Environmental Epidemiology of Type 1 Diabetes Mellitus in Prince Edward Island

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Doctor of Philosophy

in the Department of Health Management

Atlantic Veterinary College

University of Prince Edward Island

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Charlottetown, P. E. I.

June, 2006

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ISBN: 978-0-494-22835-7

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Abstract

The main objective of this thesis was to examine the relationship between type 1 diabetes mellitus (T1D) and dietary nitrate intake from both drinking water and food sources in Prince Edward Island (PEI), Canada at the ecological level, and at the individual level in a case-control study. Historical data on ground water nitrate concentrations and land use patterns were utilized to examine temporal and spatial assumptions made in these two T1D studies. Ground water nitrate concentrations were assessed temporally, and the association between ground water nitrate and local land use was assessed spatially, comparing areal aggregation methods. The relationship between average nitrate concentration in ground water and T1D incidence at the watershed level was assessed, taking into account the population-at-risk and average household income. A case-control study compared drinking water chemistry, food frequency and average dietary component (e.g. nutrients) consumption between patients diagnosed with T1D during a four year period, and their age and sex matched controls, with an emphasis on nitrate concentrations and its derivatives. Some environmental and genetic-based factors were evaluated and controlled for where appropriate.

Average monthly values in nitrate-nitrogen concentration for 54 wells across PEI over a three year period ranged from 3.80 mg L^{-1} in April to 4.08 mg L^{-1} in January, and annual values ranged from 2.12 mg L^{-1} in 1987 to 2.73 mg L^{-1} in 1983 for 167 wells over a 16-year period, with monthly measurements significantly differing over time. Local land use had a major influence on average nitrate concentrations: agricultural areas, particularly where row-crops grew, had higher nitrate concentrations than both residential and 'pristine' areas, and this was somewhat dependent on season. Specifically, potato,

grain, and hay land coverage were significantly and positively related to average ground water nitrate concentrations (compared to 'pristine' areas), regardless of method of data aggregation. Blueberry land coverage was negatively associated with ground water nitrate concentrations using two of the three aggregation methods. Watersheds were the preferred method of nitrate aggregation at the area level when compared to freeform polygons and 500 m buffer zones around each well, due to the method of creation (hydrological boundaries), being large enough to accurately determine average nitrate concentration, and being able to explain the most amount of nitrate concentration variation. The results from these historical data indicate that the assumptions made in the T1D incidence and risk studies were valid: there were minimal temporal differences but substantial spatial differences in ground water nitrate concentrations.

The incidence of T1D at the watershed level was not associated with ground water nitrate concentrations either before or after adjusting for average household income (a proxy for socioeconomic status), but a weak trend of higher nitrate concentrations being positively associated with T1D risk was present, especially after controlling for average household income.

A case-control study comprising of 57 T1D cases and 105 controls, matched by age at diagnosis and sex, concluded that the risk of T1D was increased when regular soft drinks or eggs were consumed at 'least once per week', compared to 'less than once per week'. The intake of dietary nitrate was marginally significant and positively associated with T1D ($p = 0.13$). An increased intake of total carbohydrates and caffeine were also associated with an increased risk of T1D, whereas the increased intake of vitamin A, vitamin B12, folate, and zinc were associated with a decreased risk. A family history of

T1D and having five or more infections during the first two years of life significantly increased the risk of T1D, whereas residential remoteness was associated with a reduced risk of T1D. Father's education was also related to the risk of T1D, but the direction of the relationship was not clear.

In summary, ground water nitrate concentrations in PEI were influenced by local land use more than temporal factors. Nitrate from food had a marginally significant dose-response relationship with T1D risk at the individual level, and T1D incidence was positively but not significantly associated with nitrate concentrations in ground water at the area level. Other environmental and dietary factors had a greater influence on T1D risk at the individual level.

ACKNOWLEDGEMENTS

I would like to acknowledge those who through their knowledge and support made this thesis possible. Firstly, I would like to thank my supervisor, Dr John VanLeeuwen, for providing me with the opportunity to come to beautiful PEI to pursue a PhD in epidemiology. I am extremely grateful to John for his guidance and experience with the project, and for finding both the time and patience to answer my never-ending list of questions! I am also indebted to Dr Jennifer Taylor for her wealth of knowledge in nutrition, and for her overwhelming support throughout the project. Jen has taught me a lot about nutrition, and I hope to build on this knowledge in my future years. Her constant supply of cookies always encouraged me to stop by for a chat!

Further thanks are extended to the rest of my supervisory committee: Mr George Somers has shown tremendous interest and encouragement during the evolution of this thesis, and has shared some valuable information along the way, Dr Ian Dohoo for putting me back on track when an epidemiological 'road-block' got in my way, Dr Patricia McKinney for sharing with me new and interesting information and techniques, and to Dr Linda Van Til for her endless knowledge about diabetes in PEI.

Much of this work would not have been possible without the technical assistance provided by Mrs Margaret Prouse, who interviewed many of the case-control participants. I would also like to acknowledge Margaret's friendship which brought fun and enjoyment to the project. Sincere thanks and gratitude are also given to all of the study participants who made this project a success, and welcomed us into their homes. Thanks are also due to Dr Helaine Rockett, Mr Gaetan Goddin, and Miss Sophia Wadowska for their assistance in the case-control study.

My stay in PEI would have not have been as enjoyable if it wasn't for the friends I made in PEI. They kept my mind focused on recreational topics rather than work, and introduced me to the term 'potluck'! It was wonderful to be part of an extremely social and interactive international group of friends, and learn words from many languages, with my most favourite Dutch word being 'pantoffels'! Last but not least, I would like to thank Nick for the years of love and support he has given me. He has stuck by me through thick and thin, and always manages to make me smile.

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List of Abbreviations

AIC	Akaike's Information Criteria
AR (1)	Autoregressive
ARMA (1,1)	Autoregressive moving average
BB rat	Biobreeding rat
BIC	Bayesian Information Criteria
CI	Confidence interval
CS	Compound symmetry
EU	European Union
FFQ	Food frequency questionnaire
GAD	Glutamic acid decarboxylase
GIS	Geographical information system
HLA	Human leukocyte antigen
HS-FFQ	Harvard Service Food Frequency Questionnaire
ICA	Islet cell cytoplasmic antibody
JECFA	Joint Expert Committee on Food Additives
LSM	Least square mean
MAC	Maximum acceptable concentration
NAD	Nicotinamide adenine dinucleotide
NO_3–N	Nitrate-nitrogen
NOD mouse	Non-obese diabetic mouse
OR	Odds ratio

PEI	Prince Edward Island
RFQ	Risk factor questionnaire
SD	Standard deviation
SIRR	Standardized incidence rate ratio
STZ	Streptozotocin
T1D	Type 1 diabetes mellitus
T2D	Type 2 diabetes mellitus
Th1	T helper 1 cells
Th2	T helper 2 cells
WHO	World Health Organisation
YAQ	Youth/Adolescent Questionnaire

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Chapter 1 General introduction and literature review

1.1. Introduction

This introductory chapter begins with a literature review that introduces diabetes mellitus, followed by a description of the distinction between type 1 diabetes mellitus (T1D) and type 2 diabetes mellitus (T2D). An overview of the risk factors of T1D is given, including both genetic and environmental factors. This is followed by a more detailed discussion of the primary focus of this thesis, the relationship between the incidence of T1D and exposure to nitrate, nitrite and nitrosamines from injested food and water sources. This chapter ends with a description of the subsequent chapters in the thesis.

1.2. Diabetes mellitus

Diabetes mellitus is thought to have been ancient in origin. Writings from 1550 BC report polyuria, polydipsia and fatigue (all considered to be symptoms of diabetes) among the wealthy. The ailment encountered was also described as incurable and the afflicted had sweet-tasting urine. The Greeks named the disorder diabetes mellitus, with diabetes meaning 'copious urination' and mellitus meaning 'honey' due to the sugar taste in the urine (Brink, 1987). In 1889, Oskar Minkowski and Joseph Von Mering from Strasburg, discovered that the disease was somehow caused by a disorder of the pancreas (Brothers, 1976). It was later known that it was the destruction of the pancreatic β-cells, and therefore disturbance of insulin production that lead to this chronic disorder.

Diabetes mellitus is an affliction in which the level of fasting blood glucose is consistently raised above the normal value of 7 mmol L^{-1} before a meal or over 11.1 mmol L^{-1} two hours after a meal (Meltzer et al., 1998; Canadian Diabetes Association, 2003). Hyperglycemia can occur because of a lack of insulin in the body or because of the presence of factors that prohibit the action of insulin. Without insulin function, the body cannot utilize glucose, the principal energy source, so blood sugar levels increase and fat becomes the primary intracellular energy source (Campbell, 1996). There are two main forms of diabetes mellitus: T1D and T2D. Other types of diabetes include gestational diabetes, maturity-onset diabetes in the young, and latent autoimmune diabetes in adults, but these are very uncommon and will not be discussed in this thesis.

1.2.1. Type 1 and type 2 diabetes mellitus

T1D is an autoimmune disease primarily developed during childhood, and is characterized by the self-destruction of the insulin-producing pancreatic β-cells located in the islets of Langerhans. T-lymphocytes penetrate the islets and destroy the β-cells. T1D is thought to be completely asymptomatic until at least 80% of the β-cells are destroyed (Tuch et al., 2000). The β-cells are destroyed either by genetic mutation, or damage to the cell walls. Individuals with T1D rely on exogenous injected insulin for normal growth and development (Canadian Diabetes Association, 2001). The latent period for T1D is not clearly defined, but evidence suggests that it can be from a few months (Dahlquist et al., 1990; Dahlquist et al., 1991; Pundziūtė-Lyckå et al., 2004) to several years (Helgason et al., 1984; EURODIAB Substudy 2 Study Group, 2000; EURODIAB Substudy 2 Study Group, 2002).

Life expectancy of a person with T1D is expected to be reduced by a minimum of fifteen years, and some Canadian life insurance records have shown that those diagnosed before the age of fifteen have a life expectancy shortened by up to twenty-seven years (Health Canada, 2002). T1D can lead to a number of complications and other diseases, especially if not effectively monitored and controlled. The most common complications which lead to death, are renal and heart disease. Other complications, which may not ultimately result in death, include hypoglycemia, diabetic ketoacidosis, blindness, foot ulcers (which can lead to amputation), strokes, and neuropathy (inflammation and degeneration of peripheral nerves) (Harris, 1995). In developed countries, T1D patients have an increased risk of premature death compared to the unaffected population (8-10 times higher). Conversely, in developing countries, those with T1D usually die within a few years of diagnosis due to a lack of financial support and medical expertise for disease control (WHO DIAMOND Project Group, 1990).

Conversely, T2D occurs most frequently in adults, although increasing numbers of children in high-risk populations are being diagnosed (Botero et al., 2005). T2D occurs when the pancreas is not producing enough insulin or when the body does not effectively regulate and use the insulin produced. There is clearly a genetic role in the occurrence of T2D but the environment does play a significant part (Watkins et al., 1996). Type 2 diabetes is considered less serious than T1D, and can initially be controlled by regulated exercise and a healthy specialized diet to control body weight. The most important risk factor in the development of T2D is obesity. Other risk factors include: older age, family history of diabetes, diet, alcohol consumption, physical inactivity, lower socioeconomic status, and ethnicity (such as aboriginal populations) (Rewers and Hamman, 1995). Oral

medication is often needed as the disease progresses and blood glucose becomes difficult to control, sometimes leading to the need for insulin injections.

1.2.2. Worldwide type 1 diabetes incidence

Type 1 diabetes is the second most prevalent childhood chronic disease after asthma (World Health Organization, 2006). It affects 0.5-1% of the total population during a life-time, causing approximately 10% of all diabetic cases (Rewers and Klingensmith, 1997), with the remainder being primarily T2D. In 1990, the Multinational Project for Childhood Diabetes (DIAMOND) was set up by the World Health Organization (WHO) in order to monitor incidence patterns on an international scale (WHO DIAMOND Project Group, 1990). Using direct standardization (with the standard population consisting of equal numbers of children in each of the three subgroups [grouped by ages 0-4 years, 5-9 years, and 10-14 years]) results showed that the worldwide incidence for T1D in children ranged greatly from as low as 0.1/100,000 people per year in Zunyi, China and Caracas, Venezuela to 36.8/100,000 and 36.5/100,000 people per year in Sardinia and Finland, respectively (Karvonen et al., 2000).

On a slightly smaller scale, EURODIAB (EUROpe and DIABetes) was established prior to DIAMOND in 1988 to research the epidemiology and prevention of diabetes in Europe. By 1999, 44 centers across the continent were contributing data, representing thirty million children and most European nations. Again using direct standardization (for each of the three age groups [age 0-4 years, 5-9 years, and 10-14 years, and both sex groups]), the data showed that incidence was lowest in Northern

Greece (4.6/100,000 people per year) and highest in two regions in Finland (42.9/100,000 people per year) (Green et al., 1992). The epidemiological side of the project (EURODIAB TIGER) was primarily set up to investigate a possible south-north gradient, with the hypothesis being that the further north a region, the higher the incidence rate. The results indicated that the gradient was very weak, as southern incidence data were higher than expected, and Sardinia had an incidence level comparable to that of Finland, yet Sardinia is 3,000km south of Finland (Green et al., 1992). Kuwait, a country not in the EURODIAB Study, also had surprisingly higher incidence rates than expected (Shaltout et al., 1995).

Incidence rates are typically very low in South America, Asia, and Africa, for example 4.9/100,000, 1.4/100,000, and 8.0/100,000 people per year in Monastir, Tunisia, Chiba, Japan, and São Paulo, Brazil, respectively. The highest rates seem to be in North America and Europe (Karvonen et al., 2000). However, there could be a large amount of underreporting in less developed countries that have inadequate medical systems for diagnosis and reporting. Alternatively, there may truly be an ethnic predisposition among non-Latino and non-Asian populations. Or, the variation in incidence rates could be due to different environmental conditions.

Incidence rates across regions within countries also vary considerably, for example in Italy and the United Kingdom, ranging from 6.5 to 32.6/100,000 people per year, and from 6.2 to 24.1/100,000 people per year, respectively (Karvonen et al., 2000). In Canada, the estimated incidence rates for Alberta and Prince Edward Island (PEI) were 24.0/100,000 and 24.5/100,000 people per year, respectively. These were the highest rates found in Canada (Karvonen et al., 2000), until a study in the Avalon Peninsula,

Newfoundland, estimated an incidence rate of 35.9/100,000 people per year (Newhook et al., 2004). In contrast, Montreal had an incidence rate of 9.2/100,000 people per year (Tan and MacLean, 1995).

Incidence rates depend somewhat on sex and age of the population. Typically, males are more frequently diagnosed than females (Green et al., 1992), especially in high incidence countries, and 10-14 year olds appear to be more frequently diagnosed than any other age group (Kostraba et al., 1992a). However, the incidence in younger children is gradually increasing (Atkinson and Eisenbarth, 2001). T1D incidence appears to be rising globally by approximately 3% per year (Onkamo et al., 1999).

There is currently no substantial evidence for why these diverse global variations and trends occur, but there are many theories indicating that the development of T1D depends on a combination of genetic and environmental factors.

1.2.3. Type 1 diabetes among the population of PEI

In PEI, a Diabetes Registry with the Provincial Department of Health and Social Services has existed since 1962. It is considered a reliable source of T1D incidence in the province because those who register receive government funding for medications and urine testing kits. Thus, everyone who has T1D will almost certainly be on this drug program and therefore registered (Tan et al., 1983). In order to be on the registry, a Doctor's diagnosis of T1D and a prescription for insulin is required. During 1997-1999, it was estimated that there were 15-20 new T1D cases per year and 220 cases of diabetes mellitus annually. Diagnosis of T1D in PEI is conducted according to the Canadian

Diabetes Association guidelines (Meltzer et al., 1998; Canadian Diabetes Association, 2003).

From 1966 to 1980 the prevalence of T1D cases steadily increased from 0.87% to 1.95% per year in PEI (Tan et al., 1983). Current incidence rates in PEI will be determined in this thesis, based on the data in the Diabetes Registry in PEI, and population statistics from Statistics Canada.

Over recent years, the population of PEI has been gradually increasing by approximately 0.2% per year, with the most recent population count at 138,100 (Statistics Canada, 2005). The island population is fairly static with regards to both those moving to and from the island, and movement within the island. For example, in 2001, 68% of the population resided at the same address that they lived in for the previous five years, compared to 58% in Canada and only 44% and 46% for the Northwest Territories and Nunavut, respectively (Statistics Canada, 2002). In addition, less than 1% of the population of PEI lived outside Canada for the same five years, compared with 5% for Canada as a whole, and 5% in Ontario and British Columbia (Statistics Canada, 2002). The stable population of PEI provides advantages for studying the relationship between nitrate exposure and T1D.

1.3. T1D risk factors

The following risk factors presented in this section are also shown in a causal diagram to help explain when the factors may play a role in the development of T1D, and how they may be related to each other (Figure 1-1).

1.3.1. Genetic risk factors

Although T1D may not be directly inherited, people with certain human leukocyte antigen (HLA) genes often show increased susceptibility to the disease (Watkins, 1993). The HLA genes are located in the major histocompatibility complex on the short arm of chromosome 6. The genes most closely associated with T1D susceptibility were first thought to be the Class I molecules, B8 and B15. More recently, a stronger association was discovered with the Class II DR3 and/or DR4 antigens, which are in linkage disequilibrium with B8 and B15, respectively (Thorsby and Ronningen, 1993; Nerup et al., 1994). Although DR3 and DR4 appear in 95% of Caucasian T1D patients, they can not be regarded as primary susceptibility determinants because they can also be found in up to 60% of non-T1D Caucasians. Therefore, it was proposed in 1986 that DR-associated susceptibility might be DQ-encoded (Nepom et al., 1986). This was demonstrated in a Caucasian DR4-positive population case-control study where it was discovered that the diabetic population possessed the DQB1*0302 allele 90% of the time, and the DQB1*0301 allele 10% of the time. Whereas, for the DR4-positive control population both alleles were present in equal proportions (Nepom et al., 1986). Thus suggesting that DQB1*0302 is a requirement for the disease to evolve in many T1D Caucasian patients. This was not confirmed in all ethnic groups, but it does demonstrate DR dominance. It has been demonstrated that T1D is most strongly associated with the HLA-DQA1 and DQB1 genes (Todd et al., 1987; Thorsby and Ronningen, 1993).

In addition to DQ and DR genes being promoters, there are also protective DQ gene associations involving the DR15 (a split from DR2). It is thought that protection

from T1D comes from the DQA1 locus. This has been consistently found in studies carried out on various ethnic groups (Cavan and Barnett, 1993; Cavan et al., 1993).

Genetic susceptibility appears to be a prerequisite in most but perhaps not all cases, as approximately 5% of all Caucasian T1D patients in most populations do not possess the DQ-encoded DR3 and/or DR4 class II antigens, therefore suggesting an involvement of other factors in the development of T1D (Cavan and Barnett, 1993).

1.3.2. Environmental risk factors

Epidemiological studies have shown higher T1D incidence rates in the Caucasian population when compared to African Americans or Hispanics, an increased risk at puberty, and more cases diagnosed during the winter than the summer months. As a result, viruses, nutrition, toxins, and/or socioeconomic factors may play an important role in the development of T1D. Although many possible environmental factors have been proposed using both animal models and human populations, results are still inconclusive (Dorman et al., 1995; Åkerblom and Knip, 2002). The main putative environmental risk factors are briefly discussed below, ending with an overview of nitrate, nitrite and nitrosamine compounds.

1.3.2.1. Seasonal patterns

Evidence of a seasonal pattern comes from the observation that cases seem to be diagnosed more frequently in the colder months. In Finland and Sweden, the lowest incidence of T1D was found in May, with the highest incidence reported in September, with a constant incidence rate throughout autumn and winter. No distinct seasonal

patterns were found in Estonia, Latvia, or Lithuania; however in Estonia, for the 0-14 year old age group and both sexes combined, a significant seasonal trend was evident, with the lowest incidence in May (Padaiga et al., 1999).

In Spain, the highest number of cases were diagnosed during autumn and winter (Lora-Gomez et al., 2005). In another study, both Finland and Sardinia showed a general increase in the autumn months and a decrease in incidence during the spring and summer months (Karvonen et al., 1998). Incidence in Scotland followed a similar trend (Mooney et al., 2004). In Oxford, although the trend was similar, it was not significant (Bingley and Gale, 1989). However, only two years of data were used for the analysis. The general peaking of incidence during the autumn months is hypothesized to coincide with the annual peak incidence of enteroviruses spread, such as coxsackie viruses and echviruses, thus suggesting that these infections may promote T1D onset (Honeyman, 2005).

Month of birth is also hypothesised to be related to T1D incidence. A British study including data from three registries concluded that compared to the general population, T1D patients were more likely to be born in the spring and early summer, and less likely to be born in the winter (Rothwell et al., 1996). However a large study, encompassing 15,246 patients diagnosed with T1D under 15 years of age from 19 European regions, concluded that outside of Great Britain (England, Scotland, and Wales), no seasonality of birth was evident. Sex or age at diagnosis did not play a large role in the differences in seasonal patterns found (McKinney, 2001). No month of birth effect was noted from studies in Sweden (Samuelsson and Carstensen, 2003) or Japan (Kida et al., 2000).

1.3.2.2. Viral exposure

The actual role of viruses in the development of T1D is unknown, but they are thought to either attack the β-cell directly, or interfere with the development of the immune system so that the recognition of self and non-self becomes blurred (EURODIAB Substudy 2 Study Group, 2000). Viruses are thought to be involved in the aetiology of T1D due to an increased risk of T1D in the winter months when common viral infections are more frequent (Brink, 1987). Coxsackie B4 and cytomeglovirus have most frequently been associated with T1D incidence, but these findings are not consistent (Pak et al., 1988; D'Alessio, 1992). Other viruses believed to be related to T1D onset are retrovirus, congenital rubella syndrome, and mumps (Brink, 1987; Yoon, 1990; Yoon and Park, 1993; Åkerblom and Knip, 2002). Much of the research on viruses predisposing to T1D has been conducted on animals, but there are some human studies that have had similar findings (Brink, 1987; Yoon, 1990).

Vaccinations have been identified as a possible inhibitor of the development of T1D (Šipetić et al., 2003). However, a large European case-control study consisting of seven centers evaluated nine common vaccinations and found none to be significant factors of T1D risk (EURODIAB Substudy 2 Study Group, 2000). A similar study in Denmark found no significant effect of vaccinations on T1D incidence (Hviid et al., 2004).

1.3.2.3. Autoantibodies early in life

Four autoantibodies have been proposed to be associated with the development of T1D: antibodies to islet cells (ICA), insulin autoantibodies, autoantibodies to the 65kD

isoform of glutamic acid decarboxylase (GAD), and tyrosine phosphate (related to IA-2 molecule [IA-2A]) (Virtanen and Knip, 2003). In Florida, a prospective study enrolled 9,696 non-diabetic schoolchildren all of whom were screened for ICA. A total of 57 were ICA-positive, of which 10 developed T1D. None of the negative ICA participants developed T1D (Schatz et al., 1994). Blood samples from 4,505 healthy schoolchildren living in Washington State (ages 12-18 years old) were taken to evaluate autoantibody status, and 141 children had at least one of these autoantibodies present. After eight years, only those who had multiple autoantibodies (not including ICA) at the start of the study developed T1D, representing 100% sensitivity (LaGasse et al., 2002). One concern with the study was that the median study age (age 14) was reasonably high, as many of the children who were going to develop T1D may have done so already. It was proposed that with more autoantibodies present, there is a higher risk of developing of T1D.

1.3.2.4. The hygiene hypothesis

The hygiene hypothesis works on the basis that, in infancy, limited exposure to microbial infections may elevate the risk of T1D (Parslow et al., 2001). Alternatively, it has been hypothesised that something protective in the infant's environment has been eliminated during the last fifty years (Gale, 2002). Like the brain, the immune system can only learn through experience and exposure to the environment early in life (Rook and Stanford, 1998).

The hygiene hypothesis has also been linked with childhood asthma. Both T1D and asthma are thought to occur due to lack of 'dirt' in the environment. Although both reside under the 'hygiene hypothesis', asthma is caused by an excess of Type 2 helper

cells (Th2) in the immune response, whereas T1D is caused by an excess of Type 1 helper cells (Th1). 'Normality' is considered to be when there is an equal balance of Th1 and Th2 cells. It is therefore uncommon for a child to have both asthma and T1D, however from a global perspective, both incidence rates are increasing rapidly, especially in the developed countries (Wills-Karp et al., 2001).

Animal models using the nonobese diabetic (NOD) mouse have demonstrated that diabetes is increasingly likely to occur when the mouse is housed in a sterile environment. This is perhaps because rodent pinworms (common laboratory parasites) are thought to strongly protect against the development of diabetes (Pozzilli et al., 1993). Helminths have been referred to as 'old friends' (Rook and Stanford, 1998), because the human immune system has learnt not to over-react to their presence by generating regulatory T-cells to aid in this self-suppression. In the absence of helminths and other pathogenic microbes, the helper cells may spiral out of control and cause either asthma or T1D, depending on such factors as genetic predisposition (Watts, 2004).

Human studies have shown that social contact among children at an early age helps to protect against T1D. A dose-response relationship with T1D and the number of children a child contacts was evident in a case-control study in Yorkshire, UK (McKinney et al., 2000). It was hypothesized that an increase in T1D could be due to the lack of 'germs' picked up from child to child to help boost the immune system. A meta-analysis of six case-control studies assessing the effect of daycare showed mixed results (Kaila and Taback, 2001). However, in two studies, daycare before one year of age did appear to be protective for T1D in children (Blom et al., 1989; McKinney et al., 2000).

1.3.2.5. Rapid early growth

The EURODIAB Substudy 2 Study Group evaluated early rapid growth using prospective information collected from five European centers (Vienna, Austria; Latvia; Lithuania; Luxemburg; United Kingdom, Northern Ireland) totaling 499 patients and 1,337 controls. Before diagnosis, from birth until six years of age, weight was significantly higher among cases than controls. Before they were diagnosed, patients were also significantly taller than controls after age one. The Body Mass Index was only significantly associated with T1D between 1-4 years of age, with cases prior to diagnosis scoring higher than controls. Differences in Body Mass Index between cases and controls were similar for boys and girls, but boys tended to have a larger difference than girls (EURODIAB Substudy 2 Study Group, 2002).

A Finnish study assessed early weight gain during the first year of life, and concluded that birth weight did not differ between patients and controls, but girls who later developed T1D grew faster than control girls between one and seven months of age (Hyppönen et al., 1999). The authors stated that these differences were not related to infant diet. In a subsequent Finnish study, it was also concluded that type 1 diabetic patients were consistently taller than controls during infancy and early childhood, and no difference was observed for birth measurements (Hyppönen et al., 2000). Birth weight in Norway was also found to be positively and linearly associated with T1D incidence after confounding factors such as gestational age, parity, and maternal age at delivery were controlled for (Stene et al., 2001).

1.3.2.6. Social status

The Townsend score is the most common method of assessing the level of deprivation, with higher scores equal to greater deprivation. The score is a combination of four factors; i) unemployment, ii) housing tenure, iii) household overcrowding, and iv) access to a car (Phillimore et al., 1994). Several studies determined that the Townsend score was significantly and inversely associated with the risk of developing T1D (Crow et al., 1991; Patterson et al., 1996; Staines et al., 1997; Parslow et al., 2001). An earlier study conducted in Montreal, Canada, noted that higher household income was associated with higher T1D incidence (Collé et al., 1981), and a study assessing T1D prevalence noted no association with economic deprivation (Evans et al., 2000).

At the country level, it was proposed that a wealthier life-style could influence normal growth and development, increasing the risk of T1D (Patterson et al., 2001). Thirty-four European countries with a total of 16,362 incident cases enrolled during 1989-94 were used for analysis. Results showed that national prosperity indicators could explain a significant proportion of the large variability in incidence rate ratios of T1D across Europe. Gross domestic product, liquid milk and coffee consumption, life expectancy, and latitude had significant and positive correlations with T1D incidence, and infant mortality rate was the only negative and significantly correlated indicator (Patterson et al., 2001).

1.3.2.7. Toxins

Chemical toxins may be a potential risk factor to T1D through a variety of mechanisms. These can be either by direct poisoning to the β-cells, or by triggering an

autoimmune process directed to the islets. Toxins have only been demonstrated as risk factors in experimental animal models and have yet to be studied in humans (Assan and Larger, 1993). Such toxins include alloxan and streptozotocin (STZ).

1.3.2.7.1. Alloxan

Alloxan was the first diabetogenic toxin to be discovered, in Glasgow, Scotland, in 1942 (McLetchie, 2002). Its toxicity is dose-dependent, but actual concentrations required remain unclear as there are three kinds of alloxan used in diabetic studies and it is not always stated which alloxan was used. Like STZ (below), the drug alloxan is a naturally occurring compound commonly used for inducing diabetes in animal models because of its ability to selectively destroy the β-cells and induce impairment of islet glucose oxidation and of glucose-induced insulin secretion. Alloxan stimulates superoxide generation in the pancreatic cells, thereby causing oxidative stress and cell death (Zhao, 2001).

1.3.2.7.2. Streptozotocin

Streptozotocin is a widely recognized chemical toxin known to induce diabetes in mammals. It is a glucosamine-nitrosourea compound that is chemically related to nitrosamines and demonstrates selective cytotoxicity to the β-cells. When injected into adult rats, the chemical accumulates in the islets and causes DNA fragmentation. Nitric oxide generated by STZ is thought to be involved in damaging the cells, perhaps owing to nicotinamide adenine dinucleotide (NAD) depletion. Streptozotocin is also thought to cause oxidative stress which results in β-cell depletion (Assan and Larger, 1993; Li,

2001). A study was carried out in the 1960s looking at the diabetogenic action of STZ in beagles. It was found that when given intravenously to dogs at a dose of 50 mg kg^{-1} body weight, diabetes mellitus occurred. Diabetes mellitus was reproduced using the same dosage in albino rats (Rakieten et al., 1963). A single high dose of STZ (50-200 mg) administered to an adult rat destroyed the β-cells but did not lead to insulitis (inflammation of the islets of Langerhans). Multiple low-dose injections of 35-40 mg/kg per day for five days given to mice and rats resulted in complete cell destruction and gradual development of diabetes mellitus (Assan and Larger, 1993; Dahlquist, 1995).

1.3.2.8. Diet

Typically, the incidence rate of T1D in Polynesians in Western Samoa is very low, but when they migrate to Australia or New Zealand where incidence is higher, their T1D incidence increases and matches that of the host country. One explanation for this is a noticeable dietary difference. In Western Samoa, milk and wheat are absent from the diet, whereas they play a major role in their host country's dietary intake, suggesting a link between T1D incidence and diet (Yoon, 1990). Dietary factors which have been linked to a decrease T1D incidence include: breast feeding, vitamins C, D, and E, and zinc. Introduction to cow's milk-based formula at an early age, complex carbohydrates, protein, and nitrate, nitrite, and N-nitrosamines have all been proposed as dietary factors that increase the risk of T1D.

1.3.2.8.1. Breast feeding and cow's milk protein

The most widely researched nutritional risk factors for T1D are breast-feeding and exposure to cow's milk protein in infants. In the 1980s, it was observed that breast-feeding was a protective factor against T1D incidence, with cow's milk being a promoter (Mayer et al., 1988). Although evidence is still conflicting (Kostraba et al., 1993; Šepetić et al., 2005; Malcova et al., 2006), it is thought that T1D develops in children who are introduced to cow's milk very early in life due to a short breast-feeding period. Many studies in various countries have shown that T1D incidence is highly correlated to the consumption of cow's milk protein (Mayer et al., 1988; Scott, 1990; Gerstein, 1994). The risk of T1D decreased in individuals who had a longer duration of breast-feeding. A meta-analysis of studies on breast-feeding or early exposure to cow's milk revealed that, compared to children without T1D, children with T1D were 43% more likely to have been breast-fed for less than three months from birth and 63% more likely to have been exposed to cow's milk before four months of age (Gerstein, 1994).

1.3.2.8.2. Vitamin E

The majority of the research assessing vitamin E's relation to T1D has been with animal models. Vitamin E is commonly found in nuts, fruits, and vegetables, and is considered an important free radical scavenger. Antioxidants protect cellular compounds from destruction or alteration by free radical reaction with a variety of biomolecules (e.g. lipids, carbohydrates, and proteins). This protection can be performed either by preventing formation of the free radicals, scavenging the free radicals, or promoting their decomposition. It has been proposed that oxygen-derived free radicals enhance the

autoimmune process of pancreatic β-cell destruction. Therefore, free radical scavengers such as antioxidants may play a protective role in the development of T1D (Dominguez et al., 1998). Vitamin E is also hypothesized to block the formation of nitroso compounds from dietary nitrate toxicity sources, a possible risk factor of T1D which will be discussed later.

A study assessing the role of vitamin E in the non-obese diabetic (NOD) mouse showed that although the incidence of diabetes did not decrease over a 30 week period, the date of onset was significantly delayed (Beales et al., 1994). A delay in onset of diabetes in the Biobreeding (BB) rat was also demonstrated with increasing vitamin E intake (Behrens et al., 1986). In Wistar rats, the incubation of islet cells with α-tocopherol (vitamin E) significantly increased their resistance to nitric oxide (a cytotoxic compound known to destroy islet β-cells). In this study, vitamin C and other antioxidants offered no protection to the islet cells (Burkart et al., 1995).

The possible protective effect of vitamin E against T1D was first recognized in humans when antioxidants and oxidative stress status were assessed in 54 patients and 60 matched controls in 1998 (age range 2–24 years). Results showed that antioxidants may have provided a therapeutic role, in that islets were protected from oxidative stress, possibly preventing or delaying the development of T1D (Dominguez et al., 1998). A Finnish study enrolled 17,526 males and took a blood sample at the time of interview. After a 21 year follow-up, 19 cases of T1D were diagnosed, with an average age at diagnosis of 26 (range was 21–46 years) (Knekt et al., 1999). The study showed that vitamin E did play a protective role, as the serum alpha-tocopherol concentration at baseline was lower in cases than controls. However, due to the high average age at

diagnosis and small number of cases, a larger study on children is required to be able to confirm this relationship in a younger age cohort.

1.3.2.8.3. Vitamin D

Vitamin D is found in large quantities in foods such as fish and dairy products, but is more commonly synthesised by the body through direct exposure to sunlight. Both animal and human studies have shown that vitamin D deficiency reduces insulin secretion, and the active form of vitamin D (1,25-Dihydroxyvitamin D_3) improves β-cell function, thereby improving glucose tolerance. Studies on the NOD mouse showed that 1,25-Dihydroxyvitamin D_3 prevented the development of T1D (or insulitis) in mice due to its immunomodulatory properties (Mathieu et al., 1992; Mathieu et al., 1995). In 2004, another study using the NOD mouse showed that a vitamin D deficiency early in life led to a more aggressive presentation of T1D, with an earlier onset and a higher incidence of the disease (Giulietti et al., 2004).

In 1999, a case-control study encompassing seven European countries studied the correlation between vitamin D supplementation during the first year of life and the development of T1D. They noted that vitamin D supplementation contributed to a reduced risk of T1D (EURODIAB Substudy 2 Study Group, 1999). A Finnish study followed a birth cohort for 32 years, beginning in 1966, and also showed that vitamin D supplementation decreased the risk of T1D (Hyppönen et al., 2001). Maternal cod liver oil use (high in vitamin D) during pregnancy was also associated with a lower risk in the frequency of T1D in their children (Stene et al., 2000). The use of cod liver oil during the first year of life was also inversely associated with the development of T1D (Stene and

Joner, 2003). An alternative reason for the protective effect of cod liver oil could be its high n–3 fatty acid content; these fatty acids have anti-inflammatory properties (Stene and Joner, 2003) and are believed to play a role in the prevention of several chronic diseases (Simopoulos, 1999).

1.3.2.8.4. Vitamin C

Very little information is available on the association between vitamin C and the development of T1D. A Swedish case-control study assessing food frequency found that foods rich in nitrates or nitrites may be a risk factor for T1D, but high nitrate and nitrite containing foods that were also rich in vitamin C showed no significant effect on T1D incidence (Dahlquist et al., 1990). However, Burkart et al (1995) showed that vitamin C had no significant effect on β-cells.

1.3.2.8.5. Zinc

An experimental study showed that a zinc-fortified diet significantly decreased the severity of T1D in toxin-induced diabetic mice (Ho et al., 2001). Although some studies have shown that type 1 diabetics have a decreased zinc concentration in blood plasma (Hagglof et al., 1983), others have not (Kruse-Jarres and Rukgauer, 2000). A case-control study, using area data collected three years prior to diagnosis of T1D in Sweden, concluded that higher ground water zinc concentrations decreased the risk of developing T1D (Haglund et al., 1996). No zinc concentrations were given. A study conducted in Cornwall and Devon, England, demonstrated that the incidence rate of T1D was significantly lower when zinc concentrations in drinking water were in the range of 22.27-

27.00 μg L^{-1} (compared to 15.06-22.26 μg L^{-1}) (Zhao et al., 2001), and these concentrations are much lower than the recommended value of <5,000 μg L^{-1}. A more recent study in Finland enrolled 3,564 cases over a nine year period, and concluded that zinc in drinking water at the area level was not associated with the risk of T1D (Moltchanova et al., 2004). The authors speculated that this lack of significance could have been due to aggregation bias.

1.3.2.8.6. Carbohydrates and proteins

A Swedish case-control study demonstrated that there was a dose-response relationship between the frequency of intake of carbohydrate-rich foods and an increased risk in T1D. The study showed that complex carbohydrates rather than the monosaccharides or disaccharides were related to an increase in T1D incidence (Dahlquist et al., 1990). Another Swedish study compared a high daily intake (>75th percentile) to a low daily intake (<75th percentile) of carbohydrate and was in agreement that an increased carbohydrate intake, particularly disaccharides and sucrose in this instance, led to a higher risk of T1D (Pundziūtė-Lyckå et al., 2004).

It was also discovered in the Swedish study in 1990 that protein may also be the risk factor, as a large number of foods rich in carbohydrates are also rich in wheat gliadin (Dahlquist et al., 1990). In 1988, it was hypothesed that gliadin proteins were harmful to the β-cells in BB rats (Scott et al., 1988). A study conducted in the early 1990s investigated dietary soybean as a possible trigger for T1D. Soybean meal was fed to NOD mice as the only source of protein for a specific duration. The soybean diet was slightly diabetogenic but not enough to be significant when compared with a hydrolysed casein

(no protein) diet. The incidence of diabetes in the NOD mice fed soybean meal was 43% compared to 22% to those fed hydrolysed casein (Hoorfar et al., 1993). However, in more recent studies, when soybean meal was fed to rats and then the rats were injected with STZ, many of the β-cells were not destroyed compared to those fed a normal diet and then injected. Therefore, a soybean diet may protect the β-cells from destruction by the STZ and possibly other pancreatic β-cell toxins (Lee and Park, 2000). In animal models, it has been shown that a non-protein diet can largely prevent diabetes in the spontaneous diabetic BB rat (Scott et al., 1985) and in the NOD mouse (Elliott et al., 1988).

1.3.2.8.7. Nitrate, nitrite, and nitrosamines

Nitrate and nitrite are naturally occurring chemicals that are part of the nitrogen cycle. Nitrate is used widely in inorganic fertilizers, in explosives, and as a food preservative, and is also a human metabolite. Nitrate is considered essential to life, but only in moderation. Because nitrate is a relatively stable ion, the majority of all nitrogenous materials tend to be converted to nitrate. Therefore, many forms of nitrogen should be considered as potential sources of nitrates (Health Canada, 1992). Nitrate can be measured in both total nitrate and nitrate-nitrogen (NO_3-N) concentrations, with the conversion being 4.426 mg L^{-1} nitrate equalling 1 mg L^{-1} NO_3-N.

Nitrate is reduced to nitrite in the stomach, which in turn produces nitrosamines from a nitrosation reaction between nitrites and amines (L'Hirondel and L'Hirondel, 2002). The by-products from nitrate reduction, such as nitrite and nitrosamines have the potential to cause more biological harm than the original compound (Arms, 1994). Nitrite is used predominately as a food preservative, especially in cured meats. In infants, nitrite

is believed to induce methemoglobinaemia (blue-baby syndrome), a condition which results in red blood cells losing the ability to transport oxygen. In animal studies, nitrosamines are thought to be carcinogenic (L'Hirondel and L'Hirondel, 2002), but the risks to human health remain equivocal.

1.3.2.8.7.1. Waterborne nitrate

Excessive nitrate exposure is considered a human health risk, and limits to the acceptable concentrations in drinking water are based on the end point of methemoglobinemia in infants (Gelberg et al., 1999; Van Maanen et al., 2000). The maximum acceptable concentration (MAC) for nitrate in drinking water was set by the WHO at 50.00 mg L^{-1}. The European Union (EU) also set their nitrate concentration limit at 50.00 mg L^{-1} (Van Maanen et al., 2000). In Canada, Health Canada set the MACs for nitrate at 45.00 mg L^{-1} (equivalent to approximately 10.00 mg L^{-1} NO_3–N) (Health Canada, 1992).

Nitrate has been hypothesised by various authors to induce specific cancers (for example, gastric/intestinal, colonic, bladder) (Duncan et al., 1997) and T1D (Parslow et al., 1997), affect reproductive and developmental processes (Fan and Steinberg, 1996), and cause early onset of hypertension (L'Hirondel and L'Hirondel, 2002). However, to date, the epidemiological evidence to support these theories is considered inconclusive and has not been used to change the MACs (WHO, 2003). There is the belief, by some, that the nitrate MAC may be too high, because some cancers and T1D cases have been associated with nitrate contamination in drinking water at levels less than the MAC (Choi, 1985; Kostraba et al., 1992b; Parslow et al., 1997).

1.3.2.8.7.2. Waterborne nitrate and T1D

With regards to waterborne nitrate exposure and T1D, several conflicting ecological studies exist. Results of an ecological analysis in Colorado suggested that waterborne nitrate exposure may play a role in the aetiology of T1D, because a significantly increased risk of T1D was demonstrated at concentrations between 0.77 - 8.20 mg L^{-1} NO_3–N (compared to 0.0-0.084 mg L^{-1}) (Kostraba et al., 1992a). A significant association also was found between the incidence of T1D and nitrate concentrations greater than 3.3 mg L^{-1} NO_3-N in Yorkshire, England (OR = 1.27, CI = 1.09-1.48) (Parslow et al., 1997). However, in the Netherlands, there was no association between waterborne nitrate exposure and T1D incidence in children aged 0–14. A positive association was found with concentrations above 5.56 mg L^{-1}, but this was only applicable to 15 of the study participants (Van Maanen et al., 2000). In this instance, variation in waterborne nitrate exposure was limited and areas of high nitrate concentrations (>5.56 mg L^{-1}) were few in number.

A Finnish study assessing spatial variation of T1D determined that ground water nitrate concentrations were not significantly associated with the risk of developing T1D, however a slight positive trend with increasing concentration was noted (a 0.3% increase in T1D risk with the increase of 1 mg L^{-1} of NO_3) (Moltchanova et al., 2004). Nitrate and nitrite concentration in drinking water was assessed at the area level in Finland, and no difference was found between case and control families (Virtanen et al., 1994). Moreover, in Sardinia, an earlier study demonstrated no association between T1D incidence and drinking water nitrate concentrations (Casu et al., 2000), and a more recent study reported a significant inverse relationship (Quartile risk ratios = 1.0, 0.6, 0.5, 0.6) (Muntoni et al.,

2006). Drinking water nitrate concentration was borderline significant and inversely associated with T1D incidence in an ecological study conducted in the south-west of England (Zhao et al., 2001).

No individual level study on the relationship between T1D and waterborne nitrate exposure has been published to confirm or refute the results of these ecological studies.

1.3.2.8.7.3. Foodborne nitrate and T1D

A Finnish study enrolled 684 newly diagnosed cases during a 2.5 year time frame, and 595 date-of-birth- and sex-matched controls. A food frequency questionnaire, focusing on nitrate- and nitrite-containing foods, was completed by the child (or parent if the child was too young) and both the biological father and mother, in order to collect information pertaining to six months prior to diabetic symptoms (during the time of conception for the parents). The case children and their mothers consumed more nitrite-containing foods than control children and mothers. Nitrate intake was similar between case and control children, but control mothers consumed more than case mothers at the time of conception of the child that was enrolled in the study (Virtanen et al., 1994).

A Swedish study comprising of 339 cases and 528 controls matched on age, sex, and county of residence completed a mail-out questionnaire. A significant linear association with T1D incidence was evident for foods containing nitrosamines, and there was a significant non-linear association between T1D incidence and nitrate or nitrite intake, when controlling for other factors (Dahlquist et al., 1990). Dahlquist et al. (1990) also observed that nitrosamines had a promotive effect only when vitamin C was in low concentrations in the diet. A time frame for food consumption was not given, and no

assessment of nitrate in drinking water was taken into account. Foods with low concentrations of nitrate and nitrosamines, but frequently eaten (for example, potato), were also not assessed.

1.4. Nitrate, nitrite, and nitrosamines

1.4.1. Ground water nitrate contamination

Nitrate contamination of ground water is an escalating public health concern worldwide, especially where intensive agricultural practices are applied (Kacaroglu and Gunay, 1997; Gulis et al., 2002). Nitrate is water soluble and highly mobile in soil, and is able to migrate to the water table when present in excess concentrations. Although nitrate does occur naturally in the environment as a result of the activity of nitrogen-fixing bacteria, or generation in the atmosphere by high temperatures such as those found in lightning (Arms, 1994), these sources contribute only very small concentrations of nitrate to ground water. The background concentrations for nitrate in North American ground and surface waters are generally 1-2 ppm, measured from areas classified as a 'pristine' (little or no human impact) environment (Somers, 1992; Health Canada, 1992).

Nitrate contamination of ground and surface water are primarily linked to fertilizer or manure management, soil, crop type, and farming systems. Even if these processes are carried out according to recommended guidelines, contamination to surrounding water systems could occur due to locally intensive farming practices. Major sources of nitrate contamination of ground waters arise from animal and human wastes, nitrogen-based fertilizer, and to a lesser extent, industrial wastes, and landfills (Vidal et al., 2000). In PEI,

high nitrate concentrations are more closely related to potato production intensity than to livestock (MacLeod et al., 2002).

1.4.2. Human exposure to nitrate and nitrite

The human body is exposed to nitrate primarily from the dietary intake of food and water (Health Canada, 1992). Because nitrate is an essential plant nutrient, it is found as a natural component in all fruits and vegetables (Duncan et al., 1997). It is typically in high concentration in green vegetables (McKnight et al., 1999) and is used as a preservative in some meats (Helgason and Jonasson, 1981; Dahlquist, 1993) and for curing such meats as bacon, ham, and sausages (Ministry of Agriculture, 1987). It is also found in small quantities in fish and dairy products (Health Canada, 1992). The toxicity of nitrate to humans is thought to be due to its indirect effect *in vivo* with nitrosatable substrates to form nitrite and the N-nitroso compounds, as mentioned earlier (Chilvers et al., 1984; Harrison et al., 2000). Nitrites are typically found in the same foods as nitrates, although at a lower concentration.

With regards to external sources of nitrate, vegetables are believed to account for 80% of dietary nitrate intake in the USA and 60% in the UK. In a typical diet, drinking water normally accounts for 2-25% of the nitrate intake (providing the water is not contaminated). People who drink water low in nitrate and consume very few vegetables will have an intake of about 20-25 mg NO_3^- day^{-1}, whereas a vegetarian intake can be in the region of 280 mg NO_3^- day^{-1}. Acceptable daily intake is recommended at 3.7 mg NO_3^- kg^{-1} body weight day^{-1} (e.g. 185 mg per day^{-1} for a 50 kg individual), according to both the JEFCA (Joint Expert Committee on Food Additives, WHO) and the EU Scientific

Committee. The EU Scientific Committee and JEFCA set this latest recommendation after reviewing scientific papers in 1992 and 1995, respectively. In the USA (in 1990), the Environmental Protection Agency set the acceptable daily intake at 7.1 mg NO_3^- kg^{-1} body weight day^{-1} with consideration of the risk of methemoglobinaemia in infants (L'Hirondel and L'Hirondel, 2002). In Canada, the total average daily intake of nitrate from food and water is estimated to be 51 mg, of which, 44.3 mg is derived from food and 6.8 mg from drinking water with a nitrate concentration of 4.5 mg L^{-1}. This demonstrates that nitrate from water sources only contributes approximately 13% of an adult's total dietary intake, unless contamination has occurred (Health Canada, 1992).

If elevated levels of nitrate are apparent in the water, then the proportion of nitrate intake from drinking water would be significantly increased. For example, if the water nitrate concentration was 30 mg L^{-1}, then nitrate from the water would contribute over 50% of the total dietary intake. These calculations would vary depending on the type of food consumed by the individuals (Health Canada, 1992).

1.4.3. Metabolism of nitrate

When nitrate is ingested, it is rapidly absorbed from the upper small intestine (duodenum and jejunum) and stomach, and into the plasma to circulate around the body. It is then concentrated by a factor of ten into saliva and re-secreted into the upper intestinal tract. Approximately 25% of the dietary nitrate is recirculated into the saliva and 20% of that is reduced to nitrite by bacterial enzymes produced by the microbial flora found in the oral cavity (McKnight et al., 1999; L'Hirondel and L'Hirondel, 2002). This suggests that approximately 5% of the dietary intake of nitrate is found in the form of

salivary nitrite. The majority of the nitrite is then reingested and enters the blood stream where it is readily active in the oxidation of hemoglobin to methemoglobin (aiding in the inactivation of the oxygen transport system). Nitrite may also react with amines and amides in the stomach to form the genotoxic N-nitroso compounds, nitrosamines and nitrosamides (Van Maanen et al., 2000). It is these toxic N-nitroso compounds which have deleterious effects on a number of types of cells, including the pancreatic β-cells.

1.4.4. N-nitrosamines

The toxicity of N-nitroso compounds to the pancreatic β-cells has been confirmed in both animal and human studies. The mechanism of action is believed to be either by the generation of free radicals from the N-nitroso compounds, which damage the pancreatic β-cells (Kostraba et al., 1992a), or through the reduction of NAD^+ in the cells (Dahlquist, 1995). N-nitroso compounds can be found in foods, but they are also formed naturally by the reaction of a nitrosating agent (from nitrogen oxides or nitrite salts) and an amino substance (for example amines, amides or ureas to produce N-nitrosamines, N-nitrosamides or N-nitrosureas, respectively). This reaction can also occur in the stomach and oral cavity from dietary nitrates and nitrites in consumed food and drink (Ministry of Agriculture, 1987). Nitrosamines are not frequently found in vegetables, but are often found in processed and cured meats and cheeses. They are also found in some beers, fish, tobacco smoke, rubber, leather tanning, and cosmetics (Fine, 1982; Sen et al., 1996; Sen and Baddoo, 1997). The largest nitrosamine dietary contributor is believed to be beer, followed by fish, cured meat, and then cheese. As these volatile N-nitrosamines are

derived from nitrate, nitrate consumption also needs some consideration when looking at the pathogenesis of T1D with respect to nitrosamines.

In animal studies, diabetes was induced in the Chinese hamster using N-nitrosomethylurea at a dose of 50 mg kg^{-1} body weight (Wilander and Gunnarson, 1975). Then in 1981 in Iceland, it was first hypothesised that nitrosamines may cause T1D in humans. A high incidence of T1D in male offspring born in October was reported, and believed to have been caused by the consumption of cured meats during their mothers pregnancy (Helgason and Jonasson, 1981). It was proposed that dietary nitrosamine activity was promoted by testosterone, thereby targeting the male offspring. More recently, a case-control study in Belgrade determined that the mothers of cases consumed nitrosamine-containing foods more frequently during pregnancy, and were more frequently cigarette smokers (Šipetić et al., 2004).

A Swedish case-control study assessing dietary factors the year prior to diagnosis determined that nitrosamine-containing foods were more frequently consumed by cases than controls. The TID incidence was not attributable to the high concentrations of proteins typically found in nitrosamine-containing foods (Dahlquist et al., 1990).

There was no difference in consumption of meat products high in nitrosamines between cases and controls from a study conducted in Canada (Siemiatycki et al., 1989). Similar results were also found in an Australian study assessing dietary intake during the year prior to diagnosis (Verge et al., 1994). Nitrosamine intake in a Swedish study was also not associated with T1D development (Pundziūtė-Lyckå et al., 2004).

1.5. Study area

PEI is situated on Canada's East Coast and is the smallest province in the country (Figure 1-2). The island is approximately 5,700km^2 in size (Government of PEI, 2006) and currently has a population of almost 140,000 (Statistics Canada, 2005). The island's topography is characterized by gentle rolling hills reaching to a maximum height of 120 m above sea level.

The geology in the area is characterized by a Permo-Carboniferous redbed sequence (fractured sandstone bedrock) overlain by a thin layer of glacial till. This top layer is very porous and therefore makes an excellent aquifer for potable water extraction but it also allows the ground water to be prone to contamination, especially owing to the high water table in the province (Somers, 1992; PEI Dept. Environmental Resources, 1994).

The principle industrial sectors of PEI's economy are agriculture, fisheries, and tourism. Agriculture dominates by economically contributing to over a third more than either of the other two industries. For example, in 2001, agricultural products resulted in farm cash receipts of $336 million over the year for the province, while fisheries and tourism (May 1st to October 31st) brought in $216.1 million and $228.8 million, respectively (Department of the Provincial Treasury, 2002). As these industries coexist side by side, it is clear that any negative impact from one could have an effect on the others, often at a cost (Somers, 1992). For example, nitrate contamination that enters surface water may promote eutrophication and adversely affect fish, thus negatively affecting the fishing and tourism economy. The eutrophication could lead to a foul odour in the affected water systems which would decrease tourism from the visual and odorous

impairment, as well as a cessation of water-based activities. The agricultural industry may also be jeopardized, and in some instances causing adverse health effects in the young. However, the increase in nitrate concentration is beneficial for cropped land. From a residential perspective, the drinking water could become unsuitable for consumption with possible detrimental health effects, especially to newborns and infants fed formula made from tap water, if high nitrate concentrations were to continue for a lengthy period of time. Water treatment for nitrate is rarely used in municipal systems due to its high cost. However, for private water supplies, the water system can be treated with anion exchange or reverse osmosis in order to lower nitrate concentrations.

Prince Edward Island is the only Canadian province to rely 100% on ground water for its drinking water. It is therefore imperative to keep ground water contamination to a minimum, especially in areas of intensive agricultural use where fertilizers and chemicals are applied to the land in order to improve crop yield and reduce pest infestation (Bedeque Bay Environmental Management Association, 1997). Ground water discharge comprises approximately 70% of the annual stream flow, and therefore the majority of surface water is actually comprised of ground water (Somers, 1992).

Previous research conducted in PEI (Young et al., 2002) has determined that nitrate concentrations can vary substantially, both within and between watersheds. These concentrations are predominantly influenced by well construction, location of the watershed, and proximity to sources of nitrogen. In general, ground water nitrate concentrations are progressively increasing over time, but this is often dependent on the local land use.

1.6. Thesis objectives

1.6.1. Overall goals

The overall goal of this research was to determine the relationship between the intake of total dietary nitrate and development of T1D in children and young adults in PEI. Assessment of this relationship was conducted at two levels: i) spatial clustering of T1D incidence and waterborne nitrate contamination at the area level, and ii) individual exposure of T1D cases to dietary nitrate compared to randomly selected matched controls.

1.6.2. Specific objectives of the five research chapters

The objectives of Chapter 2 were to identify annual and/or seasonal/monthly trends in nitrate concentrations in PEI ground water, while accounting for land use, clustered data, and temporal autocorrelation where possible. If ground water nitrate concentrations in PEI have remained stable over time, averaging of nitrate values within areas across years would be permissible for the area level analyses in Chapter 4.

The objectives of Chapter 3 were: i) to determine what land uses have had a significant impact on nitrate concentrations in private well water samples across PEI, adjusting for spatial autocorrelation (i.e. neighbouring nitrate concentrations are more similar [positive autocorrelation] or dissimilar [negative autocorrelation] than expected); and ii) to determine the best spatial aggregation method for assessing these nitrate factors, balancing data scarcity problems with within unit homogeneity. The best spatial aggregation method will then be utilized for the area level analyses in Chapter 4.

The objectives of Chapter 4 were to determine if: i) the incidence of T1D was associated with ground water nitrate concentrations averaged at the watershed level in

PEI, adjusting for income; and, ii) the incidence of T1D is spatially clustered at the watershed level in PEI using Bayesian methodology.

The objective of Chapter 5 was to determine if there were any differences in the frequency of consumption of individual foods (especially foods containing nitrate, nitrite, and nitrosamines) and food groups between T1D patients during the year prior to diagnosis and two matched controls during the year prior to interview, adjusting for other environmental risk factors, where appropriate.

The objectives of Chapter 6 were to determine drinking water and dietary component factors associated with the risk of T1D, and specifically, to compare the consumption of nitrate from both food and water sources at the individual level in T1D and control populations, controlling for other risk factors where applicable.

Chapters 5 and 6 are linked because both chapters present results from the same case-control study, with Chapter 5 evaluating individual food and food group consumption, and Chapter 6 evaluating food and drinking water component consumption (e.g. nutrients). The final chapter, Chapter 7, summarizes the methods and results from each of the five research chapters, how findings from each chapter relate to each other, and suggestions for further work.

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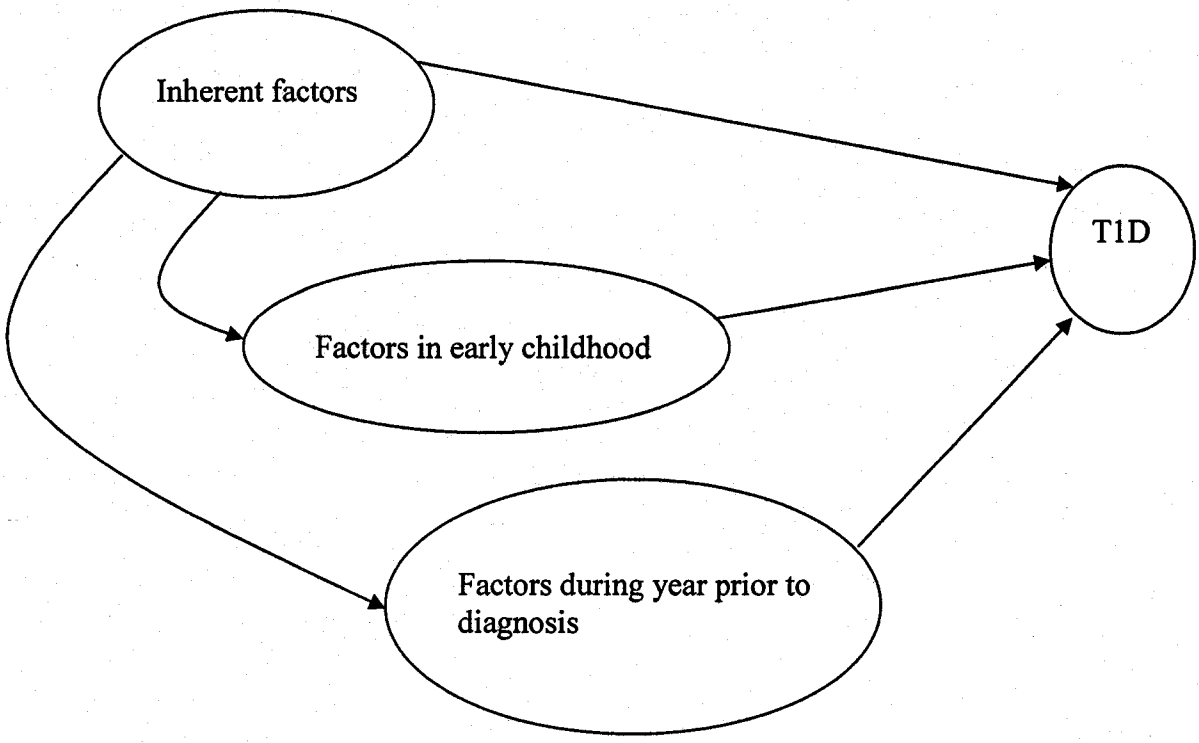
Figure 1-1 Possible causal diagrams of type 1 diabetes according to risk factors present in the literature review. Protective (-) and promotive (+) associations are shown in the diagrams.

Figure 1-1a A causal diagram of groups of factors thought to play a role in the development of type 1 diabetes.



* Not all inherent factors are possible confounders of factors acting in early childhood, or factors present during the year prior to diagnosis

Figure 1-1b A causal diagram of possible inherent risk factors of type 1 diabetes.

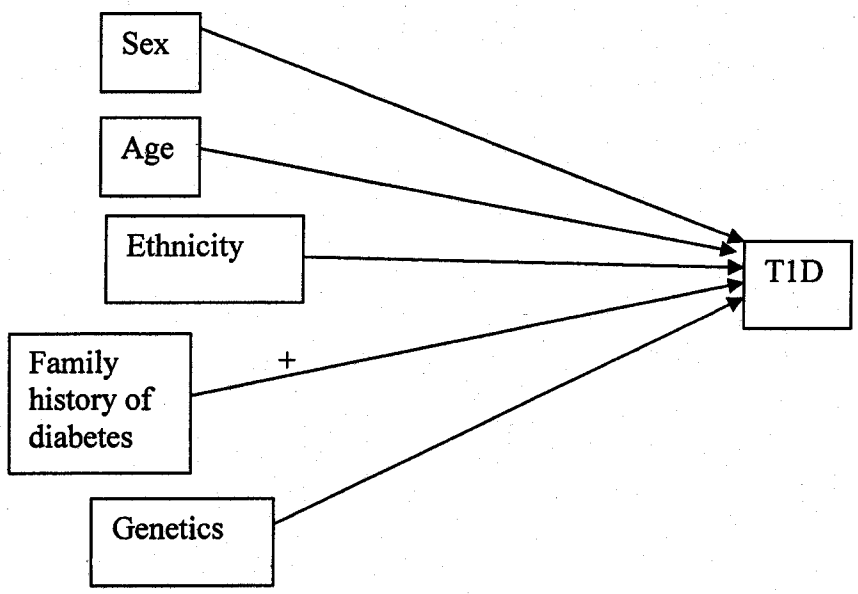


Figure 1-1c A causal diagram of possible risk factors of type 1 diabetes in early childhood

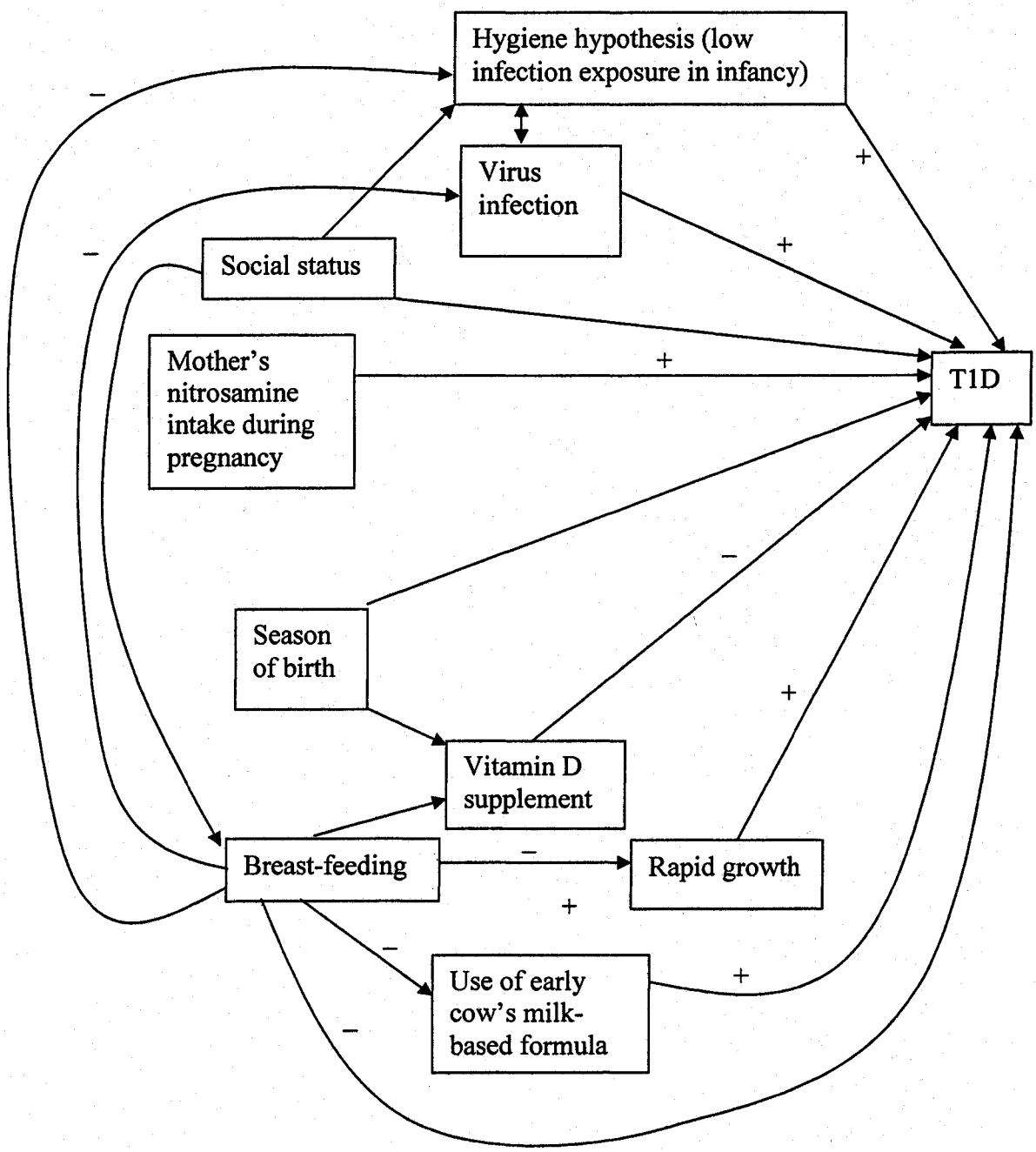


Figure 1-1d A causal diagram of possible risk factors of type 1 diabetes during the year prior to diagnosis.

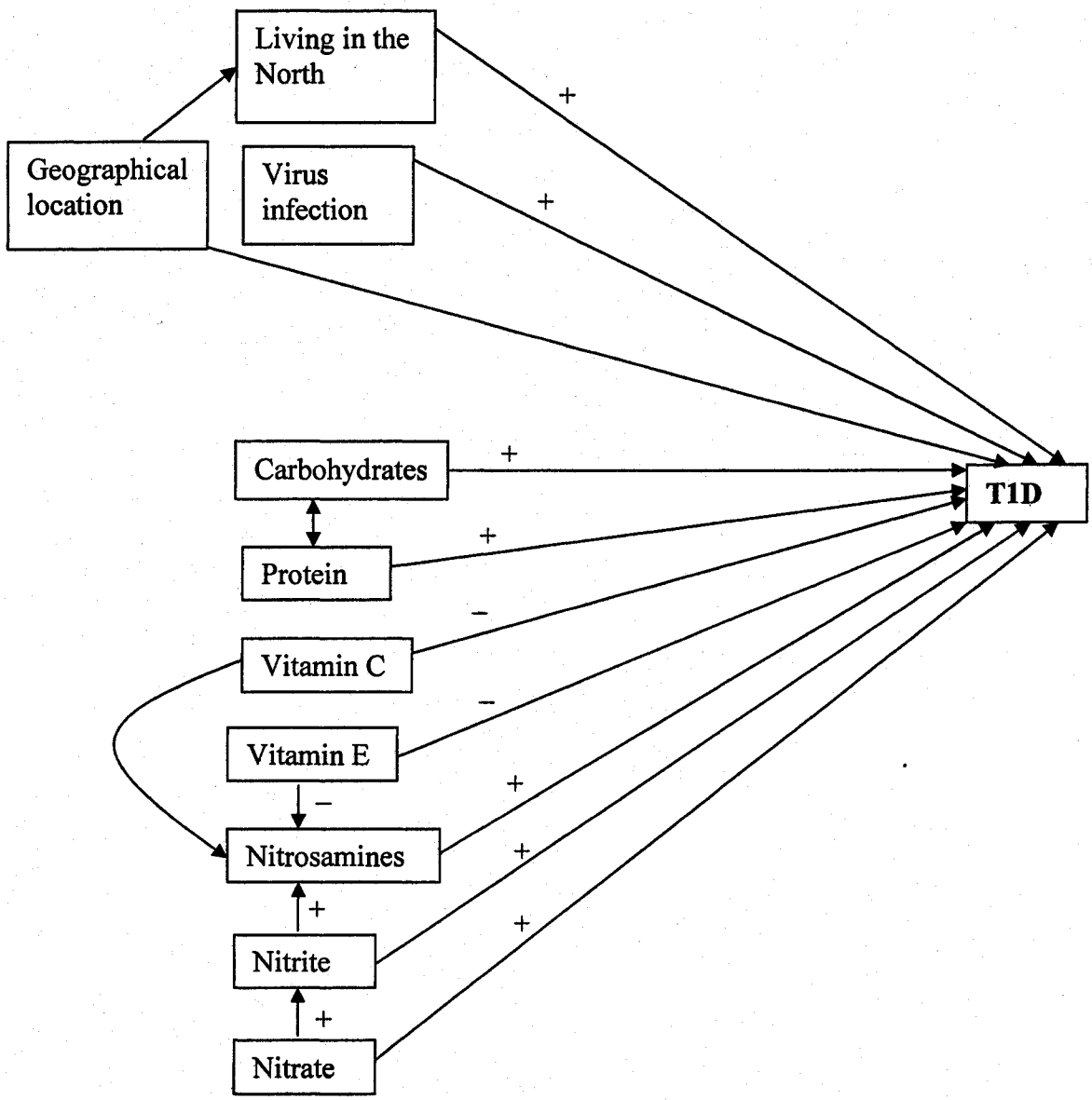
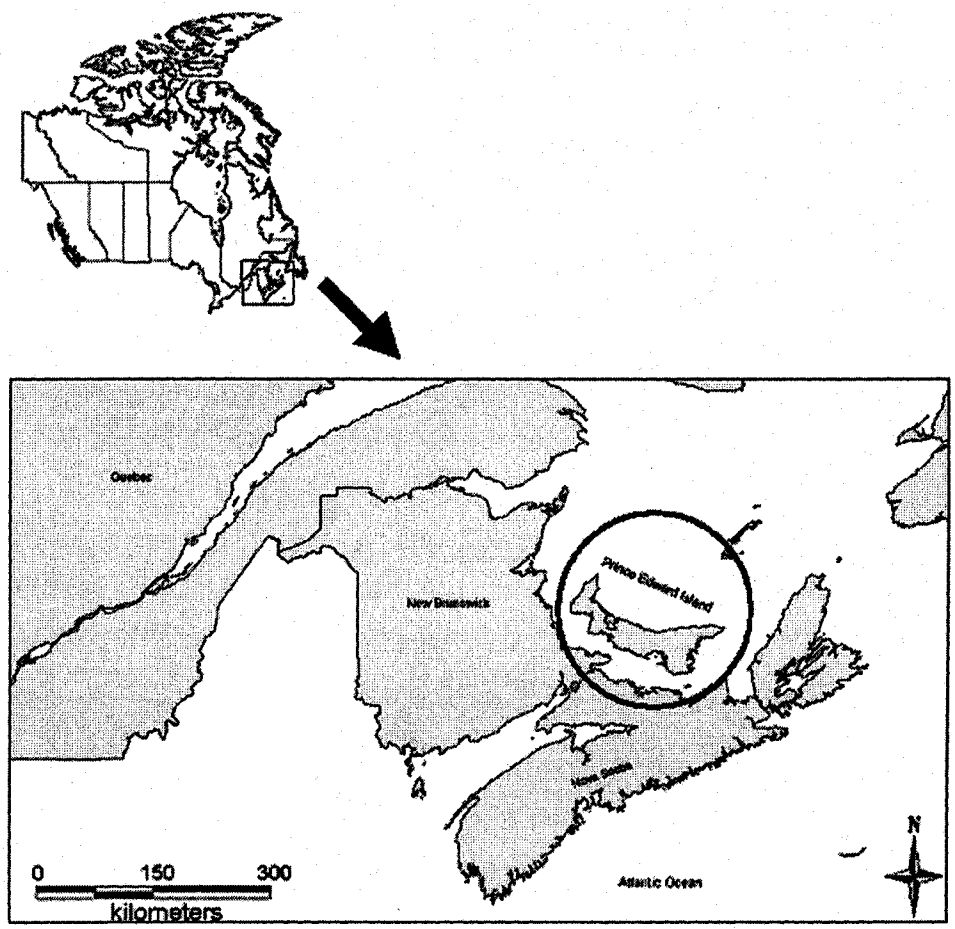


Figure 1-2 Location of study area, Prince Edward Island, Canada.



Created in MapInfo Professional 7.0 (2002)

Authors: Benson VS, VanLeeuwen JA, Stryhn H, Somers GS. Hydrogeology Journal. Accepted.

Chapter 2 Temporal analysis of ground water nitrate concentrations at the well level

2.1. Abstract

This analysis of secondary data on ground water nitrate concentrations in Prince Edward Island (PEI) indicated that in general, nitrate pollution was not a serious problem in drinking water and was not significantly increasing over time. Temporal trends in ground water nitrate concentrations in PEI were assessed annually during 1981–1996 (1,299 observations), and both seasonally and monthly during 1988–1991 (1,868 observations). All data were analyzed using linear mixed models with random effects and correlation structures. The average nitrate concentration in the monthly dataset was 4.00 mg L^{-1} NO_3-N (interquartile range = 1.90-5.20 mg L^{-1} NO_3-N), with levels in January, May, and November being higher ($p = 0.018$). There was only a seasonal effect when season was combined with land use type in an interaction term ($p = 0.004$). Wells located in agricultural areas had greater nitrate concentrations than urban areas, which in turn, had greater values than low human-impact areas. Row-cropped areas had higher ground water nitrate concentrations in the summer, whereas manure storage areas were higher in the spring and fall. Pristine areas and areas serviced by centralized sewage disposal remained relatively low and stable throughout the seasons. The average nitrate concentration in the annual dataset was 2.40 mg L^{-1} NO_3-N (interquartile range = 1.00-3.15 mg L^{-1} NO_3-N). There was no significant annual trend ($p = 0.95$), but for individual

sites, 9.6% significantly increased in nitrate concentration and 6.6% significantly decreased over time.

2.2. Introduction

Nitrate contamination of ground water is increasing in frequency and severity on a worldwide scale and is possibly the most widespread water contaminant in the world today (Gulis et al., 2002). It is an escalating public health concern, especially where intensive agricultural production is practiced. In North America and Western Europe, intensive agriculture is considered to be the main source of water pollution by nitrate (WHO, 2004). In Prince Edward Island (PEI), high nitrate concentrations appear to be more highly correlated with the use of inorganic fertilizers than manure inputs to the soils (Young et al., 2002). Unsewered urban areas may also play a substantial role in high nitrate concentrations in ground water (Barber et al., 1996).

Maximum acceptable concentrations (MAC) for nitrate in drinking water have been set at 50.00 mg L^{-1} NO_3 (equal to 11.11 mg L^{-1} NO_3-N) by the World Health Organization and 45.00 mg L^{-1} NO_3 (10.00 mg L^{-1} NO_3-N) in Canada and the United States. Hereafter, nitrate will refer to nitrate-nitrogen (NO_3-N). European Union guidelines recommended that concentrations should not exceed 5.56 mg L^{-1} NO_3-N, due to the possible health risks associated with nitrate exposure (Van Maanen et al., 2000). Elevated nitrate concentrations in drinking water have been associated with methemoglobinemia in newborns and infants less than six months of age. Links to other adverse health effects have also been proposed, but are inconclusive (Bukowski et al., 2001; Duncan et al., 1997; McKinney et al., 1997; Van Maanen et al., 2000).

North American background concentrations of nitrate are estimated to be no more than 3.00 mg L^{-1} (Spalding and Exner, 1993) in areas with relatively low human impact. In PEI, nitrate concentrations between 0.10-2.00 mg L^{-1} are thought to represent

background levels, with these values determined from low human-impact, 'pristine' watersheds (Young et al. 2002). Elevated nitrate concentrations in PEI are considered to be primarily due to agricultural practices, taking place on almost half of the island land area, with potato and livestock farming predominant (Somers, 1992).

Ground water in PEI is of particular importance not only because of the 100% reliance on this source as the drinking water supply, but because its discharge represents 60–70% of the island's surface water. Thus, protection of ground water quality is important not only for human and animal health but also for the environment due to concerns about eutrophication of surface water from nutrient enrichment. Because of the mixing of ground and surface waters, attempts to control surface water chemistry cannot be done if ground waters are left unmanaged.

Much of the scientific literature suggests that nitrate levels in ground water have been generally increasing over time, unless there has been a change in local land use practices (Somers, 1998; Trojan et al., 2003). In a recent analysis of ground water nitrate concentrations in PEI, land use type did influence nitrate concentrations, however, temporally, there was no significant annual variation (heterogeneity) within the five years of data analyzed (Chapter 4). Seasonal trends have also been evident in some studies, but, similar to annual trends, they typically depend on other factors, namely surrounding land use, ground water recharge rate, local climate, and well depth and construction (Maila et al., 2004; Scheytt, 1997; Somers, 1998). However, many of these studies have not investigated variable interactions, and have not examined these relationships while taking into account the hierarchical nature of the data (e.g. the clustering of months

within years, and years within sites), or the autocorrelation structure between months, seasons or years.

The objectives of this study were to identify annual and/or seasonal/monthly trends in nitrate concentrations in PEI ground water, while accounting for land use, clustering within the data, and temporal autocorrelation where possible. In order to reach this objective, two different datasets were used for the analyses – one assessing seasonal and monthly data over three years and one month, and the other assessing annual variation over a 16-year study period.

2.2.1. Study Area

PEI is an island located on the eastern coast of Canada, and is approximately 5,656 km^2 in area (Figure 1-2). The topography reaches a maximum height of 120 m above sea level, and is characterized by gentle rolling hills. The geology consists mainly of fractured sandstone bedrock overlain by a thin layer of fine sandy loam soils. As this top layer is very porous, it allows the ground water to be susceptible to contamination, especially considering the island's high water table and high ground water recharge rate (Government of PEI, 2004; InfoPEI, 2005; Somers, 1998).

An estimated 20,000 to 25,000 rural private wells supply water to over half of the population in PEI. The remainder of the population obtains drinking water from the same ground water source, but is serviced by central water supply systems (Young et al., 2002).

2.3. Material and methods

2.3.1. Data Acquisition

The data used to assess seasonal and monthly variation consisted of up to 37 water samples taken monthly from December 1988 to December 1991. These samples were taken from 54 different sites located across PEI and grouped by land use. The sites were originally selected to assess the effect that land use had on ground water nitrate concentrations, with nine wells in each of six land use categories. The land uses under study were 'pristine' areas (non-cropped), row cropped areas, non-row cropped areas, feedlot areas with on-site manure storage, subdivisions with on-site sewage disposal, and subdivisions with central sewage collection (Somers, 1998). Available data for each sample included site identification, nitrate concentration, land use type, month, and year. A total of 1,868 nitrate measurements were in the dataset (due to 130 missing values), for an average of 35 results per site.

For the assessment of annual ground water nitrate concentration variation, the data used were collected annually (where possible) over a 16-year period beginning in 1981. The data were originally collected as part of a routine island-wide survey assessing the drinking water chemistry of public institutions. The institutions were both privately and government-owned, and their water was supplied by a well located on the property. Institutions included schools, senior citizen homes, campgrounds, national parks, and many more. Available data for each sample included site identification, nitrate concentration, and year. No data were available for any of the sites during the year 1992. In total, 167 institutions were sampled during this time, collectively contributing 1,299 samples.

For both datasets, all samples were analyzed by flow injection analysis colorimetry (using the QuikChem Series 8000, FIA+) at the provincial laboratory of the Department of Environment, Energy and Forestry (Appendix A). The detection limit of nitrate was 0.10 mg L^{-1} N.

2.3.2. Statistical Analyses for Monthly/Seasonal Trends

A three level hierarchical model with linear mixed effects was created to assess possible monthly or seasonal effects on ground water nitrate concentrations. Each part of the model is described in turn. The top two levels accounted for clustering of years within sampling sites and clustering of monthly samples within years. The lowest level of the model represented the error term (month) of the nitrate observations. Due to limited data available within this dataset, only the following fixed effects on nitrate concentrations were investigated: land use type, year, season, and month, as well as an interaction between season and land use type. For this interaction, season was represented by averaging three months within each season. For example, the winter estimate was composed of averaging the January, February and March estimates.

Graphs of homoskedasticity (equal variance across all combinations of the predictors) and normality of the outcome variable, nitrate concentration, were assessed visually because statistical tests were not available in the software, and because it is recommended that statistical tests should only be used to supplement a graph, not represent the main assessment criteria (Dohoo et al., 2003). The top level of the hierarchy, site, was assessed first, followed by year, and then finally the residuals of the actual observations.

The natural logarithm transformation (after an addition of a constant of one to each nitrate value) was necessary to improve normality of the model residuals. This transformation also improved homoskedasticity. The residuals of the top two levels, site and year, remained normal when the log transformation of nitrate concentration was used. Without adding the constant (one), the log transformations of nitrate concentrations were not normally distributed. Variance components, explaining the proportion of the variation occurring at different levels of the hierarchy, were also assessed.

To determine if there was monthly or seasonal autocorrelation in nitrate concentrations in the above model, the residuals of one month were compared to the fitted values of the previous month in order to assess the time lags in the data. This was done using a correlation matrix in Stata 8 (Stata Corp, 2004). Autocorrelation was present between months, with a correlation value of 14% and 13% for months within one and two months of each other, respectively. Within the linear mixed model, various correlation structures were investigated to take into account the autocorrelation present, including: compound symmetry (CS), autoregressive (AR(1)), and autoregressive moving average (ARMA(1,1)). Autocorrelation was also present between seasons that were up to three lags apart, with percent correlation between the fitted values and residuals of 10.8%, 9.9%, and 6.7% for seasons one, two and three lags apart, respectively. Consequently, the same three correlation structures were also explored for seasonal autocorrelation in the following linear mixed effects regression modelling in order to improve the final model's goodness-of-fit.

Two random effects, site and year, were included in the linear mixed effects model. Model comparison of different fixed effects was assessed by likelihood-ratio tests

using the Maximum Likelihood estimation, whereas the Restricted Estimation Maximum Likelihood estimation was used for correlation matrix comparison (Venables and Ripley, 2002). The autocorrelation structure with the lowest Akaike's Information Criteria (AIC) was selected. The Bayesian Information Criteria (BIC) and -2 Log Likelihood Test were also examined for the selecting best autocorrelation structure. Interaction variables between main effects were created and investigated for their association with ground water nitrate concentrations.

To assist in understanding the relationships between nitrate concentrations and year, season, month, and land use type, the data were summarized by calculating and graphing expected nitrate concentrations by year, by season, by month, and by land use type. However, a constant of one was added to all nitrate values before they were transformed on to the natural logarithm scale, making a straightforward back-transformation impossible because the back-transformation of the effect of model coefficients is going from a multiplicative (log) to an additive (normal) scale, affecting the interpretation of that added constant of one. Therefore, expected nitrate concentrations, using estimated Least Square Means (LSM) and subtracting one, were used for the interpretative graphics. Least Square Means gave an expected value for each category within a categorical variable, with all other variables in the model being held at their mean values. The addition of one to each nitrate value also avoided the problem of working with negative log values (Rodvang et al., 2004).

2.3.3. Statistical Analyses for Annual Trends

Initially, each site was individually assessed to determine if a site increase or decrease of nitrate concentration was evident. Using a paired t-test, the average of the first three measurements from each site was compared to the average of the last three measurements to detect a significant difference. A total of 23 of the sites contained less than six measurements, so the first and last two measurements were compared.

A two level hierarchical model with linear mixed effects was created to assess possible annual effects on ground water nitrate concentrations. The top level accounted for clustering of years within sampling sites. The lowest level of the model represented the error term (year) of the nitrate observations. The residuals were again not normally distributed, and therefore nitrate, the outcome, was transformed to the natural logarithm scale (+1). Again, due to limited data within this dataset, only two variables were available for analyses, site as a random effect, and year as a fixed effect. Variance components explaining the proportion of the variation occurring at different levels of the hierarchy were again assessed.

To determine if there was annual autocorrelation in nitrate concentrations in the above model, the residuals of one year were compared to the fitted values of the previous year, using Stata 8 (Stata Corp, 2004). Autocorrelation was not present when assessing the lags, however, correlation structures were still investigated. The repeated measures correlation structures explored were the CS, AR(1) and ARMA(1,1) matrices. Model choice was achieved using AIC. The BIC and -2 Log Likelihood were also investigated for assessing model selection. Again, the data were summarized by calculating average

nitrate concentrations (using LSM), by year, in order to assist understanding the relationships between nitrate concentrations and year.

Model assumptions were assessed using the statistical package MLwiN (Beta version 2) (Rasbash et al., 2003), and all final analyses were carried out in SAS 8.02 (SAS Institute, 2001).

2.4. Results

2.4.1. Monthly/Seasonal Results

The mean and median nitrate values for all observations were 3.99 and 3.30 mg L^{-1}, respectively (interquartile range of 1.90 to 5.20 mg L^{-1}). The maximum nitrate concentration was 15.50 mg L^{-1}. By year, mean nitrate concentrations ranged from 3.86 mg L^{-1} in 1989 to 4.14 mg L^{-1} in 1990 (medians ranged from 2.80 to 3.50 mg L^{-1} for 1988 and 1990, respectively). When stratified by season, mean nitrate ranged from 3.95 mg L^{-1} in the spring to 4.03 mg L^{-1} in the summer (medians ranged from 3.20 mg L^{-1} for the summer, to 3.50 mg L^{-1} for the spring). There was also a small range of average nitrate values by month, with 3.80 mg L^{-1} in April to 4.08 mg L^{-1} in January (medians ranged from 3.10 to 3.55 mg L^{-1} for July and April, respectively). When nitrate concentrations were stratified by the six land uses, the means ranged from 1.17 mg L^{-1} for pristine areas to 6.49 mg L^{-1} for row crops (medians ranged from 1.20 to 6.00 mg L^{-1} for pristine and row crops, respectively). Finally, the mean nitrate concentrations stratified by the 54 sites ranged from 0.12 to 12.68 mg L^{-1} (medians ranged from 0.10 to 13.00 mg L^{-1}).

When month and season were assessed in separate models, autocorrelation between months or seasons was controlled, using an ARMA(1,1) correlation matrix. With

both season and month in the same model, the most appropriate correlation structure to fit the autocorrelation expressed in the data was again the ARMA(1,1) matrix, with an AIC of -1826. The other two structures, CS and AR(1), had higher AIC values (-1659 and -1820, respectively), and results from the BIC and –2 Log Likelihood Test confirmed the ARMA(1,1) as the preferred correlation structure.

With both season and month in the model, the main effects of land use type, year and month were significant factors affecting ground water nitrate concentrations in PEI (p-values of <0.0001, 0.011, and 0.018, respectively) (Table 2-1). Estimates and standard errors of nitrate concentrations for the different months were not shown in Table 2-1 because the table would be very large, and so are shown in Figure 2-1, using back-transformed LSM and then subtracting one.

Season was not significant ($p = 0.76$), however, the interaction term between season and land use was highly significant ($p = 0.004$), and therefore remained in the final model. With this interaction, land use and season should not be interpreted separately as the effect of one variable depends on the value of the other. Figure 2-2 demonstrates the land use and season interaction, using back-transformed estimated nitrate values (LSM-1). Nitrate concentrations were higher in fall and winter for locations with non-row crops and on-site sewage disposal, higher in spring and fall for locations with manure storage, and higher in summer for locations with row crops. Central sewage disposal and pristine land uses had little seasonal variation in nitrate concentrations.

Almost 92% of the variation of nitrate concentrations was between sites, with only 0.4% between years. The remainder of the variation was residual, unexplained variation between months within years.

2.4.2. Annual Results

The mean and median nitrate values for all observations were 2.40 mg L^{-1} and 1.90 mg L^{-1}, respectively, with an interquartile range of 1.00 to 3.15 mg L^{-1}. The maximum concentration was 14.00 mg L^{-1}. By year, mean nitrate concentrations ranged from 2.12 mg L^{-1} in 1987 to 2.73 mg L^{-1} in 1983 (medians ranged from 1.40 to 2.10 mg L^{-1} for 1981 and 1986, respectively). Individual site analysis showed that 9.6% of the sites had significantly increased and 6.6% significantly decreased three-year average nitrate concentrations at the $p < 0.05$ level, when comparing the first three years with the last three years of test results. For the 23 sites that did not have six years of data, no sites had significant differences in two-year average nitrate concentrations, when comparing the first two years with the last two years of test results.

The most appropriate correlation structure to fit the correlation expressed in the data was the ARMA (1,1) matrix, with an AIC of 370. The other two structures, CS and AR(1), had higher AIC values (524 and 388, respectively), and therefore fitted the data no better than the ARMA (1,1) matrix. Results from the BIC and –2 Log Likelihood confirmed the ARMA(1,1) as the most appropriate correlation structure.

With site as a random effect, a year effect across all sites was not significant (p = 0.95), as shown in Table 2-2. All years were compared to 1996, the reference value. Figure 2-3 shows estimated nitrate concentrations averaged across sites for each year, as estimated by back-transforming the LSM, and then subtracting one. Average annual nitrate concentrations in PEI remained relatively constant across the 16-year period of the study. The majority of the variance (55.2%) was between sites, with the remaining 44.8% residing as unexplained variation between years within sites (Table 2-2).

2.5. Discussion

The objective of this study was to determine if there were temporal trends in ground water nitrate concentrations in PEI. To determine yearly trends, appropriate annual water sampling and testing over a substantial number of years was required. Similarly, appropriate monthly sampling and testing was required for monthly trend determination. While these datasets were not ideal, in that there were substantial numbers of missing data in the datasets (48.6% and 6.5% for the yearly and monthly datasets, respectively) and limited other information on the sampled sites, the datasets were appropriate for determining the main goal of trend analyses.

Using up to 37 repeated measures of ground water nitrate concentrations collected from 54 wells in six different land uses in PEI, land use appeared to have a large effect on nitrate concentrations (Table 2-1). Areas influenced by substantial human activity had higher nitrate concentrations than pristine areas. However, the land use effect was dependent on season. Figure 2-2 demonstrates that agricultural areas had higher concentrations than wells close to sewered areas, while pristine areas had the lowest nitrate values. This observation could be because fertilizer is added to many of the cropped areas, especially to row-crops (potatoes). Non-row cropped areas typically require lower nitrate concentrations (Jacques Whitford Environment Limited, 2001), therefore lower nitrate values in local well water would be expected. Manure storage guidelines (Linkletter et al., 1999) are now in effect in order to limit effluent discharge, but these nitrate measurements were taken before implementation of these guidelines in PEI (1999 vs. sampling in 1988-91), confirming that manure storage sites were likely having an effect on the local drinking water. It was also expected that areas with septic

systems would produce greater ground water nitrate concentrations than areas with centralized sewage disposal systems because septic systems discharge 'on-site' while central sewer waste goes to treatment plants and is then discharged into a river or the ocean. The effect of land use on nitrate concentrations in PEI was also evident from a previous study (Chapter 3). Other studies outside of PEI have also shown an association between ground water nitrate concentrations and land use (Gardner and Vogel, 2005; Levallois et al., 1998; Trojan et al., 2003).

Seasonal effects were only significant once they were expressed in an interaction term with land use. Nitrate concentrations in the summer were higher for row-cropped areas, but lower for non-row crops and on-site sewage disposal areas. Potato crops in particular are much less efficient in nitrogen uptake than many other crops, such as grasses. This may also be because a lot of the fertilizer added to row crops will not be taken up by the crops, or held in the soil, and therefore will leach out of the soils and into the nearby water-ways. This is particularly true for row crops where there is more bare soil before, during, and after the growing season compared to hay or pasture crops that tend to hold many of the nutrients in the soil and protect the land from heavy rain that may encourage erosion and leaching. Central sewage disposal and pristine areas remained relatively constant over seasons, perhaps because contamination from these land uses is very infrequent. Nitrate concentrations from ground water near manure storage areas were higher in the spring and fall which is when the manure is typically being spread on the land and when the crops on those lands are not rapidly growing (pre-planting or post-harvest). Also, cattle are typically on pasture in the summer so a lower concentration of animals with be present in the barns. Freezing of manure in the winter may also reduce

leakage of nitrate into ground water, and frost in the ground may limit infiltration/discharge of nitrate to the underlying water table.

Seasonal effects on ground water nitrate concentrations have been observed in previous studies, but little information for monthly effects has been available. In the Gaza Strip, a seasonal effect was more noticeable in domestic wells than agricultural wells, however, only two samples were taken from each well, and just one year of data was used in the analyses (Maila et al., 2004). No seasonal effect was present in a large study conducted in Minnesota, but a slight year effect was evident, especially with a sudden land use change (Trojan et al., 2003). In another study, no overall seasonal trend was apparent, however, there were some individual wells where seasonal fluctuation in nitrate concentrations were evident (Burkart and Kolpin, 1993).

There was a significant temporal trend in the monthly dataset, both monthly and annually. However, graphically, the temporal trends, shown as least square means, were minor (Table 2-1 and Figure 2-1). No general increase or decrease was noted over time, nor was a sinusoidal pattern evident in the monthly data. Furthermore, when looking at the variance components, very little of the variation was explained by year, only 0.4%. The majority, 92%, was explained by the site itself, suggesting that land use was responsible for much of the variation between nitrate observations. Some other factor may be causing the random fluctuations seen in the temporal variables. Such unmeasured variables could be well depth, age and construction (Spalding and Exner, 1993), recharge rate or precipitation.

Using 16 years of data collected from 167 institutions in PEI, a year effect was not significant ($p = 0.95$), and autocorrelation between years was present. In this annual

dataset, average annual nitrate concentrations ranged from 1.93 mg L^{-1} in 1981 to 1.88 mg L^{-1} in 1996, but fluctuated between 1.73 and 1.96 mg L^{-1} (Figure 2-3). As no other variables were available for analysis, other factors affecting nitrate concentrations in ground water, such as land use, were not examined. Again, the majority of the variation was at the site level rather than in the residuals. Therefore, there was little evidence for an annual effect of ground water nitrate concentrations in PEI. A total of 55.2% of the random variation was at the site level, with 44.8% in the error term. When sites were individually assessed for a significant change over time, a total of 9.6% of the sites significantly increased and 6.6% significantly decreased. This suggests that site level factors such as local land use are perhaps more influential than province-wide factors.

Typically, the annual trend in ground water samples reported in other studies has been a general increase over time, but often this was dependent on a number of factors such as climate, surrounding land use, well depth and age, and recharge rates (Overgaard, 1984; Spalding and Exner, 1993; Trojan et al., 2003). In Denmark, one study showed that the overall mean concentration trebled over a 20-30 year period, with specific regional effects (Overgaard, 1984). Another study also noted a significant increase by 39% in nitrate concentrations over a six-year period (Rodvang et al., 2004).

A year effect was present within the three-year and one month dataset, but three years of data was less appropriate for assessing annual trends than our 16-year dataset. There was a slight increase in ground water nitrate concentrations during the period of 1988-91 within the annual dataset, perhaps explaining the significant annual effect in the monthly dataset, but this temporal trend was not consistent within the 16-year annual dataset, producing no significant overall annual effect.

2.6. Conclusion

In general, ground water nitrate concentrations in PEI appeared to be influenced more by short-term temporal changes, rather than yearly effects. In the dataset with monthly concentrations, a small monthly cyclic fluctuation over the three years was noted, but this pattern was not statistically significant when sine and cosine functions were added to the model. Land use greatly influenced nitrate concentrations, and this was dependent somewhat on the season. In general, agricultural land appeared to have greater nitrate concentrations than residential land, which in turn were higher than pristine (low human-impact) areas.

The annual dataset analysis showed that nitrate concentrations did fluctuate somewhat, but no specific increase or decrease was apparent for the dataset overall. At the individual site level, 9.6% of the individual sites significantly increased, and 6.6% significantly decreased over time. In general, nitrate values for the sampled institutions were low compared to the seasonal/monthly dataset and showed little variation, perhaps explaining why no specific yearly pattern was significant.

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Table 2-1 Random effects model analysis of the monthly and seasonal effect on ground water nitrate concentrations in Prince Edward Island, Canada (1988-1991).

Variable	Estimate	Standard Error [a]	*p*-value
Fixed effects[b]			
Month	-	-	0.018
Year			0.011
1988	2.97	0.15	
1989	3.10	0.14	
1990	3.25	0.14	
1991	3.16	0.14	
Land use type			<0.0001
Row-crop	5.84	0.37	
Non-row crop	3.51	0.37	
Manure storage	4.84	0.37	
On-site sewage disposal	3.35	0.37	
Central sewage disposal	2.24	0.37	
Pristine	0.92	0.37	
Land use & season interaction	-	-	0.004
Correlation parameters[c]			
Rho	0.55	0.066	
Gamma	0.37	0.031	
Random effects[d]			
Site	0.23	0.05	
Year (site)	0.001	0.0008	
Error	0.02	0.001	

[a] Standard error of estimate of variance component
[b] Least square means (LSM)
[c] ARMA(1,1) within-site correlation
[d] Variances of random effects

Table 2-2 Random effects model analysis of the annual effect on ground water nitrate concentrations in Prince Edward Island, Canada (1981-1996).

Variable	Estimate	Standard error[a]	*p*-value
Fixed effect			
Year	-		0.95
Correlation parameter[b]			
Rho	0.91	0.10	
Gamma	0.72	0.18	
Random effects[c]			
Site	0.16	0.088	
Error	0.13	0.086	

[a] Standard error of estimate of variance component
[b] ARMA(1,1) within-site correlation
[c] Variances of random effects

Figure 2-1 Average monthly ground water nitrate concentrations (including standard error bars) in Prince Edward Island, Canada (1988-1991), estimated from back-transformed least square means.

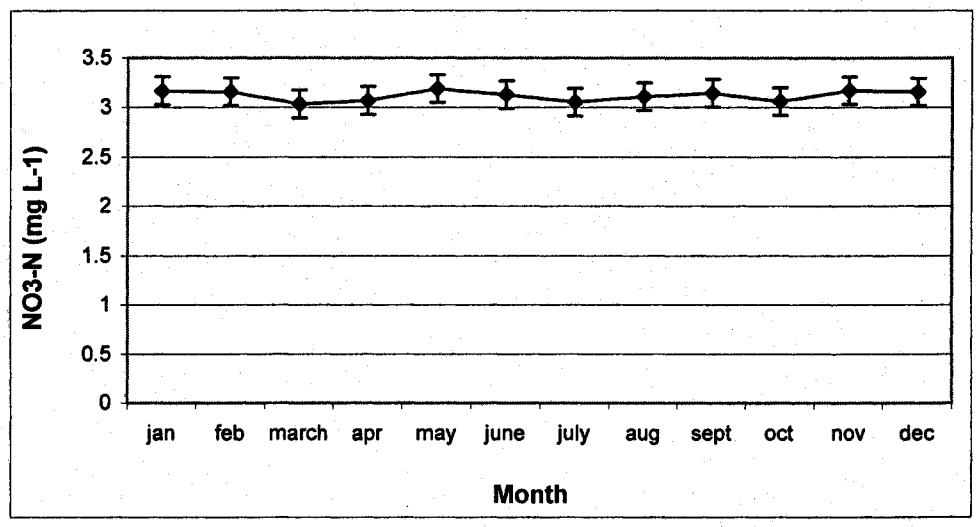


Figure 2-2 Average ground water nitrate concentrations (including standard error bars) stratified by land use type and season in Prince Edward Island, Canada (1988-1991), estimated from back-transformed least square means.

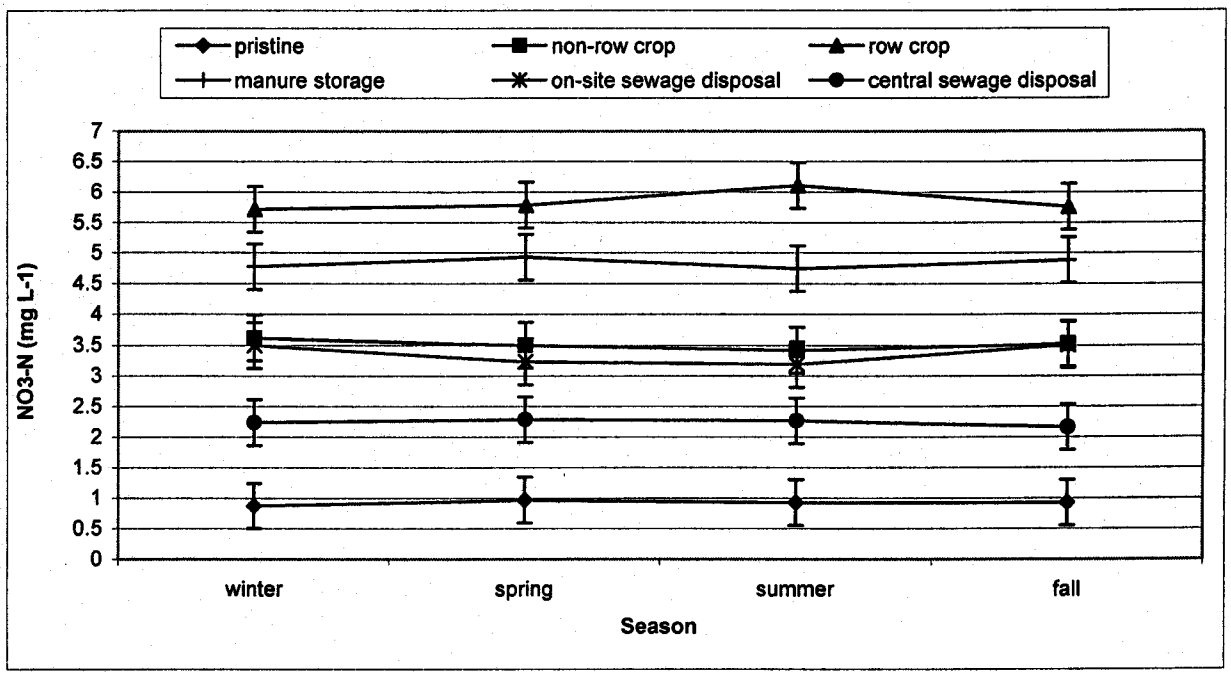
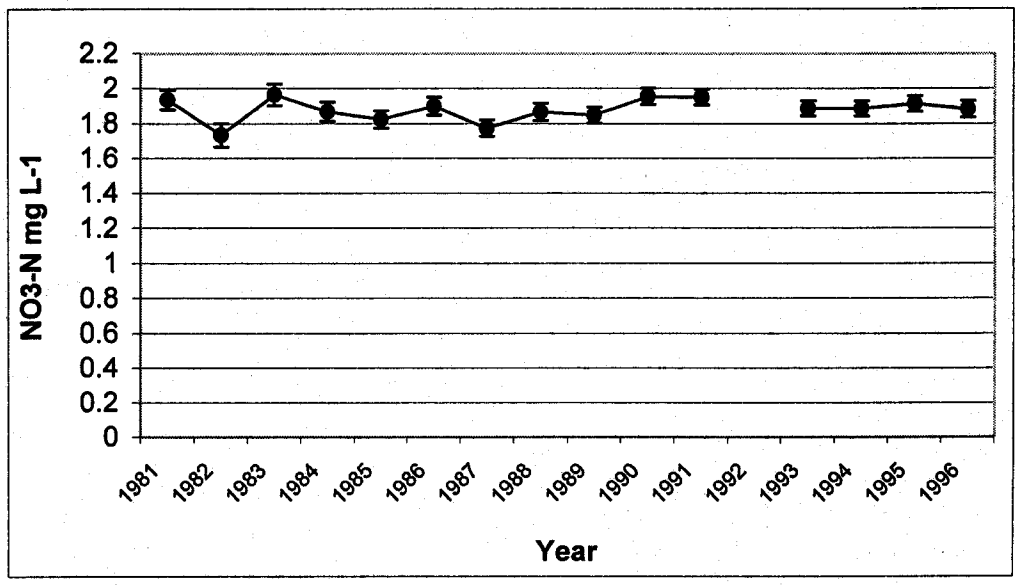


Figure 2-3 Annual ground water nitrate concentrations (including standard error bars) over a 16-year period in Prince Edward Island, Canada (1981-1996), estimated from back-transformed least square means.



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Chapter 3 Spatial analysis of land use impact on ground water nitrate concentrations

3.1. Abstract

In spatial analyses of causes or health effects of environmental pollutants, small units of analyses are usually preferred for internal environmental homogeneity reasons but can only be done when fine resolution data are available for most units. Objectives of this study were to determine which land use practices were spatially associated with ground water nitrate concentrations across Prince Edward Island (PEI), and which spatial aggregation is the preferred unit of analyses. Nitrate concentrations were determined for 4,855 samples from private wells. Validated field-by-field land use data were available. Average nitrate concentration and percentage of area for the fourteen major land use categories in PEI were determined for each of three spatial aggregations: watersheds based on topography and hydrology; freeform polygon boundaries based on similar neighbouring nitrate concentrations; and 500 meter buffer zones around each well. Results showed that the percentages of potato, grain, and hay coverage were positive predictors of ground water nitrate concentrations. Percentage of blueberry was a marginally significant negative predictor in the watershed model and significant in the freeform polygon model, and percentage of residential coverage was a positive predictor in the freeform polygon and buffer zone models. Spatial autocorrelation was present in the freeform polygon and buffer zone models even after land use was taken into account.

In conclusion, analyses based on watersheds produced the best predictive model with the percentages of land cover of potato, hay, and grain being significantly associated with ground water nitrate concentrations, and the percentages of blueberry, clear-cut woodland, and other agriculture being marginally significant.

3.2. Introduction

Nitrate contamination is possibly the most widespread contaminant of water (Gulis et al., 2002). Elevated nitrate levels in surface water or ground water can lead to numerous concerns, including nutrient enrichment of surface waters as a result of the discharge of nitrate-rich ground water; and a health concern to both animals (wild and domesticated) and humans (McLay et al., 2001). An understanding of the relative importance of various sources of nitrate is important to the development of appropriate remedial strategies, with the linkage between land use and ground water quality being a key element of this process.

Major sources of nitrate in the environment, and subsequent contamination of natural waters, include the use of nitrogen-based fertilizers, animal and human wastes, and to a lesser extent, industrial wastes, waste waters, and landfills (Vidal et al., 2000). Although nitrate does occur naturally in the environment as a breakdown product of the decomposition of organic matter, this source contributes only very small amounts of nitrate to ground water (Arms, 1994). Background concentrations of nitrate in North American ground water and surface waters have been estimated to be less than 3.00 mg L^{-1} nitrate-nitrogen (NO_3–N) (Spalding and Exner, 1993). "In the industrialized Western European and North American countries, intensive agriculture is considered to be the main source of water pollution by nitrate" (WHO, 2004).

The maximum acceptable concentration (MAC) for nitrate in drinking water in Canada and the United States is currently set at 10.00 mg L^{-1} NO_3–N (Health Canada, 1992), and the MAC recommended by the World Health Organization (WHO) and the European Union is 11.11 mg L^{-1} NO_3–N. The recommended nitrate concentration in

Europe is 5.56 mg L^{-1} NO_3–N (Van Maanen et al., 2000). In general, Canadian municipal water supplies have concentrations no higher than 4.90 mg L^{-1} NO_3–N (Health Canada, 1992) where contamination is under reasonable control.

In 2001, the concept of "human affected value" was introduced (based on previous work conducted in the early 1990s [Burkart and Kolpin, 1993; Eckhardt and Stackelberg, 1995]), whereby anything greater than the background concentration of 3.00 mg L^{-1} NO_3–N is believed to be primarily a result of human activities (McLay et al., 2001). In Prince Edward Island (PEI), nitrate levels in the range of 0.10 to 2.00 mg L^{-1} NO_3–N are considered to represent background levels for relatively un-impacted, 'pristine' watersheds (Young et al., 2002). A limited study in PEI found a mean nitrate level of 1.15 mg L^{-1} NO_3–N for ground water from wells in pristine areas (Somers, 1998).

Many studies have investigated land use factors for nitrate contamination of water resources (McLay et al., 2001; Thorburn et al., 2002; Honisch et al., 2002). However, the soil, climate, and farming systems can vary substantially from one region to another, and may exert a varying influence on the nature and extent of nitrate contamination. In a study carried out in sandy soils of Quebec, Canada, an association was found between intensive potato farming and nitrate concentrations. In this area, the ground water nitrate concentration was frequently above the MAC for human consumption (10.00 mg L^{-1} NO_3–N) (Levallois et al., 1998). Because PEI is a major potato growing area with similar climate and farming system, this finding does pose a concern for potential elevated concentrations (> 3.00 mg L^{-1}) in PEI ground water.

In PEI, all potable water is derived from ground water sources. Over half of the population resides in a rural setting, and relies on an estimated 20,000 to 25,000 private

wells as their sole source of water. The remaining population is serviced by central water supply systems, deriving their supply from the same ground water source. Private domestic water supplies in PEI rely on drilled wells with an average depth of 30 m with 12 m of casing. Water quality is generally excellent, however with high recharge rates and thin, permeable overburden, the ground water is vulnerable to contamination (Young et al., 2002). Agricultural activity is believed to be the most significant anthropogenic influence on ground water quality in PEI, and the occurrence of elevated nitrate concentrations (> 3.00 mg L^{-1}) is considered to be one of the greatest challenges to the protection of drinking water quality (Young et al., 2002).

Previous analyses in PEI have shown that nitrate concentrations in well water were associated with local land use (Young et al., 2002). In an early study aimed at characterizing well water nitrate levels under six broad land use categories, wells located in areas of row crops showed the highest mean nitrate concentrations (Somers, 1998). The study did not include field-by-field verification of land use, and therefore was subject to possible information bias. Furthermore, only a limited number of sites (54 wells) were examined, giving it questionable representativeness with respect to the entire province. In another qualitative survey, using a more representative data set (5,859 wells) than the study in 1998, it was observed that elevated ground water nitrate concentrations were more closely related to potato production intensity rather than to livestock density (MacLeod et al., 2002; Young et al., 2002). The same work suggested that average nitrate levels for 80% of the province exceeded background levels expected for relatively un-impacted watersheds (i.e. >3.00 mg L^{-1}), and 4.5% of wells had nitrate concentrations above 10.00 mg L^{-1} NO_3–N.

The primary objectives of this research were: i) to determine what land uses have a significant impact on nitrate concentrations in private well water samples across PEI, adjusting for spatial autocorrelation (neighbouring nitrate concentrations are more similar [positive autocorrelation] or dissimilar [negative autocorrelation] than expected); and ii) to determine the best spatial aggregation for assessing these nitrate factors, balancing data scarcity problems with within unit homogeneity.

3.3. Materials and methods

3.3.1. Study Site

Situated on the East Coast of Canada, PEI is approximately 5,700 km^2 in land area with 1,600 km of coastline (Somers, 1992) and a current population of almost 140,000 (Statistics Canada, 2001) (Figure 1-2). The island is divided into three counties with the most densely populated being Queen's County in central PEI (53% of the population), then Prince County in western PEI (33% of the population), and finally King's County in eastern PEI (14% of the population) (Statistics Canada, 2001).

PEI's topography is characterized by rolling hills reaching a maximum height of 120 m above sea level. Nearly half of the land base in the province is devoted to agriculture (InfoPEI, 2005a), with row crop production accounting for the largest portion of this activity. The geology in the area is comprised mainly of a Permo-Carboniferous redbed sequence (fractured sandstone bedrock) overlain by a thin generally sandy glacial till (Somers, 1992). Ground water is tapped from a highly productive sandstone aquifer, with the extent of individual ground water flow systems determined primarily by surface topography. Consequently, surface watershed boundaries provide a good approximation

of individual ground water flow system boundaries (InfoPEI, 2005b). The landmass of PEI can be divided into approximately 260 watersheds and sub-watersheds.

3.3.2. Land Use Data

Validated field-by-field land use data were available for PEI from aerial photographs taken in 2000. The data were classified into fourteen categories according to requirements of the Provincial Department of Agriculture. These categories were apple, bare soil, blueberry, clear-cut woodland, cranberry, forest (coniferous and deciduous), grain (the majority of which was barley, but also included winter and spring wheat, oats, winter rye, and occasionally corn), hay (for example, red clover, timothy grass, and alfalfa), meadow/dune, other agriculture (for example, lettuce, cabbage, and sunflowers), pasture (grazing livestock, predominantly dairy and beef cattle), potato, residential (grass, hedge/shrubs, and building/roads), and water/wetland (main areas were lakes, marshes, rivers, and beaches). Percentages of land use data within the three types of areas of analysis (watersheds, freeform polygon areas, and buffer zones) were calculated using the following steps. First, all polygons of land use that straddled an area boundary were split by that area boundary. Area boundaries in this instance were the boundaries of the watershed, freeform polygon areas, or buffer zones. Second, the percentage of each category of land use within each area was determined, based on the number of square kilometers of specific land use divided by the total number of square kilometers in the area. This procedure was carried out in Mapinfo Professional 7.0 (MapInfo Corporation, 2002). Even though crop rotation occurs in PEI, it was assumed that land use percentage within an area of analysis remained similar from one year to the next.

3.3.3. Nitrate Data

A total of 4,855 water samples from private wells sampled during 1997–2001 (part of a routine pre-mortgage sampling program in PEI) were used for the analyses. Nitrate-nitrogen (hereafter, referred to as nitrate) concentrations were determined for these samples by flow injection analysis colorimetry (using the QuikChem Series 8000, FIA+ instrument [Lachat Instruments, Milwaukee]) with a detection limit of 0.10 mg N L^{-1} (Appendix A).

If well water from a property was sampled twice or more during this time frame, then an average of the concentrations was calculated. This occurred in 7.4% of the samples. This average was only accepted if a duration of at least six months existed between the two samples. A six-month time frame was chosen in order to avoid repeat samples taken for reasons other than for a mortgage assessment (e.g., high chemistry or bacterial concentrations). If the period between two samples was less than six months, then only the first measurement was used in order to utilize the sample most likely to represent pre-treatment concentrations, assuming that any subsequent sample within six months was to determine if the treatment was effective.

3.3.4. Data Aggregation

Associations between percentages of land use and nitrate concentrations were assessed using areal and point-level data. For areal data, two methods of spatial aggregation, watershed boundaries and freeform polygon aggregations were explored. Because watersheds are defined according to hydrological properties, they were deemed to be an appropriate unit of analysis. However, within watersheds there were likely to be

areas of heterogeneity of topography, soil, land use, and nitrate concentrations. Therefore, an alternative unit of analysis was investigated, freeform polygons, with improved within unit homogeneity of nitrate concentrations compared to watersheds, as described below.

The process for determining the boundaries of the freeform polygons was carried out using BoundarySeer 1.1.9 (Jacquez et al., 2002). First, taking the spatial variation across all nitrate concentrations into account, a goodness-of-fit index (Jacquez et al., 2001) was used to calculate the appropriate number of spatially constrained contiguous polygons, to produce high within unit homogeneity of nitrate. Once an appropriate number of homogenous areas were estimated, polygons were created using fully constrained agglomerative clustering (Jacquez et al., 2001), meaning that clusters of similar nitrate concentration were grouped together, constrained so that they had to be adjacent in geographic space. The 0.9 linkage connectedness (comparing locations within one cluster to those of the neighbouring clusters) was used in order to obtain the most appropriate number of polygons, as determined by the goodness-of-fit index. While aggregating small polygons may combine two areas that have different land uses, leading to a potential bias in results, the alternative was to allow a small number of wells in that polygon to produce a potentially unrepresentative nitrate value for the polygon.

Each polygon area (watershed or freeform) was to meet the following criteria. First, areas required a minimum of five nitrate points in order to create a valid estimate for that area. Areas of aggregation with less than five observations were combined with neighbouring polygons, provided the mean difference between the two areas did not differ by more than 2.50 mg L^{-1} NO_3–N. This concentration difference was determined from the freeform polygon creation process using a goodness-of-fit index (Jacquez et al,

2001). Second, aggregated areas must not stretch over two coastlines, without the inclusion of a peninsula. This was not an issue for the watersheds because they were created with this factor in mind. A few freeform polygons spanned across the coastlines, but manually splitting the polygon into two equal parts rectified this.

After application of the two criteria mentioned above, 174 watersheds with an average size of 32.60 km^2 (range of 0.89 to 196.80 km^2) and 664 freeform polygons with an average polygon size of 8.55 km^2 (range of 0.005 to 175.63 km^2) were used for the analyses. The smaller freeform polygon areas were typically areas with one high or low nitrate concentration compared to their neighbours (differed by > 2.50 mg L^{-1} NO_3–N), and therefore could not be aggregated with neighbours to form larger areas, according to the criteria.

The point-level analyses were conducted using 500 m radius circular buffer zones of land around individual wells sampled during 2001. A 500 m buffer zone was chosen because < 500 m would predominantly be residential area (house, lawn, and road), and > 500 m increased buffer zone overlap of neighbouring buffer zones. Only one year was used in order to minimize overlapping buffers and repeated well samples, which would violate the regression assumption of independent observations. The year 2001 was chosen because the land use data were from 2000. Information on ground water flow was not available and therefore an equal distance in every direction (circular) around each well was utilized. The buffer zone method of analysis assessed the association between nitrate concentrations and land use at a more local level compared to the regional level used in the watershed and freeform polygon methodologies.

3.3.5. Statistical Analyses

Comparisons of nitrate concentrations among years were assessed using a linear mixed effects model in SAS V8 (SAS Institute, 2001) with an autoregressive (AR1) correlation structure. No significant difference in nitrate concentrations among years was revealed ($p > 0.05$), therefore, all five years of nitrate data were combined and used for the areal analyses in order to maximize the number of data points available for aggregation in each area. Although Chapter 2 reported a significant interaction between season and land use, controlling for season in this study would make the data very sparse as there would need to be at least five observations per season per watershed (criteria previously outlined).

Multiple linear regression was carried out on the data and spatial autocorrelation was controlled, if significant. Forest was chosen for the reference value because it was a common land use in the dataset, and because ground water nitrate concentrations in forests are typically stable and unaffected by human influence. Two-way interactions of all significant main effects were assessed and removed using backwards-stepwise elimination until only those that were significant ($p < 0.05$) remained in the model.

Model diagnostics were performed to detect violations of assumptions for multiple linear regression. These assumptions were homoskedasticity (equal variance) among the full model residuals, a normal distribution of residuals, a linear relationship between the response variable and each predictor, and independence of the response variable values (accounting for spatial autocorrelation when necessary). Potential outliers and influential values were assessed as well as several transformations in order to improve the model fit. As the nitrate data was not normally distributed, a Box-Cox

analysis (Box and Cox, 1964) was carried out in Stata 8.2 (StataCorp, 2004) to investigate various transformations. The natural logarithm (+1) was found to be the most appropriate transformation in order to achieve a normally distributed nitrate outcome variable.

3.3.6. Spatial Statistics

Initially, Moran's I was used to assess global autocorrelation of the nitrate concentrations for all three methods of analysis when no predictors were in the model. Values of Moran's I less than zero indicated negative spatial autocorrelation, that is, clustering of dissimilar values, while those greater than zero indicated positive spatial clustering, that is, clustering of similar values in similar areas (Wakefield et al., 2000).

For the watershed and freeform polygon analyses, a binary weights matrix was created, using Queen contiguity, identifying what areas were considered neighbours. This method takes into account those areas that share the edge to the immediate left, right, up, and down as well as taking diagonal edges into account (reflecting how a queen moves in a game of chess). In this matrix, a "one" was assigned if location *i* was neighbouring location *j*, otherwise a zero was assigned. For the buffer zone analyses, a distance weights matrix was utilized based on the inverse distance between points. The threshold distance obtained (using Euclidean Distance) was 7.57 km, representing the minimum distance required so that each observation had at least one neighbour (Anselin, 2003a).

A spatial lag model (Florax and de Graff, 2004) was run for each of the three final ordinary least squares regression models using the contiguous (polygon models) or distance (buffer zone model) weights matrix. The spatial lag model is a linear regression

model with a spatial variable incorporated to reflect spatial autocorrelation. The spatial lag variable represents the average nitrate concentration of all neighbours of each polygon, as defined by the contiguity weights matrix. Spatial autocorrelation among the areas was assessed using the Lagrange Multiplier test (Florax and de Graff, 2004; Anselin, 1995). If the *p*-value was significant and the rho (the spatial autocorrelation coefficient) was either positive or negative in value, then spatial autocorrelation was evident and needed to be controlled. All spatial analyses were conducted using GeoDa 0.9.5-i (Anselin, 2003b).

3.4. Results

All nitrate results are reported as NO_3–N. The mean and median nitrate concentrations of the 4,855 individual nitrate measurements were 3.42 and 2.87 mg L^{-1}, respectively, with a range of 0.10–27.50 mg L^{-1}. A total of 113 (2%) of the nitrate values exceeded the Canadian MAC (10.00 mg L^{-1}) and 882 (18%) exceeded the concentration recommended by the EU (5.56 mg L^{-1}).

3.4.1. Watershed Results

The average number of individual nitrate points per watershed was 29 with a range of 5 to 294 (median = 15). The mean and median nitrate concentrations at the watershed level were 3.40 and 3.24 mg L^{-1}, respectively, with a range of 0.30–9.56 mg L^{-1}. No watersheds exceeded the Canadian MAC limit of 10.00 mg L^{-1}, whereas 20 (11%) of the 174 watersheds exceeded the EU recommended concentration (5.56 mg L^{-1}).

Table 3-1 shows the significant land use variables present in the final watershed regression model on nitrate concentrations, controlling for the effects of other variables in the model. Within watersheds, percentages of potato, grain, and hay cover had very similar interquartile ranges of approximately 10% land use cover, with the percentage of hay having the highest median. Clear-cut woodland, blueberry, and other agricultural land uses were present in watersheds in very small percentages of cover and were frequently not present in watersheds.

Before land uses were incorporated into the model, spatial autocorrelation between two neighbouring watersheds was present (Moran's I of 0.416, $p = 0.001$), suggesting a reasonably strong positive autocorrelation among average nitrate values at the watershed level. Spatial autocorrelation was assessed using the Lagrange Multiplier test when all land use variables were taken into account, and was subsequently found to be non-significant ($p = 0.28$). Therefore, most spatial autocorrelation among nitrate measurements was removed once land use was incorporated into the model.

There were three significant land use predictors at the $p < 0.05$ level; potato, grain, and hay cover, and three marginally significant predictors ($p > 0.05$ but $p < 0.06$); clear-cut woodland, other agriculture, and blueberry cover (Table 3-1). All coefficients had positive coefficients except for blueberry, which had a negative association with ground water nitrate concentration. No interactions between these land uses were significant. The final regression model obtained an R^2 of 0.53. Therefore, this final regression model explained 53% of the variation found in private drinking water nitrate concentrations in PEI.

Choropleth maps of nitrate concentration in PEI and each significant land use were created to visually demonstrate the associations between nitrate and the land uses among watersheds. Figures 3-1 and 3-2 show average nitrate concentration and the percentage of potato production at the watershed level. Figure 3-1 also shows the 2001 buffer zone distribution in PEI, discussed later in this section. When assessing the distribution of potato production using the same unit of analyses, watersheds, there was clear evidence that elevated nitrate concentrations were found in areas of high potato production. A similar trend was seen with hay and grain production, while watersheds with low nitrate concentrations (< 3.00 mg L^{-1}) were often located where blueberry production was present (Figure 3-3).

3.4.2. Freeform Polygon Results

The average number of individual nitrate points per freeform polygon was 7 with a range of 1 to 154 (median = 7). The mean and median nitrate concentrations were 5.30 and 5.00 mg L^{-1}, respectively, with a range of 0.10–27.50 mg L^{-1} (see Figure 3-4 for nitrate concentration in PEI averaged at the freeform polygon level). This average is close to the European recommended concentration of 5.56 mg L^{-1} and exceeds the human affected value of 3.00 mg L^{-1}. The freeform polygon mean was particularly high because many of the higher nitrate concentrations were in polygons with only one or a few nitrate samples. A total of 76 (1%) of the freeform polygons exceeded the Canadian MAC (10.00 mg L^{-1} NO_3-N), and 290 (44%) exceeded the EU recommended value of 5.56 mg L^{-1}.

Table 3-2 shows the significant land use variables present in the final freeform polygon regression model. Again, the percentages of potato, hay, and grain coverage had very similar interquartile ranges (approximately 15%) with hay having the highest median, again (Table 3-2). The percentage of blueberry coverage had a zero interquartile range, but that was because it was grown in only 8.7% of the freeform polygons.

The freeform regression model with no land use predictors obtained a slightly negative but not significant Moran value of -0.047 ($p = 0.07$), which increased once the land use variables were incorporated in the model; therefore, a spatial lag model was assessed. Spatial autocorrelation in the full model was highly significant and negative (rho = -0.41), as the Lagrange Multiplier test reported a p-value < 0.001, suggesting that many of the neighbouring freeform polygons nitrate values differed by greater than 2.50 mg L^{-1}.

The final spatial lag model consisted of five significant land use variables: potato, hay, grain, residential, and blueberry. Again, blueberry was the only predictor with a negative coefficient. Blueberry coverage was also the strongest coefficient in the model. There were no significant interactions between any of these land use variables. Similar to watersheds, the freeform polygon maps demonstrated that high potato production was closely correlated with high nitrate concentrations and blueberry production with low concentrations. Figure 3-4 shows average nitrate concentrations at the freeform polygon level, which can be compared to Figures 3-2 and 3-3 to visualize average potato and blueberry coverage.

3.4.3. Buffer Zone Results

A total of 1,090 water samples were available during the year 2001, with mean and median nitrate concentrations of 3.50 and 2.90 mg L^{-1}, respectively (range of 0.10–17.00 mg L^{-1}). A total of 36 (3%) of the buffer zones exceeded the MAC and 218 (20%) exceeded 5.56 mg L^{-1} nitrate.

Table 3-3 shows the significant land use variables present in the buffer zone regression model. The percentage of residential areas had an interquartile range of 27%, and also a very large median. A small median of 3.3% existed for potato cover because almost 40% of the buffers did not contain any potato production.

Spatial exploration of the buffer zones produced a positive Moran statistic of 0.17 ($p = 0.001$), which was still evident once the full model was introduced. Spatial autocorrelation among neighbouring buffer zones was highly significant and positive in the spatial lag model ($\rho = 0.31, p < 0.001$). Spatial autocorrelation of the full model was highly significant as the Lagrange Multiplier test reported a p-value of < 0.001, suggesting that buffers close together (< 7.57 km) have nitrate values more similar than those farther apart. Buffer zone distribution is shown in Figure 3-1.

Three main effects, percentage cover of potato, pasture, and other agriculture, and two interaction variables, one between hay and grain and the other between hay and residential, were significant in the final buffer zone model (Table 3-3). Therefore, the coefficients for hay, grain, and residential areas cannot be interpreted independently. Figures 3-5 and 3-6 demonstrate the effects of these interactions. For both figures, the quartiles of the percentage of hay were graphed with either the percentage of grain or the percentage of residential areas on the x-axis, and predicted nitrate values on the y-axis.

Figure 3-5 shows the negative interaction on nitrate concentrations when grain and hay are both present in the same buffer zone. The slopes of the percentage of hay quartiles decrease with increasing hay cover. The percentage of hay cover has a positive but lower coefficient than the percentage of grain cover and therefore, higher hay quartile lines have a higher nitrate concentration on the y-axis at grain cover = 0%. However, as grain cover increases within a buffer zone, nitrate concentrations increase, but the amount of this increase is reduced when there is a larger percentage of hay cover in the buffer zone. The opposite trend is seen with the synergistic hay-residential interaction, with the percentage of hay quartile lines widening with increasing percentage of residential cover (expressed on the x-axis).

3.5. Discussion

This is the first detailed regression analysis of relationships between land use practices and ground water nitrate concentrations comparing these three different spatial analytical units. The mean nitrate concentration of 3.42 mg L^{-1} indicates that there is some human influence on the nitrate concentrations in ground water. Therefore, the results of this study should assist in the determination of significant sources of nitrate, aiding in the development of systems to keep the levels within acceptable limits.

3.5.1. Land Use

Regardless of unit of analysis, the percentage of potato, grain, and hay production were all significantly and positively associated with nitrate concentrations in PEI private well water. This was not surprising because these land uses frequently have nitrogen-

based fertilizers applied to improve crop yield. Potatoes are generally heavily fertilized at the start of the growing season (400 kg ha^{-1} of 18–46–0 [nitrogen, phosphorus, potassium]), and then another quantity of 500 kg ha^{-1} of 15–0–20 is added later into the growing season (PEI Government, 2000). A study in Quebec, Canada, concluded that intensive potato production on sandy soils was a significant predictor of high nitrate concentrations. They also noted that potato fields within a 2 km distance of the sampled wells were a contributing factor to this significant finding (Levallois et al., 1998).

Grain fertilization application varies depending on the type of grain, but typically spring crops are fertilized at a rate of 300 kg ha^{-1} of 18–36–18 at the start of the growing season (PEI Government, 2001), whereas hay is fertilized at a rate of 400 kg ha^{-1} of 5–10–30, with an additional amount added after each cut (300 kg ha^{-1} of 0–10–30) (PEI Government, 1998). Therefore, little nitrogen is added to hay fields, especially if the majority of the hay field contains legumes. However, a study in PEI showed that ploughing hay fields, especially in late October, enhances nitrate leaching, therefore, leading to high nitrate concentrations being associated with hay fields (Sanderson et al., 1998). It should be noted that all of these fertilizer application rates are general recommendations, because specific quantities will depend on results from a soil analysis or practices of individual farmers.

Other agriculture and residential areas were significant (or marginally significant) positive predictors in two of the three models, while pasture and clear-cut woodland areas were positive predictors in only one model each (buffer zones and watersheds, respectively). Fertilization on pastured land would typically be in the form of manure directly from the livestock (primarily cattle in PEI) grazing on the land, but amounts

would depend on stocking density. Pastures sometimes have inorganic fertilizer applied to the land, but not consistently, depending on the farm (Hovingh, 1998). Other agriculture was located in many of the spatial units, but was only represented by a very small area of the polygons. This land use would include such crops as lettuce, carrots, cabbage, and sunflowers, but due to the variety of crops included within this category, no general statements on fertilizer use can be made.

Residential areas included not only buildings and gardens, but also roads, shrubs/individual trees, and recreational grassy areas such as playing fields and golf courses. Therefore, it is possible that the association between nitrate concentrations and these land uses, designated residential, are from fertilized lawns and golf courses, as well as on-site septic system discharges. An assessment of stream water chemistry upstream and downstream from operating golf courses and those under construction, concluded that nitrate concentrations were higher downstream from golf courses compared to upstream or local un-impacted areas (Winter and Dillon, 2005). Another study noted that contamination from urban environments is a source of elevated nitrate levels (> 3.00 mg L^{-1}), but not to the same degree as from vegetable fields (Babiker et al., 2003). Clear-cut woodland, on the other hand, has no fertilizer added, but the additional nitrate in these areas is primarily due to the breakdown of organic matter left behind from tree felling. An association between organic matter and nitrate in both well water in rural areas, and spring water in urban areas was observed in Spain (Vidal et al., 2000).

Blueberry production was the only negative predictor, and was significant (or marginally significant) in two of the three aggregation methods (watersheds and freeform polygons). One possible explanation for blueberry fields having a slightly negative effect

on nitrate concentration is that wild blueberries in PEI are usually grown in acidic soils and do not require many additional nutrients. Blueberry bushes in PEI are typically only fertilized every second year and with low amounts of nitrogen (application rate of 300 kg ha^{-1} at 10–10–10) (K. Sanderson, personal communication, 2005). Because of this low-level fertilizing, very little leaches into the water table below because the majority is utilized by the bushes. If the blueberries are a new crop, then all of the available nitrogen will be used for growth and maturation. There may also be a possibility of denitrification as the plants are typically grown in areas where the water table is high and dissolved oxygen is low (Hayden, 2001).

Significant interactions among land uses were present in the buffer zone analyses. These analyses were not at the field level, but at a larger area level, with effects on the ground water from multiple farms that were likely to be the result of years of land use. With this spatial and temporal context, where crop rotations in space and time can occur, interactions between land use categories within areas are biologically possible. For example, rotations can lead to different nitrate concentrations than if the same crop were grown on the same land area year after year (Power et al., 2001). One of the strengths of regression models is the ability to investigate these interactions. However, historically, few investigators have looked for interactions among historical land use data using regression models.

The observed interactions were between hay and grain, and hay and residential land use. Interactions occur when an antagonistic (between hay and grain) or synergistic (between hay and residential) effect is present, thus implying that more of the variation in

nitrate can be explained in more detail by an interaction term rather than simply by the variables being interpreted independently.

The interpretation of the negative interaction between hay and grain could provide additional support that crop rotations lead to beneficial environmental effects. When a small amount of hay and large amounts of grain were present, there was a noticeable grain effect increasing nitrate concentration (solid line in Figure 3-5). When there were large amounts of hay and small amounts of grain (see increasing quartiles of percentage of hay along the y-axis where grain is 0%), nitrate also increased. However, there are decreasing lines for the four lines representing hay percentiles in Figure 3-5, indicating that with more hay cover in an area, increases in grain cover have a reduced impact on nitrate concentration. For the 75^{th}–100^{th} percentile, there was only a small increase in nitrate concentration with increasing grain cover. With lower quartiles of hay, there is less possibility for crop rotations to be in place. Accurate land use data for other years were not available; therefore, it cannot be confirmed that crop rotation was the reason for this observed negative interaction. In 1997–2001 crop rotation was not mandatory, only recommended, however it was frequently carried out. Frequent crop rotations practised in PEI are grain-hay rotations and potato-grain-hay rotations. It is probable that the grain-hay interaction was significant (versus potato-grain or potato-hay) because it is a part of both three-way potato farming rotations and two-way cattle farming rotations, two systems that occupy a large part of the agricultural land cover in PEI. A reduced effect on ground water nitrate from crop rotations was also noted in a study looking at corn-soybean rotations, which were effective at decreasing nitrate leachate compared to growing corn continuously (Power et al., 2001).

The interaction between hay and residential areas represents a synergist effect between these two land uses. No obvious reason can explain what is shown in Figure 3-6. There is a possibility that this interaction is spurious and simply due to chance when examining multiple interactions. Further research is warranted to determine an explanation for this finding.

While potato, grain, and hay coverage were significant variables in all three aggregation methods, there were other significant variables that were only detected in one or two of the methods. The reasons for these differences relate to the strengths and weaknesses of each of the analyses. First, the number of analytical units was highest for the buffer zone analysis and lowest for the watershed analysis. The interactions were likely detected with the buffer zone analyses because there were more units of analysis, leading to a higher power to detect significant associations if they were present. Conversely, the watershed analyses had the fewest units of analysis, and therefore the lowest power to detect significant associations.

3.5.2. Model Comparisons

With regression analysis, it was assumed that the outcome variable, average nitrate concentration of all tested wells within each watershed, was representative of each watershed. However, within each watershed and freeform polygon, there was variability around each mean nitrate concentration, and the average of the standard deviations around these means gives a measurement of this variability. The average standard deviation for nitrate concentrations within watersheds was 2.22 versus 0.93 for freeform polygons. With this improved homogeneity over the watershed units, the freeform

polygon model could have more power to detect significant predictors. It is important to be aware that the data and analyses were scale-dependent, and the interpretation of the results should depend on the scale of analysis used. Within-unit homogeneity was not an issue in the buffer zone analyses as each buffer zone represented a single nitrate measurement.

The watershed analyses actually detected more significant (including marginally significant) associations with nitrate concentration than the freeform analyses, and this may be due to another methodological difference between these analyses, boundary selection for each analysis. Watershed boundaries have been determined primarily to reflect inherent hydrological mixing of ground water within watersheds, whereas in the freeform analysis, the boundaries were created to maximize within-unit homogeneity of nitrate. However, the percentages of land uses that contributed to each freeform polygon may have been partially misclassified due to erroneous boundary allocation. For this reason, the watershed analysis may have been able to detect more significant associations than the freeform analysis.

Reliability of the watershed model was assessed using a split-sample analysis, whereby the full dataset was randomly split into two groups (60% and 40%). A linear regression model was then built on 60% of the data and predicted values were obtained and compared to the observed values using a correlation coefficient (R). The difference between the square of this correlation coefficient and the R^2 from the original model is called the "shrinkage" on cross-validation. If the shrinkage value is small, then reliability of the original model is acceptable (Dohoo et al., 2003). A small growth value (rather than a shrinkage value) of 0.06 was obtained (original R^2 of 0.53 versus 0.59), so the full

model was considered robust and reliable for predicting nitrate concentrations in PEI using land use data.

Disadvantages of the buffer zones were that only a portion of the data could be used for computational reasons, and because the buffers were small relative to the other units (watersheds and freeform polygons), many of the land uses, both significant and not, were not present in many of the buffers. For example, the buffer zone analyses did not detect blueberry coverage as a significant predictor of ground water nitrate due to the very low number of buffers zones with substantial blueberry cover. Conversely, the area analyses included the potential impacts of all blueberry fields in PEI.

3.5.3. Spatial Autocorrelation

Spatial autocorrelation among nitrate concentrations was evident when no land use predictors were present in all three regression methods, particularly for watersheds. However, when the land uses were added to the models, only the freeform polygon and buffer zone models showed remaining significant spatial autocorrelation. Spatial autocorrelation in these two methods could be due to a greater number of units used, thus adding power and enabling detection of spatial autocorrelation if it truly did exist.

Negative spatial autocorrelation (rho = -0.41) in the freeform polygons could have occurred for a number of reasons. One common explanation for negative spatial autocorrelation is due to aggregation bias; meaning positive autocorrelation exists at a smaller spatial scale than what is being analyzed (Smith, 2001). Another possibility could be due to a localized nitrate source for the high values amongst lower ones, or treatment for high nitrate levels in one well among an elevated nitrate area. However, a likely

reason is the method in which the polygons were created due to the current limitations of the software. A freeform polygon consisting of several similar nitrate concentrations can have an 'island' of a single extreme concentration within it. A physical explanation for these islands may relate to very localized effects on that well, or characteristics of the well that lead to increased contamination of nitrate, such as shallow well depth and amount of casing protecting the well.

In order to further understand the associations between ground water nitrate concentration and land use, it may be effective to consider other possible influential factors such as livestock densities, ground water depth and flow, crop rotations, and water treatment practices. Well depth and construction would also be useful when assessing nitrate concentrations in ground water. This study was somewhat limited by these absent variables, but land use was both thoroughly and efficiently evaluated with the reliable resources available and techniques utilized.

3.6. Conclusion

In conclusion, there were significant associations between several land use variables and nitrate concentrations in PEI private well water, regardless of the spatial unit of analysis used. All three regression models showed strong positive associations for the percentage of potato coverage and moderate positive associations for grain and hay. Two models, watersheds and freeform polygons, showed strong negative associations for blueberries. Although watersheds did not possess high within-unit homogeneity of nitrate concentrations, they were considered the most suitable method of data aggregation for the following reasons: they were created according to hydrological factors; they were large

enough to accurately determine average nitrate concentrations; and, they created a model which best explained the variance, according to the R^2 values of the ordinary least square regression models. The 500 m buffer zones were suitable for assessing very localized land use effects in these data, but only a sub-sample of the data could be used (Year 2001). Finally, freeform polygons showed high within-unit homogeneity, but owing to the method of their creation, significant negative spatial autocorrelation was produced.

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Table 3-1 Final model of significant land use variables associated with average nitrate concentrations in 174 watersheds in Prince Edward Island, Canada (1997-2001).

Land Use	Coefficient (ln+1)	Confidence intervals	Land use interquartile range and [median] (%)	Number of watersheds containing land use	*p*-value
Potato	0.026	0.018 – 0.034	3.7 – 13.6 [6.9]	168 (96.5%)	0.001
Grain	0.010	0.002 – 0.020	6.5 – 17.3 [10.5]	174 (100%)	0.021
Hay	0.010	0.003 – 0.017	9.9 – 22.2 [14.4]	173 (99.4%)	0.007
C-cut[a]	0.030	-0.0002 – 0.061	0.8 – 4.0 [2.0]	162 (93%)	0.051
Oth agri[b]	0.050	-0.002 – 0.102	0 – 1.1 [0.3]	127 (73%)	0.058
Bberry[c]	-0.024	-0.050 – 0.001	0 – 0.05 [0]	46 (26%)	0.059

[a] Clear-cut woodland
[b] Other agriculture (e.g., carrots, cabbage, sunflowers)
[c] Blueberry

Table 3-2 Final model of significant land use variables associated with average nitrate concentrations in 664 freeform polygons in Prince Edward Island, Canada (1997-2001).

Land Use	Coefficient (ln+1)	Confidence intervals	Land use interquartile range and [median] (%)	Number of polygons containing land use	*p*-value
Rho[a]	-0.410	-0.319 – -0.501	-	-	<0.001
Potato	0.017	0.013 – 0.0212	2.1 – 16.6 [7.1]	556 (83.7%)	<0.001
Grain	0.011	0.007 – 0.016	5.5 – 19.6 [11.9]	606 (91.3%)	<0.001
Hay	0.011	0.007 – 0.014	9.1 – 25.4 [16.6]	627 (94.4%)	<0.001
Resid[b]	0.0033	0.0001 – 0.006	7.5 – 18.9 [11.6]	664 (100%)	0.045
Bberry[c]	-0.040	-0.072 – -0.006	0 – 0 [0]	58 (8.7%)	0.0204

[a] Spatial autocorrelation among neighbouring freeform polygons, as defined by the spatial weights matrix
[b] Residential (including buildings, lawns, parks, and golf courses)
[c] Blueberry

Table 3-3 Final model of significant land use variables associated with average nitrate concentrations in 500 m buffer zones around 1,090 private wells in Prince Edward Island, Canada (1997-2001).

Land Use	Coefficient (ln+1)	Confidence intervals	Land use interquartile range and [median] (%)	Number of buffers containing land use	*p*-value
Rho[a]	0.308	0.1908 – 0.4247	-	-	<0.0001
Potato	0.017	0.0143 – 0.0205	0 – 13.3 [3.3]	668 (61.3%)	<0.001
Pasture	0.007	0.0020 – 0.0118	0 – 4.0 [0]	454 (41.7%)	0.006
Oth agri[b]	0.014	0.0046 – 0.0241	0 – 0 [0]	193 (17.7%)	<0.001
Resid[c]	0.0003	0.0002 – 0.003	13.4 – 40.1 [22.8]	1088 (99.8%)	<0.0001
Hay	0.013	0.0090 – 0.0172	5.6 – 25.8 [15.2]	977 (89.6%)	<0.0001
Grain	0.021	0.0166 – 0.026	1.1 – 19.1 [9.5]	871 (79.9%)	<0.0001
Hay&Grn[d]	-0.0004	-0.0006 – -0.0002	-	-	<0.001
Hay&Resid[e]	1.57e-005	0.000002 – 0.00003	-	-	0.023

[a] Spatial autocorrelation among neighbouring buffer zones, as defined by the spatial weights matrix
[b] Other agriculture (e.g. carrots, cabbage, sunflowers)
[c] Residential (including buildings, lawns, parks, and golf courses)
[d] Hay and grain interaction
[e] Hay and residential interaction

Figure 3-1 Average groundwater nitrate concentrations in 174 watersheds in Prince Edward Island, Canada (number of watersheds in each nitrate category in parentheses), 1997–2001. Buffer zone locations (○) are also included for the year 2001.

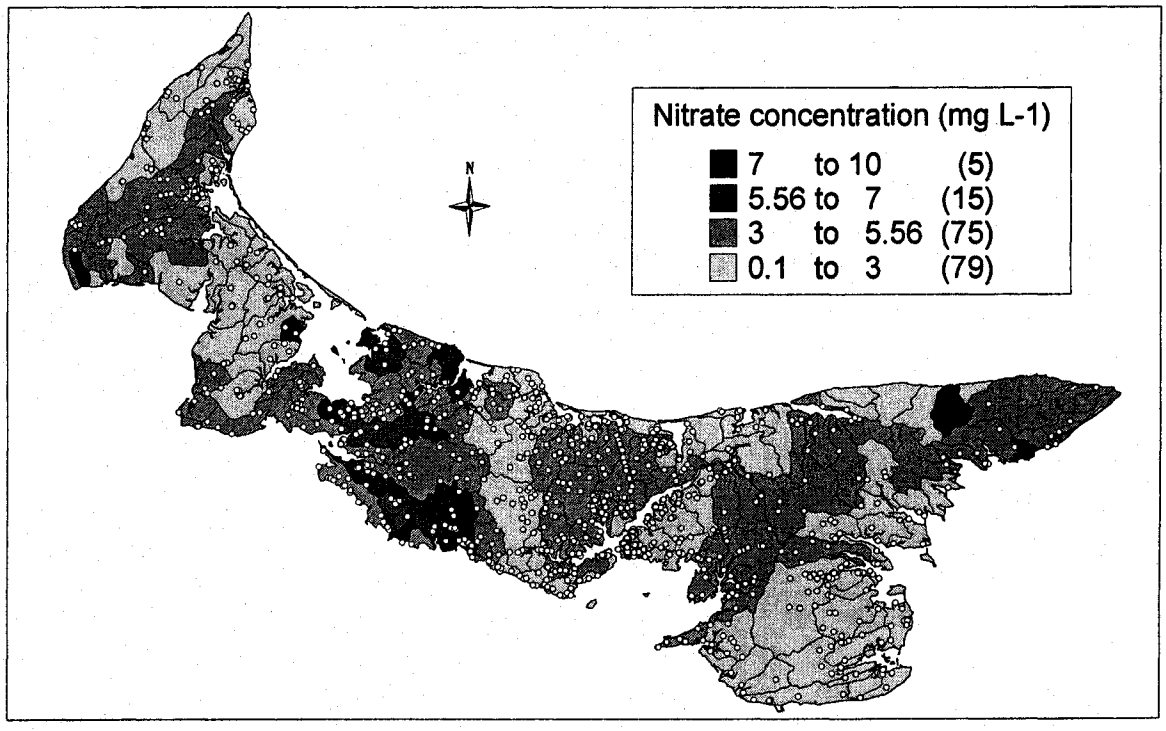


Figure 3-2 Percentage of potato cover in 174 watersheds in Prince Edward Island, Canada (number of watersheds in each nitrate category in parentheses), 2000.

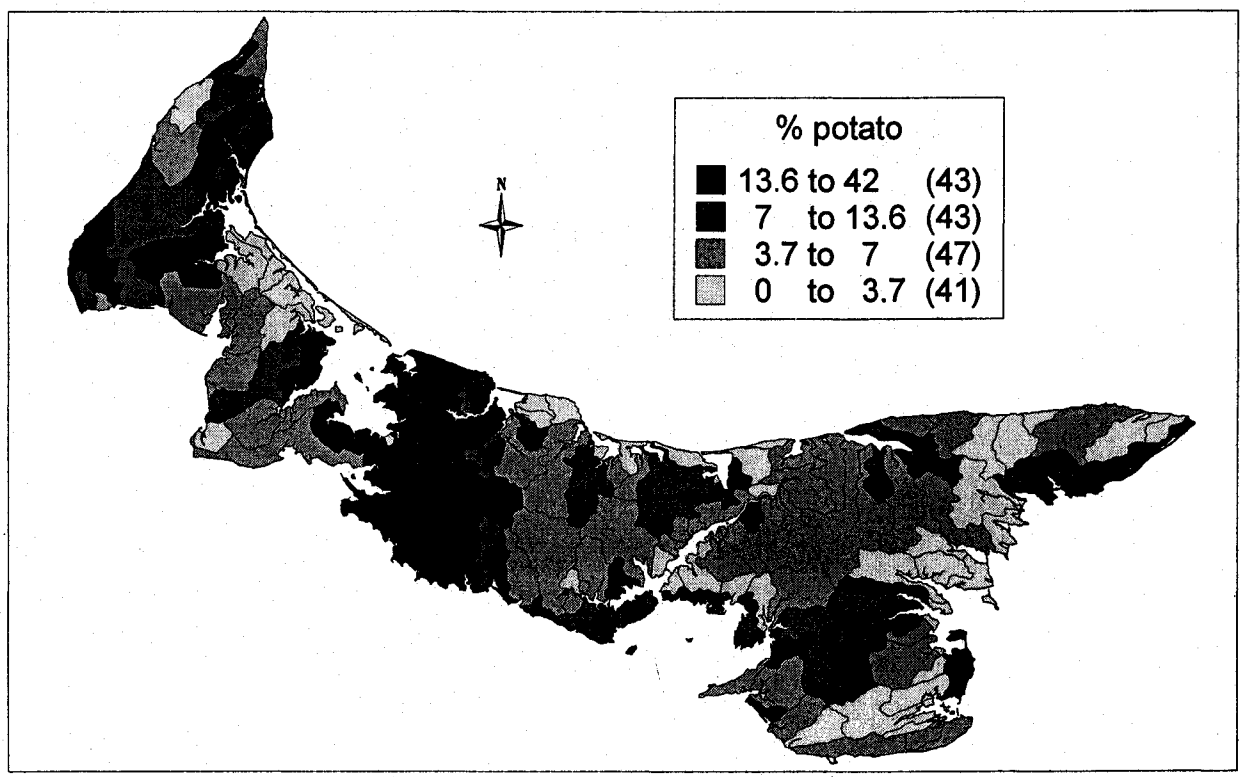


Figure 3-3 Percentage of blueberry cover in 174 watersheds in Prince Edward Island, Canada (number of watersheds in each nitrate category in parentheses), 2000.

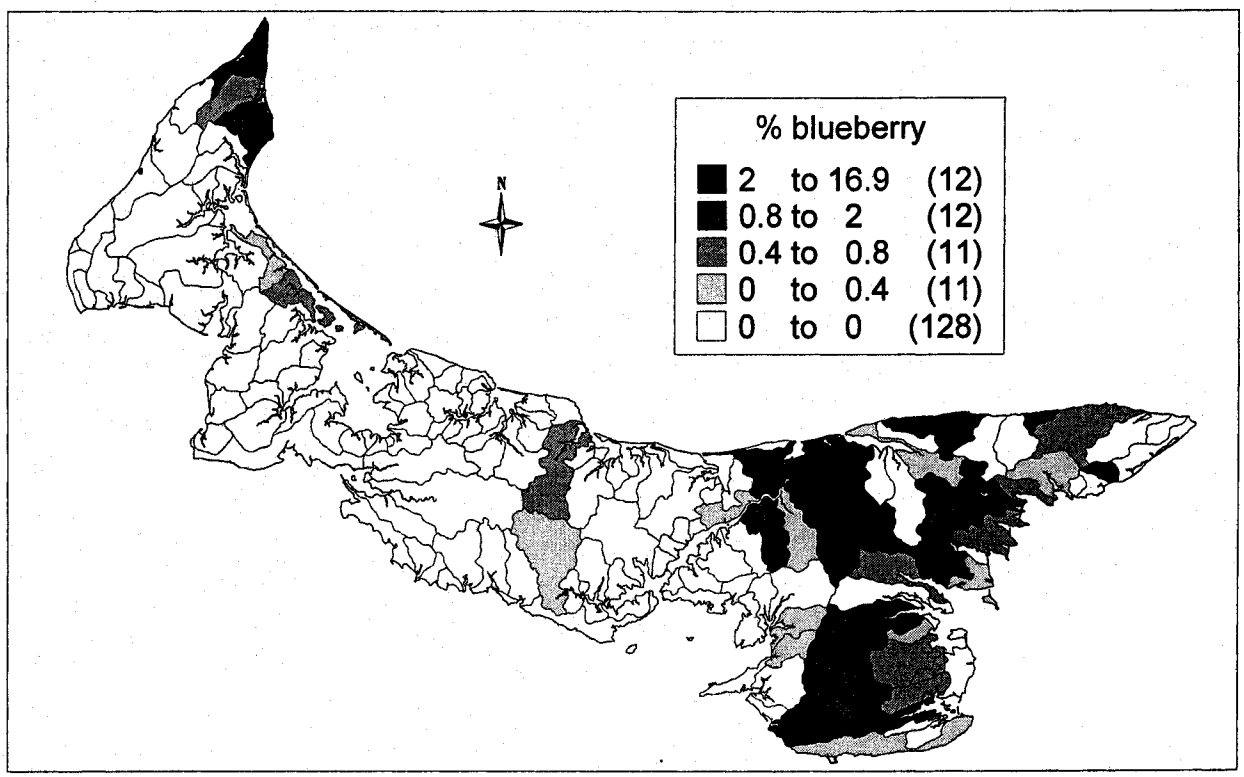


Figure 3-4 Average groundwater nitrate concentrations in 664 freeform polygons in Prince Edward Island, Canada (1997-2001), (number of freeform polygons in each nitrate category in parentheses).

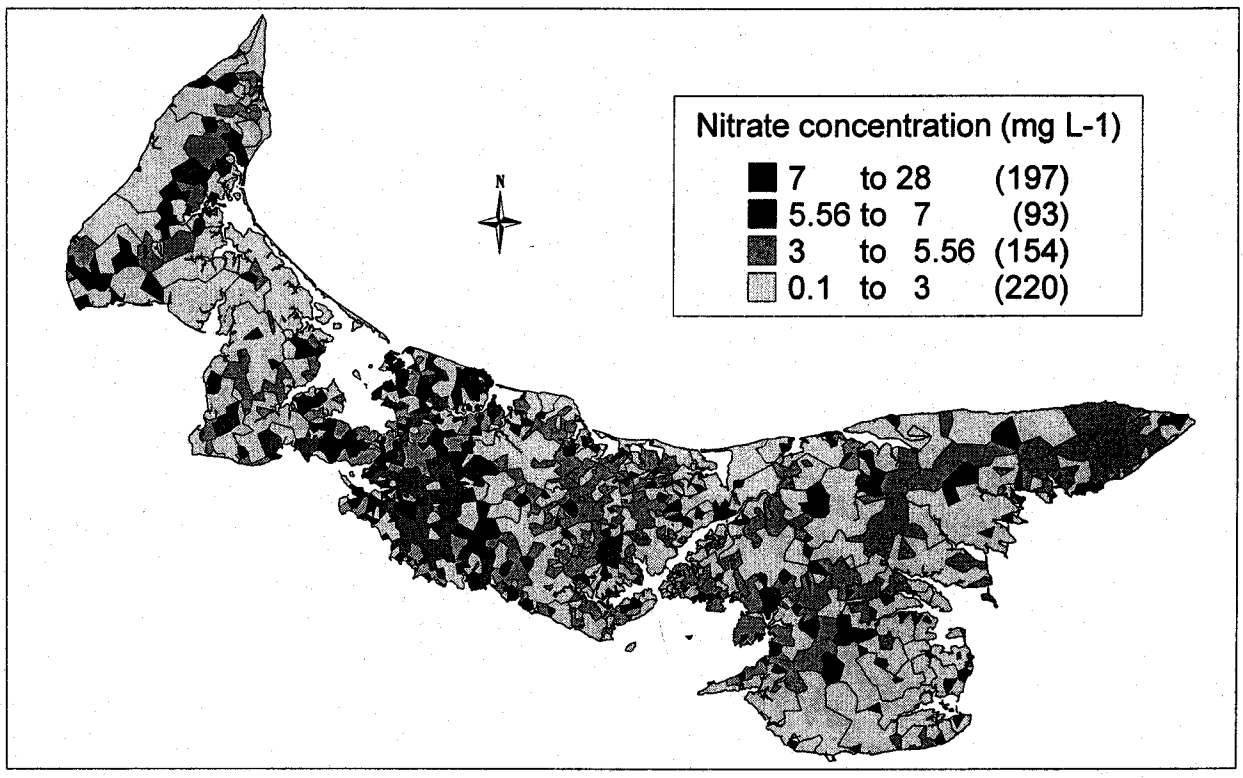


Figure 3-5 Predicted values of ground water nitrate concentrations versus percentage of grain coverage for interquartile ranges of percentage of hay, as found in buffer zone analyses in Prince Edward Island, Canada, 2001.

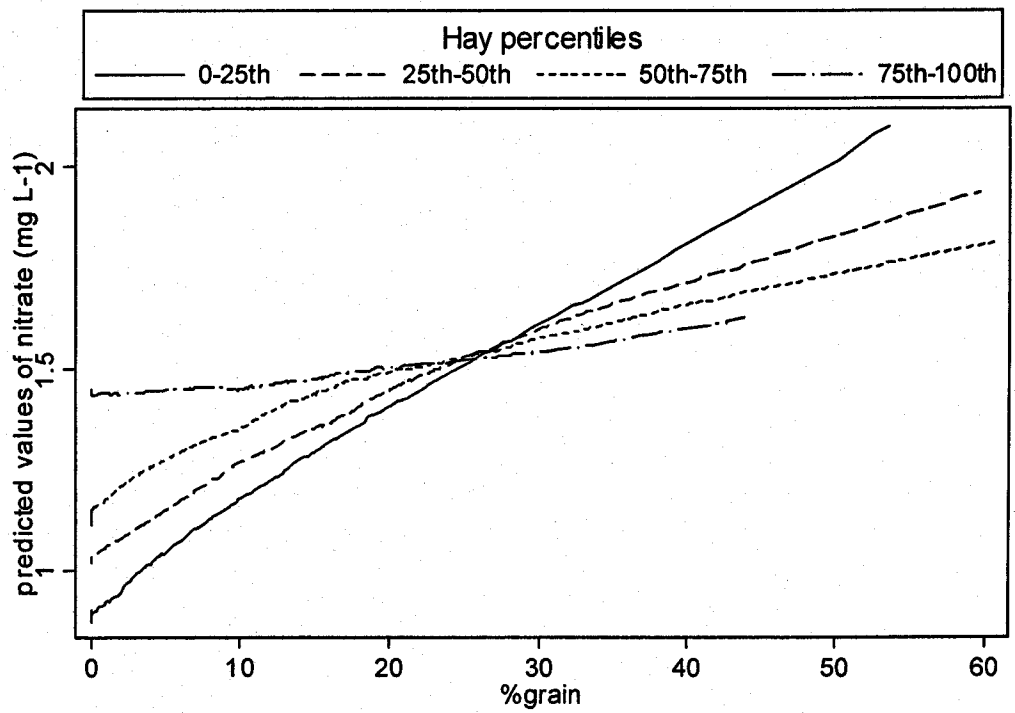
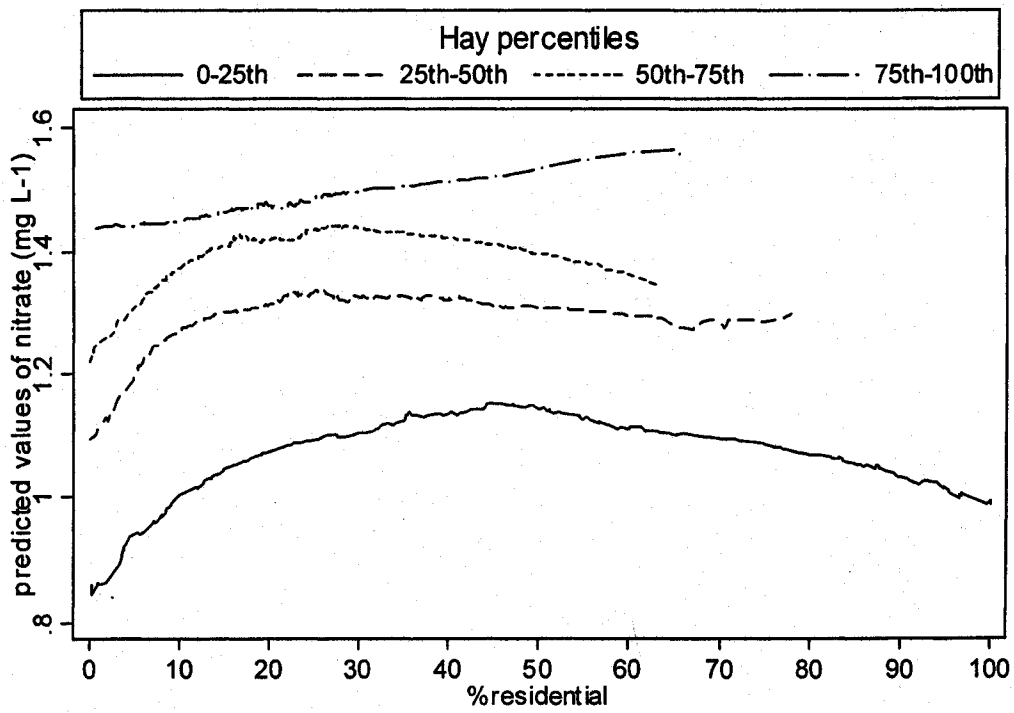


Figure 3-6 Predicted values of ground water nitrate concentrations versus percentage of residential coverage for interquartile ranges of percentage of hay, as found in buffer zone analyses in Prince Edward Island, Canda, 2001.



Authors: Benson VS, VanLeeuwen JA, Sanchez J, Somers GH, Feltbower RG, McKinney PA. Submitted.

Chapter 4 Bayesian analysis of nitrate in drinking water and the geographical variation of type 1 diabetes incidence in Prince Edward Island, Canada

4.1. Abstract

Limited evidence exists for an aetiological association between nitrates in drinking water and the risk of type 1 diabetes mellitus (T1D) in young people. Incidence of T1D is known to be high in Prince Edward Island, Canada and we aimed to investigate the relationship between T1D and drinking water nitrate concentrations using a Bayesian analysis. A total of 223 people (0–25 years) diagnosed with T1D from 1990 to 2004 were obtained from the provincial diabetes registry. Standardized incidence rate ratios (SIRRs) were estimated for each of 166 watersheds. Average drinking water nitrate levels were estimated for each watershed based on direct sampling methods. SIRRs and water nitrate levels were analysed employing hierarchical Bayesian methodology using a convolution model, adjusting for average household income. The incidence rate of T1D was 33.1 cases per 100,000 person-years, and males were more frequently diagnosed than females. Average nitrate concentration was not a significant predictor of T1D SIRRs at the watershed level. Controlling for income increased the strength of association between T1D SIRRs and nitrate concentrations in the high nitrate group (5.61–10.00 mg L^{-1}), but not significantly. A total of 75% of the random variation in SIRRs was attributable to unstructured (non-spatial) variation, with the remainder attributable to structured (spatial) variation. Neither average drinking water nitrate concentration nor income explained the

geographical variation of T1D incidence in young people at the watershed level. Larger studies including measures of exposure for individuals are necessary to explore this issue further.

4.2. Introduction

Type 1 diabetes mellitus (T1D) is an immune mediated disease, caused by the destruction of the insulin-producing pancreatic β-cells, occurring mainly in childhood (Leslie and Elliot, 1994). It affects 0.5-1% of the total population in developed countries during a life-time, causing approximately 10% of all people with diabetes (Rewers and Klingsmith, 1997). The aetiology of T1D remains unclear, and the relationship between genetic susceptibility and environmental exposures is complex (Todd, 1991; Todd, 1999). Evidence for an environmental aetiology is found in the low concordance rate for monozygotic twins (Kyvik et al., 1995), rapidly increasing incidence rates on a worldwide scale (Green and Patterson, 2001; Onkamo et al., 1999), small scale geographical heterogeneity (Staines et al., 1997; Schober et al., 2003), and migrant studies (Bodansky et al., 1992).

Nitrate has been considered as a factor leading to the onset of T1D because it can be converted to nitrosamines. The toxic properties of nitrosamines were first discovered in the 1960s when streptozotocin was specifically used as a toxin to induce T1D in animal models by damaging the pancreatic β-cells (Rakieten et al., 1963). Nitrate is reduced to nitrite in the stomach, which in turn produces nitrosamines from a nitrosation reaction between nitrites and amines (L'Hirondel and L'Hirondel, 2002). Nitrate exposure originating from both food (Dahlquist et al., 1990) and drinking water (Kostraba et al., 1992a; Parslow et al., 1997) has been significantly and positively associated with T1D in some studies, although other studies have reported no relationship (Moltchanova et al., 2004; Schober et al., 2003; Van Maanen et al., 2000; Virtanen et al., 1994).

The incidence of T1D in Prince Edward Island (PEI) may be high compared to the rest of Canada, and the world (Diabetes Epidemiology Research International Group, 1988). Using Bayesian methodology we aimed to determine if: 1) the incidence of T1D is associated with ground water nitrate concentrations averaged at the watershed level in PEI, adjusting for income; and, 2) the incidence of T1D is spatially clustered at the watershed level in PEI.

4.3. Methods

4.3.1.Study Site

Situated on the country's east coast, PEI is the smallest Canadian province. The local geology consists mainly of fractured sandstone bedrock overlain by a thin layer of fine sandy loam soils. This very porous top layer allows the ground water to be susceptible to contamination, especially due to the island's high water table and high ground water recharge rate (Agriculture and Agri-food Canada, 2003; InfoPEI, 2005; PEI Dept. Environmental Resources, 1994; Somers, 1998). An estimated 20,000 to 25,000 rural private wells supply water to over half of the population in PEI. The remainder of the population obtains drinking water from the same ground water source, but this supply is serviced by central water supply systems (Young et al., 2002). Ground water nitrate concentration in PEI varies geographically, but less so temporally, unless there is a local land use change (Somers, 1998; Chapter 3).

4.3.2. Data collection and handling

Nitrate-nitrogen (hereafter referred to as nitrate) concentrations were collected

from two database sources: privately owned wells and central water supply systems. A total of 4,855 water samples were collected from private wells during 1997–2001 as part of a routine pre-mortgage sampling program in PEI (geocoding for samples after 2001 was not available). In addition, 413 water samples were collected from 13 municipal systems during 1997–2004. Nitrate concentrations were determined using flow injection analysis colorimetry (using the QuikChem Series 8000, FIA+ instrument [Lachat Instruments, Milwaukee]) with a minimum detection limit of 0.10 mg N L^{-1} (Appendix A).

As no annual or seasonal variations were observed in nitrate values for the private well water samples, all nitrate samples from a minimum of five wells were averaged at the watershed level. If a watershed contained less than five private well samples, it was combined to a neighbouring watershed with a similar nitrate concentration (ie. did not differ by > 2.50 mg L^{-1}). This reduced the number of watersheds to 166 (from 260). Average nitrate concentrations were calculated for the 166 watersheds, weighted on the population distribution of each watershed between urban and rural homes.

The weighted average nitrate values at the watershed level were categorized into three distinct groups (0.10–3.00, 3.01–5.60, and 5.61–10.00 mg L^{-1}). These groups were chosen based on the human affected value of 3.00 mg L^{-1} (Burkart and Kolpin, 1993), whereby anything greater than this value is thought to have been influenced by human activities (McKay et al, 2001). In Europe, 5.60 mg L^{-1} has previously been recommended as a value that concentrations should not exceed, and 10.00 mg L^{-1} is recognized worldwide as the maximum acceptable concentration, above which, human health concerns are documented (Health Canada, 1992).

Type 1 diabetes in PEI is diagnosed according to guidelines developed by the Canadian Diabetes Association (Meltzer et al., 1998; Canadian Diabetes Association, 2003). The provincial Diabetes Registry contains all PEI inhabitants diagnosed with T1D who request provincial subsidization for insulin costs and diabetes education. According to the registry, a total of 244 people (ages 0–25 years) were diagnosed with T1D between 1990 and 2004. All cases were manually assigned to a watershed using the following information collected at time of diagnosis: postal codes, lot numbers (large municipal area aggregation) and civic addresses, where available. Not enough information was available to determine the appropriate watershed for 21 cases, therefore these cases were omitted from the study.

The population-at-risk for each watershed for the study period could have come from census data from 1991, 1996, and/or 2001. As no smaller censuses occurred annually between the aforementioned census years, and enumeration areas changes substantially between census years, population interpolation for the entire study period was not possible. Therefore, the census data from 1996 were obtained, being the approximate mid-point during the series of case data and used to calculate incidence rates for each watershed in PEI.

Census data were obtained at the enumeration area (small population boundaries), and re-aggregated at the watershed level using Mapinfo Professional v7 (MapInfo Corporation, 2002). The aggregated population and case data were used to calculate age- and sex-standardized incidence rate ratios, using five-year age intervals, for each watershed. Standardized incidence rate ratios (SIRRs) were calculated using (O_i/E_i)*100,

whereby O_i represented the observed number of cases for watershed i, and E_i represented the expected number of cases for watershed i, based on indirect standardization of rates.

Average household income was taken to represent socioeconomic status (SES) (Brown et al., 2005). Income was obtained from the extended 1996 census, which was completed by 20% of the population. Average income for each watershed was determined by calculating an average income for all adults (≥ 16 years old) in each enumeration area. Then, the population of adults in each enumeration area was used to create an overall weighted average per watershed. Average income was divided by 10,000 to make model coefficients of a similar order of magnitude, and enable model convergence.

4.3.3. Statistical analysis

The age and sex distribution among cases was assessed using a Chi-squared distribution. A Poisson regression was used to adjust for the population-at-risk, and to assess a possible age-sex interaction.

As T1D is a 'rare' disease, 117 of the 166 watersheds used for the analysis had zero cases, producing unstable SIRRs in many watersheds. Therefore, a hierarchical random effects model (convolution model) was implemented using the Bayesian framework in WinBUGS1.4 (Spiegelhalter et al, 2003) to evaluate the association between SIRRs and nitrate, before and after adjusting for income. The convolution model (Besag et al., 1991; Lawson et al., 2003) is represented by the following model formula:

$$ln(\theta_i) = \alpha + \beta' x_i + v_i + u_i$$

where the log relative risk ($ln(\theta_i) = ln(O_i/E_i) = lnO_i - lnE_i$, α is the intercept of the log relative risk (θ_i) for T1D in watershed i, x_i represents a vector of covariate values associated with watershed i, β is the associated parameter vector, and v_i and u_i represent random effects measuring spatial association ('between' watershed variation of neighbours) and over-dispersion (non-spatial 'between' watershed variation), respectively.

Non-informative priors with a normal distribution of mean 0 and inverse variance 0.0005, were assigned to all fixed effects, including the intercept. The unstructured random effects were assigned a normal distribution with mean 0 and inverse variance [λ]. The spatial structure was modeled assuming a conditionally autoregressive structure as described elsewhere (Besag et al., 1991; Lawson et al., 2003; Banjeree et al., 2004). The structured random effect models the log relative risk of watershed i (θ_i) conditional on the risks in all other neighbouring watersheds ($i \neq j$) as defined by a proximity matrix weights. The neighbourhood weights (w_{ij}) were binary, so that $w_{ij} = 1$ for watersheds with a common boundary, and $w_{ij} = 0$ otherwise. In our study, any given watershed had an average of four neighbours. Because no prior information was available on the variation of the relative risks of T1D across PEI, gamma(0.001, 0.001) and gamma(0.1, 0.1) non-informative prior distributions, known as hyper-priors, were assigned to [λ] and [τ], respectively, as suggested by Best and Waller (1999).

Three Gibbs sampler chains, starting from different initial values, were run for 80,000 iterations to assess model convergence to the same posterior distribution. Conversion was assessed by visual inspection of the chain path and by using the Gelman–

Rubin statistic (Brooks and Gelman, 1998). Convergence was met and considered stable after an initial burn-in of 30,000 iterations. The remaining 50,000 iterations were used for estimating SIRRs of T1D at the watershed level. Finally, to reduce autocorrelation, a thinning factor of 10 was employed to estimate the posterior parameter estimates (therefore every 10^{th} iteration was estimated).

The standard deviation (SD) of the structured and the unstructured random effects for each watershed was monitored at each iteration of the Markov Chain Monte Carlo sampler, and the relative importance of the structured random effects were computed as: $SD_v / (SD_v + SD_u)$, where SD_v and SD_u were the empirical marginal standard deviations for the structured and unstructured random effects, respectively (Best and Waller, 1999).

A sensitivity analysis was conducted based on different prior specifications to verify hyper-prior selection ([λ] and [τ]) and robustness of the model. In one model, the prior distribution of the structured random effects was changed to a more robust conditionally autoregressive structure (CAR.L1) (Best and Waller, 1999), which used a median rather than a mean SIRR of the neighbouring watersheds, with the same hyper-prior distribution (gamma[0.1, 0.1]) as the original model, and no change in the unstructured component. Another model consisted of changing hyper-priors so that the variances of both random effects were the same (gamma[0.5, 0.005]), as suggested by Lawson et al. (2003).

4.4. Results

A total of 223 people aged 0–25 years were diagnosed with T1D in PEI from 1st January 1990 to 31st December 2004, producing a mean of 1.3 cases per watershed

(median = 0, max = 25). Males were more frequently diagnosed with T1D than females ($p = 0.008$), however, this was somewhat dependent on age (Table 4-1). When the effect of gender was assessed for each age group separately, males were more frequently diagnosed in the 10–14-year old group ($p = 0.002$), and females peaked in the 5–9-year old group, but this was not significant ($p = 0.58$). When adjusting for the population-at-risk, the age-sex interaction was marginally significant ($p = 0.096$), confirming that the effect of sex does depend somewhat on age, and vice-versa. During 1990–2004, the incidence rate of males and females was 39.5 and 26.4 per 100,000 person-years, respectively, with a combined incidence rate of 33.1 cases per 100,000 persons-years, for children and adolescents up to age 25. For children age 14 years and under, the combined incidence rate was 37.0 per 100,000 person-years (43.9 and 29.9 per 100,000 person-years for males and females, respectively).

Average nitrate concentration at the watershed level was 3.37 mg L^{-1} (median = 3.13 mg L^{-1}), with an interquartile range of 2.17–4.30 mg L^{-1} (max = 9.56 mg L^{-1}). A total of 11% of the watersheds and 6% of the cases were present in the high nitrate group (5.61–10 mg L^{-1}) (Table 4-2). Average income was $19,840CAD (median = $19,800), with an interquartile range of $18,436–21,099 (max = $25,935).

Table 4-3 shows the unadjusted and adjusted medians for nitrate concentration and income from the convolution model. Results show that drinking water nitrate concentrations were not significantly associated with T1D, however, there was a trend toward the high nitrate group (5.61–10.00 mg L^{-1}) having a higher SIRR relative to the lowest nitrate group (0.10–3.00 mg L^{-1}). Adding income to the model increased the coefficient of the highest nitrate group, but not enough to consider income as a

confounder. Although not significant, income had an inverse association with T1D SIRRs. The Deviance Information Criterion (DIC) for the model with only the unstructured component was 318.4, and was not substantially improved when the structured variation was added to the model (DIC = 318.9), suggesting that structured variation at the watershed level was low. Structured variation alone however had a DIC of 341.2 showing that the fit of the model improved once the unstructured variation was included in the model.

Structured and unstructured random effects were assessed before and after adjusting for the other covariate, and were found not to change. An average of 25% (95% credible interval: 13, 43) of the variation was explained by the spatial structured variation, with the remainder of the variation occurring through unstructured random effects. Unexplained spatial variation was greatest in the northwest and northeast locations of PEI, where the population was sparser (Figure 4-1).

4.5. Discussion

Our ecological study evaluated the association between drinking water nitrate concentration and T1D incidence rates, employing Bayesian methods to overcome the problem of sparse disease counts within watershed areas and to take into account both structured (spatial) and unstructured (non-spatial) random effects. No significant association between T1D incidence and drinking water nitrate concentration was detected. Our study was consistent with ecological studies from The Netherlands (Van Maanen et al., 2000), Austria (Schober et al., 2003), Sardinia (Casu et al. 2000,) and Finland (Virtanen et al., 1994; Moltchanova et al., 2004) which also found no significant

association. More recently, a significant inverse association between TD incidence and nitrate in drinking water was reported in Sardinia (Muntoni et al., 2006). Positive findings have been reported in Yorkshire, England (Parslow et al, 1997) and Colorado, USA (Kostraba et al., 1992a), where nitrate was above 14.85 mg L^{-1} NO_3 ($\approx$ 3.30 mg L^{-1} NO_3-N) and 0.77–8.20 mg L^{-1} NO_3-N, respectively. Our study noted that concentrations above 5.60 mg L^{-1} NO3-N showed a slight trend towards increasing T1D incidence rates.

Descriptive statistics showed that in PEI, males were significantly more frequently diagnosed with T1D than females. This observation has been noted in other countries with high T1D incidence (Green et al., 1992). Among diagnosed females in PEI, there was a noticeable peak in incidence in 5–9 year olds, whereas incidence in males peaked at an older age of 10–14 years. Other studies have noted a peak in incidence in the 10-14 year old group (Kostraba et al., 1992b), and age at diagnosis may be earlier in females than males (Staines et al., 1993). It is unclear what factors contribute to these peaks, however puberty is thought to be involved, as boys tend to enter puberty later than girls, and therefore have a later pubertal growth spurt (Staines et al., 1993).

A possible reason why no significant association between nitrate concentration and T1D incidence was found in PEI could be because there was insufficient power to detect any significant differences (a low number of cases). This therefore increased the likelihood of a Type II error because 117 watersheds had no cases of T1D (producing incidence rates of 0), and only 18 (11%) watersheds had average nitrate concentrations greater than 5.60 mg L^{-1}, adding limited variation to a small sample size. Also, nitrate concentration averaged at the watershed level did not exceed 9.60 mg L^{-1}, whereas at the well level, the maximum nitrate concentration was 27.50 mg L^{-1}, thus suggesting that the

nitrate value was somewhat 'smoothed' at the watershed level. In addition, there may have been some temporal misalignment, as the nitrate and income data were taken from five and one year, respectively, and extrapolated to the 15 years of T1D data. However, the number of cases diagnosed and the nitrate data were reasonably constant over their evaluated timeframes.

In PEI, T1D SIRRs showed a non-significant decreasing risk with increasing income, in contrast to other studies which have shown strong positive links between risk of T1D and levels of affluence (Parslow et al., 1997; Patterson et al., 2001). Our explanation for the current findings is that PEI is a rural low-income province of Canada, with an average household income of just under $20,000 in 1996. At the watershed level, the range in wealth was low and narrow ($14,890–25,935), therefore our data provided limited opportunity to assess any overall relationship between social circumstances and the incidence of T1D.

The differential diagnosis between type 1 and type 2 diabetes is sometimes difficult for teenagers and young adult patients where the clinical presentation may be confusing, with some individuals having type 2 diabetes but requiring insulin treatment. It is more common for type 2 diabetics to be misdiagnosed (Fagot-Campagna et al., 2000). However, T1D may be misdiagnosed as T2D in a very small proportion of patients. A power calculation was done to determine the possible impact of including or excluding the 16–25 year olds. It was estimated from previous knowledge that 98.5% of diabetic children under age 16 would have T1D, and 82% of diabetic people aged 16 to 25 would have T1D. Based on this calculation, inclusion of all ages would not jeopardize the power

of the study to detect significant associations between T1D and nitrate exposure, therefore all cases diagnosed during the study period were included.

Unstructured random effects, representing 75% of the random variation, were much larger than structured random effects. Therefore, the variation observed in T1D incidence was likely associated with covariates at a more local level than watersheds, such as the household or individual level. A study in Yorkshire found that the unexplained variation was, on average, equally divided between the two random effects (Feltbower et al., 2005). However, when the effects of deprivation (i.e. SES) were included in their model, structured variation reduced towards zero. Therefore, SES at the area level may be an important factor for T1D incidence.

The sensitivity analysis showed that the model used was suitable, as fixed effect coefficients remained similar for all models. The proportion of structured variation ranged from 5–25% depending on the priors used, and the DIC for model fit did not substantially change depending on prior selection, thus suggesting that the selected priors could not be considered influential to the study findings.

Ecological fallacy, which arises from assuming that all individuals living within a watershed share the same characteristics of that area (Carstairs, 2000), is frequently a concern with ecological analyses. Ecological studies need to be interpreted with caution because fixed effects estimated at the small area level may not necessarily agree with those at the individual level.

4.6. Conclusion

In our study, drinking water nitrate concentrations and income averaged at the watershed level were not significant predictors of T1D SIRRs. However, controlling for income increased the apparent strength of association between T1D SIRRs and nitrate concentrations in the highest nitrate group (5.61–10.00 mg L^{-1}). A total of 25% of the unexplained variation present at the watershed level was spatially related, but significant spatial clustering was not found. The remaining 75% of variation was due to non-spatial 'between' watershed variation, the unstructured effects. These unstructured random effects did not change when average nitrate concentration and income were added to the model, suggesting that other factors are contributing to the geographical variation of T1D in PEI. Further research is needed to clarify why T1D diabetes incidence is high in certain geographical areas in PEI.

4.7. References

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Table 4-1 Age and sex distribution of type 1 diabetes incidence risk (years 1990–2004) and the population at risk (year 1996) in Prince Edward Island, Canada.

Age (years)	Male	Female	Total
	No. (%)	No. (%)	No. (%)
	Type 1 diabetes		
0–4	20 (15)	10 (11)	30 (13)
5–9	27 (20)	31 (34)	58 (26)
10–14	40 (30)	16 (18)	56 (25)
15–19	21 (16)	17 (19)	38 (17)
20–25	25 (19)	16 (18)	41 (18)
Total	133 (60)	90 (40)	223
	Population at risk		
0–4	4564 (18)	4258 (17)	8822 (18)
5–9	4869 (19)	4826 (20)	9695 (20)
10–14	5093 (20)	5022 (21)	10,115 (20)
15–19	5044 (20)	4862 (20)	9906 (20)
20–25	5536 (22)	5358 (22)	10,894 (22)
Total	25 106 (51)	24 326 (49)	49 432

Table 4-2 The number of watersheds and type 1 diabetes (T1D) cases diagnosed during 1990 – 2004, by nitrate group, in Prince Edward Island, Canada.

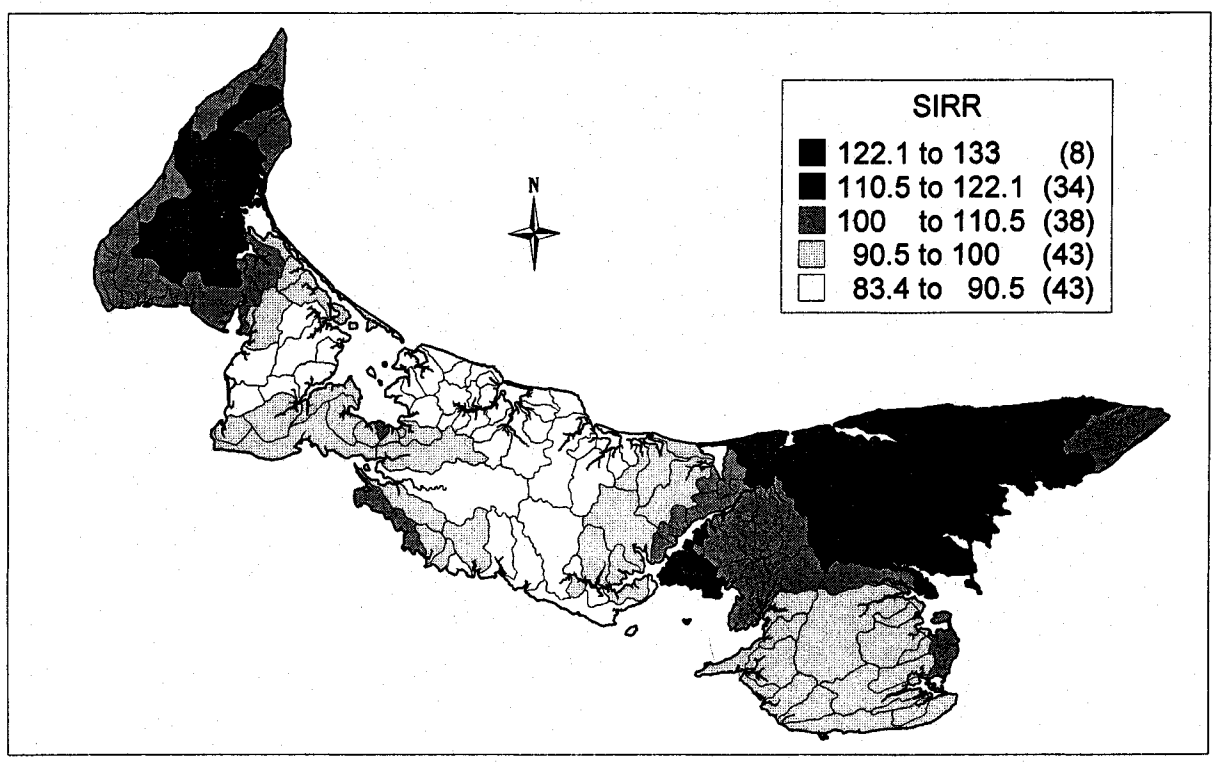
Nitrate (mg L^{-1})	Number of T1D cases (%)	Number of watersheds (%)
0.1–3.0	106 (47.5)	78 (47.0)
3.01–5.6	104 (46.6)	70 (42.2)
5.61–10.0	13 (5.9)	18 (10.8)

Table 4-3 Median and 95% credible intervals for fixed (nitrate concentration and income) and random effects from a hierarchical Bayesian convolution model averaged at the watershed level, Prince Edward Island, Canada (1990–2004).

Covariate	Model A: Nitrate		Model B: Income		Model C: Adjusted[a] Estimate	
	Coefficient	95% Credible Interval	Coefficient	95% Credible Interval	Coefficient	95% Credible Interval
Fixed Effects						
Nitrate concentration						
0–3.00 mg L^{-1}	100[b]				100[b]	
3.01–5.60 mg L^{-1}[c]	83.2	42.4, 164.0			86.7	43.7, 168.2
5.61–10.00 mg L^{-1}[c]	115.7	36.2, 371.0			133.1	40.2, 436.2
Income ($CAD)[d]			44.0	11.2, 164.3	42.2	8.9, 176.8
Random Effect						
Sigma_U[e]	1.13	0.79, 1.56	1.10	0.75, 164.3	1.13	0.80, 1.54
Sigma_V[f]	0.36	0.17, 0.90	0.36	0.17, 0.90	0.37	0.18, 0.78
Proportion of total[g] variation (%)	24.3	12.5, 42.9	24.5	12.8, 43.3	24.6	12.8, 43.3

[a] One covariate adjusted for the other covariate in the model.
[b] Reference category.
[c] Median change in standardized incidence ratio relative to the referent category.
[d] Change in standard incidence ratio for each $10,000CAD change in income.
[e] Standard deviation of the unstructured random effect.
[f] Standard deviation of the structured random effect.
[g] Percentage of structured variation.

Figure 4-1 Spatially structured standardized incidence rate ratios (SIRRs) for type 1 diabetes diagnosed at the watershed level during 1990–2004 in Prince Edward Island, Canada (number of watersheds in each category of SIRRs are shown in parentheses).



Chapter 5 Food consumption and the risk of type 1 diabetes: A population-based, case-control study in Prince Edward Island, Canada

5.1. Abstract

The aim of this study was to determine if the consumption of certain foods or food groups during the year prior to diagnosis, were associated with the risk of developing type 1 diabetes mellitus (T1D), adjusting for other environmental risk factors, where appropriate. Cases consisted of all newly diagnosed patients with T1D that registered with the Provincial Diabetes Registry during 2001 to 2004. Controls were randomly selected from the province's population, and matched to cases by age at diagnosis and sex. Face-to-face interviews were conducted with all participants (or their guardian or care-giver) to complete one of two age-appropriate food frequency questionnaires (FFQ) and a survey collecting information on other possible environmental risk factors. The age at diagnosis was nine years old, and 67% were male. For individual foods that were common to both FFQs (n = 57), the final multivariable logistic regression model showed that regular soft drinks (OR = 2.78, 95% CI = 1.21, 6.36) and eggs (OR = 2.50, 95% CI = 1.09, 5.75) were significant predictors of T1D, when consumed once per week or more often. Total beverage consumption (when consumed five or more times per day) was the only significant food group (OR = 0.32, 95% CI = 0.13, 0.77), and was inversely associated with T1D incidence. Other risk factors significantly associated with T1D were: a family history of T1D, father's education, infections during the first two years of life, and residential remoteness. For foods that were on the FFQ completed by participants aged nine years and older, the final model included popcorn (OR = 8.39, 95% CI = 2.28, 30.86), plain chocolate (OR = 4.14, 95% CI = 1.09, 15.67), and peanut butter

sandwiches (OR = 5.01, 95% CI = 1.53, 16.40) eaten at least once weekly as significant positive predictors of T1D incidence. Muffins eaten at least once per week were significantly protective (OR = 0.22, 95% CI = 0.05, 0.95). Further research is needed to confirm these observed associations.

5.2. Introduction

Type 1 diabetes mellitus (T1D) is an immune-mediated disease caused by the destruction of the pancreatic β-cells (Leslie and Elliot, 1994), typically in young people. In developed countries, it contributes to approximately 10% of all diabetic cases, affecting 0.5-1% of the total population during a life-time (Rewers and Klingensmith, 1997). Although a genetic predisposition is thought to be necessary for the development of the condition, environmental factors are believed to trigger the onset of disease (Todd, 1991). Evidence for an environmental etiology is found in the low concordance rate between monozygotic twins (Kyvik et al., 1995), rapidly increasing incidence rates on a worldwide scale (Onkamo et al., 1999; Green and Patterson, 2001), small scale geographical heterogeneity in incidence rates (Staines et al., 1997; Schober et al., 2003) and changing incidence rates in migrant studies (Bodansky et al., 1992).

Experimental studies on the Biobreeding rat and non-obese diabetic mouse demonstrated that their diet plays an essential role in development of diabetes, more specifically, protein intake (Elliott et al., 1988; Scott et al., 1997). Previous human studies have shown that nutritional factors can be both promotive (such as protein and nitrosamines) (Dahlquist et al., 1990; Helgason et al., 1992) and protective (such as vitamins D and E) (Knekt et al., 1999; Hyppönen et al., 2001) factors of T1D, but are typically studied at the nutrient level rather than at the whole food level. Assessment at the food level (both individual foods and food groupings) allows for a general assessment of nutrients and other compounds in foods, as well as being able to control for possible 'within' food interactions among nutrients. Dahlquist et al. (1990) discovered in a case-control study that nitrosamines influenced T1D risk only when vitamin C was in low concentrations.

The aim of this study was to determine if there were any differences in the frequency of consumption of individual foods and food groupings between T1D patients during the year prior to diagnosis and two matched controls the year prior to interview, adjusting for other environmental risk factors, where appropriate.

5.3. Materials and methods

Ethical approval was granted by the University of Prince Edward Island Ethics Committee before commencement of the study. All participants signed a consent form prior to participation, and had the right to withdraw from the study at any time.

5.3.1. Study population

Since 1962, the Provincial Department of Health and Social Services in Prince Edward Island (PEI), Canada, has recorded all diabetic patients residing in PEI in the Diabetes Registry. All people listed on the Diabetes Registry are also on the Provincial Medicare Registry. The Diabetes Registry was considered a reliable source of T1D incidence in the province because only those who were registered would receive government funding for medications and urine testing kits (Tan et al., 1983). In order to be on the registry, a doctor's diagnosis of T1D and prescription for insulin were required, with the diagnosis following guidelines set out by the Canadian Diabetes Association (Meltzer et al., 1998; Canadian Diabetes Association, 2003). All persons newly diagnosed with T1D in PEI during 2001 to 2004 were eligible to participate in the study as cases.

In order to obtain government funded health care, the Provincial Medicare Registry contains all persons residing in PEI, and has been frequently used for population-based

studies within the province. Two controls per case were randomly selected from this Registry, matching on age at diagnosis (within one year) and sex. Parents of study participants were initially contacted via a letter inviting them to participate, followed by a phone call to confirm their participation. If a control declined, then another was contacted in order to obtain two controls per case. If the study participant was 17 years of age or older, they were contacted directly because parental permission was not required.

5.3.2. Data collection

A face-to-face interview was conducted with each participant. All participants (or their guardian) completed two questionnaires: a food frequency questionnaire (FFQ) determining food consumption during the year prior to diagnosis (previous year for controls), and a pre-piloted risk factor questionnaire (RFQ) gathering demographic, social, and familial information for the year prior to diagnosis (previous year for controls) and during the participants' early years of life.

Two different FFQs were used to assess food consumption. Participants aged nine and older completed a self-administered, modified version of Harvard's Youth Adolescent Food Frequency Questionnaire (YAQ) (Appendix B), described and validated elsewhere (Rockett et al., 1995; Rockett et al., 1997). The YAQ normally consists of a list of 127 foods, but bacon and sausage were added because they were expected to be consumed regularly and to possibly contribute to nitrate, nitrite, and nitrosamine intake, a parameter relevant to Chapter 6.

For children less than nine years old, a surrogate reporter (usually the mother) completed the Harvard Service Food Frequency Questionnaire (HS-FFQ) (Appendix C),

explained and validated elsewhere (Blum et al., 1999). The HS-FFQ contained 85 questions pertaining to the frequency of food consumption. For children who ate a substantial amount of food away from home, such as at a day-care or at a relative's home, the care-giver also filled out a HS-FFQ. For children with more than one HS-FFQ completed, total food intake was assessed by summing the mid-value from each frequency category from each questionnaire. For example, if peas were eaten 2–4 times a week at home and at day-care, then peas were recorded as being eaten 6 times a week, therefore fitting into the 5–6 times per week category.

A total of 57 foods were common to both FFQs. Foods that were different between the two FFQs were predominantly composite foods (for example, sandwiches in the YAQ versus bread in the HS-FFQ), or when foods were grouped into a slightly different category in each FFQ (for example, popcorn and pretzels were separate in the YAQ, but combined in the HS-FFQ).

The RFQ (Appendix D) included questions related to the following: family members with T1D or type 2 diabetes (T2D), breast-feeding duration and cow's milk-based formula intake frequency under three months of age, day-care attendance under age three, and the number of infections in early childhood. Other questions pertaining to exposures in the year prior to diagnosis (year prior to interview for controls) included: the number of infections during the year prior to diagnosis (during the year prior to interview for controls), the number of smokers in the household, stressful life events, average household income, highest level of education obtained (primary, secondary, college, and university), occupational history of the participant and their parents, and location of residence (urban, rural, farm). The responses to all questions were self-reported.

5.3.3. Statistical analysis

The YAQ required participants to categorize food consumption into one of four-to-six categories ranging from 'never/less than once per month' to 'four or more times per day', with the nature and number of categories depending on the food. The HS-FFQ required participants to categorize food consumption into one of nine categories ranging from 'less than once per month' to 'six or more times per day', with all foods having the same response categories. For regression analysis (described below) of individual foods, all foods were dichotomized as 'weekly' (or more frequently) versus 'less than weekly' consumption, except for bread, cheese, yoghurt, milk, and orange juice, which were dichotomized as 'daily' (or more frequently) versus 'less than daily', as these foods were consumed more often by the participants.

Foods were aggregated into the following groups (Appendix E): total fruit and vegetable, total vegetable, total fruit (including pure fruit drinks), fresh fruit, milk and milk products, fats, grain, meats and alternatives, high sugar/high fat foods, total beverages, and other foods. The food groupings were classified according to significant nutrient content.

A total of 60% of the participants completed the YAQ and 40% completed the HS-FFQ, and there were numerous food items on the YAQ that were not on the HS-FFQ. Therefore, two sets of regression analyses were conducted: an analysis of all participants but only for the 57 foods common in both FFQs, and an analysis for all foods but only for the 97 participants who completed the YAQ.

For each analysis, unconditional logistic regression, adjusting for age, sex, and FFQ was carried out in Stata 9 (StataCorp, 2005) to estimate the odds ratios (OR) and 95% confidence intervals (CI) of significant factors associated with T1D incidence. Variables

from the FFQs and the RFQ with an unconditional association of $p \leq 0.2$ were evaluated in a multivariable logistic regression model using both a manual forward and backward elimination process. Confounders were checked throughout the model building process, and interaction terms built from variables within the final model were assessed. Regression diagnostics and predictability of the final model were also assessed and found to be suitable.

A dose-response relationship was evaluated for each individual food with a significant unconditional association ($p \leq 0.2$), as well as those foods significant in the final multivariable models. For these analyses, food consumption was categorized into either 'less than weekly', 'weekly', or 'more than weekly' consumption, or 'less than daily', 'daily', or 'more than daily' consumption, depending on the food. The dose-response relationship of all significant food groups was evaluated by the following process: Frequency of consumption of individual foods was converted to a frequency per week (for example, twice per week = 2/7 = 0.29). These standard frequencies were summed, and then the totals were reclassified into categories based on quartiles of the control group. The frequency groupings used for the individuals foods and food groupings were converted into an ordinal variable (e.g. < weekly = 0, weekly = 1, > weekly = 2) and the dose-response relationship of this ordinal variable with T1D was determined by a likelihood ratio test.

5.4. Results

Over the four-year study period, 74 cases were registered and 187 controls were contacted, of which 57 cases and 105 controls agreed to participate. Response rates for cases were higher for children under age nine years old who completed the HS-FFQ (95.7%) compared to participants who completed the YAQ (81.3%). For controls, response rates were

similar for both age groups (Table 5-1). Of all cases interviewed, the average age at diagnosis was 9.6 years old, with a median age of 9 years (interquartile range = 4 to 13). A total of 67% of the cases were male. Due to confidentiality, no information was available on the contacts that declined to participate, or were unable to be contacted.

Of those foods common between the two FFQs, 11 foods were unconditionally associated with T1D ($p \leq 0.2$) (Table 5-2). Total beverages consumed five or more times per day was the only significant food group variable (OR = 0.32, 95% CI = [0.13, 0.76], p = 0.01), whereas the intake of milk and milk products showed a protective and marginally significant dose-response (quartile OR = 1.00, 0.96, 0.83, 0.40, p = 0.12, respectively). Eight variables from the RFQ showed significant unconditional associations with T1D (Table 5-3).

The consumption of eggs, soft drinks, muffins, and hard candy showed significant (p < 0.05) or marginally significant (p = 0.05 - 0.2) dose-response associations when comparing 'less than weekly', 'weekly', and 'more than weekly' consumption. The consumption of orange juice and cheese were also marginally significant unconditionally, when comparing 'less than daily', 'daily', and 'more than daily' consumption (Table 5-4).

Interpretation of the final model of foods found in both FFQs and factors from the RFQ showed that if eggs were eaten once per week or more, then the risk of becoming a type 1 diabetic was increased by 2.5 times compared to eating eggs less than once per week (Table 5-5). The weekly or more frequent consumption of regular soft drinks (not diet) increased the risk of developing T1D by 2.8 times compared to drinking regular soft drinks less than once per week. A dose-response relationship when comparing 'less than weekly', 'weekly', and 'more than weekly' consumption was significant for regular soft drinks in the final model (OR = 1.0, 2.03, 4.02, p = 0.007). The risk of T1D was also significantly

associated with: third generation family members (grandparents, parents, siblings, parental siblings, and cousins) having T1D, the self-reported frequency of infections during the first two years of life, the father's education, and residential remoteness (self-report of living in a city, rural area, or on a farm).

According to the Pearson χ^2 goodness-of-fit test, the final multivariable model suitably fitted the data ($p = 0.61$). The predictive ability of the model was reasonable (area under the receiver operating characteristic curve of 81%), and there were no extreme residuals present.

For the participants completing the YAQ, unconditional associations showed that 19 foods were significantly associated ($p \leq 0.2$) with T1D risk, all of which were consumed once per week or more frequently, except for bread (once per day or more frequently) (Table 5-6). Dose-response relationship evaluation of unconditional associations showed that the consumption of peanut butter sandwiches, pasta, and regular soft drinks were significant and demonstrated positive associations when comparing 'less than weekly', 'weekly', and 'more than weekly' consumption, and muffins showed a significant protective association with T1D risk. The consumption of bread was also significant when comparing 'less than daily', 'daily', and 'more than daily' consumption (Table 5-7). The final multivariable model determined that if peanut butter sandwiches, popcorn, or plain chocolate were eaten at least once per week, then the risk of developing T1D significantly increased by 5.02, 5.47, and 3.60, respectively, whereas, the weekly consumption of muffins decreased T1D risk by 75% (Table 5-8). Father's education was the only environmental risk factor in the final model. Peanut butter sandwiches and muffins showed a significant dose-response relationship in the final model when comparing 'less than weekly', 'weekly', and 'more than weekly

consumption' ($p = 0.025$, and $p = 0.02$, respectively), with muffins decreasing the risk and peanut butter sandwiches increasing T1D risk. The dose-response relationships from the consumption of chocolate was positive and marginally significant ($p = 0.064$).

According to the Pearson χ^2 goodness-of-fit test, the final multivariable model suitably fitted the data ($p = 0.42$). The predictive ability of the model was reasonable (area under the receiver operating characteristic curve of 84%), and there were no extreme residuals present.

5.5. Discussion

It is widely believed that the etiology of T1D includes both environmental and genetic factors, and this study provides additional evidence. Very few studies have looked at individual food consumption in determining the etiology of a disease, especially T1D. This study is the first case-control study conducted in Canada assessing the relationship between T1D and diet during the year prior to diagnosis. This current study showed that food consumption during the year prior to diagnosis, in particular the frequent consumption ($\geq$ once per week) of regular soft drinks and eggs, may play a role in the etiology of T1D.

Due to the large number of foods evaluated, some foods could show a significant association with T1D incidence just by chance. Therefore, a dose-response relationship was assessed to determine if the foods with at least a marginally significant unconditional association ($p \leq 0.2$) could be true associations. For foods common to both FFQs, regular soft drinks, when categorized as 'less than once per week', 'once per week', and 'greater than once per week', showed a significant dose-response relationship ($p = 0.031$) in unconditional analyses, and this ordinal variable was highly significant in the final model ($p = 0.007$). The

dose-response for the consumption of eggs was marginally significant unconditionally (p = 0.15), and borderline significant in the final model (p = 0.07). Four other foods were found to have a possible dose-response relationship when categorized in the same way as regular soft drinks (hard candy, muffins, orange juice, and cheese), and these were marginally significant unconditionally (p = 0.14, 0.08, 0.12, and 0.06 respectively).

A possible link between frequent egg consumption and an elevated risk of T1D could be due to high protein consumption. Eggs are high in protein, and high levels of protein consumption have been linked to increasing T1D incidence in another case-control study (Dahlquist et al., 1990), as well as in animal models (Scott et al., 1985). Ovarian cancer has also been related to an increase in egg consumption (Pan et al., 2004), which has been hypothesed to be due to the high levels of cholesterol found in eggs (Pirozzo et al., 2002). The relationship of T1D and total cholesterol intake will be examined in Chapter 6. A previous study reported that a high consumption (> 75% percentile) of candy was associated with an increased risk of T1D, but found no association with soft drinks (Pundziūtė-Lyckå et al., 2004). The frequent consumption of orange juice may protect against the development of T1D due to its high content of vitamin C, an antioxidant. Antioxidants have previously been linked to a decrease in T1D risk due to their free radical scavenger capabilities, because oxidative stress is believed to contribute to β-cell destruction after autoimmune activation (Mandrup-Poulsen et al., 1993). No explanation for the protective role of muffins and T1D risk is known.

Because polydipsia is a known symptom of T1D and both regular and diet soft drinks were unconditionally associated with an elevated risk of T1D, total beverage consumption was assessed. Overall, controls actually drank significantly more beverages (five or more

cups per day) than cases (OR = 0.32, $p = 0.01$), but when water intake was removed from this total liquid consumption, the variable was no longer significant. Because water intake was not significant when comparing 'two or more cups' per day to 'less than two cups per day' (OR = 0.67, $p = 0.27$), the beverage consumption pattern observed was not due to water consumption alone.

The multivariable model built from the YAQ data included different foods than that generated using the combined sample dataset. Peanut butter sandwiches showed a significant dose-response relationship, possibly again due to the high protein (Dahlquist et al., 1990) content of peanuts, or wheat gluten (Scott et al., 1988; Dahlquist et al., 1990) in the bread. Bread consumed by itself rather than in sandwiches was a significant and positive predictor when eaten daily ($p = 0.010$), but it was not significant in the final YAQ model, suggesting that the peanut butter may be the food of concern, by itself or in combination with bread. Alternatively, in North America, peanut butter sandwiches typically contain jam, so perhaps the high sugar content in the jam may lead to T1D development.

In the final YAQ model, increased consumption of muffins showed an inverse association with T1D incidence. Our finding that chocolate and popcorn were significantly associated with an increase in T1D incidence was similar to a finding of the Swedish study, where snack foods were shown to significantly increase T1D incidence (Dahlquist et al., 1990). This association may also be, in part, due to recall bias, as type 1 diabetics were more likely to consume snack foods after T1D diagnosis to help regulate their glucose levels.

A dose-response evaluation of the YAQ foods showed that when comparing 'less than weekly', 'weekly', and 'more than weekly' consumption of foods, peanut butter sandwiches, regular soft drinks, muffins, and pasta were significantly associated with T1D

risk (p = 0.003, 0.049, and 0.043, respectively). Muffin consumption was significant and inversely associated with T1D risk (p = 0.018). The consumption of bread was also significant when comparing 'less than daily', 'daily', and 'more than daily' consumption (p = 0.009). Confidence intervals for the significant YAQ variables were large, suggesting imprecise estimates, so should be interpreted with caution. A high intake of carbohydrates has previously been shown to increase the risk of T1D (Dahlquist et al., 1990), and may play a role in this current study as bread, peanut butter sandwiches, soft drinks, and pasta, all high in carbohydrates, showed a significant dose-response with increased consumption. Carbohydrate consumption will be investigated in Chapter 6.

A family history of either T1D or T2D increased the occurrence of T1D, with a history of T1D being highly significant. Studies looking at only first degree relatives (parents and siblings) observed that the risk of T1D was significantly higher when a relative was previously diagnosed with T1D (Dahlquist et al., 1985; Moussa et al., 2005). A Swedish study (Dahlquist et al., 1989), also analyzed three generations of relatives, and reported an OR of 5.5 if a relative had been diagnosed with T1D, which was similar to our study (OR = 6.32, CI = [2.33, 17.10]). Other studies have also shown a higher risk of T1D incidence when T2D was reported among relatives (Dahlquist, 1985; Moussa et al., 2005), but not always (Altobelli et al., 1998). It is speculated that having a family history of either disease increases the risk of T1D, either because the two diseases are genetically related, or because similar environmental exposures are involved in both diseases (Šipetić et al., 2005).

Having at least five infections (self-reported) during the first two years of life was associated with a higher risk of T1D, but a dose-response relationship could not be evaluated due to the original grouping of infections in the questionnaire (0-5, 5-10, 10-15, or 15+

infections per year), as very few study participants had more than 10 infections per year. A higher frequency of infections during early childhood does not support the hygiene hypothesis (Parslow et al., 2001). However, the critical window for the development of the immune response is generally during the first year of life, and this current study included the first two years of life. A higher frequency of infections during the year prior to diagnosis of T1D is more commonly reported (Blom et al., 1991; Samuelsson and Stenhammar, 2005). This higher risk could be due to the increasing need for insulin in persons with frequent infections and already experiencing sub-clinical symptoms of T1D (Verge et al., 1994; Šipetić et al., 2005). Alternatively, enteroviruses may reduce insulin secretion by direct damage or inflammation to the β-cells (Roivainen, 2005).

Father's education, although highly significant in the final model, did not show a dose-response relationship with the risk of T1D incidence in either the YAQ or the combined FFQ datasets. Mother's education only showed a significant unconditional association with T1D incidence using all participants, and was somewhat correlated with the father's education ($\rho = 0.53$). When mother's education was controlled for, the ORs for father's education changed considerably in both the YAQ and the combined FFQ dataset, and the *p*-values remained similar (ORs of 1.0, 1.89, 0.65, and 5.21 for the combined dataset, and OR = 1, 2.07, 0.85, and 11.27 for the YAQ dataset for primary, secondary, college, and university education, respectively). It is probable that parent's education is a proxy for socioeconomic status (SES). An increase in the risk of T1D has been associated with high (Patterson and Waugh, 1992) and low (Crow et al., 1991; Parslow et al., 2001) SES, leaving confusion regarding the relationship. Diet may also be influenced by the educational level of parents, especially of the mother (Wachs, 2001). However, as the coefficients for the

mother's and father's education did not follow a similar association pattern with the risk of T1D, no plausible conclusions can be drawn.

Area of residence during the year prior to diagnosis played a role in the risk of T1D, whereby the more remote an area the participant lived in (farm< rural area < urban area), the lower the risk of T1D. This finding could possibly be explained by population density or SES. Although in general, PEI is not densely populated, a smaller percentage of the residents live in more rural areas of the province compared to more urban areas (Charlottetown and Summerside). A study conducted in Western Australia observed that rural areas had a lower incidence rate compared to urban areas, and this difference was independent of SES (Haynes et al., 2006). It was speculated that this finding was attributable to differences in environmental factors, and possibly population density. A study in Italy also reported a lower incidence of T1D in rural areas, and suggested that this may be due to differences in genetics (Cherubini et al., 1999). Conversely, an inverse relationship between T1D incidence and living in an urban area was reported in Finland (Rytkönen et al., 2003) and in North Yorkshire, England (Staines et al., 1997), and is likely related to population density. Alternatively, this effect may be due to SES, with the premise that less affluent residents live in more rural areas or on farms. A more accurate assessment of SES is needed to determine if SES is associated with T1D. Self-reported income categories (< $30,000CAD, $30,000 to $50,000, and > $50,000) were not associated with T1D incidence in our study ($p = 0.40$).

No association ($p > 0.2$) between breast-feeding or cow's milk-based formula and the risk of T1D was observed in our study, corroborating other studies (Siemiatycki et al., 1989; Marshall et al., 2004). However, a longer breast-feeding period (at least three months) has been associated with a lower risk of T1D (Verge et al., 1994; Šipetić et al., 2005), and an

early introduction to cow's milk-based formula (before three months of age) has been associated with a higher risk of T1D (Verge et al., 1994; Hyppönen et al., 1999). Further research is needed to further clarify these putative factors.

Surrogate reporting by parents is a valid recall method typically used for recording the diet of children under nine years of age (Klesges et al., 1987; Eck et al., 1989). Children older than age eight have reached the developmental age when they are cognitively aware of their food intake, and are able to accurately recall their diet over a short time period. It is not until age 12 that portion size and food recipes are more accurately reported (Livingstone et al., 2004). Other evidence suggests that children aged seven to 11 years of age, showed a considerable amount of knowledge regarding food detail (Sobo et al., 2000). Nonetheless, help from an interviewer or guardian/care-giver was given to the younger children who completed the YAQ.

Recall bias is frequently a concern in dietary assessment research. Typically, cases recall their current dietary intake instead of their intake prior to diagnosis. If recall bias were playing a role, then cases may have reported eating sugary foods less frequently than controls. In fact, some sugary foods were recorded as being eaten more frequently than controls, and some less frequently. If cases believed that sugary foods may have led to their development of T1D (a common assumption that diabetes mellitus is caused by high sugar intake), then cases may under-report sugary foods because they may think they should not have been eating them (Macdiarmid and Blundell, 1998). However, some sugary foods (soft drinks, chocolate bars, and raisins) appeared to be possible risk factors for the development of T1D.

Some sugary foods were consumed more frequently by cases than controls, there was a concern that some of the older T1D cases could actually be type 2 diabetics because 18% of the cases were older than age 15. The analysis was re-run omitting this age group, and similar coefficients were observed (although with slightly larger confidence intervals due to a lower sample number). Therefore, the high intake of some sugary foods among cases was not just associated with participants older than age 15.

Although obesity is a known risk factor of T2D (Rewers and Hamman, 1995), little research has investigated a possible connection with T1D. Previous studies have noted that accelerated growth (height and weight) at an early age increased the risk of being a type 1 diabetic (EURODIAB Substudy 2 Study Group, 2002; Pundziūtė-Lyckå et al., 2004), and a Finnish study linked obesity to an increased risk of T1D (Hyppönen et al., 2000). It could be that obesity increases the risk of T1D and not a direct influence from sugary foods. Unfortunately weight and height at time of diagnosis were not available for this study, so obesity could not be properly evaluated as a possible risk factor.

The families of the control population were compared to the provincial population with regards to social factors such as smoking, drinking, breast feeding, education level, and fruit and vegetable intake (Van Til, 2004). All measurable factors were similar between the two groups, therefore deeming the control population a representative sample of the base population.

Although significant associations were evident, there was not enough variability in the present sample within some categories of food to properly evaluate a dose-response relationship for some foods. These foods may have been significantly associated with T1D due to chance alone. A larger sample size could have provided more power to assess dose-

response relationships. Further research is needed to confirm or refute the role of some of the identified food factors. Also, nutrients need to be assessed to determine if the identified food factors are biologically plausible factors for developing T1D, based on their nutrient content. Nutrient analysis may also give more specific results than overall foods, and be more reflective of the possible effect of diet on T1D incidence.

5.6. Conclusion

Diet was associated with the risk of T1D. For all young people, the weekly (or more frequent) consumption of regular soft drinks significantly increased T1D risk. The weekly or more consumption of eggs also significantly increased the risk of T1D. Recall bias did not appear to influence the results. Risk factors present during the year prior to diagnosis (residential area) and in early childhood (number of infections) were significantly associated with T1D, as was a family history of T1D, and the level of education of the father. In youth older than age nine, the frequent consumption of peanut butter sandwiches, popcorn, and plain chocolate were positively associated with T1D incidence, while frequent muffin consumption was negatively associated with T1D incidence. Further research is needed to confirm these findings.

5.7. References

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Table 5-1 Participant response breakdown for cases diagnosed with type 1 diabetes during 2001–2004 and their matched controls, in Prince Edward Island, Canada.

Participation	Cases (*n*)	%	Controls (*n*)	%
	All study participants			
Contacted by letter	74		187	
Interviewed	57	86.4	105	73.4
Refused[a]	9	13.6	38	26.6
Total	66	100.0	143	100.0
Unable to contact[b]	8		44	
	Participants who completed the HS-FFQ[c] (< nine years of age)			
Contacted by letter	26		74	
Interviewed	22	95.7	43	76.8
Refused[a]	1	4.3	13	23.2
Total	23	100.0	56	100.0
Unable to contact[b]	3		18	

[a] Refused to participate
[b] Either moved house or unable to contact, or contacted and then lost contact
[c] Participants who completed the Harvard Service Food Frequency Questionnaire (age 8 and under)

Table 5-2 Unconditional associations ($p \leq 0.2$) between type 1 diabetes risk in children and youth and food items common in two food frequency questionnaires, in Prince Edward Island, Canada (2001–2004).

Food	Cases (%) $n = 57$	Controls (%) $n = 105$	Odds ratio[a] (CI[b])	p-value
Diet soft drinks[c]	16 (28)	14 (13)	2.52 (1.12, 5.71)	0.026
Raisins[c]	18 (32)	21 (20)	2.47 (1.03, 5.94)	0.043
Margarine[c]	38 (67)	53 (50)	1.96 (1.00, 3.84)	0.050
Eggs[c]	38 (67)	54 (51)	1.89 (0.97, 3.71)	0.062
Regular soft drinks[c]	31 (54)	41 (39)	1.90 (0.95, 3.78)	0.068
Muffins[c]	12 (21)	34 (32)	0.54 (0.25, 1.16)	0.114
Hard candy[c]	22 (39)	29 (28)	1.70 (0.83, 3.44)	0.145
Melon[c]	3 (5)	13 (12)	0.39 (0.11, 1.46)	0.162
Cold cereal[c]	40 (70)	84 (80)	0.59 (0.28, 1.25)	0.165
Orange juice[d]	11 (19)	30 (29)	0.57 (0.25, 1.27)	0.168
Cheese[d]	10 (18)	28 (27)	0.58 (0.26, 1.32)	0.195

[a] Adjusted for age and sex
[b] 95% confidence interval
[c] Dichotomized as weekly (or more frequently) versus less than weekly consumption
[d] Dichotomized as daily (or more frequently) versus less than daily consumption

Table 5-3 Unconditional associations ($p \leq 0.2$) between type 1 diabetes risk in children and youth and risk factor variables in Prince Edward Island, Canada (2001–2004).

Risk factor	Cases (%) $n = 57$[a]	Controls (%) $n = 105$[a]	Odds ratio[b] (CI[c])	p-value
Type 1 diabetic family[d] member	20 (36)	15 (14)	3.37 (1.57, 7.31)	0.002
Type 2 diabetic family member[d]	31 (56)	48 (46)	1.59 (0.81, 3.10)	0.177
Infections in early childhood[e]	18 (32)	21 (20)	1.93 (0.91, 4.05)	0.084
Infections in year prior[f]	14 (25)	12 (11)	2.53 (1.08, 5.95)	0.033
Fathers' education				0.013[g]
Primary	10 (17)	22 (21)	1.00	
Secondary	21 (38)	28 (27)	1.66 (0.65, 4.26)	
College	7 (13)	36 (35)	0.43 (0.14, 1.32)	
University	18 (32)	18 (17)	2.24 (0.82, 6.14)	
Mothers' education				0.131[g]
Primary	7 (13)	9 (9)	1.00	
Secondary	21 (38)	23 (22)	1.17 (0.37, 3.71)	
College	17 (30)	45 (43)	0.48 (0.15, 1.52)	
University	11 (20)	27 (26)	0.52 (0.15, 1.79)	
Same residence (n/y)[h]	46 (79)	97 (87)	0.34 (0.13, 0.92)	0.034
Residential area				0.035[g]
Urban	23 (39)	32 (29)	1.00	
Rural	33 (56)	58 (52)	0.73 (0.36, 1.48)	
Farm	3 (5)	22 (20)	0.17 (0.04, 0.65)[i]	

[a] Some variables had missing values
[b] Adjusted for age and sex
[c] 95% confidence interval
[d] Third generation family member: Parent, grandparent, siblings, parental siblings, and cousins
[e] Infections during the first two years of life. Dichotomized as <=5 versus >5. Infections included colds, flu, ear ache, and sore throat

[f] Infections during the year prior to diagnosis/interview for cases/controls, respectively. Dichotomized as <=5 versus >5. Infections included colds, flu, ear ache, and sore throat
[g] Global *p*-value for all categories of the variable (Wald test)
[h] During the year in question, did the participant move residence
[i] $P \leq 0.05$

Table 5-4 Dose-response relationships ($p \leq 0.2$) between type 1 diabetes risk in children and youth and food items common to two food frequency questionnaires, in Prince Edward Island, Canada (2001–2004).

Food	Cases (%) $n = 57$	Controls (%) $n = 105$	Consumption category	Odds ratio[a] (CI[b])	p-value[c]
Regular soft	26 (46)	64 (61)	Less than weekly	1.00	0.031
drinks	12 (21)	21 (20)	Weekly	1.43 (0.61, 3.35)	
	19 (33)	20 (19)	More than weekly	2.59 (1.08, 6.18)[d]	
Eggs	19 (33)	51 (49)	Less than weekly	1.00	0.152
	16 (28)	19 (18)	Weekly	2.27 (0.97, 5.35)	
	22 (39)	35 (33)	More than weekly	1.69 (0.79, 3.59)	
Muffins	45 (79)	71 (68)	Less than weekly	1.00	0.078
	8 (14)	19 (18)	Weekly	0.65 (0.26, 1.62)	
	4 (7)	15 (14)	More than weekly	0.40 (0.12, 1.30)	
Hard candy	35 (61)	76 (73)	Less than weekly	1.00	0.139
	12 (21)	17 (16)	Weekly	1.55 (0.67, 3.63)	
	10 (18)	12 (11)	More than weekly	1.92 (0.72, 5.10)	
Orange juice	46 (81)	75 (71)	Less than daily	1.00	0.123
	8 (14)	19 (18)	Daily	0.66 (0.27, 1.65)	
	3 (5)	11 (11)	More than daily	0.41 (0.10, 1.59)	
Cheese	47 (83)	77 (74)	Less than daily	1.00	0.062
	8 (14)	14 (13)	Daily	0.98 (0.37, 2.58)	
	2 (3)	14 (13)	More than daily	0.22 (0.05, 1.03)	

[a] Adjusted for age and sex
[b] 95% confidence interval
[c] P-value for trend (likelihood ratio test)
[d] $P \leq 0.05$

Table 5-5 Final model of significant associations between type 1 diabetes risk in children and youth and dietary and environmental factors, in Prince Edward Island, Canada (2001–2004).

Risk factor	Cases (%) $n = 57$[a]	Controls (%) $n = 105$[a]	Odds ratio[b] (CI[c])	p-value
Regular soft drinks[d]	31 (56)	40 (38)	2.78 (1.21, 6.39)	0.016
Egg[d]	36 (65)	54 (52)	2.50 (1.09, 5.75)	0.031
T1D in family[e]	19 (35)	14 (13)	6.35 (2.34, 17.22)	<0.001
Infections during early childhood[f]	18 (33)	21 (20)	2.61 (1.09, 6.28)	0.032
Fathers education				0.015[g]
Primary	10 (18)	22 (21)	1.00	
Secondary	20 (36)	28 (27)	1.63 (0.55, 4.86)	
College	7 (13)	36 (35)	0.40 (0.11, 1.41)	
University	18 (33)	18 (17)	2.62 (0.80, 8.61)	
Residence				0.019[g]
Urban	22 (39)	32 (29)	1.00	
Rural/village	32 (56)	58 (52)	0.79 (0.33, 1.90)	
Farm	3 (5)	21 (19)	0.11 (0.02, 0.53)[h]	

[a] Some variables had missing values
[b] Adjusted for age, sex, and food frequency questionnaire
[c] 95% Confidence intervals
[d] Dichotomized as weekly (or more frequently) versus less than weekly consumption
[e] Third generation family member: Parent, grandparent, siblings, parental siblings, and cousins
[f] Infections during the first two years of life. Dichotomized as <=5 versus >5. Infections included colds, flu, ear ache, and sore throat
[g] Global p-value for all categories of the variable (Wald test)
[h] $P \leq 0.05$

Table 5-6 Unconditional associations ($p \leq 0.2$) between type 1 diabetes risk in children and youth and foods present in the Youth/Adolescent Questionnaire, in Prince Edward Island, Canada (2001–2004).

Food	Cases (%) $n = 35$	Controls (%) $n = 62$	Odds ratio[a] (CI[b])	p-value
Popcorn[c]	16 (46)	11 (17)	4.61 (1.72, 12.33)	0.002
Peanut butter sandwich[c]	24 (69)	26 (42)	3.30 (1.34, 8.13)	0.009
Bread[d]	22 (63)	22 (35)	3.13 (1.31, 7.50)	0.010
Pasta[c]	31 (89)	42 (68)	3.86 (1.18, 12.59)	0.025
Pizza[c]	26 (74)	33 (53)	2.89 (1.11, 7.52)	0.030
Diet soft drinks[c]	12 (34)	10 (16)	2.73 (1.02, 7.23)	0.044
Regular soft drinks[c]	24 (69)	29 (47)	2.48 (1.03, 6.00)	0.044
Muffin[c]	6 (17)	21 (34)	0.36 (0.12, 1.05)	0.061
Plain chocolate[c]	16 (46)	18 (29)	2.25 (0.91, 5.56)	0.080
Tea[c]	1 (3)	9 (15)	0.14 (0.016, 1.29)	0.083
Hard candy[c]	13 (37)	13 (21)	2.21 (0.87, 5.62)	0.096
Chocolate bar[c]	13 (37)	14 (23)	2.11 (0.84, 5.31)	0.111
Roast beef sandwich[c]	12 (34)	12 (19)	2.12 (0.82, 5.47)	0.120
Butter[c]	10 (29)	27 (44)	0.49 (0.20, 1.21)	0.121
Melon[c]	1 (3)	9 (15)	0.18 (0.02, 1.50)	0.113
Corn[c]	18 (51)	22 (35)	2.06 (0.84, 5.02)	0.113
Cooked carrots[c]	25 (71)	34 (55)	2.00 (0.81, 4.91)	0.132
Chocolate milk[c]	16 (46)	37 (60)	0.56 (0.24, 1.31)	0.181
Low calorie salad dressing[c]	10 (29)	11 (18)	1.94 (0.71, 5.26)	0.196

[a] Adjusted for age and sex
[b] 95% confidence interval
[c] Dichotomized as weekly (or more frequently) versus less than weekly consumption
[d] Dichotomized as daily (or more frequently) versus less than daily consumption

Table 5-7 Significant ($p \leq 0.05$) dose-responses between type 1 diabetes risk in children and youth and foods present in the Youth/Adolescent Questionnaire, in Prince Edward Island, Canada (2001–2004).

Food	Cases (%) $n = 35$	Controls (%) $n = 62$	Consumption category	Odds ratio[a] (CI[b])	p-value[c]
Peanut butter	11 (31)	36 (58)	Less than weekly	1.00	0.003
sandwich	6 (17)	11 (17)	Weekly	1.97 (0.58, 6.73)	
	18 (51)	15 (24)	More than weekly	4.26 (1.58, 11.45)[d]	
Pasta	4 (11)	20 (32)	Less than weekly	1.00	0.043
	18 (51)	25 (40)	Weekly	3.73 (1.07, 12.89)[d]	
	13 (37)	17 (27)	More than weekly	4.07 (1.09, 15.18)[d]	
Regular soft	11 (31)	33 (53)	Less than weekly	1.00	0.049
drinks	9 (26)	12 (19)	Weekly	2.29 (0.75, 6.97)	
	15 (43)	17 (27)	More than weekly	2.64 (0.95, 7.37)	
Muffin	29 (83)	41 (66)	Less than weekly	1.00	0.018
	5 (14)	11 (17)	Weekly	0.58 (0.18, 1.90)	
	1 (3)	10 (16)	More than weekly	0.12 (0.01, 1.02)	
Bread	13 (37)	40 (65)	Less than daily	1.00	0.009
	6 (17)	7 (11)	Daily	2.58 (0.73, 9.12)	
	16 (46)	15 (24)	More than daily	3.42 (1.30, 8.98)[d]	

[a] Adjusted for age and sex
[b] 95% confidence interval
[c] P-value for trend (likelihood ratio test)
[d] $P \leq 0.05$

Table 5-8 Final model of significant associations between type 1 diabetes risk in youth and dietary and environmental factors present in the Youth/Adolescent Questionnaire, in Prince Edward Island, Canada (2001–2004).

Risk factor	Cases (%) $n = 35$[a]	Controls (%) $n = 62$[a]	Odds ratio[b] (CI[c])	*p*-value
Peanut butter sandwich[d]	23 (68)	26 (43)	5.01 (1.53, 16.40)	0.008
Popcorn[d]	16 (47)	11 (18)	8.39 (2.28, 30.86)	0.001
Plain chocolate[d]	16 (47)	18 (30)	4.14 (1.09, 15.67)	0.036
Muffin[d]	6 (17)	21 (34)	0.22 (0.05, 0.95)	0.042
Fathers education				0.033[e]
Primary	6 (18)	14 (23)	1.00	
Secondary	14 (41)	16 (26)	1.77 (0.39, 7.94)	
College	3 (9)	20 (33)	0.59 (0.91, 3.80)	
University	11 (32)	11 (18)	7.24 (1.27, 41.26)[f]	

[a] Some variables have missing values
[b] Adjusted for age and sex
[c] 95% confidence interval
[d] Dichotomized as weekly (or more frequently) versus less than weekly consumption
[e] Global *p*-value for all categories of the variable (Wald test)
[f] $P \leq 0.05$

Chapter 6 Drinking water and dietary components and Type 1 diabetes: A population-based, case-control study in Prince Edward Island, Canada

6.1. Abstract

The aim of this study was to determine the relationship between the risk of type 1 diabetes mellitus (T1D) and the daily intake of certain drinking water and dietary components during the year prior to diagnosis. Particular interest was in the association of nitrate and its derivatives, adjusting for other environmental and genetic risk factors where appropriate. Cases consisted of all newly diagnosed patients with T1D that registered with the Provincial Diabetes Registry during 2001 to 2004. Controls were randomly selected from the province's population, and matched to cases by age at diagnosis and sex. Face-to-face interviews were conducted with all participants (or their guardian or care-giver) to complete one of two age-appropriate food frequency questionnaires and a survey collecting information on possible genetic and environmental risk factors. A total of 14 measurements in drinking water and 28 dietary components in food were divided into quartiles based on the control population. After controlling for age, sex, and daily energy intake, nitrate intake from food sources showed a positive dose-response trend (OR = 1.00, 1.63, 1.7, 3.02, $p = 0.13$). After controlling for four significant environmental and genetic risk factors (third generation family member with T1D, the number of infections during the first two years of life, residential remoteness, and father's education), nitrate from food sources was no longer associated ($p = 0.29$). Nitrite and nitrosamine intake from food sources were not significantly related to T1D

risk. Results also indicated that a dose-response effect of an increased intake of total carbohydrates and caffeine somewhat increased the risk of T1D (OR = 1.00, 0.57, 3.36, 2.18, $p = 0.13$, and OR = 1.00, 1.49, 1.47, 4.88, $p = 0.07$, respectively), whereas a dose-response of increased concentrations of folate and zinc appeared to marginally decrease T1D risk (OR = 1.00, 0.77, 0.50, 0.28, $p = 0.13$, OR = 1.00, 0.37, 0.32, 0.31, $p = 0.20$, respectively). The daily intake of vitamin A and vitamin B12 appeared to somewhat decrease T1D risk, both demonstrating a possible threshold effect at the third quartile. The pH of drinking water showed a marginally significant positive dose-response association, even after adjusting for other factors (OR = 1.00, 0.77, 0.86, 2.18, $p = 0.10$). When food and water components were combined, total dietary zinc intake was marginally and inversely related to T1D risk (OR = 1.00, 0.64, 0.43, 0.26, $p = 0.07$), but the relationship was weakened after model adjustment (OR = 1.00, 0.37, 0.31, 0.24, $p = 0.12$). Total dietary calcium and phosphorous also showed marginally significant protective effects in the crude models (OR = 1.00, 0.89, 0.33, 0.47, $p = 0.06$, OR = 1.00, 0.61, 0.32, 0.44, $p = 0.15$), but only calcium remained marginally significant after further model adjustment (OR = 1.00, 0.82, 0.31, 0.56, $p = 0.17$). Magnesium showed a protective role in the adjusted total dietary model (OR = 1.00, 0.70, 0.24, 0.42, $p = 0.13$), with a possible threshold effect at the second quartile. Dietary components such as nitrate, total carbohydrate, caffeine, zinc, vitamin A, vitamin B12, and calcium from food and water sources combined, may influence the risk of developing T1D in children and youth.

6.2. Introduction

Type 1 diabetes mellitus (T1D) is an immune-mediated disease caused by the destruction of the pancreatic β-cells (Leslie and Elliot, 1994), occurring typically in young people. In developed countries, it constitutes to approximately 10% of all diabetic cases, affecting 0.5-1% of the population during a life-time (Rewers and Klingensmith, 1997). Although a genetic predisposition is thought to be necessary for the development of T1D, environmental factors appear to trigger the onset of disease (Todd, 1991). Evidence for an environmental etiology is found in the low concordance rate between monozygotic twins (Kyvik et al., 1995), rapidly increasing incidence rates on a worldwide scale (Onkamo et al., 1999; Green and Patterson, 2001), small scale geographical heterogeneity of T1D incidence (Staines et al., 1997; Schober et al., 2003), and varying incidence rates among immigrant populations (Bodansky et al., 1992).

Both animal and human studies indicate that diet may be associated with T1D risk. Experimental studies on the Biobreeding rat and non-obese diabetic mouse demonstrated that diet played an essential role in development of diabetes, specifically, that some protein components increased diabetes incidence in these animal models (Elliott et al., 1988; Scott et al., 1997). Previous human studies have shown that certain nutrients may be promotive (such as protein, carbohydrates, and nitrosamines) (Dahlquist et al., 1990; Helgason et al., 1992) or protective (such as vitamin D and vitamin E) (Knekt et al., 1999; Hyppönen et al., 2001) factors of T1D development. Dahlquist et al. (1990) observed in a case-control study that nitrosamines had a promotive effect only when vitamin C was in low concentrations in the diet. Ecological analysis has shown that

nitrate in drinking water may increase T1D incidence (Kostraba et al., 1992; Parslow et al., 1997), but this finding is not consistent (Van Maanen et al., 1999; Molchanova et al., 2004; Muntoni et al., 2006; Chapter 4). Similarly, the relationship between T1D and nitrate consumed from food sources has also been reported to be positive (Dahlquist et al., 1990) or non-significant (Virtanen et al., 1994). Nitrite consumption from food has also been connected with an elevated risk of T1D (Virtanen et al., 1994).

To our knowledge, no T1D risk factor analysis using drinking water information at the individual level has been previously conducted, or combined with dietary information also at the individual level. Furthermore, the relationship between T1D and nitrate, nitrite, and nitrosamine information from food sources in the same study have been examined only once before, and positive associations were reported for all three components (Dahlquist et al., 1990). In Finland, nitrate and nitrite from food at the individual level and from drinking water at the ecological level were evaluated, and nitrite was significantly more frequently consumed by cases than controls (Virtanen et al., 1994).

The primary aims of this study were to determine drinking water and dietary component factors associated with the risk of T1D, and to compare the consumption of nitrate from both food and water sources at the individual level in T1D and control populations, controlling for other risk factors where appropriate.

6.3. Materials and methods

A description of the case and control selection criteria, the two food frequency questionnaires (FFQ) utilized, and the risk factor questionnaire (RFQ) are previously described (Chapter 5). Briefly, controls were matched to cases by sex and age; age of the control was within one year of the age of the case at time of diagnosis. The RFQ collected information pertaining to possible confounders or social and familial risk factors of T1D. One of the two FFQs was administered, depending on the age of the participant, to obtain dietary intake during the year prior to diagnosis for cases, or during the year prior to interview for controls. A surrogate reporter, typically the mother, completed the Harvard Service Food Frequency Questionnaire (HS-FFQ) for children under nine years of age, and participants aged nine and older completed the Youth/Adolescent Questionnaire (YAQ), with assistance from a parent if required. Both FFQs were provided by Harvard Medical School, where they were validated for use with children and youth (Rockett et al., 1995; Rockett et al., 1997; Blum et al., 1999).

6.3.1. Food component data collection and processing

Foods in both FFQs were originally coded from foods present on the National Nutrient Database of the USDA, so each food was reassigned a corresponding code from the 2005 Canadian Nutrient File (CNF). Food frequency data from both FFQs were entered into CANDAT (Godin London Incorporated, 2006) and, utilizing the CNF, were converted from food frequencies into daily nutrient intakes, taking into account the relevant portion sizes for participants aged 0-2 years old, 3-8 years old, and nine years

and older. Portion sizes were supplied by Harvard Medical School. The consumption of vitamin and mineral supplements was not assessed in this study.

Nitrate, nitrite, and nitrosamine concentrations were not in the CNF, and therefore were added from pre-existing Canadian values, where available (Sen, 2003 [personal communication]), or from tested local foods (Appendices F and G). The locally purchased food samples were tested for these chemicals under two circumstances: 1) if there were no values available and there was no literature to suggest that those foods were unlikely to contain the chemical (e.g. vegetables generally have nitrosamine concentrations below the detection limit (Hotchkiss, 1987)); and 2) if there were values available but the literature was possibly outdated or there was indication of geographical variation in concentrations (due to nitrate fertilizer application and quantification variation from one region to the next (L'Hirondel and L'Hirondel, 2002)). All foods sampled were cooked (if that is how the food was typically eaten), chilled, and shipped overnight to the laboratories listed below for analysis.

Nitrate and nitrite concentrations, reported as sodium nitrate ($NaNO_3$) and sodium nitrite ($NaNO_2$), were analysed at the Canadian Food Inspection Agency Laboratory in Dartmouth, Nova Scotia, Canada. All concentrations were determined by ion chromatography and UV detection (Appendix H). $NaNO_3$ and $NaNO_2$ detection limits were both 0.04 $\mu g\ ml^{-1}$.

Nitrosamine concentration in foods were analyzed by the Health Canada Laboratory in Longueuil, Quebec, Canada, with a quantitative determination based on a Gas-Liquid Chromatograph–Thermal Energy Analyser (GLC-TEA) (Appendix I). The

methods are referenced elsewhere (Sen et al., 1979; Sen et al., 1987). Only volatile nitrosamines were quantified because they were thought to be a public health concern, with the most common being *N*-nitrosodimethylamine [NDMA], N-nitrosodiethylamine [NDEA], *N*-nitrosodipropylamine [NDPA], *N*-nitrosodibutylamine [NDBA], *N*-nitrosopiperidine [NPIP], *N*-nitrosopyrrolidine [NPYR], and *N*-nitrosomorpholine [NMOR]. Non-volatile nitrosamines are rarely quantified because highly specialized detection instruments are needed, and they are thought not to be harmful to human health (Kumar and McLay, 1998). Detection limits for NDMA, NDEA, NDPA, NDBA, NPIP, NPYR, and NMOR were 0.18, 0.04, 0.10, 0.10, 0.09, 0.10, 0.17 $\mu g\ Kg^{-1}$, respectively.

6.3.2. Drinking water data collection and processing

The RFQ (described in Chapter 5, and provided in Appendix D) included questions pertaining to water consumption (during the year prior to diagnosis for cases, or year prior to interview for controls), and locations of where the majority of water was drunk. Water consumption was from three possible sources: private well water; municipal water systems; or bottled water. Concentration of nitrate and other water chemistry components were obtained from these three possible sources using the following procedures: At the time of interview, a water sample was taken from all places where the participant had drunk a substantial amount of water from a private well (e.g. home or day-care) and submitted to the provincial laboratory for water chemistry testing. Water chemistry data from school wells were provided by the provincial laboratory as these wells were frequently sampled by the provincial school boards. If water was drunk from a

municipal source, then the data were also supplied by the provincial government from the routine monitoring of water chemistry of municipal supplies. If multiple water test results were available (e.g. for municipal systems), an average concentration of the water chemistry data during the year prior to diagnosis for cases, or year prior to interview for controls, was calculated. Water chemistry data from bottled water was obtained by amalgamating equal quantities of four locally drank common brands of water, which subsequently underwent analysis at the provincial laboratory.

A total of 18 water analytes were measured and the methods of analysis for alkalinity, chloride, and nitrate using flow injection analysis colorimetry are described in Appendix A. The methods of analysis of metals in ground water using an inductively coupled argon plasma spectrometer are described in Appendix J.

Daily intake of each water analyte was determined by multiplying reported consumption from different sources (on a daily basis) by the analyte concentration in the water sources, and then summing the sources. For example, if a participant drank 7 cups (250 ml) of water per week from well 1 with a nitrate concentration of 3.00 mg L^{-1}, and 18 cups per week from well 2 with a concentration of 2.10 mg L^{-1}, then the daily nitrate intake from water would be 0.75 mg from well 1 ([(7*0.250)/7)*3.00 mg L^{-1}), and 1.35 mg from well 2 ([(18*0.250)/7]*2.10 mg L^{-1}), or 2.10 mg per day.

The provincial laboratory reported water concentrations of nitrate as nitrate-nitrogen. Nitrate concentrations from food sources were therefore converted into nitrate-nitrogen, and nitrite values were converted into nitrite-nitrogen for examining total

nitrate-nitrogen and nitrite-nitrogen from food and water sources. Nitrate-nitrogen and nitrite-nitrogen will subsequently be referred to as nitrate and nitrite, respectively.

6.3.3. Statistical analysis

Dietary and water data were converted to a daily intake and categorized into quartiles based on the distribution in the control population (except for alcohol and aspartame which were dichotomized at the median concentration in the control population due to a low frequency of consumption) (Table 6-1 and Table 6-2, respectively). Components common to both food and drinking water (nitrate, calcium, zinc, magnesium, sodium, potassium, and phosphorus) were summed together to create a total dietary intake of those constituents for separate analysis. As nitrate readily reduces to nitrite during metabolism, nitrates and nitrites from both food and water sources were summed together to evaluate their overall effect. Nitrosamines were not combined with nitrate or nitrite as their contribution to this combined variable would be very small due to low concentrations in foods.

Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression in order to determine associations between the daily intake of dietary and water components and the risk of T1D. Two sets of models were assessed. Crude models assessed individual dietary and water components, while controlling for age and sex for water components, or controlling for age, sex, and total energy intake for dietary components. Then, adjusted models evaluated each dietary and water component, in turn, while adjusting for significant genetic and environmental risk factors, as well as age, sex,

and total energy intake. The four self-reported significant genetic and environmental variables from the RFQ were determined in Chapter 5 (a third generation family member previously diagnosed with T1D [siblings, parents, grandparents, parental siblings, and cousins], father's education, the number of infections during the first two years of life, and residential remoteness [urban, rural or farm]).

Tests for a dose-response were evaluated using the likelihood ratio test, whereby a statistical model containing the categorical variable as a continuous variable was compared to a model without the variable. A Wald test was used to determine overall significance of each categorical variable. Variables with a p-value ≤ 0.05 were considered significantly associated with T1D risk, whereas those with a p-value > 0.05 but ≤ 0.2 were considered marginally significant. The predictive ability and goodness-of-fit of each final model was assessed, and an evaluation of the residuals by covariate pattern was conducted. All statistical analyses were conducted using Stata 9 (StataCorp, 2005).

6.4. Results

Total participation rates for the study were 86% for cases and 73% for controls (Table 5-1). For the 57 cases and 105 controls, cadmium, iron, manganese, and nickel concentrations in drinking water were less than the detection limit in 90% of the samples, leaving 14 water variables for subsequent analyses.

For nitrate and nitrite concentration testing, 77% and 72% of the foods present on the YAQ and HS-FFQ were sampled, respectively. For nitrosamine concentration determination, 44% and 38% of the foods on the YAQ and HS-FFQ were sampled,

respectively. Approximately 7%, 7%, and 35% of the foods from both questionnaires did not have values for nitrate, nitrite or nitrosamines, respectively, because these foods were not known to contain them.

6.4.1. Crude models for the food and water components

With regards to the crude associations between dietary components in food and the risk of T1D in children and youth (Table 6-1), there was a marginally significant (p = 0.13) positive dose-response relationship between T1D and the intake of nitrate from food based on the trend test. The daily intake of nitrite and nitrosamines were not associated with T1D risk.

When evaluating other crude models for food components (Table 6-1), the intake of caffeine had a marginally significant positive dose-response relationship with T1D risk based on the trend test. Conversely, the following variables had marginally significant protective dose-response relationships with T1D risk based on the trend test: vitamin C, riboflavin, vitamin B12, folate, phosphorus, and zinc. The daily intake of fat, vitamin B12, and calcium had marginally significant negative relationships with T1D risk based on the Wald's test. In particular, the third quartile for the daily intake of vitamin B12 was significantly lower than the first quartile (p = 0.01). Also, the second quartile of daily fat intake was significantly lower than the first quartile (p = 0.03).

When evaluating crude models for water analytes (Table 6-2), the trend test determined that water pH had a marginally significant positive dose-response relationship with T1D risk, and potassium was negatively associated with T1D risk. Both potassium

and sodium had marginally significant relationships with T1D risk based on the Wald's test, however the negative association was less clear.

When evaluating crude models for components common to water and food (Table 6-3), calcium, phosphorus, and zinc had marginally significant negative dose-response relationships with T1D risk, based on the trend test. The intake of nitrate was positive and marginally significant to T1D risk, according to the Wald test, as was nitrate and nitrite intake combined, and the intake of calcium was negatively associated. In addition, total nitrate intake and total nitrate and nitrite intake combined, showed possible threshold effects at their second quartiles, and in the case of nitrate consumption, indicated that compared to a daily intake of < 7.20 mg per day, the intake of 7.20-9.86 mg per day significantly increases the risk of T1D by 3.17 times (Quartile *p*-values for nitrate, and nitrate and nitrite combined = 0.03 and 0.05, respectively). The intake of calcium also exhibited a possible threshold effect at the second quartile ($p = 0.04$).

6.4.2. Adjusted models for the food and water components

When evaluating the adjusted models for food components (Table 6-4), nitrate, nitrite, and nitrosamines were not associated with T1D risk. However, total carbohydrates and caffeine had marginally significant positive dose-response relationships with T1D risk based on the trend test. Total carbohydrate intake was positively and significantly ($p = 0.05$) related to T1D risk according to the Wald test, possibly showing a threshold effect at the third quartile, and caffeine was positive and marginally significantly associated with T1D risk. Conversely, the dose-response relationships between folate and

zinc and T1D risk were marginally significant and inversely associated. According to the Wald test, vitamin A was significantly and inversely related with T1D risk. In particular, the second quartile for the daily intake of vitamin A was significantly lower than the first quartile ($p = 0.01$). The intake of calcium and vitamin B12 had marginally significant negative relationships with T1D risk, with a possible threshold effect at the third quartile for vitamin B12 ($p = 0.02$). The inversely associated dose-response effects of vitamin C and riboflavin and the risk of developing T1D were still apparent, but marginal significance was not present after adjustment for the other risk factors (Table 6-4). The daily intake of fat was no longer associated with T1D risk.

Water component analyses (Table 6-5) showed that water pH was the only water analyte shown to have a marginally significant positive dose-response association with T1D risk based on the trend test. Whereas the Wald test determined that both calcium and sodium had marginally significant associations with T1D risk, but the direction of association was not clear.

Finally, when evaluating adjusted models for components common to both food and water (Table 6-6), calcium, magnesium, and zinc were negatively and marginally significantly associated with T1D risk when using the trend test. The Wald test reported that nitrate had a positive and marginally significant relationship with T1D risk, and magnesium decreased T1D risk, with a possible threshold effect at the third quartile ($p = 0.05$). The effect of phosphorus was no longer apparent after model adjustment.

The predictive ability for each model was satisfactory when evaluating the dietary and water components, before and after controlling for the four genetic and

environmental factors. Goodness-of-fit tests confirmed that the models fit the data, and no extreme residuals were present.

6.5. Discussion

To our knowledge this is the first study to combine nitrate values measured at the individual level from both food and water sources when evaluating for possible risk factors of T1D during the year prior to diagnosis. There was some evidence that a dose-response effect of the daily intake of nitrate from food sources was positively and marginally significantly related to the risk of T1D ($p = 0.13$) from the crude associations. The combination of nitrate from both food and water sources (total nitrate) was also marginally significant (global $p = 0.07$), and no dose-response trend was apparent as a result of the very weak inverse relationship between nitrate in water and T1D risk. After adjustment for the four significant environmental and genetic factors, the positive dose-response trend for nitrate from food sources and T1D risk was removed ($p = 0.29$), and the association of total nitrate intake and T1D risk was weakened (global $p = 0.10$).

Limited research has been conducted evaluating the relationship between nitrate from food sources and T1D risk at the individual level. A Finnish case-control study reported no association between nitrate obtained from food sources at the individual and drinking water sources at the area level and T1D risk (Virtanen et al., 1994), whereas a Swedish study reported a significant positive relationship (Dahlquist et al., 1990). Previous ecological studies have shown that nitrate in drinking water increased the risk of T1D (Kostraba et al., 1992; Parslow et al., 1997), but other studies have reported no

effect (Van Maanen et al., 1999; Casu et al., 2000), or more recently, a negative effect (Zhao et al., 2001; Muntoni et al., 2006).

It has been suggested that nitrate in the diet may increase T1D risk because nitrate is reduced to nitrite in the gastrointestinal tract, and then N-nitroso-compounds are formed by a chemical or bacterial nitrosation reaction with amino compounds. It is these nitrosamines which are believed to be toxic to the pancreatic β-cells (Rakieten et al., 1963; Assan and Larger, 1993). Our study provides additional evidence, albeit weak, in favour of a relationship between T1D risk and nitrate intake. However, in our study, no dose-response association between T1D risk and the total daily intake of nitrate and nitrite concentration combined was evident, and the daily intake of nitrite or nitrosamines from food sources was not associated with T1D risk. Positive associations between these compounds and T1D risk have been reported in previous studies (Dahlquist et al, 1990; Virtanen et al,. 1994).

After model adjustment of the four significant genetic and social factors, the intake of total carbohydrates was positively associated with an overall significant (global $p = 0.05$) increase in T1D risk, even after controlling for energy intake. The dose-response effect was marginally significant ($p = 0.13$), possibly due to a potential threshold effect present at the third quartile (289-352 g per day). This study supports a previous study which suggested that total carbohydrates increased the risk of T1D, also reaching a potential threshold (Dahlquist et al., 1990). Another Swedish study compared the first and fourth quartile of the daily intake of carbohydrate and was also in agreement that an increased carbohydrate intake, particularly disaccharides and sucrose in this

instance, led to a higher risk of T1D (Pundziūtė-Lyckå et al., 2004). Total sugar intake was evaluated in our study, and found not to influence on T1D risk in both the crude and adjusted analyses. A possible reason for the positive association between T1D risk and the intake of total carbohydrates is that an increased intake of carbohydrates may cause stress on the β-cell, thereby fitting the hypothesis that the cytotoxic action of certain immune cells in experimental models is more frequent in β–cells stimulated by glucose (Nerup et al., 1988).

Although no dose-response trend was noted, the significant effect of vitamin A according to the Wald test was reasonable ($p = 0.02$) after model adjustment, and appeared to protect against the development of T1D, showing a potential threshold effect at the second quartile (502-654 μg per day). As oxidative stress is believed to contribute to β-cell destruction after autoimmune activation, it is hypothesized that the antioxidant properties found in vitamin A (beta-carotene) may decrease the risk of T1D due to their free radical scavenger abilities (Mandrup-Poulsen et al., 1993).

For the crude analysis, there was a somewhat marginally significant dose-response relationship between vitamin C and T1D risk, however, this negative association was removed after adjustment for other factors. A previous study noted that vitamin C was also inversely related to T1D risk, but was considered a confounder as the effect of vitamin C was removed when stratified for nitrates and nitrites (Dahlquist et al., 1990). Vitamins E (Knekt et al., 1999) and D (EURODIAB Substudy 2 Study Group, 1999) have been previously linked to a possible decrease in T1D risk, but these observations were not supported by this study. However, the previously reported

protective association was for vitamin D supplementation in early life. Experimental studies have reported that the intake of protein was associated with an increased risk in T1D, but this observation (Scott et al., 1985) was also not supported by our study.

The effect of total daily zinc intake (from food and water sources) showed a somewhat marginally significant dose-response effect in the crude ($p = 0.07$) and adjusted model ($p = 0.12$), whereby the risk of T1D decreased with increased zinc concentrations. To our knowledge, the association between zinc and T1D has only been reported in ecological analyses evaluating the effect of zinc in drinking water, and never before at the individual level. Zinc consumed from drinking water showed a protective role in T1D development in previous studies (Haglund et al., 1996; Zhao et al., 2001), but not always consistently (Molchanova et al., 2004) or significantly (Stene et al., 2002). In our study, zinc consumed from water alone was low in concentration, possibly explaining why the inverse marginal association with T1D risk was only evident when combined with zinc concentrations derived from food. Zinc is an essential element required by the human body and is believed to protect against T1D development by protecting the insulin-producing pancreatic β-cells from destruction by free radicals (Ohly et al., 2000). An experimental study using Wistar BB rats determined that a diet supplemented with zinc delayed or decreased the onset of diabetes, possibly due to the improved glucose tolerance in rats fed a high zinc diet compared to rats fed a normal zinc diet (Tobia et al., 1998). Previous studies have shown that zinc in the urine, serum or blood plasma of newly diagnosed type 1 diabetic patients is significantly lower than in controls (Hagglof

et al., 1983; Bideci et al., 2005), however, it is not known as to whether these differences in zinc concentration are a cause or a consequence of T1D development.

The effect of water pH has been investigated in a Norwegian study where a lower pH actually increased the risk of T1D (Stene et al., 2002), whereas our study reported a trend in the opposite direction. In our study, the first quartile ranged from a pH of 6.4-7.67 and the fourth quartile ranged from 7.94 to 8.3, making the evaluation of acidic water impossible. The Norwegian study did report that acidic water may not be causally related to T1D, but may be a marker for other factors. This may also be true in our study, as no explanation for this finding is apparent.

Adjusting the regression models of food components for total energy was important for a number of reasons. It is widely accepted that energy intake is related to disease risk in western societies (Willett et al., 1997). Adjusting for energy intake also accounts for possible differences in physical activity, body size, and metabolic efficiency between participants (Willett et al., 1997). Many nutrients are positively correlated with energy intake, because typically, individuals who consume more calories also consume more nutrients. There are several ways to control for energy intake, but as energy intake is not documented as being a strong risk factor of T1D, controlling for it in a multivariable model was considered appropriate (Willett et al., 1997).

An important strength of the study was that many of the foods present in both FFQs were sampled for nitrate, nitrite, and nitrosamine concentration. This extensive sampling provided the study with recent estimates for locally produced foods, something which is currently limited.

The families of the control population were compared to the provincial population with regards to social factors such as smoking, drinking, breast feeding, education level, and fruit and vegetable intake (Van Til, 2004). All measurable factors were similar between the two groups, therefore suggesting that the control population is a representative sample of the base population.

Although both FFQs were previously validated, a FFQ is a crude instrument of nutritional assessment. Measurement error of dietary intakes may have introduced misclassification of exposure, but this error would likely have been random and non-differential, moving the ORs toward the null. Also, the number of foods on the two FFQs was different (34% more on the YAQ), and because FFQs are prone to over-reporting, the YAQ could have over-estimated total dietary intake relative to the HS-FFQ (Willett, 1994). With age-matched controls, it is likely that these differences would have affected the cases and controls equally, leading to minimal bias, with the results again being directed towards the null.

As only a limited number of participants were available for study enrolment (due to a small base population), it is likely that insufficient power was available to determine significance between T1D risk and food and water components in some instances, unless the relationship between T1D and the predictor was strong. This may explain the observation that although nitrate intake from food appeared to be related to an increased T1D risk, significance was not attained. A larger sample size may have also generated smaller confidence intervals around the ORs, thereby estimating the risk with more precision. Nonetheless, this study was beneficial in determining possible water and

dietary risk factors for T1D in PEI, especially the association between nitrate and it derivatives and T1D. More research is required to confirm these interesting results.

6.6. Conclusion

Nitrate intake from food was marginally significant and positively associated with the risk of T1D in children and youth in crude associations using a trend test. However, nitrate from both food and drinking water combined was not significantly related to the risk of T1D overall, although the second quartile showed a significant increased risk of T1D compared to the first quartile. A positive effect from the intake of total carbohydrates was associated with increased risk of T1D, after adjustment for other factors, with a possible threshold effect at the third quartile. Higher total dietary intake of zinc was marginally associated with a decreased risk of T1D. The intake of vitamin A and vitamin B12 were significantly associated with T1D risk, with a possible threshold effect at the second and third quartile, respectively. Diet may play a role in the development of T1D, however more research is needed to confirm these current findings.

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Table 6-1 Crude associations between type 1 diabetes in children and youth and dietary intake from food (n = 162), in Prince Edward Island, Canada (2001-2004).

Food component (unit)	Concentration of daily intake	Cases (%) n = 57[a]	Controls (%) n = 105[a]	Odds ratio[b] (CI[c])	p for trend[d]	Global p-value[e]
Nitrate	<5.66	10 (18)	26 (25)	1.00	**0.134**	0.451
(mg)	5.66-7.27	14 (25)	26 (25)	1.63 (0.58, 4.63)		
	7.28-9.00	13 (23)	26 (25)	1.71 (0.54, 5.40)		
	≥9.01	20 (35)	27 (26)	3.02 (0.78, 11.74)		
Nitrite	<1.83	12 (21)	26 (25)	1.00	0.772	0.841
(mg)	1.83-3.26	16 (28)	27 (26)	1.15 (0.42, 3.14)		
	3.27-4.81	17 (30)	26 (25)	1.23 (0.43, 3.53)		
	≥4.82	12 (21)	26 (25)	0.82 (0.24, 2.81)		
Nitrosamine	<0.01	21 (37)	35 (33)	1.00	0.808	0.901
(μg)	0.01-0.029	17 (30)	36 (34)	0.73 (0.32, 1.69)		
	0.03-0.039	6 (11)	12 (11)	0.77 (0.24, 2.44)		
	≥0.04	13 (23)	22 (21)	0.86 (0.32, 2.35)		
Protein	<67.64	17 (30)	27 (26)	1.00	0.411	0.772
(g)	67.64-82.12	12 (21)	26 (25)	0.61 (0.23, 1.67)		
	82.13-97.63	13 (23)	26 (25)	0.61 (0.20, 1.81)		
	≥97.64	15 (26)	26 (25)	0.53 (0.12, 2.43)		
Fat	<62.21	19 (33)	27 (26)	1.00	0.616	**0.163**
(g)	62.21-72.87	6 (11)	26 (25)	0.27 (0.09, 0.88)[f]		
	72.88-96.37	17 (30)	26 (25)	0.70 (0.23, 2.12)		
	≥96.38	15 (26)	26 (25)	0.48 (0.10, 2.42)		
Total	<233.93	13 (23)	27 (26)	1.00	0.278	0.285
carbohydrate	233.93-289.43	8 (14)	25 (24)	0.74 (0.24, 2.30)		
(g)	289.44-351.99	21 (37)	26 (25)	2.08 (0.62, 6.99)		

	≥352.00	15 (26)	27 (26)	1.69 (0.30, 9.68)		
Total sugars	<120.57	19 (33)	27 (26)	1.00	0.700	0.288
(g)	120.57-140.03	7 (12)	26 (25)	0.35 (0.12, 1.03)		
	140.04-171.41	15 (26)	26 (25)	0.72 (0.26, 1.98)		
	≥171.42	16 (28)	26 (25)	0.66 (0.17, 2.59)		
Total fibre	<11.5	7 (12)	23 (22)	1.00	0.408	0.279
(g)	11.5-15.09	21 (37)	29 (28)	2.65 (0.88, 7.94)		
	15.10-18.59	11 (19)	26 (25)	1.63 (0.46, 5.78)		
	≥18.60	18 (32)	27 (26)	2.82 (0.67, 11.87)		
Vitamin A	<501.80	17 (30)	27 (26)	1.00	0.706	0.203
(μg)	501.80-653.99	7 (12)	25 (24)	0.38 (0.13, 1.14)		
	654.00-951.99	20 (35)	26 (25)	1.00 (0.38, 2.61)		
	≥952.00	13 (23)	27 (26)	0.55 (0.17, 1.78)		
Vitamin E	<3.21	13 (23)	26 (25)	1.00	0.733	0.343
(mg)	3.21-4.06	12 (21)	26 (25)	0.78 (0.28, 2.16)		
	4.07-5.35	21 (37)	26 (25)	1.26 (0.45, 3.54)		
	≥5.36	11 (19)	27 (26)	0.50 (0.12, 2.14)		
Vitamin D	<5.14	14 (25)	26 (25)	1.00	0.289	0.474
(μg)	5.14-8.72	19 (33)	27 (26)	1.28 (0.50, 3.27)		
	8.73-10.86	15 (26)	26 (25)	1.05 (0.39, 2.81)		
	≥10.87	9 (16)	26 (25)	0.58 (0.19, 1.77)		
Vitamin C	<81.20	18 (32)	27 (26)	1.00	**0.172**	0.490
(mg)	81.20-130.89	15 (26)	26 (25)	0.69 (0.27, 1.77)		
	130.90-203.89	11 (19)	26 (25)	0.44 (0.15, 1.30)		
	≥203.90	13 (23)	26 (25)	0.49 (0.16, 1.52)		
Thiamin	<1.31	11 (19)	27 (26)	1.00	0.653	0.840
(mg)	1.31-1.70	15 (26)	25 (24)	1.49 (0.52, 4.23)		
	1.71-2.07	17 (30)	27 (26)	1.61 (0.48, 5.44)		
	≥2.08	14 (25)	26 (25)	1.41 (0.28, 7.11)		
Riboflavin	<2.01	16 (28)	27 (26)	1.00	**0.097**	0.280

(mg)	2.01-2.52	19 (33)	25 (24)	1.04 (0.40, 2.71)		
	2.53-2.95	9 (16)	26 (25)	0.45 (0.15, 1.36)		
	≥2.96	13 (23)	27 (26)	0.46 (0.12, 1.80)		
Niacin	<29.30	14 (25)	27 (26)	1.00	0.723	0.954
(NE)	29.30-36.92	12 (21)	26 (25)	0.89 (0.32, 2.43)		
	36.93-43.89	15 (26)	26 (25)	1.12 (0.37, 3.46)		
	≥43.90	16 (28)	26 (25)	1.24 (0.28, 5.35)		
Vitamin B6	<1.31	13 (23)	27 (26)	1.00	0.971	0.976
(mg)	1.31-1.64	15 (26)	27 (26)	1.09 (0.40, 2.99)		
	1.65-1.95	15 (26)	25 (24)	1.14 (0.38, 3.42)		
	≥1.96	14 (25)	26 (25)	0.93 (0.21, 3.99)		
Vitamin B12	<4.09	20 (35)	26 (25)	1.00	**0.121**	**0.093**
(μg)	4.09-5.02	15 (26)	25 (24)	0.69 (0.27, 1.75)		
	5.03-6.23	6 (11)	28 (27)	0.24 (0.08, 0.73)[f]		
	≥6.24	16 (28)	26 (25)	0.59 (0.20, 1.74)		
Folate	<333.20	16 (28)	26 (25)	1.00	**0.127**	0.432
(μg)	333.20-414.99	14 (25)	26 (25)	0.66 (0.25, 1.74)		
	415.00-544.29	16 (28)	27 (26)	0.59 (0.20, 1.73)		
	≥544.30	11 (19)	26 (25)	0.29 (0.07, 1.27)		
Calcium	<1024.10	14 (25)	27 (26)	1.00	0.304	**0.156**
(mg)	10024.10-1392.99	21 (37)	25 (24)	1.64 (0.62, 4.32)		
	1393.00-1672.79	7 (12)	26 (25)	0.50 (0.16, 1.53)		
	≥1672.80	15 (26)	27 (26)	0.93 (0.28, 3.16)		
Iron	<10.00	11 (19)	26 (25)	1.00	0.537	0.440
(mg)	10.00-13.27	18 (32)	27 (26)	1.72 (0.62, 4.76)		
	13.28-16.11	9 (16)	25 (24)	0.99 (0.28, 3.50)		
	≥16.12	19 (33)	27 (26)	2.10 (0.48, 9.10)		
Magnesium	<228.50	14 (25)	26 (25)	1.00	0.281	0.697
(mg)	228.50-295.49	17 (30)	26 (25)	0.97 (0.36, 2.63)		
	295.50-354.99	12 (21)	26 (25)	0.61 (0.20, 1.91)		

	≥355.00	14 (25)	27 (26)	0.53 (0.12, 2.37)		
Phosphorus	<1350.30	18 (32)	26 (25)	1.00	**0.163**	0.358
(mg)	1350.30-1690.99	14 (25)	26 (25)	0.62 (0.23, 1.65)		
	1661.00-1897.99	9 (16)	26 (25)	0.34 (0.11, 1.10)		
	≥1898.00	16 (28)	27 (26)	0.44 (0.11, 1.79)		
Potassium	<2639.00	15 (26)	26 (25)	1.00	0.295	0.744
(mg)	2639.00-3245.99	16 (28)	26 (25)	0.90 (0.34, 2.36)		
	3246.00-3852.99	12 (21)	26 (25)	0.60 (0.20, 1.80)		
	≥3853.00	14 (25)	27 (26)	0.54 (0.13, 2.18)		
Sodium	<2131.00	12 (21)	26 (25)	1.00	0.368	0.616
(mg)	2131.00-2889.69	18 (32)	27 (26)	1.14 (0.41, 3.18)		
	2889.70-3557.99	15 (26)	25 (24)	0.86 (0.25, 2.95)		
	≥3558.00	12 (21)	27 (26)	0.50 (0.10, 2.56)		
Zinc	<8.47	17 (30)	25 (24)	1.00	**0.089**	0.388
(mg)	8.47-10.27	15 (26)	28 (27)	0.57 (0.21, 1.52)		
	10.28-13.12	11 (19)	25 (24)	0.39 (0.12, 1.22)		
	≥13.13	14 (25)	27 (26)	0.30 (0.06, 1.36)		
Selenium	<76.30	14 (25)	26 (25)	1.00	0.969	0.897
(μg)	76.30-94.69	15 (26)	28 (27)	0.95 (0.36, 2.48)		
	94.70-116.19	11 (19)	25 (24)	0.76 (0.25, 2.32)		
	≥116.20	17 (30)	26 (25)	1.09 (0.28, 4.30)		
Alcohol	0	53 (93)	94 (90)	1.00	-	0.242
(g)	>0	4 (7)	11 (10)	0.42 (0.10, 1.79)		
Caffeine	<3.36	14 (25)	27 (26)	1.00	**0.182**	0.278
(mg)	3.36-8.67	8 (14)	25 (24)	0.70 (0.23, 2.09)		
	8.68-21.99	13 (23)	26 (25)	1.08 (0.39, 3.01)		
	≥22.00	22 (39)	27 (26)	2.08 (0.59, 7.36)		
Aspartame	0	39 (68)	77 (73)	1.00	-	0.554
(mg)	>0	18 (32)	28 (27)	1.24 (0.60, 2.56)		
Cholesterol	<179.00	17 (30)	26 (25)	1.00	0.258	0.443

(mg)	179.00-219.99	11 (19)	26 (25)	0.55 (0.21, 1.47)
	220.00-287.99	17 (30)	26 (25)	0.74 (0.28, 2.01)
	≥288.00	12 (21)	27 (26)	0.39 (0.11, 1.45)

[a] May not total 100% due to rounding to nearest whole number
[b] Adjusted for age, sex, and energy intake (Kcal)
[c] 95% confidence interval
[d] *P* for trend (likelihood ratio test)
[e] Global *p*-value (Wald test)
[f] $P \leq 0.05$

Table 6-2 Crude associations between type 1 diabetes in children and youth and drinking water chemistry (n = 162), in Prince Edward Island, Canada (2001-2004).

Water component (unit)	Concentration of daily intake	Cases (%) n = 57[a]	Controls (%) n = 105[a]	Odds ratio[b] (CI[c])	p for trend[d]	Global p-value[e]
Nitrate	<0.78	15 (26)	26 (25)	1.00	0.268	0.416
(mg)	0.78-1.61	16 (28)	26 (25)	0.98 (0.39, 2.44)		
	1.62-3.27	17 (30)	26 (25)	1.09 (0.44, 2.68)		
	≥3.28	9 (16)	27 (26)	0.48 (0.17, 1.39)		
Water pH[f]	<7.668	10 (18)	26 (25)	1.00	**0.096**	0.359
(pH unit)	7.678-7.851	16 (29)	34 (32)	1.21 (0.47, 3.13)		
	7.852-7.933	9 (16)	19 (18)	1.21 (0.41, 3.58)		
	≥7.934	21 (38)	26 (25)	2.15 (0.84, 5.45)		
Alkalinity	<47.53	16 (28)	26 (25)	1.00	0.452	0.400
(mg)	47.53-84.05	12 (21)	27 (26)	0.66 (0.25, 1.69)		
	84.06-121.88	19 (33)	26 (25)	1.08 (0.44, 2.61)		
	≥121.89	10 (18)	26 (25)	0.50 (0.17, 1.46)		
Calcium	<11.91	16 (28)	26 (25)	1.00	0.363	0.361
(mg)	11.91-23.68	13 (23)	27 (26)	0.73 (0.29, 1.83)		
	23.69-39.87	19 (33)	26 (25)	1.12 (0.46, 2.71)		
	≥39.88	9 (16)	26 (25)	0.48 (0.17, 1.37)		
Chloride	<6.79	13 (23)	26 (25)	1.00	0.516	0.881
(mg)	6.79-12.77	12 (21)	27 (26)	0.88 (0.34, 2.30)		
	12.78-20.71	14 (25)	25 (24)	1.11 (0.43, 2.86)		
	≥20.72	18 (32)	27 (26)	1.28 (0.49, 3.35)		
Copper	<0.009	13 (23)	27 (26)	1.00	0.720	0.858
(mg)	0.009-0.017	15 (26)	25 (24)	1.23 (0.48, 3.14)		
	0.018-0.039	12 (21)	27 (26)	0.92 (0.35, 2.39)		
	≥0.040	17 (30)	26 (25)	1.34 (0.51, 3.51)		

Magnesium (mg)	<2.64	13 (23)	27 (26)	1.00	0.977	0.940
	2.64-7.37	15 (26)	26 (25)	1.16 (0.46, 2.93)		
	7.38-13.84	16 (28)	26 (25)	1.23 (0.49, 3.12)		
	≥13.85	13 (23)	26 (25)	0.96 (0.35, 2.59)		
Phosphorus (mg)	<0.010	12 (21)	28 (27)	1.00	0.799	0.862
	0.010-0.020	17 (30)	26 (25)	1.49 (0.59, 3.75)		
	0.0210-0.036	13 (23)	25 (24)	1.17 (0.43, 3.19)		
	≥0.037	15 (26)	26 (25)	1.26 (0.46, 3.46)		
Potassium (mg)	<0.597	17 (30)	26 (25)	1.00	**0.099**	**0.126**
	0.597-1.053	15 (26)	27 (26)	0.81 (0.33, 1.96)		
	1.054-1.640	19 (33)	26 (25)	1.02 (0.43, 2.44)		
	≥1.641	6 (11)	26 (25)	0.29 (0.09, 0.91)[g]		
Sodium (mg)	<3.56	16 (28)	27 (26)	1.00	0.348	**0.126**
	3.56-6.72	12 (21)	26 (25)	0.75 (0.29, 1.93)		
	6.73-12.36	22 (39)	26 (25)	1.34 (0.57, 3.15)		
	≥12.37	7 (12)	26 (25)	0.40 (0.13, 1.18)		
Sulfate (mg)	<2.46	14 (25)	27 (26)	1.00	0.556	0.277
	2.46-4.75	14 (25)	26 (25)	0.98 (0.39, 2.48)		
	4.76-8.54	20 (35)	25 (24)	1.48 (0.61, 3.59)		
	≥8.55	9 (16)	27 (26)	0.55 (0.19, 1.58)		
Zinc[f] (mg)	<0.007	13 (23)	27 (26)	1.00	0.870	0.994
	0.007-0.013	14 (25)	25 (24)	1.10 (0.42, 2.86)		
	0.014-0.027	14 (25)	27 (26)	1.01 (0.39, 2.64)		
	≥0.028	15 (27)	26 (25)	1.12 (0.43, 2.95)		

[a] May not total 100% due to rounding to nearest whole number
[b] Adjusted for age and sex
[c] 95% confidence interval
[d] *P* for trend (likelihood ratio test)
[e] Global *p*-value (Wald test)
[f] Missing value (case)

[g] $P \leq 0.05$

Table 6-3 Crude unconditional associations between type 1 diabetics in children and youth and common dietary components from food and water (total dietary intake) ($n = 159$), in Prince Edward Island, Canada (2001-2004).

Total dietary component (unit)	Concentration of daily intake	Cases (%)[a] $n = 57$	Controls (%)[a] $n = 105$	Odds ratio[b] (CI[c])	p for trend[d]	Global p-value[e]
Nitrate	<7.20	8 (14)	27 (26)	1.00	0.930	**0.072**
(mg)	7.20-9.86	24 (42)	26 (25)	3.17 (1.12, 9.01)[f]		
	9.87-11.88	9 (16)	25 (24)	1.18 (0.34, 4.09)		
	≥11.89	16 (28)	27 (26)	1.99 (0.53, 7.52)		
Nitrate and nitrite	<9.56	8 (14)	27 (26)	1.00	0.937	**0.176**
(mg)	9.56-13.20	22 (39)	25 (24)	2.90 (1.02, 8.25)[f]		
	13.21-16.72	13 (23)	27 (26)	1.56 (0.48, 5.07)		
	≥16.73	14 (25)	26 (25)	1.67 (0.40, 6.88)		
Calcium	<1065.5	18 (32)	26 (25)	1.00	**0.058**	**0.163**
(mg)	1065.5-1439.36	18 (32)	26 (25)	0.89 (0.35, 2.23)		
	1439.37-1707.90	7 (12)	26 (25)	0.33 (0.11, 0.97)[f]		
	≥1707.91	14 (25)	27 (26)	0.47 (0.14, 1.55)		
Magnesium	<233.60	14 (25)	26 (25)	1.00	0.281	0.717
(mg)	233.60-303.04	17 (30)	27 (26)	0.92 (0.34, 2.48)		
	303.05-367.92	12 (21)	26 (25)	0.59 (0.19, 1.86)		
	≥367.93	14 (25)	26 (25)	0.51 (0.11, 2.36)		
Phosphorus	<1350.30	18 (32)	26 (25)	1.00	**0.147**	0.299
(mg)	1350.30-1691.00	14 (25)	26 (25)	0.61 (0.23, 1.62)		
	1691.01-1898.02	9 (16)	27 (26)	0.32 (0.10, 1.03)		
	≥1898.03	16 (28)	26 (25)	0.44 (0.11, 1.77)		
Potassium	<2640.79	15 (26)	26 (25)	1.00	0.272	0.742
(mg)	2640.79-3247.33	16 (28)	27 (26)	0.85 (0.33, 2.20)		

	3247.34-3854.30	12 (21)	25 (24)	0.60 (0.20, 1.80)		
	≥3854.31	14 (25)	27 (26)	0.50 (0.12, 2.01)		
Sodium	<2148.40	12 (21)	27 (26)	1.00	0.338	0.532
(mg)	2148.40-2890.71	18 (32)	25 (24)	1.26 (0.45, 3.50)		
	2890.72-3572.18	15 (26)	27 (26)	0.80 (0.24, 2.71)		
	≥3572.19	12 (21)	26 (25)	0.50 (0.10, 2.58)		
Zinc[g]	<8.48	17 (30)	27 (26)	1.00	**0.068**	0.358
(mg)	8.48-10.31	15 (26)	26 (25)	0.64 (0.24, 1.69)		
	10.32-13.16	12 (21)	25 (24)	0.43 (0.14, 1.32)		
	≥13.17	13 (23)	27 (26)	0.26 (0.06, 1.20)		

[a] May not total 100% due to rounding to nearest whole number
[b] Adjusted for age, sex, energy intake (Kcal), family member with type 1 diabetes, infections during the first two years of life, father's education, and residential remoteness
[c] 95% confidence interval
[d] *P* for trend (likelihood ratio test)
[e] Global *p*-value (Wald test)
[f] $P \leq 0.05$
[g] Missing value (case)

Table 6-4 Adjusted associations between type 1 diabetes in children and youth and dietary intake from food, controlling for significant genetic and environmental factors ($n = 159$), in Prince Edward Island, Canada (2001-2004)

Food component (unit)	Concentration of daily intake	Cases (%)[a] $n = 55$	Controls (%)[a] $n = 104$	Odds ratio[b] (CI[c])	p for trend[d]	Global p-value[e]
Nitrate	<5.66	9 (16)	26 (25)	1.00	0.286	0.611
(mg)	5.66-7.27	13 (24)	25 (24)	1.01 (0.28, 3.61)		
	7.28-9.00	13 (24)	26 (25)	1.19 (0.31, 4.52)		
	≥9.01	20 (36)	27 (26)	2.25 (0.45, 11.14)		
Nitrite	<1.83	11 (20)	26 (25)	1.00	0.624	0.949
(mg)	1.83-3.26	15 (27)	27 (26)	0.94 (0.28, 3.18)		
	3.27-4.81	17 (31)	26 (25)	1.24 (0.35, 4.47)		
	≥4.82	12 (22)	25 (24)	1.30 (0.30, 5.59)		
Nitrosamine	<0.01	19 (35)	35 (34)	1.00	0.510	0.742
(mg)	0.01-0.029	17 (31)	35 (34)	0.57 (0.21, 1.57)		
	0.03-0.039	6 (11)	12 (12)	0.66 (0.18, 2.45)		
	≥0.04	13 (24)	22 (21)	0.62 (0.19, 2.00)		
Protein	<67.64	15 (27)	26 (25)	1.00	0.666	0.448
(g)	67.64-82.12	12 (22)	26 (25)	0.40 (0.12, 1.36)		
	82.13-97.63	13 (24)	26 (25)	0.51 (0.14, 1.87)		
	≥97.64	15 (27)	26 (25)	0.70 (0.12, 4.05)		
Fat	<62.21	17 (31)	26 (25)	1.00	0.764	0.513
(g)	62.21-72.87	6 (11)	26 (25)	0.37 (0.10, 1.42)		
	72.88-96.37	17 (31)	26 (25)	0.64 (0.18, 2.33)		
	≥96.38	15 (27)	26 (25)	0.71 (0.11, 4.57)		
Total	<233.93	11 (20)	27 (26)	1.00	**0.129**	**0.050**
carbohydrate	233.93-289.43	8 (15)	24 (23)	0.57 (0.15, 2.23)		
(g)	289.44-351.99	21 (38)	26 (25)	3.36 (0.80, 14.14)		
	≥352.00	15 (27)	27 (26)	2.18 (0.29, 16.47)		

Total sugars (g)	<120.57	17 (31)	27 (26)	1.00	0.917	0.351
	120.57-140.03	7 (13)	25 (24)	0.34 (0.10, 1.17)		
	140.04-171.41	15 (27)	26 (25)	0.74 (0.23, 2.42)		
	≥171.42	16 (29)	26 (25)	0.79 (0.17, 3.70)		
Total fibre (g)	<11.5	6 (11)	23 (22)	1.00	0.867	0.343
	11.5-15.09	21 (38)	28 (27)	2.15 (0.56, 8.33)		
	15.10-18.59	11 (20)	26 (25)	0.90 (0.20, 4.12)		
	≥18.60	17 (31)	27 (26)	1.63 (0.28, 9.52)		
Vitamin A (µg)	<501.80	16 (29)	26 (25)	1.00	0.868	**0.015**
	501.80-653.99	7 (13)	25 (24)	0.10 (0.02, 0.44)[f]		
	654.00-951.99	19 (35)	26 (25)	0.63 (0.18, 2.19)		
	≥952.00	13 (24)	27 (26)	0.49 (0.12, 2.06)		
Vitamin E (mg)	<3.21	11 (20)	26 (25)	1.00	0.340	0.628
	3.21-4.06	12 (22)	25 (24)	0.87 (0.26, 2.89)		
	4.07-5.35	21 (38)	26 (25)	0.83 (0.24, 2.86)		
	≥5.36	11 (20)	27 (26)	0.40 (0.08, 2.06)		
Vitamin D (µg)	<5.14	13 (24)	25 (24)	1.00	0.478	0.572
	5.14-8.72	19 (35)	27 (26)	1.50 (0.51, 4.39)		
	8.73-10.86	15 (27)	26 (25)	1.13 (0.37, 3.49)		
	≥10.87	8 (15)	26 (25)	0.65 (0.17, 2.44)		
Vitamin C (mg)	<81.20	16 (29)	27 (26)	1.00	0.445	0.848
	81.20-130.89	15 (27)	26 (25)	0.73 (0.25, 2.17)		
	130.90-203.89	11 (20)	26 (25)	0.58 (0.17, 1.97)		
	≥203.90	13 (24)	25 (24)	0.61 (0.16, 2.34)		
Thiamin (mg)	<1.31	10 (18)	26 (25)	1.00	0.817	0.970
	1.31-1.70	14 (25)	25 (24)	1.33 (0.38, 4.62)		
	1.71-2.07	17 (31)	27 (26)	1.33 (0.33, 5.42)		
	≥2.08	14 (25)	26 (25)	1.26 (0.19, 8.17)		
Riboflavin (mg)	<2.01	15 (27)	26 (25)	1.00	0.252	0.643
	2.01-2.52	18 (33)	25 (24)	0.83 (0.26, 2.62)		

	2.53-2.95	9 (16)	26 (25)	0.48 (0.14, 1.63)		
	≥2.96	13 (24)	27 (26)	0.52 (0.10, 2.59)		
Niacin	<29.30	13 (24)	26 (25)	1.00	0.927	0.987
(NE)	29.30-36.92	11 (20)	26 (25)	0.82 (0.24, 2.78)		
	36.93-43.89	15 (27)	26 (25)	0.86 (0.24, 3.07)		
	≥43.90	16 (29)	26 (25)	0.93 (0.17, 5.15)		
Vitamin B6	<1.31	11 (20)	26 (25)	1.00	0.917	0.956
(mg)	1.31-1.64	15 (27)	27 (26)	0.93 (0.28, 3.11)		
	1.65-1.95	15 (27)	25 (24)	0.78 (0.21, 2.88)		
	≥1.96	14 (25)	26 (25)	1.02 (0.19, 5.59)		
Vitamin B12	<4.09	20 (36)	25 (24)	1.00	0.340	**0.072**
(μg)	4.09-5.02	14 (25)	25 (24)	0.61 (0.20, 1.86)		
	5.03-6.23	5 (9)	28 (27)	0.18 (0.04, 0.69) [f]		
	≥6.24	16 (29)	26 (25)	0.85 (0.23, 3.18)		
Folate	<333.20	15 (27)	26 (25)	1.00	**0.130**	0.500
(μg)	333.20-414.99	14 (25)	25 (24)	0.77 (0.24, 2.48)		
	415.00-544.29	15 (27)	27 (26)	0.50 (0.14, 1.79)		
	≥544.30	11 (20)	26 (25)	0.28 (0.05, 1.58)		
Calcium	<1024.10	13 (24)	26 (25)	1.00	0.544	**0.169**
(mg)	10024.10-1392.99	20 (36)	25 (24)	1.47 (0.46, 4.73)		
	1393.00-1672.79	7 (13)	26 (25)	0.39 (0.11, 1.44)		
	≥1672.80	15 (27)	27 (26)	1.23 (0.29, 5.24)		
Iron	<10.00	10 (18)	26 (25)	1.00	0.958	0.936
(mg)	10.00-13.27	17 (31)	26 (25)	1.19 (0.34, 4.20)		
	13.28-16.11	9 (16)	25 (24)	0.87 (0.20, 3.75)		
	≥16.12	19 (35)	27 (26)	1.22 (0.22, 6.85)		
Magnesium	<228.50	13 (24)	25 (24)	1.00	0.257	0.305
(μg)	228.50-295.49	16 (29)	26 (25)	0.73 (0.22, 2.45)		
	295.50-354.99	12 (22)	26 (25)	0.31 (0.08, 1.23)		
	≥355.00	14 (25)	27 (26)	0.59 (0.10, 3.66)		

Phosphorus (µg)	<1350.30	16 (29)	25 (24)	1.00	0.389	0.433
	1350.30-1690.99	14 (25)	26 (25)	0.45 (0.14, 1.46)		
	1661.00-1897.99	9 (16)	26 (25)	0.36 (0.09, 1.40)		
	≥1898.00	16 (29)	27 (26)	0.51 (0.10, 2.58)		
Potassium (µg)	<2639.00	13 (24)	25 (24)	1.00	0.561	0.766
	2639.00-3245.99	16 (29)	26 (25)	0.55 (0.17, 1.79)		
	3246.00-3852.99	12 (22)	26 (25)	0.55 (0.15, 2.01)		
	≥3853.00	14 (25)	27 (26)	0.60 (0.11, 3.20)		
Sodium (µg)	<2131.00	11 (20)	26 (25)	1.00	0.326	0.734
	2131.00-2889.69	17 (31)	26 (25)	1.01 (0.30, 3.46)		
	2889.70-3557.99	15 (27)	25 (24)	0.60 (0.14, 2.47)		
	≥3558.00	12 (22)	27 (26)	0.48 (0.07, 3.11)		
Zinc (µg)	<8.47	15 (27)	24 (23)	1.00	**0.198**	0.361
	8.47-10.27	15 (27)	28 (27)	0.37 (0.11, 1.23)		
	10.28-13.12	11 (20)	25 (24)	0.32 (0.08, 1.29)		
	≥13.13	14 (25)	27 (26)	0.31 (0.05, 1.89)		
Selenium (µg)	<76.30	13 (24)	26 (25)	1.00	0.830	0.611
	76.30-94.69	15 (27)	27 (26)	0.90 (0.28, 2.87)		
	94.70-116.19	10 (18)	25 (24)	0.54 (0.15, 2.00)		
	≥116.20	17 (31)	26 (25)	1.09 (0.22, 5.50)		
Alcohol (g)	0	51 (93)	94 (90)	1.00	-	0.354
	>0	4 (7)	10 (10)	0.45 (0.08, 2.43)		
Caffeine (µg)	<3.36	13 (24)	27 (26)	1.00	**0.068**	**0.180**
	3.36-8.67	8 (15)	25 (24)	1.49 (0.40, 5.58)		
	8.68-21.99	13 (24)	25 (24)	1.47 (0.42, 5.09)		
	≥22.00	21 (38)	27 (26)	4.88 (1.00, 23.86)[f]		
Aspartame (µg)	0	38 (69)	77 (74)	1.00	-	0.707
	>0	17 (31)	27 (26)	1.17 (0.51, 2.68)		
Cholesterol (µg)	<179.00	15 (27)	25 (24)	1.00	0.669	0.421
	179.00-219.99	11 (20)	26 (25)	0.42 (0.13, 1.38)		

220.00-287.99	17 (31)	26 (25)	0.88 (0.27, 2.92)
≥288.00	12 (22)	27 (26)	0.54 (0.12, 2.41)

[a] May not total 100% due to rounding to nearest whole number
[b] Adjusted for age, sex, energy intake (Kcal), family member with type 1 diabetes, infections during the first two years of life, father's education, and residential remoteness
[c] 95% confidence interval
[d] *P* for trend (likelihood ratio test)
[e] Global *p*-value (Wald test)
[f] $P \leq 0.05$

Table 6-5 Adjusted associations between type 1 diabetes in children and youth and drinking water chemistry components ($n = 159$), in Prince Edward Island, Canada (2001-2004).

Water component (unit)	Concentration of daily intake	Cases (%)[a] $n = 55$	Controls (%)[a] $n = 104$	Odds ratio[b] (95% CI)[c]	p for trend[d]	Global p-value[e]
Nitrate	<0.78	14 (25)	26 (25)	1.00	0.830	0.972
(mg)	0.78-1.61	15 (27)	26 (25)	1.09 (0.38, 3.08)		
	1.62-3.27	17 (31)	26 (25)	1.08 (0.38, 3.03)		
	≥3.28	9 (16)	26 (25)	0.83 (0.24, 2.84)		
Water pH[f]	<7.668	9 (17)	26 (25)	1.00	**0.103**	0.226
(pH unit)	7.678-7.851	16 (30)	34 (33)	0.77 (0.25, 2.38)		
	7.852-7.933	8 (15)	18 (17)	0.86 (0.24, 3.15)		
	≥7.934	21 (39)	26 (25)	2.18 (0.73, 6.55)		
Alkalinity	<47.53	15 (27)	26 (25)	1.00	0.758	0.744
(mg)	47.53-84.05	12 (22)	27 (26)	0.73 (0.24, 2.21)		
	84.06-121.88	18 (33)	26 (25)	1.14 (0.40, 3.28)		
	≥121.89	10 (18)	25 (24)	0.64 (0.18, 2.26)		
Calcium	<11.91	15 (27)	26 (25)	1.00	0.925	**0.142**
(mg)	11.91-23.68	12 (22)	27 (26)	0.51 (0.17, 1.55)		
	23.69-39.87	19 (35)	26 (25)	1.82 (0.59, 5.54)		
	≥39.88	9 (16)	25 (24)	0.70 (0.20, 2.48)		
Chloride	<6.79	12 (22)	26 (25)	1.00	0.203	0.487
(mg)	6.79-12.77	11 (20)	27 (26)	0.81 (0.26, 2.47)		
	12.78-20.71	14 (25)	25 (24)	1.20 (0.38, 3.77)		
	≥20.72	18 (33)	26 (25)	1.94 (0.61, 6.22)		
Copper	<0.009	13 (24)	27 (26)	1.00	0.723	0.957
(mg)	0.009-0.017	15 (27)	25 (24)	0.91 (0.31, 2.61)		
	0.018-0.039	11 (20)	27 (26)	0.73 (0.24, 2.25)		
	≥0.040	16 (29)	25 (24)	0.86 (0.27, 2.76)		

Magnesium (mg)	<2.64	12 (22)	27 (26)	1.00	0.959	0.602
	2.64-7.37	15 (27)	26 (25)	1.86 (0.62, 5.54)		
	7.38-13.84	15 (27)	26 (25)	0.92 (0.31, 2.72)		
	≥13.85	13 (24)	25 (24)	1.21 (0.39, 3.73)		
Phosphorus (mg)	<0.010	11 (20)	28 (27)	1.00	0.572	0.808
	0.010-0.020	17 (31)	26 (25)	1.35 (0.46, 4.00)		
	0.0210-0.036	13 (24)	25 (24)	1.01 (0.31, 3.23)		
	≥0.037	14 (25)	25 (24)	1.62 (0.47, 5.54)		
Potassium (mg)	<0.597	16 (29)	26 (25)	1.00	0.248	0.556
	0.597-1.053	14 (25)	27 (26)	0.83 (0.30, 2.35)		
	1.054-1.640	19 (35)	26 (25)	0.85 (0.31, 2.33)		
	≥1.641	6 (11)	25 (24)	0.40 (0.11. 1.46)		
Sodium (mg)	<3.56	15 (27)	27 (26)	1.00	0.399	**0.094**
	3.56-6.72	11 (20)	26 (25)	0.63 (0.21, 1.94)		
	6.73-12.36	22 (40)	26 (25)	1.48 (0.52, 4.21)		
	≥12.37	7 (13)	25 (24)	0.37 (0.10, 1.31)		
Sulfate (mg)	<2.46	13 (24)	27 (26)	1.00	0.941	0.341
	2.46-4.75	13 (24)	26 (25)	0.75 (0.26, 2.18)		
	4.76-8.54	20 (36)	25 (24)	1.64 (0.57, 4.69)		
	≥8.55	9 (16)	26 (25)	0.66 (0.19, 2.27)		
Zinc[f] (mg)	<0.007	12 (22)	27 (26)	1.00	0.837	0.958
	0.007-0.013	14 (26)	25 (24)	0.85 (0.27, 2.64)		
	0.014-0.027	13 (24)	26 (25)	0.87 (0.28, 2.70)		
	≥0.028	15 (28)	26 (25)	1.10 (0.35, 3.47)		

[a] May not total 100% due to rounding to nearest whole number

[b] Adjusted for age, sex, energy intake (Kcal), family member with type 1 diabetes, infections during the first two years of life, father's education, and residential remoteness

[c] 95% confidence interval

[d] *P* for trend (likelihood ratio test)

[e] Global *p*-value (Wald test)

[f] Missing value (case)

Table 6-6 Adjusted associations between type 1 diabetes in children and youth and common dietary components from food and water (total dietary intake) (n = 162), in Prince Edward Island, Canada (2001-2004).

Total dietary component (unit)	Concentration of daily intake	Cases (%)[a] n = 55	Controls (%)[a] n = 104	Odds ratio[b] (95% CI)[c]	p for trend[d]	Global p-value[e]
Nitrate	<7.20	7 (13)	27 (26)	1.00	0.611	**0.104**
(mg)	7.20-9.86	23 (42)	26 (25)	3.22 (0.93, 11.17)		
	9.87-11.88	9 (16)	24 (23)	1.02 (0.23, 4.46)		
	≥11.89	16 (29)	27 (26)	2.81 (0.60, 13.23)		
Nitrate and nitrite	<9.56	7 (13)	27 (26)	1.00	0.594	0.440
(mg)	9.56-13.20	21 (38)	25 (24)	2.70 (0.77, 9.43)		
	13.21-16.72	13 (24)	26 (25)	1.66 (0.42, 6.58)		
	≥16.73	14 (25)	26 (25)	2.39 (0.46, 12.37)		
Calcium	<1065.5	17 (31)	25 (24)	1.00	**0.170**	0.288
(mg)	1065.5-1439.36	17 (31)	26 (25)	0.82 (0.27, 2.45)		
	1439.37-1707.90	7 (13)	26 (25)	0.31 (0.09, 1.07)		
	≥1707.91	14 (25)	27 (26)	0.56 (0.14, 2.23)		
Magnesium	<233.60	13 (24)	25 (24)	1.00	**0.131**	**0.196**
(mg)	233.60-303.04	16 (29)	27 (26)	0.70 (0.21, 2.37)		
	303.05-367.92	12 (22)	26 (25)	0.24 (0.06, 1.00)[f]		
	≥367.93	14 (25)	26 (25)	0.42 (0.06, 2.70)		
Phosphorus	<1350.30	16 (29)	25 (24)	1.00	0.359	0.361
(mg)	1350.30-1691.00	14 (25)	26 (25)	0.44 (0.14, 1.45)		
	1691.01-1898.02	9 (16)	27 (26)	0.33 (0.08, 1.27)		
	≥1898.03	16 (29)	26 (25)	0.51 (0.10, 2.62)		
Potassium	<2640.79	13 (24)	25 (24)	1.00	0.592	0.697
(mg)	2640.79-3247.33	16 (29)	27 (26)	0.48 (0.15, 1.59)		
	3247.34-3854.30	12 (22)	25 (24)	0.59 (0.16, 2.19)		
	≥3854.31	14 (25)	27 (26)	0.56 (0.10, 2.99)		

Sodium (mg)	<2148.40	11 (20)	27 (26)	1.00	0.261	0.586
	2148.40-2890.71	17 (31)	24 (23)	1.11 (0.33, 3.75)		
	2890.72-3572.18	15 (27)	27 (26)	0.55 (0.14, 2.26)		
	≥3572.19	12 (22)	26 (25)	0.45 (0.07, 2.84)		
Zinc[g] (mg)	<8.48	15 (27)	26 (25)	1.00	**0.115**	0.326
	8.48-10.31	15 (27)	26 (25)	0.37 (0.11, 1.21)		
	10.32-13.16	12 (22)	25 (24)	0.31 (0.08, 1.26)		
	≥13.17	13 (24)	27 (26)	0.24 (0.04, 1.45)		

[a] May not total 100% due to rounding to nearest whole number
[b] Adjusted for age and sex
[c] 95% confidence interval
[d] *P* for trend (likelihood ratio test)
[e] Global *p*-value (Wald test)
[f] $P \leq 0.05$
[g] Missing value (case)

Chapter 7 Summary and general discussion

7.1. Introduction

The overall hypothesis of this research was that nitrate from food and drinking water sources was positively associated with the risk of type 1 diabetes mellitus (T1D). The main objective was to examine the relationship between T1D and dietary nitrate intake from both drinking water and food sources in Prince Edward Island (PEI) at the ecological level, and at the individual level. Historical data on ground water nitrate concentrations and land use patterns were utilized to examine temporal and spatial assumptions made in these two T1D studies. Ground water nitrate concentrations were temporally assessed (Chapter 2), and the association between ground water nitrate and local land use was spatially evaluated, comparing several areal aggregation methods (Chapter 3). Average nitrate concentration in ground water and T1D incidence at the watershed level were compared, taking into account the population-at-risk and average household income (Chapter 4). A case-control study compared drinking water chemistry, food frequency, and average dietary component (e.g. nutrients) consumption between patients diagnosed with T1D during a four year period, and their age and sex matched controls, with an emphasis on nitrate concentrations and its derivatives (Chapters 5 and 6). Some environmental and genetic-based factors were evaluated and controlled for where appropriate.

The following chapter summarizes each of the five research chapters in this thesis, highlighting the main components of the materials and methods used for the

analyses, the results obtained, and the conclusions drawn from the studies. Following the chapter summaries is a section describing how the chapters link together, and how nitrate exposure may be related to T1D in a causal pathway. Finally, suggestions for future research are made, with a particular emphasis on the case-control study.

7.2. Temporal evaluation of ground water nitrate concentrations

The specific objective of this study was to identify monthly, seasonal or annual trends in historical nitrate concentrations in PEI ground water, taking into account local land use, clustering of data, and temporal autocorrelation, where possible. Results from this study would help to determine if the temporal distribution of ground water nitrate concentrations should be controlled for when assessing the effect of land use on local ground water nitrate concentrations (Chapter 3) and ground water nitrate as a possible risk factor of the development of T1D at the area level (Chapter 4) and at the individual level (Chapter 6).

Part of the temporal data consisted of 37 months of monthly ground water samples from December 1988 to December 1991, taken from 54 different sites, grouped by land use, totalling 1,868 samples. The six land use categories were 'pristine' areas (non-cropped), row cropped areas, non-row cropped areas, feedlot areas with on-site manure storage, subdivisions with on-site sewage disposal, and subdivisions with central sewage collection (Somers, 1998). The annual assessment of nitrate concentrations was evaluated using yearly ground water samples from 167 institutions sampled over a 16-

year period, totalling 1,299 samples. Institutions included schools, senior citizen homes, and national and provincial park facilities, to name a few.

The monthly data were analysed using a hierarchical model. The top two levels accounted for years clustered within sites, and months clustered within years, and the lowest level was the error term (month). The annual nitrate samples were analysed using a two level hierarchical model with the top level being sampling sites, and the bottom level being the error term (year).

Results from the monthly dataset showed that the mean and median nitrate values for all observations were 4.0 mg L^{-1} and 3.3 mg L^{-1}, respectively, (interquartile range of 1.9 to 5.2 mg L^{-1}). The maximum nitrate concentration was 15.5 mg L^{-1}. Land use was strongly associated with the variation in ground water nitrate concentrations, and this effect was somewhat dependent on season. Nitrate concentrations were higher in the autumn and winter for residential locations with on-site sewage disposal and agricultural areas without row-crops, higher in the spring and autumn for areas with on-site manure storage, and higher in the summer for locations with row crops. Residential areas with central sewage disposal and pristine areas showed little seasonal variation in nitrate concentrations. Monthly fluctuations were evident, however over the three years, the range in average monthly nitrate concentration (as nitrate-nitrogen) was small (3.8 to 4.1 mg L^{-1} in April and January, respectively). In general, areas with agricultural land uses had greater average nitrate concentrations than areas with residential land uses (6.5, 4.0, and 5.4 mg L^{-1} for row crops, non-row crops, and feedlot areas with on-site manure storage, respectively, versus 4.3 and 2.6 mg L^{-1} for residential areas with on-site sewage

disposal areas or central sewage collection, respectively), which in-turn were higher than pristine (low human-impact) areas (1.1 mg L^{-1}).

For the annual nitrate dataset, the mean and median nitrate values in the dataset were 2.4 mg L^{-1} and 1.9 mg L^{-1}, respectively (interquartile range of 1.0 to 3.2 mg L^{-1}). The maximum concentration was 14.0 mg L^{-1}. When comparing the first three sampling years to the last three sampling years, nitrate concentrations in 9.6% of the sites significantly increased over time, and 6.6% significantly decreased. There was no overall significant annual effect with ground water nitrate concentrations.

From these findings, it was concluded that in general, ground water nitrate concentrations in PEI did not greatly change over time. Some monthly temporal fluctuation was significant, but the range at which this occurred was very small. Therefore, the temporal distribution of nitrate concentrations did not need to be controlled for in further analyses assessing the relationship between land use and nitrate, and the incidence of T1D and ground water concentrations at area and individual levels. Furthermore, current nitrate concentrations could be utilized to represent nitrate concentrations prior to the diagnosis of T1D (up to two years before being interviewed – Chapter 6), as possible misclassification of nitrate concentrations would likely be limited. A more thorough analysis of land use was conducted to determine its role in ground water nitrate concentrations (Chapter 3).

7.3. Spatial variation of ground water nitrate concentrations

The primary objective of this study was to determine what land uses had a significant impact on the nitrate concentrations of private well water systems in PEI, adjusting for spatial autocorrelation where present. The secondary objective was to determine the best method of spatial aggregation for assessing impacts of land use on nitrate concentrations, balancing data scarcity problems with within unit homogeneity of nitrate concentrations. Results of this study would assist in the determination of the best method of unit of aggregation to effectively assess the relationship between nitrate in ground water and T1D incidence at the area level in Chapter 4. Also, a better understanding of impacts of land use on nitrate concentrations in ground water would lead to better interpretation of ground water test results from the homes of study participants in Chapter 6, according to the changes in local land use surrounding the homes.

A total of 4,855 nitrate samples taken from a routine pre-mortgage ground water sampling program during 1997 to 2001 were used in the analyses. Land use categories were taken from detailed areal photographs from the summer of 2000, and were allocated into 14 categories: apple, bare soil, blueberry, clear-cut woodland, cranberry, forest, grain, hay, meadow/dune, other agriculture, pasture, potato, residential, and water/wetland. Three different units of spatial aggregations were investigated: watersheds based on topography and hydrology systems; freeform polygon boundaries created based on similar neighbouring nitrate values; and 500 m buffer zones created around wells sampled during one year (insufficient computational power to analyse more than one

year). Average nitrate concentrations were determined for each watershed and freeform polygon with a minimum of five nitrate values per area unit. If an area contained less than five nitrate values, then it was combined with a neighbouring unit of similar average nitrate concentration (difference < 2.5 mg L^{-1}). A total of 174 watersheds remained, with an average size of 32.6 km^2 (range of 0.89 to 196.8 km^2), and 664 freeform polygons remained, with an average size of 8.55 km^2 (range of 0.005 to 175.63 km^2). Percent of each category of land use was determined for all units of analysis for the three spatial aggregations methods.

For the watershed and freeform polygon analyses, a binary weights matrix was created, using Queen contiguity, identifying which areas were considered neighbours. For the buffer zone analyses, a distance weights matrix was utilized based on the inverse distance between points. Using the Moran's I statistic to assess global spatial autocorrelation of nitrate concentrations between spatial units for each aggregation method (not taking land use into account), it was determined that watersheds and buffer zones showed positive spatial autocorrelation, whereby wells with high nitrate concentrations were clustered together, and wells with low concentrations were clustered together. Freeform polygons showed negative spatial autocorrelation, whereby low nitrate concentrations were neighbouring high nitrate concentrations, and vice-versa, but this may have been due to the aggregation methodology.

Ordinary least squares regression modelling was conducted for each spatial aggregation method to determine associations between nitrate concentrations and percent land use. Then, a spatial lag model (Florax and de Graff, 2004) was run for each final

model using the contiguous (polygon models) or distance (buffer zone model) weights matrices. The spatial lag model is a linear regression model with a spatial variable incorporated to adjust for lack of independence of the units of analysis due to spatial autocorrelation.

The mean and median nitrate concentrations of the 4,855 individual nitrate measurements were 3.4 and 2.9 mg L^{-1}, respectively, with a range of 0.1 to 27.5 mg L^{-1}. When land use was taken into account, spatial autocorrelation was no longer significant for watersheds, but remained for the other two aggregations. Regardless of the aggregation method, the percentage of potato, grain, and hay production were significantly and positively associated with nitrate concentrations in PEI well water, compared to the baseline land use of forested areas. Residential areas, and those with "other agriculture" were significant positive predictors in two of the three methods of aggregation, while pasture and clear-cut woodland areas were positive predictors in only one aggregation unit each (buffer zones and watersheds, respectively). Blueberry production was the only negative predictor, and was marginally significant in the watershed and significant in the freeform polygon models.

Although watersheds did not possess high within-unit homogeneity of nitrate concentrations, they were the preferred method of aggregation because: they were created according to hydrological factors; they were large enough to accurately determine average nitrate concentrations because each watershed contained most land use categories; and they explained the largest amount of variation between nitrate concentrations, according to the R^2 of each final model.

Results of this study determined which land uses had significant relationships with nitrate concentrations in ground water in PEI, confirming the importance of collecting data on the land use surrounding the homes of case-control study participants in Chapter 6. This information should be utilized, in conjunction with current and historical ground water nitrate test results, in order to adjust observed nitrate values where recent land use changes may have led to misclassifications of ground water nitrate values. Furthermore, watersheds were found to be the preferred unit of analysis and therefore should be used to assess the relationship between nitrate concentrations in ground water and T1D incidence at the area level in Chapter 4.

7.4. Relationship between standardized incidence rate ratios of T1D and nitrate in ground water

Because of the reasonably high incidence of T1D in PEI, as well as high ground water nitrate concentrations, an assessment of the relationship between the two factors at the ecological level was conducted. The objectives of this study were to determine if the incidence of T1D was associated with ground water nitrate concentrations averaged at the watershed level, adjusting for income, and to determine if the incidence of T1D was spatially clustered at the watershed level.

According to the Provincial Diabetes Registry, a total of 244 cases were diagnosed with T1D during 1990 to 2004. The specific watershed locations of 223 of these cases were identified using postal codes, lot numbers, and civic addresses, where available. The population-at-risk for each watershed for the study period came from the

1996 census data. Census data were originally obtained at the enumeration area (small population boundaries), and re-aggregated at the watershed level. The aggregated population and case data were used to calculate age- and sex-standardized incidence rate ratios, using five-year age intervals, for each watershed.

A total of 4,855 water samples from private rural wells, along with 413 samples from 13 municipal systems sampled during 1997 to 2004, were utilized for nitrate concentration determination. Average nitrate concentrations were calculated for 166 watersheds for each of the two datasets (10 watersheds from the original 174 had very low populations so were amalgamated with neighbouring watersheds to create stable standardized incidence rate ratios). Then, average weighted nitrate concentrations were calculated for the watersheds, weighted on the population distribution of each watershed between town and rural homes. The weighted average nitrate values at the watershed level were categorized into three distinct groups (0.10–3.00, 3.01–5.60, and 5.61–10.00 mg L^{-1}). Total household income was also available from the 1996 census, re-aggregated from enumeration area to watersheds, and then also averaged at the watershed level.

Over the 15-year period, the incidence rate of T1D was 33.1/100,000 person-years, with males more frequently being diagnosed than females (60% of cases versus 40%). However, the effect of sex did depend somewhat on age. Males were significantly more frequently diagnosed in the 10–14-year old group, and female incidence peaked in the 5–9-year old group. As T1D is considered a 'rare' disease, a convolution model using a Bayesian framework was implemented in order to effectively cope with 70% of the watersheds having zero cases. Results showed that average ground water nitrate

concentrations were not associated with the spatial distribution of T1D, however, there was a trend toward the high nitrate group (5.61–10.00 mg L^{-1}) having a higher standardized incidence rate ratio compared to the lower nitrate group (0.10–3.00 mg L^{-1}). Adding income to the model increased the coefficient of the higher nitrate group, but not enough to consider income as a confounder. Although not significant, average household income was inversely associated with T1D standardized incidence rate ratios. A total of 25% of the random variation of the T1D standardized incidence rate ratios was attributable to spatially structured variation, with the remainder of the variation occurring through unstructured (non-spatial) random effects. A sensitivity analysis showed that the non-informative priors were appropriate for the model, as they were robust enough to not substantially influence the study results.

Even though the spatial analysis did not show a significant relationship between T1D incidence and nitrate, there is still a need to examine the relationship at the individual level because spatial analyses can present difficulties in their interpretation. With aggregation of data into larger spatial units, the number of units of analysis is lower, and there is less variability between units of analysis due to the averaging of data among individuals within units, producing less power to detect differences if they were present. Furthermore, ecological fallacy may confound the interpretation, whereby the exposure assessed to the area may not be representative of the exposures of those with the disease in the area, particularly if there is substantial within unit variability. Therefore, a study of the relationship between T1D incidence and ground water nitrate concentrations (and

other potential risk factors) was also conducted at the individual level using a case-control study design (Chapter 6).

7.5. Relationship between the risk of T1D and food consumption patterns

The objectives of the last two substantive chapters of the thesis were to determine the relationships between T1D incidence and food consumption patterns, and dietary component distribution patterns (including drinking water components), respectively. Initial analyses utilized food consumption data to determine which foods or food groups may be predisposing to, or protective of, T1D incidence, and these are reported in this section. The relationships of specific nutrients and other dietary components relationships with T1D incidence are reported in the next section.

Cases were identified from the Provincial Diabetes Registry. In order to be on the registry, a doctor's diagnosis of T1D and prescription for insulin were required, with the diagnosis following guidelines set out by the Canadian Diabetes Association (Meltzer et al., 1998; Canadian Diabetes Association, 2003). All cases diagnosed during 2001 to 2004 were eligible to participate in the case-control study. Two controls per case were randomly selected from the Provincial Medicare Registry, matching on sex and age at diagnosis (within one year).

A face-to-face interview was conducted with each participant. All participants (or their guardian) completed two questionnaires: a food frequency questionnaire (FFQ) determining food consumption during the year prior to diagnosis (previous year for controls), and a pre-piloted risk factor questionnaire (RFQ) gathering familial,

environmental, and social information for the year prior to diagnosis (previous year for controls) and during the participants' early years of life.

With regards to food consumption, participants aged nine and older completed a self-administered, modified version of Harvard's Youth/Adolescent Food Frequency Questionnaire (YAQ). For children less than nine years old, a surrogate reporter (usually the mother) completed the modified Harvard Service Food Frequency Questionnaire (HS-FFQ). The validity of results from both of these FFQs has been previously assessed and reported elsewhere (Rockett et al., 1995; Rockett et al., 1997; Blum et al., 1999)

Food consumption was re-categorized from the original FFQ categories into 'weekly or more often' consumption versus 'less than weekly' consumption, except for some commonly eaten foods which were categorized as either eaten 'daily or more often' or 'less than daily' consumption. Food frequency by food group was also examined after foods were aggregated into the following groups: total vegetable, fresh fruit, all fruit (including pure fruit drinks), all fruit and vegetables, dairy, grain, meats and alternatives, sugary foods, total beverages, and other foods. Dose-response relationships for both individual foods and food groups were evaluated based on their frequency distribution in the control population.

The RFQ included questions related to the following: family member with T1D or type 2 diabetes (T2D); breast-feeding duration and cow's milk-based formula intake frequency under three months of age; day-care attendance under age three; and the number of infections in infancy. Other questions pertaining to exposures in the year prior to diagnosis (year prior to interview for controls) included: the number of smokers in the

household; stressful life events; average self-reported household income; highest level of education obtained (primary, secondary, college, and university); occupational history of participants and their parents; location of residence (urban, rural non-farm, farm); and a change in local land use.

Using logistic regression, unconditional associations with T1D incidence were first analysed for each food, food group, and RFQ variable, and then those that achieved a p-value ≤ 0.2 were evaluated in a multivariable logistic regression model using both a manual forward and backward elimination process. All analyses were conducted controlling for age, sex, and FFQ. A larger number of participants completed the YAQ (60%) than the HSFFQ, and there were numerous food items on the YAQ that were not on the HSFFQ. So, two sets of regression analyses were conducted: an analysis of all participants but only for the 57 foods common to both FFQs, and an analysis for all foods but only for the 97 participants who completed the YAQ.

During the 4-year study period (2001-2004), 74 cases were diagnosed, 66 were contactable, and 57 agreed to participate. A total of 105 controls agreed to participate (86% and 73% case and control response rate, respectively).

Foods unconditionally associated with a decreased risk of T1D incidence were, a daily or more intake of orange juice or cheese, and the weekly or more consumption of muffins, melon, or cold cereal. At least weekly consumption of regular soft drinks, diet soft drinks, eggs, raisins, margarine, or hard candy increased the risk of T1D. Of all food groups evaluated, an intake of five or more beverages per day (including water)

significantly decreased the risk of T1D, confirming that the effect of cases drinking more regular and diet soft drinks than controls was not due to polydipsia, a symptom of T1D.

In the final multivariable logistic regression model, at least weekly consumption of regular soft drinks and eggs significantly increased the risk of T1D by 2.78 and 2.50 times, respectively. A dose-response for regular soft drinks and T1D incidence was significant in the final model when comparing 'less than weekly', 'weekly', and 'more than weekly' consumption. The risk of T1D was also increased by a three generation family member (parent, grand parent, sibling, parental sibling, or cousin) being previously diagnosed with T1D, and cases were more likely to have had five or more infections during the first two years of life. Compared to living in an urban area, living in a village or on a farm significantly decreased the risk of T1D. Father's education also influenced the risk of T1D, but the direction of association was not clear.

For the participants completing the YAQ, unconditional associations showed that 19 foods were significantly associated with T1D incidence ($p \leq 0.2$), all of which were consumed weekly or more frequently, except for bread (daily or more frequently). The final multivariable model determined that if peanut butter sandwiches, popcorn, and plain chocolate were eaten at least once per week, then the risk of developing T1D significantly increased by 5.01, 8.39, and 4.14, respectively, whereas, at least weekly consumption of muffins decreased the risk by approximately 75%. Father's education was the only environmental risk factor in the final model, and as with analysis using all participants, this variable did not show a dose-response relationship with T1D incidence. Peanut butter sandwiches and muffins showed a positive and negative significant dose-

response, respectively, when comparing 'less than weekly', 'weekly', and 'more than weekly' consumption, which was still significant in the final model.

Although significant associations were evident, there was not enough variability within some categories of food to properly evaluate a dose-response relationship for some foods that may have been significantly associated with T1D due to chance alone. A larger sample size would have provided more power to more accurately assess these relationships. An assessment of nutrient intake is needed to confirm the associations found with whole food intake.

7.6. Relationship between the risk of T1D and dietary and drinking water components

The primary objectives of this study were to determine dietary component factors related to the risk of T1D and to compare the consumption of nitrate from both food and drinking water sources in a T1D and a control population, adjusting for other factors where applicable. The study population described in section 7.5 was also utilized for this study. In addition to the food frequency and risk factor data on this study population, drinking water samples were collected at the time of interview for participants who drank from a private well. Routine test data collected by the provincial government was used for participants who drank from a municipal supply.

Food frequency data collected from the YAQ (completed by participants aged nine and above) and the HS-FFQ (completed by parents of children under age nine) were converted from food frequencies into a daily intake of nutrients and other dietary components using the 2005 Canadian Nutrient File and a conversion program called

CANDAT (Godin London Incorporated, 2006). Specific portion sizes for participants aged 0-2 years, 3-8 years, and nine years and older were supplied by Harvard Medical School, the source of both FFQs. Nitrate, nitrite, and nitrosamine data was obtained from published data, where available (Sen, 2003 [personal communication]*). If no suitable information was available for a food, then that food was purchased locally and sent to a laboratory for chemical analysis. For the 57 cases and 105 controls, the final dataset contained 14 water chemicals and 30 dietary food components for statistical analysis.

Unconditional logistic regression was used to assess the differences in diet and water intake between the case and control groups. Associations were investigated using a crude model adjusting for age and sex for evaluation of water components, and for age, sex, and daily energy intake for the food components. Finally, an evaluation using adjusted models which also controlled for four significant genetic and environmental factors (from the RFQ) that were determined in Chapter 5: a third generation family member (siblings, parents, grandparents, parental siblings, and cousins) being previously diagnosed with T1D; five or more infections during the first two years of life; residential remoteness (urban, rural, and farm); and father's education.

Results showed that the crude association between nitrate from food and the risk of T1D in children and youth had a positive and marginally significant (OR = 1.0, 1.63, 1.71, 3.02, $p = 0.13$) dose-response relationship. Calcium, folate, vitamin B12, and zinc had marginally significant negative relationships with T1D risk based on the Wald test. The daily intake of fat, calcium, and vitamin B12 had marginally significant negative relationships with T1D risk based on the Wald's test, with a possible threshold effect at

* Dr Sen was manager (now retired) of the Nitrosamine Health Canada Laboratory, Ottawa, where the majority of their work was reported in internal documents.

the second quartile for fat, and the third quartile for vitamin B12. Negative dose-response associations that were marginally significant in the crude models and not in the adjusted models were: vitamin C, phosphorus, and riboflavin. When evaluating dose-response associations between drinking water analytes and T1D risk, water pH was positively associated with T1D risk. Potassium was negatively associated with T1D risk using the trend and Wald test, and sodium had a marginally significant relationship with T1D risk based on the Wald test. When evaluating crude models for components common to water and food, calcium, phosphorus, and zinc had marginally significant negative dose-response relationships with T1D risk (trend test), and the intake of nitrate, nitrate and nitrite combined, and calcium were negatively associated with T1D risk (Wald test).

When evaluating the adjusted models for food components, total carbohydrates ($p = 0.13$), and caffeine ($p = 0.07$) had marginally significant ($p < 0.20$) positive dose-response relationships with T1D risk. The daily intake of folate was weakly inversely related to T1D risk ($p = 0.13$), and to a lesser extent, zinc ($p = 0.20$), according to the trend test. Total carbohydrate intake and caffeine were also marginally significantly related to T1D risk according to the Wald test, with the intake of total carbohydrates possibly showing a threshold effect at the third quartile. According to the Wald test, vitamin A was significantly inversely related with T1D risk ($p = 0.02$), and as in the crude analysis, the second quartile was significantly lower than the first quartile. The intake of vitamin B12 had a marginally significant negative relationship with T1D risk, with a possible threshold effect at the third quartile and although calcium was also marginally significant (according to the Wald test) the direction of the association was

unclear. Water pH was the only water analyte shown to have a marginally significant (p = 0.10) positive dose-response association with T1D risk based on the trend test. Conversely, the Wald test determined that both calcium and sodium had marginally significant associations with T1D risk (p = 0.14 and p = 0.09, respectively), but the direction of the association was not clear. Finally, when evaluating adjusted models for components common to both food and water, calcium, magnesium, and zinc had a negative and weak marginally significant association with T1D risk when using the trend test (p = 0.17, 0.13, and 0.12, respectively). The Wald test reported that nitrate and magnesium had a positive and marginally significant relationship with T1D risk (p = 0.10 and 0.2, respectively).

Results from Chapter 5 and Chapter 6 agree that foods containing caffeine (e.g. soft drinks) and foods containing carbohydrates (e.g. bread and pasta), although not significant, appeared to increase the risk of T1D. The association between individual foods and nitrate intake was less evident as foods containing high concentrations of nitrate (e.g. hotdogs), were not associated with T1D risk. However, a study that calculated the intake of nitrate from food sources observed that children and adolescents in Finland obtain 86% of their daily nitrate intake from vegetables and potatoes (a food containing low concentrations of nitrate, but frequently consumed) (Laitinen et al., 1993). Both Chapters 4 and 6 concluded that nitrate from drinking water was not significantly associated with the development of T1D. However, as only a limited number of participants were available for study enrolment (due to a small base population), it is

likely that insufficient power was available to determine significance, if it truly does exist.

7.7. Overall conclusions

Conclusions from Chapter 2 suggested that there was no significant temporal annual variation of ground water nitrate concentrations across PEI. Thus, it was appropriate to aggregate nitrate concentrations over several years to represent nitrate exposure at the time of T1D diagnosis in Chapter 4. These findings also indicated that a water sample taken one year could be representative of several years before or after that time without the concern of incorrect exposure status of nitrate from drinking water, assuming the land use does not change. This lack of an annual trend was also beneficial for the case-control study because nitrate samples taken at the time of interview could be assumed to be appropriate for representing nitrate exposure at the time of diagnosis (up to two years before the interview) (Chapter 6), if the land use did not change over the time frame in question.

Findings from Chapter 3 determined that the best method of spatial aggregation of private well water nitrate concentrations was at the watershed level (compared to freeform polygons [created based on similar neighbouring nitrate concentrations] and 500 m buffer zones around individual wells). Chapter 3 also determined that ground water nitrate concentrations were greatly influenced by local land use, therefore the social risk factor questionnaire used in the case-control study was right to include questions related to land use change between the year prior to diagnosis and the time of interview in order

to confirm that current water sample results would be representative of the exposures at the time of diagnosis.

One interesting finding was that at both the watershed level (Chapter 4) and at the individual level (Chapter 6), nitrate exposure from drinking water was not associated with T1D development. However, nitrate exposure from food sources evaluated at the individual level (Chapter 6) showed a marginally significant dose-response relationship, whereby the increased daily intake of nitrate during the year prior to diagnosis was associated with an increased risk of T1D. Thus, although not significant, exposure to nitrate is more likely to be considered a promoter of T1D, rather than an initiator, meaning that nitrate likely accelerates the process by which T1D is already developing in an individual who is going to develop T1D at some point. Evidence for this stems from the premise that the development of T1D may take many years to develop, and that several environmental influences in infancy/early childhood have been significantly related to T1D development (EURODIAB Substudy 2 Study Group, 2002; McKinney et al., 2000). However, previous studies have shown that nitrosamine intake by mothers at the time of conception may increase the risk of T1D in their children (Helgason and Jonasson, 1981; Šipetić et al., 2004), therefore suggesting that nitrogen-based compounds could also be considered initiators as well as promoters of the disease.

It is widely believed that a genetic predisposition is necessary for T1D to occur, and that environmental factors are sufficient to cause T1D, providing the individual is genetically susceptible. Environmental factors can act on their own or with other factors as components of a sufficient cause of T1D (Dohoo et al., 2003). Evidence from our

research suggests that nitrate exposure from food sources during the year prior to diagnosis is likely a component cause of T1D, because after controlling for other significant factors, the marginally significant association between foodborne nitrate and T1D was no longer present.

7.8. Future research

Very little research on the association between T1D and environmental exposures has been conducted in Canada, and never before in PEI, despite the fact that there is a high incidence rate in the province and relatively high and variable ground water nitrate concentrations. Several previous studies to evaluate the association between T1D and waterborne nitrate concentrations have been conducted elsewhere, but all were at the ecological level. Furthermore, nitrate consumption from both foodborne and waterborne sources have not previously been taken into account in the same study at the same level, making the current study unique in its approach. In addition, nitrate, nitrite, and nitrosamine concentrations from food sources were also taken into account in order to estimate total dietary intake of nitrate and its derivatives.

However, a limited sample size did not allow for a thorough assessment of the dose-response relationships between food consumption and the risk of T1D in order to confirm or contest the current study findings. A continuation of the study in PEI and/or expansion of the case-control study to neighbouring provinces would be beneficial to increase the sample size. Expansion to other jurisdictions would also determine if the current findings are unique to PEI, or if they are appropriate to other parts of Canada.

However, this relies on neighbouring provinces having a diabetes register (only Nova Scotia has a register at this time).

In the current case-control study, controls were matched to cases by an age of within one year of diagnosis. Previous studies have matched by age to within one or two days of date of birth (Marshall et al., 2004). A closer matching by age could reduce possible misclassification of exposure data because participants of a similar age would have a similar understanding of dietary intake and portion sizes, and therefore be more comparable. However, due to a small population base in PEI, one-year matching was the most appropriate. For example, one potential problem with the current study was that a seven day old case was matched with an 11 month old control. In this situation, both diet and environmental exposures were very different, as the case was directly exposed to very little in the first seven days of life. Ideally, this case should have been matched to a younger control and their mothers food consumption compared, as there is evidence to support that the foetus in utero is influenced by the mothers diet, consequently indirectly affecting a child's risk of developing T1D (Helgason and Jonasson, 1981).

It would be beneficial for future studies to analyse more foods for nitrate, nitrite, and nitrosamine concentrations, as limited data on these constituents are available in Canada. A complete evaluation of all foods on both FFQs would eliminate the use of literature values or missing values, and make the analysis more relevant to Canadian residents, especially those in PEI. These values could be made available in the Canadian Nutrient File for other research groups using either of the FFQs used in this study to assess diet and disease, as well as general dietary assessment studies.

One important omission from the case-control study was non-familial genetic susceptibility. As the onset of T1D is presumed to be related to both genetic and environmental factors, the genetic profile of all participants in a study would aid in understanding the interaction between environmental factors and genetic susceptibility. As the population of PEI is small, and migration in and out of the province is low compared to the rest of the country (Statistics Canada, 2005), there is a possibility that there may be a limited gene pool which may contribute to the high T1D incidence in PEI. Genetics may also help to explain the geographical variation in T1D incidence rates, as PEI families may reside closer together.

Finally, for the area level analyses of T1D incidence, information on only two possible risk factors of T1D incidence were collected: ground water nitrate concentrations during an eight year period and household income from one year. These predictors were used to represent the entire 15-year sampling period of cases. Nitrate concentrations for the entire sampling period of the cases (15 years) could improve the area assessment by providing more detailed predictor variable data without extrapolating concentrations to other years. A more detailed analysis of T1D at the area level could also include more predictor variables (for example, residential remoteness or population density [calculated by dividing the respective populations for each area unit by the area of that unit]), or perhaps using a different unit of aggregation, such as municipal boundaries, for which census data are available.

In conclusion, this thesis provides some evidence for a relationship between the risk of T1D in PEI and total nitrate exposure at the individual level (from food sources).

This association was not supported in drinking water nitrate concentrations averaged at the ecological level. T1D is also related to several other environmental and dietary factors at the individual level. More research remains to be conducted to confirm these findings.

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APPENDICIES

APPENDIX A Methods to determine alkalinity and nitrate and chloride concentration in PEI ground water

P.E.I. Analytical Laboratories

Section: Water Chemistry Lab

Method Title: Determination of Alkalinity (Methyl Orange), Chloride, and High Level Nitrate (NO_3-) in Surface, and Wastewaters by Flow Injection Analysis Colorimetry .

Prepared by: _____Date: November 12, 2002
QA/QC Officer

Last revised by: _____Date: March 10, 2003

QA/QC Officer

Reviewed by: _____Date: March 10, 2003

Laboratory Manager

QA approval by: _____Date: March 10, 2003
QA/QC Officer

Final Approval by: _____Date: March 10, 2003
Laboratory Manager

Based on the method validation data this method is fit for intended use.
The approval of this document is valid for 2 years at which time it is subject to review to determine if any updates or modifications are warranted.

Original Copy Holder: Quality Assurance/Quality Control Officer

DETERMINATION OF ALKALINITY (METHYL ORANGE), CHLORIDE, AND HIGH LEVEL NITRATE (NO_3-) IN SURFACE, AND WASTEWATERS BY FLOW INJECTION ANALYSIS COLORIMETRY .

References

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Scope and Application

This purpose of this method is a combined method used to determine alkalinity, chlorides and nitrates in surface and wastewater, and domestic and industrial wastes. Alkalinity, Chlorides and Nitrates may be determined independently of each other in which case the standards used would not be combined.
The combined method has the capacity to analyse 60 samples per hour.
The method detection limits and applicable ranges are as follows:

Method Detection Limits:

Alkalinity	2.81 mg $CaCO_3$/L
Chlorides	0.5 mg Cl/L
Nitrogen as NO_3-	0.1 mg N/L

Applicable Ranges:

Alkalinity	5 to 500 mg $CaCO_3$/L
Chlorides	1.0 to 300.0 mg Cl/L
Nitrogen as NO_3-	0.2 to 10.0 mg N/L

Principle

CHLORIDES

Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms the highly colored ferric thiocyanate, of which the absorbance is proportional to the chloride concentration. Ferric thiocyanate absorbs strongly at 480 nm. The calibration curve fits a second order polynomial.

NITRATES

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride (NED). The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone can be determined by removing the cadmium column.

ALKALINITY

Methyl orange is used as a color reagent for this method because its pH range is the same as the pH of the equivalence point for a total alkalinity titration. The methyl orange indicator is in a dilute pH 3.1 buffer which is just below its color change pH. When an alkaline sample is injected, the poorly buffered methyl orange changes color in proportion to the change in pH of the weak buffer, and thus in proportion to the alkalinity of the sample which absorbs at 550 nm.

Interferences

Sample turbidity may interfere. Turbidity can be removed by filtration through a sera-clear filter prior to analysis.

Low results would be obtained for sample that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.

Residual chlorine can interfere by oxidizing the cadmium column.

Equipment and Supplies

Certified Balance (analytical, capable of accurately weighing to the nearest 0.0001 g)

Glassware (Class A volumetric flasks and pipettes)

Plastic storage containers

QuikChem Series 8000 (Flow Injection Analysis Equipment)

Sampler

Multichannel proportioning pump

Reaction unit or manifold
Colorimetric detector
Data system (FIA software)
Peristaltic Pump
0 mm pathlength, 80 µL, glass flow cell
550 nm interference filter (alkalinity)
520 nm interference filter (nitrate)

480 nm interference filter (chloride)
Lachat Sample Preparation Module with UV-254 lamp
Cadmium Reduction Column
PVC pump tubes
Drying Oven (as stated in reagent/standard preparation)
x5, x10 dilutors

Lab safety is performed according to procedure WCL_07P of the Standard Operating Procedures Manual. Review Material Safety Data Sheets for detailed explanation on potentially and highly toxic or hazardous effects of chemicals used.Collect waste from QuikChem line and dispose according to WCL_26P.

Reagents
All solutions require deionized water. Ensure the resistivity is ≥17 megohms/cm. All reagents are stored at room temperature in Room 114, unless specified.

CHLORIDES

Combined Color Reagent
Prepare Solution A and Solution B .

Solution A
Stock Mercuric Thiocyanate Solution
In a 1 L volumetric flask, dissolve 4.17 g mercuric thiocyanate ($Hg(SCN)_2$) in about 500 mL methanol. Dilute to the mark with methanol and invert to mix. Prepare every 6 months.
CAUTION: Mercuric thiocyanate is toxic. Wear gloves!

Solution B
Ferric Nitrate Reagent, 0.5 M
In a 1 L volumetric flask, dissolve 202 g ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) in approximately 800 mL water. Add 25 mL conc. nitric acid and dilute to the mark. Invert to mix. Prepare every 6 months.

Combined Color Reagent
In a 1 L volumetric flask, mix 150 mL stock Mercuric Thiocyanate Solution (Solution A) with 150 mL Stock Ferric Nitrate Reagent (Solution B) and dilute to the mark with water. Invert to mix. Vacuum filter through a 0.45 micrometer membrane filter. Prepare monthly.

Standards

Stock Standard (1000 mg Cl/L)

In a 105° C oven, dry 3 g primary standard grade sodium chloride (NaCl) overnight. In a 1 L volumetric flask, dissolve 1.648 g primary grade sodium chloride in about 500 mL water. Dilute to the mark and invert to mix. Stable for 1 year.

Intermediate Standard (600 mg Cl/L)

To a 1 L volumetric flask, add 600 mL of Stock Standard , above. Dilute to the mark with DI water and invert to mix. Prepare fresh.

HIGH LEVEL NITRATE (NO_3-)

15 N Sodium Hydroxide

Add 150 g NaOH very slowly to 250 ml of water. CAUTION: The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

Ammonium Chloride buffer, pH 8.5

In a 1 L volumetric flask, dissolve 85.0g ammonium chloride (NH_4Cl) and 1.0 g disodium ethylenediamine tetraacetic acid dihydrate ($Na_2EDTA \square 2H_20$) in about 800 ml water. Dilute to the mark and invert to mix. Adjust the pH to 8.5 with 15 N sodium hydroxide solution. Prepare monthly.

Sulfanilamide Color Reagent

To a 1 L volumetric flask add about 600 ml water.
Then add 100 ml of 85% phosphoric acid (H_3PO_4), 40.0 g sulfanilamide, and 1.0 g N-(1-Naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet, and stir for 30 min. to dissolve. Dilute to the mark, and invert to mix. Store in a dark bottle. This solution is stable for one month.

Standards

Stock Nitrate Standard, 500 mg N/L as NO3-

In a 1 L volumetric flask dissolve 3.610 g potassium nitrate (KNO_3) in about 600ml water. Dilute to the mark and invert to mix. This solution is stable for one year.

Intermediate Nitrate Standard, 50 mg N/L as NO3-

In a 1 L volumetric flask, add 100 ml of Stock Nitrate Standard . Dilute to the mark and invert to mix. Prepare fresh.

Working Nitrate Standards and Chloride standards are prepared in the same solution. See Table 1.

Table 1

Working Chloride/Nitrate Standards Prepare every 3 months. Dilute Intermediate Standard to 1 L with D H_2O.						
Working Standard Conc Cl/N mg/L	1 300/10	2 150/5	3 60/1.0	4 30/.5	5 6.0/0.1	6 0/0
Add (ml) Intermediate Cl std	500	250	100	50	10	0
Add (ml) Intermediate N std	200	100	20	10	0.2	0

ALKALINITY

Carbon dioxide-free water

Bring required volume of deionized water (2 - 6L) to a boil for five minutes to remove carbon dioxide. Cool before using to avoid error in the volumetric measurements.

Hydrochloric Acid (HCL), 0.1M

In a 1L volumetric flask containing about 800ml carbon dioxide-free water, add 8.3 ml concentrated hydrochloric acid. Dilute to the mark with carbon dioxide-free water. Invert to mix. Store in glass bottle.

KHP Buffer,pH 3.1, 25.0 mM

In a 1L container, dissolve 5.0g potassium acid phthalate [potassium hydrogen phthalate, potassium biphthalate, KHP, $(HOOCC_6H_4COOK)$] in 900 ml carbon dioxide-free water. Add 85.0 ml 0.1 M HCL, then add acid (no more then 5.0 ml) to bring the pH to 3.1 +/- 0.05. If greater then 5.0 ml of 0.1 M HCL is necessary, start over in the preparation of this reagent. If this fails, use Standardized, 0.1 M HCL. Store in dark glass bottle for up to two months.

Methyl Orange Reagent

In a 1 L volumetric flask, dissolve 0.1313 g methyl orange (indicator grade, Aldrich #11,451-0,) in about 700 ml carbon dioxide-free water. Dilute to the mark and invert to mix. Store in dark glass bottle for 1 month. Prepare fresh bimonthly.

Standards

Note: There are 12 working standards in the combined method.

Chloride and Nitrate Working Standards are # 1 - 6 and Alkalinity Working Standards are # 7 -12.

Stock Standard 2500 mg $CaCO_3$/L as Na_2CO_3
In a 1 L volumetric flask dissolve 2.650 g anhydrous primary standard grade sodium carbonate (Na_2CO_3) that has been dried for four hours at 250°C in about 900 ml carbon dioxide-free water. Dilute to the mark and invert to mix. Store in tightly covered glass bottle to prevent absorption of carbon dioxide. Stable for 1 year.

Table 2

Working Alkalinity Standards Prepare every 3 months. Dilute to 500 ml with CO_2 free D H_2O						
Working Standards (Conc $CaCO_3$ mg/L)	7 (500)	8 (375)	9 (250)	10 (125)	11 (25)	12 (0)
Add (ml) Stock Standard.	**100**	**75**	**50**	**25**	**5**	**0**

PROCEDURE

1. **Sample Collection, Preservation, and Storage**
Sample receipt, handling, storage and disposal is performed according to standard operating procedure WCL_03P

2. **Sample Analysis**

The basic procedural sequence of steps according to WCL_01P, standard operating procedure for the Flow Injection Analyzer (FIA) QuikChem Series 8000 are followed and steps specifically required for this determination are explained in detail below.

2.1 **Analyzer Start-up**

Check that sample line (green) is connected to the alkalinity port # 6 and sample outlet is connected to port# 5.
Take the outlet line from alkalinity port #5 and insert into nitrates port #6. The outlet line from nitrate port# 5 is inserted into chloride port #6. The outlet line from chloride port# 5 is inserted into the waste line.

Note: DI water is the carrier for all methods **except** alkalinity which uses CO_2-free water. Keep the container covered.

2.2 Computer Start-up

Click Run and screen appears which require three filenames:

i.e.	Method 1.	com-phb.met	
i.e.	Tray	2.	com0116a.tra
i.e.	Data file	3.	com0116a.fdt

2.3 Sample Tray Set-up

The samples are analysed in the following sequence:

2 CRMs (both straight)
Standard 1 or CRM (automatically diluted 1:10)
Set of 20 samples
1 Duplicate (first sample in each set of 20)
Working Standards (Standards 1, 5, 7 and 11).

The sequence is repeated as follows:
Set of 20 samples
1 Duplicate (first sample in each set of 20)
Working Standards (Standards 1, 5, 7 and 11).

2.4 Printing Data Report
(See procedure WCL_01P)

2.5 Quality Control Range Check

A table is posted on the wall adjacent to the QuikChem in Room 114 stating the acceptable range for the current Certified Reference Material.

2.6 Shut Down
(See procedure WCL_01P)

2.7 Trouble Shooting
(See procedure WCL_01P)

3. Reporting of Test Results

Test results are faxed to the Water Resources Office. Authorized Water Resource staff enter the test results to the Water Quality Report via the WATSIS program. The Water Chemistry Lab analyst verifies the test results entered on the report. The date verified and verifier is recorded electronically.

Quality Assurance

Quality Control is monitored by the use of Certified Reference Materials (CRM) and standard rechecks. Guidelines are stated in standard operating procedure WCL-25P.
The standard rechecks must not be beyond ± 2 standard deviation of the mean, consecutively.
The duplicate sample value must be within ± 20% of the mean duplicate value as stated in standard operating procedure WCL-21P.
When values fall outside the acceptable range trouble shooting must be performed according to standard operating procedure WCL-01P and the analysis must be repeated.

Note:
If build-up of suspended matter in the reduction column restricts sample flow, the samples may be pre-filtered.

Method Revision History:

Version 1 : 05/30/2002
Original document.

Version 2.0 : 11/12/2002
Revisions made due to routine errors and omissions.

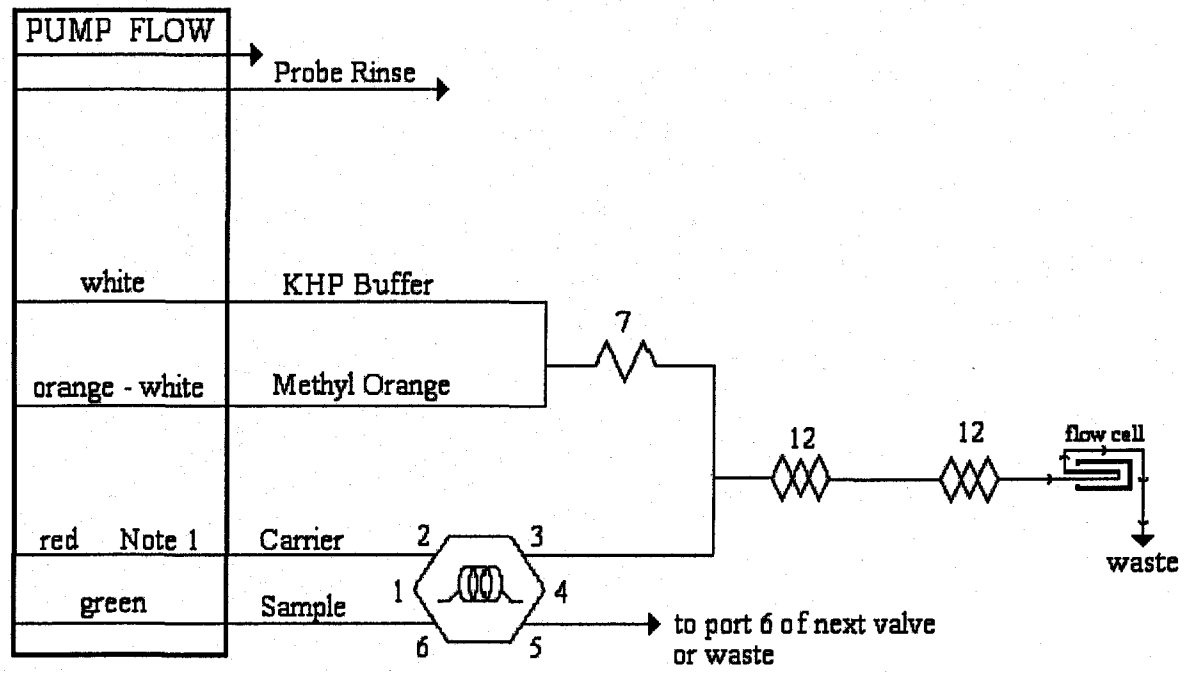
Version 2.1 : 03/10/2003
Revisions made to meet requirements of ISO/IEC 17025 Standard as indicated in external audit performed by SCC/CAEAL on 12/17/02.The revision includes authorization of the method and revisions and reference to the standard operating procedures.

The following standard operating procedures apply to this method.

WCL#	SOP
1	Operation of Quikchem
2	Reagent Receipt, Storage, Preparation and Use
3	Sample Collection, Handling, Receipt, Storage, Log In, Analysis, Reporting and Disposal
4	Orientation of Water Chemistry Lab Trainee
WCL#	SOP

5	Storage of Files
6	Calibration
7	Safety
8	Security
9	Quality Control of Reagent Water
12	Housekeeping of Water Chemistry Lab
13	Preparation of CRM
14	Instrument validation
15	Method validation
16	pH Meter Operation, Maintenance and Monitoring
17	Estimation of Uncertainty Measurement
18	Receipt of Materials and Supplies
19	Chain of Custody
20	Proficiency Testing
21	Precision Testing
24	Bottle Washing
25	Quality Control
26	Disposal of Quikchem waste
30	Authorization of Test Methods and Procedures
35	Reporting of Non-Conformance by the Staff
36	Water Sample Collection Pick-up

ALKALINITY MANIFOLD DIAGRAM



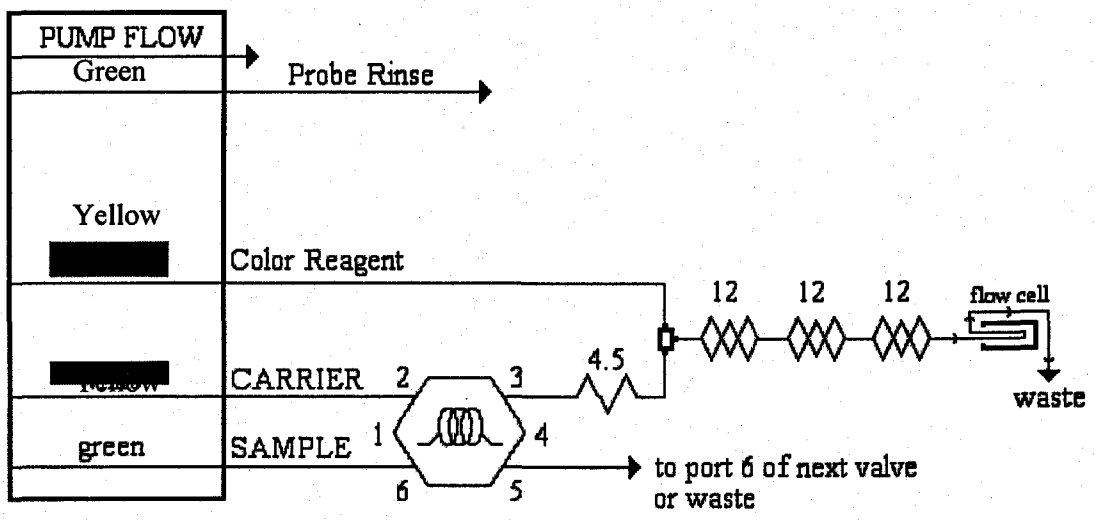
Carrier: DI water (CO_2 free)
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 L/cm.
AE Sample Loop: 100 cm of 0.8 mm (0.032 in) i.d. This is 5.2 L/cm
QC8000 Sample Loop: 100 cm of 0.8 mm (0.032 in) i.d. This is 5.2 L/cm
Interference Filter: 550 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

7: 135 cm of tubing on a 7 cm coil support
12: 255 cm of tubing on a 12 cm alternating coil support

Note 1: Remove the steel pins from the carrier reagent line.
Note 2: PVC PUMP TUBES MUST BE USED FOR THIS METHOD.

CHLORIDE MANIFOLD DIAGRAM

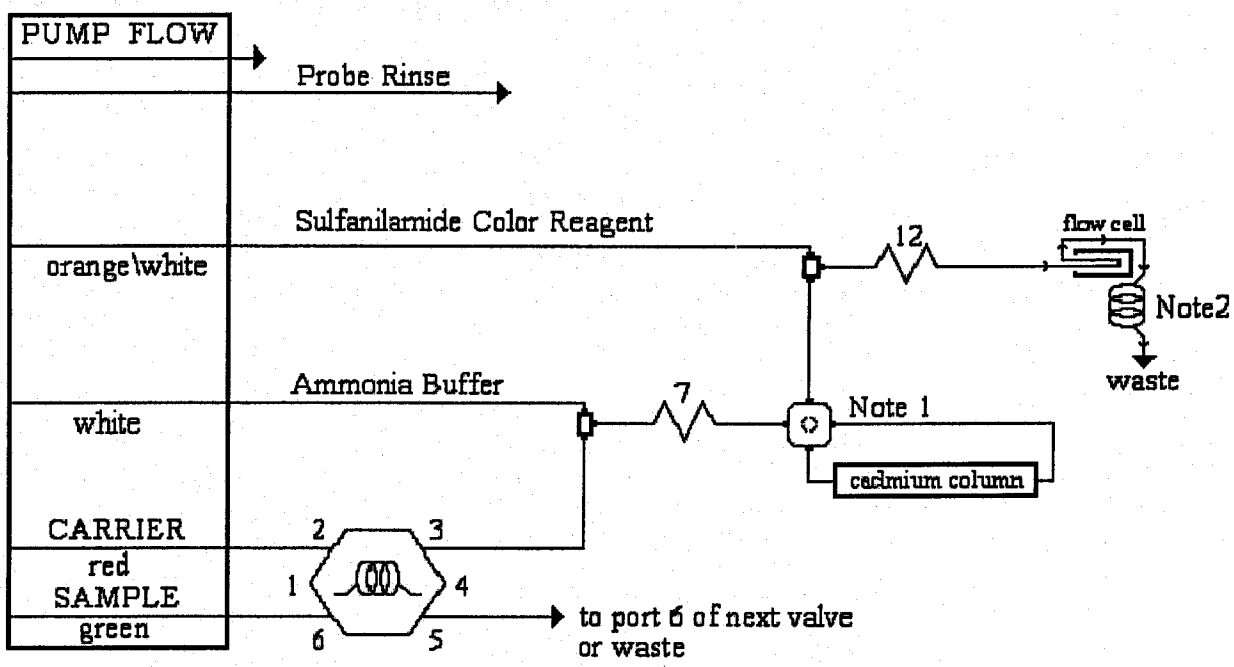


Carrier: DI water
Manifold Tubing: 0.5 mm (0.022 in) i.d. This is 2.5 L/cm.
AE Sample Loop: 8.5 cm
QC8000 Sample Loop: 13 cm x 0.5 mm i.d. (0.022 in) i.d.
Interference Filter: 480 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

4.5: 70 cm of tubing on a 7 cm coil support
12: 255 cm of tubing on a 12 cm alternating coil support

NITRATE/NITRITE MANIFOLD DIAGRAM



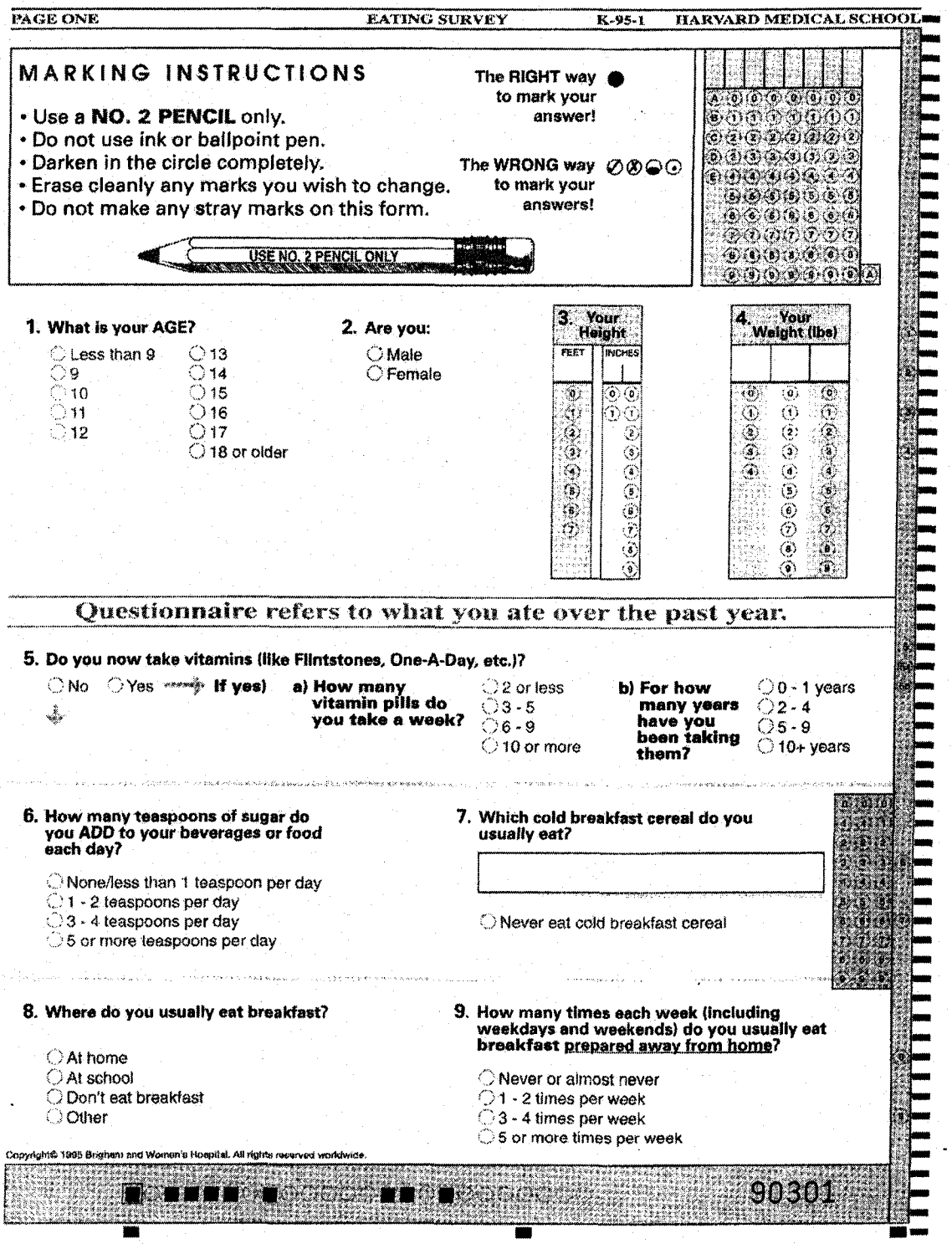
Carrier: DI water
Manifold Tubing: 0.5 mm (0.022 in) i.d. This is 2.5 L/cm.
AE Sample Loop: Microloop
QC8000 Sample Loop: Microloop
Interference Filter: 520 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

Data System	Alkalinity	Chlorides	Nitrates
Sample Throughout	60 samples/h, s/sample	60 samples/h, 60 s/sample	60 samples/h, 60 s/sample
Pump Speed	35	35	35
Cycle Period	60	60	60
Inject to start of Peak Period			s
Analyte Data			

Concentration Units	**mg $CaCO_3$/L**	**mg Cl/L**	**mg N/L**
Peak Base Width	**22.493 s**	**10 s**	**17 s**
% Width Tolerance	**30**	**30**	**30**
Threshold	**60000**	**6800**	**1315**
Inject to Peak Start	**33 s**	**6 s**	**27 s with column**
Chemistry	**Inverse**	**Direct**	**Direct**
Calibraton Data			
Calibration fit type	**3 rd order Polynomial**	**3 rd order Polynomial**	**1 st order Polynomial**
Calibration Rep. Handling	**Average**	**Average**	**Average**
Weighing Method	**None**	**1/X**	**None**
Concentration Sealing	**None**	**None**	**None**
Force through Zero	**No**	**No**	**No**
Sampler Timing	**Alkalinity**	**Chlorides**	**Nitrates**
Min. probe in Wash Period	**9.0 s**	**9.0 s**	**9 s**
Probe in Sample Period	**25 s**	**25 s**	**25 s**
Data System			
Valve Timing			
Load Time			
Load Period	**20 s**	**20 s**	**20 s**
Inject Period	**40 s**	**40 s**	**40 s**

APPENDIX B Youth/Adolescent Questionnaire



PAGE ONE EATING SURVEY K-95-1 HARVARD MEDICAL SCHOOL

MARKING INSTRUCTIONS

- Use a **NO. 2 PENCIL** only.
- Do not use ink or ballpoint pen.
- Darken in the circle completely.
- Erase cleanly any marks you wish to change.
- Do not make any stray marks on this form.

The RIGHT way ● to mark your answer!

The WRONG way ⊘ ⊗ ◒ ⊙ to mark your answers!

USE NO. 2 PENCIL ONLY

1. What is your AGE?

○ Less than 9 ○ 9 ○ 10 ○ 11 ○ 12 ○ 13 ○ 14 ○ 15 ○ 16 ○ 17 ○ 18 or older

2. Are you:

○ Male ○ Female

3. Your Height FEET INCHES

4. Your Weight (lbs)

Questionnaire refers to what you ate over the past year.

5. Do you now take vitamins (like Flintstones, One-A-Day, etc.)?

○ No ○ Yes → **If yes)** **a) How many vitamin pills do you take a week?** ○ 2 or less ○ 3 - 5 ○ 6 - 9 ○ 10 or more **b) For how many years have you been taking them?** ○ 0 - 1 years ○ 2 - 4 ○ 5 - 9 ○ 10+ years

6. How many teaspoons of sugar do you *ADD* to your beverages or food each day?

○ None/less than 1 teaspoon per day
○ 1 - 2 teaspoons per day
○ 3 - 4 teaspoons per day
○ 5 or more teaspoons per day

7. Which cold breakfast cereal do you usually eat?

○ Never eat cold breakfast cereal

8. Where do you usually eat breakfast?

○ At home
○ At school
○ Don't eat breakfast
○ Other

9. How many times each week (including weekdays and weekends) do you usually eat breakfast prepared away from home?

○ Never or almost never
○ 1 - 2 times per week
○ 3 - 4 times per week
○ 5 or more times per week

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10. How many times each week (including weekdays and weekends) do you usually eat lunch prepared away from home?

- ○ Never or almost never
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

11. How many times each week do you usually eat after-school snacks or foods prepared away from home?

- ○ Never or almost never
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

12. How many times each week (weekdays and weekends) do you usually eat dinner prepared away from home?

- ○ Never or almost never
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

13. How many times per week do you prepare dinner for yourself (and/or others in your house)?

- ○ Never or almost never
- ○ Less than once per week
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

14. How often do you have dinner that is ready made, like frozen dinners, Spaghetti-O's, microwave meals, etc.

- ○ Never/less than once per month
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

15. How many times each week (including weekdays and weekends) do you eat late night snacks prepared away from home?

- ○ Never/less than once per month
- ○ 1 - 2 times per week
- ○ 3 - 4 times per week
- ○ 5 or more times per week

16. How often do you eat food that is fried at home, like fried chicken?

- ○ Never/less than once per week
- ○ 1 - 3 times per week
- ○ 4 - 6 times per week
- ○ Daily

17. How often do you eat fried food away from home (like french fries, chicken nuggets)?

- ○ Never/less than once per week
- ○ 1 - 3 times per week
- ○ 4 - 6 times per week
- ○ Daily

DIETARY INTAKE

How often do you eat the following foods:

Example If you drink one can of diet soda 2 - 3 times per week, then your answer should look like this:

E1. Diet soda (1 can or glass)

- ○ Never
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ● 2 - 6 cans per week
- ○ 1 can per day
- ○ 2 or more cans per day

BEVERAGES

FILL OUT ONE BUBBLE FOR EACH FOOD ITEM

18. Diet soda (1 can or glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ○ 2 - 6 cans per week
- ○ 1 can per day
- ○ 2 or more cans per day

19. Soda - not diet (1 can or glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ○ 2 - 6 cans per week
- ○ 1 can per day
- ○ 2 or more cans per day

20. Hawaiian Punch, lemonade, Koolaid or other non-carbonated fruit drink (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 4 glasses per week
- ○ 5 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 or more glasses per day

21. Iced Tea - sweetened (1 glass, can or bottle)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 - 4 glasses per week
- ○ 5 - 6 glasses per week
- ○ 1 or more glasses per day

22. Tea (1 cup)
- ○ Never/less than 1 per month
- ○ 1 - 3 cups per month
- ○ 1 - 2 cups per week
- ○ 3 - 6 cups per week
- ○ 1 or more cups per day

23. Coffee - not decaf. (1 cup)
- ○ Never/less than 1 per month
- ○ 1 - 3 cups per month
- ○ 1 - 2 cups per week
- ○ 3 - 6 cups per week
- ○ 1 or more cups per day

24. Beer (1 glass, bottle or can)
- ○ Never/less than 1 per month
- ○ 1 - 3 cans per month
- ○ 1 can per week
- ○ 2 or more cans per week

25. Wine or wine coolers (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 or more glasses per week

26. Liquor, like vodka or rum (1 drink or shot)
- ○ Never/less than 1 per month
- ○ 1 - 3 drinks per month
- ○ 1 drink per week
- ○ 2 or more drinks per week

Example If you eat:

3 pats of margarine on toast
1 - 2 pats of margarine on sandwich
1 pat of margarine on vegetables

5 - 6 pats total all day

then answer this way →

E2. Margarine (1 pat) - not butter
- ○ Never
- ○ 1 - 3 pats per month
- ○ 1 pat per week
- ○ 2 - 6 pats per week
- ○ 1 pat per day
- ○ 2 - 4 pats per day
- ● 5 or more pats per day

DAIRY PRODUCTS

27. What TYPE of milk do you usually drink?
- ○ Whole milk
- ○ 2% milk
- ○ 1% milk
- ○ Skim/nonfat milk
- ○ Don't know
- ○ Don't drink milk

28. Milk (glass or with cereal)
- ○ Never/less than 1 per month
- ○ 1 glass per week or less
- ○ 2 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 - 3 glasses per day
- ○ 4+ glasses per day

29. Chocolate milk (glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 6 glasses per week
- ○ 1 - 2 glasses per day
- ○ 3 or more glasses per day

30. Instant Breakfast Drink (1 packet)

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

31. Whipped cream

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

32. Yogurt (1 cup) - Not frozen

- ○ Never/less than 1 per month
- ○ 1 - 3 cups per month
- ○ 1 cup per week
- ○ 2 - 6 cups per week
- ○ 1 cup per day
- ○ 2 or more cups per day

33. Cottage or ricotta cheese

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

34. Cheese (1 slice)

- ○ Never/less than 1 per month
- ○ 1 - 3 slices per month
- ○ 1 slice per week
- ○ 2 - 6 slices per week
- ○ 1 slice per day
- ○ 2 or more slices per day

35. Cream cheese

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

36. What TYPE of yogurt, cottage cheese & dairy products (besides milk) do you use mostly?

- ○ Nonfat
- ○ Lowfat
- ○ Regular
- ○ Don't know

37. Butter (1 pat) - NOT margarine

- ○ Never/less than 1 per month
- ○ 1 - 3 pats per month
- ○ 1 pat per week
- ○ 2 - 6 pats per week
- ○ 1 pat per day
- ○ 2 - 4 pats per day
- ○ 5 or more pats per day

38. Margarine (1 pat) - NOT butter

- ○ Never/less than 1 per month
- ○ 1 - 3 pats per month
- ○ 1 pat per week
- ○ 2 - 6 pats per week
- ○ 1 pat per day
- ○ 2 - 4 pats per day
- ○ 5 or more pats per day

39. What FORM and BRAND of margarine does your family usually use?

- ○ None
- ○ Stick
- ○ Tub
- ○ Squeeze (liquid)

WHAT SPECIFIC BRAND AND TYPE (LIKE "PARKAY CORN OIL SPREAD")?

Leave blank if you don't know.

40. What TYPE of oil does your family use at home?

- ○ Canola oil
- ○ Corn oil
- ○ Safflower oil
- ○ Olive oil
- ○ Vegetable oil
- ○ Don't know

MAIN DISHES

41. Cheeseburger (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 - 4 per week
- ○ 5 or more per week

42. Hamburger (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 - 4 per week
- ○ 5 or more per week

43. Pizza (2 slices)

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

44. Tacos/burritos (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ One per week
- ○ 2 - 4 per week
- ○ 5 or more per week

45. Which taco filling do you usually have:

- ○ Beef & beans
- ○ Beef
- ○ Chicken
- ○ Beans

46. Chicken nuggets (6)

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

47. Hot dogs (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

48. Peanut butter sandwich (1) (plain or with jelly, fluff, etc.)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 - 4 per week
- 5 or more per week

49. Chicken or turkey sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

50. Roast beef or ham sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

51. Salami, bologna, or other deli meat sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

52. Tuna sandwich (1)
- Never/less than 1 per month
- 1 - 3 per month
- One per week
- 2 or more per week

53. Chicken or turkey as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

54. Fish sticks, fish cakes or fish sandwich (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

55. Fresh fish as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

56. Beef (steak, roast) or lamb as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

57. Pork or ham as main dish (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

58. Meatballs or meatloaf (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

59. Lasagna/baked ziti (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

60. Macaroni and cheese (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

61. Spaghetti with tomato sauce (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 - 4 times per week
- 5 or more times per week

62. Eggs (1)
- Never/less than 1 per month
- 1 - 3 eggs per month
- One egg per week
- 2 - 4 eggs per week
- 5 or more eggs per week

63. Liver: beef, calf, chicken or pork (1 serving)
- Never/less than 1 per month
- Less than once per month
- Once per month
- 2 - 3 times per month
- Once per week or more

64. Shrimp, lobster, scallops (1 serving)
- Never/less than 1 per month
- 1 - 3 times per month
- Once per week
- 2 or more times per week

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65. French toast (2 slices)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

66. Grilled cheese (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

67. Eggrolls (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

MISCELLANEOUS FOODS

68. Brown gravy
- ○ Never/less than 1 per month
- ○ Once per week or less
- ○ 2 - 6 times per week
- ○ Once per day
- ○ 2 or more times per day

69. Ketchup
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

70. Clear soup (with rice, noodles, vegetables) 1 bowl
- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 or more bowls per week

71. Cream (milk) soups or chowder (1 bowl)
- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 - 6 bowls per week
- ○ 1 or more bowls per day

72. Mayonnaise
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once per day

73. Low calorie/fat salad dressing
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once or more per day

74. Salad dressing (not low calorie)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once or more per day

75. Salsa
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ Once or more per day

76. How much fat on your beef, pork, or lamb do you eat?
- ○ Eat all
- ○ Eat some
- ○ Eat none
- ○ Don't eat meat

77. When you have chicken or turkey, do you eat the skin?
- ○ Yes
- ○ No
- ○ Sometimes

BREADS & CEREALS

78. Cold breakfast cereal (1 bowl)

- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 - 4 bowls per week
- ○ 5 - 7 bowls per week
- ○ 2 or more bowls per day

79. Hot breakfast cereal, like oatmeal, grits (1 bowl)

- ○ Never/less than 1 per month
- ○ 1 - 3 bowls per month
- ○ 1 bowl per week
- ○ 2 - 4 bowls per week
- ○ 5 - 7 bowls per week
- ○ 2 or more bowls per day

80. White bread, pita bread, or toast (1 slice)

- ○ Never/less than 1 per month
- ○ 1 slice per week or less
- ○ 2 - 4 slices per week
- ○ 5 - 7 slices per week
- ○ 2 - 3 slices per day
- ○ 4+ slices per day

81. Dark bread (1 slice)

- ○ Never/less than 1 per month
- ○ 1 slice per week or less
- ○ 2 - 4 slices per week
- ○ 5 - 7 slices per week
- ○ 2 - 3 slices per day
- ○ 4+ slices per day

82. English muffins or bagels (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

83. Muffin (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 muffins per month
- ○ 1 muffin per week
- ○ 2 - 4 muffins per week
- ○ 5 or more muffins per week

84. Cornbread (1 square)

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more per week

85. Biscuit/roll (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

86. Rice

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

87. Noodles, pasta

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

88. Tortilla - no filling (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

89. Other grains, like kasha, couscous, bulgur

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

90. Pancakes (2) or waffles (1)

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

91. French fries (large order)

- ○ Never/less than 1 per month
- ○ 1 - 3 orders per month
- ○ 1 order per week
- ○ 2 - 4 orders per week
- ○ 5 or more orders per week

92. Potatoes - baked, boiled, mashed

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

FRUITS & VEGETABLES

93. Raisins (small pack)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

94. Grapes (bunch)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

95. Bananas (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

96. Cantaloupe, melons (1/4 melon)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 per week
- ○ 2 or more times per week

97. Apples (1) or applesauce
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

98. Pears (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

99. Oranges (1), grapefruit (1/2)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

100. Strawberries
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

101. Peaches, plums, apricots (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 or more per week

102. Orange juice (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 or more glasses per day

103. Apple juice and other fruit juices (1 glass)
- ○ Never/less than 1 per month
- ○ 1 - 3 glasses per month
- ○ 1 glass per week
- ○ 2 - 6 glasses per week
- ○ 1 glass per day
- ○ 2 or more glasses per day

104. Tomatoes (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

105. Tomato/spaghetti sauce
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

106. Tofu
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

107. String beans
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

108. Beans/lentils/soybeans
- ○ Never/less than 1 per month
- ○ Once per week or less
- ○ 2 - 6 times per week
- ○ Once per day

109. Broccoli
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

110. Beets (not greens)
- ○ Never/less than 1 per month
- ○ Once per week or less
- ○ 2 or more times per week

111. Corn
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

112. Peas or lima beans
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

113. Mixed vegetables
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

114. Spinach
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once a week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

115. Greens/kale
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

116. Green/red peppers
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once a week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

117. Yams/sweet potatoes (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once a week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

118. Zucchini, summer squash, eggplant
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

119. Carrots, cooked
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

120. Carrots, raw
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

121. Celery
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

122. Lettuce/tossed salad
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 6 times per week
- ○ One or more per day

123. Coleslaw
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

124. Potato salad
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

Think about your usual snacks. How often do you eat each type of snack food.

Example If you eat poptarts rarely (about 6 per year) then your answer should look like this:

E3. Poptarts (1)

- ● Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 - 6 per week
- ○ 1 or more per day

SNACK FOODS/DESSERTS

125. Fill in the number of snacks (food or drinks) eaten on school days and weekends/vacation days.

Snacks	School Days					Vacation/Weekend Days				
	NONE	1	2	3	4 OR MORE	NONE	1	2	3	4 OR MORE
Between breakfast and lunch	○	○	○	○	○	○	○	○	○	○
After lunch, before dinner	○	○	○	○	○	○	○	○	○	○
After dinner	○	○	○	○	○	○	○	○	○	○

126. Potato chips (1 small bag)

- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ One small bag per week
- ○ 2 - 6 small bags per week
- ○ 1 or more small bags per day

127. Corn chips/Doritos (small bag)

- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ One small bag per week
- ○ 2 - 6 small bags per week
- ○ 1 or more small bags per day

128. Nachos with cheese (1 serving)

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 or more times per week

129. Popcorn (1 small bag)

- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 - 4 small bags per week
- ○ 5 or more small bags per week

130. Pretzels (1 small bag)

- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 small bags per week
- ○ 2 or more small bags per week

131. Peanuts, nuts (1 small bag)

- ○ Never/less than 1 per month
- ○ 1 - 3 small bags per month
- ○ 1 - 4 small bags per week
- ○ 5 or more small bags per week

132. Fun fruit or fruit rollups (1 pack)

- ○ Never/less than 1 per month
- ○ 1 - 3 packs per month
- ○ 1 - 4 packs per week
- ○ 5 or more packs per week

133. Graham crackers

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 - 4 times per week
- ○ 5 or more times per week

134. Crackers, like saltines or wheat thins

- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ 1 - 4 times per week
- ○ 5 or more times per week

135. Poptarts (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 poptarts per month
- ○ 1 - 6 poptarts per week
- ○ 1 or more poptarts per day

136. Cake (1 slice)
- ○ Never/less than 1 per month
- ○ 1 - 3 slices per month
- ○ 1 slice per week
- ○ 2 or more slices per week

137. Snack cakes, Twinkies (1 package)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ Once per week
- ○ 2 - 6 per week
- ○ 1 or more per day

138. Danish, sweetrolls, pastry (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

139. Donuts (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 donuts per month
- ○ 1 donut per week
- ○ 2 - 6 donuts per week
- ○ 1 or more donuts per day

140. Cookies (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 cookies per month
- ○ 1 cookie per week
- ○ 2 - 6 cookies per week
- ○ 1 - 3 cookies per day
- ○ 4 or more cookies per day

141. Brownies (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 4 per week
- ○ 5 or more per week

142. Pie (1 slice)
- ○ Never/less than 1 per month
- ○ 1 - 3 slices per month
- ○ 1 slice per week
- ○ 2 or more slices per week

143. Chocolate (1 bar or packet) like Hershey's or M & M's
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 - 6 per week
- ○ 1 or more per day

144. Other candy bars (Milky Way, Snickers)
- ○ Never/less than 1 per month
- ○ 1 - 3 candy bars per month
- ○ 1 candy bar per week
- ○ 2 - 4 candy bars per week
- ○ 5 or more candy bars per week

145. Other candy without chocolate (Skittles) (1 pack)
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

146. Jello
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

147. Pudding
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

148. Frozen yogurt
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

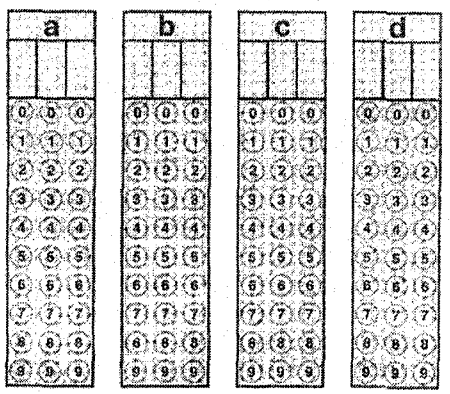
149. Ice cream
- ○ Never/less than 1 per month
- ○ 1 - 3 times per month
- ○ Once per week
- ○ 2 - 4 times per week
- ○ 5 or more times per week

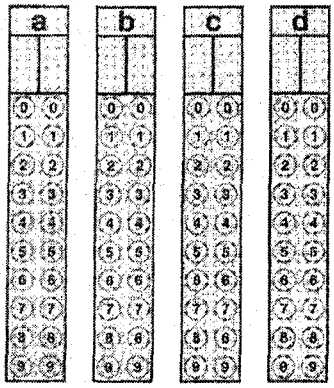
150. Milkshake or frappe (1)
- ○ Never/less than 1 per month
- ○ 1 - 3 per month
- ○ 1 per week
- ○ 2 or more per week

151. Popsicles
- ○ Never/less than 1 per month
- ○ 1 - 3 popsicles per month
- ○ 1 popsicle per week
- ○ 2 - 4 popsicles per week
- ○ 5 or more popsicles per week

152. Please list any other foods that you usually eat <u>at least once per week</u> that are not listed (for example, coconut, hummus, falafel, chili, plantains, mangoes, etc. . .)

FOODS	HOW OFTEN?
a) _____	a) _____
b) _____	b) _____
c) _____	c) _____
d) _____	d) _____





THANK YOU FOR COMPLETING THIS SURVEY!

APPENDIX C Harvard Service Food Frequency Questionnaire

Children's Nutrition Questionnaire

What Have You Been Eating Lately?

"During the past 4 weeks, how often did you eat a serving of each of the foods listed here?"

Mark only one X for each food

Name: _____

ID: _____

Date ___/___/___

DOB: ___/___/___

Age: _____

Respondent: *(please check)*

☐ Mother

☐ Other _____

Example:

	last 4 weeks		each week			each day			
Number of times	0	1–3	1	2–4	5–6	1	2–3	4–5	6+
Milk				X					
Hot chocolate	X								

	last 4 weeks		each week			each day			
Number of times	0	1–3	1	2–4	5–6	1	2–3	4–5	6+
Milk									
Hot chocolate									
Cheese (plain or in sandwiches)									
Yogurt									
Ice cream (cones, sandwiches, sundaes)									
Pudding									

0 1 2 3 4 5 6 7 8

What kind of milk does your child usually drink? (Check one)

1 ☐ breastmilk 3 ☐ whole 5 ☐ 1% 7 ☐ Chocolate Milk

2 ☐ formula 4 ☐ 2% 6 ☐ skim 8 ☐ other _____

	last 4 weeks		each week			each day			
Number of times	0	1–3	1	2–4	5–6	1	2–3	4–5	6+
Orange juice or grapefruit juice									
Other juice									
Fruit drinks (Hi-C, Kool-Aid, lemonade, sports drink)									
Banana									
Peaches									
Fruit cocktail, mixed fruit									
Orange or grapefruit									
Apple or pear									
Applesauce									
Grapes									
Strawberries									
Melon									
Pineapple									
Raisins or prunes									

0 1 2 3 4 5 6 7 8

Continued on next pa

Mark only one X for each food.
How often did you eat a serving of these foods during the past 4 weeks?

	last 4 weeks		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Corn									
Peas									
Tomatoes, tomato sauce, salsa									
Peppers (green, red or hot)									
Carrots									
Broccoli									
Green beans									
Spinach									
Mixed vegetables									
Squash, orange or winter									
Zucchini/yellow squash									
French fries, fried potatoes, tater tots									
Potatoes (baked, boiled, or mashed)									
Sweet potatoes or yams									
Cabbage, coleslaw or cauliflower									
Lettuce salad									
Salad dressing									
Mayonnaise									
	0	1	2	3	4	5	6	7	8

	last 4 weeks		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Chips (potato, corn or others)									
Popcorn or pretzels									
Crackers									
Nuts									
Cookies or brownies									
Cake or cupcake									
Pie									
Jello									
Chocolate or candy bar									
Other candy (not chocolate)									
Coffee or tea									
Soda, soft drink, pop (not sugar free)									
Soda, soft drink, pop (sugar free)									
	0	1	2	3	4	5	6	7	8

	last 4 weeks		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Beans (baked, chili, or other)									
Rice									
Spaghetti or other pasta									
Pizza									
Tacos, burritos									
Macaroni and cheese									
Hot dogs									
Sausage									
Hamburger (prepared any way)									
Canned tuna									
Fried fish, fish sticks									
Other fish									
Cold cuts (baloney, ham, salami)									
Fried chicken, chicken nuggets									
Other chicken or turkey									
Pork or ham									
Roast beef or steak									
Liver, organ meats									
Peanut butter									
Bread (slice) toast, roll, or pita									
Butter (not margarine)									
Margarine									
	0	1	2	3	4	5	6	7	8

	last 4 weeks		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Vegetable soup									
Other soup									
Cornbread or tortilla									
Eggs									
Bacon									
Hot cereal, grits									
Cold cereal									
Donut									
Sweet roll or muffin									
Pancake, waffle, or french toast									
English muffin or bagel									
Biscuit									
	0	1	2	3	4	5	6	7	8

1. What type of bread does your child usually eat:
   ☐ white bread ☐ whole wheat or dark bread ☐ about half and half ☐ DON'T EAT BREAD

2. What type of margarine does your child usually use:
   ☐ stick ☐ tub ☐ squeeze ☐ DON'T USE MARGARINE
   Is this margarine:
   ☐ corn oil ☐ nonfat ☐ other

3. If your child eats cold breakfast cereal, what type:
   ☐ high fiber (eg. All Bran) ☐ unsweetened (eg. Corn Flakes) ☐ sweetened (eg. Cap'n Crunch)

4. Does your child take a multi-vitamin pill (Flintstones, TriViFlor):
   ☐ no ☐ yes
   If yes, how often:
   ☐ Every day ☐ 4–6 times a week ☐ 1–3 times a week ☐ Less than one time a week

5. Does your child take a separate iron pill (not in the multi-vitamin pill above):
   ☐ no ☐ yes

6. Does your child take a separate fluoride supplement (not in the multi-vitamin pill above):
   ☐ no ☐ yes

7. Does your child eat fried food at home:
   ☐ no ☐ yes
   If yes, how often:
   ☐ Every day ☐ 4–6 times a week ☐ 1–3 times a week ☐ Less than one time a week
   If yes, what type of fat do you use to fry at home:
   ☐ butter ☐ margarine ☐ crisco ☐ corn oil ☐ canola oil ☐ olive oil ☐ other vegetable oil

9. Do you bake cookies, cake or pies at home:
   ☐ no ☐ yes
   If yes, how often does your child eat home-baked cookies, cake or pies?
   ☐ Every day ☐ 4–6 times a week ☐ 1–3 times a week ☐ Less than one time a week
   If yes, what type of fat do you use to bake at home:
   ☐ butter ☐ margarine ☐ crisco ☐ corn oil ☐ canola oil ☐ olive oil ☐ other vegetable oil

APPENDIX D Risk Factor Questionnaire. Control participants completed the same questionnaire except the time frame was the year prior to interview, and not the year prior to diagnosis.

Exposures Questionnaire for Nitrate and Type I Diabetes Project (CASES)

Respondent Name:_____ **Respondent Number:**
Survey Date (m/d/y):_____ **Location of interview:**
Date of Birth (m/d/y):_____ **Interviewer:**
Date of Diagnosis (m/d/y): _____

Please provide information for the following survey.

If the participant is a child, the child should be assisted by his/her parent/guardian. All questions refer to the child, unless otherwise indicated (ie. survey question refers to parent).

General questions:

1. Extended family member* has/had Type 1 diabetes - ❑ yes ❑ no ❑ not sure

** Extended family member defined as brother/sister, grandparent, parent, child, first cousin, or related aunt/uncle (not aunt/uncle by marriage)*

If yes, describe which family members have/had been diagnosed and describe the number of family members

a) Diagnosed family members_____

_____

b) Number of family members with and without diabetes:

Family member	Number
Brothers	
Sisters	
Maternal aunts/uncles	
Paternal aunts/uncles	
Maternal cousins	
Paternal cousins	

2. Extended family member* has/had Type 2 diabetes - ❑ yes ❑ no ❑ not sure

Questions related to early childhood (0-2 years of age):

3. Breast-feeding duration _____months

4. Cow's milk-based formula regularly fed before 3 months of age ❑ yes ❑ no

_____ (record frequency)

5. Regular (at least 1 day/week) day care attendance prior to age 3 ❑ yes ❑ no

6. Standard vaccination against measles ❑ yes ❑ no

7. Number of infections (cold, flu, etc.) per year ❑ <5 ❑ 5-10 ❑ 11-15 ❑ >15

Questions related to the 12 months prior to Type 1 diagnosis

Set time frame for respondent: from _____ to _____ (month year).

8. Oral hygiene (teeth brushing frequency - # times/day) _____/day

9. Number of infections (cold, flu, etc.) ❑ <5 ❑ 5-10 ❑ 11-15 ❑ >15

10. History of stomach/pancreas problems ❑ yes ❑ no
If yes, describe _____

11. History of chronic urinary bladder infections ❑ yes ❑ no
If yes, describe

12. Stressful life events during the 12 months prior to Type 1 diagnosis
(Eg. * immediate family member death, parents getting divorced) ❑ yes ❑ no

If yes, describe

_____

_____

* Immediate family member defined as brother/sister, grandparent, parent, or child

13. Smoking: a) Were you/your child a daily smoker during the year prior to diagnosis ❑ yes ❑ no

If yes, for how long and how many did you/your child smoke a day?

b) Relative living in same home smoked daily during the year prior to diagnosis

❑ yes ❑ no

If yes, for how long and how many did they smoke a day?

_____

14. Alcohol consumption during _____ (dates above)
    a) participant - average number of drinks/week _____/week
    b) father - average number of drinks/week _____/week
    c) mother - average number of drinks/week _____/week

15. Circle the highest level of education obtained (completed only)
    a) participant - primary/secondary/college/university/NOT APPLICABLE
    b) father - primary/secondary/college/university
    c) mother - primary/secondary/college/university

16. Occupational history in primary industry*
    a) participant - ❑ yes ❑ no
    b) father - ❑ yes ❑ no
    c) mother - ❑ yes ❑ no

Describe timing/duration:_____

*Primary industry includes farming, fishing, mining (working on the land or sea)

17. Circle combined household income category
    <$30,000/yr $30,000-$50,0000 >$50,000

Answer the following questions for the residence in which you lived <u>during the year prior to diagnosis</u>

18. Residence location at time of diagnosis same as current residence ❑ yes ❑ no

19. If no, what was the residence location at time of diagnosis?

_____
(Include exact civic address number)

20. Family owned the house in which you lived during year prior to diagnosis - ❑ yes ❑ no

21. Check residential type for the year prior to diagnosis

❑ Urban = Charlottetown/Summerside
❑ Rural = Village or town
❑ Farm = House is not part of a cluster

22. Did you/your child drink the tap water? ❑ yes ❑ no

23. Does the house have a municipal water supply?
❑ yes → Go to #31 ❑ no ❑ don't know → Go to #26

24. If no, is the well the same compared to the year prior to diagnosis?
❑ yes ❑ no ❑ don't know

25. If no, how is it different now from then?

---

26. Is the current land use on and immediately adjacent to the residence (within 100 metres) similar now compared to the year prior to diagnosis?

❑ yes ❑ no ❑ don't know

27. If no, how is it different now from then?

---

28. Has water been taken from your house to test for nitrate in the last 10 years?
❑ yes ❑ no → Go to #31 ❑ don't know → Go to #31

29. What was the nitrate concentration?
_____mg/dl ❑ don't know

30. What was the date of that test?
_____ ❑ don't know

31. Were you attending **SCHOOL** during the year prior to diagnosis? ❑ yes ❑ no

32. If no, go to **# 43**. If yes, which one?

Answer the following questions for the school that you attended during the year prior to diagnosis

33. How many hours of the day were you at this school? _____/day

34. Did you/your child consume the tap water while at the school?
❑ yes ❑ no → Go to #43 ❑ don't know

35. Was the school on a municipal water supply?
❑ yes → Go to #43 ❑ no ❑ don't know → Go to #38

36. If no, is the well the same compared to the year prior to diagnosis
❑ yes ❑ no ❑ don't know

37. If no, how is it different now from then?

---

38. Is the current land use immediately adjacent to the school (within 100 metres) similar now compared to the year prior to diagnosis ❑ yes ❑ no ❑ don't know

39. If no, how is it different now from then?

_____

40. Has water been taken from your school to test for nitrate in the last 10 years?
❑ yes ❑ no →Go to #43 ❑ don't know → Go to #43

41. If yes, what was the nitrate concentration?
_____mg/dl ❑ don't know

42. If yes, what was the date of that test?
_____ ❑ don't know

43. Were you attending a **DAYCARE** during the year prior to diagnosis? ❑ yes ❑ no

44. If no, go to # 55. If yes, what is the name and address of the daycare provider

_____

Answer the following questions for the daycare that you attended during the year prior to diagnosis

45. How many hours of the day were you at this daycare? _____/day

46. Did you/your child consume the tap water while at daycare?
❑ yes ❑ no → Go to #55 ❑ don't know

47. Was the daycare on a municipal water supply?
❑ yes → Go to #55 ❑ no ❑ don't know → Go to #50

48. If no, is the well the same compared to the year prior to diagnosis?
❑ yes ❑ no ❑ don't know

49. If no, how is it different now from then?

_____

50. Is the current land use on and immediately adjacent to the daycare (within 100 metres) similar compared to the year prior to diagnosis?
❑ yes ❑ no ❑ don't know

51. If no, how is it different now from then?

52. Has water been taken from the daycare to test for nitrate in the last 10 years?
❑ yes ❑ no →Go to #55 ❑ don't know →Go to #55

53. What was the nitrate concentration?
_____mg/dl ❑ don't know

54. What was the date of that test?
_____ ❑ don't know

55. Were you **EMPLOYED** during the year prior to diagnosis? ❑ yes ❑ no

56. If no, go to #68. If yes, what is the name and address of the employer?

Answer the following questions for the employer where you worked during the year prior to diagnosis

57. Was employment seasonal? ❑ yes ❑ no

58. How many hours of the day were you at this employment? _____/day

59. Did you consume the tap water from the place of employment?
❑ yes ❑ no →Go to #68 ❑ don't know

60. Was employment on a municipal water supply?
❑ yes → Go to #68 ❑ no ❑ don't know → Go to #63

61. If no, is the well the same compared to the year prior to diagnosis
❑ yes ❑ no ❑ don't know

62. If no, how is it different now from then?

63. Is the current land use on and immediately adjacent to the employment similar compared to the year prior to diagnosis
❑ yes ❑ no ❑ don't know

64. If no, how is it different now from then?

65. Has water been taken from your place of employment to test for nitrate in the last 10 years?
❑ yes ❑ no →Go to #68 ❑ don't know →Go to #68

66. What was the nitrate concentration?
_____mg/dl ❑ don't know

67. What was the date of that test?
_____ ❑ don't know

68. Racial background (percent - eg. 100% White OR 50% White and 50% Hispanic). Answers must total 100%.

White	
Hispanic	
Asian	
Black	
Aboriginal	
Other, please specify	

69. Height/length at time of Type 1 diabetes diagnosis: below average average above average

70. Current height/length _____Feet

71. Current weight_____Pounds

72. Explanation of rationale and expected findings of research:

Many human and non-human inhabitants of agricultural areas such as Prince Edward Island (PEI) are being exposed to nitrate contamination of drinking water. The objective of this research is to understand if, and at what level, nitrate contamination of drinking water is related to Type 1 diabetes among Island inhabitants. It will also be determined if Type 1 diabetics have been exposed to high nitrate levels from waterborne and foodborne sources compared to non-diabetics. This research will allow us to more fully inform individual households with unsafe nitrate levels regarding the human health risks associated with drinking their tap water.
Thank you for your participation in this research project.

73. Reminder to take current water sample obtained

74. Reminder to do Food Frequency Questionnaire

APPENDIX E Foods from both food frequency questionnaires used to create food groups

Vegetables
vegetable soup[ψ], potato[ψ‡], sweet potato[ψ‡], string bean[ψ‡], broccoli[ψ‡], beetroot[‡], corn[ψ‡], peas[ψ‡], mixed vegegetables[ψ‡], spinach[ψ‡], kale[‡], peppers[ψ‡], zucchini[ψ], squash[ψ], zucchini and squash combined[‡], cooked carrot[‡], raw carrot[‡], carrots[ψ], celery[‡], lettuce[ψ‡], coleslaw[‡], potato salad[‡], cabbage[ψ]

Fresh fruit
grape[ψ‡], banana[ψ‡], melon[ψ‡], apple[‡], pear[‡], apple and pear combined[ψ], orange[ψ‡], strawberry[ψ‡], tomato[‡], pineapple[ψ], peach[ψ‡]

Total fruit
grape[ψ‡], banana[ψ‡], melon[ψ‡], apple[‡], pear[‡], apple and pear[ψ] , orange[ψ‡], strawberry[ψ‡], tomato[ψ‡], tomato sauce[‡], salsa[‡], tomato, tomato sauce and salsa combined[ψ], peach[ψ‡], raisin[ψ‡], orange juice[ψ‡], other juice[ψ‡], fruit cocktail[ψ], apple sauce[ψ], pineapple[ψ]

Milk and milk products
milk[ψ‡], chocolate milk[‡], instant breakfast drink[‡], ice-cream[ψ‡], yoghurt[ψ‡], cottage cheese[‡], cheese[ψ‡], frozen yoghurt[‡], milkshake[‡], pudding[ψ‡], cream soups[ψ‡]

Fats
whipped cream[‡], cream cheese[‡], butter[ψ‡], margarine[ψ‡], regular salad dressing[‡], low calorie salad dressing[‡], salad dressing[ψ], mayonnaise[ψ‡]

High sugar/high fat foods
ice-tea[‡], fruit punch[ψ‡], regular soft drinks[ψ‡], pound cake[‡], snack cake[‡], all cake[ψ], pudding[ψ‡], fruit rollups[‡], sweet roll[‡], donut[ψ‡], cookie[‡], brownie[‡], cookie and brownie[ψ] , plain chocolate[‡], chocolate bar[‡], plain chocolate and chocolate bar[ψ], hard candy[‡], popsicle[‡], jello[ψ‡], poptart[‡], pie[ψ‡]

Liquid
milk[ψ‡], chocolate milk[‡], milkshake[‡], water[ψ‡], tea[‡], coffee[‡], tea and coffee[ψ], wine[‡], beer[‡], orange juice[ψ‡], other pure juice[ψ‡], ice-tea[‡], fruit punch[ψ‡], regular soft drinks[ψ‡], diet soft drinks[ψ‡], hot chocolate[ψ]

Meats and alternatives
ham[ψ‡], sausage[ψ‡], bacon[ψ‡], coldcuts[ψ], coldcut sandwich[‡], hotdog[ψ‡], tuna[ψ], tuna sandwich[‡], fresh fish[ψ‡], fish sticks[ψ‡], shellfish[ψ‡], cheese burger and bun[‡], burger and bun[‡], ground beef[ψ], chicken nuggets[ψ‡], chicken sandwich[‡], roast beef sandwich[‡], chicken[ψ‡], pork[ψ‡], beef[ψ‡], meatballs[‡], liver[ψ‡], egg[ψ‡], baked beans[ψ‡], peanut butter sandwich[‡], peanut butter[ψ]

Grain

clear soup [rice, noodle, veg][‡], rice[Ψ‡], pizza[Ψ‡], tacos[Ψ‡], macaroni and cheese[Ψ‡], hot cereal[Ψ‡], cold cereal[Ψ‡], muffin[Ψ‡], English muffin or bagel[Ψ‡], biscuit[Ψ‡], burger and bun[‡], cheese burger and bun[‡], chicken sandwich[‡], roast beef sandwich[‡], coldcut sandwich[‡], tuna sandwich[‡], peanut butter sandwich[‡], lasagne[Ψ‡], spaghetti[Ψ‡], French toast[‡], grilled cheese sandwich[‡], white bread[‡], dark bread[‡], bread[Ψ], cornbread[Ψ‡], pasta[Ψ‡], tortilla[‡], grain[‡], pancake[Ψ‡], graham cracker[‡], other crackers[‡], cracker[Ψ]

Other

ice-cream[Ψ‡], pudding[Ψ‡], mayonnaise[Ψ‡], plain chocolate and chocolate bars[Ψ], plain chocolate[‡], chocolate bars[‡], hard candy[Ψ‡], peanut butter[Ψ‡], donut[Ψ‡], eggroll[Ψ‡], gravy[‡], ketchup[‡], regular salad dressing[‡], low calorie salad dressing[‡], salad dressing[Ψ], potato chips[‡], corn chips[‡], all chips[Ψ], nachos[‡], popcorn[‡], pretzel[‡], popcorn and pretzel, nuts[Ψ], fruit roll-ups[‡], poptart[‡], pound cake[‡], snack cake[‡], all cake[Ψ], sweet roll[‡], cookie[‡], brownie[‡], cookie and brownie[Ψ], jello[Ψ‡], frozen yoghurt[‡], milkshake[‡], popsicle[‡]

[‡] Food on the Youth/Adolescent Questionnaire
[Ψ] Food on the Harvard Service Food Frequency Questionnaire

APPENDIX F Harvard Service Food Frequency Questionnaire: Nitrate, nitrite and nitrosamine concentrations in food sampled from Prince Edward Island, or published values where available.

FOOD LIST	Sodium nitrate mg/100g[a]	Sodium nitrite mg/100g[a]	Nitrosamines mg/100g[a]
Milk	0.6	0.004	.
Hot chocolate	3.673	0.004	.
Cheese	1.169[b]	0.004[b]	2E-06[b]
Yogurt	2.6	0.004	2E-06[b]
Ice cream	3.3	0.004	2E-06
Pudding	0.8	0.004	2E-06
Orange, grapefruit juice	0.004	0.004	.
Other juice	1.3	0.004	.
Fruit drinks	1.1	2.4	2E-06
Banana	23.8	0.004	.
Peaches	0.004[d]	0.004[d]	.
Fruit cocktail, mixed fruit	0.004	0.004	.
Orange or grapefruit	0.004	0.004	.
Apple or pear	1.3	0.004	.
Applesauce	1.3	0.004	.
Grapes	0.004	0.004	.
Strawberries, raspberries	20.1	0.004	.
Melon	0.004[d]	0.004[d]	.
Pineapple	0.004	0.004	.
Raisins or prunes, plums (incl. dried)	0.004	0.004	.
Corn	.	.	.
Peas	.	.	.
Tomato, tomato sauce, salsa	4	0.004	.
Peppers (green, red, or hot)	.	.	.
Carrots	1	0.004	.
Broccoli	6.9	0.004	.
Green beans	.	.	.
Spinach	.	.	.
Mixed vegetables	1[d]	0.004[d]	.
Squash, orange or	1.1[d]	10.7[d]	.

winter			
Zucchini, yellow squash	68.3	0.004	.
French fried potatoes, tater tots	12.7	0.004	2E-06
Potatoes, (baked, boiled, or mashed)	1.1	10.7	.
Sweet potatoes or yams	1.1[d]	10.7[d]	
Cabbage coleslaw	14.2	12.8	2E-06
Lettuce			
Salad dressing	6.6	101.5	2E-06[b]
Mayonnaise	4.36	70.1	2E-06
Chips (potato, corn, or other)	40.8	0.004	2E-06
Popcorn or pretzels	9.8	3.3	2E-06
Crackers	2.5	0.4	5.1E-05
Nuts (incl. peanuts)	1.6	0.004	
Cookies or brownies	0.6	4.2	2E-06
Cake or cupcake	1.5[d]	0.004[d]	6.5E-05
Pie	1	8.5	2E-06
Jello	0.4	0.004	2E-06
Chocolate or candy bar	1.6	0.004	2E-06
Other candy (not chocolate), suckers	0.004	0.004	2E-06
Coffee or tea	.	.	.
soft drinks, soft drink, pop (not sugar free)	2.7	0.004	
soft drinks, soft drink, pop (sugar free)	2.7[d]	0.004[d]	.
Beans (baked, chilli, other)	0.004	0.004	
Rice	7.2	0.004	2E-06
Spaghetti or other pasta	4.5	0.004	2E-06
Pizza	4.2	0.7	2E-06
Tacos, burritos	1.535[c]	0.420[c]	6E-06[c]
Macaroni and cheese	1.737[c]	0.011[c]	2E-06[c]
Hot dogs	2.315[b]	1.109[b]	2E-06[b]
Sausage	0.066[b]	0.519[b]	0.00005[b]
Hamburger (prepared any way)	0.42[b]	0.074[b]	0.00001[b]
Canned tuna	0.206[b]	0.013[b]	2E-06[b]
Fried fish, fish sticks	0.135[b]	0.005[b]	2E-06[b]

Other fish	0.135[b]	0.005[b]	2E-06[b]
Cold cuts (bologna, ham, salami)	2.285[b]	0.917[b]	7.5E-06[b]
Fried chicken, chicken nuggets	0.353[b]	0.211[b]	2E-06[b]
Other chicken or turkey	0.004	0.004	2E-06
Pork or ham	3.8	5.3	6.9E-05
Roast beef or steak	0.004	0.004	2E-06
Liver, organ meats	.	.	.
Peanut butter	0.004	0.004	2.1E-05
Bread (slice), toast, roll, pita	7.9	0.004	2E-06
Butter (not margarine)	0.558[b]	0.009[b]	2E-06[b]
Margarine	0.392[b]	0.004[b]	2E-06[b]
Vegetable soup			2E-06[b]
Other soup (with meat), canned	0.479[b]	0.004[b]	2E-06[b]
Cornbread or tortilla	7.9	0.004	2E-06
Eggs	3.6	0.004	2.3E-05
Bacon	3.6	3.37[b]	0.00137[b]
Hot cereal, grits	6.4	0.004	2E-06[b]
Cold cereal	9.9	0.7	2E-06
Donut	3.7	0.004	2E-06
Sweet roll	2	7.8	2E-06
Pancake, waffle, or french toast	0.325[b]	0.311[b]	2E-06[b]
English muffin or bagel	7.9[d]	0.004[d]	2E-06[d]
Biscuit	2.3	0.004	2E-06

[a] Based on test results of locally sampled foods, unless otherwise indicated

[b] Book values provided by Sen 2003 (personal communication)

c Average nitrate, nitrite, or nitrosamine concentrations were a composite of both book values and/or sampled foods (eg taco shells were sampled locally, and hamburger values were provided by Sen)

[d] Nitrate, nitrite, or nitrosamine value imputed from a similar food

APPENDIX G Youth/Adolescent Questionnaire nitrate, nitrite and nitrosamine concentrations in food sampled from Prince Edward Island, and published values where available.

FOOD LIST	Sodium nitrate mg/100g[a]	Sodium nitrite mg/100g[a]	Nitrosamines mg/100g[a]
Diet soft drinks	2.7[d]	0.004[d]	.
Soft drinks	2.7	0.004	.
Fruit drink	1.1	2.4	2E-06
Iced tea - sweetened	1.1	0.004	2E-06
Tea	.	.	.
Coffee	.	.	.
Beer	1	0.004	1E-05[a]
Wine or wine coolers	.	.	.
Liquor	.	.	.
Milk	0.6	0.004	.
Chocolate milk	0.6[d]	0.004[d]	.
Instant breakfast drink	0.528[b]	0.004[b]	.
Whipped cream	0.558[bd]	0.009[bd]	2E-06[bd]
Yogurt	2.6	0.004	2E-06[b]
Cottage or ricotta cheese	0.9[b]	0.004[b]	2E-06[b]
Cheese	1.169[b]	0.004[b]	2E-06[b]
Cream cheese	0.558[b]	0.009[b]	2E-06[b]
Butter	0.558[b]	0.009[b]	2E-06[b]
Margarine	0.392[b]	0.004[b]	2E-06[b]
Cheeseburger	0.447[b]	0.062[b]	2E-06[b]
Hamburger	0.42[b]	0.074[b]	2E-06[b]
Pizza	4.2	0.7	2E-06
Tacos / burritos	1.535[c]	0.403[c]	6E-06[c]
Chicken nuggets	0.353[b]	0.211[b]	4E-06[b]
Hot dogs	2.315[b]	1.109[b]	4E-06[b]
Peanut butter sandwich	3.109[c]	0.003[c]	5E-06[c]
Chicken or turkey sandwich	2.356[c]	7.133[c]	2E-06[c]
Roast beef or ham sandwich	2.523[c]	7.715[c]	2E-06[c]

Deli meat (salami, bologna)	3.654[c]	8.167[c]	4.7E-06[c]
Tuna sandwich	1.716[c]	0.009[c]	1.8E-06[c]
chicken or turkey	0.004	0.004	2E-06
Fish sticks, cakes, or sandwich	0.135[b]	0.005[b]	2E-06[b]
Fresh fish	0.135[b]	0.005[b]	2E-06[b]
Beef or lamb	0.004	0.004	2E-06
Pork or ham	3.8	5.3	6.9E-05
Meatballs or meatloaf	1.098[c]	0.050[c]	4.7E-06[c]
Lasagna	3.831[c]	0.004[c]	2E-06[c]
Macaroni and cheese	1.737[c]	0.011[c]	2E-06[c]
Spaghetti with tomato sauce	4.5	0.004	2E-06
Eggs	3.6	0.004	2.3E-05
Liver	.	.	.
shellfish	0.260[b]	0.043[b]	2E-06[b]
French toast	4.520	0.004	1.2E-05
Grilled cheese	5.180	0.008	2.1E-06
Eggrolls	37.1	0.004	2E-06
Brown gravy	3.8	0.004	2E-06
Ketchup	2.8	0.004	.
Clear soup (with rice, veg, noodles)	0.480[b]	0.004[b]	2E-06[b]
Cream soups or chowder	13.6	0.004	.
Mayonnaise	4.36	70.1	2E-06
Low calorie/fat salad dressing	6.6[d]	101.5[d]	2E-06[bd]
Regular salad dressing	6.6[d]	101.5[d]	2E-06[bd]
Salsa	4	0.004	2E-06[b]
Cold cereal	9.9	0.5	2E-06
Hot cereal (oatmeal)	6.4	0.004	2E-06[a]
White bread or pita	7.9	0.004	2E-06
Dark bread	2.9	0.004	2E-06
English muffin or bagel	7.9[d]	0.004[d]	2E-06[d]

muffin	2.7	0.004	2E-06
Cornbread	7.9	0.004	2E-06
Biscuit or roll	2.3	0.004	2E-06
Rice	7.2	0.004	2E-06
Noodles or pasta	7[d]	0.004[d]	2E-06[d]
Tortilla	7.9[d]	0.004[d]	2E-06[d]
Other grains like couscous	.	.	.
Pancakes or waffles	0.325[b]	0.311[b]	2E-06[b]
French fries	12.7	0.004	2E-06
Potatoes	1.1	10.7	.
Raisins	0.004	0.004	.
Grapes	0.004	0.004	.
Bananas	23.8	0.004	.
Cantaloupe, melon	0.004[d]	0.004[d]	.
Apples or applesauce	1.3	0.004	.
Pears	1.3[d]	0.004[d]	.
Oranges or grapefruit	0.004	0.004	.
Strawberries	20.1	0.004	.
Peaches, plums, apricots	0.004	0.004	2E-06
Orange juice	0.004	0.004	.
Apple juice	1.3	0.004	.
Tomatoes	4.0[d]	0.004[d]	.
Tomato/spaghetti sauce	4.5	0.004	2E-06[b]
Tofu	.	.	.
String beans	.	.	.
Beans / lentils	0.004	0.004	.
Broccoli	6.9	0.004	.
Beets	.	.	.
Corn	.	.	.
Peas or lima beans	.	.	.
Mixed vegetables	1.0[d]	0.004[d]	.
Spinach	.	.	.
Greens	.	.	.
Green/red pepper	.	.	.
Yams, sweet	0.8[d]	7.1[d]	.

potato			
Zucchini	68.3	0.004	.
Carrots (cooked)	1.0[d]	0.004[d]	.
Carrots (raw)	1.0	0.004	.
Celery	.	.	.
Lettuce	.	.	.
Coleslaw	14.2	12.8	2E-06
Potato salad	1.884	16.633	3.8E-06
Potato chips	40.8	0.004	2E-06
Corn chips / doritos	14.4	4.7	5.5E-05
Nachos with cheese	5.592[c]	1.568[c]	2E-05[c]
Popcorn	9.8	3.3	2E-06
Pretzels	9.5	0.004	2E-06
Peanuts, nuts	1.6	0.004	.
Fun fruit/fruit roll up	10.6	0.004	.
Graham crackers	2.5[d]	0.4[d]	5.1E-05[d]
Crackers	2.5	0.4	5.1E-05
Poptarts	7.9	0.004	1.9E-05
Cake	1.5	0.004	6.5E-05
Snack cakes	8.5	0.004	6.5E-05
Danish	2	7.8	2E-06
Donuts	3.7	0.004	2E-06
Cookies	0.6	4.2	2E-06
Brownies	5.9	0.004	2E-06
Pie	1	8.5	2E-06
Chocolate (hershey's, M & M's)	1.6	0.004	2E-06
Other candy bars	0.004	0.6	2E-06
Other candy (no chocolate)	0.004	0.004	2E-06
Jello	0.4	0.004	2E-06
Pudding	0.8	0.004	2E-06
Frozen yogurt	2.6[d]	0.004[d]	2E-06[bd]
Ice cream	3.3	0.004	2E-06
Milkshakes	0.6[d]	0.004[d]	.
Popsicles	1.1	2.4	2E-06
Bacon	3.6	3.37[b]	0.001[b]
Sausage	0.066[b]	0.519[b]	5E-05[b]

[a] Based on test results of locally sampled foods, unless otherwise indicated
[b] Book values provided by Sen 2003 (personal communication)

c Average nitrate, nitrite, or nitrosamine concentrations were a composite of both book values and/or sampled foods (eg taco shells were sampled locally, and hamburger values were provided by Sen)

[d] Nitrate, nitrite, or nitrosamine value imputed from a similar food

APPENDIX H Methods for nitrate and nitrite concentration determination in foods

This document is CONFIDENTIAL when completed

Section and Section Code: Chemistry CHE

Standard Operating Document Number: SOM-DAR-CHE-009-02 **Standard**

Operating Document Title: Nitrate and Nitrite Contents in Foods

SIGNATURE PAGE

The signatures below indicate that those so identified have read, understood and agree to abide by the contents of this document. If additional signature pages are required, the continuation will be indicated on the last line of the this table.

Name	Signature	Initials	Date

1. PURPOSE

1.1 To give the specific information required for the determination of Nitrate and Nitrite Content in food and food products.

2. REFERENCES

2.1 Determination of Sodium Nitrite and Sodium Nitrate in Foods by Ion Chromatography; CFIA (Appendix 1)

3. SCOPE

3.1 Only trained and authorized analysts shall perform this analysis.

4. DEFINITIONS

4.1 Refer to the referenced method.

5. EQUIPMENT AND MATERIALS REQUIRED

5.1 Refer to the referenced method.

6. SAFETY PRECAUTIONS

6.1 Refer to the referenced method.

7. POLICY

7.1 Refer to the referenced method.

8. INSTRUCTIONS

8.1 Perform analysis as given in the referenced method.

DETERMINATION OF SODIUM NITRITE AND SODIUM NITRATE IN FOODS BY ION CHROMATOGRAPHY

01/10/22

1. PURPOSE:

1.1 To outlines the steps to be taken to determine the concentrations of sodium nitrite and sodium nitrate in foods by ion chromatography.

2. SCOPE:

2.1 This method is for use by analysts in the Chemistry Section of the Canadian Food Inspection Agency, Dartmouth Laboratory.

2.2 The method is applicable to the analysis of nitrate and nitrite in foods and food products.

3. PRINCIPLE OF THE METHOD:

3.1 Samples are extracted with water, centrifuged and filtered. Anions in the filtrate are determined by ion chromatography and UV detection.

4. DEFINITIONS:

4.1 Not Applicable

5. REAGENTS and STANDARDS:

5.1 Zinc sulfate.

5.1.1 Zinc sulfate solution (0.42 M): Dissolve 120 grams of $ZnSO_4.7H_2O$ in water and dilute to 1000ml.

5.2 Sodium hydroxide.

5.2.1 Sodium hydroxide solution: Prepare a 2% solution in water w/v.

5.3 Sodium nitrite.

5.3.1 Sodium nitrite stock solution (0.5 mg/mL): Dissolve 250 mg of the salt in water in a 500 mL volumetric flask and dilute to volume. Keep refrigerated and prepare fresh weekly.

5.4 Sodium nitrate.

5.4.1 Sodium nitrate stock solution(0.5 mg/mL): Dissolve 250mg of the salt in water in a 500 mL volumetric flask and dilute to volume. Keep refrigerated and prepare fresh weekly.

5.5 Mixed sodium nitrite and sodium nitrate intermediate solution (100 ug/mL): Add10 mL of sodium nitrate and sodium nitrite stock solutions to a 50 mL volumetric flask and dilute to volume with water. Prepare fresh daily.

5.6 Working standards(0 to 20 ug/mL): Pipette 0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 ml of the working solution into separate 50-ml volumetric flasks and make to volume with water.

5.7 Mixed nitrate/nitrite spiking solution (0.30 mg each/mL)

5.7.1 Accurately weigh 150mg each of sodium nitrate and sodium nitrite into a 500mL volumetric flask and dissolve and dilute to volume with deionized water.

5.8 Control sample:

5.8.1 Place about 10 grams of ground beef into a 150ml beaker. Add 65 ml of water , 12 ml of 2% NaOH and 5.0 ml of the mixed spike stock solution. Polytron for 2 minutes.

5.8.2 Proceed as from Section 7.1(2) below.

5.9 Mobile phase (15 mM sodium chloride):

5.9.1 Weigh 0.87 grams of sodium chloride into a 1000 mL volumetric flask, dissolve and dilute to volume with water.

6. EQUIPMENT and MATERIALS:

6.1 Dionex HPLC ion chromatography system consisting of an autosampler equipped with a 20 ul loop, a degas module, a gradient pump, a variable wavelength UV detector a Liquid Chromatograph Module-3 and a data handling system and recording device. The pumps must be capable of a flow of 1.0 mL/minute and the detector must be capable of measurement at 225 nm. Equivalent equipment from other manufacturers should be acceptable.

6.2 Ion chromatography column: Dionex IonPAC AS12 analytical column, 4 × 250 mm equipped with a Dionex IonPAC AG12 guard column, 4 x50 mm.

6.3 25 mm Nylon membrane filter discs 0.45 μ.

6.4 47 mm Nylon membrane filters, 0.45 μ.

6.5 Polytron homogenizer or equivalent.

6.6 Food processor or equivalent.

7. SAMPLE PREPARATION:

7.1 Prepare a homogeneous, finely divided sample using a food processor.

7.2 Transfer the blended sample to a polypropylene cup and refrigerate until analysis.

8. ANALYTICAL PROCEDURES:

8.1 Extraction of Sample:

8.1.1 Accurately weigh about 10 grams of homogenate into a 200 mL beaker. Add 70 mL of water and 12 mL of 2% NaOH and homogenize with a Polytron for about 2 minutes.

8.1.2 Rinse the generator with 30 to 50 mL of water into the beaker and mix.

8.1.3 Take 2 drops of the suspension and test with pH paper. If pH is less than 8 add additional amounts of 2% NaOH to bring the pH between 8 and 10.

8.1.4 Transfer suspension to a 200 mL flask, with water washes.

8.1.5 Heat in a water bath until the temperature of the suspension reaches between 50 to 60 $_{o}$C and maintain temperature for about 15 minute with occasional swirling.

8.1.6 Add 10 mL of $ZnSO_4$ and mix. If no white precipitate of $Zn(OH)_2$ becomes visible add 2 to 5 ml of 2% NaOH (avoiding excess) and mix.

8.1.7 Cool to room temperature dilute to volume with water and mix thoroughly.

8.1.8 Filter through Whatman #4 or equivalent paper, into a polypropylene cup discarding the first 20 mL of filtrate, .

8.2 Ion chromatography parameters:

8.2.1 Isocratic analysis using 15mM sodium chloride mobile phase.

8.2.2 Flow Rate 1.0 mL/minute.

8.2.3 Detector set at 225 nm.

8.2.4 Run time 12 minutes.

8.2.5 Turn on the degas module as per instructions in the manual. Sparge for 5 minutes with

helium then tighten the eluent caps. Pressurize for 5 minutes at a setting of 5.0.

8.2.6 Run eluent through the column for 10 minutes before proceeding with analysis.

9. EXPRESSION and INTERPRETATION of RESULTS:

9.1 Prepare a calibration curve of absorbance area versus concentration using the working standards.

9.2 Determine the concentrations of nitrate and nitrite in the samples by comparing the sample absorbance area with the calibration curve.

9.3 Determine the nitrate/nitrite concentration in the original sample using the following formula:

Sample concentration (ug/g) = Solution concentration x 200/Sample Weight (g)

10. QUALITY CONTROL/RESULTS VERIFICATION:

10.1 Critical Control Points:

Item	Acceptable Control
Pipettes	Class "A"
Top-loading Balance	Accurate to 0.01 gram
Analytical Balance	Accurate to 0.0001 gram
De-ionised Water	Conductivity $=10^{-18}$ mho

11. METHOD VALIDATION and IMPLEMENTATION:

11.1 Repeatability: Duplicate controls should differ by no greater than 5% relative from each other based upon recovery values.

11.2 Accuracy: The percent recoveries of both sodium nitrite and sodium nitrate should fall within 90% and 110% of the theoretical weights added to the control specimen.

11.3.Quantitative Detection Limit: The detection limit is 0.04 ug/mL

11.4 Working Analytical Range: The working analytical range is from 0.04 ug/mL to 20 ug/mL.

11.5 Acceptability Criteria: Analytical results are acceptable if the percent recovery of both sodium nitrite and sodium nitrate fall between 90% and 110% of the amount spiked and the calibration curve has a correlation coefficient of 0.99 or better.

11.6 Interlaboratory Check Samples: The Laboratory participates in the analysis of Canadian Food Inspection Agency interlaboratory proficiency samples.

11.7 Linear Range: The calibration curves are linear over the entire working range.

12. REMARKS:

12.1 Not Applicable.

13. ASSOCIATED DOCUMENTS:

13.1 DX-300 Series Chromatography System Manual

14. BIBLIOGRAPHY:

14.1 Colorimetric Determination of Nitrate and Nitrite in Foods. Health Protection Branch Laboratories, Bureau of Chemical Safety, Ottawa, LPFC-126, December, 1983.

14.2 Determination of Nitrate and Nitrite in Meat using High-Performance Anion Exchange Chromatography. Dionex Application Note 112.

15. APPENDICES:

15.1 Figure 1: Standard sodium nitrite and sodium nitrate (2.0 ug/mL) run on a Dioxex IonPAC AS12 250 mm x 4 mm id column equipped with a 50 mm x 4 mm id Dionex IonPAC AG 12 guard column run isocratically at 1.0 mL.

15.2 Figure 2: Food sample containing sodium nitrite and sodium nitrate at the 2.0 ug/mL level. Conditions as per Figure 1.

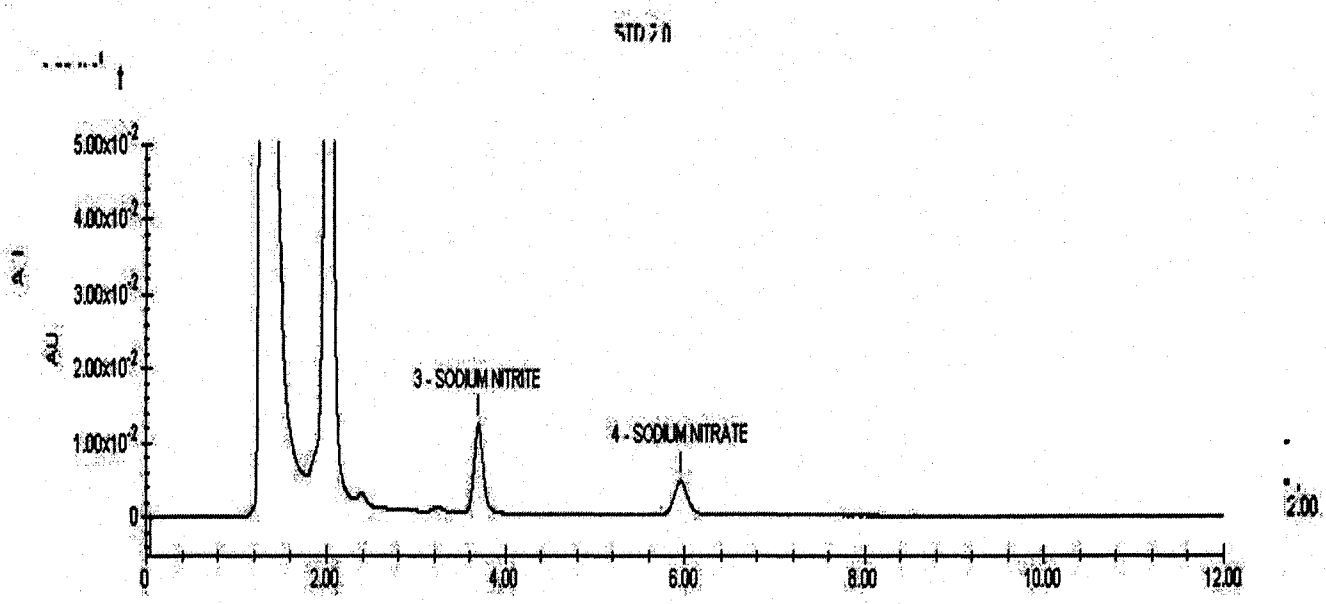


Figure 1

Standard (2.0 ug/mL) sodium nitrite (1) and sodium nitrate (2) run on a Dioxex IonPAC AS12 250 mm x 4 mm id column equipped with a 50 mm x 4 mm id Dionex IonPAC AG 12 guard column run isocratically at 1.0 mL.

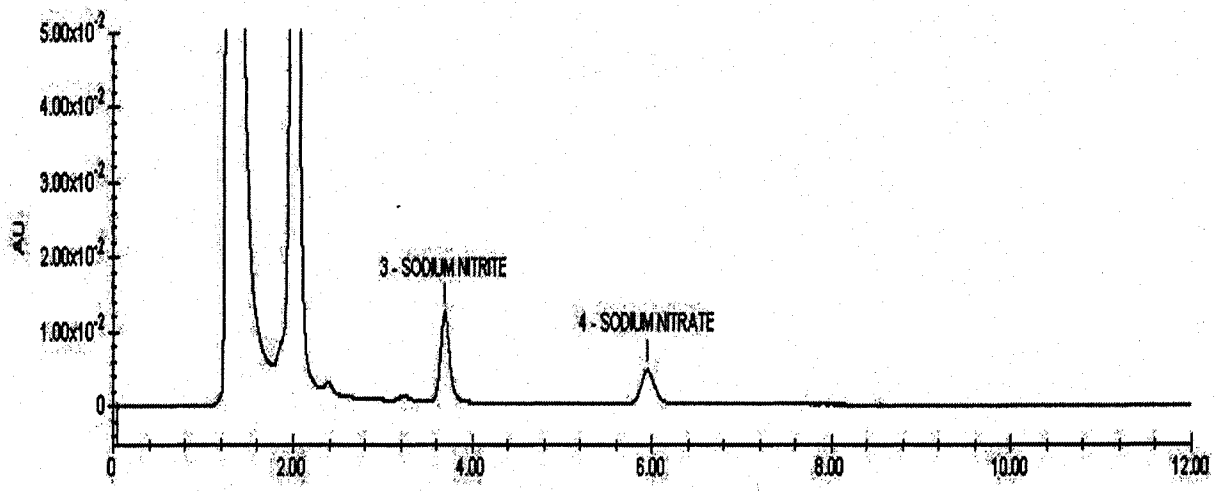


Figure 2

Food sample containing sodium nitrite and sodium nitrate at the 2.0 ug/mL level. Conditions as per Figure 1.

APPENDIX I Methods used for the determination of volatile nitrosamines in foods

DETERMINATION OF VOLATILE NITROSAMINES IN FOODS

by N.P. SEN

1. SCOPE AND FIELD OF APPLICATION

The method is applicable to a variety of foods such as cured meats, fried bacon, cooked-out bacon fat, cheese, alcoholic beverages, skim milk powder, powdered soup mixes, mushrooms, fruit juices, and many more items. It has also been used for the analysis of fish meal, malt, human blood, etc.

The final quantitative determination is based on GLC-TEA analysis. The method works well for NDMA, NDEA, NDPA, NDBA, NPIP, NPYR, and NMOR. (see note 'g' on p.9)

2. PRINCIPLE

A 20 g aliquot of sample is distilled under vacuum (at 45°C) from 200 ml (180 ml for liquid samples) of 3N KOH or 1% sulfamic acid. The nitrosamines in the distillate are extracted into CH_2Cl_2, extract washed with glycine buffer and dilute KOH solution. The extract is finally dried over anhydrous Na_2SO_4, filtered, and concentrated (using Snyder columns) to 1.0 ml. A 5-10 µl aliquot is then analyzed by GLC-TEA.

3. REAGENTS

Dichloromethane (glass-distilled)

Sodium sulfate, anhydrous (granular)

Boileezers (Fisher Scientific Co.)

KOH 3N, and 0.3N

Sulfamic acid (1% in water) - Store at 4°C

Glycine-HCl buffer - Dissolve 22 g glycine in 200 ml 1 N HCl, dilute to 1000 ml with water, adjust pH to 2.1 ± 0.1 with 1 N HCl or 1 N NaOH.

Dry ice or ordinary ice cubes (dry ice preferable).

I. APPARATUS

Distillation apparatus - All glass flash evaporator with provision for circulating ice-cold water through the condenser.
- preferably vertical standing type such as the one by Buchler Instruments. The one by Büchi Inc. is also suitable.

Immersion-type water pump - Used for circulating ice-cold water through condenser. Take a small plastic (preferably insulated) bucket, fill it half with water. Place the pump inside water and connect to condenser of the flash evaporator. Add enough dry ice or ice cubes so that temp. of circulating water stays within 0-5°C at all times (monitor with a thermometer).

Evaporative concentrator	-	(a) 35 ml micro concentration flask, graduated to 1.0 ml (Fig. 1) (b) 1000 ml flask graduated to 20.0 ml (Fig. 2) (c) Snyder column - micro with 3 sections and 14/20 joints (Kontes Glass Co.) (d) Snyder column - large with 3 sections and 24/40 joints (Kontes Glass Co.) Other Kuderna - Danish-type concentrators can be used instead.
Gas chromatograph	-	Varian Aerograph model 2700 or equivalent.
GC column	-	Stainless steel 6' or 9' (1/8" o.d.) Carbowax 20M, 20% on Chromosorb P 60-80 mesh with 2% NaOH or equivalent.

5. DISTILLATION

[Two methods are available. Occasionally the same sample is analyzed by the two methods and results are compared. This is done to ensure that no artefact formation is taking place. For routine work use either one - whichever you find works better in your own hand. see note 'g' on p.9].

...4

(a) KOH METHOD - Weigh 20 g sample into a 2L round bottom flask. Add 200 ml 3N KOH (180 ml if sample is liquid). Add 200 ng NDPA (internal standard) and 100 µg (in 1 ml water solution) di-n-butylamine (to check against artefact formation). [Do not use any antifoam. If absolutely necessary, use 1 g lard as antifoam; analyze 20 g lard for nitrosamine contamination before use. If necessary, add a few boileezers.]

Attach distilling flask to flash evaporator (water bath 45°C), and a 250 ml r.b. flask on collector. Immerse collection flask in ice-water or dry-ice water (avoid freezing) and monitor temp. of ice-water mixture to keep it within 0-5°C. Turn on vacuum (water aspirator) and adjust vacuum release valve so that excessive foaming does not occur. At the same time turn on immersion pump to circulate cold water through condenser. After a few minutes when the foaming ceases or becomes less critical put on near-full vacuum (adjust vacuum to just maintain condensation about 1/3rd the way up the condenser). Keep both flasks covered with Al foil during evaporation. Stop distillation when about 180 ml distillate has been collected. During last 10-15 min of distillation apply full vacuum and if necessary increase the water bath temp. to 50°C. The total distillation time is about 1 hr.

Remove 250 ml collection flask containing the distillate. In its place connect a 500 ml round bottom flask. Disconnect the distillation flask (containing the sample), add 400 ml CH_2Cl_2 into the flask, connect it again to the flash evaporator and continue distillation under a low vacuum. Adjust the vacuum in such a way that the CH_2Cl_2 vapors rise to about $\frac{3}{4}$ the way up the condenser. Occasionally shut off the circulating water pump for a minute or so, and watch the CH_2Cl_2 vapor rise to the top of the column. As soon as the vapor rises to the top, turn on the water pump. Distill all the CH_2Cl_2 and use the distillate in step 6 below. [The purpose of this distillation is to rinse the condenser with CH_2Cl_2. Failure to do this may lead to lower recovery results].

(b) SULFAMIC ACID METHOD - Operate exactly as 5 (a) (KOH method) except use 200 ml (or 180 ml for liquids) 1% sulfamic acid instead of 3N KOH solution.

When using sulfamic acid method you can collect more distillate and get highly improved recoveries. For liquid samples (e.g. beer, fruit juices, etc.) you can continue distillation to near dryness (stop when 1-5 ml liquid left in the distillation flask). For solid samples this may be difficult but collect at least 180 ml distillate and more (if possible)

provided it does not take too long. Avoid increasing the water bath temp. over 50°C - watch for too much frothing on bumping (this will contaminate distillate). [If you notice any artefact formation of NDBA (from the added dibutylamine) then acidify the mixture of sample + sulfamic acid to ph <4 with 1N HCl then continue distillation in the dark. This may be necessary because sulfamic acid is most effective in destroying nitrosating agents under acidic conditions. If no NDBA formation is detected proceed without acidification].

6. EXTRACTION AND DRYING

Basify aqueous distillate in 250 ml flask with 5 ml 3N KOH and transfer to a 500 ml separatory funnel. Rinse flask with ½ of CH_2Cl_2 that was saved from step 5 above and pour into funnel. Extract vigorously and collect organic layer into another separatory funnel (500 ml). Extract water layer (in sep funnel #1) again with remaining CH_2Cl_2 (from step 5) and again collect organic layer into the 2nd funnel. Extract the combined CH_2Cl_2 phase with 50 ml glycine buffer and retain CH_2Cl_2 phase. Discard aqueous layer. Extract CH_2Cl_2 layer again with 50 ml 0.3N KOH and discard aqueous phase. Finally, dry organic layer over anhydrous sodium sulfate in a glass-stoppered Erlenmeyer flask for 1 hr (or overnight) shaking occasionally.

. CONCENTRATION

Filter through Whatman #1 filter paper and collect filtrate in 1L concentration flask (Fig. 2). Rinse Erlenmeyer twice with 40 ml CH_2Cl_2 and use this to wash Na_2SO_4 in flask, and pour on the filter paper.

Add small boiling chip to concentration flask. Attach 3-ball Snyder column on top and put flask in a deep water bath [a 4L beaker can be used] at 50-55°C. Concentrate to 6-8 ml, remove from water bath, rinse Snyder column with 2 ml CH_2Cl_2 and disconnect.

Quantitatively transfer concentrate to a micro concentration flask (Fig. 1). Rinse large flask twice with ≈ 2 ml CH_2Cl_2 and add the rinsings to the micro flask. Using one very small boiling chip and a small Snyder column concentrate extract to 1.0 ml (do not concentrate less than 0.8 ml at any stage). Raise flask from water bath and allow condensed liquid in the column to come down. If necessary, add a few drops of CH_2Cl_2 on top of Snyder column and let it drain down to flask. Finally make up volume to 1.0 ml. Do not use nitrogen stream to concentrate extract. Stopper flask and store at 4°C, and analyze as soon as possible.

. GLC-TEA ANALYSIS

Operate instrument according to the manual. Calibrate instrument with standard nitrosamine mixture. You should be able to detect 25 pg NDMA at an attenuation setting of 1. For the cold trap (TEA) use a mixture of liquid N_2 and n-propanol or liquid N_2 and n-pentane (flammable).

Analyze a 5-10 µl aliquot of extract (from step 7) using an attenuation setting of 1, 2 or 4 depending on the sensitivity desired and background noise. Inject varying amounts of nitrosamine standard mixtures that give peak heights close to that of the sample peak. Calculate results by comparison of peak heights.

GLC conditions: Column - see section 4
Carrier gas flow - Argon, 30-40 ml/min
Injector - 220°C
GLC furnace - 170°C or 180°C isothermal

9. DISCUSSION

(a) BETTER CLEAN-UP - Since temp. of distillation is low (45-50°C) there is less pyrolysis and practically no charring or browning - therefore, cleaner extract. No contamination with mineral oil either. May need only minor additional clean-up for GLC-MS confirmation.

(b) LESS CHANCE OF ARTEFACT FORMATION

Because - of lower temperature of distillation;
- reaction of nitrite and amines is minimum in 3N KOH;
- there is a chance of reaction of NO_2 and amines but it is checked by adding dibutylamine. Moreover, NO_2 + amine reactions are more rapid in mineral oil, especially at high temperature which is usually employed for mineral oil distillation;

...9

- occasional double-checking by the two distillation techniques (3N KOH & 1% sulfamic acid) - each with added dibutylamine virtually eliminates the possibility of artefact formation. During analysis always add dibutylamine after you have added 3N KOH or 1% sulfamic acid and mixed the sample.

(c) IMPROVED AND CONSISTENT RECOVERIES OF NDPA (EVEN FOR MALT)

- 80-90%; most often >90%.

(d) WORKS WELL WITH A VARIETY OF PRODUCTS, EVEN FOR DRY SAMPLES SUCH AS MALT, FISH MEAL

(Use smaller sample size if high levels of nitrosamines are present).

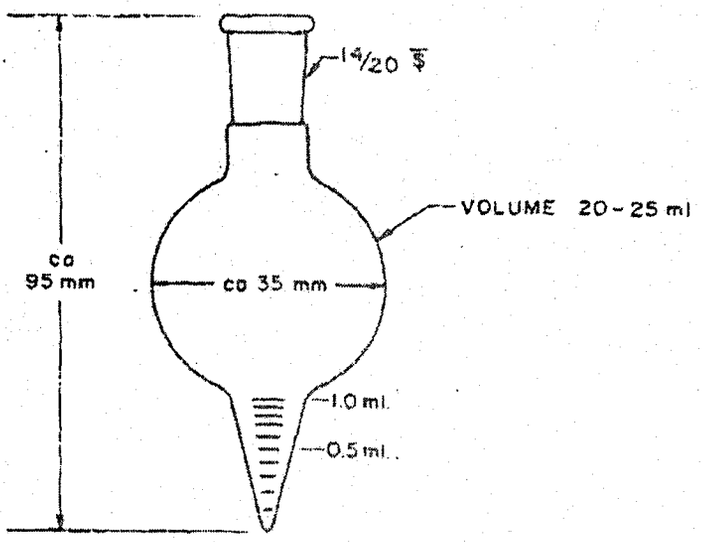
(e) CARRY OUT REAGENT BLANKS TAKEN THROUGH ALL THE STEPS

- Check all reagents including water, before use.
- For Na_2SO_4 - dissolve 50 g in water, basify, extract with CH_2Cl_2, dry CH_2Cl_2 extract, concentrate, and then analyze.
- Rinse flash evaporator with glass-distilled acetone and then with water after each use.

(f) If lower recoveries of NDPA are obtained check distillate volume, efficiency of rinsing the condenser.

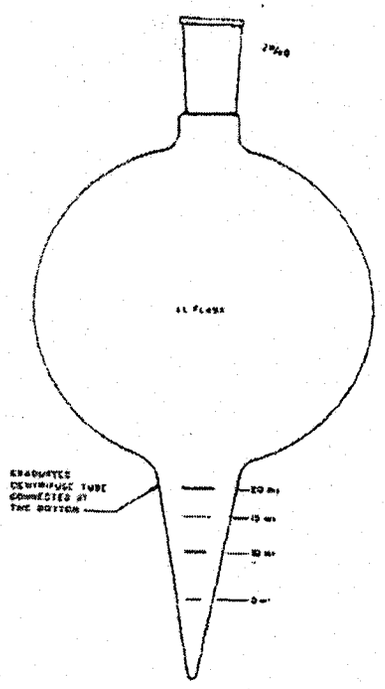
(g) The sulfamic acid method has not yet been tested extensively, and it gives lower recoveries for NDBA, NPYR, NPIP, NMOR, etc. Therefore, do not use the sulfamic acid method if the sample contains any of these nitrosamines.

FIG. 1



SEN

FIG. 2



APPENDIX J Methods to determine the concentration of metals in PEI ground water

P.E.I. Analytical Laboratories

Section: Water Chemistry Lab

Method Title: Metals in Water by ICAP

Section: Water Chemistry Lab

Prepared by: Date: November 12, 2002
QA/QC Officer

Last revised by: Date: March 10, 2003
QA/QC Officer

Reviewed by: Date: March 10, 2003
Laboratory Manager

QA approval by: Date: March 10, 2003
QA/QC Officer

Final Approval by: Date: March 10, 2003
Laboratory Manager

Based on the method validation data this method is fit for intended use.
The approval of this document is valid for 2 years at which time it is subject to review to determine if any updates or modifications are warranted.

Original Copy Holder: Quality Assurance/Quality Control Officer

Water Method
Metals in Water by ICAP

Reference

National Water Quality Data Bank (NAQUADAT) - Dictionary of Parameter Codes 1986 - Data Systems Section, Water Quality Branch, Environment Canada, Ottawa

NAQUADAT Codes	ICAP Spectrometer
48311	mg/L extractable Cadmium
20311	mg/L extractable Calcium
24311	mg/L extractable Chromium
29311	mg/L extractable Copper
26311	mg/L extractable Iron
82311	mg/L extractable Lead
12311	mg/L extractable Magnesium
25311	mg/L extractable Manganese
28311	mg/L extractable Nickel
N/A	mg/L extractable Ortho-Phosphate
19311	mg/L extractable Potassium
11311	mg/L extractable Sodium
16311	mg/L extractable Sulfate
30311	mg/L extractable Zinc

Scope

The IRIS Optical Emission Spectrometer is an inductively coupled argon plasma (ICAP) optical emission spectrometer (OES) which uses Echelle optics and a unique Charge Injection Device (CID) solid state detector to provide complete and continuous wavelength coverage over the typical analytical wavelength range. Typically, OES can analyze samples in the concentration range from low ppb to % levels. This method uses an ICAP unit combined with an ultrasonic nebulizer (USN) to concentrate the sample for trace metal analysis. Raw samples are acidified with nitric acid prior to analysis.

Principle

An inductively coupled plasma is an electrical discharge formed in a stream of argon gas flowing through a series of quartz tubes surrounded by a coil. During plasma ignition, the gas stream is seeded with electrons

from an external source, such as a small spark. These electrons are accelerated and collide with argon atoms to form more electrons and argon ions, which in turn are accelerated. This process continues until the gas becomes highly ionized (a plasma). Liquid samples are introduced onto the face of the piezoelectric transducer of the ultrasonic nebulizer where it is converted to a fine, dense aerosol. The nebulizer gas flow transports the wet aerosol through the heated U-tube where the solvent is vaporized.

Solvent vapors are then condensed by the thermo-electric cooler and removed by the drain pump. The sample output is a dry, analyte-laden aerosol which is introduced to the plasma. This sample is carried through the quartz tubes, passes through the plasma discharge, and the excited atoms emit light characteristic of their wavelengths. The IRIS CID detector then measures the intensity of this light emitted by the elements in a sample. The intensity is converted to concentration by comparing it with intensities emitted by known standard concentrations of the same elements.

Interferences:

Inter-element interference recorded in instrument Validation Log.

Equipment

IRIS Advantage/1000 Axial ICAP Spectrometer
U-5000AT+ Ultrasonic Nebulizer
Standard Computer System with specifications as listed in the IRIS Advantage Hardware Guide, Section 2, page 14
13*100 mm Polystyrene Round Bottom Test Tubes
x5, x10 dilutors

Safety

The waste sample liquid which drains from the spray chamber is collected in a polyethylene container. To avoid acid fumes forming, always place marble chips in the waste container to neutralize acidic waste.

The high voltage power supply generates the 4,000 volts required to amplify the RF signal and energize the work coil within which the plasma is created. The internal workings of this supply are potentially lethal.

UV radiation is produced by the plasma and during operation is screened by both the outer door to the sample compartment and by the inner, interlocked door which provides access to the torch and work coil. Removal of this inner

door will immediately cause the plasma to be extinguished. Under no circumstances should any attempt be made to defeat the interlocks.

During operation, the torch exhaust gases must be vented (this vent is a permanent installation above the unit) to prevent the introduction of poisonous corrosive materials into the laboratory air. Proper venting also reduces arc-over problems within the torch compartment.

Standards and reagents are acidified to a 1% HNO_3 solution.

Reagents

- High purity concentrated nitric acid (HNO_3)
- Use deionized water (>17 megohm) for all solutions.

Remember: Add acid to water.

1. 1% Nitric Acid (HNO_3)
   In a 1L volumetric flask, add about 500 mL of deionized water. Add 10 mL of concentrated high purity HNO_3. Fill to the 1L mark with deionized water and mix.

2. 5% Yttrium + 1% HNO_3
   In a 1L volumetric flask, add about 500 mL of deionized water. To that add 5 mL of 1000 ppm Yttrium and 10 mL of concentrated high purity HNO_3. Bring up to the 1L volume with deionized water and mix.

Standards, Reagents
Certified Stock Solutions
Concentrated High Purity Nitric Acid (HNO_3)

Prepararion of IRIS Standards Method : PEIWY

1. Pipette the specified mls of certified stock solution (or 100ppm (1000ppm for S) intermediate standard) for each analyte given in Table 2 into a 1000ml volumetric flask.

2. Add 10mls of concentrated HNO_3 to the 1000ml volumetric flask.

3. Bring the final volume up 1000ml using deionized water.

4. Mix to form a homogenous solution which is acidified to 1% HNO_3.

Table 1. Final concentrations (in ppm) of each analyte in each standard.

Analyte	Water 1	Water 2	Water 3	Water 4	Water 5
Ca	0	5	20	50	100
Mg	0	5	20	50	100
K	0	2.5	10	25	50
Cd	0	0.05	0.2	0.5	1
Cu	0	0.05	0.2	0.5	1
Mn	0	0.05	0.2	0.5	1
Pb	0	0.05	0.2	0.5	1
Zn	0	0.05	0.2	0.5	1
Fe	0	0.25	1	2.5	5
Na	0	5	20	50	100
Ni	0	0.05	0.2	0.5	1
P	0	0.25	1	2.5	5
Cr	0	0.05	0.2	0.5	1
SO_4	0	5	20	50	100

Table 2. Preparation of Water Standards using PEIWY Method.

Analyte	Cert.	#mls to prepare	# mls of Certified Stock to add to 1000ml volumetric flask

	Stock Conc.	100ppm sol'n (use 100 ml vol. flask)	PEIWY Water 1	PEIWY Water 2	PEIWY Water 3	PEIWY Water 4	PEIWY Water 5
Ca	10000	-	0	0.5	2	5	10
Mg	10000	-	0	0.5	2	5	10
K	10000	-	0	0.25	1	2.5	5
Cd	1000	10	0	0.5	2	5	10
Cu	1000	10	0	0.5	2	5	10
Mn	1000	10	0	0.5	2	5	10
Pb	1000	10	0	0.5	2	5	10
Zn	1000	10	0	0.5	2	5	10
Fe	1000	10	0	2.5	10	25	50
Na	10000	-	0	0.5	2	5	10
Ni	1000	10	0	0.5	2	5	10
P	10000	1	0	2.5	10	25	50
Cr	1000	10	0	0.5	2	5	10
SO4	10,000 (ppm S)	10 (1000ppm S)	0	1.7	6.7	16.7	33.3

Procedure for Analyzing Water

1. Make sure that the water samples have been acidified with high purity concentrated HNO3. Acidity to 1% HNO_3 (see procedure WCL-03P).

See Procedure WCL-22P for operation of the IRIS.

2. Sample Tray set-up
   CRM's (set of 5) trace metals; minerals; hardness; 27.2 nutrient simple
   Set of 20 samples
   1 duplicate (first sample in each set of 20)
   TM 27.2
   The sequence from samples onward is repeated until all samples have been analyzed.

3. Print a report.

Note:
Water samples with reported lead values greater than the method detection limit are reanayzed.

A dissolved iron is preformed on each water sample with a reported Iron value of 0.3 ppm or greater. For dissolved iron analysis the unacidified portion of the sample is filter through a 0.45um Membrane filter using a millipore filtering system.A plastic adapter is attached to the end of the filtering base and the IRIS sample tube is attached to collect the filtered sample.

Reported zinc values greater than 1.0 ppm will be diluted 1:10 and rerun.

4. Shut down the IRIS (See Procedure WCL-22P).

Determination of Hardness

Hardness is determined from the test values obtained from the IRIS.
Total Hardness is defined as the sum of the calcium, magnesium, iron, zinc and manganese concentrations expressed in mg/L.

The Watsis program automatically calculates the hardness value.

The following formula is used to calculate hardness:

(Reference - NAQUADAT No. 10606)

Hardness =
(([Ca]*2.497)+([Mg]*4.116)+([Fe]*1.792)+([Zn]*1.531)+([Mn]*1.822))

Quality Assurance
Quality Control is monitored by the use of Certified Reference Materials (CRM) and standard rechecks.
Guidelines are stated in standard operating procedure WCL-25P.
The values obtained must fall within 2 sd of the mean CRM mean value.
The standard rechecks must not be beyond $\pm$ 2 standard deviation of the mean, consecutively.
The duplicate sample value must be within $\pm$ 20% of the mean duplicate value as stated in standard operating procedure WCL-21P.
When values fall outside the acceptable ranges, trouble shooting must be performed according to equipment manual, room 114 and the analysis must be repeated.
The QC sample values are stored in a QC file.

Method Revision History:

Version 1 :05/30/2002
Original document.

Version 2.0 : 11/12/2002
Revisions made due to routine errors and omissions.

Version 2.1 : 03/10/2003
Revisions made to meet requirements of ISO/IEC 17025 Standard as indicated in external audit performed by SCC/CAEAL on 12/17/02.The revision includes authorization of the method and revisions, preparation of standards, Quality Assurance section and reference to standard operating procedures. Also addition of hardness calculation, rerun due to lead value and dissolved iron analysis.

The following procedures apply for this method.

WCL #	Standard Operating Procedure (SOP)
2	Reagent Receipt, Storage, Preparation and Use
3	Sample Collection, Handling, Receipt, Storage, Log In, Analysis, Reporting and Disposal
4	Orientation of Water Chemistry Lab Trainee
5	Storage of Files
6	Calibration
7	Safety
8	Security
9	Quality Control of Reagent Water
10	Recording and Verification of IRIS test results
12	Housekeeping of Water Chemistry Lab
13	Preparation of CRM
14	Instrument validation
15	Method validation

17	Estimation of Uncertainty Measurement
18	Receipt of Materials and Supplies
19	Chain of Custody
20	Proficiency Testing
21	Precision Testing
22	IRIS
24	Bottle Washing
25	Quality Control
30	Authorization of Test Methods and Procedures
35	Reporting of Non-Conformance by the Staff
WCL #	**Standard Operating Procedure (SOP)**
36	Water Sample Collection Pick-up
37	Cleaning of IRIS Torch