to be 1185 and 128 mg/L (cycle 1), 421 and 0 mg/L (cycle 2) and 527 and 46 mg/L (cycle 3) (Bennett et al., 1974). Of the FWAIs in the laundry effluent, it is anticipated that for DAS 1 and DSBP, the main isomers discharged in the washing liquor to the septic tank would be in the parent or E and EE forms respectively. In general, laundry effluent from the machines to the drainfield via septic
tanks is not exposed to any lighting, and thus any photo degradation processes should be minimal or none existant.

(ii) Isomerization and photodegradation

In general, FWAs are not exposed to light during washing process and in the sewers, however, in the sewage treatment plant, the FWAs are exposed to sunlight and photoisomerization occurs. In filtered sewage effluents, a steady-state isomeric composition consisted of 75% (Z) to 25% (E) for DAS1 and 14% (E,Z) to 86% (E,E) for DSBP after 3-5 minutes of irradiation with sunlight. This indicates that DSBP isomerizes less readily to the non-fluorescent Z form than DAS1 (Poiger et al., 1996).

The main degradation routes for all FWAs in the environment are direct photochemical reactions by sunlight in surface waters. In fact, Stoll et al., (1998) reported that photodegradation contributed to 53% and 81% of the overall removal of DAS 1 and DSBP respectively from lake water. Kramer et al., (1996) reported the photodegradation rates for FWAs in lake water at 25°C, during summer noon were $t_{1/2} = 278$ and $t_{1/2} = 87$ minutes for DAS 1 and DSBP respectively. Stoll et al., (1997) observed that due to photodegradation, the reductions of DAS 1 and DSBP in lake water within 28 days were 2-24% and 11-61% respectively. Additionally, DSBP was found to photochemically degrade three times faster than DAS1 because of a higher rate of sunlight absorption by the DSBP isomer mixture. The maximum absorption spectra for the E,E-isomer of DSBP and E-isomers of the DAS-type FWAs were found to be similar at ~350 nm (Kramer et al., 1996).

(iii) Microbial and chemical degradation

Zinkernagel, (1975) reported that biodegradation is not an important pathway for the removal of FWAs in activated sludge treatment of wastewater. Modern FWAs are unlikely to be readily biodegradable because they possess a high degree of aromaticity. A biodegradation test of FWAs in activated sludge resulted in a slow biodegradation rate of the compounds during the first 15 days due to the adaptation period of the activated sludge.
Poiger et al., (1998) also reported that FWAs were not readily biodegraded by anaerobic or activated sludge treatment. The authors observed that anaerobic digestion of sludge from a primary clarifier at a STP in Zürich resulted in the removal of only 4% for DAS 1; however, there was a + 3% increase in DSBP concentration in the digestor. These results were based on grab samples, and may not represent average situations.

Similarly, Hayashi et al., (2002), found that microbial and chemical degradation does not have a large impact on the reduction FWAs in aquatic environments. In a laboratory experiment conducted in a dark room at 25°C and using estuarine water, the authors found no decrease of DSBP and DAS1 concentrations during 4 days of incubation. The authors concluded that due to poor bioavailability, FWAs may be useful tools for tracking and tracing dilution and movement of sewage input in aqueous environments.

(iv) Adsorption

At wastewater treatment plants, the moderate removal of FWAs from secondary influents (primary effluents) can be attributed to activated sludge treatment. For a given sampling day at four municipal sewage treatment plants in Switzerland, the removal rates for DSBP and DAS 1 from the secondary influent ranged between 27-60% and 51-77% respectively. According to Zinkernagel (1975), FWAs in sewage treatment plants may be completely removed by adsorption rather than biodegradation. Typical concentrations of DAS 1 and DSBP in raw sewage, primary effluent and secondary effluent were reported to be 23, 12 and 3 μg/L and 18, 14 and 6 μg/L respectively (Poiger et al., 1996).

Poiger et al., (1996) conducted a study on adsorption of isomerised FWAs in a wastewater treatment plant. Prior to sampling, the raw sewage, primary and secondary effluents were exposed to sunlight for a few seconds, more than one hour and more than two hours respectively. According to Figure 2.3, there is a decrease in the fraction of FWAs isomers adsorbed to suspended solids with decreasing solids content from raw sewage (63 mg/L) to primary effluent (30 mg/L) to secondary effluent (5.1 mg/L). There is also a strong tendency for DAS 1 to adsorb more onto suspended solids than DSBP.
In rivers and coastal waters adsorbed FWAs are a minor portion (~5%). Only $5.2\% \pm 7.2\%$ of DSBP and $3.1\% \pm 3.4\%$ of DAS1 were found in the particulate phase (adsorbed FWAs) out of 14 river water samples (Hayashi et al., 2002). In comparison, Stoll et al., (1997) observed that FWAs exhibited a conservative behaviour in the freshwater sediments, and as a result, the FWAs can be useful markers for domestic wastewater. The FWAs were strongly sorbed to particles and removed from the water column through rapid sedimentation, since horizontal
mixing was at a minimum in the lake. In agreement with Poiger et al., (1996), the authors also observed higher sorption behaviour and thus more enrichment of the parent $E$ (DAS 1) and $E,E$ (DSBP) isomers in the lake sediments than the $Z$ (DAS 1) and $E,Z$ (DSBP) photoisomers.

(v) Flushing

Stoll et al. (1998) determined that flushing was an important mechanism for the elimination of FWAs from an aqueous environment. Based on the average concentrations of FWAs in the top 5 m of a lake and the outflow, the authors determined that flushing accounted for the overall reduction of 27% and 10% of the contribution of DAS 1 and DSBP respectively.

(vi) Dissolution

The dissolved fraction of FWAs in sewage treatment plants may vary, but generally the concentrations are in the lower microgram per litre range. Hayashi et al., (2002) determined the range of dissolved FWA concentrations in raw sewage to be 11.1-21.9 μg/L for DSBP and 2.9-8.2 μg/L for DAS1. Lower concentrations of dissolved FWAs in the secondary effluent (DSBP: 1.8-12.5 μg/L and DAS1: 0.68 – 4.9 μg/L) were expected due to adsorption to activated sludge. However, the range of dissolved FWAs in primary effluent was similar to those in the raw sewage. Therefore, it is expected that there would be untreated FWAs in the effluent from a septic tank (due to only primary settling), thus the possibility of FWAs leaching through the drain field into the aquifer. This application favours FWAs as potential markers.

Due to the relatively low removal efficiency in wastewater treatment plants, dissolved FWAs may be found in the environment. Hayashi et al., (2002) found concentrations of dissolved DSBP and DAS1 ranging from 0.1 μg/L to 6.4 μg/L, and ~1μg/L respectively in river water.
2.6.3 Sources of FWAs in the environment

(i) Domestic sewage

Poiger et al., (1998) found that initial setting of the solids in the raw influent at a STP, resulted in the removal of an estimated 69 and 23% of DAS 1 and DSBP respectively from the wastewater. The remaining FWAs in the primary effluent may be detected in the low microgram per litre range. For example, typical DAS 1 and DSBP concentrations in the primary effluent at various STP plants in Zürich were reported to be 3.8 - 11.4 µg/L and 5.4 - 21.3 µg/L respectively. Although, there may be significant variability of FWA concentrations in sewage on a daily basis, the typical ratios of DAS 1 to DSBP is 1:1 (Poiger et al. 1996; Poiger et al., 1998). Overall, Kramer (1992) reported that of the two FWAs, DAS 1 was the most widely used.

In the subsurface environment of the septic tanks, it is expected that the behaviour of DAS 1 and DSBP in the soil would be fairly conservative due to the absence of photodegradation and minimum biodegradation. However, adsorption within the tile field and aquifer materials, and dilution within the aquifer may be important mechanisms for the reduction of FWAs.

The fate of the FWA in the subsurface environment may vary between the type (DAS 1 or DSBP) and isomer (E, EE, Z or EZ) and their solid-liquid partitioning behaviour. As mentioned earlier, and based on mass flows of FWAs in STPs, it is anticipated, that approximately 31% and 77% of DAS 1 and DSBP may be in the effluent after primary clarification (Poiger et al., 1998) in the septic tank. Furthermore, an estimation of the state (adsorbed and dissolved) of the DAS 1 and DSBP isomers in the primary effluent of a septic tank was determined based on the fate of the FWAs in the primary effluent of a STP. For example, based on primary effluent from a STP, Poiger et al., (1996) determined that approximately 20% of the E (DAS 1) isomer was adsorbed to suspended solids and another 20% was in the dissolved form. The Z photoisomer comprised about 59% of DAS 1 and was predominantly in the dissolved form, whereas, only about 1% of the photoisomer was adsorbed to suspended solids. Similarly, the compositions of DSBP in the EE isomeric forms comprised of ~73% and 10% in dissolved and
adsorbed states respectively. Whereas, about 12% of the DSBP which was in the photoisomeric EZ form, was completely in the dissolved state.

Ignoring photoisomers, which were not expected to occur in the septic tank due to the lack of light, the solid-liquid partitioning behaviour of only the parent isomers in STPs generally indicated that because DAS 1 and DSBP can exist in the dissolved state, it is thus possible that the mobility of the FWAs can be increased from the septic tank into the groundwater. In contrasts, any strong sorption of the FWAs in the tile field soil may result in the persistence of DAS 1 and DSBP through repeated application (Poiger et al., 1998) of the septic tank effluent.

The occurrence of an FWA in the subsurface environment of a septic tank system belonging to a single family home in rural Minnesota, USA, has been observed by Fay et al., (1995). The authors detected a DAS type (DASC-4) FWA in the drainfield influent with a concentration of 8.4 μg/L, whereas, at distances of 30.5, 61 and 91.5 cm below the drainfield, the concentrations of DAS were 0.3, 1.6 and 3.2 μg/L. This increasing trend could be attributed to processes such as flushing and adsorption, and the aquifer’s materials providing a sink for the FWAs.

(ii) Aqueous environment

According to Stoll and Giger, (1998), 13(±4) % of the FWAs consumed by Swiss householders during a year can be found in the rivers. The averaging loading per capita of DAS1 and DSBP from Swiss households to surface waters in 1995 was calculated to be 1.8(±0.5) and 1.3(±0.4) mg d⁻¹ inhabitant⁻¹ respectively, which corresponds to a total yearly load of 4.6(±1.3) t DAS 1 and 3.3(±1.0) t DSBP. Typical concentrations of DAS 1 and DSBP in lake water, river water and sediments were reported to be ~0.05-0.1 μg/L, 0.4 μg/L and ~ 0.6-1.4 (mg/kg dry matter) and ~0.01-0.07 μg/L, 0.8 μg/L and ~ 0.4-1 (mg/kg dry matter) respectively (Poiger et al., 1996; Stoll and Giger, 1997). In contrasts, Kramer, (1992) calculated the concentration of FWAs in surface water to be about 3 μg/L. In comparison to data on FWAs in surface water, there have been few findings of FWAs in groundwater. Close et al., (1989) detected a FWA with a maximum concentration of 3.3 μg/L in a well water sample, which was taken from a householder, who lives in a semi-rural and unsewered region in Yaldhurst, near Christchurch, New Zealand.
2.6.4 Analytical methods

(i) Solid phase extraction

Poiger et al., (1996), investigated a method for the determination of FWAs in sewage and river water samples. This method was suitable for both the determination of total concentrations of FWAs and the individual isomers (Z and E,Z) at the low micrograms per liter. Solid-phase extractions (SPE) were conducted using C\textsubscript{18} SPE disks for a number of simultaneous extractions. According to Fritz, (1999), the solutes or analytes in SPE are extracted from a liquid phase into a solid phase, and the stationary phase might consist of small, porous silica materials with a bonded organic phase or of an organic polymer. The analytes are retained in the solid phase due to the affinity of specific functional groups for the former in the coated region of the extraction device (MacGillivray, 1999).

(ii) Elution

SPE materials are usually in the form of cartridges and disks. However, unlike the cartridges, the SPE disks are much less prone to clogging by suspended particulate matter because of their large surface area (Poiger et al., 1996). SPE cartridges were used by Hayashi et al., (2002) to extract FWAs from river and sea water. Prior to extraction, the water samples were first filtered through glass fibre filters. According to Poiger et al., (1996), another advantage of SPE disks over cartridges is that the disks can be a combination of filtration and enrichment into one step, thereby, eliminating a filtering step if cartridges were to be used.

For elution, the adsorbed substances can be removed with an appropriate solvent. Typically, organic analytes can be eluted from a SPE column with an organic solvent such as acetonitrile or methanol (Fritz, 1999). In the method by Poiger et al., (1996), the solvents used for preconditioning and elution of the C\textsubscript{18} disks included methanol and 0.05 M tetrabutylammonium hydrogen sulphate (TBA). High recoveries of 75-90% were obtained for the determination of FWAs in primary effluent using only 6 mls of 0.05M TBA in methanol. Elution of the C\textsubscript{18} SPE disks with only methanol resulted in lower recoveries of 50-70%. The use of TBA in methanol facilitated a more efficient extraction of TBA-FWAs ion pairs from suspended matter in the
sample (Poiger et al., 1996). Hayashi et al., (2002) used only methanol (20 mls) to elute the FWAs from the cartridges, however, like Poiger et al., (1996), TBA in methanol was used to elute the FWAs from the particles contained on the glass fiber filters.

(iii) **High-performance liquid chromatography (HPLC)**

Analyses of the FWAs by Poiger et al., (1996) were performed using High-Performance Liquid Chromatography (HPLC) connected to a post –column UV irradiation apparatus. According to Gilbert, (1987), the HPLC is a commonly used analytical technique, due mainly to the ability to improve the selectivity of the system by the interactions of the stationary phase and the mobile phase. The mobile phase solvents comprised of acetonitrile/methanol (1:1) (eluent A) and 0.1 M aqueous ammonium acetate buffer of pH 6.5 (eluent B). For elution, a linear gradient from 30%A/70%B to A70%/30%B for 25 minutes was used for the FWAs (Poiger et al., 1996). A gradient elution means that the composition of the solvents A and B (mobile phase) changes within a predetermined time period (Issaq, 2002). Generally, in liquid chromatography the three gradient shapes generally used are linear, convex, or concave. Prior to detection, the samples were irradiated for 5 seconds by the column in order to achieve a constant ratio of (E)- and (Z) isomers. The quantification of the (Z) isomers is done by exposing standards containing the (E) and (E,E) FWAs to direct sunlight for 1 minute. A fluorescence detector was used to monitor the irradiated column effluent at an excitation wavelength of 350 nm and an emission wavelength of 430 nm.

For the determination and behaviour of individual FWA isomers, all sample preparation steps were conducted in a windowless room, equipped with special lamps (Phillips TLD36/16Yellow), however, all procedures can be carried out in normal laboratory light if only the total FWA concentrations were to be investigated (Poiger et al., 1996).

The method by Poiger et al., (1996) using SPE disks achieved recoveries of 76 to 96% for FWAs from raw sewage, primary effluent, secondary effluent, and river water. The precision of the method indicated by the standard deviation ranged from 1 to 11% and the limit of quantification was 0.03 μg/L. Hayashi et al., (2002) also received excellent recoveries of 92% for DSBP and ~ 94% for DAS1 using cartridges for the extraction of spiked sea water.
Typical limit of quantitation for DAS 1 and DSBP were reported as 0.003 and 0.0032 µg/L (Stoll and Giger, 1997), and 0.005 and 0.0004 µg/L (Hayashi et al., 2002) respectively in distilled water.

2.7 Sulfamethazine

Sulfamethazine (4-amino-N-(4,6-dimethyl-2-ptyimidinyl)benzenesulfonamide), which are yellowish colored crystals, belongs to a group of sulfonamides that were the first antimicrobial (antibacterial) drugs to be of clinical use. In general, sulfonamides, which are anti-infective drugs, do not kill organisms but restrict their reproduction and growth, as a result, the defence mechanisms of the host determines the success of the treatment. Sulfonamides are generally used for the treatment of various infectious diseases to animals; some of these infections include respiratory and urinary, foot root and mastitis (Spinelli and Enos, 1978).

2.7.1 Specific use

(i) Drug product database (DPD)

Drugs approved for use in Canada, are listed in the Drug Product Database (DPD), which is managed by Health Canada, and includes human pharmaceutical and biological drugs, veterinary drugs and disinfectant products. The DPD contains about 23,000 marketed products, which were notified by companies. A search of the online DPD with sulfamethazine as the active ingredient, returned 49 sulfamethazine/ drug combinations, all classed for veterinary use. In contrasts, sulfamethoxazole, a drug in the same class as sulfamethazine returned 17 sulfamethoxazole/ drug combinations all classed for human use (Health Canada, 2007). Thus, in Canada, sulfamethazine is approved solely for veterinary use.
At the Federal level, non-prescription antimicrobials for animal feed are approved by Health Canada, and listed in the Canadian Compendium of Medicated Ingredients Brochure (CMIB) (Health Canada, 2002). In general, the CMIB specifies the livestock species, the level of medication, instructions for feeding, the reason for which each medicating ingredient may be legally used and the brand name of each medicating ingredient, which is approved for use in Canada. Overall, all medicated feed manufactured, used or sold in Canada must be prepared according to the specifications in the CMIB. The only exception is feeds prepared according to a veterinarian’s prescription (CFIA, 2007).

According to the CMIB, only drug and drug combinations, which are specifically listed, may be used in feed unless accompanied by a veterinary prescription. For example, according to Health Canada, there are three CMIBs for sulfamethazine/drug combinations, which are #38, 49 and 67 (Health Canada, 2007). These three Medicated Ingredients Brochure (MIB) have been approved for use in meal or pellet feed for swine (#38 and 67) and beef cattle (#49) (Table 2.1). As observed in the previously discussed land use study and reported by the Township of Langley, beef cattle and swine are key agricultural activities in Langley (TOL, 2007b) thus, it is possible that feed containing sulfamethazine have been fed to the cattle and swine, particularly during periods of stress, due to weaning, shipping or handling, and diseases.

As mentioned earlier, each CMIB also describes the type of treatment approved for the sulfamethazine/drug combination (Table 2.2). Essentially, a drug, which has been approved for therapeutic or medicinal use, cannot be used as a growth promoter, even under a veterinary prescription. However, several of the growth promotion claims levels also overlap the therapeutic claims (Health Canada, 2002), thus increasing the use of sulfamethazine as a drug. For example, according to CMIB #38 (chlortetracycline hydrochloride, sulfamethazine and procaine penicillin), there are four claims listed for growth promotion (1, 2, 3, and 4) and two claims (5 and 6) for therapeutic purposes in swine (Health Canada, 2007).
### Table 2.1 Federal approved use for sulfamethazine in medicated feed

<table>
<thead>
<tr>
<th>Drug/ Drug combination</th>
<th>CMIB #</th>
<th>Approved use</th>
<th>Approved Claims</th>
<th>Level of sulfamethazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>38</td>
<td>In meal or pellet feed for swine.</td>
<td>1. For swine pre-starters and starters (claim 1,2,5)</td>
<td>110 mg/kg (0.011%) in the complete feed.</td>
</tr>
<tr>
<td>hydrochloride, sulfamethazine and procaine penicillin</td>
<td></td>
<td></td>
<td>2. For other swine feeds (claim 3, 4, and 6).</td>
<td></td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>49</td>
<td>In meal or pellet feed for beef cattle.</td>
<td>1. For beef cattle (claim 1).</td>
<td>350 mg/head/day.</td>
</tr>
<tr>
<td>hydrochloride and sulfamethazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tylosin phosphate and sulfamethazine</td>
<td>67</td>
<td>In meal or pellet feed for swine.</td>
<td>1. For swine starter feeds (claim 1).</td>
<td>110 mg/kg (0.011%).</td>
</tr>
</tbody>
</table>

**Source: Health Canada (2007)**

### Table 2.2 Federal approved claims for sulfamethazine in medicated feed

<table>
<thead>
<tr>
<th>CMIB #</th>
<th>Claim #</th>
<th>Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>1,3</td>
<td>As an aid in maintaining growth rate and feed efficiency in the presence of Atrophic Rhinitis in swine.</td>
</tr>
<tr>
<td></td>
<td>2,4</td>
<td>As an aid in maintaining weight gains and stimulating appetite during periods of stress caused by moving, vaccination, extreme temperature changes and castration in swine.</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>As an aid in the prevention of Bacterial Enteritis (including <em>Salmonella choleraesuis</em> and Swine (Vibrio) Dysentery).</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>As an aid in the treatment of Bacterial Enteritis (including <em>Salmonella choleraesuis</em> and Swine (Vibrio) Dysentery).</td>
</tr>
<tr>
<td>49</td>
<td>1</td>
<td>As an aid in the maintenance of weight gains and feed efficiency in cattle during periods of stress, due to weaning, shipping or handling.</td>
</tr>
<tr>
<td>67</td>
<td>1</td>
<td>For the prevention of Swine Pneumonia caused by bacterial pathogens (<em>Pasteurella multocida</em> and / or <em>Corynebacterium pyogenes</em>) sensitive to tylosin and/or sulfamethazine.</td>
</tr>
</tbody>
</table>

**Source: Health Canada (2007)**
In addition to swine, sulfamethazine has also been registered for therapeutic use in other animals including cattle, horse, turkey, breeder, broiler, sheep, duck, goat, cat and dog, and disease prevention, prophylaxis and/or control in cattle, sheep, swine and horse. Essentially, in therapeutic treatment, individual animals may be treated; however, it is more feasible and efficient to treat a group of animals by medicating the food or water. Treatments for prophylaxis are for the most part used during high risk periods for disease (after weaning or transport) (Health Canada, 2002). As mentioned earlier, horse, beef cattle, poultry and swine were key agricultural activities within Langley, all livestock, which may need sulfamethazine drugs for therapeutic or disease prevention, prophylaxis and/or control.

In addition to the antimicrobial specific use as indicated on the label, veterinarians sometimes use their prudence to prescribe the antimicrobial drug for use other than that indicated on the label, (often called “extra-label or off – label use”). Extra label drugs may be used in the treatment to animals, when no approved drugs or doses are effective for specific animals or conditions, due to the limited availability of approved drugs for minor species, such as fish, goats, llamas and sheep (Health Canada, 2002). Thus, antimicrobial drugs for extra label use, may add to the inventory of sulfonamides used in Canada.

2.7.2 Frequency of use

(i) Sales and purchases

Overall, in Canada, the sale of drugs is regulated by Health Canada through the Food and Drugs Act and Regulations, and the Controlled Drug and Substance Act (Health Canada, 2002). Essentially, Health Canada reviews the drugs to assess their safety, efficacy and quality before the drug product are authorized for sale in Canada. The legislations for the drugs used by humans are administered mainly through the Therapeutic Products Directorate (TPD), and the federal authority, which regulates all pharmaceuticals meant for human use in Canada, is the Health Products and Food Branch (HPFB). According to the TPD, it is mandatory that a manufacturer present scientific evidence of a product’s safety, efficacy and quality according to the Food and Drugs Act and Regulations (Health Canada, 2007). The legislations for antimicrobials and other veterinary drugs for food animals are administered mainly through the
Veterinary Drugs Directorate (VDD), formerly Bureau of Veterinary Drugs (BVD). The veterinary antimicrobials are divided by Federal regulations into sale by prescription and sale without prescription or over the counter. Antimicrobials may be sold by pharmacists, veterinarians and approved layperson outlets (Health Canada, 2002).

Once the drugs are approved at the federal level, each province in Canada, through its regulatory body, has the right to regulate more severely, but not more leniently, the sale of drugs. For example, drugs are regulated through the Pharmacists Act of British Columbia, and administered by the Chief Veterinarian with the Animal Health Branch of British Columbia’s Minister of Agriculture, Fisheries and Food (BCMAFF). The Chief Veterinarian under the regulation licenses established properties to sell veterinary drugs. Licensed properties may include feed mills to mix and sell medicated feed, feed dealers to mix and sell medicated feed, or for retail outlet to sell veterinary drugs (Health Canada, 2002). According to the British Columbia Pharmacists, Pharmacy Operations, and Drug Scheduling Act for Veterinary Drug and Medicated Feed (Schedule A, Table 2), sulfamethazine, which is listed as a chemotherapeutic drug, can be sold by the holders of the appropriate Veterinary Drug licenses, therefore, no public access is allowed to these drugs (BCMAL, 2009).

As established earlier, sulfamethazine was approved for use in animals, which includes some of the key livestock in Langley such as horse, sheep, poultry, beef and swine. However, on a local, municipal, regional or national scale, data (purchases or sales) supporting the use of sulfamethazine or any other antimicrobial in Canada was difficult to obtain for this study. A report by the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health stated that in Canada, there are no mechanisms in place for the collection, analysis and reporting of data for antimicrobial consumption in food producing animals. As a result, the quantities of various antimicrobials used in animals are not known (Health Canada, 2002). Recently, Carson et al., (2008) reported that currently there are few studies about antimicrobial use on beef cattle farms in Canada or the USA, which are analogous.

An attempt was made by Fraser et al., (2004) to estimate the weight of antimicrobial purchased in British Columbia for animal use, however, the sources of animal drug use data for the project was limited or hindered by confidentiality concerns. For example, under the authority of the
British Columbia Veterinary Drug and Medicated Feeds Regulation, licensed lay drug outlets and licensed feed mills are required to submit records of their drug purchases annually to BCMAFF, however, the records have generally not been compiled and analyzed (except for a salmon farm study in 1996) due to staffing shortages. The authors reported that the BCMAFF had started to implement a new medicated feed database program to collect from feed mills more detailed information, such as species, production class, dose and duration.

Based on handwritten provincial lay drug outlet and feed mill purchase records from the BCMAFF for 1997 and 1998, Fraser et al., (2004) reported that for 1997 and 1998, out of a total of 32 and 45.7 tons of antimicrobials purchases by licensed veterinary drug outlets and feed mills in British Columbia respectively, around 1 and 2 tons respectively were sulfonamides. However the authors cautioned that the data compiled for 1997 and 1998 were underestimated, due to missing or unavailable data.

(ii) Cattle feedlots

Radostits and Stockdale (1980) reported that the treatment of beef cattle using sulfonamides in Western Canada had been widely practised due to a common disease called coccidiosis, which may affect 25-50% of the herd. Young calves between the ages of six months to 12 months of age are vulnerable, particularly during the fall and winter months, when the calves are confined in farms or commercial feedlots. Sulfamethazine is one of the drugs, which had been reported to successfully control bovine coccidiosis.

A study on steer calves was conducted at an experimental feedlot in Lethbridge, Alberta by Inglis et al., (2005). The authors reported that the antimicrobial agents including sulfamethazine were chosen for the study due to their widespread use in the Canadian feedlot industry. The feeding regime, which is typical for the Canadian feedlot industry, consisted of forage based diets containing antimicrobials, which was fed to the steers 18 days after arrival at the feedlots. The forage based diets were fed to the steers for 56 days, after which, antimicrobials were removed from the diet for 91 days, and then reintroduced with the grain based diet for 42 days. Subsequently, the steers were slaughtered 315 days after arrival at the feedlot.
On a commercial scale, the current use of sulfamethazine in large feedlots (> 16,000 animals) in Canada was confirmed in a study by Inglis et al., (2006). The authors reported that at four cattle feedlots in south – central Alberta, under standard industry practices, the cattle feed were supplemented with antimicrobials including chlortetracycline with sulfamethazine (Aureo S-700 G; 350 mg/head/day), in order to control liver abscesses, bacterial pathogens and to serve as growth promoters. In addition to reducing mortality, it has been reported that the overall use of sulfamethazine as a feed additive has had economic benefits. Gallo and Berg (1995) reported a savings of $7.20 per head for cattle fed chlortetracycline (350 mg per head per day) and sulfamethazine (350 mg per head per day) for 56 days.

Aust et al., (2008) also reported the use of sulfamethazine at both experimental and commercial beef cattle feedlots in Alberta. At the experimental feedlot, which comprised of 24 pens each containing 10 head of beef cattle, it was reported that the livestock were fed antibiotics in a manner similar to the commercial feedlot. The sulfamethazine/chlortetracycline (SMZ/CTC) drug combination AS 700, which contained 77 mg SMZ kg\(^{-1}\) and 77 mg CTC kg\(^{-1}\) was administered to the cattle at a dose of 350 mg (head day\(^{-1}\)). The authors reported that the antibiotics, which were fed to the beef cattle in a premix for a period of 211 days, were chosen due to the widespread use in the industry. At the commercial feedlot, sulfamethazine was administered to cattle for a period of 20 years; however, the last drug administration was in 2004 for a period of 40 days at a dose of 350 mg (head day\(^{-1}\)).

(iii) Swine farms

Dunlop et al., (1998) conducted a study in 1992, to determine the antimicrobial usage rates of farrow to finish swine farms in Ontario, Canada. Overall, sulfonamides, in particular sulfamethazine were used in 25 out of 34 swine farms. In 2000, a survey of 90 swine farms in Alberta representing 25% of the province’s market swine production revealed that the chlortetracycline/sulfamethazine/penicillin drugs combination were given to the swine. Out of 76 (weaners), 88 (growers), 76 (lactating sows) and 76 (dry sow) farms in Alberta; the number of farms using the chlortetracycline/sulfamethazine/penicillin drugs combinations through feed were 45 (59.2%), 5 (5.7%), 6 (7.9%) and 3 (3.9%) respectively. Whereas, for the same numbers of farms, 3 (3.9%), 1 (1.1%) and 1 (1.1%) of the weaning phase, growing phase and finishing
phase were administered sulfamethazine through water. In this study, there were no reports of sulfamethazine being administered to the swine through injections. Overall, the survey reported that most pigs were given antibiotics in feed after weaning since they are most vulnerable to infectious diseases during this phase (Rajić et al., 2006).

(iv) Residue in meat

In general, the Canadian Food Inspection Agency (CFIA) monitors sulphonamides in meat products including beef, chicken, horse, mutton, pork, sow, turkey and veal. A compliance summary for sulfonamides residues in meat from domestic animals for a five year period revealed that there were 27 instances of meat products, which contained sulfonamides above the maximum residue level, and therefore not in compliance with Canadian standards (Table 2.3) (CFIA, 2006).

In addition, on site sulfonamides tests by veterinarians and front line inspectors in slaughter plants reported positive testing of sulfonamides in pork, which consisted of 10 urine positives, 6 confirmed liver violations and 3 confirmed muscle violations. Laboratory testing of positive sulfonamides samples from slaughter plants confirmed sulfamethazine in the tissues of pork and beef with maximum concentrations of 801 and 167 µg/L respectively. Violations are samples, which exceeded the maximum residue levels (Table 2.4) (CFIA, 2006). In Canada, sulphonamide residues (sulfamethazine and sulfamerazine) have also been detected in egg samples in a screening test conducted by the CFIA. Over a two year period (1997-1999), sulphonamide residues were tested potentially positive in egg samples from Alberta (n = 2), British Columbia (n = 1), Manitoba (n = 1) and Ontario (n = 1). However, only one egg sample, which tested positive and originated from Manitoba, was confirmed to contain sulfamethazine residue at an extremely low level of < 15 µg/L (Quon, 2000).
### Table 2.3  Canadian domestic product testing for sulfonamides residues in meat

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Pork</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Sow</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Horse</td>
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<td>1</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>Other</td>
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<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7</strong></td>
<td><strong>5</strong></td>
<td><strong>2</strong></td>
<td><strong>10</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

*Source: Canadian Food Inspection Agency (2006)*

### Table 2.4  Laboratory confirmations of sulfamethazine positive animal tissue

<table>
<thead>
<tr>
<th>Meat</th>
<th>Residue found</th>
<th>Tissue</th>
<th>Mean ug/L</th>
<th>Minimum ug/L</th>
<th>Maximum ug/L</th>
<th>Violations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork</td>
<td>Sulfamethazine</td>
<td>Liver</td>
<td>236</td>
<td>13</td>
<td>801</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Sulfamethazine</td>
<td>Muscle</td>
<td>120</td>
<td>17</td>
<td>364</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Sulfamethazine</td>
<td>Unknown</td>
<td>326</td>
<td>326</td>
<td>326</td>
<td>1</td>
</tr>
<tr>
<td>Beef</td>
<td>Sulfamethazine</td>
<td>Liver</td>
<td>167</td>
<td>167</td>
<td>167</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sulfamethazine</td>
<td>Muscle</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

*Source: Canadian Food Inspection Agency (2006)*

### 2.7.3  Fate of sulfamethazine

#### (i)  Animal digestion and waste

In general, sulfonamides including sulfamethazine are removed from the animal's body as unchanged parent compounds or as metabolites after extensive metabolism. Depending upon their physicochemical nature, both the parent drug and metabolites may be excreted via kidney and/or bile (Rehm *et al.*, 1986). For example, (Halling-Sørensen *et al.*, 2001) reported that 90%
of the sulfamethazine, which are administered through the diets of beef cattle will be excreted. Sulphonamide concentrations totalling 1,380 mg/L have been reported in the urine of two calves, immediately following the administration sulfamethazine at a therapeutic dose of 99 mg/kg per day. In addition to urine, sulphonamide residues have also been detected in the muscle, liver, kidney, fat and serum of the aforementioned two calves at concentrations of about 30, 55, 43, 19 and 107 mg/L respectively (Righter et al., 1971).

Similar to calves, sulfamethazine compounds have also been detected in the blood, urine and tissues of lambs. For example, after the intravenous administration of sulfamethazine to 13 lambs, the average plasma concentration after 0.5 hours was 28.2 mg/100 ml, with about 1.4% of the dose excreted in the urine. However, after 48 hours of the initial dose, sulfamethazine was for the most part cleared in the 6 tested lambs with only an average plasma concentration of 0.1 mg/100 ml, and about 70% of the dose excreted in the urine. Interestingly, six hours after dosing, the sulfamethazine concentration in the tissues of a slaughtered lamb was highest in the kidney (124 mg/L), as compared with other tissues including the liver (69 mg/L), heart (83 mg/L), and leg muscle (57 mg/L). The highest concentration of sulfamethazine in the kidney tissues suggests rapid urinary clearance of the parent drug (Bevill et al., 1977a).

The amount of sulfamethazine present in animals’ waste may be dependent on the drug’s route of administration. For example, in a study on turkey poults, as much as 17% of the intravenous sulfamethazine dose was recovered in the urine and feces of 11 female and 3 male poults, after 72 hours of drug withdrawal. However, a lesser 8.6% of the oral dose (143 mg/kg) was recovered in the urine and feces of 11 female poults. Overall, the study found that sulfamethazine was quickly eliminated from blood and various tissues in the turkey poults, however, small concentrations of the parent drug may be retained in the liver, kidney and skin, at concentrations ranging from 0.1 to 0.4 mg/L (Heath et al., 1975).

The metabolites of sulfamethazine have also been detected in animal waste. Sulfamethazine and three metabolites have been detected in the urine of lambs, up to the time of slaughter. An estimated 70% of the administered dose (intravenous) was excreted in the urine as sulfamethazine (18%), hydroxylated metabolite (18%), polar metabolite (24%) and acetylated metabolite (11%) (Bevill et al., 1977a). Essentially, metabolites have a higher polarity and
hydrosolubility than their parent compound; as a result, renal excretion is generally favoured (Rehm et al., 1986).

Similar to the parent compound, the amount of sulfamethazine metabolites found in the animal’s wastes, may also depend upon the drug’s route of administration. For example, three experiments, which involved the use of an oral solution, an oral sustained release bolus and an injection, were conducted on three 1 year old calves. The oral dosages, which comprised of a solution of 12.5% sulfamethazine sodium (107 mg/kg), and a sustained release bolus (67.5 g) were administered to each calf through a stomach tube and a balling gun respectively. The intravenous dose, which comprised of 107 mg/kg of a solution of 12.5% solution of sulfametazine was rapidly injected into the jugular vein of each calf. After 48 hours, the average cumulative percentages of the parent sulfamethazine excreted in the urine of the three calves were 17.84, 12.61 and 2.97% for the injection, oral solution and sustained bolus respectively. The average cumulative percentages of the polar metabolite were 13.65, 9.93 and 3.30% respectively. For the hydroxy and the acetyl metabolites, the average cumulative percentages were 11.73, 10.69 and 2.87%, and 16.22, 23.09 and 5.25 % respectively. Based on the results, the major excretion product for the oral administrations were the acetyl metabolites, which could be attributed to increase metabolism as a result of greater exposure of the sulfamethazine drugs to the enzymes of the gut and liver. Overall, in many animals, N-acetylation is the major metabolic pathway for most sulfonamides (Rehm et al., 1986).

In Canada, antimicrobials may be administered to animals via feed, water or by injection (Health Canada, 2002). For example, in 1992, based on a study on drug use practices on 34 swine farms in Ontario, it was reported that sulfamethazine was administered to the swine through feed or water (12 farms), oral or injection (8 farms) and by both treatments (5 farms); only 9 swine farms did not use sulfamethazine. Interestingly, most farms used sulfamethazine in combination with other antimicrobials in the rations for weaning pigs (12 farms) and piglets (10 farms), compared with growers (2 farms) and finishers (1 farm) (Dunlop et al., 1998).

Similarly, in Alberta, the results of interview questionnaires from weaning phase of 76 farrow-to-finish farms, growing phase of 88 mixed finishing farms, lactating sow phase of 76 farrow-to-finish farms and dry sow phase of 76 farrow-to-finish farms suggested that sulfamethazine
was administered to the swine predominantly through feed and after weaning, when the young swine are highly vulnerable to infectious diseases. For example, the percentages of farms, which administered sulfamethazine in combination with chlortetracycline/penicillin through feed were 59.2% (weaners), 5.7% (growers), 7.9% (lactating sows) and 3.9% (dry sows). The percentages of farms, which administered sulfamethazine through water, were 3.9% (weaners), 1.1% (growers), and 1.1% (finishers). There were no reports of sulfamethazine being administered to the swine through injections (Rajić et al., 2006). In general, sulfamethazine is one of the antimicrobials, which is in greatest use for the therapeutic treatment of pigs. Most pigs receive sulfamethazine in their starter rations or water after weaning due their vulnerability to infectious diseases caused by viruses, mycoplasma and bacteria (Health Canada, 2002).

Due to the extensive use of sulfamethazine on cattle and swine farms in Alberta, and its therapeutic use in horses, it is assumed that most farms within Langley would also use the sulfamethazine drug to reduce mortality, in particular pigs (weaners) and calves. As a result, it is possible that sulfamethazine from these farm animals may be transported to groundwater via manure and urine.

(ii) Livestock manure

Since livestock is a key agricultural land use activity within Langley, then one major pathway for the possible release of sulfamethazine into the Hopington Aquifer is through livestock manure. Sulfamethazine had been detected at a concentration of about 1 mg/kg in swine liquid manure \((n=2)\), and observed at a concentration of < 0.1 mg/kg \((n=2)\) in cattle liquid manure from North Rhine- Westphalia (Northwestern Germany) (Christian et al., 2003). Another study in Germany also reported sulfamethazine in swine manure at a concentration ranging from 11-62 \(\mu g/kg\) (Pfeifer et al., 2002). In general, sulfamethazine is solely used for veterinary purposes in Germany (Christian et al., 2003).

In Washington County (Idaho, USA), sulfamethazine was detected at a concentration of 43 \(\mu g/L\) in the waste lagoon of a confined animal feeding operation (CAFO), which housed beef cattle (Batt et al., 2006). At an experimental beef cattle feedlot in Alberta, Canada, sulfamethazine was detected in solid manure at a maximum concentration of 9990 \(\mu g/kg\) (Aust et al., 2008).
Sulfamethazine and its main $^4N$-acetyl-sulfamethazine metabolite were detected in the manure pits of pigs (five grab samples) and fattening calves (1 grab sample) in Switzerland. The concentrations of sulfamethazine and $^4N$-acetyl metabolite residues in the manure of the pigs (mother pigs with farrows and fattening pigs) ranged from 0.13 – 8.7 mg/kg and < 0.1 – 2.6 mg/kg (wet sample) respectively. In the manure of the fattening calves, the concentrations of sulfamethazine and $^4N$-acetyl metabolite were 3.2 and < 0.1 mg/kg (wet sample) respectively. Overall, in the five grab samples, the concentrations of the $^4N$-acetyl metabolites were 2 to 50 times lower than the concentrations of the parent sulfamethazine, and in one grab sample, the $^4N$-acetyl metabolite was not detected, it is possible that sulfamethazine remains primarily in the parent form in manure (Haller et al., 2002). According to Christian et al., (2003), the acetyl metabolites in the manure can be transformed to the original active ingredient, as a result of cleaving.

(iii) Soil

The spreading of contaminated manure or slurries onto soil is the main pathway by which veterinary antimicrobials enter the environment (Accinelli et al., 2007). However, few studies have focused on the fate of veterinary antimicrobials in the environment (Hamscher et al., 2005).

Sulfonamides are generally moderately water soluble and polar compounds, and as a result, sorption is pH dependent (Accinelli et al., 2007). The pKa values for sulfamethazine have been reported as 2.07 and 7.49 (Qiang and Adams, 2004). At slightly acidic pH to neutral pH levels, sulfonamides may predominantly exist in the neutral form, and thus can readily partition into the organic fraction of the soils (Yong et al., 1992).

Various sorption experiments were carried out with sulfamethazine and a predominantly sandy North Carolina soil (77%), which had a small organic content of 1%. The sorption values ($K_D$) were determined to be 2.5, 3.9 and 30 at pH levels of 7.4, 5.6 and 2.3 respectively. The significant differences between the $K_D$ values were attributed to rapid dissociation of the sulphonamide (Kurwadkar et al., 2007). Low sorption levels for sulfamethazine were also observed in a predominantly sandy Princeton soil (93.5%), which had a neutral pH of 7.2, and an organic carbon content of 0.94% (Accinelli et al., 2007). In general, Thiele-Bruhn et al.,
(2004) reported that the adsorption values ($K_D$) for sulfonamides are small in comparison to other antibiotics including tetracyclines and fluoroquinolones. Thus, according to Accinelli et al., (2007, the low sorption levels suggest that sulfamethazine is for the most part highly mobile in soil.

In contrast, fertilised soils with livestock manure can contain high concentrations of organic matter including carbon, which should increase the adsorption of sulfamethazine, depending upon the pH. However, Thiele-Bruhn et al., (2004) reported a low $K_D$ value of 3.8 for the sand fraction of a fertilised (farmyard manure) Chernozem soil, which had a pH and organic content of 6.7 and 3.8% respectively. In the above experiment, based on a slightly acidic soil, the neutral sulphonamide species would have predominated. In addition, given the increase in organic content from 1 to 3.8%, it was expected that the ($K_D$) value would have increased; however, the ($K_D$) value remained quite low. As mentioned earlier, a low ($K_D$) value of 2.5 (L kg$^{-1}$) was reported for sulfamethazine based on a North Carolina Loamy Sand at a neutral level of pH 7.4, and an organic carbon content of 1% (Kurwadkar et al., 2007).

In addition to the soil organic content, sulfamethazine can also adsorb unto the clay fraction of soil, particularly under acidic conditions. For example, Gao and Pedersen (2005) observed that between pH ~ 5 and 7, pH dependence of sulfamethazine adsorption to montmorillonite clays was minor, due to the neutral species (unionized) predominating. However, below pH ~ 5, the authors reported a significant increase in sulfamethazine adsorption to the clays. Similarly, Kurwadkar et al., (2007) also reported a low cation exchange capacity of 6.8 (cmol/kg) for the North Carolina loamy sand, which also had a slightly acidic pH of 5.3, and silt and clay fractions of 18 and 5.2% respectively.

Given the relatively low ($K_D$) values for sulfamethazine, it is expected that the antimicrobial will be relatively mobile particularly in soils, which have a neutral or basic pH. According to Boxall et al., (2002), an increase in pH value may result in a decrease in the apparent $K_D$ value of the sulphonamide. For example, in the anionic form (> pH 7.49), adsorption of the species is reduced, due to the lack of cationic exchange or neutral adsorption into the soil organic matter. Based on batch sorption tests of the speciated sulfamethazine (cationic, neutral and anionic) on North Carolina loamy sand at pH range 2.3 to 7.4; the $K_D$ value for the anionic species was ~ 0
L/kg, compared with $K_D$ 70 and 4.8 (L/kg) for the cationic and neutral species respectively (Kurwadkar et al., 2007). In a similar sulfamethazine adsorption experiment, Gao and Pedersen (2005), reported $K_D$ values of ~0 L/kg for montmorillonite clays, at a pH above 8.

The transport of sulfamethazine from manure to soil has been observed at experimental and commercial beef cattle feedlots in Alberta, Canada. Sulfamethazine was detected in the 0 to -10 cm sections of the underlying soil at maximum concentrations of 72 and 10.4 µg/L respectively. Solid manure layers with thicknesses ranging from 15-40 cm and 50-65 cm had developed above the soil sections in the pens. In general, the manure and soil at the experimental and commercial feedlots had basic pH levels of 7.9 and 8.5, and 8.0 and 8.1 respectively. At a slightly basic pH (>7.49), sulfamethazine exists in the predominantly anionic species, which is highly mobile. Interestingly, sulfamethazine was also detected at an average concentration of 710 µg/kg in the manure layers in the control pen, where antimicrobials had not been administered to the animals. The detection of sulfamethazine in the control pen was attributed to horizontal transportation (Aust et al., 2008).

According to Stoob et al., (2006), sulphonamides may persist for several months following soil fertilisation with manure. For example, seven months after fertilisation with swine manure, Christian et al., (2003) observed sulfamethazine at a concentration of about 15 µg/kg in soil. As a result, the authors suggested that the sulphonamide was highly stable in soil. Similarly, Aust et al., (2008) reported that sulfamethazine was persistent in soil. The authors observed sulfamethazine at a concentration of 10.4 µg/kg dry matter in the soil section of a commercial feedlot 1 year after the drug was administered to the cattle.

(iv) Biodegradation

In general, sulfonamides are not readily biodegradable, and thus, there is the potential for these antimicrobials to persist in the environment. Ingerslev and Halling-Sorensen (2000) reported that twelve different sulfonamides biologically degraded after an initial lag phase of 7 to 10 days in an activated sludge system using aerated reactors. Following the lag phase, elimination of the sulfonamides in the reactors occurred within 5 to 10 days.
In a degradation experiment, which used a predominantly sandy Princeton soil (93.5%), it was observed that sulfamethazine was reduced to about 27% of its initial level at the end of a 40 day incubation period with no lag phase. In addition, the average half life values of sulfamethazine in non autoclaved vs autoclaved soils were about 20 and 65 days respectively. Interestingly, when the Princeton soil was amended with a liquid swine slurry (5% w/v), which contained about 20% organic carbon; there was a decrease in sorption, which correlated with an increase in biodegradation of the sulfonamides. In fact, it was reported that there was a significant increase in the degradation rates of sulfamethazine, possibly due to the occurrence of a large quantity of microbial organisms in the swine slurry. These results suggested that biological degradation was the main mechanism for the dissipation of sulfamethazine in the soil (Accinelli et al., 2007). In contrast, Dolliver et al., (2007) observed less degradation of sulfamethazine in a predominantly sandy (71%) soil mixture, which had been amended with sulfamethazine spiked swine manure. After 45 days, the sulfamethazine was reduced to more than 70% of its initial level.

In another study, low concentrations of sulfamethazine have been observed in a predominantly sandy soil (91.50%), which had been fertilised with liquid pig manure over a period of three years (2001-2003). Sulfamethazine was detected in one sample at a concentration of around 2 μg/kg in the top soil (0-10 cm), and observed in 12 soil samples (0- 40 cm) below the detection limit of 2 μg/kg. In one soil sample, sulfamethazine was observed (<2 μg/kg) at a depth of 30-40 cm. Essentially, the low concentrations of sulfamethazine were attributed to the soil’s potential to degrade or remove the antimicrobial (Hamscher et al., 2005).

(v) Groundwater

As discussed earlier, sulfamethazine has the potential to be adsorbed to soil organic matter, diluted or biodegraded. However, according to Batt et al., (2006) the sulphonamide still has high soil mobility due to the low octanol-water partition coefficient (Log Kow), which was determined to be 0.25 (Liu et al., 2003). As a result, it is possible for the sulphonamide to be leached into the groundwater, which makes it favourable as a molecular marker.
In general, data on sulfamethazine in groundwater is limited, although antimicrobials have been widely detected in wastewater and surface water (Batt et al., 2006). To a lesser degree, due to predominantly animal use, sulfamethazine has also been detected in wastewater treatment plants (WWTPS) at concentrations of about 0.018 – 0.039 µg/L (1 out of 2 WWTPS) in the canton St Gall, Switzerland (Göbel et al., 2004), 0.11- 0.21 µg/L (2 out 7 WWTPS) in Wisconsin, USA (Karthikeyan and Meyer, 2006) and 0.363 µg/L (1 out of 8 WWTPS) in Canada (Miao et al., 2004). In surface waters such as streams, creeks and rivers, sulfamethazine had been detected at concentrations of 0.02 – 0.22 µg/L (Kolpin et al., 2002), 0.002 – 0.007 µg/L (Christian et al., 2003) and about 0.06 µg/L (Yang et al., 2004). In contrasts, there are a few studies, which reported the occurrence of sulfamethazine in groundwater.

In northern Germany, sulfamethazine has been detected in groundwater samples from a predominantly sandy soil (91.50%), which had been previously fertilised with liquid pig manure over a period of three years (2001-2003). Out of 28 groundwater samples, which were sampled from four different sites during the period of 2002 to 2003, sulfamethazine was detected in 13 samples at concentrations ranging from 0.05 to 0.24 µg/L, and observed in 9 samples below the limit of quantification (0.05 µg/L). In fact, the authors reported that there was a relationship between the detection of sulfamethazine in the groundwater and the liquid manure, which had been previously applied. (Hamscher et al., 2005). In another study in Germany, also in an agricultural region, sulfamethazine was detected in two groundwater samples at concentrations of 0.08 and 0.16 µg/L; however, these results could not have been confirmed a couple of weeks later from the same or similar sites (Hirsch et al., 1999).

In Idaho, USA, sulfamethazine had been detected in six private well water samples at concentrations ranging from 0.076 to 0.215 µg/L. The private wells were located down-gradient or nearby (< 1600 ft) to a confined animal feeding operation (CAFO), which housed beef cattle, and contained two wastewater lagoons. Since sulfamethazine was detected in higher concentrations in the CAFO waste lagoon (43.353 µg/L) and CAFO well water (0.310 µg/L) (Batt et al., 2006), the likely source of antimicrobial contamination was from agricultural use.

Overall, the detection of sulfamethazine in groundwater is attributed to its leaching potential (Hamscher et al., 2005), particularly in a sand type soil, where the sulphonamide is expected to
be highly mobile. In addition, the occurrence of the sulfonamide in the environment is an indication of contamination from animal sources, given that it is not derived from natural sources, and for the most part, it is approved for animal uses in some countries including Canada (Batt et al., 2006). Historic data ranging from pH 5.6 to 9 for 73 wells from the Hopington aquifer (Gartner Lee Limited, 2000) suggests that sulfamethazine will generally be mobile to highly mobile in the groundwater due to the neutral and anionic species respectively dominating.

2.7.4 Analytical methods

(i) Solid phase extraction

Several researchers have used various types of SPE devices for the extraction of sulfamethazine from an aqueous media including XAD-4 resins (styrene-divinylbenzene) and Oasis HLB cartridges poly(divinylbenzene-co-N-vinylpyrrolidone). Unlike the C-18 and C-8 cartridges, both XAD-4 and Oasis HLB do not contain silica but organic polymers; in addition, the polymeric sorbents are more retentive than the C-18 reversed phase sorbents (Thurman and Mills, 1998).

Polymeric sorbents generally have a hydrophobic surface, large surface areas, typically 750 m²/g and 830 m²/g for XAD-4 and HLB respectively. In general, polymeric sorbents, which have been in use since the 1970s, and have particular applications in reversed-phase SPE, have been widely used in the extraction of both drugs and environmental compounds (Thurman and Mills, 1998).

(ii) XAD-4

Some of the benefits of using XAD-4 resins include the ability of the polymeric resins to retain a large quantity of the analyte without any breakthrough. In general, polymers have high capacities for polar organic compounds (Thurman and Mills, 1998); also, polymeric resins have a higher surface area than that of silica particles (e.g. C-8 and C-18), as a result, there is more complete uptake of organic analytes, with no interfering silanol groups, as with C-8 and C-18
adsorbents (Fritz, 1999). In addition, XAD resins may be used over a pH range of 0-14, with a maximum usage temperature of 480°F (Sigma–Aldrich, 1998).

Cox and Krzeminski (1982) obtained good recoveries of sulfamethazine using XAD resins, although the media was pork tissue compared with a water media used in this current study. The authors obtained an average recovery of about 86% using XAD-2 resin. In general, the XAD-2 polymeric resin, which is used to remove hydrophobic compounds up to 20,000 MW (molecular weight), has a surface area of 330 sq. m/g and a pore diameter of 90 Angstroms. Whereas, the XAD-4 polymeric resin, which is used to remove small hydrophobic compounds, and is widely used in pharmaceutical manufacturing, has a larger surface area of 725 sq. m/g and a pore diameter of 50 Angstroms (Sigma–Aldrich, 1998).

Although the XAD-4 resin is a promising method of extraction for sulfamethazine from an aqueous media; during loading, samples may become contaminated as the water passes through the XAD-4. Essentially, the resins may contain leachable impurities acquired during the polymerization process (Fritz, 1999). As a result, the XAD resins should be washed thoroughly usually by extensive Soxhlet extraction (Sigma–Aldrich, 1998).

(iii) Oasis HLB cartridges

Similar to the older XAD, which is a styrene-divinylbenzene copolymer, the Oasis HLB sorbent is a high surface area copolymer of [poly(divinylbenzene –cos-N-vinylpyrrolidone)]. The HLB sorbent is the only polymer, which has the property of conditioning with only water, as methanol is not needed (Thurman and Mills, 1998).

(iv) pH adjustment

Göbel et al., (2004), reported that of all the parameters involved in sample extraction, the pH of a sample was the most significant variable for sulfonamides, due to their amino groups. In general, sulfonamides are classified as amphoteric drugs (Babić et. al., 2006), which means that they are either cationic or anionic depending on the pH (Thurman and Mills, 1998). For example, sulfonamides are positively charged at acidic conditions, neutral between pH 2.5 and 6,
and negatively charged at alkaline conditions (Babić et al., 2006). In addition, specific compounds have \( pK_a \) of 5-8 for the sulfamino groups (\( pK_a \, 1 \)) and a \( pK_a \) of 2-2.5 for the arylamin (Göbel et al., 2004), as a result, sulfonamides are highly water soluble (Lindsey et al., 2001). Other amphoteric drugs may also be zwitterions (both positively and negatively charged at the same time) (Thurman and Mills, 1998). For sulfamethazine, the specific \( pK_a \, 1 \) and \( pK_a \, 2 \) values have been reported as 2.28 and 7.42 respectively (Lin et al., 1997).

Lindsey et al., (2001) obtained acceptable sulfamethazine recoveries of up to 130% for sulfamethazine spiked distilled water (123 mL), which was adjusted to a pH of 2.5. Similarly, Yang et al., (2004) also observed high recoveries of sulfamethazine spiked in two different water matrix, which was adjusted to pH <3.0. The average recoveries were around 100 and 97% for the spiked deionised and river water respectively. The similar average recoveries for the different water samples were due to the minimal matrix effects in the river water.

In contrasts, Göbel et al., (2004) observed the highest recoveries at pH 4 for sulfonamides, based on an enrichment tests conducted between pH 2 and 6. Average recoveries of 98% for primary, secondary and tertiary were obtained for effluent spiked with sulfamethazine. Essentially, the interaction with the cartridge material was strongest at a pH of around 4 for the analytes in the unchanged forms. Babić et al., (2006) also reported that a pH of 4 was optimum for the extraction of sulfamethazine; the authors obtained an average sulfamethazine recovery of 94.5% for spiked wellspring water. Overall, Lindsey et al., (2001) reported that sulfonamides mainly depend upon hydrophobic interactions with SPE adsorbents.

(v) Conditioning and elution

Based on the SPE method by Lindsey et al., (2001), conditioning of the HLB cartridge involved the use of 3 mls of 0.5 N HCl. Several studies have used HLB cartridges to simultaneously recover different types of antibiotics such as tetracyclines and sulfonamides from an aqueous media. The use of 0.5 N HCl has been used to wash off residual metals on SPE cartridges. Essentially, tetracyclines can adsorb to residual metals on SPE cartridges resulting in irreversible binding to the cartridges, thus lowering recoveries; however, there were no observations reported of sulfonamides binding to residual metals. In addition, the use of HCl
was reported to not affect the extraction efficiency of sulfonamides (Yang et al., 2004; Lindsey et al., 2001). For the elution of sulfamethazine from the cartridges, Lindsey et al., (2001) tested acetone, acetonitrile, ethyl acetate, 2-propanol, and methanol. Overall, each solvent showed acceptable recovery with a minimum of 60%; however, the authors reported a recovery of about 130% for methanol.

(vi) Instrumentation

In general, most contaminants, which are constantly monitored, are determined by gas chromatography/mass spectrometry (GCMS). However, using GC/MS for the analyses of antimicrobials including sulfamethazine requires derivatization of the polar moieties. For the most part, developing a universal derivatization procedure, which is applicable for all substances, is quite difficult, since analyte groups have different properties and functional groups. As a result, high-performance liquid chromatography (HPLC) together with a tandem MS detector was reported to be the instrument of choice for the separation, identification and quantification of polar compounds (Hirsch et al., 1998). In addition, Lindsey et al., (2001), reported that antimicrobials respond well to positive electrospray ionization ESI (+), due to many being non-volatile and having high molecular weight. ESI(+) is also sensitive enough for the detection of sulfamethazine; as a result, liquid chromatography / mass spectrometry (LC/MS) was reported to be a superb choice for separation and identification.

Yang et al., (2004) also reported that LC/MS, LC/MS/MS and ion trap LC/MS were good instruments for the quantitation of trace levels of antibiotics. Other benefits of using these instruments include good quality full scan MS/MS data and its lower cost compared with triple quadrupole mass spectrometer. Similarly, Batt et al., (2006) observed the distinctive fragment ions of sulfamethazine in all their water samples, and concluded that there was great confidence using the MS/MS method even at trace levels of the analytes.

For the determination of sulfamethazine in surface water, Yang et al., (2004) used LCQ Duo ion trap LC/MS/MS with ESI(+) and selected reaction monitoring (SRM). The authors stated that the use of SRM increases the analytical sensitivity and selectivity in complex matrices, due to
the use of a mass spectrometer to acquire and record ion current at only selected mass-to-charge ratio (m/z) values.

Typical method detection limits and limits of quantitations reported for sulfamethazine in aqueous samples, which were analysed by LC/MS or LC/MS/MS instrumentation include 0.02 µg/L (Hirsch et al., 1998; Batt et al., 2006; Karthikeyan et al., 2006), 0.05 µg/L (Yang et al., 2004; Hamscher et al., 2005), 0.1 µg/L (Lindsey et al., 2001).

In comparison to the frequent use of MS, few researchers have used ultraviolet detection (UV) for the determination of sulfamethazine in aqueous samples. de Zayas-Blanco et al., (2004) used LC/UV for the determination of sulfamethazine in milk samples. Using a UV 2000 ultraviolet detector at a wavelength of 264 nm, the authors reported well separated sulfamethazine peaks, no interferences from the matrix and low detection limits of 3 and 10 µg/kg for the LOD and LOQ respectively. Similarly, Jen et al., (1998), used HPLC with a Waters 484 UV tunable detector, which operated at a wavelength of 260 nm for the determination of sulfonamides residues in swine wastewater. Overall, the authors reported sharp, symmetrical and well separated sulphonamide peaks using a UV detector.

In terms of economics, Babić et al., (2006) stated that LC/MS is expensive for the routine analysis of pharmaceuticals in aqueous samples. For example, the estimated costs for LC MS/MS is $150,000, compared with approximately $60,000 for LC UV.

2.8 Summary

Based on historical data for the Hopington Aquifer, elevated nitrate-N concentrations above the drinking water quality limit of 10 mg/L have been observed in the wells of private residences. The continuous input of nitrates from non point sources into the unconfined portion of the aquifer has been an ongoing issue with managers and residents of the aquifer.

Livestock operations and septic systems have been identified as key land use activities within the Township of Langley, and thus possible contributors of nitrate-N to the unconfined Hopington Aquifer. Confirming the main source(s) of nitrate-N in the Hopington Aquifer may
assists with the overall management of the aquifer, and the mitigation of nitrate contamination. DAS 1 and DSBP (septic systems), and sulfamethazine (livestock) were suggested as potential molecular markers for this study by Dr Ken Hall (Emeritus Professor at UBC) and Dr Mervyn Wetzstein (Manager, Livestock Health Management and Regulation Unit, B.C. Ministry of Agriculture and Lands).

Published analytical methods on SPE followed by HPLC UV will be investigated for the determination of DAS 1, DSBP and sulfamethazine from an aqueous media. In addition, method development of key analytical components including instrumentation (columns, mobile phases and wavelength) and SPE (cartridges and solvents) will be investigated in order to optimize recoveries of the markers.
3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

The fluorescent whitening agents Tinopal CBS-X (distyryl biphenyl derivative (DSBP) and Tinopal AMS-GX (triazole stilbene brightener (DAS) were obtained in the form of sodium salts from Ciba Specialty Chemical Corporation. Sulfamethazine was purchased from Sigma-Aldrich. The purities of DSBP, DAS and sulfamethazine by weight were 89-91%, 84-86% and 99% respectively. Tetrabutylammonium hydrogen sulfate (TBA) 97% was purchased from Sigma-Aldrich. The solvents methanol, acetonitrile (HPLC grade), ammonium acetate (laboratory grade), glacial acetic acid (reagent grade) and N, N – dimethyl formamide (DMF) were all purchased from Fisher Scientific. Reagent grade water was obtained from an Alpha-Q Ultra Pure Water System (Millipore). All HPLC solvents were filtered with 0.45 μm HA (50 mm) Millipore membrane filters prior to use.

3.1.2 Cartridges and resins

3M Empore High Density (HD) disposable C18 extraction disk cartridges (10 mm) and Waters 60- mg hydrophilic-lipophilic balance (HLB) cartridges were used for the extractions of the FWAs and sulfamethazine respectively. Sigma Amberlite™ XAD-4 polymeric resins were also used for the extraction of sulfamethazine. ‘Baker’ -10 SPE System consisting of a stainless steel vacuum basin was used to process the cartridges for both the FWAs and sulfamethazine extractions.
3.2 Methods

3.2.1 Standard solutions

Standard stock solutions of FWA (1000 mg/L) were prepared in DMF/Water (1:1). The standard solutions were diluted in 0.2 M TBA in DMF/water (1:1) to concentrations of 0.005, 0.01, 0.02, 0.04 and 0.05 mg/L. Standard sulfamethazine stock solution (1000 mg/L) was prepared in methanol and stored in an amber vial at 4°C, this stock should be stable for at least two months (de Zayas-Blanco et al., 2004). Working solutions of 10, 1, 0.1, 0.01 and 0.005 mg/L were diluted in mobile phase (water/methanol/glacial acetic acid 79:20:1). For the detection of the DAS 1 (Z) and DSBP (EZ) photoisomers, standards comprising of the parent E and EE isomers respectively were exposed to direct sunlight for approximately 1 minute.

3.2.2 Calibration standards

Standard solutions with concentrations of 0.005, 0.01, 0.02, 0.04 and 0.05 mg/L, and 0.005, 0.01, 0.05, 0.02, 0.1 and 1 mg/L respectively were used for the construction of calibration curves for the FWAs and sulfamethazine respectively. Quantitation was performed by comparing the peak areas of the unknown sample with that of the external calibration. The linearity of the calibration curves for DAS 1, DSBP, and sulfamethazine were \( r^2 > 0.999 \).

3.2.3 Solid phase extraction of fluorescent whitening agents

In general, DAS 1 and DSBP were extracted and analyzed according to adapted methods of Stoll and Giger (1997) and Poiger et al (1993, 1996). The disk cartridges were cleaned with 2 mLs of 0.05 M TBA in methanol and preconditioned using 10 mLs of methanol followed by 30 mLs of DI water. During the above cleaning and pre-conditioning steps, the disk cartridges remained wet. In order to determine the total (adsorbed and dissolved) concentration of FWAs, the samples were unfiltered and homogenized by shaking. A volume of 400 mLs sample was passed through the extraction disk cartridges by vacuum. For the recovery test, 500 mLs of reagent grade water was used. The cartridges were dried for 5 minutes by vacuum to remove excess water in the disk. For elution, methanol was first allowed to soak into the disks for 3
minutes. A total of 20 mLs of methanol was eluted from each cartridge and collected in round bottom flasks by vacuum. The eluent was rotoevaporated to dryness using a Flash-Evaporator (Buchler Instruments) under mild heating of 50°C. For reconstitution and analysis, a 0.5 mL mixture of water and DMF (1:1) was added to the flask and mixed.

3.2.4 Solid phase extraction of sulfamethazine

Sulfamethazine was extracted and analyzed according to adapted methods by Lindsey et al., (2001) and Waters Corporation, (2002).

(i) Cartridges

The HLB cartridges were preconditioned with 3 mLs of methanol, 3 mLs of 0.5 N HCl, and 3 mLs of reagent grade water (Lindsey et al., 2001), there was a moderate flow of the solvents through the cartridges under gravity, therefore the vacuum was turned off during the preconditioning step. All samples were adjusted to ~ pH 2.5 with 40% sulfuric acid (H₂SO₄) prior to extractions. The pH adjusted samples were passed through the cartridges with the aid of 75 mLs reservoirs at a flow rate of 5 mLs per minute. The cartridges were rinsed with 3 mLs of reagent grade water after the samples had completely passed through. The analytes were eluted with 5 mLs of methanol, concentrated to dryness under a flow of N₂ and reconstituted with mobile phase (water/methanol/glacial acetic acid 79:20:1).

(ii) XAD-4 resins

A glass minicolumn approximately 15 cm x 11 mm in length was filled to about ¾ of the length with Sigma Amberlite™ XAD-4 polymeric resins, which was previously washed with methanol. The tip of the column was stuffed with Supelco glass wool to prevent both the XAD-4 beads from escaping and to provide a suitable flow rate for the sample. Three successive elutions, each comprising of 5 mLs of methanol were used to recover the sulfamethazine from the resins.
3.2.5 HPLC UV-fluorescent whitening agents

All analyses were performed using a HPLC system, which comprised of a Waters 600E Multisolvent Delivery System, a Waters 486 Tunable Absorbance Detector and a Waters 717 plus Autosampler. A 50 µL aliquot of the reconstituted sample in a 1 mL vial was injected unto the HPLC.

The FWAs were separated using a 4.6 x 100 mm SymmetryShield™ RP8 column with a 3.5 µm pore size (donated by the Waters Corporation) operated at 25°C with an eluent flow rate of 1 mL/min. The mobile-phase solvents consisted of a 2:3 mixture of methanol and acetonitrile (eluent A) and 0.1 M aqueous ammonium acetate buffer of pH 6.5 (eluent B). A 15 minute linear gradient from 35%A/65%B to 60%A/40%B, followed by a 2 min linear gradient to 90%A/10%B, was used to analyse the FWAs. The washing time was 9 minutes, after which the initial eluent composition was reestablished by a 3 minute linear gradient, followed by an equilibration time of 5 min (Table 3.1). The UV detector was operated at a wavelength of 350 nm.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Flow (mL/min)</th>
<th>A %</th>
<th>B %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>35.0</td>
<td>65.0</td>
</tr>
<tr>
<td>15.00</td>
<td>1.0</td>
<td>60.0</td>
<td>40.0</td>
</tr>
<tr>
<td>17.00</td>
<td>1.0</td>
<td>90.0</td>
<td>10.0</td>
</tr>
<tr>
<td>18.00</td>
<td>1.0</td>
<td>90.0</td>
<td>10.0</td>
</tr>
<tr>
<td>26.00</td>
<td>1.0</td>
<td>90.0</td>
<td>10.0</td>
</tr>
<tr>
<td>29.00</td>
<td>1.0</td>
<td>30.0</td>
<td>70.0</td>
</tr>
<tr>
<td>34.00</td>
<td>1.0</td>
<td>30.0</td>
<td>70.0</td>
</tr>
</tbody>
</table>
3.2.6 **HPLC UV- sulfamethazine**

Sulfamethazine was also separated using a 4.6 x 100 mm SymmetryShield™ RP8 column with a 3.5 µm pore size (donated by the Waters Corporation). The mobile phases A and B comprised of 1% acetic acid in reagent grade water (A), and methanol (B). The isocratic composition of the solvents was 80% A and 20% B for 20 minutes operating at a wavelength of 264 nm, a temperature of 25°C and a flow rate of 1.0 mL/min (Waters Corporation, 2002).

3.2.7 **Nitrate, ammonia, chloride and phosphate**

Nitrate, ammonia, chloride and phosphate were analyzed by 4500 NO₃⁻ I. Cadmium Reduction Flow Injection Method (Proposed), 4500 NH₃ H. Flow Injection Analysis (Proposed), 4500 Cl⁻ G. Mercuric Thiocyanate Flow Injection Analysis (Proposed) and 4500 P G. Flow Injection Analysis for Orthophosphate (Proposed) respectively (Clesceri et al., 1998). The flow injection analysis was performed on a Lachat QuikChem 8000 instrument at the UBC Environmental Engineering Laboratory.

3.3 **Quality assurance and quality control**

3.3.1 **Sample collection and preservation**

All field aqueous samples were collected in baked (400°C) 1 L amber bottles and transported in a cooler packed with ice. At the laboratory, the samples were immediately refrigerated at 4°C and extracted and/or analysed within one week of collection. In the field, some samples were either preserved with H₂SO₄ (pH < 2) for the analyses of ammonia-N or phenylmercuric acetate for the analyses of phosphate and nitrate-N.

3.3.2 **Blanks**

Blanks, which were comprised of reagent grade water were used to determine if there was any contamination of the samples during sampling, transportation and solid-phase extraction. The
reagent used for reconstitution of the extracted analytes was also analysed as a blank with each run on the HPLC.

3.3.3 Instrument reproducibility

Instrument reproducibility was determined by injecting known concentrations of DAS 1 (0.01 mg/L), DSBP (0.01 mg/L) and sulfamethazine (0.005 mg/L) into the HPLC seven times. The relative standard deviations (RSDs) were determined for the respective retention times or \( k \) values, and the peak areas of the repetitive injections.

3.3.4 Recoveries and precisions

The reproducibility of the entire method for the FWAs and sulfamethazine, including sample preparation, SPE and HPLC/UV were validated by conducting recoveries and precisions tests. Seven replicates of reagent grade water (500 mls) were spiked with DAS 1 and DSBP at concentrations of \( 4 \times 10^{-5} \) and \( 2 \times 10^{-5} \) ug/L. For sulfamethazine, reagent grade water was spiked with the analyte at concentrations of 0.01 (500 mls, \( n = 1 \)), 0.05 (100 mls, \( n = 6 \)), and 0.5 ug/L (100 mls). Groundwater samples (100 mls, \( n = 4 \)) were also spiked with 0.05 ug/L of sulfamethazine. Recoveries were expressed as a percentage (%) of the initial spiked samples, and precisions were represented by the relative standard deviation (RSD).

3.3.5 Method detection limits

The MDL was calculated according to the USEPA (2003) formula: \( \text{MDL} = t(n-1, \text{1-alpha} = 0.99) \) \((S)\), where: \( \text{MDL} = \) the method detection limit \( t(n-1, \text{1-alpha} = .99) \) = the student’s value appropriate for a 99% confidence level and alpha standard deviation \( S = \) standard deviation of the replicate analyses.

In order to determine the method detection limit (MDL), an estimate of the detection limit was done according to the USEPA (2003) method. Various known concentrations (500 \( \mu \)g/L, 100 \( \mu \)g/L, 50 \( \mu \)g/L, 20 \( \mu \)g/L, 10 \( \mu \)g/L and 5 \( \mu \)g/L) of FWAs and sulfamethazine were analysed until
a signal/noise ratio of 5:1 was obtained; the USEPA (2003) recommends a range of 2.5 to 5. This was done by observing the height of the analyte peak (signal) against the noise level.

Laboratory standards were then prepared in reagent grade water at the USEPA’s (2003) recommended range of one to five times the estimated detection limit. A minimum of six aliquots of the sample were processed through the entire analytical method. All computations were made according to the above EPA’s formula.

3.4 Sampling sites

3.4.1 Locations

The Hopington Aquifer is located in the Township of Langley (Figure 3.1), approximately 50 km east of Vancouver (Wernick et al., 1998). The aquifer, which underlies the Salmon River Uplands, is comprised of glacial outwash sediments up to 50 metres in thickness. The upper unconfined portion of the aquifer (Hopington AB), which is the main site in this study, covers about 30 square kilometres, and consists of thick permeable sand and gravel deposits. The unconfined Hopington AB Aquifers, which is somewhat close to the surface, is an important groundwater recharge area (TOL, 2001; TOL, 2008), and thus is susceptible to contamination from overlying land use activities.

Two control sites were chosen for the study, namely Hopington C and Abbotsford aquifers. The Hopington C is a confined aquifer, which underlies the Hopington AB and extends further south and west covering an area of 50 km². The Abbotsford aquifer is located in south-western British Columbia (approximately 60 km from Vancouver) and north-western Washington State (Figure 3.1). Out of approximately 200 aquifers in the Lower Fraser Valley, the Abbotsford aquifer is the largest covering an area of approximately 100 km² in British Columbia, and about 100 km² in Washington State. Similar to the Hopington AB, the Abbotsford aquifer is largely unconfined and consists of extensive sand and gravel deposits (Liebscher et al., 1992).
3.4.2 Well selection

Based on historic data, 22 private residences with nitrate-N generally ≥3 mg/L in their well water from the Hopington AB aquifers were selected for this study; however, only 5 agreed to participate. The five wells (sites 4–8), which had well depths ranging from 2.67 to 33 meters, were generally located in Langley between 50 – 63rd Avenue and 235 – 248 Street.

The control samples comprised of 6 private residences (Hopington C) and 9 municipal wells (Abbotsford Aquifer). The private residences (sites 9 – 14), which were located in the vicinity of 232nd Street and 34A Avenue, had wells depths in the range of 7.6 m to 51.8 m. The municipal wells (sites 15 – 23), which were located in the vicinity of Ross Road and Short Road, and 0 Avenue and King Road, had wells with depths in the range of 2.6 – 21.7 m. Well water samples from all the private residences were collected through taps; whereas, the municipal well water samples were collected via piezometers.
3.4.3 Surface waters

Two small streams namely the Union Creek (site 1) and the Coghlan Creek (site 2), which are tributaries of the Salmon River were also selected for this study. The sampling site at Union Creek, which is located approximately 20 m upstream of the Creek’s confluence with the Salmon River, is fed predominantly by groundwater from adjacent small springs. In addition, the springs, which are located downgradient of private residences, have been impacted by septic systems resulting in elevated nitrate-N (per communication J. Atwater). The Coghlan Creek site is located on the upper boundary of Williams Park. According to Schreier et al., (1996), the Coghlan Creek is fed predominantly by groundwater from the Hopington Aquifer especially in summer. Water samples were collected approximately 30 cm below the surfaces of the two creeks.

3.4.4 Domestic wastewater

Domestic wastewater samples were obtained from primary and secondary effluents at a biological nutrient removal (BNR) pilot plant. The BNR plant (site 24) is located at the University of British Columbia (UBC) in the vicinity of West 16th Avenue and Southwest Marine Drive, Vancouver. The small scale pilot plant treats approximately 5% of the domestic sewage generated on campus. The wastewater samples were obtained primarily to determine if DAS 1 and DSBP were components of local domestic wastewater.
3.4.5 Laundry detergent

Two local studies in Vancouver, British Columbia were undertaken in order to determine specifically if DAS 1 or DSBP were components of laundry detergents used by local householders, and thus components of domestic wastewater at the study sites in Langley. Firstly, a small survey was carried out among a total of 15 staff and students at UBC, to determine the brand names of laundry detergents generally being used in the region. In addition, most of these detergents were analysed to determine whether they contained DAS 1 or DSBP.

3.5 Land use study

A land use study was conducted on the Township of Langley, in particular, the Hopington Aquifer region to determine the main activities, which were possible sources of nitrate contamination in the aquifer. The information obtained for the land use study including data and maps was mainly obtained from Township of Langley Agricultural Land Use Inventory, 2001 (BCMAFF, 2002).
4 RESULTS AND DISCUSSION

4.1 Land use study

4.1.1 Hopington AB Aquifer

Figure 4.1 illustrates a map of the Hopington AB Aquifer superimposed over an agricultural land use inventory map produced by the British Columbia Ministry of Agriculture, Food and Fisheries (BCMAFF) and the Township of Langley in 2001. The land use activities overlying the Hopington AB Aquifer can be classified into inside and outside the Agriculture Land Reserve (ALR), and the urban rural interface (inside and outside the ALR edge). Both agriculture and residential use are major land use activities inside the ALR; whereas, residential use predominate outside the ALR including the urban rural edge (Table 4.1).

Table 4.1 Primary land use activities on the inside and outside edge of the ALR

<table>
<thead>
<tr>
<th>Primary land use activity</th>
<th>Inside ALR edge</th>
<th>Outside ALR edge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of parcels, inside edge</td>
<td>Average parcel size (ha)</td>
</tr>
<tr>
<td>Residential Use</td>
<td>294</td>
<td>2.5</td>
</tr>
<tr>
<td>Agriculture</td>
<td>134</td>
<td>8.9</td>
</tr>
<tr>
<td>Hobby Farm (includes a residence)</td>
<td>75</td>
<td>3.7</td>
</tr>
<tr>
<td>Golf Course</td>
<td>5</td>
<td>22.4</td>
</tr>
<tr>
<td>Other* **</td>
<td>46</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Total parcels</strong></td>
<td>554</td>
<td>4.7</td>
</tr>
</tbody>
</table>


** = Not in Use outside the ALR edge has a total parcel size of 409 ha.

(Source: Township of Langley Agricultural Land Use Inventory, 2001, page 15, BCMAFF, 2002).
Figure 4.1  Inside and outside ALR boundaries and edge, and approximate locations of the Hopington AB and C aquifers. Source: Township of Langley Agricultural Land Use Inventory, 2001 BCMAFF, 2002. Copyright © Province of British Columbia. All rights reserved. Reprinted with permission of the Province of British Columbia.
The primary agricultural activities include extensive livestock, forage and pasture, nursery or trees, berry and vine crops and intensive livestock. Extensive livestock operations, which include horse and beef cattle, comprised of structures such as barns, paddocks and manure storage facilities. Intensive livestock refers to livestock (poultry, fur, swine and game bird) which are exclusively raised inside a building. Some dairy and non grazing beef cattle and horses may be classified as intensive livestock. Overall, extensive livestock, which include horse and beef cattle operations, was identified as the main agricultural activity within the ALR, and the urban rural interface. Details of the horse and beef cattle operations, which were in close proximity to the Hopington Aquifer, will be discussed in a later chapter.

4.1.2 Hopington C Aquifer

Land use activities, which overlay the Hopington C are similar to the Hopington AB and include private residences and agricultural activities. However, due to the low-permeability surface sediments of the Hopington C (TOL, 2001; TOL, 2008), contamination of the groundwater by FWAs or nitrate-N should be at a minimum or non existent.

4.1.3 Abbotsford Aquifer

The Abbotsford Aquifer was chosen for this study because of its overlying land use activity, which is predominantly agriculture, although there is an increasing urban development located on the northern portion of the aquifer (Hii et al., 1999). In general, only about 20% of the aquifer's surface is covered by urban areas (Liebscher et al., 1992). Previous studies on the Abbotsford Aquifer have reported nitrate-N levels above the maximum acceptable concentration of 10 mg/L in many groundwater samples (Liebscher et al., 1992; Wassenaar, 1995; Hii et al., 1999), and have generally attributed these high concentrations due to agricultural activities on the surface of the aquifer (Liebscher et al., 1992; Wassenaar, 1995; Zebarth et al., 1998). Overall, in British Columbia, high nitrate concentrations above the drinking water limit have been observed in well water samples from areas that are in predominately intensive agricultural or septic tank locations (BCM0E, 2007).
4.2 Quantification

Sulfamethazine, DAS 1 and DSBP concentrations were quantified by the external standard method based on peak area. The linearity of the calibration curve ($r^2 > 0.999$) for sulfamethazine, and DAS 1 and DSBP were generally based on 0.005, 0.01, 0.02, 0.05, 0.1 and 1 mg/L (Figure 4.2) and 0.005, 0.01, 0.02, 0.04 and 0.05 mg/L (Figure 4.3) standards respectively.

![Figure 4.2 Typical sulfamethazine 5-point calibration graph in this study.](image)

$R^2 = 0.9996$
Figure 4.3  Typical DAS 1 and DSBP 5-point calibration graphs in this study.
4.3 Quality control

4.3.1 HPLC UV-instrument reproducibility

In order to determine whether or not the HPLC system was generating reproducible results of acceptable accuracy and precision, system suitability tests were performed. These used relative standard deviations (RSD) of the respective retention times or $k$ values, and the peak areas of repetitive injections of FWAs (0.01 mg/L) and sulfamethazine (0.005 mg/L) standards. The RSD (%) for the retention times were ±0.67, ±0.71 and ±0.44 for sulfamethazine, DSBP and DAS 1 respectively (Table 4.2). These values are well within the typical standard deviations of retention times for the HPLC, which are in the order of 0.2%-2%. This range is also within the precision necessary for the identification of peaks (Johnson and Steveson, 1978).

Table 4.2  Retention time and peak areas for chemical markers using a C8 column

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Sulfamethazine (n=7)</th>
<th>DSBP (n=7)</th>
<th>DAS 1 (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (seconds)</td>
<td>360</td>
<td>691</td>
<td>876</td>
</tr>
<tr>
<td>Standard Deviation (SD)</td>
<td>±2.42</td>
<td>±4.88</td>
<td>±3.82</td>
</tr>
<tr>
<td>Relative Standard Deviation (RSD) %</td>
<td>±0.67</td>
<td>±0.71</td>
<td>±0.44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak Area</th>
<th>(n=5)</th>
<th>(n=5)</th>
<th>(n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>863</td>
<td>3415</td>
<td>1745</td>
</tr>
<tr>
<td>Standard Deviation (SD)</td>
<td>±23.37</td>
<td>±63.34</td>
<td>±42.64</td>
</tr>
<tr>
<td>Relative Standard Deviation (RSD) %</td>
<td>±3</td>
<td>±2</td>
<td>±2</td>
</tr>
</tbody>
</table>
4.3.2 Method detection limits

Based on a signal to noise ratio of 5:1, the estimated detection limits were found to be 5 μg/L for DSBP (Figure 4.4) and 10 μg/L for both DAS 1 and sulfamethazine (Figure 4.5 a and b); which had peak heights in the range of the USEPA's (2003) 2.5 to 5. The calculated MDL, based on a single operator, a single instrument and a $T$ value for 7 replicates, 3.143 was 0.01 μg/L for both DAS 1 (S, 0.0027 μg/L) and DSBP. For sulfamethazine, the calculated MDL based on a $T$ value for 6 replicates, 3.365 was 0.04 μg/L (S, 0.0035 μg/L). The USEPA (2003) recommends that the spike concentrations be 1-5 times the expected MDL.

Figure 4.4 Signal-to-noise (S/N’) ratio for DSBP (5 μg/L) = 5:1.
Figure 4.5  Signal-to-noise ($S/N'$) ratio using standards (10 $\mu$g/L) = 5:1 for (a) DAS 1 and (b) sulfamethazine.
Therefore, the MDLs were determined at 0.02 µg/L (DSBP), 0.04 µg/L (DAS1) and 0.05 µg/L (sulfamethazine), which, were ~ 1, 2 and 4 times the calculated MDLs for sulfamethazine, DSBP and DAS1 respectively. Typical concentrations of FWA and sulfamethazine in groundwater were reported as 3.3 µg/L (Close et al., 1989) and 0.05 – 0.310 µg/L (Hamscher et al., 2005; Batt et al., 2006) respectively. The MDLs obtained in this study were below the concentrations of FWAs and sulfamethazine obtained in the environment by other researchers, which suggested that the overall SPE and HPLC UV methods were suitable for the extraction and analyses of these chemical markers from an aqueous environment.

4.3.3 Fluorescent whitening agents recoveries

Recoveries and precisions tests using 500 mLs of spiked reagent grade water were conducted to validate the reproducibility of the entire method, including sample preparation, SPE and HPLC/UV.

The mean recoveries for the two FWAs varied significantly from 50 to 94% (n = 7) for DAS1 and DSBP respectively (Table 4.3). SPE recoveries obtained for DAS1 and DSBP in aqueous samples (river or lake water) from various authors included 88% and 87% (Stoll and Giger, 1997), 84% and 96% (Poiger et al., 1996), and ~ 78% and 89% respectively (Shu and Ding, 2005).

Table 4.3 Recoveries for DAS1 and DSBP

<table>
<thead>
<tr>
<th></th>
<th>DAS1 (Total)</th>
<th>DSBP (Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 7</td>
<td>n = 7</td>
</tr>
<tr>
<td>Spike before concentrating (µg/L)</td>
<td>4 x 10⁻⁵</td>
<td>2 x 10⁻⁵</td>
</tr>
<tr>
<td>Spike after concentrating (µg/L)</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean recovery (µg/L)</td>
<td>0.0191</td>
<td>0.0189</td>
</tr>
<tr>
<td>Mean recovery (%)</td>
<td>50</td>
<td>94</td>
</tr>
<tr>
<td>Minimum recovery (%)</td>
<td>40</td>
<td>79</td>
</tr>
<tr>
<td>Maximum recovery (%)</td>
<td>60</td>
<td>125</td>
</tr>
<tr>
<td>Standard deviation (µg/L)</td>
<td>±0.0027</td>
<td>±0.0035</td>
</tr>
<tr>
<td>Relative Standard Deviation (%)</td>
<td>14</td>
<td>19</td>
</tr>
</tbody>
</table>
The poor recovery of DAS 1, which to some extent was anticipated due to the lower recoveries in the earlier elution tests, as possibly a result of its highly retentive behaviour, may have been further exacerbated by poor extraction and recovery processes, and the potential to readily isomerise. In general, this was due to the intermittent vacuum pressure throughout the SPE box, probably due to broken seals as a result of wear. Thus flow rates were uneven, resulting in alternating fast and slow flow rates during loading of the sample solution and elution of the FWAs. Thurman and Mills (1998) reported that during SPE, there is a drastic decrease in percent recovery when flow rates are increased because of the non occurrence of an equilibrium sorption and desorption. In particular, during elution, the flow rate should be reduced to allow enough time for the solvent to penetrate into the sorbent thus making sufficient contact for desorption.

DAS 1 compared with DSBP has more binding sites, and thus the potential to increase its retentiveness and decrease it’s desorption capabilities on the SPE membranes. The highly retentive nature of DAS 1 was first observed in the elution experiments, where 1-2 mL of methanol resulted in recoveries of only 1-2% (n = 2) for this FWA. In contrast, higher recoveries of 69-81% were obtained for DSBP using the same 1-2 mL (n = 2) of methanol respectively. There is a notion in this study that due to the excessive transferring of solution containing DAS 1 from glassware to glassware, during the preparation, loading, elution, rotovapilation, nitrogen drying and reconstitution stages, then it was possible that the poor recoveries of DAS 1 may have been attributed to loss of the highly sorbing analyte during the transferring stages. Therefore, it is highly recommended that in future work, transferring of the FWAs should be minimised as much as possible.

In addition to the results of this study, several authors have also reported on the highly sorbing behaviour of DAS 1 from STPs to the aquatic environment. For example, Poiger et al., (1996) observed that DAS 1 was more adsorbing unto suspended solids than DSBP in a STP. Due to the high retentive behaviour of DAS 1 on the C-18 membrane, it was possible that erratic flow rates during elution was not optimum for the recoveries of this highly sorbed FWA. According to Dean (1998), fast flow rates may result in poor recovery of the analyte due to less time for interaction between the analyte and the sorbent material.
As a result of the uneven vacuum pressure in the SPE box, the total SPE processing time for eight cartridges and 4000 mLs of sample was > 12 hours. A new SPE system has since replaced the old system; therefore, it is recommended that SPE recovery tests should be repeated for DAS 1, in order to troubleshoot the poor recovery.

One significant effect of the long SPE processing time was the observance of photoisomerisation, notably of DAS 1 under normal laboratory lighting. Photoisomerisation resulted in the partial conversion of $E$ to $Z$ photoisomer in the eluate of all the samples. The range of isomers as percentages of the total DAS 1 recovered for the $E$ and $Z$ isomers were 69%-78% and 22%-31% respectively (Figure 4.6). Poiger et al., (1996) also reported changes in isomeric compositions during sample preparation under normal laboratory light; however, unlike the poor recoveries for DAS 1 in this study, these authors achieved excellent recoveries of 96% for this FWA. Since DSBP has a longer fluorescence lifetime than DAS 1 by a factor of 2-3 (Canonica and Kramer, 1997), then it is possible that the former did not significantly isomerise enough to be detected by UV.

Photodegradation of the FWAs due to long exposure of laboratory lighting was not expected to contribute in a large way to the poor recoveries of DAS 1. Kramer et al., (1996) observed that the photodegradation rate for DSBP was three times faster than DAS 1 while exposed to laboratory lighting. However, the authors reported that excellent recoveries of 79-125% were still obtained for DSBP, even though exposed to laboratory lighting for a long period. In this current study, excellent recoveries were also obtained for DSBP ($\sim$ 94%), which implied that there was no significant reduction of DAS 1 due to photodegradation.
4.3.4 Fluorescent whitening agents precisions

As a result of photoisomerisation of the FWAs in the water samples, the results of the precision test also included the photoisomers. In general, the precision, which is represented by the relative standard deviation (RSD) was within 14-20% (Table 4.4). Also, it was observed that there was a decreasing RSD trend with increasing isomer concentration. Stoll and Giger (1997) reported that where concentrations are near the detection limits as in this study, precisions are usually poorer. Typical SPE precisions (RSD) for FWAs in aqueous samples included 1-12% (Stoll and Giger, 1997), 2-3% (Poiger et al., 1996) and 12% (Uchiyama, 1979).
4.3.5 Sulfamethazine recoveries

In order to validate the reproducibility of the entire method, including sample preparation, SPE and HPLC/UV, accuracy and precision tests were conducted on Millipore water spiked at concentrations of 0.01 μg/L (500 mLs), 0.05 μg/L (100 mLs) and 0.5 μg/L (100 mLs), and a spike of 0.05 μg/L (100 mLs) for groundwater (Table 4.5). Maximum recoveries were between 74 and 125% for the Millipore water and 112% for the groundwater samples. Typical SPE average recoveries obtained from various researchers for sulfamethazine spiked in deionized water, river water, distilled water and groundwater samples were 98, 97.6 (Yang et al., 2004), 130 and 100% respectively (Lindsey et al., 2001).

Table 4.4     Precisions for DAS 1 and DSBP

<table>
<thead>
<tr>
<th>FWA</th>
<th>Iso-</th>
<th>Mean Conc.</th>
<th>Relative Standard Deviation</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mer</td>
<td>(μg/L)</td>
<td>Concentration (μg/L)</td>
<td>Concentration (μg/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>DAS 1</td>
<td>E</td>
<td>0.014</td>
<td>0.002</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>0.005</td>
<td>0.001</td>
<td>20</td>
</tr>
<tr>
<td>DSBP</td>
<td>EE</td>
<td>0.019</td>
<td>0.003</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 4.5     Recoveries for sulfamethazine

<table>
<thead>
<tr>
<th></th>
<th>Millipore water</th>
<th>Groundwater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples (n)</td>
<td>n = 1</td>
<td>n = 6</td>
</tr>
<tr>
<td>Spike before concentrating (μg/L)</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Spike after concentrating (μg/L)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean recovery (μg/L)</td>
<td>-</td>
<td>0.053</td>
</tr>
<tr>
<td>Mean recovery (%)</td>
<td>-</td>
<td>106</td>
</tr>
<tr>
<td>Minimum recovery (%)</td>
<td>74</td>
<td>79</td>
</tr>
<tr>
<td>Maximum recovery (%)</td>
<td>74</td>
<td>125</td>
</tr>
<tr>
<td>Standard deviation (μg/L)</td>
<td>-</td>
<td>0.008</td>
</tr>
<tr>
<td>Relative Standard Deviation (%)</td>
<td>-</td>
<td>15.1</td>
</tr>
</tbody>
</table>
4.3.6 Sulfamethazine precisions

The precisions for the spiked Millipore water were within 6.5 – 15.1% relative standard deviation (RSD). Although in this study it was observed that the recoveries were generally not concentration dependent, the precision indicated by a lower RSD (6.5%) was better at a higher concentration (0.5 µg/L), which is not unusual. The RSD value obtained for the groundwater sample was 26.7%, indicating a poorer precision than the Millipore water. Prior to SPE, all water samples were first filtered with glass fibre filters (0.5 µm), however, during the reconstitution step, small particles, which were probably extracted from the groundwater samples and retained on the HLB sorbent bed were eluted together with the sulfamethazine in the methanol. Once taken up by the injector, these particles in the sample can irreversibly partition onto and rapidly reduce the efficiency of the column (Sadek, 2000); as a result, the reconstituted sulfamethazine extract, which had a volume of only 0.5 mL, may have incurred losses during a second filtration. Perhaps a filter less than 0.5 µm should be investigated for the removal of impurities and small particulate matter from the water samples. For example, Yang et al., (2004), and Kim and Carlson, (2007) used 0.2 µm glass fibre filters for the filtering of water samples.

Typical recoveries and RSD values for sulfamethazine obtained by various authors who also used SPE and Waters Oasis HLB cartridges included 94.5 ± 5.2% for well spring water (Babić et al., 2006) and 130 ± 17% for distilled water, (Lindsey et al., 2001).

The precision of the entire method (reproducibility) in this study was represented by the relative standard deviation (RSD) of six samples. The RSD value for the recovery and analysis of sulfamethazine spiked in Millipore and groundwater was ± 25%. Similar to the FWAs, which also had high RSDs values ranging from ± 14 to 20%, it was possible that the poor precision may have occurred during sample preparation and extraction, since it was proven earlier that analyses of FWAs and sulfamethazine on the HPLC system were reproducible. As mentioned earlier, one consistent problem observed during the sample extraction was due to the different flow rates through each SPE cartridge, as a result of the uneven vacuum pressure throughout the SPE box. The net result would be varying extraction efficiencies of the cartridges; however, this observation needs further investigation.

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4.3.7 Blanks

In this study, blanks, which consisted of Millipore water, were used to determine if there was any contamination of the samples during sampling, transportation, solid-phase extraction, reconstitution and analysis by HPLC UV. In general, the sampling and transportation blanks for nitrate-Ns, ammonia, chlorides and phosphate were below the detection limits. The sampling, transportation, solid–phase extraction, reconstitution and HPLC blanks did not contain any FWAs or sulfamethazine.

4.4 Method optimisation – fluorescent whitening agents

4.4.1 Mobile Phase

The HPLC gradient method by Stoll and Giger (1997) (Table 4.6) yielded high resolution of the FWAs peaks based on a 10 mg/L standard, and a 10 ul injection of DAS 1 and DSBP into a C-18 column (Figure 4.7).

Table 4.6 Typical HPLC solvent gradient composition used in this study

<table>
<thead>
<tr>
<th>Author</th>
<th>Time (minutes)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoll and Giger (1997)</td>
<td>0</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>This Study</td>
<td>0</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>
Figure 4.7  Chromatograms of (a) DSBP and (b) DAS 1 using a C-18 column, and based on the HPLC gradient composition by Stoll and Giger (1997).
The value of resolution is determined by the narrowness of the peak and the distance between the peaks (Johnson and Stevenson, 1978). Although the FWAs eluted in ~18 and ~19 minutes for DSBP and DAS 1 respectively, it was felt that the total processing time of 40 minutes per sample was too long, especially when a large number of samples have to be analysed; therefore, the linear gradient composition of the mobile-phase was changed for earlier elution (see Table 4.6). Essentially, a gradient elution is where the strength of the mobile phase is increased during a chromatographic analysis; the ultimate result is that the compounds which are strongly retained on the column would elute earlier. In general the optimum gradient is chosen by trial and error (Johnson and Stevenson, 1978). The modified HPLC method by Stoll and Giger (1997) resulted in retention times of ~15 minutes for DSBP and ~16 minutes for DAS 1, which is somewhat close to the retention times first obtained in this study. For earlier elution of the FWAs, the methanol/acetonitrile solvent (mobile-phase A) was increased from 30% to 35% (Table 4.6), which resulted in retention times of ~11.9 and 13.4 minutes for DSBP and DAS 1 respectively (Figure 4.8 and Table 4.7).
Figure 4.8. Chromatograms of DAS 1 and DSBP using a C-18 column, and based on the HPLC modified gradient composition by Stoll and Giger (1997).

Table 4.7: Retention times of DAS 1 and DSBP using a C18 column

<table>
<thead>
<tr>
<th>Concentration Mg/L</th>
<th>Retention Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DSBP</td>
</tr>
<tr>
<td>0.005</td>
<td>11.894</td>
</tr>
<tr>
<td>0.01</td>
<td>11.905</td>
</tr>
<tr>
<td>0.1</td>
<td>11.741</td>
</tr>
<tr>
<td>1</td>
<td>11.808</td>
</tr>
</tbody>
</table>
In order to minimise the constant changing of C-18 with C-8 columns and vice versa for the analysis of FWAs and sulfamethazine respectively, a 1 mg/L standard of DAS 1 and DSBP was analysed on the HPLC using the C-8 column to determine if the column can also be used for the analysis of FWAs. According to Figure 4.9, excellent resolutions were obtained with the C-8 column for both FWAs. The retention times of the FWAs using the C-8 column was similar to that of the C-18 column of ~ 12 minutes and ~ 15 minutes for DSBP and DAS 1 respectively, therefore, the C-8 column was used for the analysis of both FWAs and sulfamethazine in aqueous samples for the duration of the study. Reproducibility of the C-8 column was discussed earlier in this chapter. Generally, a C-8 column is similar to but slightly less retentive than the rugged, highly retentive C-18 column (Snyder et al., 1997).

![Figure 4.9. HPLC determination of DAS 1 and DSBP using a C-8 Column.](image)

4.4.2 UV detector

As mentioned earlier, excellent resolution was obtained for the FWAs using HPLC with UV detection. However, as observed in Figure 4.9, the chromatograms generated in this study have consistently illustrated lower peak heights for DAS 1 than DSBP, by a factor of ~ x2 at a wavelength of 350 nm. In order to increase the peak heights, and thus the sensitivity of the FWAs in particular DAS 1, standards comprising each of 0.5 mg/L concentration of DAS1 and DSBP were separately scanned on a UV 300 Spectrometer (Spectronic UNICAM) to ensure
that 350 nm was the optimum wavelength for elution of the peaks on the HPLC. There were two peaks observed for DAS 1 at the 200-600 nm wavelength scanned; peak 1 at ~240nm and peak 2 at ~350nm, with a slightly increased height observed for peak 1 at 240 nm (Figure 4.10). For DSBP, the optimum peak height occurred at 350 nm (Figure 4.11).

![Figure 4.10. Identification of DAS 1 major peaks at wavelengths of 240 and 350 nm.](image1)

![Figure 4.11. Identification of DSBP major peak at a wavelength of 350 nm.](image2)
In order to confirm whether 240 nm was the optimum operating wavelength for the analysis of DAS 1 due to slightly better resolution, a 1 mg/L concentration of the FWA was tested at this wavelength on the HPLC, and the results were compared with the same concentration analysed at 350 nm; DSBP was also analysed for comparison purposes. Using the same scales on both the chromatograms for Figures 4.12 (a) and (b), there was little difference observed between the peak heights of DAS 1 at 240 nm compared with 350 nm. In agreement with the UV scan, the optimum peak height for DSBP occurred at 350 nm (Figure 4.12 a).

In terms of noise/signal and other peaks, it was observed that the chromatogram at 350 nm had a less noisy baseline than at 240 nm. Essentially, in gradient elution, the weaker solvent (A) which dominates the mobile phase composition (%) at the start of the chromatographic run, may contain impurities. Often water (solvent A) even though purified is the main source of these impurities, which can result in baseline irregularities in reverse phase work. Early in the chromatography, these impurities, which can be detected by UV are strongly retained on the head of the column; subsequently, the impurities elute from the column when the solvent strength (B) increases, to produce peaks and baseline fluctuations. When run under the same conditions, these impurities are not visible at a higher wavelength (Johnson and Stevenson, 1978), as observed in this current study.

A second explanation for the noise/signal differences at 350 nm and 240 nm was possibly due to the cutoff ranges (the wavelength at which the absorbance of the solvents is equal to 1 AU) of the mobile phase solvents. The cutoffs for the mobile-phases used in this study were 205 nm (methanol and ammonium acetate) and 190 nm (acetonitrile), which were closer to the operating wavelengths of 240 nm than 350 nm. In general, using a wavelength near or below the cutoff reduces the ability to detect the compound and increases baseline noise due to the solvent being absorbed into the chromatogram (Waters Corporation, 1991), which was possibly the case of DSBP (Figure 4.12 b). Therefore, all analysis of both DAS 1 and DSBP in this study was conducted at a wavelength of 350 nm on the HPLC.
Figure 4.12 Chromatograms of DSBP and DAS 1 using UV detection at (a) 350 nm and (b) 240 nm.

4.4.3 Solid phase extraction

Initially, in this study, the adapted SPE method by Stoll and Giger (1997) yielded poor recoveries for DSBP (46%) and even poorer for DAS 1 (9%) in reagent grade water. In order to improve the recoveries of the FWAs, several optimization tests were performed in an attempt to troubleshoot the problems.
(i) **Breakthrough test**

One method of determining whether or not there is a loss of the analyte of interest during the processing stage (loading), particularly during SPE, is to perform a breakthrough test. Generally, the capacity of the sorbent material particularly in a short SPE column should exceed the total mass of the analyte in the sample (Fritz, 1999). Poor recoveries can occur when the analyte is not retained on the sorbent bed due to overloading, and is passed through the column with the sample solution.

Breakthrough tests were conducted by loading standard solutions each comprising of 100 mLs of 20 µg/L and 10 µg/L of DAS 1 and DSBP respectively through individual SPE cartridges. Four samples of the eluant, each consisting of 25 mLs, were taken during the extraction process and analyzed for FWAs with a 10-AU-005-CE Fluorometer (Turner Designs Sunnyvale, California). There was < 1% DAS 1 or DSBP present in the eluant, indicating that the C-8 sorbent bed had good retention of the analytes; therefore, the loss of the FWAs and poor recoveries were not due to breakthrough. This is in agreement with Poiger et al., (1996) who also conducted breakthrough tests of the FWAs by stacking C-18 SPE disks together and processing each disks separately. The authors did not detect any FWAs in the second disk and concluded that the bonding between C-18 SPE disks and the FWAs were probably strong enough for isolation of the optical brighteners.

Similar to this current study, breakthrough tests using C-18 cartridges were conducted by Shu and Ding (2005) and Hayashi et al., (2002), the results based on tandem cartridges indicated that there were no significant amounts of FWAs (<3%) in the eluate of the second cartridge. The authors attributed the excellent recoveries of the FWAs due to the strong interaction between the ion paired FWAs and the C-18 cartridges.
(ii) Salting out

Even though there was no loss of DAS 1 or DSBP observed during the earlier breakthrough tests, salting out of the FWAs were still performed in order to perhaps increase the polarity of the solution, which would force out the FWAs on the reversed phase C-18 sorbent (Thurman and Mills, 1998). The addition of 5% sodium chloride salt to the Millipore water containing the FWAs did not increase the poor recoveries of DAS 1 and DSBP. Poiger et al., (1996) reported that the retention of the FWAs by the C-18 disks was sufficient without the addition of salt.

(iii) Elution solvents

The elution solvents comprising of 0.05M TBA in methanol (2 mLs) resulted in excellent recoveries of ~ 88% for both DAS 1 and DSBP in the study conducted by Stoll and Giger (1997). However, in this study one aliquot comprising of 0.05M TBA in methanol (2 mL), resulted in low recoveries ranging from 1-9% and 41-48% for DAS 1 and DSBP respectively using C-18 cartridges. Therefore, in order to optimize the recoveries of the FWAs, troubleshooting was performed using a variety of cartridges, elution solvents and spiked concentrations.

One of the main differences which possibly affect the recovery rates between the SPE methods of Stoll and Giger (1997) and this study was the use of discs versus cartridges. The discs used by Stoll and Giger (1997) have larger diameters, typically 25 mm compared with the 10 mm extraction disc cartridges used in this study. The efficiency of using a larger surface area is that in addition to allowing a faster flow and reduced channelling, a smaller volume of elution solvent is required (Thurman and Mills, 1998). In addition, Poiger et al., (1996) reported that SPE cartridges are more prone to clogging by suspended particulate matter than SPE disks due to the latter’s large surface area.

Generally, as part of method development, smaller volumes increasing to larger volumes of solvents are used for elution of the analyte. This is to ensure that the least volume is required for optimum recovery. Poiger et al., (1996), obtained 84-96% recoveries of DAS 1 and DSBP respectively using 6 mLs of 0.05M TBA in methanol. Therefore, in this study, the volume of
the elution solvent was optimized by increasing the number of elutions using separate vials, drying the eluate solution with nitrogen gas under mild heat, and finally reconstituting the eluate with DMF and water (1:1), before being injected into the HPLC. Using Supelco LC-18 cartridges, recoveries of DAS 1 and DSBP for the first, second and third elutions ranged from 1-9% and 41-48%, 0-5% and 0-2%, and 1 and 0% respectively. Overall, the total recoveries of FWAs using up to 6 mL of 0.05M TBA in methanol still resulted in low recoveries of up to 14% for DAS 1 and 49% for DSBP (Table 4.8).

In order to optimize the recoveries of FWAs, a solvent elution test was conducted using a C-18 endcapped (EC) cartridge instead of a C-18 cartridge, and three different solvents; Table 4.8 is a summary of the results. For the solvent elution test, an Isolute C-18 EC cartridge was conditioned and loaded with a spiked concentration of Millipore water containing 10 μg/L of DAS 1 and DSBP. The three solvents comprising of 0.05M TBA in methanol, methanol (without TBA), and methylene chloride were each successively eluted from the cartridge into separate vials, dried with N₂ gas and reconstituted with 0.5 mL of DMF and water (1:1). This test was performed to determine the optimum solvent polarity (p'), which will elute the FWAs from the C-18 sorbent. Basically, nonpolar solvents with mutual solubility with the silica surfaces are used to release the non polar forces (Thurman and Mills, 1998) that retain the FWAs from the bonded phase C-18 cartridges.

In terms of polarity, the p' for water, methanol and methylene chloride is 10.2, 6.6 and 3.4 (Zief and Kiser, 1987) respectively, with water being the most polar and methylene chloride the least. The first elution with 0.05M TBA in methanol resulted in only 4% recovery for DAS 1, but as much as 74% recovery for DSBP. The second elution using only methanol resulted in 14% for DAS 1 and only 1% for DSBP. The recoveries for DAS 1 and DSBP using methylene chloride were very low at 1% and 0% respectively. Since 4mL of methanol with or without 0.05M TBA resulted in the same 14% recovery for DAS 1, then all further method development experiments were conducted with only methanol. In general, for reversed-phase sorbents, the solvents normally used are restricted to water, methanol, isopropyl alcohol and acetonitrile (Dean, 1998).
Table 4.8  SPE optimisation tests by cartridge type and elution solvent for FWAs

<table>
<thead>
<tr>
<th>Cartridge Type</th>
<th>Solvent</th>
<th>Volume of solvent per elution (mL)</th>
<th>n</th>
<th>Spiked Concentration (µg/L)</th>
<th>Recovery (%)</th>
<th>DAS 1</th>
<th>DSBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supelco</td>
<td>TBA/Methanol</td>
<td>2</td>
<td>1</td>
<td>0.1</td>
<td>46</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>LC 18</td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Supelco</td>
<td>TBA/Methanol</td>
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<td>1</td>
<td>0.1</td>
<td>48</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>LC 18</td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Supelco</td>
<td>TBA/Methanol</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>41</td>
<td>n/a</td>
<td>0</td>
</tr>
<tr>
<td>LC 18</td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supelco</td>
<td>TBA/Methanol</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>n/a</td>
<td>3</td>
<td>n/a</td>
</tr>
<tr>
<td>LC 18</td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Supelco</td>
<td>TBA/Methanol</td>
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<td>1</td>
<td>10</td>
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<td>1</td>
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<tr>
<td>LC 18</td>
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<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Isolute</td>
<td>TBA/Methanol</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td>74</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>C-18 EC</td>
<td>Methanol</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methylene Chloride</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Empore</td>
<td>Methanol</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td>n/a</td>
<td>1</td>
<td>n/a</td>
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<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
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<td>Methanol</td>
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<td>1</td>
<td>10</td>
<td>n/a</td>
<td>69</td>
<td></td>
</tr>
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<td>2</td>
<td></td>
<td></td>
<td>8</td>
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<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Empore</td>
<td>Methanol</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>81</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C-18 EC</td>
<td></td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td>23</td>
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<td></td>
<td></td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

n = elution number
A series of elutions \((n = 3)\) using only 1mL aliquots of methanol were performed on Empore C-18 EC cartridges, which had previously been loaded with Millipore water spiked with 20 \(\mu\)g/L DAS 1 and 10 \(\mu\)g/L of DSBP. The purpose of using a small volume (1mL) of methanol was that the eluate which was compatible with mobile phase can be directly analysed by the HPLC without the need for evaporation and reconstitution. The results showed that the third elution produced the highest recovery of 10% for DAS 1, whereas, for DSBP, the highest recovery of 69% was obtained with only the first elution.

The next step in the optimization of the FWAs, involved increasing both the volume of methanol (2 mLs) and the number of elutions \((n=5)\). The purpose of this experiment was to determine if perhaps the DAS 1 was strongly retained onto the sorbent, and therefore, needed additional elution. Since 2 mLs of methanol was used for elution, then the drying and reconstituting steps were employed. Based on spiked concentrations of 10 \(\mu\)g/L for DAS 1 and DSBP, the results showed that 81% recovery was obtained for DSBP from the first elution, however, significant recoveries of DAS 1 (~ 20%) were obtained only from the third elution, with a total of 72% for DAS 1. Since the fifth and final elution resulted in 20% recovery for DAS 1, then it was possible that there was a significant amount of this FWA still sorbed to the C-18 (Table 4.8). A final elution test using 20 mL of methanol was used for all further elutions; since a second elution did not contain any more FWAs.

The large volume of methanol (20 mLs) used for the elution of FWAs from the SPE cartridge, seemed to be consistent with the methods of other researchers. For example, Hayashi et al., (2002), also used 20 mLs of methanol to elute FWAs from a SPE cartridge after loading with filtered water samples; however, the authors used up to 40 mL of 0.03 M tetrabutylammonium/methanol to extract the FWAs from the filtrate samples. Poiger et al., (1996) observed that the use of solvents such as methanol and acetonitrile are suitable for the elution of FWAs, but large volumes of 10-15 mLs were necessary and recoveries were 50-70%. The authors found that the addition of 0.05 M TBA in methanol resulted in recoveries of 75-90 %, using only 6 mLs of solvents. This increase in recovery was due to the ion pairing properties of TBA-FWA from suspended matter in the samples. However, Shu and Ding (2005) observed that only 5 mLs of methanol without TBA was sufficient as an elution solvent for FWAs, which was contrary to the observations made by Poiger et al., (1996) and this study.
(iv) **Encapping**

During the SPE optimization tests, it was observed that the use of C-18 endcapped (EC) cartridges resulted in better recoveries for the FWAs, as compared with C-18 non endcapped cartridges (see Table 4.8). This was particularly noticeable for DSBP, (based on 2 mL of elution solvent) where low recoveries (41-48%, \( n = 4 \)) were obtained using C-18 non endcapped cartridges, compared with the higher recoveries (77-81%, \( n = 2 \)) obtained using C-18 encapped cartridges.

In general, SPE sorbents consists of a 40- to 60-\( \mu \)m silica particle (silica gel), and chemically bonded to the silica gel are functional groups such as octadecyl (C-18) which provides the mode of action for a reversed-phase sorbent. For example, in reversed phase SPE, the packing material or the stationary phase is more hydrophobic than the sample or the mobile phase, and the types of interactions are nonpolar or hydrophobic interactions. However, with non endcapped cartridges, the silica particles contain unreacted silanols or hydroxyl groups (HO) where there is no chemical bonding or derivitization by the C-18. Therefore, it is possible to have unwanted secondary polar interactions between the analytes and the free silanols. For example, on the silica surface, the hydroxyl groups are the donors of hydrogen to the hydrogen-bonding, and the atoms, which accept a hydrogen atom include nitrogen and oxygen, the latter which are most present on the structures of the FWAs. The hydrogen bonding of the analytes to the hydroxyl groups creates an additional retention, in addition to the reversed phase sorption to the C-18 (Thurman and Mills, 1998).

The final result is that with non-endcapped polar interactions are taking place between the FWAs and the silica surfaces. Endcapping or the addition of a typical chemical such as trimethylsilane will bond to any available free silanols, which have not been derivatized by the C-18, thereby, eliminating the polar interactions and creating a more homogeneously nonpolar stationary phase (Thurman and Mills, 1998). However, endcapping may only result in the decrease of unmodified silanols to <50% (Sadek, 2000), therefore, it is possible to still have silanols present on the silica.
4.5 Method optimisation - sulfamethazine

Initially at the start of this project, several methods for the determination of sulfamethazine specifically by HPLC UV were attempted; however, only two of these methods by de Zayas – Blanco et al (2004) “Determination of sulfamethazine in milk by solid phase extraction and liquid chromatographic separation with ultraviolet detection” and the Waters Corporation (2003) “Sulfa Drugs in Serum” would be discussed in detail.

4.5.1 Method 1 - de Zayas- Blanco (2004)

For the determination of sulfamethazine, which had been previously extracted from milk and reconstituted in 1 M hydrochloric acid/water (0.5 mL), de Zayas – Blanco et al (2004) used HPLC fitted with a 5 cm x 4.6 mm i.d stainless steel guard column packed with 40 µm Pelligard LC-18 guard column followed by a 15 cm x 4.6 i.d. stainless steel analytical column packed with 5 µm Spherisorb ODS2 (Waters). The mobile phase conditions were 10 minute isocratic elution with 35:65 methanol/40 mM ammonium acetate in water. Using a flow rate of 1 mL/min and ultraviolet detection of 264 nm, the sulfamethazine peak eluted in 4.5 minutes.

In this current study, using only a Novapak 3.9 x 150 mm C18 column and the same elution, flow rate and ultraviolet conditions as de Zayas – Blanco et al (2004), a 10 mg/L sulfamethazine standard eluted in 1.950 minutes. Overall, the resolution of the sulfamethazine peak was poor, firstly due to possible interference from the earlier eluting large solvent or system peak, which had a close retention time of 1.169 minutes (Figure 4.13a). This phenomenon may be more pronounced if the sulfamethazine peak preceding the large solvent peak was small. According to Johnson and Stevenson (1978), a small peak may be very difficult to quantify if it elutes after a large peak.

The second problem with the resolution of the sulfamethazine peak was due to the occurrence of a shoulder, caused by the overlapping of two or more peaks, which may cause difficulties with identification and quantification of the sulfamethazine peak. In chromatography, the ideal shape of a peak is symmetrical; however, a shoulder may result in an asymmetrical peak (Johnson and Stevenson, 1978) as illustrated in Figure 4.13a.
Figure 4.13  Isocratic separation of sulfamethazine by HPLC UV. Mobile phase conditions: methanol (35%) and 40 mM ammonium acetate in water (65%). (a) 10 mg/L standard; (b) 1 mg/L standard.

In order to determine whether or not a lower concentration of sulfamethazine was also impacted by poor resolution, a standard comprising of 50 μl of a 1 mg/L concentration was injected into the HPLC using the above isocratic elution composition. The retention time of the 1 mg/L sulfamethazine standard was 1.917 minutes, which was close to the 1.950 minutes retention time of the 10 mg/L standard. However, as illustrated in Figure 4.13b, it appeared that the slightly earlier elution of the solvent peak at 1.1866 minutes coupled with a very large area of 297091 may also have an interfering effect on the elution of the sulfamethazine peak (1 mg/L), which had area of 176233. Although the sulfamethazine peak had a more symmetrical shape at
a lower concentration (1 mg/L), peak overlapping was still observed as illustrated in Figure 4.13b.

As a result of poor resolution of the sulfamethazine peak, three elution tests were conducted in an attempt to both increase the retention time of the sulfamethazine peak and separate the overlapping peaks. These elution tests consisted of changing the isocratic compositions of the binary mobile phases 40 mM ammonium (%A) and methanol (%B) to 70/30%, 75/25% and 80/20% respectively (Table 4.9).

Essentially in order to obtain a later retention time for the sulfamethazine peak, it is necessary to reduce the methanol (%B) mobile phase composition. Snyder et al., (1997) also reported that the main effect of a decrease in %B solvent is to increase the retention time for all the sample components, and as a result, there may be distinct changes with the spacing of the adjacent peaks. In general, at high methanol concentrations the stationary phase is not fully utilized, as a result, the analyte which is hardly retained by the column elutes close to the solvent peak. The net result is low resolution due to poor separation of the peaks (Johnson and Stevenson, 1978), as in the case of the solvent and sulfamethazine peaks in this study.

<table>
<thead>
<tr>
<th>Isocratic mobile phase composition</th>
<th>Retention time (minutes)</th>
<th>Peak Height</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isocratic mobile phase composition</strong></td>
<td><strong>A (%)</strong></td>
<td><strong>B (%)</strong></td>
<td><strong>Retention time (minutes)</strong></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>-----------------</td>
</tr>
<tr>
<td>40mM ammonium acetate methanol</td>
<td>65</td>
<td>35</td>
<td>1.917</td>
</tr>
<tr>
<td>70</td>
<td>70</td>
<td>30</td>
<td>2.358</td>
</tr>
<tr>
<td>75</td>
<td>75</td>
<td>25</td>
<td>3.212</td>
</tr>
<tr>
<td>80</td>
<td>80</td>
<td>20</td>
<td>5.282</td>
</tr>
</tbody>
</table>
As shown in Table 4.9 and Figures 4.14 a, b and c, the reduction of the methanol resulted in increased retention times for the 1 mg/L concentration of up to 5.282 minutes for the 80/20% composition; however, the overall resolution of the overlapping peaks was still poor due to non separation. As a result of the continuing poor resolution, all future developments of the HPLC method by de Zayas – Blanco et al (2004) were terminated. One possible optimization method, which could have been explored, involved the changing of the strong solvent (B). Snyder et al., (1997) reported that changing solvent B for the most part can result in the spacing of previously overlapped peaks. For example, instead of using either methanol or acetonitrile as solvent B, the authors suggested mixing these two strong solvents together, perhaps 25% acetonitrile and 25% methanol.

4.5.2 Waters Corporation (2003)

The second of the two HPLC methods, which was utilised in this study, was initially used for the analysis of sulfa drugs (sulfadiazine, sulfathiazole and sulfamerazine) in serum. The isocratic elution composition of the mobile phase consisted of 1% glacial acetic acid and 4% methanol in water. The column used was a Symmetry® C18, 3.9 x 150 mm, 5 μm, flow rate was 1.0 mL/min and the UV detector was set at 254 nm (Waters Corporation, 2003). In this current study, the same mobile phase, isocratic elution composition and UV wavelength was used. The column used was a Novapak 3.9 x 150 mm C18 column and the run time was 30 minutes.
Figure 4.14. Isocratic separation of sulfamethazine (1 mg/L) as a function of solvent strength (40 mM ammonium acetate: methanol). (a) 70:30%, (b) 75:25% and (c) 80:20%.
As illustrated in Figure 4.15, the sulfamethazine peak did not elute during the run time of 30 minutes, based on the method by the Waters Corporation (2003); as a result, several changes were made in order to optimize the method. Firstly, the mobile phase composition was changed from a single mixed mobile phase consisting of 1% glacial acetic acid and 4% methanol in water to binary mobile phases, which ran on an isocratic elution composition of 96 % mobile phase A (glacial acetic acid) and 4 % mobile phase B (methanol) respectively.

Secondly, the run time was increased to 45 minutes in order to determine whether or not the sulfamethazine peak possibly eluted beyond the original run time of 30 minutes. Thirdly, a scan of a sulfamethazine standard (1 mg/L) was performed using a UV 300 Spectrometer (Spectronic UNICAM) to determine the optimum wavelength for elution of the sulfamethazine peak using a UV detector.

![Figure 4.15. Isocratic separation of sulfamethazine by HPLC UV. Other operating conditions include a C-18 column and a single mixed mobile phase: 1 % glacial acetic acid and 4 % methanol in water (Waters Corporation 2003). Sulfamethazine did not elute during the run time of 30 minutes.](image)
Figure 4.16 Identification of a major sulfamethazine peak at a wavelength of 263 nm.

As illustrated in Figure 4.16, the optimum wavelength for the elution of sulfamethazine was around 263 nm; therefore, all future sulfamethazine analyses on the HPLC system were conducted at a UV wavelength of 263 nm. The combination of wavelength and mobile phase changes resulted in the elution of a fairly sharp sulfamethazine peak with a retention time of 18.086 minutes, a height of 8794 and an area of 318374 (Figure 4.17a).

Although the retention time of the sulfamethazine peak increased, which meant that there was less chance of interference from the system peak; the overall run time of 45 minutes was considered too long for the processing of one sample. For this study, the best resolution with the shortest retention and run times was desired; as a result, several elution tests were conducted by increasing the composition of the methanol mobile phase (%B) and decreasing the run time. According to Johnson and Stevenson (1978), the retention of the analyte is decreased by increasing the concentration of the organic solvent, which is methanol in this study.
Figure 4.17 Isocratic separation of sulfamethazine as a function of solvent strength. Binary mobile phase compositions (1% glacial acetic acid: methanol). (a) 96:4%; (b) 85:15%; (c) 80:20%.
Based on the fact that solvent strength increases as solvent polarity decreases (Snyder et al., 1997), the mobile phase compositions (%) for the 1% glacial acetic acid/methanol, which were arbitrarily chosen for the elution tests, comprised of 90/10%, 89/11%, 85/15% and 80/20% respectively (Table 4.10). Frequently, mobile phase conditions, which involve the adjustment of solvent strength, are chosen by trial and error (Gilbert, 1987). As can be seen in Table 4.10 and Figures 4.17b and c, as the percentages of methanol increased the retention times also decreased, subsequently the run times were also decreased.

**Table 4.10 Retention time as a function of mobile phase composition**

<table>
<thead>
<tr>
<th>Isocratic mobile phase composition</th>
<th>Run time (minutes)</th>
<th>Retention time (minutes)</th>
<th>Peak height</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Glacial acetic acid Methanol</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>96 4 45 18.086 8794 318374</td>
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<td>90 10 20 12.552 12696 324524</td>
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<tr>
<td>89 11 20 10.968 14105 319726</td>
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<tr>
<td>85 15 20 6.938 21665 330785</td>
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<tr>
<td>80 20 15 4.440 32177 345930</td>
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</tr>
</tbody>
</table>

In addition, with decreasing retention time, the resolution in terms of peak height also improved. Essentially, the greater the peak height the lower the detection limit as observed in the earlier chapter on detection limits. Although the best resolution was achieved for the 80/20 mobile phase composition, which had a retention time of only 4.440 minutes, all future HPLC method developments were undertaken with the 85/15 composition, which had a slightly longer retention time of 6.938 minutes and thus less chance of system peak interference. As demonstrated in this current study, and in general, Johnson and Stevenson, (1978) reported that the HPLC conditions which are optimized are between speed and resolution.

### 4.5.3 HPLC UV – determination of sulfamethazine from reagent grade water

Several samples, including an extraction blank and a sulfamethazine spiked sample, which were previously extracted from reagent grade by solid phase extraction process, were injected into the
HPLC for analysis. The isocratic mobile phase composition consisted of 85% glacial acetic acid (1%) and 15% methanol. Peak retention times for the sulfamethazine standard and the sulfamethazine spiked sample were 6.915 and 6.934 minutes respectively. Overall, the resolution of the sulfamethazine peak for the spiked sample was good in terms of peak height (Figure 4.18c), however, quantification of the peak area may have been affected by a contaminant, which appeared in the extraction blank, and had a close retention time of 7.010 minutes (Figure 4.18b).

As part of the quality assurance protocol in this study, a blank comprising of the solvent used for dilution of standards and the reconstitution of the dried samples following SPE was also analysed with every run on the HPLC. The contaminant did not appear in the chromatogram of the solvent blank, which eliminates any possibility of contamination from the HPLC system, in particular the column.
As a precaution, the HPLC system was subsequently purged, all equipment including the SPE system were decontaminated and new sets of standards and SPE samples were prepared. However, contamination of the extraction blank samples continued after repeated solid phase extractions and analysis of the samples on the HPLC. Examples of contaminated blanks (peak

Figure 4.18   Isocratic separations of extracted (SPE) samples from reagent grade water. (a) sulfamethazine standard (0.01 mg/L); (b) SPE blank; (c) sulfamethazine spike (0.01 mg/L).
areas) before (run 1) and after (run 2) decontamination of the HPLC and all the equipment are represented in Table 4.11).

### Table 4.11 Analyses of SPE reagent grade water samples with a used C18 column

<table>
<thead>
<tr>
<th>Run #</th>
<th>Sample</th>
<th>Mobile phase</th>
<th>Mobile phase composition (%)</th>
<th>Peak retention time</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>Extraction blank</td>
<td>Isocratic</td>
<td>85</td>
<td>7.010</td>
<td>562</td>
</tr>
<tr>
<td></td>
<td>Standard (0.01 mg/L)</td>
<td>Isocratic</td>
<td>85</td>
<td>7.047</td>
<td>1273</td>
</tr>
<tr>
<td>2</td>
<td>Extraction blank</td>
<td>Isocratic</td>
<td>85</td>
<td>7.126</td>
<td>937</td>
</tr>
<tr>
<td></td>
<td>Standard (0.01 mg/L)</td>
<td>Isocratic</td>
<td>85</td>
<td>6.988</td>
<td>1716</td>
</tr>
<tr>
<td>3</td>
<td>Extraction blank</td>
<td>Gradient</td>
<td>85</td>
<td>6.646</td>
<td>462</td>
</tr>
<tr>
<td></td>
<td>Standard (0.01 mg/L)</td>
<td>Gradient</td>
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<td>6.459</td>
<td>1372</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 4.5.4 Mobile phase

Due to the re-occurrence of the interfering peak in the extraction blank even after decontamination of the equipment, it was thought that perhaps the interfering peak was not due to sulfamethazine contamination but rather to an unknown contaminant. As a result, optimization to maximise resolution of both the sulfamethazine and the interfering peaks was attempted by changing the mobile phase composition from isocratic to gradient. For the most part, gradient elution can be used when the resolution of the components in a mixture is not adequately determined using an isocratic mobile phase composition (Issaq, 2002). Essentially, with gradient elution, the solvent strength is increased as the separation progresses (Run 3, Table 4.11). In respect to time and resolution, gradient elution is particularly useful to optimize separations (Johnson and Stevenson, 1978), thus it is anticipated that the unknown peak would elute either earlier or later than the sulfamethazine peak. However, the gradient mobile phase composition did not result in the separation of the unknown peak; as a result, the extraction
blank was still contaminated with a peak, which had a retention time of 6.646 minutes (Run 3, Table 4.11).

4.5.5 Columns

Snyder et al., (1997) reported that a column may be changed for the purpose of improving separation if mobile phase optimisation has failed. The Novapak C-18 column, which was partially used for the analysis of the FWAs and sulfamethazine in this study and other compounds in prior studies, may have lost its efficiency, thereby resulting in the inability to separate the sulfamethazine and contaminant peaks. In general, separation occurs within the column, as a result it is the most important component of any liquid chromatography system (Gilbert, 1987), and the outcome of the analysis depends upon the type of column and the appropriate operating conditions. Although the column is reusable, it may degrade in time (Johnson and Stevenson, 1978). For clean samples, 1000 to 2000 analyses per column is expected (Snyder et al., 1997). As a result, the Novapak C-18 column in this study was replaced firstly with a Zorbax C-8 column. As mentioned earlier in the chapter on FWA method optimization, the C-8 column is similar to but slightly less retentive than the rugged, highly retentive C-18 column (Snyder et al., 1997).

The Zorbax C-8 column was first tested on the HPLC by injecting a 50 μL sulfamethazine standard (0.1 mg/L) into the system. In this study, a higher concentration of the analyte, typically 10 mg/L is generally chosen for optimum peak identification, however, for this particular run, a higher concentration of sulfamethazine may have further exacerbated the ongoing contamination problem. The binary mobile phases consisted of 1% acetic acid / methanol, and ran on a gradient elution starting with 85/15 % (Run 3, Table 4.11). A second run was also performed starting with a 90/10 % composition. For both runs 1 and 2, there were no elution of sulfamethazine peaks on the chromatograms (Figure 4.19 b and c). In general, once a column is changed, the mobile phases should be reoptimized to suit the new column (Snyder et al., 1997), thus, it is possible that reoptimization of the mobile phases was not adequately performed, which resulted in the non elution of sulfamethazine peaks.
Figure 4.19 Determination of sulfamethazine using a Zorbax C-8 column. Gradient mobile phase composition: 1% acetic acid:methanol. (a) SPE blank; 85/15%; (b) 0.1 mg/L standard; 85/15%; (c) 0.1 mg/L standard; 90/10%. Sulfamethazine did not elute.

As a result of the poor resolution of the sulfamethazine peak, the Zorbax C-8 column was replaced with a new SymmetryShield™ RP8 3.5 µm 4.6 x 100 mm column. Identification of
the sulfamethazine peak using the new C-8 column was performed by injecting 10 μL of a 10 mg/L sulfamethazine standard into the HPLC system. The binary mobile phases A and B consisted of 1% acetic acid and methanol respectively, and ran on a gradient elution composition from 80 and 20% to 65 and 35% respectively in 19 minutes; equilibration time was 11 minutes, making the total run time 30 minutes, and flow rate was 1 ml/minute. Since the column was new and possibly contained some contaminating material, the slightly stronger composition (20%) was chosen for solvent B, in order to flush out the column. As illustrated in Figure 4.20a, the sulfamethazine peak, which eluted in 6.062 minutes, had excellent resolution including peak symmetry and height. Although the C-8 column is less retentive than the C-18 column, the peak height and area for a sulfamethazine standard (10 mg/L) obtained with the former column were comparable with the measurements obtained for the same concentration using a Novapak C-18 column (Table 4.12).

<table>
<thead>
<tr>
<th>Column Type</th>
<th>Type of Elution</th>
<th>Retention Time</th>
<th>Peak Height</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
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<td>6.938</td>
<td>21665</td>
<td>330785</td>
</tr>
<tr>
<td>Symmetry RP8</td>
<td>Gradient</td>
<td>6.062</td>
<td>28370</td>
<td>320597</td>
</tr>
</tbody>
</table>
Figure 4.20 Comparison of a SymmetryShield™ RP8 column vs. Novapak C-18 column. (a) 10 mg/L sulfamethazine standard (RP8); (b) and (c) SPE blanks (RP8); (d) SPE blank (C-18).
The contaminated SPE blank (Figure 4.20d), which had been previously analysed (Run 1, Table 4.10) and a new SPE blank were both run on the HPLC system using the new Symmetry RP8 column. The chromatograms for both samples illustrated that there were no interferences from unwanted peaks, probably due to better separation of the peaks by the Symmetry RP8 column (Figures 4.20b and c). The rising or upward drift in the baselines in Figures 4.20b and c are somewhat common during gradient elution and with UV detectors, due to solvent A and B differences in terms of UV absorbance. In general, the UV absorbance of organic solvent (B) is always higher than that of water; thus during separation in reversed phased gradient elution, the concentration of the organic solvent B increases, resulting in a rise in the baseline. In addition, baseline drift is more visible with a lower detector wavelength, as observed earlier with the FWAs at a wavelength of 240 nm. Overall, baselines obtained from gradient elution are generally problematic (Snyder et al., 1997).

### 4.5.6 HPLC UV – determination of sulfamethazine from groundwater

Following SPE and reconstitution with mobile phases A and B, groundwater samples from 5 sites (4-8) in Langley were analysed for sulfamethazine by HPLC with a UV detector. The binary mobile phases, which consisted of 1% acetic acid (A) and methanol (B) ran on a gradient elution composition from 80 and 20% to 65 and 35% respectively in 19 minutes, after which there was an equilibration time of 11 minutes, making a total run time of 30 minutes. Other operating conditions included a flow rate of 1 mL/minute and a Symmetry RP8 column.

Overall, the chromatograms of the groundwater samples illustrated that there were no elution of any sulfamethazine peaks, and baseline separation of the surrounding unidentified peaks was generally acceptable (Figure 4.21). However, there were two notable observations associated with the analysis of environmental samples such as groundwater, which can hinder the detection of the analyst of interest or interfere with the elution of the analyte's peak.
Figure 4.21 Analyses of HLB extracted groundwater samples using a new Symmetry RP8 column. (a) sulfamethazine standard (0.01 µg/L); (b) SPE blank; (c) sulfamethazine spike (0.05 µg/L).
The first observation was the occurrences of two unidentified peaks with similar retention times at 5.398 to 6.903 minutes (Figure 4.22a) and 5.888 to 6.843 minutes (not shown) in the chromatograms for the extracted groundwater samples. The retention times of the two unidentified peaks were relatively close to the retention time of ~6 minutes for a typical sulfamethazine peak based on previous analyses of standards. As a result, an elution test was performed on one of the groundwater sample in order to confirm firstly that the unidentified peaks was not sulfamethazine, and secondly to investigate whether or not the two unidentified peaks may hinder or interfere with the elution of a sulfamethazine peak. The elution test was performed following the SPE (HLB) of a groundwater sample (100 mLs) spiked with a known concentration (0.05 μg/L) of sulfamethazine.

Based on the elution test, the blank groundwater sample also contained two unidentified peaks with retention times of 5.679 and 6.491 minutes (Figure 4.21b), which was close to the retention time of the sulfamethazine standard (0.01 mg/L) at 6.039 minutes (Figure 4.21a). The chromatograms for the spiked groundwater sample illustrated the elution of a sulfamethazine peak in 6.066 minutes, and two unidentified peaks in 5.681 and 6.520 minutes, which confirmed that neither one of the unidentified peaks was sulfamethazine (Figure 4.21c). In addition, it appeared that there were no interfering effects on the elution of the sulfamethazine peak in the spiked groundwater sample by the two unidentified peaks. Overall, the resolution of the sulfamethazine and the two unidentified peaks were good, and it appeared that the Symmetry RP8 column was adequate in the separation of the sulfamethazine peak from adjacent possibly interfering peaks, which may be found in actual samples.
Figure 4.22 Determination of sulfamethazine in extracted (SPE) groundwater samples as a function of 30 minutes run time (a) run 1 (site 8); (b) run 2 (site 5); (c) run 3 (site 5 duplicated).
The second observation was the occurrences of large unidentified peaks in the chromatograms of two extracted groundwater samples from site 5, possibly due to incomplete elution from a prior run (site 8). For example, Figure 4.22a illustrates a chromatogram with a run time of 30 minutes (run 1) for an extracted groundwater sample from site 8, which contains numerous unidentified peaks. The chromatograms for the duplicate groundwater samples from site 5 (runs 2 and 3), which were analysed following run 1, illustrated large wide peaks or bands (peak 1 and Peak 2), which eluted in 14.861 minutes (run 2, Figure 4.22b) and 2.786 minutes (run 3, Figure 4.22c) respectively. Of particular concern though, is peak 2, which had a retention time of 2.786 minutes but did not complete elution until around 7 minutes after the start of the run (Figure 4.22c). As a result, there may be peak overlapping with a possible eluting sulfamethazine peak at ~ 6 minutes, which can possibly interfere with the detection and quantification of the analyte.

Similar to this study, Snyder et al. (1997) reported that sometimes the method developed for the initial sample may not be suitable for later samples, due to new interference, impurity, and metabolite or degradation products occurring in the new samples. For example, samples generally used for method development compose of pure standards, whereas actual samples often contain late eluting interferences. These late eluters may contaminate the column or interfere with later separations, if the final mobile phase gradient composition (%B) is selected to correspond with the elution of the last analyte of interest. Subsequently, column efficiency and retention may be changed, resulting in alteration in the separation of the samples. The result is overlapping of the analyte in the chromatogram by the unwanted component in the later sample. In order to resolve this situation, the authors suggested to either run the sample until separation of the analyte has been achieved or adjust the HPLC conditions such as the mobile phase, pH or temperature.

Column efficiency may be improved by holding the B mobile phase at 100% at the end of the gradient elution or quickly adjusting the gradient to 100% B. In general, the strong solvent (B), will flush the columns; ideally 2 to 5 column volumes should be used for the cleansing. However, it was suggested that running the sample until separation of the analyte has been achieved, often results in the required separation with the least amount of work. Subsequently,
the samples from run 1 and run 2 were re run under the same operating conditions but with a longer run time of 60 minutes.

Figure 4.23 Determination of sulfamethazine in extracted (SPE) groundwater samples as a function of 60 minutes run time. No sulfamethazine was detected. (a) run 1 (site 8); (b) run 2 (site 5).

In the chromatogram for run 1, an unknown peak eluted in 46.596 minutes (Figure 4.23a), which most probably was the same unwanted peak (peak 1) that appeared in run 2 (see Figure 4.22b). This assumption was based on the total run time needed for the unwanted peak to elute. For example, according to the chromatogram for run 1 (see Figure 4.22a), peak 1 did not elute
during the run time of 30 minutes. Whereas, in the chromatogram for run 2 (see Figure 4.22b),
the unwanted peak eluted in ~ 15 minutes, when added together (30 +15), then the unwanted
peak should have eluted in ~ 45 minutes, which was relatively close to the elution time of
46.596 minutes, when the sample was re run for 60 minutes. Based on the same above principle,
peak 2, which appeared in run 3 (see Figure 4.22c) with a retention time of 2.786 minutes,
should have eluted in ~ 63 minutes (30 + 30 + 2.786), however, it was possible that peak 2
eluted at the end of the 60 minutes run time, even though it was not illustrated in the
chromatogram (Figure 4.23a).

4.5.7 Solid phase extraction – XAD-4 resins

Two different types of SPE devices, namely XAD-4 resins (styrene-divinylbenzene) and Oasis
HLB cartridges poly(divinylbenze-co-N-vinylpyrrolidone) were investigated for the extraction
of sulfamethazine from an aqueous media. Unlike the C-18 and C-8 cartridges, both XAD-4
and Oasis HLB do not contain silica but organic polymers; in addition, the polymeric sorbents
are more retentive than the C-18 reversed phase sorbents (Thurman and Mills, 1998).

Overall, excellent sulfamethazine recoveries totalling 97% was achieved using the XAD-4
resins, based on a spiked concentration of 0.5 mg/L in Millipore water (100 mLs) and a
concentrating factor of 200. In addition, most of the sulfamethazine was recovered with the first
(61%) and second (35%) elutions. Figure 4.24 illustrates the chromatogram for one of the
elutions (61%); the concentration of the recovered sample was 60 mg/L (after concentrating) or
0.3 mg/L for the spiked sample. As observed in this study, a benefit of using XAD-4 is the
ability of the polymeric resins to retain a large quantity of the analyte without any breakthrough.
In general, polymers have high capacities for polar organic compounds (Thurman and Mills,
1998); also, polymeric resins have a higher surface area than that of silica particles (e.g. C-8 and
C-18), as a result, there is more complete uptake of organic analytes, with no interfering silanol
groups, as with C-8 and C-18 adsorbents (Fritz, 1999).
Similar to this study, Cox and Krzeminski (1982) also obtained good recoveries of sulfamethazine using a XAD resin, although the media was pork tissue compared with a water media used in this current study. The authors obtained an average recovery of about 86% using XAD-2 resin.

Although the XAD-4 resin is a promising method of extraction for sulfamethazine from an aqueous media; during loading, samples may become contaminated as the water passes through the XAD-4. Essentially, the resins may contain leachable impurities acquired during the polymerization process (Fritz, 1999). Figure 4.25 illustrate the chromatograms of Millipore water samples (blanks), which were extracted (SPE) by both HLB cartridge (Figure 4.25a) and XAD-4 (Figure 4.25b). All samples were analysed with the used Novapak C-18 column, which was subsequently replaced with a new Symmetry RP8 column. However, for comparison purposes, the chromatograms for the XAD-4 extractions illustrated more unwanted peaks than the chromatogram for the HLB extraction. In addition, in the chromatogram for the XAD-4 extraction, a very large peak was observed at around 8.5 minutes (Figure 4.25b), which most likely was a contaminant from the XAD-4 resin.
Of particular concern though for the XAD-4, was a band of unknown peaks, which may interfere with a possibly eluting sulfamethazine peak. For example, Figure 4.26a illustrates the chromatogram of a 0.1 mg/L sulfamethazine standard, which had a retention time of 4.378 minutes and an area of 15488. In comparison, a blank sample, which had been previously extracted with methanol washed resins contained an unknown band with a retention time of 4.132 minutes, start and end run times of 3.859 and 4.645 minutes respectively, and an area of 4631. As a result, the unknown band may interfere with a possibly eluting sulfamethazine peak due to overlapping elution times.
Figure 4.26 Identification of unwanted compounds previously extracted from Millipore water with XAD-4 resins. (a) sulfamethazine standard (0.1 mg/L); (b) XAD-4 extraction blank.

Although the XAD-4 resins may have contributed to the interfering peaks, it should be noted that there were also similar unwanted peaks in the chromatogram for the HLB extraction (see section 4.5.1), thus, it was possible that these peaks may have been due to impurities from polymeric resin or the water. However, an attempt to remove the interfering band was performed by both adjusting the HPLC mobile phase composition, and further purification of the resins using different solvents. In general, due to the nature of the polymers, successive washing of the resins with a large volume of solvent may be required over a few days. Due to leachable impurities in the resins (Fritz, 1999), XAD resins should be washed thoroughly usually by extensive Soxhlet extraction (Sigma–Aldrich, 1998).
In this study, several attempts were made to purify the XAD-4 resins by soaking and washing the resins with different solvents. For example, one purification method involved soaking the XAD-4 resins for two days in solvents comprising of 50% methylene chloride and 50% methanol, followed by repeated Soxhlet extractions with methanol until the solvent appeared cleaner, usually the latter process lasted up to 2 days. As illustrated in the chromatogram (Figure 4.27), the soaking of the XAD-4 resins in methylene chloride and methanol followed by extensive Soxhlet washing in methanol resulted in a slightly cleaner sample than the initial washing of the resins using only methanol, however, the interfering peaks with retention times of 4.092 and 5.021 were still present.

![Chromatogram of SPE of Millipore water using XAD-4 previously washed with methylene chloride and methanol using a Soxhlet.](image)

Due to the existing unwanted peaks in the chromatograms even after Soxhlet washing, a recovery test was performed to determine if the unknown peaks would interfere with an eluting sulfamethazine peak. For the recovery test, XAD-4 resins, which had been previously purified using the above methylene chloride and methanol Soxhlet washing method, were used to extract 100 mls of Millipore water spiked with sulfamethazine at a concentration of 0.05 µg/L. Similar to the above study, two unknown peaks with retention times of 4.092 and 4.070 (Peak 1, Figure 4.28b and c), and 5.021 minutes and 5.019 respectively (Peak 2, Figures 4.28b and c) were also observed in the chromatograms for the extraction blank and the spiked sulfamethazine samples.
Overall, recovery of the sulfamethazine sample using the XAD-4 resins was good at 101%, however, one of the unknown peaks (Peak 1) interfered with the elution of the sulfamethazine peak, as a result, both peaks were fused (Figure 4.28c).

In order to separate the fused or overlapping peaks, the gradient mobile phase composition for A (0.1% acetic acid) and B (90% acetic acid and 10% acetonitrile) was changed from 85 (A) and 15% (B) to 96 (A) and 4% (B). Although, the overlapping peaks separated (not shown), the resolution of the peak decreased resulting in poor peak shape and sensitivity; however, it should be noted that the old C-18 column was used for the analysis of the XAD-4 extracts, and perhaps the new C-8 column may offer a better separation.

4.5.8 Solid phase extraction - HLB cartridges

Overall, excellent average recoveries for sulfamethazine spiked Millipore water (106%, n = 10) and groundwater (90%, n = 4) samples were achieved using Waters Oasis (HLB) cartridges, indicating that there was no discernable matrix effect particularly from the groundwater samples. In general, the HLB cartridges were effective for the removal of sulfamathzine from both types of water samples. Similar to the older XAD, which is a styrene-divinylbenzene copolymer, the Oasis HLB sorbent is a high surface area copolymer of [poly(divinylbenzene –cos-N-viny1pyrrolidone)]. The HLB sorbent is the only polymer, which has the property of conditioning with only water, as methanol is not needed (Thurman and Mills, 1998).
Figure 4.28. Chromatograms illustrating XAD-4 recovery of sulfamethazine from Millipore water (a) sulfamethazine standard (10 µg/L), (b) blank sample, (c) sulfamethazine spiked sample (10 µg/L).
Similar to this study, Yang et al., (2004) also observed high recoveries of sulfamethazine in two different water matrix also using HLB cartridges for SPE. Average recoveries of around 100 and 97% were obtained for sulfamethazine spiked in deionised and river water respectively. The similar average recoveries for the different water samples were due to the minimal matrix effects in the river water.

(i) pH

Göbel et al., (2004), reported that of all the parameters involved in sample extraction, the pH of a sample was the most significant variable for sulfonamides including sulfamethazine, due to their amino groups. In this study, sulfamethazine extractions using HLB cartridges were performed at a pH of around 2.5, and acceptable maximum recoveries obtained for spiked Millipore water and groundwater were 125 and 112% respectively. Lindsey et al., (2001) also obtained acceptable sulfamethazine recoveries of up to 130% for sulfamethazine spiked distilled water at a pH of 2.5. In contrasts, Göbel et al., (2004) observed the highest recoveries at pH 4 for sulfonamides, based on an enrichment tests conducted between pH 2 and 6. Average recoveries of 98% for primary, secondary and tertiary were obtained for effluent spiked with sulfamethazine. Essentially, the interaction with the cartridge material was strongest at a pH of around 4 for the analytes in the unchanged forms. Babić et al., (2006) also reported that a pH of 4 was optimum for the extraction of sulfamethazine; the authors obtained an average sulfamethazine recovery of 94.5% for spiked spring water.

Sulfonamides mainly depend upon hydrophobic interactions with SPE adsorbents (Lindsey et al., 2001), and as mentioned earlier in this study, all water samples were first adjusted to an unionized or neutral form at pH 2.5 for sulfamethazine extraction with the HLB cartridges. In contrasts, the pH of water samples containing sulfamethazine to be extracted by XAD-4 were not adjusted and yet excellent recoveries (>90%) were obtained using this resin. The pH of Millipore water is generally between 6 and 6.9, which falls within the neutral range of about 2.3 to 7.4 for sulfamethazine, as a result, hydrophobic interactions between the XAD-4 and the sulfamethazine was effective. Lindsey et al., (2001) were also able to extract sulfonamides from water at both pH < 3.0 and neutral pH using HLB cartridges.
(ii) Conditioning

Based on the SPE method by Lindsey et al., (2001), conditioning of the HLB cartridges in this study was performed using 3 mLs of 0.5 N HCl. However, HCl was not used as part of the conditioning process for the XAD-4, and excellent recoveries were still obtained. For the most part, several studies have used HLB cartridges to simultaneously recover different types of antibiotics such as tetracyclines and sulfonamides from an aqueous media. The use of 0.5 N HCL has been used to wash off residual metals on SPE cartridges. Essentially, tetracyclines can adsorb to residual metals on SPE cartridges resulting in irreversible binding to the cartridges, thus lowering recoveries; however, there were no observations reported of sulfonamides binding to residual metals. In addition, the use of HCl was reported to not affect the extraction efficiency of sulfonamides (Yang et al., 2004; Lindsey et al., 2001). Perhaps all future SPE of sulfamethazine should be attempted without the use of HCl as a conditioner for the cartridges.

4.6 Data analysis

Table 4.13 contains the site numbers, sampling dates, 24 sampling locations and results of all parameters analysed in this study.
Table 4.13 Summary of analytical results for all site locations in this study

<table>
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<th>Site #</th>
<th>Date</th>
<th>Site Location</th>
<th>Type</th>
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<th>NH₃-N mg/L</th>
<th>P mg/L</th>
<th>Cl mg/L</th>
<th>DAS I μg/L</th>
<th>DSBP μg/L</th>
<th>S μg/L</th>
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<td>NA</td>
<td>ND</td>
</tr>
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</tr>
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<td>0.13</td>
<td>0.79</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>8/2/05</td>
<td>Hop. C</td>
<td>PW</td>
<td>0.005</td>
<td>0.06</td>
<td>0.18</td>
<td>0.5</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>15</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>16.35</td>
<td>0.03</td>
<td>ND</td>
<td>12.30</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>16</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>23.05</td>
<td>0.03</td>
<td>0.01</td>
<td>12.10</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>17</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>32.45</td>
<td>0.02</td>
<td>ND</td>
<td>12.85</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>18</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>12.65</td>
<td>0.02</td>
<td>0.01</td>
<td>11.65</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>19</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>25.90</td>
<td>0.02</td>
<td>0.01</td>
<td>9.96</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
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<tr>
<td>20</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>4.60</td>
<td>0.03</td>
<td>ND</td>
<td>8.52</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>21</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>36.75</td>
<td>0.04</td>
<td>0.01</td>
<td>22.60</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>22</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>0.003</td>
<td>0.03</td>
<td>ND</td>
<td>7.89</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>23</td>
<td>14/2/05</td>
<td>Abbotts. MW</td>
<td>PW</td>
<td>20.75</td>
<td>0.03</td>
<td>ND</td>
<td>7.63</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>24</td>
<td>27/09/05</td>
<td>Van. PP(I)</td>
<td>PW</td>
<td>&lt;0.010</td>
<td>0.022</td>
<td>&lt;0.01</td>
<td>21.8</td>
<td>1.79</td>
<td>NA</td>
<td>7.84</td>
</tr>
<tr>
<td>25</td>
<td>27/09/05</td>
<td>Van. PP(E)</td>
<td>PW</td>
<td>10.4</td>
<td>0.022</td>
<td>&lt;0.01</td>
<td>21.8</td>
<td>1.79</td>
<td>NA</td>
<td>7.84</td>
</tr>
</tbody>
</table>

Method Detection Limit 0.002 0.01 0.01 0.5 0.01 0.01 0.04

S = Sulfamethazine  MW = Monitoring Well  ND = Not Detected  PP = Pilot Plant
NO₃-N = Nitrate-N  PW = Private Well  NA = Not Analysed  I = Influent
NH₃-N = Ammonia-  Hop. = Hopington  N/L = Nitrogen per Litre  E = Effluent
P = Phosphorus  Abbotts. = Abbotsford  P/L = Phosphate per Litre  St. = Stream
Cl = Chloride  Van. = Vancouver  * = Result based on one sample.
4.6.1 Nitrate-N

(i) Statistical analysis

In this study, all nitrate-N – nitrite (N) values will be reported as nitrate-N. Nitrate-N concentrations ≥ the detection limit of 0.002 mg/L were found in 100% of all water samples which comprised of surface water (n = 3), private wells (n = 11) and monitoring wells (n = 9) (Table 4.13, Figure 4.29). Statistical analysis of the sampling data, which was summarised in Table 4.14, indicated that the maximum concentration of nitrate-N (36 mg/L) was observed in water samples taken from a monitoring well in Abbotsford. Minimum concentrations of nitrate-N which were 0.003 and 0.004 mg/L were obtained from a monitoring well in the Abbotsford and a private well in the Hopington C regions respectively.

(ii) Water quality guidelines

In British Columbia, the ambient level of nitrate-N concentrations is very low in groundwater, usually less than 1 mg/L, therefore, nitrate-N in groundwater ≥ 3 mg/L usually indicate anthropogenic activities on the aquifer (BCMoE, 2007). Of the 20 groundwater samples (private and monitoring wells) and 1 spring water sample analysed, nitrate-N concentrations ≥ 3 mg/L were detected in 62% of the samples (Figure 4.29).
Table 4.14  Nitrate-N statistical analyses

<table>
<thead>
<tr>
<th>Water Sample</th>
<th>n</th>
<th>Nitrate-N Concentrations (mg/L)</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface Water</td>
<td>3</td>
<td></td>
<td>17.50</td>
<td>4.38</td>
<td>9.43</td>
<td>± 7.07</td>
</tr>
<tr>
<td>Private Wells</td>
<td>11</td>
<td></td>
<td>14</td>
<td>0.004</td>
<td>2.10</td>
<td>± 4.11</td>
</tr>
<tr>
<td>Monitoring Wells</td>
<td>9</td>
<td></td>
<td>36.75</td>
<td>0.003</td>
<td>19.32</td>
<td>± 11.45</td>
</tr>
<tr>
<td>Total number of samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total number of samples: 23

Figure 4.29  Nitrate-N concentration ranges for surface and well water.
Of concern, are the high levels of nitrate-N concentrations found in 43% of the groundwater and spring water samples, which have exceeded the maximum acceptable concentration of 10 mg/L (nitrate-N-nitrogen) for drinking water (Figure 4.30), according to the Canadian Drinking Water Quality Guidelines (CDWQG), 2006 (Health Canada, 2006) and the British Columbia Approved Water Quality Guidelines (BCAWQG) 2006 (BCMoE, 2006). In one private residential well, the nitrate-N concentration was as high as 14 mg/L.

![Bar chart showing nitrate-N concentrations](chart)

**Figure 4.30 Maximum Acceptable Concentration Limit (MAC) for drinking water.**

In addition to the water quality guidelines for human consumption, there are also nitrate-N guidelines for the protection of riverine fish. Union Creek which flows into Salmon River (Figure 4.31) had been classified ‘A’, which means that it is a fish bearing watercourse with the potential that there might be the year round presence of fish in the stream (TOL, 2002). The importance of this classification is that the nitrate-N concentrations in Union Creek were below the interim limit for the protection of freshwater life, which is 13 mg/L (Environment Canada, 2005).
Surface water samples taken from Union Creek (Site 1) and Coglan Creek (Site 3) contained nitrate-N concentrations of 6.40 mg/L and 4.38 mg/L respectively (Figure 4.32). According to the Canadian Water Quality Guidelines, only the Coglan Creek samples had concentrations of nitrate-N near the limit of < 4 mg/L for most Canadian rivers (Environment Canada, 2005). Wernick et al., (1998), also detected similar nitrate-N concentrations in Coglan Creek; the authors found a maximum nitrate-N concentration of ~ 3 mg/L during high flow events in the month of February, which was comparable with the 4.38 mg/L detected in the month of January in this study.
4.6.2 Molecular markers

(i) Statistical analysis

Sulfamethazine was not observed in any of the water samples analysed (n = 8, Hopington AB). Out of the 21 water samples analysed from three locations (Hopington AB and C, and Abbotsford), FWAs were observed in only the Hopington AB water samples. Out of the two FWAs analysed in this study, DAS 1 had the highest maximum concentration of 0.13 µg/L, whereas, the minimum concentrations for DAS 1 and DSBP were generally ≤ the detection limit of 0.01 µg/L (Table 4.15). The concentrations of DAS 1 and DSBP in the Pilot Plant secondary influent (primary effluent) were 7.84 µg/L and 2.36 µg/L respectively. In the final effluent, the concentrations of DAS 1 and DSBP were 3.14 µg/L and 0.05 µg/L respectively following secondary, and tertiary treatment with membrane filtration.
Table 4.15 FWAs statistical analyses

<table>
<thead>
<tr>
<th>Statistical Analysis</th>
<th>DAS 1 µg/L</th>
<th>DSBP µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean</td>
<td>0.044</td>
<td>NA</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>± 0.051</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = Not Applicable
n = Number of samples.

(ii) Water quality guidelines

Currently, there are no CDWQG or BCAWQG for FWAs and sulfamethazine. In general, DAS 1 and DSBP are considered non-toxic due to their occurrence in the low milligram per Litre range in surface and groundwater, and based on the vast quantities that are needed to cause any acute oral toxicity (DAS 1: LD 50 > 10,000 mg/kg and DSBP: LD 50 > 5000 mg/kg) to rats (Thomann and Krüger, 1975). DSBP toxicity values for other organisms include (a) zebra fish, 96 hour LC50 76 mg/L, (b) trout, 96 hour LC50 130 mg/L, and (c) Daphnia magna, 24 hour LC50 > 1000 mg/L (CIBA, 2004).

Although there are no CDWQG for sulfamethazine, there are maximum residue levels allowed for this antimicrobial in some food products from treated animals. Antimicrobial residues in animal based food products, which are consumed by human, may cause potential health hazards including antimicrobial resistance (AMR). Thus, the new maximum residue limits for sulfamethazine in milk and edible tissues in calves are 0.01 mg/L and 0.1 mg/L respectively (Health Canada, 2002).
4.6.3 Ammonium-N, chloride and phosphorus

(i) Statistical analysis

Ammonia and phosphorus concentrations were very low in all the water samples from the Hopington AB and C, and Abbotsford locations, generally ≤ 0.10 mg/L and ≤ 0.34 mg P/L respectively. However, maximum chloride concentrations of 57.3 mg/L and 22.6 mg/L were observed in the Hopington AB and Abbotsford locations respectively; whereas, almost non detectable concentrations of chloride in the Hopington C location were generally ≤ 0.85 mg/L (Table 4.16).

Table 4.16 Chloride statistical analyses

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>Chloride Concentrations mg/L</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>Hopington AB</td>
<td>8</td>
<td>57.30</td>
<td>3.50</td>
</tr>
<tr>
<td>Hopington C</td>
<td>6</td>
<td>0.85</td>
<td>0.49</td>
</tr>
<tr>
<td>Abbotsford</td>
<td>9</td>
<td>22.60</td>
<td>7.63</td>
</tr>
<tr>
<td>Total number of samples</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = number of samples.

(ii) Water quality guidelines

Currently, there are no CDWQG numerical guidelines for ammonia in drinking water because the available data suggest that it is not a health hazard or aesthetic problem at the concentrations found in drinking water in Canada (Health Canada, 2004). For phosphorus, there are no current BCAWQG or CDWQG maximum levels in drinking water taken from streams or groundwater.

In this study, the maximum concentration of chloride (57.30 mg/L), which was obtained from a private well water sample (site 5), was far below the recommended guideline (aesthetic objective) of ≤ 250 mg/L (BCMoE, 2006; Health Canada, 2004). In general, chloride
concentrations above 250 mg/L in drinking water may cause an unwanted tastes and corrosion in the distribution system (Health Canada, 2004).

Based on the British Columbia Approved Water Quality Guidelines 2006, the maximum concentration of total ammonia nitrogen for the protection of aquatic life (mg/L) for Coglan Creek and Union Creek, should be less than 15.5 mg/L and 15.7 mg/L, based on the creeks water temperatures of 3°C and 2°C respectively, and a pH of ~ 7.42. In this study, ammonia concentrations of 0.02 mg/L and 0.01 mg/L for Coglan Creek and Union Creek respectively were below the above mentioned BCAWQG values. There are no proposed total phosphorus BCAWQ guidelines for aquatic life in streams (BCMoE, 2006).

4.7 Fluorescent whitening agents - local occurrences

4.7.1 Detergents

Two local studies in Vancouver, British Columbia were undertaken in order to determine specifically if DAS 1 or DSBP were components of laundry detergents used by local householders, and thus components of domestic sewage at the study sites in Langley. Firstly, a small survey was carried out among a total of 15 staff and students at UBC, to determine the brand names of laundry detergents generally being used in the region. In addition, most of these detergents were analysed to determine whether they contained DAS 1 or DSBP.

Overall, there were five different brands of laundry detergents used by the staff and students at UBC, which consisted of Sunlight, Tide, ABC, Purex and a No Name brand. Out of the five brands of laundry detergents, 33 and 47 % of the participants used Sunlight and Tide respectively, whereas 7% of the participants used Purex, ABC or No Name. Only four of the detergents (Tide, Purex, ABC and No Name) were analysed for FWAs. Both Tide and ABC contained DAS 1, whereas DSBP was detected in Purex. Neither DAS 1 nor DSBP was detected in the No Name detergent (Figure 4.33). In the current study, it was observed that most of the analysed laundry detergents (75%) contained FWA. Hayashi et al., (2002) also reported that in Japan both DAS 1 and DSBP are major FWAs presently found in laundry detergents. In general,
DAS 1 and DSBP are the two most used FWAs (Stoll and Giger, 1998), with worldwide consumptions of an estimated 3000 and 14000 t/yr respectively in 1990 (Poiger et al., 1996).

4.7.2 Domestic wastewater

In the second study, an attempt was made to confirm the detection of FWAs in domestic sewage. According to Poiger et al., (1998), out of an annual consumption of FWAs by householders in Zürich, an estimated 38 and 97% of DAS 1 and DSBP respectively were reported to be discharged with household wastewater. The authors suggested that a large proportion of the remaining fraction of DAS 1 and DSBP from householders’ wastewater were perhaps adsorbed to the laundry during washing, where they replace the FWAs lost during wear.

![Graph showing laundry detergent brands](image)

**Figure 4.33** Laundry detergents by brand name used by householders in Vancouver, BC.

Due to the unavailability of a septic tank sewage sample, the primary and secondary effluents at a biological nutrient removal (BNR) pilot plant in Vancouver was analysed for FWAs; a small
percent of UBC’s domestic sewage is treated at this wastewater facility. DAS 1 and DSBP were observed in the primary effluent and secondary effluent at concentrations of 7.84 µg/L and 2.36 µg/L, and 3.14 µg/L and 0.05 µg/L respectively. Similar to the FWA results obtained from the pilot plant effluent, DAS 1 and DSBP concentrations in the low microgram per litre range have also been detected in the primary effluent at various STP plants, in Zürich at 3.8 to 11.4 µg/L and 5.4 to 21.3 µg/L respectively (Poiger et al. 1996; Poiger et al., 1998). Contrary to the lower concentrations of DAS 1 observed in the primary effluent from the above studies, Kramer (1992) reported that of the two FWAs, DAS 1 was the most widely used.

Based on the aforementioned information, the concentration of DAS 1 obtained in the primary effluent in this study, was in the range of the data obtained by Poiger et al. (1996, 1998). However, the concentration of DSBP was somewhat lower than the minimum concentrations obtained by Poiger et al. (1996) and Poiger et al., (1998) by factors of ~3 and 2 respectively. There are several reasons which could possibly explain these differences including method of sampling, FWAs loading and photodegradation. In this study, one grab sample was taken from the BNR pilot plant and therefore, these results do not necessarily reflect the normal or average daily loading of FWAs in the influent. According to Poiger et al. (1996), there may be significant variability of FWA concentrations in sewage on a daily basis. In addition, typical 1:1 ratios of DAS 1 to DSBP have been observed in primary effluent (n = 3) by Poiger et al. (1996), unlike this study where the ratio was about 3:1. Poiger et al., (1998) observed that daily mass flows of FWAs in primary effluent varied by a factor of 2-3, which can possibly explain the 3:1 ratio for DAS 1 to DSBP obtained in this study.

Overall, photodegradation of DSBP was not expected to make a large contribution towards the degradation of the parent EE isomers in the primary effluent thus resulting in lower concentrations, mostly due to limited exposure of the wastewater to direct sunlight during storage and clarification at the pilot plant. Typical half lives based on direct sun exposure for DAS 1 and DSBP were reported as ~3h and ~2h respectively (Hayashi et al., 2002).
4.8 Molecular markers and sources of nitrate-N

In the primary and secondary effluents at the UBC Pilot Plant, DAS 1, DSBP, ammonia-N and nitrate-N were observed at concentrations of 7.84 μg/L, 2.36 μg/L and 21.8 mg/L and <0.010 mg/L, and 3.14 μg/L, 0.05 μg/L, 0.022 mg/L 10.4 mg/L respectively. Overall, the detection of molecular markers (FWAs) in the laundry detergent and the Pilot Plant effluent confirmed that there was a specific link to the human source of nitrate-N, which was domestic wastewater. In agreement with the results of this study, Eganhouse, (1997) reported that source specificity is one of the main criteria for an ideal molecular marker.

As determined earlier, nitrate-N concentrations above the background level of 3 mg/L was observed in the unconfined Hopington AB and Abbotsford aquifers, suggesting anthropogenic impacts. Land use studies for these two regions generally indicated that the main activities, which overlay the Hopington and the Abbotsford aquifers were agriculture and/or residential use.

4.9 Aquifer materials and sources of nitrate-N

4.9.1 Unconfined aquifers

Out of the three aquifers in this study (Hopington AB, C and Abbotsford), the most significant occurrences of nitrate-N concentrations, which were greater than the background level of 3 mg/L and drinking water level of 10 mg /L, were observed in the well water samples from the Hopington AB, and the Abbotsford aquifers. In these aquifers, maximum nitrate-N concentrations reached 14 mg/L and 36.75 mg/L respectively. As illustrated in Figure 4.34a and b, the most significant relationship between nitrate-N and a molecular marker was observed only in the Hopington AB aquifer.
Figure 4.34 Nitrate vs FWAs observances in this study: (a) DAS 1 and (b) DSBP.
Out of six sites (2, 4, 5, 6, 7, and 8) in the Hopington AB Aquifer, both nitrate-N concentrations > 3 mg/L and at least one FWA were observed in the well water samples from sites 2, 4, 7 and 8. At site 5, only nitrate-N was observed at a concentration of 4.91 mg/L; whereas, at site 6, one FWA was observed, but the nitrate-N was < 3 mg/L. For the most part, the Hopington AB, and the Abbotsford aquifers are unconfined, and consist predominantly of sand and gravel materials. In agreement with this study, Close et al., (1989) also reported that aquifers particularly unconfined and in semi-rural areas are frequently subjected to pollution from various sources, including septic tanks, farming and agricultural activities; therefore, well water abstracted from these aquifers particularly for domestic use may pose health problems. One of the many contaminants in elevated concentrations, which can adversely affect groundwater quality and ultimately human health, is nitrate.

The Abbotsford Aquifer was chosen for this study because of its overlying land use activity, which was predominantly agriculture, although there is an increasing urban development located on the northern portion of the aquifer (Hii et al., 1999). In general, only about 20% of the aquifer’s surface is covered by urban areas (Liebscher et al., 1992). Studies on the Abbotsford Aquifer have reported nitrate-N levels above the maximum acceptable concentration of 10 mg/L in many groundwater samples (Liebscher et al., 1992; Wassenaar, 1995; Hii et al., 1999), and have generally attributed these high concentrations due to agricultural activities on the surface of the aquifer (Liebscher et al., 1992; Wassenaar, 1995; Zebarth et al., 1998). For example, Hii et al., (1999) reported nitrate-N concentrations ranging from 0.01 to 55.30 mg/L and 0 to 50 mg/L for sites 15 – 23 in this current study for the sampling periods 1996 and 1997 respectively. Therefore, it was anticipated that the chemical markers FWAs, which for the most part are used by householders, and can be found in septic tank wastewater via laundry effluent, would not be observed in groundwater samples from the predominantly agricultural regions of the Abbotsford Aquifer. In addition to FWAs, the Abbotsford Aquifer was also chosen to determine if there were any background concentrations of naturally occurring fluorescence matter existing in groundwater, which can cause interference or result in false positives.
4.9.2 Confined aquifer

In contrasts, all the groundwater samples collected from sites 9-14, which were located in the Hopington C, generally had very low concentrations of nitrate-N (< 3 mg/L) ranging from 0.004 - 0.323 mg/L, with no detections of FWAs (Figure 4.34). Historic low nitrate-N concentrations were also reported for five unspecified well locations within the study area in Hopington C; the data was summarised as follows: <0.01 mg/L (year unknown), 0.0 – 0.051 mg/L (1990), 0.4 - 1.62 mg/L (1995) and 0.004 – 0.184 mg/L (2001) (Scovill and Zubel, 2002).

The low concentrations of nitrate-N < 3mg/L indicated that there was no anthropogenic impact on the groundwater quality, primarily due to a confined aquifer. The Hopington C aquifer, which consists of low permeability surface sediments (clay/silt/till), underlies the unconfined area and extends further south and west covering an area of 50 km² (Gartner Lee Limited, 2000; TOL, 2001; TOL, 2008).

Hatch et al., (2002) reported that due to the negative charges of clay minerals, ammonium is bounded tightly and thus becomes relatively immobile and less harmful. As a result, due to the low permeability nature of the clay soil, historic and current low nitrate-N data, then in agreement with Scovill and Zubel, (2002), it is unlikely that nitrogen originating from septic tank systems or any other sources situated above the clay layer would easily infiltrate this stratum and reach the porous sand and gravel aquifer. Since FWAs have similar structures in terms of polarity with nitrate, then the non detection of this marker in the confined aquifer, supports its usefulness as a molecular marker.

4.10 Groundwater flow and sources of nitrate-N

4.10.1 Septic systems and FWAs

Figure 4.35 illustrates groundwater piezometric contours of the Hopington AB and C Aquifers, and the approximate locations of the sampling sites in this study. Based on groundwater contour lines, the flow of the water was generally from south to north and from high to low topography. Up gradient of the Hopington AB, nitrate-N was observed below the drinking
water limit of 10 mg/L at sites 4-7. However, nitrate-N concentrations (3.20-8.47 mg/L) above
the background level of 3 mg/L were detected at most of the sites (4, 5, 7 and 8), which
indicated an anthropogenic impact on the groundwater quality, possibly from human and/or
agricultural source(s). Sites 4-7 were private residences with septic tank systems. In only one
well (site 6), the nitrate-N concentration (0.1 mg/L) was less than the background limit of 3
mg/L. According to TOL (2001), it is not unusual to find wells in close proximity with high and
low concentrations of nitrate.

In addition, during sampling, several hobby farms including a vineyard (site 4) and three cows
(opposite site 4) were observed at or in close proximity to the sampling sites. Behind site 5, it
was reported that there was approximately ½ acre of hay cultivation. For the most part, sites 4
(8.47 mg/L) and 5 (4.91 mg/L), had the highest nitrate-N concentrations upgradient of the
groundwater flow. Overall, the observance of trace concentrations of FWAs (<0.01-0.02 µg/L)
in these water samples suggested that septic systems were a source of nitrate contamination in
the groundwater.
Figure 4.35 Groundwater Contours and Site Locations in Langley. Source: Golder Associates, (2005). Reproduced with permission of the Township of Langley.
In the well water samples, the highest concentrations of nitrate-N (14 mg/L) and FWAs (0.13 µg/L as DAS 1) were observed at site 8, which was located downgradient (>180 m) of sites 1-7. Based on historic data obtained from the Township of Langley and Gartner Lee Limited (1998), high nitrate concentrations were also observed for site 8. For example, average and maximum nitrate-N concentrations of 22.5 mg/L \( (n=8) \) and 48.10 mg/L respectively were reported for site 8. At two other sites, which were located upgradient of site 8; average and maximum historic nitrate-N concentrations for each site were reported as 15 and 20.7 mg/L and 0.8 and 6.19 mg/L. For the sites with average nitrate-N above the drinking water quality limit, including site 8, there was the possibility of cumulative nitrate-N concentrations downgradient of septic systems. The site with the lower nitrate concentration was less impacted probably because the well was deeper at 82 m, compared with 26 m (site 8) and 42-57 m for the other two sites.

Similar to the results in this study, Batt et al., (2006) also observed the highest concentration of nitrate-N (39.1 mg/L) in groundwater, which was taken from a well located at a distance of 487 m down gradient from a confined animal feeding operation (CAFO). At the other sites, the nitrate-N concentrations and the distances downgradient of the CAFO ranged from 1.28 – 21.6 mg/L and 76 – 225 m respectively.

Nitrate-N at a concentration of 4.38 mg/L has also been detected at Coglan Creek (site 3), which was also located downgradient of septic systems, and in close proximity to site 8 (Gartner Lee Limited, 1998). Similarly, Wernick et al., (1998) observed nitrate-N (7.1 mg/L) in the Coglan Creek. Using septic tank density, the authors were able to conclude that in the Coglan region, a high septic system density (0.88 ha⁻¹) was generally responsible for the nitrate in the Coglan Creek. In addition, Wernick et al., (1998) suggested that groundwater discharge might have contributed largely to the Coglan Creek flow. According to Gartner Lee Limited (1998), the base flow for many streams in the watershed including the Coglan Creek and the Salmon River were provided by groundwater discharged from the Hopington Aquifer.

In agreement with Wernick et al., (1998), the downgradient locations of the Coglan Creek (site 3) and site 8, together with the recurring high nitrate-N concentrations and the highests concentration of FWAs particularly at site 8, suggests that source of the contamination at sites 3
and 8 were from the upgradient septic systems. In fact, site 8 may have acted as a sink for these mobile analytes.

Nitrate-N, DAS 1 and DSBP concentrations of 17.5 mg/L, 0.05 μg/L and 0.02 μg/L respectively were detected in spring (site 2) water samples in the Hopington AB aquifer study area. The spring water samples were thought to be predominantly septic tank effluent; as prior to the sampling day (14th January) there was snowfall, and the air temperature which averaged 3.5°C (Abbotsford Station) may not have been warm enough to facilitate any significant snowmelt. Usually, surface runoff is the predominant source of water to the streams during the winter months (Wernick et al., 1998). The small springs were located approximately 180 m down gradient of private residences with septic tanks systems.

In a Hopington Aquifer Study, Schreier et al., (1996) suggested that nitrate discharged into the Salmon River and the Coghlan Creek via groundwater was a concern to fish-bearing streams. In agreement with Schreier et al., (1996), evidence of groundwater contaminated with nitrate-N discharged directly into a fish-bearing stream was observed in this study. Nitrate-N concentrations as high as 17.5 mg/L together with FWAs in groundwater were discharged into the Union Creek (site 1) via springs (site 2). The detection of FWAs in the spring water indicated that septic systems were a source of the nitrate. Union Creek, which is located at the base of site 2, has been classified “A”, which means that it is a fish bearing watercourse with the potential that there may be year round presence of fish in the stream (TOL, 2002). However, the nitrate-N concentration in Union Creek, which was 6.40 mg/L, was below the interim limit for the protection of freshwater life, which was 13 mg N/L (Environment Canada, 2005).

In the spring and well water samples, there were more occurrences of DAS 1 (n = 5) than DSBP (n = 1), which were ≥ 0.01 μg/L. The well water samples taken from sites 4 and 6 contained only one type of FWA. For these homeowners, which were situated up gradient of the groundwater flow, this could have implied the preferential use of detergents containing DAS 1. However, the detection of both FWAs in the spring water samples may have been the results of septic tank effluents combining down gradient from various homeowners, as the groundwater flowed through the Township. Similar to site 2, DAS 1 and trace amounts of DSBP (<0.01 μg/L)
were both observed in the well water samples from sites 7 and 8, which were also situated down
gradient of the ground water flow.

4.10.2 Septic systems and impact of well depth

In terms of depth, Walker et al., (1973b) reported that nitrate-N concentrations are usually
highest in the upper portion of the aquifer, thus private residences depending upon groundwater
should use deep wells which are cased. For example, the authors observed high nitrate-N
concentration of 40 mg/L in the upper 30 cm of the aquifer adjacent to the system, whereas, at a
depth of 1.5 m, the nitrate-N concentration was still high at 15 mg/L. Roberston et al., (1991)
also observed high nitrate-N concentrations of around 39 mg/L in plume core groundwater
samples from shallow wells with depths of 2.6 to 3.5 m. The plume which originated from the
beneath a single family's septic system, extended to about 20 m downgradient and had a high
nitrate-N contour gradient of 30 mg/L.

In contrasts, in this current study, groundwater samples taken from 2 out of 3 deep wells with
depths of 26 m (site 8) and 27 m (site 4) had nitrate-N concentrations of 14 and 8.47 mg/L
respectively; whereas, in agreement with Walker et al., (1973b), very low nitrate-N
concentrations (0.1 mg/L) were observed in only one deep well (site 6), which had a depth of 33
metres. Low nitrate-N concentrations (4.91 and 3.2 mg/L), which were still higher than the
background level were observed in shallow wells, which had depths of 8.33 (site 5) and 2.67 m
(site 7) respectively. Schreier et al., (1996) also observed that shallow wells in the Hopingtn
aquifer were more contaminated than deep wells.

There are several possible reasons for the low nitrate-N concentrations in the shallow wells at
sites 5 and 7, including denitrification in the saturated zone as a result of anaerobic activities. In
addition, several authors have reported the occurrence of non contaminated groundwater
recharge, which overlay a nitrate-N plume, thus low concentrations of nitrate-N may be present
in shallow wells. For example, Robertson et al., (1991) observed a nitrate-N plume
downgradient of a septic drainfield, which was overlay by non contaminated ground water
recharge through an overlying lawn. Whereas, Harman et al., (1996), reported a thick wedge
(0.4 to 0.8 m) of non contaminated water which overlay a nitrate-N plume in the region of 50-100 m downgradient from the drainfield.

In respect to the relatively high nitrate-N concentrations observed in the deep wells (sites 4 and 8), Gooddy et al., (1997) reported that pumping from deep wells may cause vertical hydraulic gradients, which can result in groundwater containing nitrate-N to be leaked from the shallow horizon. As mentioned earlier, fairly high nitrate-N concentrations in deep wells may also be the result of nitrate-N sources upgradient (Mitchell et al., 2003). Both theories on vertical hydraulic gradient and up gradient sources may also explain the occurrence of FWA in deep wells, in particular site 8, which was located downgradient of septic tanks on the groundwater contour map, and had both the highest concentration of nitrate-N and FWAs in the Hopington AB Aquifer.

As mentioned earlier, Roberston et al., (1991), observed a nitrate-N plume which extended at a considerable distance downgradient of a septic tank system, and dropped to a depth of only 2 m below the water table in the vicinity of the drainfield. However, it must be noted that the individual results of the plume studies by both Walker et al., (1973b) and Roberston et al., (1991) represented only a single family household. Thus, the length and depth of the plumes may be an underestimate of the actual size of a typical nitrate-N plume developed from a number of septic tank sources within the study area. An example of a large plume was observed at a 44 year old school, which was situated in an unconfined sand aquifer in Ontario, Canada. The plume had a width of 20 metres, thickness of 1 to 2 metres, and extended more than 110 m downgradient of the tile bed (Harman et al., 1996).

In general, nitrate-N concentrations may be highest in wells located down gradient and not far from septic systems as observed by Walker et al., (1973b) and Roberston et al., (1991), however, dilution may be possible with the groundwater flow, resulting in lower concentrations farther away from the system. One major limitation to the reduction of nitrate-N concentrations in an aquifer through dilution may occur in areas where there are high densities of homes using septic tanks for the disposal of on-site liquid waste. As a result, background concentrations of nitrate-N in the groundwater may already be high if there are other septic systems present up gradient.
(Walker et al., 1973b). In general, the above results indicated that a large body of water adjacent to septic systems may be contaminated with nitrate-N

For the Township of Langley, it is possible that over the years that septic tank systems have been in existence, a nitrate-N plume may have developed in the saturated zone of the Hopington A aquifer. In addition, with the downward water table gradient existing in the aquifer, then within the plume, high nitrate-N concentration contours may have reached site 8, which would explain the consistently high nitrate-N concentrations in this deep well.

In general, a soluble contaminant such as nitrate-N may have an increased concentration relative to the background concentration in a groundwater flow, as the contaminant infiltrate into the water table. This zone of contamination in the groundwater may form a very large plume due to the presence of several plumes overlapping and uniting, particularly from non point sources such as agricultural or septic tank fields (Freeze et al., 1994). For example, at two study sites also located in sandy soils, Walker et al., (1973b), reported nitrate-N in groundwater, each with concentrations of 10 mg/L located at distances of approximately 30 m and 70 m down gradient from individual septic tank systems.

In a similar study conducted by Roberston et al., (1991), and based on a predominantly silty sand aquifer \(K = 1.3 \times 10^{-3}\), the authors reported nitrate-N concentration contours of 10, 20 and 30 mg/L in a plume which extended to about 17 m down gradient of the tile bed and arrived after 300 days of the system operation. However, unlike the presence of nitrate-N in the stream water samples in this current study, Roberston et al., (1991) observed drastic reductions in nitrate-N concentrations from about 20 mg/L to <0.5 mg/L in the last m of a 20 m plume before it discharged into the river. The nitrate-N in the plume, which was mobile and occurred in uniformly high concentrations, was attenuated in the riverbed sediments as a result of prevailing anaerobic conditions. In addition, the authors reported that the riverbed sediments contained an organic carbon (foc) level, which was 60 times higher than that of the aquifer sand, which would stimulate denitrification.

One limitation to the expansion of a contaminant plume can be due to the abstraction of groundwater from deep wells. For example, in a pumping well 200 m deep, which had a daily
total abstraction rate of 20000 m\(^3\), Guanghe et al., (1997) observed an artificial groundwater valley of 3 m deep, which caused the flow of the groundwater to change resulting in the halt of an expanding pollution plume.

4.10.3 Livestock operations and sulfamethazine

In this current study, sulfamethazine was not detected in any of the five Hopington AB well water samples, which were analysed for the antimicrobial; although the aquifers were surrounded by agricultural activities. In addition, some private residences had hobby farms on their properties.

Figures 4.36a and b illustrates maps of the Hopington AB aquifer superimposed over a portion of the Township of Langley’s ALR. From the maps, it is observed that there are numerous horse (Figure 4.36a) and beef cattle (Figure 4.36b) operations, which are located on the urban rural interface and in close proximity to the aquifer. Missing from the maps were livestock operations situated within the urban region of the Township, which overlay the Hopington AB Aquifers. Other types of livestock operations also situated within the urban rural interface and the Hopington AB Aquifer included poultry, sheep and goats.

Of particular interests, are the beef cattle and horse operations, which were situated upgradient of all the residential sites in this study, which may be contributors of nitrate-N to the groundwater. Thus, one of the reasons why sulfamethazine was not detected in the groundwater samples was possibly because the livestock farmers did not use the drug, although it was being used frequently in Alberta. Perhaps an antimicrobial survey could be carried out in Langley to corroborate this hypothesis.
Figure 4.36 Approximate locations of the five residential sites to (a) horse operations (b) beef cattle operations on the Hopington AB Aquifers. Source: Township of Langley Agricultural Land Use Inventory, 2001 (BCMAFF, 2002). Copyright © Province of British Columbia. All rights reserved. Reprinted with permission of the Province of British Columbia.
Overall, the non-detection of sulfamethazine in the five well water samples does not necessarily imply that agriculture has not contributed to the overall nitrate-N contamination in the Hopington AB aquifer. In fact, the well water samples (sites 4-8) were taken from a predominantly residential area with some hobby farms. However, upgradient of the residential area (sites 4-8); there exists numerous horse and cow operations on the urban rural fringe, which could possibly contribute to the nitrate contamination of the groundwater, particularly downgradient of the groundwater flow. Based on a nutrient budget model, Schreier et al., (1996) reported that agriculture contributed largely to nitrogen in the Hopington Aquifer. For example, out of an average surplus of 68 kg/ha/year of nitrogen applied to the Hopington Aquifer land surface, agriculture, hobby farms and septic systems accounted for 49, 18 and 33% respectively of the surplus.

For livestock operations, there are factors, which can affect the occurrence of sulfamethazine in the environment, including size of operation and grazing vs confined operations. For the most part, the sizes of the livestock operations on the urban rural fringe were not very large and as a result, any impact to the groundwater may be minor and localized. For example, out of an estimated 20 beef cattle operations surrounding the Hopington AB Aquifer, 90% were classified as small size (1 – 4 cattle); whereas only 10% were classified as medium size (4 – 50 cattle) (Figure 4.36b). Surrounding the aquifer, horses seem to have more operations than beef cattle, and may possibly contribute a larger portion of nitrate-N to the aquifer, similar to the beef cattle, most of the horse operations were classified as small (1-10 horses) (Figure 4.36a). In addition, grazing livestock on a pasture, particularly from small operations may have less of an impact to the aquifer; since nitrate-N may be absorbed by the plants. For the most part, few studies have reported findings on the impact of antimicrobials in the environment from grazing cattle; most studies have focused on soil application or amendment with manure or slurry (Christian et al., 2003; Hamscher et al., 2005; Dolliver et al., 2007).

Several studies have also linked the non detection and the detection of sulfamethazine in the environment to either a minor/non agricultural input or a predominantly agricultural input. As a result, this antimicrobial to some extent can be an important tool for determining agricultural sources of nitrate-N contamination in the environment. For example, Christian et al., (2003) observed sulfamethazine in 6 out of 40 samples in a creek in Northwestern Germany. The low
sulfamethazine concentrations, which ranged from 0.002 to 0.007 μg/L, were attributed to a relatively small agricultural input.

In contrasts, Hirsch et al., (1998) did not detect any sulfamethazine in a small river in Bielefeld, Germany. Instead, five antimicrobials (erythromycin, roxithromycin, clarithromycin, sulfamethoxazole and trimethoprim), which were possibly human medication were detected. The authors attributed the detection of the human medication to a municipal sewage treatment plant in the vicinity. In a similar study, sulfamethazine was not detected in the pristine and the urban fringe sections of the Poudre River in northern Colorado, USA. The non detection of sulfamethazine in the up stream part of the river was attributed to non and minimal agricultural impacts respectively. However, downstream of the Poudre River, which had the highest agricultural impact, sulfamethazine was detected at a concentration of 0.06 μg/L (Yang et al., 2004).

In comparison, larger livestock operations, particulary confined or housed may have a greater impact on groundwater quality. For example, Batt et al., (2006) reported nitrate-N above the drinking water quality limit of 10 mg/L in 3 out of 6 private wells, which were located downgradient of a confined animal feeding operation (CAFO) in Washington County, Idaho. According to C. Tesch (personal communication, 2008), at its peak, the CAFO housed approximately 4000 beef cattle compared with a maximum of 50 beef cattle at a livestock operation in Langley.

Sulfamethazine was detected in the CAFO wastewater lagoon and CAFO well water at concentrations of 43.353 and 0.310 μg/L respectively. In all six private wells, sulfamethazine was also detected at concentrations ranging from 0.076 – 0.215 μg/L, even at two well locations, where the nitrate-N concentrations were lower (<0.10 – 6.83 mg/L). Four of the wells, which had nitrate-N concentrations ranging from 1.28 – 39.1 mg/L, were located at distances ranging from 76 – 487 m downgradient of the CAFO. Similar to the results in this study, the highest nitrate-N concentration of 39.1 mg/L was also detected furthest downgradient (487 m) of the CAFO (Batt et al., 2006). Overall, the detection of sulfamethazine in the CAFO wastewater lagoon at relatively high concentrations indicates source specificity, which makes it potentially useful as a molecular marker for the detection of animal waste. Similar to Canada,
sulfamethazine is approved for use as a feed additive in beef cattle, and not approved for human use in the USA.

4.11 Fate of fluorescent whitening agents - photochemical processes

A summary of the FWA data for water samples in this study by isomer types $E$ and $Z$ (DAS 1) and, $EE$ and $EZ$ (DSBP) are represented in Figure 4.37a and b. Out of the five well water samples taken from sites 4-8, the $E$ isomer (DAS 1) was observed in one water sample taken from site 6, whereas, the $Z$ isomer was detected in four water samples taken from sites 4, 6, 7 and 8. The DSBP isomer $EE$ was observed in three well water samples taken from sites 6, 7 and 8, however, the $EZ$ isomer was not detected in any well water samples. The spring water sample (site 2) which was not analysed for the $Z$ and $EZ$ isomers contained both the $E$ and $EE$ isomers.

At the commencement of this study, it was not anticipated that the $Z$ isomer, the result of photoisomerisation of the $E$ isomer, would be present in groundwater due to the fate of DAS 1 in the environment. Essentially, the purpose of FWAs in laundry detergents is to make the faded fabric appear whiter; therefore, the active florescence isomers in the washing process are the $E$ and $EE$ isomeric forms. As mentioned earlier, one of the main deterioration pathways for the FWAs is photoisomerisation, where the $E$ and $EE$ isomeric forms under some sort of irradiation are converted to the non fluorescence $Z$ and $EZ$ isomeric forms. Due to the limited or non exposure of the FWAs in the washing liquor to light during the washing stage (provided that machines are used), septic tank, drainfield and groundwater, then photoisomerisation of the $E$ and $EE$ isomeric forms should not occur. Poiger et al., (1996) also reported that FWAs are not exposed to light from the washing process to the sewers. Thus in theory, if groundwater contained FWAs, it should only be in the $E$ and $EE$ isomeric forms.
Figure 4.37 Isomers and photoisomers obtained in groundwater and wastewater in this study (a) DAS 1 and (b) DSBP.
There are several possible explanations as to the occurrence of the Z isomeric form in the groundwater samples in this study. First and foremost, the water samples were collected in amber coloured bottles and stored in a sealed box in the refrigerator, thereby limiting exposure to light; however, neither sampling nor the solid phase extraction processes were conducted in a controlled, light free environment. For example, the water samples were taken from taps outside the respective houses on a sunny summer day in August, thus photoisomerisation of the FWAs may have occurred. Poiger et al., (1996) observed that under direct sunlight, FWAs in standard solutions can be readily isomerised to the Z and EZ forms in only 1 minute. Kramer (1992) added that low concentrations of FWAs in the microgram per litre range are relatively unstable with light exposure, thus facilitating a quick reduction in fluorescence due to the E to Z isomerisation.

Initially, upon isomerisation, there is a distinct composition or ratio of the isomers which may vary with length and intensity of light exposure and temperature. In the Hopington A water samples, the percentage compositions for E/Z and EE/EZ where peaks were observed even if below the detection limit of 0.01 μg/L, were 0/100% (n = 4) and 100/0% (n = 3) respectively. The predominance of the Z photoisomer generally indicated that steady state had been reached for DAS 1. However, for DSBP, the absence of the EZ photoisomers confirmed the generally lower isomerisation rate for this FWA, thus it was possible that steady state was not achieved.

Kaschig (2003) reported steady state conditions for FWAs occurred in less than a minute, which resulted in isomeric compositions of 17% (E), 83% (Z), and 85% (EE), 15% (EZ) for DAS 1 and DSBP respectively. Similar steady state compositions consisting of 25% E to 75% Z and 86% EE to 14% EZ for DAS 1 and DSBP respectively were observed for sewage effluent after 3-5 minutes of irradiation with sunlight (Poiger et al., 1996). Based on the steady state isomeric compositions observed by Kaschig (2003) and Poiger et al., (1996), then it was possible that in approximately 30 seconds of sample collection under sunlight, steady state occurred and resulted in the transformation of the E parent isomer to the predominant Z photoisomer in the water samples. In contrasts DSBP remained almost unaffected in terms of fluorescence loss, resulting in predominantly the parent EE isomer.
The isomer compositions obtained by Kaschig (2003) and Poiger et al., (1996) indicated that both parent and photoisomers were present upon isomerisation, unlike the results of this study, where either one or the other predominated (sites 4, 6, 7 and 8). The non detection of the parent E isomer (DAS 1) or EZ photoisomer (DSBP) may not necessarily imply that photoisomerisation due to light exposure during sampling or the extraction processes did not occur. The possibility exists that these isomers may have been present but at concentrations too low to be detected by the UV; perhaps, increasing the sample volume from 500 to 1000 mLs for SPE, may increase the overall detection of the FWAs.

Under normal laboratory lighting, long exposure of the FWAs during the extraction process particularly in dilute solutions, may also result in isomerisation. Poiger et al., (1996) also observed changes in isomeric compositions of the FWAs during sample preparation, which was conducted under normal laboratory light. In this current study, SPE average processing time for both the recovery tests and the Hopington samples were similar > 12 hours. In the earlier recovery tests it was observed that the long SPE process under laboratory lighting resulted in the isomerisation of DAS 1 and the occurrence of an average of 24% Z isomers in seven samples. However, since steady state was not achieved for DAS 1 during the recovery tests, then it could be concluded that laboratory lighting was not exclusively responsible for the predominant Z isomers (steady state conditions) in the Langley samples.

According to Stoll and Giger (1997), photoisomerisation of the FWAs is reversible and will not occur in the absence of light, therefore, the ratios of the various isomeric forms will remain constant or photostationary. Thus, in order to investigate individual FWA isomers, then precaution should taken to minimise the samples to UV or light emitted by normal laboratory light sources such as blue visible light. These authors prepared their samples in a windowless room with a special lamp fitting (Philips TLD 36W/16 yellow).

As illustrated in Figure 4.38, and similar to sites 4 and 8, isomerisation of the FWAs also occurred in the primary and secondary effluents from a pilot plant due to natural and normal laboratory lighting. For example, the primary effluent had been previously stored in semi transparent feed storage tanks situated outside of the pilot plant, and thus partially exposed to about 36 hours of natural lighting, 40-45 minutes in the primary clarifier and >12 hrs of normal
laboratory lighting during SPE. Despite the long exposure to light, the compositions of the photoisomers and isomers for DAS 1 and DSBP in the primary effluent were 25% Z and 75% E, and 0% EZ and 100% EE respectively, indicating that steady state conditions had not been reached. In terms of the overall isomeric composition, it was observed that the percentage of the Z photoisomers in the primary effluent for DAS 1 was higher than the EZ photoisomers for DSBP. Poiger et al., (1996) also observed that the effect of photoisomerization on DSBP was limited compared with DAS 1, due to the small fraction of the EZ formed when raw sewage, primary effluent and secondary effluent were exposed to sunlight for a few seconds, < 1 hr and > 2 hrs respectively.

Poiger et al., (1996) also observed photoisomers and isomers in primary effluent comprising of about 60% Z and 40% E and about 15% EZ and 85% EE respectively. The higher percentage of the Z and EZ photoisomers in the primary effluent observed by Poiger et al., (1996) as compared with this study, was possibly due to direct exposure to sunlight for more than 1 hour during primary clarification. However, the authors reported that despite the sunlight exposure, steady state conditions had not been quite achieved, due to a large quantity of particulate matter in the primary effluent, which can deter penetration of light through far reaches of the water column. In addition, UV energy does not penetrate very far into the water column.

In the current study, the primary effluent had been further exposed to an estimated total of 10 hrs of light exposure during anaerobic, anoxic and aerobic treatments, and > 12 hours of SPE processing under normal laboratory lighting. Isomerisation of DAS 1 and DSBP resulted in photoisomers and isomers in the tertiary effluent which comprised of about 26% Z and 74% E, and 17% EZ and 83% EE respectively. Typical isomeric conditions for DAS 1 and DSBP in the secondary effluent after exposure to > 2 hours of sunlight were observed to be about 70% Z and 30% E, and 16% EZ and 84% EE respectively.
Figure 4.38 Typical HPLC chromatograms of DSBP (EE and EZ) and DAS 1 (E and Z): (a) standard mixture, (b) site 4 well water, (c) site 8 well water, (d) UBC Pilot Plant secondary effluent and (e) UBC Pilot Plant primary effluent.
Thus, direct exposure to sunlight as compared with laboratory lighting can more readily result in the transformation of isomers to photoisomers, as might have been the reason for the Z photoisomers in the Hopington AB samples (Figure 4.38). Contaminated glassware or equipment during spiking and extraction processes with the parent isomers, may not have produced any significant quantities of Z isomers under normal laboratory lighting. For example, in this study, the most significant concentration of Z isomer above the detection limit was recovered from duplicate samples each with a concentration of ~ 0.13 µg/L with no E isomer. Based on the earlier recovery experiments, then contamination with the E isomer exposed to laboratory lighting should have resulted in only about 30% Z isomer, the remaining 70% in E isomers. Therefore, contamination of the SPE apparatus was not likely to be the source of the Z isomers in the groundwater samples.

Another possible source of the Z isomers in the well water samples may have originated from the removal of photoisomers from previously whitened fabrics into the washing liquor. Kaschig (2003) reported that most of the textile mills use DAS type FWAs for whitening cotton, and exposure to sunlight results in the fabric becoming a major sink for FWAs. In addition, between the two FWAs in this study, fabric containing DAS 1 when exposed to sunlight is more readily photoisomerised, resulting in the loss of whiteness caused by the loss of fluorophores. However, Hilfiker (1996) observed (as cited in Canonica and Kramer, 1997) that upon irradiation with sunlight, the fraction of DAS 1 on cotton was determined to be predominantly in the E form (>90%) at the photostationary state.

Historic data to support the occurrence of photoisomers in the washing liquor could not be obtained, because most studies if not all have been focused on the fate of photoisomers in STPs (Poiger et al., 1996), surface water environments such as marine (Managaki and Takada, 2005) and rivers (Poiger et al., 1996; Poiger et al., 1999). Therefore, further research is needed to either accept or refute this claim. In the interim though, the resulting Z photoisomers compared with the parent E isomers are highly soluble in water and are less absorbing onto cellulose fibres (Kaschig, 2003), thus, it is quite possible that the photoisomers are easily removed from the fabric during the washing process and are essential components of the washing liquor. A possible limitation to the availability of Z photoisomers from the fabrics in the washing liquor is photodegradation. According to Kaschig (2003), photodegradation follows isomerization upon
further irradiation of the FWAs. In the absence of information about the presence of photoisomers in the washing liquor, this study will assume that the the Z isomer in the groundwater samples originated from exposure to sunlight during sampling.

Arguably, if all the E, EE, Z and EZ forms of isomers are discharged with the washing liquor into the drainfield, then both forms, particularly the E isomer should be present in the groundwater samples in higher concentrations, since a photostationary state was expected due to the lack of photodegradation. However, another main removal mechanism for the FWAs in the environment is adsorption to sediments, which can possibly result in uneven compositions of the isomers in the groundwater, depending upon the solid liquid partitioning effects of each type of isomer. Firstly, between the parent E and EE isomers, the former isomer was observed to be more adsorbing unto suspended solids in a STP (Poiger et al., 1996). Secondly, Stoll et al., (1997) observed that both the E and EE isomers have higher sorption coefficients than the Z and the EZ isomers; therefore, in sediments there is a tendency for enrichment of E and EE isomers.

Often when considering chemical markers to source various contaminants in the environment, there is the possibility that these markers are present in trace amounts, often below detection limits or not detected at all. Depending upon the fate, deterioration of the parent marker such as the E and EE isomers may produce photoisomers, such as the Z and EZ isomers respectively. Thus, parent and photoisomers when combined may increase the overall detection of the chemical markers. In this study it was observed that in only one site location (6) and with DAS 1, the combination of the parent E isomer (0.01 μg/L) and the Z photoisomer (0.01 μg/L) increased the overall detection, with a total concentration of 0.02 μg/L.

The detection of FWAs in the pilot plant wastewater (this study) and drainfield influent generally confirmed this study’s theory, that septic tank sewage may contain FWAs. In addition, both septic tank and drainfield treatment processes will not effectively remove all the FWAs from the septic tank sewage, as observed in drainfield water. This is in agreement with the observation made by Hayashi et al., (2002), that FWAs may be used as molecular markers due to the lack of removal during sewage treatment. In addition, the occurrence of FWAs in groundwater overlain with septic tank systems suggests that the highly sorbing DAS 1 and DSBP may be effectively transported through the drainfield into the saturated zone either in the
dissolved state or through desorption by flushing. Overall, FWAs may be particularly useful to track contamination of groundwater possibly as a result of poorly maintained septic systems. Schreier et al., (1996) reported that out of 80 householders who live on the Hopington AB aquifers and participated in a septic tank survey, about 1/3 reported that their septic systems were not adequately functioning. As a result, if tanks are not pumped out on a regular schedule, the solids build up, which could (1) adsorb the FWAs or (2) short circuit the FWAs through the tank to the drainfield.

An example of a failing septic system resulting in groundwater pollution has been observed by Viraraghavan and Warnock (1976). The authors reported a maximum total suspended solids concentration of up to 120 mg/L in groundwater samples below the drainfield, whereas background total suspended solid concentration was reported as 40 mg/L. The significance of the high suspended solid concentration in the groundwater was that the solids percolating through the drainfield untreated may essentially provide binding sites for adsorption of FWAs, thus increasing the mobility and efficiency for the transport of the adsorbed DAS 1 and DSBP into the water table. Viraraghavan and Warnock (1976) concluded that the soil below a septic tank system may lose the ability to retain percolating contaminants through the soil before reaching groundwater. As a result, according to Fay et al., (1995), due to the strong sorption behaviour of the FWAs, they can be used as indicators of failing septic tank systems, particularly where the soil sorption capacities have been exceeded.

Overall, the detection of FWAs in the well water samples suggested that septic systems contributed to the nitrate-N concentrations for those specific sites. There are 6,600 rural residents with 1,871 septic systems located on the Hopington aquifers (Schreier et al., 1996). According to Eganhouse (1997), if a molecular marker is found in the environment, then it is possible to identify the source of the contaminating material.
5 SUMMARY AND CONCLUSIONS

Elevated nitrate-N concentrations above the background limit of 3 mg/L have been detected in 4 out of 5 private wells indicating anthropogenic impacts to the water quality of the Hopington AB aquifers. In addition, harmful nitrate-N concentrations above the background limit of 10 mg/L have been detected in one residential well (14 mg/L) and spring water (17 mg/L), which may be a concern for residents of the Hopington AB aquifers, located in Langley, B.C.

Land use activities, which overlay the vulnerable Hopington AB aquifers, generally indicated that residential use (septic systems) and livestock operations were key activities and possible contributors of nitrate-N to the aquifers. Molecular markers are useful tools for the identification of a specific source of contaminant in the environment, especially in areas where there is a mixture of land use activities. Knowing the source of contamination, managers can effectively implement rules, regulations or fines to mitigate the adverse effect to the groundwater quality.

DAS 1 and DSBP are fluorescent whitening agents, which have been detected in 3 out of 4 popular laundry detergents used in the Fraser Valley. In addition, both DAS 1 and DSBP have been detected at concentrations of 7.84 and 2.36 µg/L respectively in the primary effluent at a BNR wastewater treatment pilot plant. These results suggested that the FWAs are effectively discharged at detectable concentrations with laundry effluent from households, and thus, should be found in septic systems. Sulfamethazine, which is an antimicrobial approved solely for veterinary use in Canada, is widely used in the livestock industry, particularly as a feed additive for beef cattle and swine. Beef cattle and horse operations were major livestock activities, which overlay or were in close proximity to the Hopington AB Aquifers.

Adapted SPE and HPLC methods for the analyses of DAS 1, DSBP and sulfamethazine resulted in good maximum recoveries of 60, 125 and 125% respectively. In addition, excellent instrument reproducibility and low method detection limits, suggested that HPLC with UV detection were adequate low cost instruments, compared with HPLC with MS/MS or fluorescence detectors for the determination of low concentrations of these analytes particularly in environmental samples.
In addition to the primary effluent, DAS 1 and DSBP have also been detected in the secondary effluent of the BNR pilot plant at concentrations of 3.14 and 0.05 µg/L respectively, which had two implications. Firstly, that there was a direct link between the FWAs and the source, which makes the analytes good molecular markers in terms of source specificity. Secondly, DAS 1 and DSBP should resist septic system treatment processes including clarification, and biological degradation (aerobic and anaerobic); thus exhibiting good conservative behaviour. As a result, once released from septic systems, DAS 1 and DSBP can persist in the environment. As predicted, neither DAS 1 nor DSBP were detected in any of the 15 wells at the two control sites (Hopington C and Abbotsford), which implied that there was no background interference from natural fluorescent minerals, and thus the possibility of false positives.

Out of the 5 wells located on the Hopington AB Aquifers, DAS 1 (0.01 – 0.13 µg/L) and/or nitrate-N (3.2 - 14 mg/L) above the background level (>3 mg/L) were detected in 4 wells. Using a groundwater flow map, it was observed that the greatest impact to the vulnerable Hopington AB Aquifers in terms of nitrate-N concentrations was observed at both a private residence (14 mg/L) and spring (17 mg/L), which were located down gradient of a predominantly residential area intermingled with beef cattle and horse operations. Both DAS 1 (0.05 µg/L) and DSBP (0.02 µg/L) were detected in the spring water, which implied mixing of the detergents down gradient of septic systems. In addition, the highest concentration (0.13 µg/L) of a FWA (DAS 1) in well waters in this study was also observed at the residential site. The overall detection of FWAs in the well water samples suggested that residential use via septic systems contributed to the elevated nitrate-N concentrations in the groundwater, particularly down gradient of the aquifer.

In this study, there were several observations concerning the fate of FWAs. Firstly, there were more occurrences of the Z (DAS 1) photoisomers than the EZ (DSBP) photoisomers in the samples. The Z isomer was observed at 6 sites compared with the observance of the EZ isomer at only 1 site. In addition, the Z isomer was also detected in all the standards (n = 6) during the recovery experiments. The preferential photoisomerization of DAS 1 suggested that this FWA was more affected by natural lighting than DSBP. Secondly, it appeared that DAS 1 was also more adsorbing than DSBP, since a total of 10 mLs of methanol was required to elute up to 72 % of
the analyte from the SPE cartridge, compared with only 2 mLs of methanol, which eluted over 80% of DSBP.

Although sulfamethazine was not detected in any of the five wells from the Hopington AB aquifers, it does not necessarily imply that livestock has not contributed to the overall nitrate in the aquifers. In fact, further investigation is needed to clarify if the non detection of the sulphonamide was due to non use, minor inputs into the aquifer from livestock activities, removal or degradation by the time transportation to the aquifer would occur.
6 RECOMMENDATIONS

The FWAs detected in the groundwater samples in this study, have been generally near or below the detection limit of 0.01 μg/L. Therefore, in order to increase its detection in the environment, the sample volume for SPE should be increased from 400-500 mLs to about 1L.

The results of this study based on the analyses of 23 water samples from the Fraser Valley for fluorescent whitening agents suggest that FWAs are definitely useful molecular markers for sourcing nitrate contamination in groundwater. It is therefore recommended that monitoring of FWAs and nitrate-N in the Hopington AB groundwater continues. Furthermore, in order to confirm the relationships between the molecular markers and their sources in the Hopington AB aquifer, it is recommended that number of sampling sites should be increased from the current 5 to about 20-25. Frequent sampling (> 2 per year) and long term monitoring for nitrate-N and FWAs in the groundwater together with any trends on a groundwater flow map should provide useful information about septic system sources.

The identification of the source, which appears to be septic systems at the sites investigated is only the first step in the mitigation of elevated concentrations of nitrate-N in the Hopington AB aquifers.

Sulfamethazine was not observed in any of the five water samples analysed. In general, there was a lack of data or information in this study to confirm that farmers in Langley actually used the antimicrobial, although there was evidence that this sulfonamide was widely used across Canada. Therefore, in order to conclude whether sulfamethazine was an effective molecular marker for the identification of nitrate-N from livestock sources, further studies including interviews or surveys of farmers could be conducted about the use of the drug.
BIBLIOGRAPHY


British Columbia Ministry of Agriculture, Food and Fisheries (BCMAFF) (2002). *Township of Langley Agricultural Land Use Inventory, 2001*.


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