Fluoride intakes of 9-10-year-old children living in fluoridated and non-fluoridated areas of Dunedin and Timaru – A pilot study

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ABSTRACT

Background: Fluoride plays an important role in preventing dental caries, however ingesting too much can cause dental and skeletal fluorosis. Determining the impact of community water fluoridation (CWF) is important as changes in the regulation of CWF in New Zealand are in progress. Previous research has focused on the fluoride intakes of children <8 years, as they are at a higher risk of fluorosis resulting in a lack of data on older children. This pilot study aimed to measure fluoride intakes of 9-10-year-old children living in fluoridated and non-fluoridated regions, while evaluating the methods required to get an accurate estimate.

Objectives: To evaluate the use of diet records and duplicate diets to determine fluoride intake from dietary sources, including fluoridated water; to evaluate the collection method of expectorated toothpaste to determine fluoride intake from toothpaste ingestion, and to evaluate the feasibility of 24-hour urinary collections to determine urinary fluoride excretion.

Design: Fluoride intakes were estimated for 10 children from Dunedin (fluoridated water) and 10 from Timaru (non-fluoridated water). Data collection involved 24-hour duplicate diets, 24-hour diet records, 24-hour urine samples, estimation of toothpaste ingestion and toenail samples. Children completed either one or two days of data collection on weekend days or in the school holidays.

Results: The mean total daily fluoride intake was 1.66 ± 0.56mg/d and 1.20 ± 0.60mg/d in Dunedin and Timaru, respectively, below the adequate intake of 2.0mg/d. No participant exceeded the upper limit of 10mg/d. The mean fluoride intake from diet alone, was 0.71 ± 0.36mg/d in Dunedin and 0.21 ± 0.06mg/d in Timaru, with toothpaste contributing an additional 0.95 ± 0.41mg/d and 0.99 ± 0.52mg/d, respectively. Water was the most common source of dietary fluoride intake, contributing 75% and 38% of total dietary fluoride intake in Dunedin and
Timaru, followed by breads and cereals, at 15% and 35%, respectively. The daily urinary fluoride excretion was 0.378 ± 0.115mg/d in Dunedin and 0.249 ± 0.122mg/d in Timaru, which equates to 23% and 21% of total daily fluoride intake, respectively. Parents reported the methods to be acceptable, with the majority of children not changing eating habits during the study or changing drinking habits to avoid using the toilet. The results of the duplicate diets and toenails are not presented in this thesis.

**Conclusions:** Fluoride intake from toothpaste ingestion was the biggest contributor to total daily fluoride intake, providing approximately 50% of the adequate intake in both cities. The effect of CWF in Dunedin was evident, with Dunedin children consuming more fluoride from dietary sources, namely water. The children in both Dunedin and Timaru are at low risk of dental fluorosis, as the upper limit was not exceeded, and consequently may be at higher risk of dental caries. The methods used in this study are feasible and with refinement would be suitable to include in a larger scale study.

**Key Words:** fluoride, children, New Zealand, community water fluoridation
The FLuOride in School-children Study (FLOSS) was conducted by the Department of Human Nutrition, University of Otago. The research project was developed by the candidate’s supervisor, Associate Professor Sheila Skeaff, who was responsible for applying for funding, ethical approval and the study protocol.

The FLOSS project was undertaken by two candidates to meet the requirements for a Masters of Dietetics degree who undertook the work as a team. The aim of the project was to determine the fluoride intake of 9-10-year-old children, living in areas with fluoridated and non-fluoridated water, of Dunedin and Timaru.

As part of the team, the candidate was responsible for:

- Writing the ethics application including Information for Participants Form and Consent Forms.
- Contributing to study protocol.
- Recruitment and follow up of participants via phone and email.
- Preparation for data collection, assembling information and equipment packs, organising and ordering equipment.
- Corresponding and organising dates with participating families, meeting with participants to explain the study, and collecting samples.
- Obtaining and categorising unpublished fluoride values from the 2016 New Zealand Total Diet Study authors and preparing values for input into Kai-culator (University of Otago diet analysis software).
- Creating SurveyMonkey questionnaires: modifying the “Oral Health Questionnaire for Children,” developed by WHO and creating a FLOSS follow-up survey.
• Entering half of diet records into Kai-culator.

• Processing food and urine samples.

• Exporting, cleaning and coding data from SurveyMonkey and cleaning data from Kai-culator.

• Analysis of urine and toothpaste samples with help from Associate Professor Sheila Skeaff, Michelle Harper (Human Nutrition), and Pauline Bandeen (Chemistry).

• Statistical analysis using Excel.
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Associate Professor Sheila Skeaff for being an excellent supervisor. Your continuous support, feedback and ability to keep us calm throughout the process will always be highly appreciated.

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Liz Fleming, thank you very much for your time and patience in entering all the fluoride data into Kai-culator and handling all of the issues that came along with it.

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Participants and their families, thank you for inviting us into your homes and for the huge effort you put into this study. The support and interest you showed in our project is highly appreciated.

Mum and Dad, for all your support throughout the past six years of university. I would never have come this far without your help and generosity.

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<table>
<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BMR</td>
<td>Basal Metabolic Rate</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CNS02</td>
<td>Children’s National Survey 2002</td>
</tr>
<tr>
<td>CWF</td>
<td>Community Water Fluoridation</td>
</tr>
<tr>
<td>DUFE</td>
<td>Daily Urinary Fluoride Excretion</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>EI</td>
<td>Energy Intake</td>
</tr>
<tr>
<td>F-</td>
<td>Non-fluoridated</td>
</tr>
<tr>
<td>F+</td>
<td>Fluoridated</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>FLOSS</td>
<td>FLuOride in School-children Study</td>
</tr>
<tr>
<td>HF</td>
<td>Hydrogen fluoride</td>
</tr>
<tr>
<td>IQ</td>
<td>Intelligent Quotient</td>
</tr>
<tr>
<td>mg/d</td>
<td>Milligrams per day</td>
</tr>
<tr>
<td>mg/kg</td>
<td>Milligrams per kilogram</td>
</tr>
<tr>
<td>mg/kg/d</td>
<td>Milligrams per kilogram body weight per day</td>
</tr>
<tr>
<td>mg/L</td>
<td>Milligrams per litre</td>
</tr>
<tr>
<td>NNS97</td>
<td>National Nutrition Survey 1997</td>
</tr>
<tr>
<td>NRV</td>
<td>Nutrient Reference Value</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>NZTDS</td>
<td>New Zealand Total Diet Study</td>
</tr>
<tr>
<td>PAL</td>
<td>Physical Activity Level</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>TDFI</td>
<td>Total Daily Fluoride Intake</td>
</tr>
<tr>
<td>TISAB III</td>
<td>Total ionic strength adjustment buffer III solution</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>UL</td>
<td>Upper Level</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>

Note that ppm is equal to mg/kg
1 INTRODUCTION

Fluoride is a nutrient found naturally in water, soil, plants and animals (1) that is stored predominantly in teeth and bones (2). Optimum intakes of fluoride help to prevent and repair tooth decay by reforming and inhibiting demineralisation of the enamel (1, 3). In New Zealand, tooth decay is a widespread problem with almost 50% of children aged 5-11 years with dental caries in their primary teeth (4). Conversely, ingesting too much fluoride can lead to fluorosis, a condition of the teeth that causes white patches in minor cases, to enamel loss in severe cases (3). Finding the balance between consuming enough fluoride to prevent dental caries while avoiding fluorosis has been difficult to determine (5).

Many studies have assessed the relationships between fluoride intake, dental caries and fluorosis. Regardless of population or location, the results of these studies consistently find that in areas with community water fluoridation (CWF) there are fewer dental caries (6-11), but a higher prevalence of fluorosis (6, 10-13). A recent Australian review reported that fluoridated populations had a 26-44% reduction in dental caries prevalence and severity (11). No association was found between CWF and cancer, IQ, mortality or musculoskeletal effects (11).

The most common sources of fluoride intake are water, toothpaste and food (1). A large intake assessment study conducted in New Zealand found that the only group to exceed the adequate intake (AI) for fluoride were 6-12-month-old infants living in areas with CWF and using high fluoride toothpaste (1). Other New Zealand studies have also found infants to be at the highest risk of exceeding intakes, due to excessive use of high fluoride toothpaste when living in areas with fluoridated water (14) and exclusive formula feeding (15). This is a cause of concern because fluorosis occurs during the first 8 years of life when teeth are forming (5). However, in
New Zealand, the vast majority of cases have only mild fluorosis (4), which has no impact on tooth function and little impact on appearance (11). Due to the risk of fluorosis in children <8 years, the majority of studies have focused on this age group, leaving little data on fluoride intake in children above 8 years.

In New Zealand, the concentration of natural fluoride in water supplies is low at around 0.15mg/L (3). The Ministry of Health recommends a fluoride concentration of 0.7-1mg/L of drinking water (16). Currently, an estimated 52% of the New Zealand population reside in areas with fluoridated water supplies (1, 4) with an average fluoride concentration of 0.8-0.9mg/L (3). There are many people that oppose CWF due to a perceived risk to public health (5) and the lack of recent data on New Zealand fluoride intake does little to alleviate this controversy.

The primary aim of this thesis is to investigate the feasibility of assessing fluoride intake in 9-10-year-old children living in areas with fluoridated and non-fluoridated water, in Dunedin and Timaru, respectively.
2 LITERATURE REVIEW

2.1.1 Search strategy
Searches were made using Medline, PEN, Science Direct, CINAHL, AMED and Google Scholar using the terms listed in Table 2.1 individually and in different combinations. Repeat searches were done throughout the research process.

Table 2.1. Search terms

<table>
<thead>
<tr>
<th>Search terms</th>
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<tbody>
<tr>
<td>Fluoride intake</td>
</tr>
<tr>
<td>Water fluoridation</td>
</tr>
<tr>
<td>Children</td>
</tr>
<tr>
<td>New Zealand</td>
</tr>
<tr>
<td>Sources</td>
</tr>
<tr>
<td>Metabolism</td>
</tr>
<tr>
<td>Dental caries</td>
</tr>
<tr>
<td>Fluorosis</td>
</tr>
</tbody>
</table>

2.2 FLUORIDE BACKGROUND

2.2.1 Fluoride metabolism
Fluoride is ingested from multiple sources, including water, food and dental products (5). When fluoride enters the acidic environment of the stomach it is converted to hydrogen fluoride (HF) (17), where HF is passively absorbed (18). Approximately 20-25% of dietary fluoride is absorbed in the stomach, with the remainder absorbed in the proximal small intestine (18, 19). Roughly 80-90% of all ingested fluoride is absorbed by the body, however, absorption decreases in the presence of calcium, phosphorus, magnesium and aluminium (18, 19). On an empty stomach, almost 100% of fluoride from sodium fluoride tablets is bioavailable, which decreases to 70%
when taken with a glass of milk and further drops to 60% when taken with a calcium-rich breakfast (20). Fluoride absorption increases with increased dietary fat or protein intake, due to slower gastric emptying and increased gastric acidity, respectively (21). There is no difference in bioavailability when fluoride is ingested from natural fluoride versus fluoride compounds that are added to water in the water fluoridation process (5, 19).

Once absorbed, fluoride is rapidly circulated throughout the body. Ten minutes after ingestion, plasma fluoride has increased reaching its peak concentration in 20-60 minutes (18, 19). In children younger than seven years, around 55% of ingested fluoride is distributed to calcified tissues and approximately 1% into soft tissues, with the remaining fluoride excreted (18). Adults have fully developed bones and teeth, and therefore the calcified tissues only absorb around 40% of ingested fluoride (18). Of all the fluoride contained in the body, 99% is found in bones and teeth (2, 17, 19, 22).

The kidneys provide the main route of fluoride excretion (18, 19). Excretion is altered by the pH of urine, which can be affected by diet, drugs, physical activity, altitude and renal impairment, where an increased pH leads to decreased urinary fluoride excretion (19). A common alteration in excretion is seen in those consuming a vegetarian diet, which can result in a higher urinary pH and less fluoride excretion (19, 23). In both adults and children, approximately 10% of fluoride intake is excreted in faeces (19, 24).

2.2.2 Fluoride and dental health

2.2.2.1 Caries prevention

Dental caries are cavities formed in the teeth when enamel is dissolved (5, 25). Teeth are coated by bacteria-containing plaque, which produce acids when the bacteria metabolise sugars (25, 26).
These acids can dissolve the enamel of the tooth in a process called demineralisation (25). A high sugar intake increases acid production by the bacteria leading to increased decay (4). The process of decay can be stopped by decreasing plaque through brushing teeth, decreasing sugar intake and with fluoride (4).

Fluoride is widely known for its protective effect on dental health and is a factor in three different mechanisms of action on teeth (5, 27, 28). Firstly, fluoride can inhibit demineralisation when fluoride is incorporated into the structure of teeth, creating stronger enamel that is more resistant to breakdown from the bacterial acids (5, 27, 28). The enamel made in tooth development is carbonated hydroxyapatite. Demineralisation causes the loss of carbonate ions from hydroxyapatite, making the enamel vulnerable to attack by acids (25). When fluoride comes into contact with enamel from topical sources such as fluoridated water and toothpaste, it can replace the hydroxide ions in the hydroxyapatite to form fluorapatite, which is more resistant to breakdown from bacterial acids (25, 28). Secondly, remineralisation of enamel can occur when fluoride is present in saliva, helping to repair early caries (4, 5, 27). Fluoride enhances remineralisation due to its ability to attract calcium ions to the teeth, strengthening enamel (27, 28). Thirdly, fluoride can interfere with the metabolism of plaque bacteria, reducing the production of acids that dissolve enamel (4, 27, 28).

### 2.2.2.2 Fluorosis

Dental fluorosis, a manifestation of chronic fluoride toxicity, is caused by ingesting excess fluoride during tooth formation (29). Fluorosis only occurs when teeth are still growing, which is why the majority of studies investigate fluoride intake during the tooth developmental period between the ages of 0-8yrs (5, 29). When teeth are developing, excess fluoride can disrupt enamel-forming proteins resulting in hypomineralisation, known as fluorosis (5, 29). Mild
fluorosis is diagnosed as white flecks on the teeth, moderate fluorosis presents as opaque enamel with some discolouration and pitting, and in severe fluorosis the teeth have brown stains, pitting and enamel loss (29). The severity of fluorosis increases with increasing fluoride intake and time of exposure (29).

Skeletal fluorosis occurs with much higher intakes of fluoride over longer periods of time (>20 years) than dental fluorosis (5). This is prevalent only in countries with naturally high levels of fluoride in the water and soil, such as India, China, Tanzania and South Africa, and causes the bone diseases of osteomalacia, osteoporosis and osteosclerosis (5).

2.2.3 Sources of fluoride
Fluoride is an abundant element, present naturally in water, soil, animals and plants. The natural concentration of fluoride in water supplies varies widely around the world (20). Water is the most frequently encountered source of fluoride intake and because of its benefits for dental health, areas that have naturally low levels of fluoride in the water may add fluoride to the water, known as fluoridation (5).

A range of dental products have fluoride added to them, including toothpaste, varnishes, gels and mouth rinses (3). Topical mouth rinses for daily use usually contain 230-500mg fluoride/L and mouthwashes for use once or twice per week have 900-1000mg/L (20). After water, the most common source of fluoride intake is toothpaste, with the majority of toothpastes having a fluoride concentration of 1000mg/kg or ppm, as per recommendations from various health organisations such as the New Zealand Ministry of Health, Australian National Health and Medical Research Council and Public Health England (5). There is an increase in the availability of higher strength toothpastes, with fluoride concentrations of 1450mg/kg and lower strength toothpaste is also available at 400-500mg/kg, promoted for children 0-6 years (5). Fluoride tablets were a common
form of fluoride intake used in areas without water fluoridation from the 1950s (3). However, since the early 1990s, fluoride tablets are no longer used as a public health measure in New Zealand and Australia and are recommended on an individual basis only, due to the risk of fluorosis in young children and lack of compliance in the wider population (3).

The concentration of fluoride in food is low, with commonly eaten foods available in New Zealand and the United Kingdom (UK) ranging from 0.07-0.83mg F/kg (30, 31). While most foods are low in fluoride, the concentration of fluoride can increase significantly when some foods are cooked in fluoridated water, such as rice and pasta (20). Differing fluoride concentrations in soil and water between regions and processing methods also cause variation in the fluoride content of foods (20). Tea has relatively high concentrations of fluoride (5), with values ranging between 1.2-6.5mg/kg (20, 31-33). Tea plants accumulate fluoride from the soil and the fluoride concentration of tea further increases with brewing time (12). Seafood also has relatively high concentrations of fluoride (5), with values ranging from 0.18-4.57mg/kg (20, 30, 31, 33). This is because seawater has more fluoride than fresh water (i.e. 1.2-1.5mg/L in seawater, 0.01-0.3mg/L in fresh water) (14, 20), and the fluoride ingested from seafood increases when bones are eaten, as fluoride is stored in skeletal tissues (14, 17). The 2016 New Zealand Total Diet Study (NZTDS) reported a high average fluoride concentration in almonds, at 5.66mg/kg (31), whereas the concentration in pecans and peanuts have been reported at 0.1mg/kg and undetectable, respectively (14, 33).

Cressey et al. estimated fluoride intake in New Zealanders, using 24-hour recall data from the New Zealand Children’s Nutrition Study 2002 (CNS02), National Nutrition Survey 1997 (NNS97) and average food fluoride content from previous Total Diet Studies (1). The main contributors to fluoride intake in children aged 7-10 years living in areas with fluoridated water
were water, followed by bread, tea, carbonated beverages and fruit drink. For those children in areas with non-fluoridated water bread was the most important contributor, followed by water, carbonated beverages and tea (1). Although low in fluoride, bread is an important contributor as it is a staple food in children’s diets (1).

2.2.4 Nutrient Reference Values (NRVs)

An adequate intake (AI) and upper level (UL) for fluoride intake is given due to its role in preventing dental caries and prevention of fluorosis (5). The NRVs for fluoride are summarised in Table 2.2. The NRVs for children aged 0-8 years were recently updated by the New Zealand Ministry of Health and the Australian National Health and Medical Research Council (22). The 2006 NRVs for people over 9 years are still used by Australia and New Zealand and are also recommended by the US Institute of Medicine (5), the European Food Safety Authority (18, 34) and the United Kingdom Department of Health (35).

Table 2.2. Summary of adequate intakes (AIs) and upper levels (ULs) for New Zealand, by age group (5, 22).

<table>
<thead>
<tr>
<th>Age group</th>
<th>AI (mg/day)</th>
<th>AI (mg/kg/d)</th>
<th>UL (mg/day)</th>
<th>UL (mg/kg/d)</th>
</tr>
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<tbody>
<tr>
<td>Infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-6 months</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
<td>0.2</td>
</tr>
<tr>
<td>7-12 months</td>
<td>0.50</td>
<td>0.05</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Children &amp; adolescents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 years</td>
<td>0.6</td>
<td>0.05</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>4-8 years</td>
<td>1.1</td>
<td>0.05</td>
<td>4.4</td>
<td>0.2</td>
</tr>
<tr>
<td>9-13 years</td>
<td>2.0</td>
<td>0.05</td>
<td>10.0</td>
<td>0.1</td>
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<tr>
<td>14-18 years</td>
<td>3.0</td>
<td>0.05</td>
<td>10.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women 19+ years</td>
<td>3.0</td>
<td>0.05</td>
<td>10.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Men 19+ years</td>
<td>4.0</td>
<td>0.05</td>
<td>10.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>
An AI of 0.01mg/d for infants aged 0-6 months was originally determined from the average concentration of fluoride in breastmilk of mothers living in areas with fluoridated water, multiplied by the average breastmilk intake (5, 36). For all other age groups, an AI of 0.05mg/kg/day was chosen based on extensive data from children living in areas with fluoridated water offering the highest level of prevention of dental caries without adverse effects (18, 36). This estimate was then multiplied by the average weight of children and adults in specified age groups. The UL for children aged up to 8 years, at 0.10mg/kg body weight, was based on the prevention of moderate dental fluorosis (36, 37), whereas the UL for older children and adults, at 10mg/day, was set based on prevention of skeletal fluorosis. Because of the discrepancies seen between the percentage of those with moderate fluorosis and the percentage exceeding the UL, some of the NRVs for fluoride have been recently revised (37).

A recent review on NRVs in Australia and New Zealand was published in 2017, updating values for those aged 0-8 years, who are at the highest risk of fluorosis (22). The update removed the original AI for 0-6-month-olds, due to ambiguity between studies on the fluoride concentration that prevents both dental caries and fluorosis in this age group (22). The AI for 1-3 years changed from 0.7mg/d to 0.6mg/d, and for 4-8 years changed from 1.0mg/d to 1.1mg/d. Larger increases were made in the UL of 0-8 year olds, doubling from 0.10mg/kg/d to 0.20mg/kg/d (22). More recent bodyweight data were also used to update daily recommendations for each age group from 0-8 years, however, the NRVs for children aged 9-10 years were unchanged as children over 8 years were beyond the scope of the review (22).
2.3 GLOBAL FLUORIDE STATUS

As previously mentioned, the naturally occurring fluoride present in water and soil varies around the world, resulting in a wide variety of fluoride intakes globally (20). The World Health Organization (WHO) recommends CWF at 1.5 mg/L, which should be altered according to regional climate and dietary intake (17). Around 30 countries have implemented CWF in some areas reaching around 370 million people (5, 23, 38). Many European and some Asian, Caribbean and South American countries choose to fluoridate salt, due to the complex or unsuitable supply of water in these regions (5, 23, 39). The United Kingdom, Russia, Chile, Peru and Thailand have programmes providing fluoridated milk to children (23, 39). A further 50 million people live in countries with naturally occurring optimum levels of fluoride (0.5-1mg/L) (23), and on the other hand, areas of China, Africa and India have very high levels of fluoride in the water, causing widespread fluorosis (5).

Table 2.3 presents a summary of fluoride intakes around the world for children aged 1-12 years. Almost half (47%) of the groups, differentiated by water fluoridation concentration, met or exceeded the AI (12, 13, 40-48). Only 18% of the groups exceeded the UL, and more than half of these groups had water fluoride concentrations at least double the recommended maximum 1.5mg/L (12, 13, 17, 40, 47). The remaining groups not meeting the AI had water fluoride concentrations ranging between <0.05-1.5mg/L (41, 45-55).

Contradictory to many other studies, Zohouri et al. observed a higher total fluoride intake in areas with non-fluoridated water (45). However, this was due to fluoride intake from toothpaste; the fluoride intake from dietary sources was higher in areas with fluoridated water, highlighting the influence dental products can have on fluoride intake. Another study of interest is one by Rojas-Sanchez et al., who determined fluoride intake in two non-fluoridated regions (0.3mg/L)
and one fluoridated region (0.8-1.2mg/L) (44). One of the non-fluoridated regions had the same fluoride intake as the fluoridated region (0.965mg/d) and this was reported to be the result of the halo effect, as this particular non-fluoridated region was surrounded by regions with optimal water fluoridation. The halo effect is predictable due to the common consumption of imported foods and beverages, which may have been produced in areas with water fluoridation (42). Of the studies undertaken in Western countries, the fluoride intake of children over 8 years old has not been investigated.
Table 2.3.

*Summary of global fluoride intakes and assessment methods including children aged 1-12 years.*

<table>
<thead>
<tr>
<th>Author and Location</th>
<th>Age (years)</th>
<th>Methods</th>
<th>Fluoride water concentration (mg/L)</th>
<th>Average Fluoride Intake (mg/day)</th>
<th>Common sources of fluoride intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akpata et al., 2014 (49). Kuwait, Middle East</td>
<td>1-9</td>
<td>Estimated intake of 2016 children, based on samples of tap water and brands of beverages consumed. Fluoride excretion estimated in 400 children based on spot urine samples.</td>
<td>0.04</td>
<td>0.365</td>
<td>Water, bottled water, fruit juices and milk</td>
</tr>
<tr>
<td>Buzalaf et al., 2011 (48), Rodrigues et al., 2009 (39). Brazil and Peru</td>
<td>4-6</td>
<td>Estimated intake of 121 children, based on 24-hour duplicate diets, 24-hour urine samples, finger- and toenail samples and toothpaste ingestion.</td>
<td>0.6-0.8</td>
<td>1.43*</td>
<td>Water, fluoridated milk, fluoridated salt, toothpaste</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.6-0.9</td>
<td>1.85**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>180-200 - salt</td>
<td>1.94**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.25 – milk</td>
<td>1.94**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&quot;No fluoride&quot;</td>
<td>0.594</td>
<td></td>
</tr>
<tr>
<td>Craig et al., 2015 (40). Upper East Ghana</td>
<td>5-10</td>
<td>Estimated intake of seven children based on water samples from 57 wells and 4 water purification companies, house-to-house surveys and daily water consumption.</td>
<td>1.0</td>
<td>1.9*</td>
<td>Water, tea, canned mackerel</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0</td>
<td>3.7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.0</td>
<td>7.4**</td>
<td></td>
</tr>
<tr>
<td>Haftenberger et al., 2001 (50). Germany</td>
<td>3-6</td>
<td>Estimated intake of 11 children based on duplicate diets, diet records, toothpaste ingestion and 24-hour urine samples.</td>
<td>0.25 + salt and tablets</td>
<td>0.931</td>
<td>Water, fluoridated salt, beverages, toothpaste</td>
</tr>
<tr>
<td>Kimura et al., 2001 (51). Japan</td>
<td>1-6</td>
<td>Estimated intake of 29 children based on 3-days of duplicate diets.</td>
<td>&lt;0.05</td>
<td>0.28</td>
<td>Seaweed, fish, tea</td>
</tr>
<tr>
<td>Levy et al., 2003 (52). Iowa, USA</td>
<td>3-6</td>
<td>Estimated intake of 785 children based on questionnaires every 4-6 months (including a food frequency questionnaire, questions on toothpaste use and fluoride supplements) and samples of water or state health department public water data.</td>
<td>&lt;0.3-1.5</td>
<td>0.782</td>
<td>Water, beverages, toothpaste</td>
</tr>
<tr>
<td>Author and Location</td>
<td>Age (years)</td>
<td>Methods</td>
<td>Fluoride water concentration (mg/L)</td>
<td>Average Fluoride Intake (mg/day)*</td>
<td>Common sources of fluoride intake</td>
</tr>
<tr>
<td>---------------------</td>
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<td>----------------------------------</td>
</tr>
<tr>
<td>Maguire et al., 2007 (41) North East England</td>
<td>6-7</td>
<td>Estimated intake of 29 children based on 3-day food diaries, 24-hour urine samples, food and drink samples and toothpaste ingestion.</td>
<td>0.08</td>
<td>0.736</td>
<td>Toothpaste, water</td>
</tr>
<tr>
<td>Mardic et al., 2009 (13). Serbia</td>
<td>12</td>
<td>Estimated intake of 76 children based on water, soil, potato and bean samples and previous dietary intake data.</td>
<td>0.1 11.0</td>
<td>3.364* 15.482**</td>
<td>Water, potato, beans.</td>
</tr>
<tr>
<td>Nohno et al., 2006 (53). Japan</td>
<td>2-8</td>
<td>Estimated intake of 38 children based on 24-hour duplicate diets and diet records.</td>
<td>&lt;0.1 0.555</td>
<td>0.266 0.494</td>
<td>Rice, water, tea,</td>
</tr>
<tr>
<td>Omid et al., 2015 (54) North East England</td>
<td>4-6</td>
<td>Estimated intake of 61 children based on 3-day food diaries and 2-day duplicate diets.</td>
<td>0.9</td>
<td>0.558</td>
<td>Food and toothpaste</td>
</tr>
<tr>
<td>Omid et al., 2017 (42) North East England</td>
<td>4-6</td>
<td>Estimated intake of 61 children based on 2x 24-hour duplicate diets, 2x 24-hour diet records, toothpaste ingestion, 2x 24-hour urine samples, home and school tap water samples.</td>
<td>1.0</td>
<td>1.166*</td>
<td>Food and toothpaste</td>
</tr>
<tr>
<td>Paiva et al., 2003 (43) Brazil</td>
<td>1.6-3</td>
<td>Estimated intake of 71 children based on 2-day duplicate diets and toothpaste ingestion.</td>
<td>0.7</td>
<td>1.192*</td>
<td>Water, beverages prepared with water, toothpaste</td>
</tr>
<tr>
<td>Rojas-Sanchez et al., '999 (44). United States of America</td>
<td>1.3-3.3</td>
<td>Estimated intake of 54 children based on 2 or 3-day duplicate diets and toothpaste ingestion.</td>
<td>&lt;0.3 &lt;0.3 0.8-1.2</td>
<td>0.767* 0.965* 0.965*</td>
<td>Water, diet, toothpaste</td>
</tr>
<tr>
<td>Viswanathan et al., 2010 (12). South India</td>
<td>3-10</td>
<td>Estimated intake of children from 68 villages based on water, cow’s milk, solid and liquid food samples and household surveys determining the quantities of food and liquid consumed.</td>
<td>0.74 1.73 3.12</td>
<td>1.366* 3.796* 6.919**</td>
<td>Water, rice, tea</td>
</tr>
<tr>
<td>Author and Location</td>
<td>Age (years)</td>
<td>Methods</td>
<td>Fluoride water concentration (mg/L)</td>
<td>Average Fluoride Intake (mg/day)</td>
<td>Common sources of fluoride intake</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------</td>
<td>---------</td>
<td>-----------------------------------</td>
<td>---------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Zohouri et al., 2013 (46). North East England</td>
<td>6-7</td>
<td>Estimated intake of 33 children based on 24-hour duplicate diets, 24-hour diet record, toothpaste ingestion and 24-hour urine samples.</td>
<td>0.3</td>
<td>1.06</td>
<td>0.945</td>
</tr>
<tr>
<td>Zohouri et al., 2013 (45). Iran</td>
<td>2.5-3.5</td>
<td>Estimated intake of 29 children based on 2-day duplicate diets, toothpaste ingestion and samples of urine and faeces.</td>
<td>0.6-0.8</td>
<td>0.3</td>
<td>0.653</td>
</tr>
<tr>
<td>Zohouri et al., 2006 (55). North East England</td>
<td>6-7</td>
<td>Estimated intake of 33 children based on 3-day diet records and, samples of food and drink collected.</td>
<td>&lt;0.3</td>
<td>0.3-0.7</td>
<td>0.188</td>
</tr>
<tr>
<td>Zohouri et al., 2000 (47). Iran</td>
<td>4</td>
<td>Estimated intake of 103 children, based on 3-day diet records followed by private interviews by a nutritionist, and food and fluid samples.</td>
<td>0.3-0.35</td>
<td>0.6</td>
<td>0.413</td>
</tr>
</tbody>
</table>

* Results from studies given in mg/kg were converted to mg/d using either 1) the reported average weight of children in the study, or 2) average weights reported in the most recent update of bodyweight reference data (22)
* Meeting respective AI reported in the study
** Exceeding respective UL reported in the study
F- non-fluoridated
2.4 New Zealand’s Fluoride History

2.4.1 Community Water Fluoridation

The average natural fluoride concentration of water in New Zealand is low, at around 0.15mg/L (3). Because of its role in the prevention of dental caries, the New Zealand Ministry of Health recommends fluoridating water at 0.7-1mg/L (16). In 1954 New Zealand began fluoridating water supplies (5, 56) and currently 52% of the population live in areas with community water fluoridation at around 0.8-0.9mg/L (3-5). In New Zealand, the decision to fluoridate community water currently lies with the local government, however, it is proposed that in the next few years this authority will be shifted to District Health Boards (38). As the Ministry of Health governs District Health Boards and recommends water fluoridation, this shift in regulation may result in the extension of CWF in New Zealand, reaching up to 1.45 million more people (38).

It is well documented that areas with CWF experience lower rates of dental caries with benefits more noticeable in those with poorer dental health (4, 38). The percentage of caries-free children aged 5 years in areas with and without CWF are 59% and 55% for European children, 40% and 32% for Maori children, and 34% and 28% for Pacific children, respectively (4).

Despite the clear disparities between ethnicities, the differences in dental caries between areas with fluoridated and non-fluoridated water are not huge. One explanation for this is the halo effect, where those in regions with non-fluoridated water are consuming food and beverages produced in areas with fluoridated water (42). A comprehensive cost-effectiveness study found that the cost of CWF in New Zealand is lower than the cost of treating decay in communities with more than 1000 people (56). This cost-effectiveness is even higher in areas with a greater proportion of lower socio-economic status, Maori or children (56).
2.4.2 Fluoride intake in New Zealand

As seen in Table 2.4., most New Zealanders are not consuming enough fluoride to meet the AI. In 1990, Chowdhury et al. compared the fluoride intakes of 60 11-13-month-old infants living in Timaru, Oamaru (non-fluoridated) and Dunedin (fluoridated) using duplicate diets and samples of food, fluid, toothpaste and fluoride tablets (57). The average fluoride intake of the 11-13-month olds was below the AI, and only one child living in a fluoridated region and using fluoridated toothpaste exceeded the UL. In 1996, Guha-Chowdhury et al. conducted a similar study, using areas with fluoridated and non-fluoridated water of Otago to assess fluoride intake of 84 3-4-year-old children over a 12-month period (58). Three days of duplicate diets and diet records were used along with the assessment of fluoride consumed from brushing teeth, and again found the average intake of 0.49mg/d and 0.68mg/d to be below the former AI of 0.7-1.0mg/d, in areas with non-fluoridated and fluoridated water.

Existing data from the 1997 National Nutrition Survey (NNS97), 2002 National Children’s Nutrition Survey (CNS02) and the 2003-2004 New Zealand Total Diet Study (NZTDS) was used more recently to estimate fluoride intake in a wider age range from 6 months to 25+ years (1). New Zealanders aged 6 months and older had their fluoride intake estimated based on both actual dietary intake - using data from the CNS02 and NNS97, and simulated dietary intake - using data from the NZTDS (1). Dietary intake data were combined with average fluoride concentrations in food to get estimates of fluoride intake. Even in areas with optimal water fluoridation the estimated average dietary intakes did not meet the AI, except for 6-12-month-olds living in areas with CWF (1). None of the fluoride intakes exceeded the UL (1). The assessment of actual fluoride intake in New Zealand children has not been carried out in children over 6 years.
Although Cressey et al. estimated fluoride intake for 7-10-year olds, this was based on simulated diets, rather than actual intakes (1).

Table 2.4.

<table>
<thead>
<tr>
<th>Study population</th>
<th>NRVs (mg/d) (5)</th>
<th>Average fluoride intake (mg/d)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AI</td>
<td>UL</td>
<td>F+</td>
</tr>
<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-12-month-old infants(^a)</td>
<td>0.5</td>
<td>1.8</td>
<td>0.71(^*)</td>
</tr>
<tr>
<td>11-13-month-old infants(^a)</td>
<td>0.5-0.6</td>
<td>1.8-2.4</td>
<td>0.305</td>
</tr>
<tr>
<td>1-3-year-old toddlers(^a)</td>
<td>0.6</td>
<td>2.4</td>
<td>0.57</td>
</tr>
<tr>
<td>3-4-year-old children(^a)</td>
<td>0.6-1.1</td>
<td>2.4-4.4</td>
<td>0.68</td>
</tr>
<tr>
<td>5-6-year-old children(^a)</td>
<td>1.1</td>
<td>4.4</td>
<td>0.86</td>
</tr>
<tr>
<td>7-10-year-old children(^b)</td>
<td>1.1-2</td>
<td>4.4-10</td>
<td>0.99</td>
</tr>
<tr>
<td>11-14-year-old females(^a)</td>
<td>2-3</td>
<td>10</td>
<td>0.9</td>
</tr>
<tr>
<td>11-14-year-old males(^a)</td>
<td>2-3</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Adults</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-24-year-old males(^a)</td>
<td>4</td>
<td>10</td>
<td>1.37</td>
</tr>
<tr>
<td>25+ year-old males(^a)</td>
<td>4</td>
<td>10</td>
<td>2.1</td>
</tr>
<tr>
<td>25+ year-old females(^a)</td>
<td>3</td>
<td>10</td>
<td>2.07</td>
</tr>
</tbody>
</table>

\(^a\) Meeting respective AI  
\(^b\) based on actual dietary intake from CNS02 and NNS97  
\(^c\) based on simulated dietary intake from NZTDS  
F+ Fluoridated regions  
F- Non-fluoridated regions
2.4.3 Oral health in New Zealand

In New Zealand dental caries are one of the most common chronic diseases in all age groups (4, 5). The 2009 New Zealand Oral Health Survey is the most recent and comprehensive insight into our dental health, as routine oral health data is only collected at age 5 and 12-13 years (4). This survey reported that the dental health of New Zealand children has improved since the 1980s, however only two-thirds of children and adults were following the Ministry of Health guidelines of brushing teeth twice a day, and only 43% of children were doing this using fluoridated toothpaste (i.e. ≥1000mg/kg) (4). Despite free dental care offered to children up to 18 years of age, 50.7% of children and adolescents aged 2-17 years presented with caries in primary or permanent teeth (4). There are significant disparities with Māori, Pacific and those living with low socio-economic status. Children and adults in these groups are less likely to access oral health services, less likely to be meeting Ministry of Health tooth brushing guidelines and therefore, are more likely to have dental caries (4).

No cases of severe dental fluorosis have been reported in New Zealand as of 2009. A report by Food Standards Australia New Zealand using previous data from Australia and New Zealand found that the prevalence of very mild and mild fluorosis in children was 10-25% (37).

2.5 Assessment of Fluoride Status in Humans

Nutrition assessment enables us to estimate the nutrient intake of individuals and populations. The four components of nutritional assessment are anthropology,
biochemical, clinical and dietary assessment (59, 60). To measure fluoride intake, biochemical and dietary assessment can be used.

2.5.1 Dietary assessment

There are various methods to assess the dietary intake of fluoride. A common method for fluoride intake is the diet record. This method can estimate an individual’s fluoride intake, as well as determine the food source of the fluoride. Weighed diet records provide the most accurate estimate of food and nutrient intake, however, participants may alter or reduce dietary intake to lessen the burden (59). Because the fluoride content of most foods is low, there is a lack of data on the fluoride content of a wide range of foods in many national food composition tables, limiting the value of the diet record (18, 54). An approach to manage this lack of fluoride data is to ‘map’ the fluoride content of food items that have been analysed, onto similar foods that have not been analysed (1). This creates an estimate of fluoride content for a wider range of foods, however, reduces the accuracy of estimating fluoride intake from diet records.

Food frequency questionnaires (FFQ) have been used in studies with larger populations to assess fluoride intake (12). Because most foods are low in fluoride, only foods and fluids known to have high fluoride concentrations are included in these FFQs (8, 10, 12, 40). Questionnaires determining the consumption of fluoride in toothpaste and other supplements are also common, as these products can make a large contribution to the intake of fluoride (4, 8, 49). Additionally, there is an equation to determine the amount of fluoride ingested from brushing teeth (41, 58), which is often used in young children as they tend to swallow more toothpaste (61).
Duplicate diets are frequently used to assess fluoride intake, to make up for the lack of data on the fluoride content in foods. A duplicate diet involves collecting a duplicate of everything eaten and drunk in a specified time, which is then homogenised before analysis (54, 58). This can provide very accurate results when collected correctly, however it is associated with a time and cost burden for the participant (54). A big limitation of the duplicate diet is that you cannot determine the individual food sources of the fluoride, as all the food and drink is mixed together (54). To address this issue, it is common for diet records to be used alongside duplicate diets, both to determine the source and to act as validation, however, the burden of this can decrease participation (50, 54). To reduce participant burden, some studies collect water, food and beverage samples of products that were commonly consumed in the diet records, as an alternative to individual duplicate diets (40, 55).

2.5.2 Biochemical assessment

Urine is the most common biomarker when assessing fluoride intake, as it is a promising tool for estimating the level of recent fluoride intake at a population level (23, 24, 62). These estimates can be used to predict the risk of fluorosis, as standard fluoride excretion values associated with low, optimal and high fluoride intakes are available (63). Villa et al. conducted a meta-analysis with data from 212 children (0-7 years) and 269 adults and found a significant linear relationship between fluoride intake and urinary excretion in both children ($R^2 = 0.76$) and adults ($R^2 = 0.94$) (24). The same meta-analysis found that on average 35% and 54% of dietary fluoride was excreted in the urine of children and adults, respectively (24). The percentage of fluoride excreted in children <7 years ranged widely between the studies, between approximately 30-80% (24, 42). Twenty-four-hour
Urine samples show the most potential as a biomarker for fluoride intake (18), however, a strong correlation ($r = 0.76$) has been found between 24-hour samples and morning spot urine samples using the fluoride/creatinine ratio in children aged 12-36 months (64).

There is a linear relationship between the dose of fluoride and the concentration of fluoride in plasma (62). However, as urine is a much simpler and a less invasive method of collection, plasma fluoride is not commonly used in research. Plasma fluoride is also affected by various factors such as age and altitude, and there are no normal reference values (23, 62). While saliva is simple to collect, the variability in fluoride concentration between an individual’s different salivary glands means there are no reference values currently available (62).

Urine samples provide information on recent fluoride consumption, in contrast to nail clippings that provide a long-term index (65). Fingernail fluoride concentrations increase on average 3.4 months following an increase in fluoride intake, whereas toenail concentrations increase after an average of 4 months (66). Nail clippings approximately 1.5mm in length provide sufficient weight for analysis (66). Nail clippings are less burdensome than 24-hour urine samples and have been shown to correlate better with total fluoride intake than urinary excretion at the individual level in 4-6-year-olds (48). However, both correlations were weak, at $r = 0.28$ for urinary fluoride excretion and $r = 0.36$ for fluoride in toenails (48). Toenail samples are recommended, as fingernails are more likely to be exposed to environmental contamination (65, 66).

Clippings of hair are unsuitable as previous analysis has been conflicting. Furthermore, it may be unacceptable to participants as the hair needs to be cut close to the scalp (65). Of all the above methods, urine is the most researched and can be useful to
determine fluoride exposure in groups of people (62). Further investigation and method refinement is required before nail clippings can predict fluoride intake, however, they should continue to be used in research (23, 65).
3 OBJECTIVE STATEMENT

Fluoride intakes between regions with and without water fluoridation have been compared in many studies, both to determine the impact of community water fluoridation and to determine those at higher risk of developing dental caries or fluorosis (18). However, because dental fluorosis develops in children aged below 8 years, most studies focus their attention on younger children (5, 29). This is no data on the actual fluoride intakes of 9-10-year-old children in New Zealand. There are various methods to estimate fluoride intake, including diet records, duplicate diets, urine samples and nail samples (18). It is essential to evaluate the methods required to obtain an accurate estimate of chronic fluoride intake while minimising respondent burden.

The primary aim of this thesis was to investigate the feasibility of assessing fluoride intakes in 9-10-year-old children living in areas with fluoridated and non-fluoridated water, in Dunedin and Timaru, respectively.

The objectives of the current study are to:

1. Evaluate the use of diet records and duplicate diets to determine fluoride intake from dietary sources, including fluoridated water.
2. Evaluate the collection method of expectorated toothpaste to determine fluoride intake from toothpaste ingestion.
3. Evaluate the feasibility of 24-hour urinary collections to determine urinary fluoride excretion.
4 Participants and Methods

4.1 Outline of Study Design

This was a feasibility study that aimed to measure fluoride intakes in 9-10-year-old children. Ten children from Dunedin and 10 children from Timaru participated, to allow for comparison of fluoride intakes between regions with fluoridated water (Dunedin: 0.754mg/L) and non-fluoridated water (Timaru: 0.120mg/L), respectively (31). The University of Otago Health Ethics Committee approved the study (reference HE17/001) (Appendix A).

4.2 Participants and Recruitment

4.2.1 Recruitment of children and exclusion

Families were recruited through word of mouth. In Dunedin, this included information being emailed to University of Otago staff and posted on the “Dunedin Mums” Facebook page, and in Timaru five primary schools were emailed. Inclusion criteria were as follows: healthy, 9 to 10 years old, no identified food intolerances or allergies. Siblings were excluded. All interested families were given an information pack (Appendix B).

4.2.1.1 Deviations from protocol

Due to the time constraints, children with allergies and food intolerances were accepted. An amendment to ethics was sought and approved for this.
4.3 DATA COLLECTION

Data were collected on two days, approximately one week apart, from March-May 2017, to reduce daily variation. Four home visits took place between the researchers and participants. At the first meeting, the study was explained to the family and any questions were answered before consent forms were signed by a parent and the child. Families were provided with sample collection containers for food and urine, written instructions (Appendix C) and a diet record form (Appendix D). All methods were explained verbally with written instructions provided to each family. Researchers were available via phone and email to assist with data collection if required.

4.3.1 Anthropometric measurements

Children had their height and weight recorded at the first meeting. Weight was measured on Seca scale (Alpha Model 770, Hamburg, Germany), with the child standing upright and looking straight ahead. Shoes and heavy clothing were removed and weight was recorded to the nearest 100g. Height in centimetres was recorded using a tape measure with children standing against a wall. BMI (kg/m²) was calculated for each child.

4.3.2 Diet records

A 1-day weighed diet record was completed on each data collection day. Parents and children were asked to weigh food using an electronic kitchen scale (Salter Vista Model 3010) whenever possible and alternatively provide values in cups, millilitres or spoons. Recipes cooked for the family were written in a separate section of the diet record booklet, and parents noted the weight of the recipe eaten in the main section. It was requested that the diet record booklet be nearby at all times, particularly if the child ate away from home.
4.3.2.1 **Kai-culator**

Diet records were entered into Kai-culator (v1.15k), a nutrient analysis programme developed by the University of Otago (67). Plant and Food Research Food Files (2010) and nutrient data from the 2008/09 Adult Nutrition Survey were used to analyse the energy, protein and calcium content of the diets. The fluoride concentration of 75 foods from the 2016 New Zealand Total Diet Study (NZTDS) was entered into Kai-culator (31). The 75 foods analysed included a range of fruit and vegetables, breads and cereals, and beverages including water, soft drink, fruit juice, tea and alcohol. Beans, dried fruit, almonds, coconut cream, soya milk, tofu, soup, tomato sauce, plain biscuits and cracker biscuits were also included. No meat or dairy products were included in the database as they were not analysed for the NZTDS because the fat interferes with fluoride determination (A. Pearson, Ministry for Primary Industries, personal communication). Because the NZTDS used distilled water to prepare foods, foods that absorb water such as rice and pasta had the respective fluoridated or non-fluoridated water added separately as a recipe in Kai-culator (31). These recipes and all further assumptions made when entering diets into Kai-culator are presented in Appendix E.

As only 75 foods were analysed for fluoride in the NZTDS, and the majority of foods in Kai-culator had no fluoride values. To increase the number of foods with fluoride data, a mapping process similar to that used by Cressey et al. was utilised (1). This involves mapping the fluoride value of foods that were analysed in the NZTDS, onto similar foods with no fluoride data. For example, ‘apple’ in the NZTDS was mapped onto all fresh apples, and ‘white bread’ was mapped onto all white bread, buns and rolls, in Kai-culator. Foods with no fluoride data were assumed to have a concentration of 0.0001mg/kg.
Energy intakes were compared with estimated energy requirements to identify potential underreporting (60). The basal metabolic rate (BMR) for each participating child was calculated using Schofield equations (60), using gender, height and weight. Because physical activity level (PAL) was not assessed, a minimum PAL of 1.55 was used, as suggested by WHO (68). Under-reporters or under-eaters were identified using the Goldberg underreporting cut-offs, set at <90% of the ratio of EI:BMR, calculated by dividing energy intake (EI) by estimated BMR (69).

4.3.3 Duplicate diets

On data collection days, all food and drink consumed by the child was duplicated by the parents through observing and replicating the actual amount consumed. Parts of food not consumed, such as bones, fruit skins or leftovers were not included in the duplicate diet container. Duplicate diets were homogenised in a Waring commercial blender (Conair Corporation, Connecticut, United States) until smooth. In Dunedin, the blender was rinsed three times with deionised water between samples, to remove any fluoride contamination from rinsing water. Aliquots at 50g and 200g were frozen at -20 °C and the remainder of the diet was discarded; results of the duplicate diet are not included in this thesis.

4.3.4 24-hour urine collection

Children were provided with a 2L bottle, a choice of two collection bowls and a funnel for the 24-hour urine collection. On the days of data collection, the first void of the day was discarded and the time noted. Following this, all urine for that day was collected, including the first void on the next day. Once collected, urine samples were weighed and two aliquots of approximately 10 ml were frozen at -20°C until analysis with remaining
urine discarded. The volume of each child’s 24-hour urine sample was checked against the lower limit of >9ml/hr for completeness, suggested by WHO (70).

4.3.5 Toothpaste
Using their own toothpaste and toothbrush, children brushed their teeth as usual and a calculation was used to estimate toothpaste ingestion. The fluoride concentration of the toothpaste and all branding was recorded. The toothbrush was weighed before and after toothpaste was dispensed, on Sartorius scales (Model 1475 MP8-2, Germany). All expectorated toothpaste and saliva was collected during tooth brushing along with any water used to rinse the mouth. Samples were mixed and frozen at -20°C until analysis. Ingested fluoride was calculated by subtracting the recovered amount from the original amount used and then multiplied by the amount of times per day the child brushed their teeth.

4.3.6 Toenails
It was requested that the children refrain from trimming their toenails or wearing nail polish for at least two weeks prior to data collection. Either at the first meeting or in their own time during data collection the child - with or without help from their parent - trimmed their toenails, which were collected and frozen at -20°C. Due to the small size of children’s toenails, clippings were taken from all toenails to ensure an adequate weight for analysis; results of the toenail clippings are not included in this thesis.

4.3.7 Questionnaires
The “Oral Health Questionnaire for Children,” developed by WHO was modified (Appendix F) and completed by children at the first meeting (71). Alterations to the questionnaire included removal of questions not relevant to the current study and aims,
and rewording of long questions. The questionnaire was completed on an iPad via
SurveyMonkey and parents assisted where necessary. A follow-up survey (Appendix G)
was sent out to parents via email after the study’s completion, for feedback on the
methods.

4.3.8 Follow-up meetings

The second meeting took place the morning following data collection. At this short
visit, the diet record was checked by researchers for missing information, participants
were asked for feedback on the methods, and the completed samples were collected. A
third home visit was then scheduled, to provide new collection containers, a new diet
record form and to briefly remind participants of each process. The final visit was similar
to the second visit, and parents were given a thank you card with a $30 grocery voucher
and children provided with two movie vouchers as a token of appreciation.

To simplify the process, collection days only occurred on weekends in Dunedin and
during the school holidays in Timaru, removing the need to coordinate with schools.
Again, due to time constraints and public holidays, 9 children only completed one day of
data collection.

4.4 BIOCHEMICAL ANALYSIS

All analyses were completed in the Department of Chemistry, University of Otago.

4.4.1 Analysis of urine samples

A standard curve was established on a log scale, from 0.1-25µg fluoride/mL, shown in
Appendix H. Standards were made up using a fluoride standard (Sigma-Aldrich
1000mg/L, Missouri, United States) and distilled, deionised water, according to CDC
methodology (72). Five mL of this solution was mixed with 5mL of total ionic strength adjustment buffer (TISAB III) (Sigma-Aldrich, Missouri, United States) (73).

Urine samples were analysed using a fluoride-ion selective electrode (EDT directION Fluoride Flow Plus ISE, Dover, United Kingdom) coupled to a potentiometer (Eutech Instruments pH 2700, Thermo Fisher Scientific, Massachusetts, United States). Samples were thawed and vortexed, with 5mL of urine added to 5mL of TISAB III buffer solution and analysed (72). Millivolt readings were taken from the potentiometer, and the standard curve was used to determine fluoride content.

4.4.2 Analysis of expectorated toothpaste

A standard curve was established on a log scale, from 0.001-100µg fluoride/mL, shown in Appendix I. Standards were made up using a fluoride standard (Sigma-Aldrich 1000mg/L, Missouri, United States) and distilled, deionised water, according to CDC methodology (72).

Toothpaste samples were also analysed using the fluoride-ion selective electrode coupled to a potentiometer. Samples were thawed, mixed and brought up to 100mL using distilled, deionised water. This diluted sample was mixed with distilled, deionised water in a 1:4 ratio before analysis. Millivolt readings were taken from the potentiometer, and the standard curve was used to determine fluoride content.

4.5 Statistical analysis

Microsoft Excel for Mac (Version 15.34) was used to calculate means and standard deviations. When a child had completed both days of data collection, the average of both
days was taken and used in mean and standard deviation calculations. To calculate intakes per kilogram of body weight, values were divided by each child’s body weight.
5 Results

5.1 Participant Characteristics

Twenty children participated in the study, 10 from Timaru and 10 from Dunedin, with similar demographics (Table 5.1). The average age of participants was 9.5 and 9.6 years for Dunedin and Timaru, respectively. More boys (70%) participated in the study than girls. The ethnicity distribution was also similar between cities, with 90% NZ European, 10% Māori and 5% Asian overall, comparable to the distribution seen in the 2013 NZ Census, with 74% NZ European, 15% Maori and 12% Asian (74). While the average height was identical in both Timaru and Dunedin, the weight was heavier in Dunedin resulting in a higher BMI. The BMI for Dunedin was 18.4 kg/m², close to the 85th percentile observed on the WHO BMI-for-age boys and girls charts (60). In Timaru, the BMI was 16.6 kg/m², close to the 50th percentile on the same charts. The average NZDep of the participant’s households was similar in both cities at 4.4 – falling between NZDep 1 (i.e. least deprived) and NZDep 10 (i.e. most deprived) (75). A quarter (25%) of children reported taking a vitamins or mineral supplement, and 15% reported food allergies or intolerances.
Table 5.1. *Summary of socio-demographic characteristics of participating children*

<table>
<thead>
<tr>
<th></th>
<th>Dunedin</th>
<th>Timaru</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of participants</strong></td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>9.5</td>
<td>9.6</td>
<td>9.55</td>
</tr>
<tr>
<td><strong>Gender (n)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td><strong>Ethnicity (n)</strong> a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ Euro</td>
<td>8</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Māori</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pacific</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>37.03</td>
<td>33.39</td>
<td>35.21</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>18.4</td>
<td>16.6</td>
<td>17.5</td>
</tr>
<tr>
<td><strong>NZ Dep Index</strong> b</td>
<td>4.6</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Taking vitamins/minerals (n)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasionally</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Regularly</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>Food allergies or intolerances (n)</strong></td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*Ethnicities are prioritised as per New Zealand Ministry of Health (76). Participants could nominate more than one ethnicity*

*As determined by Statistics New Zealand 2013 (75)*

### 5.2 Estimated Dietary Fluoride Intake from Diet Records

The mean energy, protein, calcium and fluoride intakes are shown in Table 5.2. The average energy intake in Dunedin was 7481kJ and in Timaru was 7074kJ. Only one child (5%) under-reported on the first day of data collection, but not on the second day, thus their data were included with the other participants. Protein intakes were above the estimated average requirement (EAR) for 9-10-year-old girls and boys, with slight variation between data collection days. The average calcium intake in Dunedin of
865mg/d met the EAR of 800mg/d. The average intake in Timaru was 704mg/d, below the EAR.

Fluoride intakes derived from the diet record (i.e. do not include toothpaste) did not meet the AI of 2.0mg/d at any time. Average intakes in Dunedin were higher than in Timaru, at 0.71mg/d in Dunedin and 0.21mg/d in Timaru. The range of fluoride intakes was 0.14-1.19mg/d in Dunedin and 0.15-0.33mg/d in Timaru. In Dunedin, children were consuming 0.095mg fluoride per 1000kJ, and in Timaru 0.030mg fluoride per 1000kJ.

Table 5.2. Mean ± standard deviation of 20 participant’s energy, protein, calcium and fluoride intakes from diet records

<table>
<thead>
<tr>
<th></th>
<th>Dunedin (n=10)</th>
<th>Timaru (n=10)</th>
<th>Girls</th>
<th>Boys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/d)</td>
<td>7481 ± 1136</td>
<td>7074 ± 1646</td>
<td>6475-8870b</td>
<td>7812-10480b</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>69 ± 25</td>
<td>63 ± 16</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>865 ± 399</td>
<td>704 ± 289</td>
<td>800c</td>
<td>800c</td>
</tr>
<tr>
<td>Fluoride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/d</td>
<td>0.71 ± 0.36</td>
<td>0.21 ± 0.06</td>
<td>2.0d</td>
<td>2.0d</td>
</tr>
<tr>
<td>mg/kg/d</td>
<td>0.018 ± 0.008</td>
<td>0.006 ± 0.001</td>
<td>0.05d</td>
<td>0.05d</td>
</tr>
</tbody>
</table>

* Nutrient Reference Values for New Zealand and Australia (36)
* Estimated energy requirements calculated using height, weight and gender of participants with Schofield equations, a PAL of 1.55 and rounded
* EAR
* AI

### 5.3 Food Groups Contributing to Fluoride Intake

Figure 5.1. shows the percentage contribution of food groups towards fluoride intake in Dunedin and Timaru. Water, followed by breads and cereals were the top contributors in both areas with fluoridated and non-fluoridated water. Children in Dunedin were getting almost 75% of their dietary fluoride intake from fluoridated water, compared to less than 40% in Timaru. This affects the contribution to dietary fluoride from breads and cereals,
which was 15% in Dunedin and 35% in Timaru. Milk and dairy products contributed very low fluoride intake in Dunedin (<1%) but was 12% in Timaru.
Figure 5.1: Percentage contribution of main food groups to fluoride intake in Dunedin (fluoridated) and Timaru (non-fluoridated)
5.4 Estimated fluoride intake from toothpaste

Table 5.3 shows the fluoride present in the toothpaste used each day, the fluoride measured in the expectorated saliva and the estimated fluoride ingestion. On average, the amount of fluoride in the toothpaste used per day by children in Dunedin was slightly higher than in Timaru, at 1.58mg/d and 1.21mg/d respectively. However, Dunedin children on average, used a lower strength of toothpaste, at 1045mg/kg, compared to 1170mg/kg in Timaru. The mean frequency of toothbrushing was similar in both cities, at 1.7 and 1.8 times per day in Dunedin and Timaru respectively, ranging from once a day to three times a day. On average, Dunedin children expectorated more fluoride following tooth-brushing than Timaru children, at 0.43mg/d and 0.23mg/d. However, there were four children in Timaru who swallowed the toothpaste, rather than expectorating, and no children in Dunedin who swallowed toothpaste. The estimated fluoride ingestion from toothpaste was similar between cities, at 0.95mg/d in Dunedin and 0.99mg/d in Timaru. This equates to the children ingesting 60% and 82% of the fluoride in toothpaste in Dunedin and Timaru, respectively.

Table 5.3. Mean ± standard deviation of fluoride content in toothpaste used, fluoride content of expectorated saliva and estimated fluoride ingestion from toothpaste

<table>
<thead>
<tr>
<th></th>
<th>Dunedin (n=10)</th>
<th>Timaru (n=9 a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride in toothpaste used (mg/d)</td>
<td>1.58 ± 0.60</td>
<td>1.21 ± 0.61</td>
</tr>
<tr>
<td>Fluoride in expectorated saliva (mg/d)</td>
<td>0.43 ± 0.24</td>
<td>0.23 ± 0.26</td>
</tr>
<tr>
<td>Estimated fluoride ingestion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/d</td>
<td>0.95 ± 0.41</td>
<td>0.99 ± 0.52</td>
</tr>
<tr>
<td>mg/kg/d</td>
<td>0.038 ± 0.015</td>
<td>0.030 ± 0.013</td>
</tr>
</tbody>
</table>

*One outlier with estimated fluoride ingestion from toothpaste of 8.33mg/d is excluded from these means. The average fluoride ingestion from toothpaste in Timaru including this participant is 1.70mg/d.
### 5.5 Total Fluoride Intake

In Dunedin, 36% of the AI for fluoride was achieved through diet alone, and in Timaru only 11% of the AI was achieved, shown in Table 5.4. Fluoride intake from toothpaste contributed 48% and 50% of the AI in Dunedin and Timaru respectively. When dietary fluoride intake is combined with fluoride intake from toothpaste, Dunedin children were consuming an average of 1.66mg/d and 1.20mg/d in Timaru, neither meeting the AI of 2.0mg/d. However, when the AI is expressed per kg body weight, Dunedin children were meeting the AI of 0.05mg/kg/d. No children exceeded the UL of 10mg/d.

#### Table 5.4. Daily fluoride intake from diet, toothpaste and combined fluoride intakes of 20 children, with comparison to AI

<table>
<thead>
<tr>
<th>Mean ± SD Daily Fluoride Intake</th>
<th>Diet</th>
<th>Toothpaste</th>
<th>Diet + Toothpaste</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dunedin (n=10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/d</td>
<td>0.71 ± 0.36</td>
<td>0.95 ± 0.41</td>
<td>1.66 ± 0.56</td>
<td>2.0</td>
</tr>
<tr>
<td>mg/kg/d</td>
<td>0.018 ± 0.008</td>
<td>0.038 ± 0.015</td>
<td>0.056 ± 0.015</td>
<td>0.05</td>
</tr>
<tr>
<td>% of AI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36</td>
<td>48</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td><strong>Timaru (n=10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/d</td>
<td>0.21 ± 0.06</td>
<td>0.99 ± 0.52</td>
<td>1.20 ± 0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0</td>
</tr>
<tr>
<td>mg/kg/d</td>
<td>0.006 ± 0.001</td>
<td>0.030 ± 0.013</td>
<td>0.032 ± 0.018&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td>% of AI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>50</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Percentages calculated using mg/d
<sup>b</sup>One outlier with a daily F intake from toothpaste of 8.33mg/d is excluded from these means

### 5.6 Fluoride Excretion

The average urine volume of children in Dunedin and Timaru was 790mL/d and 908mL/d, presented in Table 5.5. One child was excluded from these calculations, as the total urine volume was below the 9mL/hr cut-off, suggested by WHO (70). The daily urinary fluoride excretion (DUF E) was higher in Dunedin children, at 0.378mg/d, compared to 0.249mg/d in Timaru. The
average fractional urinary fluoride excretion was similar in Dunedin and Timaru at 23% and 21% respectively. Figure 5.2 presents the relationship between DUFE and total daily fluoride intake (TDFI), with $R^2$ values of 0.112 and 0.357 for Dunedin and Timaru, respectively.

### Table 5.5. Mean ± standard deviation of average daily urine volume, total daily fluoride intake (TDFI), daily urinary fluoride excretion (DUFE) and fractional urinary fluoride excretion (FUFE) of 19 children

<table>
<thead>
<tr>
<th></th>
<th>Dunedin (n=10)</th>
<th>Timaru a (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average urine volume (mL)</td>
<td>790 ± 244</td>
<td>908 ± 430</td>
</tr>
<tr>
<td>DUFE mg/d</td>
<td>0.378 ± 0.155</td>
<td>0.249 ± 0.122</td>
</tr>
<tr>
<td>DUFE mg/kg/d</td>
<td>0.010 ± 0.003</td>
<td>0.008 ± 0.004</td>
</tr>
<tr>
<td>FUFE (%) b</td>
<td>23</td>
<td>21</td>
</tr>
</tbody>
</table>

- One outlier was excluded due to an incomplete urine volume
- $FUFE = (DUFE/TDFI) \times 100$

**Figure 5.2.** Relationship between DUFE and TDFI for 19 participants. The dashed lines are the linear trend line, with $R^2$ values corresponding to the trend lines.
5.7 Feedback on Study Methods

The parents of 15 children responded to the follow-up survey. When asked what was the easiest part of the study, 10 parents chose urine collection, 2 chose duplicate diet, 1 chose diet record and no one chose “none of the above”. One parent said all 3 methods were easy, and another said both the urine collection and diet record were the easiest. Six parents said the most difficult part of the study was the duplicate diet, 4 chose diet record, no one chose urine collection and 5 chose “none of the above”. All 15 parents reported that their child did not drink less to avoid going to the toilet. One parent reported making meals that were easier and another reported making meals that were cheaper, with the remaining 13 parents reporting they did not change how they ate on collection days. Additional feedback included that it was good to run the study during the holidays and weekends as the parents were not rushed with work and school, so could better supervise their children. Many parents and some children felt wasteful having to provide food that would not be eaten. Overall, the majority of parents reported that the burden of participating was not as great as it initially appeared after the first meeting.
Fluoride plays an important role in preventing dental caries, one of the most common chronic diseases in New Zealand (5). Community water fluoridation (CWF) is a public health measure to increase fluoride intakes in areas where natural fluoride levels in the water are low (5). It is important to determine the impact of community water fluoridation, especially as changes in the regulation of CWF in New Zealand are expected in the near future (38). This study was the first to measure actual fluoride intake in 9-10-year-old children, living in areas of New Zealand with fluoridated and non-fluoridated water.

6.1 Fluoride Intake of 9-10-Year Olds in Dunedin and Timaru

This study found that 9-10-year-old children living in Dunedin and Timaru were not meeting the AI for fluoride of 2.0mg/d (36). The children in the non-fluoridated region of Timaru were consuming an average of 1.20mg/d (60% of AI), while the children in the fluoridated region of Dunedin were consuming an average of 1.66mg/d (83% of AI). However, when expressing the AI per kg body weight, Dunedin children were meeting the AI at 0.056mg/kg/d. This is due to the average weight of Dunedin children (37.03kg) being lower than the reference weight (40kg) used to calculate the AI per kg body weight (5). The fluoride intakes are higher than Cressey et al.’s estimated fluoride intakes of 7-10-year olds using simulated diets (1). They reported total fluoride intakes of 1.29mg/d and 0.75mg/d in areas with fluoridated and non-fluoridated water, respectively (1). Fluoride intakes of 6-7-year-olds in England with water fluoridation concentrations similar to those in Timaru, had a lower fluoride intake (0.736mg/d) than the present study (1.20mg/d) (41). Conversely, the current findings are slightly lower than previous studies undertaken in Brazil with water fluoride concentrations similar to that in Dunedin (0.754mg/kg). The 4-6-year-olds had fluoride intakes from diet and toothpaste at 0.065mg/kg/d (48), and the 1-3-
year-olds at 0.089mg/kg/d (43), both meeting the respective AIs. These previous findings, along with the present data, is consistent with findings from the Iowa Fluoride Study, that found that as children grow, the less likely they are to be meeting the AI in terms of mg fluoride per kg body weight (52).

Diet alone contributed 36% of the AI in Dunedin and only 11% in Timaru. Cressey et al. estimated fluoride intake in 7-10-year-olds in New Zealand, and reported that approximately 50% of the AI was achieved by dietary intake in areas with fluoridated water, and 23% was achieved in areas with non-fluoridated water (1). Other studies in Brazil, Germany and the UK also reported a higher percentage of the AI achieved by dietary intake in both areas with fluoridated and non-fluoridated water (41, 42, 46, 48, 50).

The dietary intake of fluoride in this study is likely to be an underestimate, as NZ data used to determine dietary fluoride intake was not complete. Only 75 foods were analysed as part of the NZTDS, and no meat or dairy products were included (31). Using the database of food and beverages in the UK (77), which include a wider range of foods, the contribution of additional fluoride from meat and dairy products could be estimated. The most common meat and dairy products consumed by the participants were sausages, milk and cheese. Adding these foods to the intakes determined in this study, total fluoride intake from dietary sources would change from 0.71mg/d to 0.72mg/d in Dunedin, and from 0.21mg/d to 0.22mg/d in Timaru, which makes little impact on the overall fluoride intake.

The most important source of fluoride intake in Dunedin was water (75%), followed by breads and cereals (15%), with the remaining food groups contributing less than 4%. These findings are similar to that of Cressey et al., where they found water, followed by bread, to be the most important contributors in 7-10-year-olds living in areas with fluoridated water (1). In Timaru water was still the most important contributor to fluoride intake (38%), followed by breads and cereals (35%), milk and milk products (12%) and fruit (6%). A slightly different contribution was found
by Cressey et al. in areas with non-fluoridated water, with bread the most important (15%) followed by water (9%).

Ingestion of fluoride from toothpaste made up around half of the AI, at 0.95mg/d and 0.99mg/d in Dunedin and Timaru, respectively. These findings are considerably higher than the assumption of 0.3mg/d made by Cressey et al. (1). Studies using similar methods of collecting expectorated toothpaste report between 25-113% of the AI achieved for fluoride (41, 42, 46, 48, 50). The estimated toothpaste ingestion in the present study may have been overestimated, as the children’s toothbrushes were not rinsed following tooth-brushing, as was done in several previous studies (41, 43, 58). This means the residual toothpaste may have been left on the toothbrush and this was not subtracted from the total weight of toothpaste used, but assumed to be swallowed.

6.2 Urinary Fluoride Excretion

The daily urinary fluoride excretion (DUFIE) in Dunedin was 0.378mg/d, and 0.249mg/d in Timaru, which is 23% and 21% of total daily fluoride intake (TDFI), respectively. The percentage of fluoride intake excreted in children <7 years varies widely and has been reported to be between 30-80% (24, 42). One possible explanation for the lower percentage of urinary fluoride excretion in this study could be the high protein intakes of the children, shown in Table 5.2. Fluoride absorption has been shown to increase with increased protein intake (21), and increased fluoride absorption could lead to decreased excretion. Another possibility is due to the growth in children. If the bones are growing, they require more fluoride, therefore absorbing more fluoride and excreting less (18). A common reason for a decreased fluoride excretion is a vegetarian diet (19, 23), however, no children in the present study were consuming a vegetarian diet. The relationship between TDFI and DUFIE in the present study ($R^2 = 0.112$ in Dunedin, $R^2 = 0.357$ in Timaru) was not as strong as that found by Villa et al. ($R^2 = 0.76$) (24), however the small sample size in the present study is likely to explain some of this discrepancy.
6.3 Feasibility of Methods

The current pilot study should be considered feasible, as the methods produced complete data for almost all participants and participants found them acceptable. However, it is essential to analyse the remaining samples (i.e. duplicate diets, toenails), to determine if all these would be required in a larger study. If the duplicate diet has a fluoride content that is similar to the diet record, then it is strongly recommended to exclude the duplicate diet from future studies, as it was considered the most difficult aspect of the study and many commented on the wasteful nature of this method. A study of 61 4-6-year-olds found no difference between the fluoride intake estimated by diet records and duplicate diets at a group level (54).

The majority of diet records were completed by the parents, however, some children completed parts of it. While it was helpful to have the children involved in the process, parental involvement was essential to obtain the detail required. Once the obstacle of entering the new fluoride data into the Kai-calculator nutrient database was achieved, entering the diets into Kai-calculator was a simple process.

Expectorated saliva collection was well accepted by most children, however, from a researcher’s perspective, was sometimes difficult as the children needed reminders to stop and weigh the toothbrush before brushing their teeth. Collecting rinsing water following tooth brushing would produce more accurate results, however, would require very strict supervision as it was a natural response for most children to rinse their toothbrush immediately after brushing. Collecting rinsing water would be easier in younger children when the parent has more control. The analysis of expectorated toothpaste was straightforward; samples were diluted so that the same standard solution could be used to analyse both urine and expectorated samples.

Urine collection was a key barrier when recruiting children, especially in females, possibly contributing to the higher number of male participants. However, in most cases, this age group was
capable of collecting urine on their own, which is likely to be the reason parents selected urine collection as the easiest method.

Because no statistical analyses took place in the current study, it cannot be concluded whether there was a significant difference between data from the two collection days in the 11 children who completed both days. A previous study found that fluoride intake did not differ significantly within children on different days, suggesting that one collection period is sufficient (42, 54).

6.4 STRENGTHS AND LIMITATIONS

6.4.1 Strengths

The fluoride data used to analyse dietary fluoride intake was taken from the latest, very recent 2016 NZTDS. The foods analysed were from four different locations around New Zealand, using four brands of each food, in two different seasons (31), and then averaged for use in the present study. Because the level of fluoride in the soil and water varies between countries (5), it is important to use data analysed from the same location.

The dietary intake and urine collection data should be accurate, as 13 of 15 parents reported children not changing how they ate. The two parents reporting a change in eating were still consuming their usual diet, just changing the day on which they had certain meals. All 15 parents reported that their child did not drink any less to avoid going to the toilet, suggesting that the urine volumes were complete.

As the dietary intake data were effectively collected twice – using the diet record and duplicate diet, each method could be used to check the other in terms of total weight consumed and food included. In one instance, this check identified missing water in the duplicate diet, as the participant was drinking from a drink bottle over the day, and had forgotten to add in the duplicate amount at the end of the day.
The similarities between socio-demographic characteristics of the participants from each city is another strength, simplifying the comparison of results between cities. The aim of 10 participants from each city was achieved, with a similar number of children from each city completing the second day of data collection.

6.4.2 Limitations

As previously mentioned, the fluoride data used to analyse dietary intake was incomplete, in particular fluoride values were lacking for meat and dairy products. Additionally, the 75 foods with fluoride contents that were analysed in the NZTDS were mapped onto similar foods where appropriate, and this may have decreased the accuracy of the dietary intake findings.

Data collection days only took place on weekends or during the school holidays, where dietary intake may be different to weekday consumption (59). This limits the generalisability of the dietary intake findings, however, the estimated ingestion of fluoride from toothpaste should be similar on all days of the week.

The estimated fluoride intake from toothpaste ingestion is likely to be over-estimated as the rinsing water used to rinse the toothbrushes following tooth-brushing was not collected. This means that any fluoride left on the toothbrush would have been washed away. However, not all children rinsed their brush, meaning there would always be fluoride left on the brush, and therefore rinsing the brush in these children would have led to an underestimation of ingestion.

No external reference standard for fluoride was used to check the accuracy of laboratory methods. The final limitation is that there was not enough time for all children to complete two days of data collection. Approximately half of participants completed a second day, five children in Dunedin and six in Timaru, which helped to reduce variation in the results.
6.5 Recommendations for Future Research

It is recommended that the duplicate diet and toenail samples are analysed and compared with the dietary data presented in this thesis, to determine the necessity of including these samples in a future study. If the duplicate diet is required, it is suggested that water and other beverages be collected separately from the food, to make the dietary collection more pleasant. To account for the missing fluoride data in the NZTDS, it is recommended that values from another database from a country with similar water fluoride concentrations are used, to fill in any gaps. Because toothpaste was such a major contributor to daily fluoride intake, the method of collecting expectorated toothpaste should be revised to include the collection of rinsing water.

A larger study is now required to ascertain the fluoride intakes of a larger number of children in New Zealand, perhaps including a wider age range. Due to previous research with a larger sample size finding no significant difference in results between two collection days, it would be recommended to use only one day of data collection. This would reduce the burden for participants, making it more appealing and therefore a larger sample size could be achieved.

6.6 Conclusions

The results from this pilot study found that 9-10-year old children living in Dunedin (fluoridated) and Timaru (non-fluoridated) were not meeting the AI for fluoride of 2.0mg/d. Dunedin children consumed more fluoride (1.66mg/d) than Timaru children (1.20mg/d), a result of the CWF in Dunedin. Water, followed by breads and cereals, were the main contributors to dietary fluoride intake in both cities, however, fluoride ingestion from toothpaste was the main contributor to total fluoride intake. The data collection methods are feasible, in particular, the urine and expectorated toothpaste analysis methods reported in this thesis. The children observed in this study are at low risk of dental fluorosis, however, may be at an increased risk of dental caries as the AI had not been met.
7 APPLICATION OF RESEARCH TO DIETETIC PRACTICE

Dietitians must use the latest evidence-based research to guide their practice and therefore must have awareness of how research is carried out. This research project has deepened my understanding of the research process, in particular recognising the level of involvement in developing study protocol, consolidating results and appreciating the need for pilot studies. The research carried out in this thesis can be applied to both clinical and public health dietetics.

Dietary assessment is an important aspect of clinical dietetics, and as a dietitian, it is important to understand how difficult it can be for the client. The process of entering the participant’s diet records into Kai-culator reinforced the difference in perception between us as a dietitian, and the participant as an everyday client. Although it seemed that participants were clearly instructed to record as many details as possible about the foods and drinks consumed, more often than not, these details were missing and assumptions had to be made when choosing a food in the database. Obtaining a diet history occurs more frequently in a dietetic consultation as opposed to a diet record, however the missing details observed by the candidate while entering the participants diet records has emphasised the importance of effective questioning and communication while obtaining a diet history. In situations where diet records are required from a patient, it will be important to emphasise the level and type of information required, tailored to each individual.

The results of this thesis have highlighted the impact of nutrient fortification. While neither the fluoridated nor non-fluoridated region met the AI for fluoride, the fluoridated region was much closer, without having to alter their diet in any way. Completing this thesis has reinforced how policy can affect the health of many. Dietitians need to be informed about the effect of nutrient fortification so that they can be successful advocates in public health. It is essential to be aware of the impact of large-scale fortification on different age groups, and when it is appropriate to
recommend fortification in place of supplementation. Effective communication skills and the
ability to persuade and motivate is also extremely important, especially in order to overcome the
ever-increasing advice given by those with alternative opinions.
REFERENCES


77. Zohoori FV, Maguire A. Database of the Fluoride (F) content of Selected Drinks and Foods in the UK. Newcastle University and Teesside University 2015.
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<thead>
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<th>Description</th>
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<td>I</td>
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ASSOCIATE DEAN (RESEARCH) – ETHICAL APPROVAL

Assoc. Prof. S Skeaff
Department of Human Nutrition
Division of Sciences

15 March 2017

Dear Assoc. Prof. Skeaff,

I am again writing to you concerning your proposal entitled “Fluoride intakes of primary school children living in fluoridated and non-fluoridated areas of the lower South Island: a pilot study”, Ethics Committee reference number HE17/001.

Thank you for your response of 3rd March 2017 addressing the issues raised by the Committee.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

The standard conditions of approval for all human research projects reviewed and approved by the Committee are the following:

Conduct the research project strictly in accordance with the research proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee.

Inform the Human Research Ethics Committee immediately of anything which may warrant review of ethics approval of the research project, including: serious or unexpected adverse effects on participants; unforeseen events that might affect continued ethical acceptability of the project; and a written report about these matters must be submitted to the Academic Committees Office by no later than the next working day after recognition of an adverse occurrence/event. Please note that in cases of adverse events an incident report should also be made to the Health and Safety Office:

http://www.otago.ac.nz/healthandsafety/index.html

Advise the Committee in writing as soon as practicable if the research project is discontinued.

Make no change to the project as approved in its entirety by the Committee, including any wording in any document approved as part of the project, without prior written approval of the Committee for any change. If you are applying for an amendment to your approved research, please email your request to the Academic Committees Office;
Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval or an extension of approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

The Human Ethics Committee (Health) asks for a Final Report to be provided upon completion of the study. The Final Report template can be found on the Human Ethics Web Page http://www.otago.ac.nz/council/committees/committees/HumanEthicsCommittees.html

Yours sincerely,

[Signature]

Mr Gary Witte  
Manager, Academic Committees  
Tel: 479 8256  
Email: gary.witte@otago.ac.nz

c.c. Professor S Samman  
Department of Human Nutrition
APPENDIX B – INFORMATION PACK

Information Sheet (FOR PARENTS/CAREGIVERS)

<table>
<thead>
<tr>
<th>Study title:</th>
<th>Fluoride intakes of primary schoolchildren: a pilot study</th>
</tr>
</thead>
</table>
| Principal investigator: | Dr Sheila Skeaff  
Department of Human Nutrition  
Associate Professor  
Contact phone number: 03-479-7944 |
| Study cell phone number: | 021 114 2155 |
| Study email address: | kids.2017@otago.ac.nz |

Introduction

Thank you for showing an interest in this project. Please read this information sheet carefully. Take time to consider and, if you wish, talk with relatives or friends, before deciding whether or not to participate.

If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the aim of this research project?
Dental caries is the most prevalent disease in New Zealand. Around half of the New Zealand population have access to fluoridated tap water, however, research into actual fluoride intakes of the New Zealand population is minimal. Because fluoride is critical in the prevention of dental caries in childhood, it is important to determine if fluoride intakes are adequate in children aged 9-10 years. This is a pilot study (i.e. a small study designed to see if the study is practical for parents and their children to undertake) that aims to measure the amount of fluoride consumed by children.

Who is funding this project?
This project is being funded by the University of Otago.

Who are we seeking to participate in the project?
We need healthy school children between the ages of 9 and 10 years. Siblings will be excluded from the study.

If you participate, what will you be asked to do?
Parents/caregivers will be contacted by telephone and assigned a time for researchers to visit that is convenient for both the child and parent.

At the first meeting:
- researchers will measure the weight and height of the child
- the family will be given all the equipment and instructions they require
- a brief questionnaire asking about sociodemographic information, and the use of fluoride toothpaste and supplements will be given
- oral and written instructions will be provided on how to collect samples of food, diet records, urine samples
- the child will be asked to brush their teeth and collect any toothpaste/saliva spat out
- the child will be asked to provide their toenail clippings (refrain from clipping your child’s toenails and allowing them to wear toenail polish for 2-3 weeks)

We require the following samples to be collected over a 24-hour period. This will be repeated again within 1-2 weeks.
- Parents will be asked to provide researchers with duplicate portions of all food and drink consumed within 24 hours by observing and replicating the amounts actually ingested by your child.
  - This involves making two meals for the child. One meal will be given to the child. Whatever the child eats will be removed from the duplicate meal and put into a sample container to give to the researcher. Until the researcher is able to collect these duplicate meals, the duplicate meal portion should be stored in the fridge.
- Parents will be required to provide a written diet record. This involves weighing all food consumed by the child within the 24-hour time period.
- 24-hour urine samples need to be collected from the child. This means that all urine voided by the child needs to be collected in the provided containers.

If you do agree to take part, participants will receive $30 to reimburse for the cost of participating.

**Is there any risk of discomfort or harm from participation?**
Your child will collect urine in a plastic container for 24 hours, which may cause some inconvenience to normal activities. Spillage of urine can occur, but this is rare, however it is recommended it is cleaned up using gloved hands and a 10% bleach solution to disinfect the area. Once the 24-hour urine has been collected we will then arrange for the samples to be picked up by one of the research students.

**What specimens, data or information will be collected, and how will they be used?**
The urine samples will be analysed for fluoride. Leftover urine will be stored in a freezer in a locked room in the Department of Human Nutrition. The diet records and duplicate diets will be analysed to determine the amount of fluoride eaten each day. These results will be stored alongside your height, weight and fluoride toothpaste/supplement use on a password-locked computer. Access to your information will be granted only to researchers from the Department of Human Nutrition. Once all the results are collected, the information will be used to determine how much fluoride is typically consumed in the diet of New Zealand children, and to guide public health recommendations about the fluoridation of water supplies.
What about anonymity and confidentiality?
The information that you provide that can identify you and your child is totally confidential and will not be disclosed to anyone without your permission, except if required by law. Information that identifies you personally (such as name and address) will be kept in a separate location from all your other results, and will only be linked back to your results if we need to contact you personally. Your name and address will only be available to the Masters students and Dr Sheila Skeaff.

Any publications of the findings of the study will be made in such a way that you cannot be identified. This includes any journal articles, and thesis reports. Data obtained as a result of the research will be retained for 5 years in secure storage. Any personal information held on the participants [such as name and contact details] will be destroyed at the completion of the research. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but no identifying information will be included in the results published.

If you agree to participate, can you withdraw later?
You and your child may withdraw from participation in the project at any time and without any disadvantage to yourself.

Any questions?

If you have any questions now or in the future, please feel free to contact either:

<table>
<thead>
<tr>
<th>Name</th>
<th>Contact phone numbers:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Sheila Skeaff</td>
<td>03-479-7944</td>
</tr>
<tr>
<td>Department of Human Nutrition</td>
<td></td>
</tr>
<tr>
<td>Associate Professor</td>
<td></td>
</tr>
<tr>
<td>Chontelle Watts</td>
<td>Study Cell phone#:</td>
</tr>
<tr>
<td>Department of Human Nutrition</td>
<td>021 114 2155</td>
</tr>
<tr>
<td>Masters of Dietetics Student</td>
<td></td>
</tr>
<tr>
<td>Caitlin Davenport</td>
<td>Study Cell phone#:</td>
</tr>
<tr>
<td>Department of Human Nutrition</td>
<td>021 114 2155</td>
</tr>
<tr>
<td>Masters of Dietetics Student</td>
<td></td>
</tr>
</tbody>
</table>

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256 or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Fluoride Fun

Who We Are

About Us
Our names are Chontelle and Caitlin and we study at the University of Otago. This is our last year before we graduate to become dietitians.

Contact Us
Phone: 021 114 2155
Email: kids.2017@otago.ac.nz

This study was approved by the University of Otago Health Ethics Committee - reference HE17/001

The Fluoride in School-children Study
FLOSS - The Fluoride in School-children Study

Background

Fluoride helps make your teeth strong and protects them from bad bacteria. You will find fluoride in your toothpaste and some cities have fluoride added to the tap water. Fluoride is also found in some foods.

Why do we want your help?

We want to know how much fluoride 9-10 year olds get in their foods and drinks. At the moment there is no information about this!

What do you need to do?

- Have your height and weight taken
- Fill out a questionnaire
- Show us how you brush your teeth
- Give us some toenail clippings
- Collect all your urine in a bottle for 2 days
- Eat and drink what you usually do

Important Information

- Participation is voluntary (your choice) and both you and your parents will need to agree to take part
- All your information will be kept private
- Anytime I want to stop, that's okay
- If you have any questions you can talk to Caitlin and Chontelle

For being so helpful, we will give you 2 movie vouchers!! 😊

Where do you live?

Water Fluoridation status

- Fluoridated (>70%)
- Less than 50% public supply
- Mix (10%–70%)
- Not Fluoridated (<10%)

Is there fluoride in the water where you live?
CONSENT FORM FOR PARENTS/GUARDIANS

Following signature and return to the research team this form will be stored in a secure place for ten years.

Name of Child: .................................................................

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people of my choice about participating in the study.
3. I confirm that my child meets the criteria for participation which are explained in the Information Sheet.
4. All my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
5. I know that my child’s participation in the project is entirely voluntary, and that my child is free to withdraw from the project at any time without disadvantage.
6. I know that as a participant my child will have his/her height and weight measured, will provide two 24-hour urine samples, two 24-hour weighed diet records, and two 24-hour duplicate diets and a sample of toothpaste and spit.
7. I understand the nature and size of the risks of discomfort or harm which are explained in the Information Sheet.
8. I know that when the project is completed all personal identifying information will be removed from the paper records and electronic files which represent the data from the project, and that these will be placed in secure storage and kept for at least ten years.
9. I understand that the results of the project may be published and be available in the University of Otago Library, but I agree that any personal identifying information will remain confidential between myself and the researchers during the study, and will not appear in any spoken or written report of the study.
10. I know that no commercial use will be made of the data.
11. I understand that the urine and saliva samples will be stored in locked freezers at the Department of Human Nutrition, University of Otago, Dunedin until analysed.

Signature of Parent/Guardian: Date:

Name of Parent/Guardian (Printed) Date:
Fluoride intake of primary school children

Principal Investigator: Dr Sheila Skeaff, sheila.skeaff@otago.ac.nz, 479-7944

CONSENT FORM FOR CHILDREN

I have read and understood the information sheet about this study. I have talked about the study with my parents and understand what it is about. Any questions I have about the study have been answered in a way that makes sense.

I know that:

1. Taking part in the study is my choice, which means that I do not have to take part if I don’t want to and nothing will happen to me. I can stop taking part at any time and don’t have to give a reason;

2. Anytime I want to stop, that’s okay;

3. If I have any worries or other questions, then I can talk about these with the researcher that comes to my school and home;

4. My name, birthdate, height, weight, answers to questions about brushing my teeth, urine samples, will only be seen by the researcher and the people she is working with. They will keep this information private.

5. Taking part in this study is private. Results of the study may be written up for the researchers’ university work but my name will not be on anything.

Child:

I _________________________________ (print your full name), agree to take part in this study.

I go to _________________________________ School.

Date: ___________ Signature: _____________________
APPENDIX C – WRITTEN INSTRUCTIONS FOR PARTICIPANTS

Duplicate Diet & Urine Collection Instructions

We welcome any comments on your experience!
**Duplicate Diet**

A duplicate diet involves making 2 identical portions (for example, a snack, meal or drink) for your child.

Give one portion to your child to eat as usual and keep the other portion separate. We would like you to carefully observe what your child eats and drinks. From the identical uneaten portion, take the exact amount that your child did eat or drink, and put this in the bucket.

In the diet record booklet, write down the food or drink, including the weight and description of what your child ate or drank (e.g. 1 slice of wholegrain bread, 10 grams of tomato, 15 grams of cheddar cheese, 250ml of blue top milk)

Do not include anything in the bucket that your child does not eat, such as:
- Bones
- Fruit stones
- Banana peel
- Leftovers

**Urine**

Collection of 24-hour urine samples will happen on the same day as the duplicate diet and diet record. We would like you to make a note of the first time your child goes to the toilet that day. The urine from this first bathroom void does not need to be collected.

From then onwards, all urine for the next 24 hours will need to be collected, including the urine from the first bathroom break the next morning.
1-Day Diet Record

Department of Human Nutrition
University of Otago
Instructions

We would like you to record in this booklet everything you eat and drink for a period of 24 hours.

It can be annoying to write down everything you eat and drink, but please try not to change what you eat and drink because you are keeping a record.

Describing food and drinks

We need as much detail as possible about the food and drinks you consume.

- Please record the brand name of each food, drink or cooking ingredient where possible.

- Please describe each item, including cooking details and any salt, sugar, oils, spices and sauces you have added before eating.
  e.g. chicken breast with fat, bone and skin removed, sprinkled iodised salt

- Don’t forget to include any drinks or snacks between meals
  e.g. water, soft drink, juice, tea, biscuits, crisps, peanuts, slices, muffins, lollies

Tips

We have given you kitchen scales and we would like you to weigh food wherever possible. If you are adding multiple items, for example, to a sandwich, you can use the ‘zero’ function between food items.

For mixed food dishes, it will be easier to list the total ingredients at the back of this book, then describe the proportion of this recipe you consumed on the diet record.

e.g. 200g of spaghetti carbonara recipe, or ¼ of spaghetti carbonara recipe
**Sample diet record**

<table>
<thead>
<tr>
<th>Time</th>
<th>Food or Drink</th>
<th>Brand and details</th>
<th>Preparation/Cooking</th>
<th>Weighed amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7am</td>
<td>2 pieces toast</td>
<td>Couplands wholegrain</td>
<td>Toasted</td>
<td>76g</td>
</tr>
<tr>
<td></td>
<td>Margarine</td>
<td>Pams Canola – low salt</td>
<td>-</td>
<td>16g</td>
</tr>
<tr>
<td></td>
<td>Raspberry jam</td>
<td>Craigs</td>
<td>-</td>
<td>25g</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>Tap</td>
<td>-</td>
<td>250g</td>
</tr>
<tr>
<td>10am</td>
<td>Banana</td>
<td></td>
<td>Fresh</td>
<td>120g</td>
</tr>
<tr>
<td>12pm</td>
<td>Apple</td>
<td></td>
<td>Fresh</td>
<td>105g</td>
</tr>
<tr>
<td></td>
<td>Sandwich – 2 slices bread</td>
<td>Couplands wholegrain</td>
<td></td>
<td>76g</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>Iceberg</td>
<td>Fresh</td>
<td>16g</td>
</tr>
<tr>
<td></td>
<td>Shredded chicken</td>
<td>Deli, no flavour, no skin</td>
<td></td>
<td>20g</td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>Mainland Cheddar</td>
<td>-</td>
<td>20g</td>
</tr>
<tr>
<td>6pm</td>
<td>Pasta – carbonara</td>
<td>Homemade – see recipe sheet</td>
<td></td>
<td>200g</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>Tap</td>
<td>-</td>
<td>250g</td>
</tr>
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</table>

**Example recipe**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Brand and details</th>
<th>Weight (g)</th>
<th>Preparation/cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasta</td>
<td>Budget spaghetti</td>
<td>400g dry weight</td>
<td>Pasta boiled</td>
</tr>
<tr>
<td>Bacon</td>
<td>Hellers</td>
<td>50g</td>
<td>Bacon fried in 2T canola oil</td>
</tr>
<tr>
<td>4 Eggs</td>
<td>Pams size 7</td>
<td>150g</td>
<td>Everything then combined in pot, except for half of cheese which was sprinkled on top</td>
</tr>
<tr>
<td>Cheese</td>
<td>Mainland Cheddar</td>
<td>100g</td>
<td></td>
</tr>
<tr>
<td>Mixed veg</td>
<td>McCain – peas, corn, carrot</td>
<td>500g</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Food or Drink</td>
<td>Brand and details</td>
<td>Preparation/Cooking</td>
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<tr>
<td>Time</td>
<td>Food or Drink</td>
<td>Preparation/Cooking</td>
<td>Weighed amount (g)</td>
</tr>
<tr>
<td>Recipes</td>
<td>Preparation/cooking</td>
<td>Weighed amount (g)</td>
<td>Brand and details</td>
</tr>
<tr>
<td>---------</td>
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<td>------------------</td>
</tr>
<tr>
<td>Recipe 1: Food or Drink</td>
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<td></td>
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<tr>
<td>Recipe 2: Food or Drink</td>
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</tbody>
</table>
We appreciate the time you have taken to complete this diet record!

We welcome any comments:
## APPENDIX E – KAI-CULATOR ASSUMPTIONS

<table>
<thead>
<tr>
<th>Diet Record Food:</th>
<th>Entered into Kaiculator as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate hot cross bun</td>
<td>White bread roll (A189)</td>
</tr>
<tr>
<td>Hot cross bun</td>
<td></td>
</tr>
<tr>
<td>Scottish bap</td>
<td></td>
</tr>
<tr>
<td>Plain bread</td>
<td></td>
</tr>
<tr>
<td>46g Cheese rolls</td>
<td>40g white bread, 6g edam cheese</td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>Wholemeal bread roll (A52)</td>
</tr>
<tr>
<td>Juice – strawberry and kiwifruit</td>
<td>Kiwifruit juice (HN000072)</td>
</tr>
<tr>
<td>Hamburger bun</td>
<td>White bread roll commercial (A40)</td>
</tr>
<tr>
<td>Weet-bix</td>
<td>Breakfast cereal, wholegrain wheat biscuit, weet-bix (D1056)</td>
</tr>
<tr>
<td>Honey grain bread</td>
<td>Bread, mixed grain, light, sliced, composite (A157)</td>
</tr>
<tr>
<td>Kit Kat</td>
<td>Biscuit, wafers, chocolate &amp; vanilla (A219)</td>
</tr>
<tr>
<td>Neopolitan ice-cream</td>
<td>Ice cream, chocolate standard, composite (F1070)</td>
</tr>
<tr>
<td>Hot dog bun</td>
<td>White bread roll (A174)</td>
</tr>
<tr>
<td>Birthday cake ice-cream</td>
<td>Rocky road ice-cream</td>
</tr>
<tr>
<td>White bread</td>
<td>Bread, white, sliced, prepacked (A1007)</td>
</tr>
<tr>
<td>Countdown crusty Vienna bread</td>
<td></td>
</tr>
<tr>
<td>Lemonade juice</td>
<td>Lemonade ice-bloc quencher (R4219)</td>
</tr>
<tr>
<td>Nuttelex butter</td>
<td>Olivani 75% fat (J1009)</td>
</tr>
<tr>
<td>Beauty and the Beast cereal</td>
<td></td>
</tr>
<tr>
<td>Nequik cereal</td>
<td>Cocoa pops (D1041)</td>
</tr>
<tr>
<td>Strawberry milkshake powder</td>
<td>Strawberry syrup, McDonalds (H167)</td>
</tr>
<tr>
<td>French onion soup</td>
<td>Onion powder (P43)</td>
</tr>
<tr>
<td>Healtheries potato stix</td>
<td>Potato Krispa (U18)</td>
</tr>
<tr>
<td>Chilean guava</td>
<td>Dried cranberry (L1023)</td>
</tr>
<tr>
<td>Jason’s bongo corn puffs</td>
<td>Corn snacks, cheese flavour (U16)</td>
</tr>
<tr>
<td>Dilmah tea</td>
<td>Green tea (C1039)</td>
</tr>
<tr>
<td>Cookie bear choc chippie</td>
<td>Cookie time smart cookie (A1073)</td>
</tr>
<tr>
<td>Oxtail soup packet</td>
<td>Oxtail soup (V12)</td>
</tr>
<tr>
<td>Free from gluten chicken twists</td>
<td>Potato crisps flavoured (U8)</td>
</tr>
<tr>
<td>Gluten free chocolate cake baking mix</td>
<td>Cake, chocolate, baked, topped with butter icing (R5435)</td>
</tr>
<tr>
<td>with buttercream icing</td>
<td></td>
</tr>
<tr>
<td>Iced bun</td>
<td>Bread roll, white (A230). Took 10g off bun weight, and added 10g icing (R3852)</td>
</tr>
<tr>
<td>Rice crackers</td>
<td>Cracker, rice, rice cracker plain, composite (A1034)</td>
</tr>
<tr>
<td>Fruit and vege juice, pineapple</td>
<td>Juice, pineapple and carrot (C135)</td>
</tr>
<tr>
<td>Lemonade popsicle</td>
<td>Lemonade soft drink (C17)</td>
</tr>
<tr>
<td>Bliss ball</td>
<td>Snack bar, bumper bar (R4910)</td>
</tr>
<tr>
<td>Night’n’day sundae</td>
<td>Wendy’s sundae</td>
</tr>
<tr>
<td>M&amp;M’s</td>
<td>Chocolate bar plain (W4)</td>
</tr>
<tr>
<td>Porridge</td>
<td>Oats, wholegrain, raw, composite (E24)</td>
</tr>
<tr>
<td>Wholemeal wrap</td>
<td>Wholemeal bread (A23)</td>
</tr>
<tr>
<td>Green beans</td>
<td>Mixed vegetables (R3180)</td>
</tr>
<tr>
<td>4 bean mix</td>
<td>Baked beans (X1004)</td>
</tr>
<tr>
<td>Eskimo lollies</td>
<td>Marshmallows, pink and white (W40)</td>
</tr>
<tr>
<td>Chilli beans</td>
<td>Baked beans + chilli sauce</td>
</tr>
</tbody>
</table>
## Quantities:

<table>
<thead>
<tr>
<th>Issue</th>
<th>Assumption made</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amount of cucumber given (FLOSS101)</td>
<td>Assumed to be 20g, the same amount as the carrot and cheese used in the meal.</td>
</tr>
<tr>
<td>Left over amount given for a mixed meal</td>
<td>Took amount out of each ingredient evenly</td>
</tr>
<tr>
<td>Missing weight for Chilean guava</td>
<td>Assumed amount to be the same as last time it was eaten</td>
</tr>
<tr>
<td>No weight given for rice crackers</td>
<td>Assumed to be 25g (recommended serving size, and similar to other children’s amounts)</td>
</tr>
<tr>
<td>No weight given for roast chicken</td>
<td>Assumed to be 100g</td>
</tr>
<tr>
<td>No cheese type given</td>
<td>Assumed to be edam</td>
</tr>
<tr>
<td>No milk type given</td>
<td>Assumed to be standard</td>
</tr>
<tr>
<td>No type of sausage</td>
<td>Assumed to be beef</td>
</tr>
</tbody>
</table>

**FLOSS 208:**
1. some weights included bowl and glass
2. no weight given for main meal (total weight given but not the weight the child ate)

**Rice and Pasta Calculations** (to account for fluoridated water used during cooking):

- Boiled pasta – 61.4g water per 100g
- Dry pasta – 10.8g water per 100g
- Difference = 50.6g water per 100g
  - Where dry weight given – add 50.6g Dunedin or Timaru water per 100g
  - Where cooked weight given – take away 50.6g per 100g of weight given, and then add this weight back on in Dunedin or Timaru water

- Boiled rice – 66.0g water per 100g
- Dry rice – 13.9g water per 100g
- Difference = 52.1g per 100g
  - Where dry weight given – add 52.1g Dunedin or Timaru water per 100g
  - Where cooked weight given – take away 52.1g per 100g of weight given, and then this weight back on in Dunedin or Timaru water
APPENDIX F – ORAL HEALTH QUESTIONNAIRE FOR CHILDREN

1. What is your name?

2. How old are you TODAY?
   - 9
   - 10

3. When is your birthday?
   - DD / MM / YYYY

4. Are you a boy or a girl?
   - Boy
   - Girl

5. What is the name of your school?

6. What year are you in?
   - Year 4
   - Year 5
   - Year 6
   - Year 7
7. What ethnicity are you?

☐ New Zealand European/Pakeha
☐ Maori
☐ Pacific
☐ Asian
☐ Other (African, Spanish)

8. Do you take any pills or tablets like vitamins or minerals?

☐ Yes, regularly (more than once a week)
☐ Yes, Occasionally (less than once a week)
☐ No

Please list any pills/tablets you have taken in last 2 months.

9. How often do you brush your teeth?

☐ Never
☐ Once a day
☐ Two times a day
☐ Three times a day
☐ More than three times a day

10. How often do you use dental floss?

☐ Never
☐ Once a day
☐ Two times a day
☐ Three times a day
☐ More than three times a day
11. Do you have any food allergies or intolerances?
   - Yes
   - No

   If yes, please specify:

12. Do you visit the school dental nurse and/or a dentist each year?
   - Yes
   - No

13. What was the reason for your last visit to the dentist/dental nurse?
   - Pain or trouble with teeth, gums or mouth
   - Treatment/follow-up treatment e.g. fillings
   - Routine check-up
   - I don't know/don't remember

14. Have you had trouble with any of the following?
   - I do not like the look of my teeth
   - I often avoid smiling and laughing because of my teeth
   - If my teeth are sore, it changes how I eat
   - I have trouble biting hard foods
   - I have trouble chewing
   - I have not had trouble with any of these

15. What kind of toothpaste do you use?

16. What is your height?

17. What is your weight?
FLOSS Follow Up Survey

1. What was the easiest part of this study?
   - Duplicate diet
   - Diet record
   - Urine collection
   - None of the above

2. What was the most difficult part of this study?
   - Duplicate diet
   - Diet record
   - Urine collection
   - None of the above

3. Did your child drink less to avoid going to the toilet?
   - Yes
   - No

4. Did you make meals that were easier/cheaper to prepare?
   - Yes, I made meals that were easier
   - Yes, I made meals that were cheaper
   - No, I didn’t change how we ate

5. Do you have any ideas for improving this study?
APPENDIX H – STANDARD CURVE FOR URINE ANALYSIS

Standard Curve using TISAB III

\[ y = 23.124 \ln(x) + 409.54 \]
APPENDIX I – STANDARD CURVE FOR TOOTHPASTE ANALYSIS

\[ Y = 19.826 \ln(x) + 466.86 \]