FLOSS
Fluoride in Schoolchildren Study: A Pilot Study

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A thesis submitted in partial fulfilment of the requirements for the degree of
Master of Dietetics

At the University of Otago, Dunedin, New Zealand

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Abstract

**Background:** Fluoride plays an essential role in maintaining good dental health, especially during childhood when permanent teeth are forming. Fluoride helps prevent tooth decay by promoting tooth remineralisation and preventing tooth demineralisation. There are no data on the actual intakes of fluoride in New Zealand children 5 years or older.

**Objective:** To trial the feasibility of measuring actual fluoride intakes of 9-10-year-old New Zealand children living in the fluoridated and non-fluoridated cities of Dunedin and Timaru, respectively.

**Design:** This study aimed to recruit 10 children from each city. Dietary fluoride intake was measured by 24-hour diet records and duplicate diets. Expectorated toothpaste and saliva samples were used to measure fluoride from toothpaste ingestion. Twenty-four-hour urine samples were collected to analyse urinary fluoride excretion.

**Results:** The mean (SD) total daily fluoride intake of Dunedin (n=10) and Timaru (n=10) children was 1.7 (0.6) mg/day and 1.2 (0.6) mg/day, respectively, with 1.0 (0.4) mg/day and 1.0 (0.5) mg/day contributed from toothpaste; the AI for fluoride is 2mg/day. Fractional urinary fluoride excretion for Dunedin and Timaru children was 23% and 21%, respectively.

**Conclusion:** Diet records, expectorated toothpaste and saliva, and 24-hour urine samples are feasible methods to determine the daily fluoride intakes of 9-10-year-old children. Toothpaste was the primary source of fluoride for children in both cities and fluoridated water was an important source of fluoride for Dunedin children.
Preface

The research project undertaken in this thesis was conceived by the candidate’s supervisor, Associate Professor Sheila Skeaff who was responsible for submitting the research proposal, applying for funding, and obtaining ethical approval.

The Fluoride in Schoolchildren Study (FLOSS) was managed by Masters of Dietetic two candidates, both from the Department of Human Nutrition. Responsibility for every aspect of the study was shared between the candidates, as listed below.

- Contributed to the pilot study design.
- Assisted with the ethical application including drafts of Information for Participants and Consent Forms.
- Developed an information pamphlet for children.
- Sourced and placed orders for equipment required for the study.
- Recruited participants i.e. contacting primary schools and university departments via email, and parents via word of mouth and Facebook, and corresponded with participants to arrange visiting times.
- Made up study packs for participants including kitchen scales, 4L plastic bucket, 2L screw-top bottle, funnel, bowl, small pink urine pottles, diet record and bin liner.
- Developed FLOSS baseline and follow-up survey Survey Monkey and contributed to the cleaning of data set from Survey Monkey.
- Provided verbal and written instructions to parents and children on how to complete sample collection.
• Contacted Roncalli College in Timaru to gain permission to access the school kitchen for use during the time in Timaru.

• Processed samples i.e. weighing samples, blending food, taking aliquots and freezing samples at -20°C until analysis.

• Sourced fluoride food and beverage data from the 2016 Total Diet Study New Zealand.

• Entered fluoride data for 75 foods into the nutrient lines of Kaiculator.

• Entered 15 diet records into Kaiculator and followed up on missing data from caregivers.

• Completed sample analysis (urine and toothpaste) with assistance from Michelle Harper (Human Nutrition) and Pauline Bandeen (Chemistry).

Associate Professor Skeaff proofread and provided feedback on the candidate’s written work.
Acknowledgements

I would like to thank Associate Professor Sheila Skeaff for your support, expertise and calming words. Your guidance through this process has been hugely appreciated and it has been a privilege to work with you.

Chontelle Watts, I could not have asked for a better person to do this with. I have been very thankful to have someone to laugh, blend and share ideas with. I wish you all the best for your future and hope we remain friends for a long time!

Liz Fleming, thank you for the time and effort you put into our project and for teaching us the do’s and don’ts of Kaiculator.

Thank you, Michelle, Pauline and Ash, for assisting us in the lab. A special thank you to Michelle for receiving and storing our samples that we sent from Timaru and for solving any issues we had with our results.

Kieran Columb, thank you for helping us find the equipment we needed for our project (and for keeping those old blenders!).

Frances Moseley, thank you for making equipment/voucher ordering so easy for us.

To Chris Comeau, Rose Struthers and Roncalli Staff Members for allowing us to access to Roncalli school kitchen during our time in Timaru. It was nice to spend time in such a familiar setting and was the perfect place to blend our food samples.

MDiet 2016/17 class thank you so much for the time we have spent together. I am so grateful to have such a great group of people to share this experience with and know I have made some lifelong friends. I wish you all the best of luck for the future.

Finally, thank you to my family and Ben. Your ongoing support, love and belief in my abilities mean a lot.
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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>bw</td>
<td>body weight</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>FUFE</td>
<td>fractional urinary fluoride excretion</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>kJ</td>
<td>kilojoules</td>
</tr>
<tr>
<td>L</td>
<td>litres</td>
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<tr>
<td>m</td>
<td>metres</td>
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<td>µg</td>
<td>micrograms</td>
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<td>milligrams</td>
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<td>millilitres</td>
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<tr>
<td>mm</td>
<td>millimetres</td>
</tr>
<tr>
<td>NRV</td>
<td>Nutrient Reference Value</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended Dietary Intake</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>TDFI</td>
<td>total daily fluoride intake</td>
</tr>
<tr>
<td>TISAB</td>
<td>total ionic adjustment buffer</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
1 Introduction

Fluoride is a trace element occurring naturally in soil, water, and most foods at low levels (1). Inorganic sources of fluoride include fluoridated toothpaste, community water fluoridation (CWF) and fluoride tablets (1). Fluoride is involved in the mineralisation of teeth and protects teeth from dental caries by inhibiting bacterial growth, preventing surface demineralisation and promoting enamel remineralisation (2). Until recently, many countries did not recognise fluoride as an essential nutrient because deficiency does not cause life-threatening disease (3). However, low fluoride intake may result in suboptimal dental health (4).

Dental caries is among the most prevalent diseases in New Zealand and is the most common childhood disease worldwide (5). As a prevention strategy, the New Zealand Ministry of Health strongly recommends community water fluoridation at levels of 0.7-1.0ppm (6). However, CWF is currently at the discretion of district councils, thus some areas of New Zealand have fluoridated water (52%) and others have non-fluoridated water (48%) (6). Alongside fluoridated water, dentifrice is a primary source of fluoride (7, 8), especially in children who tend to ingest more than adults. Other sources of fluoride include diet, particularly bread (4, 9) and rice, pasta and vegetables cooked with fluoridated water (9), and fluoride tablets (10). As is becoming more common, fluoride intakes of those living in non-fluoridated and fluoridated areas may not differ significantly
because food and beverages produced in fluoridated areas may be transported to non-fluoridated areas, and vice-versa (11, 12).

Much of the research conducted on fluoride focuses on its effect on dental health, hence there is less data surrounding the intake of fluoride. The majority of fluoride intake data that is available is conducted internationally, meaning it may not be applicable to the New Zealand population. The most recent data available on fluoride intake in New Zealand has been estimated from the New Zealand Total Diet Study, and is not actual intake; the study reported that fluoride intake for the majority of the population, except 6-12month-old infants, was below the level of Adequate Intake (AI) regardless of whether they had access to fluoridated water or not (4). The current fluoride intake of New Zealand children is unknown.

This pilot study aims to trial the feasibility of measuring actual fluoride intakes of New Zealand children living in fluoridated and non-fluoridated areas of the South Island by assessing:

- The methods used to determine the contribution of the diet to total fluoride intakes;
- The methods used to determine the contribution of fluoride from toothpaste inadvertently swallowed.
2 Literature Review

A literature review was conducted to gather and review the existing information on the role of fluoride in dental health, fluoride sources and intake, and the methods used to analyse fluoride intake. To find key papers, several search engines and keywords were used. Medline and Scopus search engines were accessed through the Otago University Library website. Google Scholar was also used to provide a more general search. Further papers were found by looking at the reference list of papers that had already been accessed.

Table 1 Keywords used in literature search

<table>
<thead>
<tr>
<th>Keywords</th>
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<tbody>
<tr>
<td>Fluoride</td>
</tr>
<tr>
<td>Children</td>
</tr>
<tr>
<td>Intake</td>
</tr>
<tr>
<td>New Zealand</td>
</tr>
<tr>
<td>Dental caries</td>
</tr>
<tr>
<td>Fluorosis</td>
</tr>
<tr>
<td>Metabolism</td>
</tr>
<tr>
<td>Sources</td>
</tr>
</tbody>
</table>
2.1 Fluoride Background

2.1.1 Fluoride Metabolism

Fluoride is the ionic form of fluorine (13). Gastric absorption of fluoride is pH dependent but absorption in the small intestine is not (14). The presence of large quantities of calcium, magnesium or aluminium can result in the formation of fluoride compounds, preventing it from being fully absorbed due to low solubility (1). With low levels of these dietary components present, almost all soluble fluoride is completely absorbed (1, 14). Peak plasma concentration of fluoride will occur 20 to 60 minutes post-ingestion (14). Following absorption, the majority of fluoride is taken up by the bones and teeth, with a small proportion distributed to soft tissues and bodily fluids (1, 15). Approximately 99% of absorbed fluoride accumulates in calcified bones, where it forms part of the hydroxyapatite crystal lattice (1, 2). Fluoride not taken up by the skeleton is primarily filtered by the kidneys then excreted in urine (16), although a small amount is also lost via faeces and sweat (3).

2.1.2 Fluoride and Dental Health

Dental caries, more commonly known as tooth decay, is an irreversible disease that is progressed by a diet high in fermentable carbohydrate (17) and a lack of dental hygiene (5, 18). Dental caries can occur at any age, regardless of demographic variations (5) and is often first seen as a small, white spot or lesion on the tooth surface, or enamel (2). The surface of the tooth is coated in enamel which, in mature adults, is mainly made up of non-stoichiometric hydroxyapatite (calcium phosphate mineral), as well as an organic matrix
and water (19). Under the enamel lies calcified tissue called dentine (3, 6), which is more susceptible to dental caries than enamel (18). Tooth enamel and dentine serve to protect the core of the tooth (19). Demineralisation of the tooth can occur when plaque builds up on the tooth surface. The bacteria in the plaque produce acid through anaerobic metabolism of dietary sugars and starches (20, 21), causing the pH level in the plaque to fall below the critical level of 5.5 (2, 21, 22). If this acidic environment is maintained, a dental cavity may form (18, 23). However, demineralisation can be reversed in its early stages via remineralisation which occurs when calcium and phosphate mineral in saliva, or in remineralising solutions, is deposited in and on tooth enamel (19, 21) at an oral pH of 5.5 or higher (18). When fluoride is incorporated in the hydroxyapatite being deposited (i.e. fluorhydroxyapatite), the remineralised enamel is less porous and more resistant to future plaque damage than the original enamel (5).

Fluoride is an important catalyst in promoting the uptake of calcium and phosphate mineral into the teeth (21), meaning fluoride plays a crucial role in preventing, and reversing, dental caries (13). When bacteria build up on the tooth surface, the bacteria metabolises sugar and starch which produces acid and causes the pH in the plaque to drop (2). Fluoride can attach to hydrogen in this acid to form hydrogen fluoride which can rapidly enter the cariogenic bacteria on the tooth surface. Once this occurs, hydrogen fluoride dissociates and releases the fluoride (2) which disrupts acidogenic bacteria metabolism by interfering with essential enzyme activity, thereby preventing demineralisation of enamel crystals and promoting remineralisation (2). In the majority of people, the cycle between demineralisation and remineralisation occurs several times a day, eventually leading to either a cavity, remineralised enamel, or maintenance of the
current tooth structure (21). Fluoride provides the most benefit to dental health when it is constantly present at low levels in saliva (1), thus in continuous contact with teeth (24).

Whilst suboptimal fluoride intake may result in dental caries, excessive intake may also lead to adverse dental health effects in the form of fluorosis. Fluorosis is the only proven side effect of excessive fluoride intake (18). Dental fluorosis usually presents as a cosmetic defect rather than a health issue (25) and is caused by consuming high quantities of fluoride in the first eight years of life when teeth are in the critical stages of development (3, 25). Fluorosis is a permanent change in the tooth mineralisation, resulting in a more porous tooth surface and subsurface (25). The majority of fluorosis cases are mild, have no effect on teeth function, and commonly present as white spots on the tooth surface, or may cause no visible appearance (26). However, some forms of fluorosis are more severe and can result in the pitting and staining of teeth (1, 27). A rare form of fluorosis is skeletal fluorosis, a bone and joint condition that also ranges in severity (27). This condition may occur from long-term exposure to fluoride either by ingestion or inhalation and results in a variety of symptoms including bone deformity, bone weakness and joint stiffness (1, 27). Whilst fluoride generally promotes the strengthening of bones, in the case of skeletal fluorosis, bone mineralisation appears to be slowed, eventually resulting in weaker, more porous bones (1). A number of factors influence the amount of fluoride deposited in the bones including age, kidney function and calcium intake (1).

2.1.3 Sources of Fluoride

The most common sources of fluoride can be arranged into the following three groups; water, food and other beverages; dentifrice; and fluoride supplements (9). Beverages
typically provide the greatest amount of fluoride to the diet, however, this is dependent on the concentration of fluoride in the water supply (3). Natural water supplies vary in fluoride content, commonly from 0.1ppm-5ppm (3). Water collected from wells and boreholes are typically higher in fluoride content than surface waters (3). In some areas of the world, community water fluoridation (CWF) has been initiated due to the proven beneficial effects of fluoride on dental health, the concentration of which generally ranges from 0.7-1.2 ppm (1, 3).

In New Zealand, local councils currently hold the responsibility for deciding whether or not the community water supply should be fluoridated (28). For those living in fluoridated areas, the water supplies children with about one-quarter of their average daily fluoride intake, and it supplies adults with about half of their daily intake. The concentration of fluoride in drinking water directly influences the fluoride content of any food or drink made using the fluoridated water (3).

Plant foods generally contain more fluoride than animal foods, with the exception of ocean fish which contain about 1-3ppm of fluoride (3, 29). The concentration of fluoride in plant foods depends on the fluoride content of the soil it was grown in (29). Tea leaves, thus tea drinks, are also a primary source of fluoride in the diet (3) since tea plants have a high uptake of fluoride and accumulate fluoride in their leaves (29). Previous studies found that tea brewed in water contain fluoride concentrations of 0.4-3.4ppm (30, 31).

In New Zealand, bread and potatoes, beverages (tea and beer), and fluoridated toothpaste (4, 32) are the main non-water sources of fluoride. Infant formula is the main source of fluoride for infants (33).
Fluoride-containing dentifrice includes toothpaste and mouth rinses. Almost all toothpaste contains fluoride at concentrations of 1000-1500ppm (29), with the minority containing lesser amounts designed for children who inadvertently swallow more toothpaste. (4, 33, 34). In non-fluoridated areas of New Zealand, toothpaste intake is the main source of fluoride (32). Fluoride tablets containing approximately 0.5mg of fluoride (35) may be available to children living in low-fluoride areas to promote good dental health but are generally not recommended for population treatment in New Zealand (3, 6).

2.1.4 Nutrient Reference Values for New Zealand and Australia

The Ministry of Health recommends CWF at a level of 0.7-1.0ppm in order to provide optimal dental benefits (5). The Adequate Intake (AI) for fluoride varies based on life stage and gender and is adjusted as necessary with the goal of preventing dental caries (15). The reference values for children aged 0-8-years-old have recently been reviewed since dietary estimates of fluoride intake for this age group often exceed the Upper Limit of Intake (UL) but without any evidence of widespread moderate or severe dental fluorosis (36). Such findings indicated that the adequate and upper limits of intake for fluoride needed to be reviewed. The AI for 1-3-year-olds decreased from 0.7mg/day in 2006 to 0.6mg/day in 2017 and for 4-8-year-olds, the AI increased from 1.0 mg/day to 1.1 mg/day. The upper limit of intake for 1-8-year-olds doubled from 0.1mg/kg body weight/day in 2006 to 0.2mg/kg body weight/day in 2017 (36).
Table 2 Nutrient Reference Values for Fluoride: Children and Adolescents

<table>
<thead>
<tr>
<th>Age</th>
<th>Adequate level of intake (mg/day)</th>
<th>Upper limit of intake (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 months</td>
<td>-</td>
<td>1.2</td>
</tr>
<tr>
<td>7-12 months</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>1-3 years</td>
<td>0.6</td>
<td>2.4</td>
</tr>
<tr>
<td>4-8 years</td>
<td>1.1</td>
<td>4.4</td>
</tr>
<tr>
<td>9-13 years</td>
<td>2.0</td>
<td>10.0</td>
</tr>
<tr>
<td>14-18 years</td>
<td>3.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*New Zealand Nutrient Reference Values for Children and Adolescents (37)*

2.2 New Zealand’s Fluoride Intake

In New Zealand, dental caries continues to be one of the most prevalent chronic diseases affecting the population at all ages, despite the oral health of the nation improving over several decades (6). The prevalence of dental caries in New Zealand is higher in areas without fluoridated water than in areas with fluoridated water (38, 39). Children in New Zealand can receive free oral health care from birth until they reach their eighteenth birthday.

2.2.1 Fluoride Intake in New Zealand (children)

In 1990, a study measuring actual fluoride intakes of 11-13 month infants living in New Zealand found that infants living in fluoridated areas were consuming an average of 0.263mg of fluoride per day via diet. This was half the recommended level of 0.5 and 0.7mg/day for 7-12months and 1-3-year-olds, respectively. Those living in non-fluoridated
areas had a mean dietary fluoride intake of 0.082mg of fluoride per day which was 16% and 12% of the acceptable intake (33). When intake of fluoridated toothpaste and tablets were included, fluoride intake increased to 0.305mg/day and 0.195mg/day for those living in fluoridated and non-fluoridated areas, respectively. (33).

In 1996, Chowdhury et al (32) examined the fluoride content of 3- and 4-year-old children’s diets and found the contribution of toothpaste and diet to average daily fluoride intake was variable. Those living in non-fluoridated areas had a mean fluoride intake of 0.15mg/day which was significantly lower than those living in fluoridated areas, who had an average intake of 0.36mg/day (32). The results suggest that on average, 3-year-old children living in fluoridated areas met their recommended level of intake but 4-year-olds did not (15). On average, children living in non-fluoridated areas did not meet the recommended level of fluoride intake for their age group (15). For those living in non-fluoridated areas, the contribution of toothpaste and diet to average daily fluoride intake decreased significantly as age increased (32) which was likely due to decreased fluoride intake from ingested toothpaste, as the child grew older and swallowed less toothpaste during brushing. This decrease in toothpaste consumed impacted significantly on daily fluoride intake because toothpaste were the primary source of fluoride in non-fluoridated areas (32).

More recently, Cressey et al (4) conducted a study in which the mean daily fluoride intake of different age groups was estimated by assessing dietary information collected from several New Zealand nutrition and diet surveys. Information on the rates of toothpaste intake for children was not available so values were assumed, as was the number of times children brushed their teeth per day. They found that the estimated
average daily fluoride intake in all age groups, except infants aged 6-12 months, was below the Adequate Intake (AI). Fluoride intake was markedly higher in areas with fluoridated water supplies, although this was not significantly different to intakes from non-fluoridated areas.

2.3 Global Fluoride Status/History

As shown in Table 3, children living in an area without naturally high or artificially fluoridated water are less likely to be meeting the recommended level of fluoride intake (4, 7, 9, 32, 33, 40-43). Many children who live in areas with artificially fluoridated water also do not meet the optimal range for fluoride intake (4, 7, 9, 32, 33, 40). Fluoride tablets used to be a popular way to increase fluoride intake in areas without CWF (10), however, there is concern over children exceeding the UL of fluoride intake if they are already consuming optimally fluoridated water and toothpaste (1). A number of places worldwide, including New Zealand, have implemented CWF as another method of increasing population fluoride intake to meet the optimum range of 0.05-0.07mg fluoride/kg body weight/day. Approximately 380 million (5.7%) people worldwide have access to CWF (44), including approximately 211 million Americans (45) and 12 million Western Europeans (46). Some countries, such as Brazil and Iran (40, 43), have regions where fluoride is found naturally in water at high enough concentrations to be a primary source of fluoride. In many areas of the world, CWF is unavailable due to political or climatic reasons, so other approaches to community fluoridation have been proposed, such as salt and milk fluoridation (40).
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Country</th>
<th>Age (years)</th>
<th>Methods used to estimate TDFI (total daily fluoride intake)</th>
<th>Intake levels (daily average)</th>
<th>Sources of fluoride</th>
</tr>
</thead>
</table>
| Chowdhury et al. (1990) | New Zealand | 11-13 months | F vs NF areas  
**Diet:** Duplicate diet and breast milk samples were collected.  
**Analysis:** F content was measured using an F-specific electrode. | **F areas:** 0.130-0.679mg F/kg body weight via diet  
**NF areas:** 0.036-0.281mg F/kg body weight  
High F sources (tea and soy milk infant formula) or introducing F toothpaste and tablets increased intake to near the ‘optimal’ level for F. | Dietary sources containing high F include soy milk infant formula and tea. Non-dietary sources include toothpaste and F tablets. |
| Chowdhury et al. (1996) | New Zealand | 3-4 | F vs NF areas  
**Diet:** x3 24-hour duplicate diets  
**Clinical:** Expectorated toothpaste  
**Analysis:** Ion specific electrode. F content of the diet was calculated by multiplying dietary F content (mg F/kg of food) by the amount of food eaten (kg/day). The amount of ingested F from toothpaste was estimated by subtracting expectorated F from the amount of toothpaste put onto the toothbrush. | **F areas:** 0.68 ± 0.27mg/day  
- 3-year-olds  
- 4-year-olds  
**NF areas:** 0.49 ± 0.25mg/day | **F areas:** Diet in conjunction with toothpaste  
**NF areas:** Toothpaste |
<table>
<thead>
<tr>
<th>Author (year) Country</th>
<th>Age (years)</th>
<th>Methods used to estimate TDFI (total daily fluoride intake)</th>
<th>Intake levels (daily average)</th>
<th>Sources of fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cressy et al. (2010)</td>
<td>All age groups</td>
<td><strong>Diet:</strong> Food composition data and simulated diet information from two New Zealand Total Diet Studies, National Nutrition Survey and 2002 National Children’s Nutrition Survey. F area was based on a water concentration of 1.0mg F. NF area was based on a water concentration of 0.1mg F.</td>
<td><strong>All age groups:</strong> F intake was below the level of adequate intake. F intake was lower in NF areas than F areas.</td>
<td>7-10-year-old children:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>F areas:</strong> Water (41%), bread (7%), tea (5%), carbonated beverages (4%) and fruit drink (4%).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>NF areas:</strong> Bread, water, carbonated beverages and tea</td>
</tr>
<tr>
<td>Zohouri et al. (2006)</td>
<td>6 n = 33</td>
<td>F vs NF areas were categorised into three groups depending on their home tap water F concentration:</td>
<td><strong>Optimal F areas:</strong> 0.591mg/day (0.027mg/kg body weight/day)\downarrow</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td></td>
<td>• Optimal F (≤0.7ppm)</td>
<td><strong>Sub-optimal F areas:</strong> 0.349mg/day (0.016mg/kg body weight/day)\downarrow</td>
<td><strong>Optimal and suboptimal F areas:</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sub-optimal F (≥0.3 to ≤0.7ppm)</td>
<td><strong>NF areas:</strong> 0.188mg/day (0.008mg/kg body weight/day)\downarrow</td>
<td>Beverages made up with F tap water e.g. cordial, food cooked with water i.e. pasta, rice, vegetables.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• NF (≤0.3ppm).</td>
<td></td>
<td><strong>NF areas:</strong> Carbonated beverages and bread</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-day diet records and food/beverage samples were collected and analysed.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maguire et al. (2007)</td>
<td>6 - 7  n = 33</td>
<td>F vs NF schools.</td>
<td><strong>Optimally F areas:</strong> 0.047 ± 0.008mg/kg body weight/day\downarrow</td>
<td>F toothpaste is an important contributor to daily fluoride intake in children, especially for those living in NF areas.</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>NF areas: 18 Sub-optimally F areas: 9 Optimally F areas: 6</td>
<td><strong>Diet:</strong> 3-day food diaries, food and drink samples <strong>Biochemical:</strong> 24-hour urine samples <strong>Clinical:</strong> Expectorated toothpaste/ saliva analysis <strong>Analysis:</strong> F content of food and drink was analysed using direct and indirect silicon-facilitated diffusion methods.</td>
<td><strong>Sub-optimally F areas:</strong> 0.038 ± 0.038mg/kg body weight/day\downarrow</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NF areas:</strong> 0.031 ± .025 mg/kg body weight/day\downarrow</td>
<td></td>
</tr>
<tr>
<td>Author (year) Country</td>
<td>Age (years)</td>
<td>Methods used to estimate TDFI (total daily fluoride intake)</td>
<td>Intake levels (daily average)</td>
<td>Sources of fluoride</td>
</tr>
<tr>
<td>-----------------------</td>
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</tr>
<tr>
<td>Omid et al. (2017)</td>
<td>4 - 6</td>
<td>F area Two meetings, one week apart.</td>
<td>4-year-olds: 1.062 ± 0.699mg/day (0.057mg/kg body weight/day)</td>
<td>Toothpaste is a major source of F for this age group. On average, for 4-, 5- and 6-year-olds, toothpaste provided them with 46%, 45% and 42% of their daily F intake.</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>n = 61</td>
<td><strong>Diet:</strong> Duplicate diets, 3-day diet records. <strong>Clinical:</strong> Toothpaste/saliva expectorate. <strong>Analysis:</strong> Daily F intake was calculated by combining dietary and toothpaste intake. 24-hour urine samples were collected to estimate daily F excretion. Weekly variation in F intake and excretion were assessed.</td>
<td>5-year-olds: 1.223 ± 0.827mg/day (0.060mg/kg body weight/day) 6-year-olds: 1.202 ± 0.549mg/day (0.050mg/kg body weight/day)</td>
<td></td>
</tr>
<tr>
<td>Rodrigues et al. (2009)</td>
<td>4 - 6</td>
<td>Four communities in Brazil and Peru, each with different sources of F.  • Bauru, Brazil: artificially F water (AFW)  • Brejo dos Santos: naturally F water (NFW)  • Lima, Peru: F salt (FS)  • Trujillo, Peru: F milk (FM)  • Control community: NF  <strong>Diet:</strong> x2 duplicate diets  <strong>Analysis:</strong> An ion-selective electrode was used to analyse dietary F content of the diets.</td>
<td>All in mg/kg body weight/day:  <strong>Bauru, Brazil (AFW):</strong> 0.04 ± 0.01  <strong>Brejo dos Santos, Brazil (NFW):</strong> 0.06 ± 0.02  <strong>Lima, Peru (FS):</strong> 0.05 ± 0.02  <strong>Trujillo, Peru (FM):</strong> 0.06 ± 0.01  <strong>Control community (NoF):</strong> 0.01</td>
<td>Depending on the location, primary sources of F include water, milk and salt.</td>
</tr>
<tr>
<td>Brazil and Peru</td>
<td>n = 21-26 (in each of the four communities)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kimura et al. (2001)</td>
<td>1 - 6</td>
<td><strong>Diet:</strong> x3 24-hour duplicate diets (covering all seasons). The fluoride concentration in tap water was &lt;0.05ppm.  <strong>Analysis:</strong> The diets were homogenised and F content was analysed using an F electrode.</td>
<td>F: 0.28 ± 0.18mg/day with a range of 0.04-1.24mg/day</td>
<td>Japan does not have a water F program, nor do they use F supplements, which means that food and toothpaste are the main sources of F.</td>
</tr>
<tr>
<td>Japan</td>
<td>n = 29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author (year) Country</td>
<td>Age (years)</td>
<td>Methods used to estimate TDFI (total daily fluoride intake)</td>
<td>Intake levels (daily average)</td>
<td>Sources of fluoride</td>
</tr>
<tr>
<td>-----------------------</td>
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<td>-------------------------------------------------------------</td>
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</tr>
</tbody>
</table>
| Nohno et al. (2006) Japan | 2 - 8 | Moderately F areas (MFA): 0.555ppm vs low F areas (LFA): 0.040-0.131ppm. **Diet:** 24-hour duplicate diet | **MFA (mg/kg body weight/day):**  
- 2-5-years-old: 0.0252↓  
- 6-8-years-old: 0.0254↓  
**LFA (mg/kg body weight/day):**  
- 2-5-years-old: 0.0126↓  
- 6-8-years-old: 0.0144↓  
The difference in F intake between the two areas was only significant in 2-5-year-old children. | Some areas of Japan have water that is F naturally to a moderate or low level. Rice that is boiled using naturally F water is an important source of F in these areas. |
| Haftenberger et al. (2001) Germany | 3 - 6 | **Diet:** Duplicate diet  
**Biochemical:** 24-hour urine samples  
**Clinical:** Toothpaste/saliva expectorate.  
**Analysis:** F determined by ionometer coupled to a fluoride-specific electrode | F: 0.931 ± 0.392 mg/day  
(0.053 ± 0.021 mg/kg body weight/day)  
**% contribution to daily F intake:**  
F tablets: 48.8%  
Toothpaste: 29.4%  
Diet: 21.8% (partly due to F salt) | |
| Zohouri et al. (2000) Iran | 4 | **LFA:** 0.3-0.35ppm  
**MFA:** 0.6ppm  
**HFA** (high fluoride area): 4.0ppm  
**Diet:** x2 3-day diet records. >117 items of food and drink were collected.  
**Analysis:**  
Food and drink samples: silicon-facilitated diffusion method in un-ashed samples.  
Diet records: Applied F content of food and drink samples and F data from several food composition tables. | **LFA:** 0.413 mg/day  
**MFA:** 0.698 mg/day  
**HFA:** 3.472 mg/day | Primary sources of F in all areas was water and drinks made with water i.e. tea.  
White rice and white bread contributed 5-8% dietary F in all areas. |
<table>
<thead>
<tr>
<th>Author (year) Country</th>
<th>Age (years)</th>
<th>Methods used to estimate TDFI (total daily fluoride intake)</th>
<th>Intake levels (daily average)</th>
<th>Sources of fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rojas-Sanchez et al. (1999) USA, Puerto Rico</td>
<td>16 – 14mo</td>
<td><strong>Optimal F area:</strong> Indianapolis, Indiana (0.8-1.2ppm) <strong>LFA:</strong> Connersville, Indiana and San Juan, Puerto Rico (&lt;0.3ppm) <strong>Diet:</strong> x2-3 duplicate diets <strong>Clinical:</strong> Expectorated toothpaste/saliva samples <strong>Analysis:</strong> Hexamethyldisiloxane microdiffusion</td>
<td><strong>Indianapolis:</strong> 0.965 ± 0.009 mg/day (0.07 ± 0.007 mg F/kg body weight/day) <strong>Connersville:</strong> 0.965 ± 0.009 mg/day (0.073 ± 0.007 mg F/kg body weight/day) <strong>San Juan:</strong> 0.767 ± 0.0075 mg/day (0.056 ± 0.0004 mg F/kg body weight/day)</td>
<td><strong>All areas:</strong> Toothpaste <strong>Indianapolis and Connersville:</strong> Water <strong>San Juan:</strong> Connersville is surrounded by optimal F areas, which impacted on F intake due to the ‘halo’ effect.</td>
</tr>
</tbody>
</table>

F= fluoride/fluoridated, NF= non-fluoridated
ppm= parts per million
↓= below optimal level of intake
Optimal level of intake based on 0.05-0.07mg F/kg bw/day (36, 47)
2.4 Assessment of Fluoride Status in Humans

Fluoride intakes in children have been assessed using a combination of dietary, biochemical, and clinical assessment methods.

2.4.1 Dietary Assessment

Duplicate Plate Method:

The majority of studies that assessed dietary intake of fluoride in children, did so using the duplicate plate method, as described by Chowdhury et al (33) because it measures actual fluoride intake (11, 48). For this method, parents collected duplicates of meals and beverages consumed over a set period of time, typically 1 to 3 days. This was often repeated a short time later to account for dietary variations, such as on weekends (48, 49). The importance of maintaining the child’s usual diet was stressed (32, 50). Parents were provided with collection containers. Some beverages may have been collected separately, such as fluoridated water and/or milk samples (40, 42). Parents were asked to wait until the child had finished eating their meal, then remove any parts of the meal not eaten by the child from the duplicate meal before putting it in the collection container for storage (32, 51). Commonly removed parts of food included bones, seeds, skin and cores (32). To assess the fluoride content of meals, the duplicate portions were pooled together, weighed and homogenised with a known amount of deionised water (32, 48). Aliquots of these samples were then frozen until fluoride analysis (42, 51). The main limitation of the duplicate plate method includes the burden of extra cost associated with producing two meals instead of one, which may result in a reduced response rate or cause individuals to
alter their usual dietary habits, resulting in reporting bias (48). If the duplicate plate method alone is used to assess fluoride intake, it will be impossible to assess the main sources of fluoride in the diet because the collected food and beverages are homogenised into one sample to assess fluoride content (48).

*Diet Method:*

Diet records only provide an estimate of fluoride intake because the data generated by diet records are usually based on information from food composition tables (7, 48). In New Zealand, Cressey et al (4) used fluoride values from two New Zealand Total Diet Studies. It is not uncommon that there is limited data on fluoride (52), thus very few studies have used the food diary method in isolation to assess dietary fluoride intake in children. In contrast to the duplicate plate method, food records allow some individual sources of fluoride in the diet to be identified and are less burdensome for individuals to complete (48). The majority of studies looking at fluoride intake used diet records in conjunction with the duplicate plate method to validate the accuracy of the data collected in the diet record (10, 48). Where scales could not be provided, individuals were asked to estimate the portions of food eaten using household measures, such as measuring cups and spoons (10, 48).

**2.4.2 Toothpaste Analysis**

Recent studies (11) have calculated intake of fluoride from toothpaste using a method as described by Maguire et al (7). This involves asking the children to brush their teeth as they usually would. The toothbrush is weighed before and after toothpaste is added to it, in
order to calculate the amount of toothpaste usually used. Any expectorated saliva and toothpaste is collected in containers, as is any water used to rinse the toothbrush or mouth. Any toothpaste left on the face or toothbrush is also collected for analysis using a spatula. These samples are weighed and homogenised to a slurry before being frozen until fluoride analysis. The frequency of brushing per day is recorded, as is the brand and concentration of toothpaste used. Fluoride ingested from toothpaste can be estimated by calculating the difference between the fluoride content of the toothpaste and water used, and the fluoride content of expectorated saliva and toothpaste recovered after brushing (10). The fluoride concentration of expectorated saliva/toothpaste samples is measured using a fluoride specific electrode (8). Total daily fluoride intake from tooth brushing is calculated by multiplying fluoride ingested during brushing by the frequency of brushing per day (11).

2.4.3 Biochemical Assessment

*Urine as a biomarker for fluoride intake*

The collection of urine samples is a non-invasive method that indirectly assesses fluoride intake (53). The World Health Organisation (WHO) have developed basic methods to guide the assessment of fluoride intake via urine (54). Twenty-four-hour urine samples are required since the urinary fluoride concentration fluctuates during the day (55). Often the first void of the day is not included (53, 54), but every subsequent void for the next twenty-four hours, including the first void of the next day, is collected and pooled (11, 53). The total amount of urine is weighed to assess the total daily excretion volume (11) and from this sample, aliquots are taken and frozen until analysis (53). In children, daily fluoride
excretion varies between 30-80% (8, 11). The high variability is most likely due to age, dietary intake and oral hygiene habits (8). The fluoride concentration of urine is also influenced by glomerular filtration rate and urinary pH (13). A primary limitation of collecting 24-hour urine samples from children is the possible lack of compliance (53). Urine has been used as an indirect measure of individual fluoride intake for many years, however, the World Health Organisation (54) states that using urinary fluoride excretion to estimate individual fluoride intake is inappropriate, as there is an absence of standard correlation between intake and excretion of fluoride. Urine samples are primarily assessed by using a fluoride specific electrode and potentiometer after the sample has been mixed with total ionic adjustment buffer (TISAB) I, II or III (8).

Nails as Biomarkers for Fluoride Intake

Several studies have used nails as biomarkers to monitor varying levels of exposure to fluoride (56-58). In contrast to urine, fingernails represent the average intake of fluoride over the past three months (59) rather than the fluoride intake over recent days, thus nails may be a better indicator of chronic individual fluoride intake (53). No guidelines or reference values have yet been developed for the process of using nails as biomarkers (53). Nail clippings of at least 1.5mm in length are needed to be able to analyse their fluoride content (60). Buzalaf et al (53) and Fukushima et al (58) contacted parents two weeks prior to when the nail samples would be taken and asked them to avoid cutting their child’s nails for this time to allow for adequate growth. Buzalaf et al (56) compared finger and toenails and concluded that the big toenail is the most suitable biomarker of fluoride intake due to
its faster growth rate and reduced exposure to environmental contaminants. Nails must be cleaned and unpolished to be used (59).

**Conclusion**

Fluoride plays an essential role in maintaining good dental health, especially during childhood. In areas where the water is fluoridated, tap water and toothpaste are the primary sources of fluoride. In areas without fluoridated water, diet and toothpaste are the primary sources of fluoride. Usually areas without fluoridated water have a lower daily intake of fluoride, however, this may begin to change due to food and beverage products from fluoridated areas being sold in non-fluoridated areas and vice versa. Studies conducted in New Zealand estimated that children living in fluoridated and non-fluoridated areas are not meeting the AI for fluoride. The most appropriate methods for assessing fluoride intake in children are 24-hour duplicate diets, diet records and urine samples, although nail sampling has emerged as being an indicator of long-term fluoride intake.
3 Objective Statement

Only two New Zealand studies have directly measured fluoride intake; both were conducted in the 1990’s, one in infants (33) and the other in 3-4-year-old children(32). In 2010, Cressey et al (4), estimated the fluoride intake for the New Zealand population, at all ages, by assessing dietary information collected by two New Zealand Total Diet Studies, the 1997 National Nutrition Survey and the 2002 National Children’s Nutrition Survey. The estimated fluoride intake for all age groups, except 6-12-month infants, fell below the AI. Internationally most studies focus on children aged 0-8-years because this is when permanent teeth are formed, but oral health is important across the lifespan. There are no published data on actual fluoride intakes in older children and adolescents.

Aim: To trial the feasibility of measuring actual fluoride intakes of 9-10-year-old New Zealand children living in the fluoridated and non-fluoridated cities of Dunedin and Timaru, respectively.

In order to obtain this aim, the objectives of this study are to:

i. Assess the methods used to determine the contribution of diet to total fluoride intakes;

ii. Assess the methods used to determine the contribution of fluoride from toothpaste inadvertently swallowed.
4 Methods

4.1 Outline of Study Design

This pilot study aimed to look at the feasibility of the methods used to determine the fluoride intake of 9- and 10-year-old children in New Zealand. This research took place in the cities of Dunedin and Timaru, in the South Island of New Zealand. Dunedin has a fluoridated water supply whilst Timaru does not. Data collection took place between March-May 2017. Ethical approval was obtained from the University of Otago Health Ethics Committee (reference HE17/001) (Appendix A).

4.2 Participants and Recruitment

The inclusion criteria for participation was as follows: healthy children aged 9- and 10-years-old at the time of the study. Siblings were excluded. Ten children from each area were recruited into the study for a final sample size of 20 children. In Dunedin, children were recruited via word of mouth and by advertising on Facebook. In Timaru, children were recruited via word of mouth and through two local primary schools known to the candidate. The schools were contacted by email and asked to distribute the study information pamphlet and letter to children of suitable ages.

Interested children and their parents were each provided with an Information for Participants form and Consent form (Appendix B and C). Caregivers and children who
returned a signed consent form were then contacted by phone or email to arrange a suitable time for the researchers to visit their home for data collection.

4.3 Data Collection

Eleven children participated in two data collection periods each lasting 24 hours, which took place 7-21 days apart. Nine children participated in one day of data collection, rather than two due to time constraints. Data collected during each period included; an expectorated toothpaste and saliva sample, a 24-hour urine sample, a 24-hour weighed diet record and a 24-hour duplicate diet. Caregivers were contacted prior to the first visit and asked to not clip the child’s toenails to allow adequate time for the nails to grow. Toenail clippings were collected during either the first or second 24-hour period.

The researchers, caregivers and children met on two or four occasions. On the first occasion, the researchers visited the children’s homes to explain the study and provide instruction on sample collection. The children completed the online survey (Appendix D) about their tooth-brushing habits, dental history and socio-demographic characteristics. This survey was an altered version of the “Questionnaire on the use of fluoride supplements and toothpaste” for children, created by the World Health Organisation (61). The child’s weight was measured using a Seca electronic scale and height was measured using a tape measure and results entered into the survey. Researchers provided caregivers with all the necessary urine and food sample collection vessels, electronic kitchen scales, a form for completing the 24-hour diet record, and a booklet with instructions for the duplicate diet and urine collection. Verbal instructions on how and when to collect samples
were given, and the importance of the children following their usual diet and drinking habits was emphasised. At the end of the visit, children were asked to brush their teeth and expectorated saliva/toothpaste was collected, described in more detail later. The second visit took place on the day following the sample collection, when researchers picked up the first set of completed 24-hour urine sample, diet record, duplicate diet bucket. One set of toenail clippings from each child were collected by the researchers at the second visit, and frozen at -20°C. The third and fourth visit followed a similar pattern, with a second 24-hour urine sample, expectorated saliva/toothpaste sample, diet record, and duplicate diet obtained from each participant collected at the fourth visit. Children were given two movie tickets and parents were given a $30 supermarket voucher as a token of appreciation. A follow-up questionnaire was sent out to caregivers following completion of the study to ask about their experience of the study (Appendix D).

*Dietary Assessment*

Dietary fluoride intake was determined by the duplicate plate and 24-hour diet record methods. Caregivers were asked to make duplicate portions of all their child’s food and drink for 24-hours. The caregiver observed what the child ate and replicated this by removing and discarding any uneaten parts of the meal from the duplicate portion, including commonly discarded leftovers such as bones, fruit/vegetable cores and skins. All duplicate portions of food and drink were placed in a plastic 2L bucket, with a lid, and caregivers asked to keep the bucket stored in a cool place, such as the refrigerator.
Caregivers were also given a diet record form to fill out over the 24-hour time period. Details about the food or drink including brand, preparation or cooking method, and weight were noted in the record booklet at the time of consumption. For recipes, all ingredients were weighed and recorded in total, and once the meal was assembled the caregivers were asked to weigh the proportion of the recipe eaten by the child.

24-hour urine collection
Children were provided with a 2L screw-top bottle, along with a funnel and bowl to assist with urine collection. The children collected their urine over the same 24-hour time period being used to collect weighed diet records and duplicate diets. The time of the first void of the day was recorded by caregivers and this urine was discarded. All urine for the remaining 24-hours, including the first void of the following morning, was collected into the 2L bottles; any missing void was noted.

Expectorated toothpaste and saliva:
At the first visit, the contribution of toothpaste to total daily fluoride intake was estimated using the method of Maguire et al (7) as follows. The child’s toothbrush was weighed before and after toothpaste was applied by the child, using a scale (Sartorius type 1475 MP 8-2) and the weight of the toothpaste noted. The fluoride content of the toothpaste was recorded and it was assumed that the concentration of fluoride in the toothpaste was as stated on the tube. The child was then asked to brush their teeth as normal, with all expectorated toothpaste and saliva collected in the potte, as was any residue on the face. If
the child completed two periods of data collection, this process was repeated at the third visit.

4.4 Sample Preparation and Analysis

The day following data collection, researchers weighed the duplicate diet, subtracting the weight of the bucket, to determine the weight of food eaten by the child. The food was homogenised by an industrial-strength Waring blender until smooth. Known amounts of deionised water used to rinse the bucket of any food residue were added to the blender. Once homogenised, researchers took 50g and 200 g samples that were frozen at -20°C until required for analysis. Due to time constraints, the fluoride content of duplicate diet and nail samples will not be analysed in this study.

Information on the fluoride content of 75 food and beverages was obtained from the 2016 New Zealand Total Diet Study (62). The fluoride content (mg/100g) was entered into the nutrient lines of Kaiculator, a food analysis programme developed at the University of Otago. The fluoride values from the 2016 Total Diet Study were mapped to all similar foods (4). For example, ‘wheatmeal bread’ fluoride values were mapped to all types of wheatmeal bread, including bread rolls. Any foods without a fluoride value were assigned a fluoride value of 0.0001mg/100g. The fluoride concentration of Dunedin water was 0.75ppm, and was 0.12ppm in Timaru (62). Each diet record was entered into Kaiculator and was analysed for daily fluoride, calcium, energy and protein intake. The food concentration values in Kaiculator for calcium, energy and protein were sourced from the New Zealand Food Composition Database (Foodfiles) (63). Energy underreporting was
analysed by dividing the reported energy intake by the estimated energy expenditure for 9-10-year-old boys and girls (64). If the value calculated was less than 0.9 the child was classified as underreporting (64). If a child underreported during one data collection period, but not the other, they were not excluded as this may have been due to under-eating rather than under-reporting. For Dunedin diets, the fluoride found in cooked pasta and rice, due to high water retention, was accounted for by adding fluoridated water to the recipe (Appendix F). Several assumptions were made about one participant’s diet record, as detailed in Appendix F.

At the end of the 24-hour collection, the total volume (weight) of urine was recorded, and two 10mL aliquots of urine were placed in a test tube and frozen at -20°C until analysis. Completeness of 24-hour urine samples was assessed by comparing them to the lower limit of 9mL/hour for children >6-years-old (65). Any sample that did not meet this criterion was excluded from analysis. The fluoride content of the urine was measured by a fluoride ion specific electrode, after the total ionic adjustment buffer (TISAB) III had been added to the urine in a 1:1 ratio (66). The fluoride content of the urine samples was calculated using a standard curve. The fluoride standards used to create the curve ranged from 0.1 – 25µg/mL (66). The standards were made using fluoride standard (1000mg/L) and deionised water (66). TISAB III was mixed in a 1:1 ratio with the fluoride standard and deionised water (67).

Expectorated toothpaste/saliva samples were weighed and frozen at -20°C until required for analysis. Toothpaste samples were diluted to 100mL with deionised water, then diluted again in a 1:4 ratio with deionised water. The fluoride content of the
toothpaste samples was analysed by a fluoride-specific electrode. The fluoride concentration of the expectorated toothpaste and saliva samples were calculated using a set of standards to the one mentioned above, based on the matrix of the solutions (i.e. urine diluted with TISAB and expectorated saliva diluted with deionised water). The fluoride standards used to create the curve ranged from 0.01-100µg/mL and were made using fluoride standard (1000mg/L) and deionised water. The amount of fluoride ingested from toothpaste was determined by subtracting the amount of fluoride found in the expectorated toothpaste/saliva sample from the total amount of fluoride contained in the toothpaste first applied to the toothbrush. This value was then multiplied by the number of times a day children brushed their teeth (as noted in the survey) to determine total daily fluoride intake from toothpaste.

For diet, toothpaste and urine statistical analysis, values for children who completed two periods of data collection were averaged before being combined with the values for children who completed one period of data collection.
5 Results

In Dunedin, 10 participants were recruited via word of mouth and the ‘Dunedin Mums’ Facebook page. Five Timaru primary schools were contacted, but only two replied and were willing to circulate study information to children and parents. In Timaru, 10 participants were recruited via word of mouth and by contacting local primary schools.

As shown in Table 4, the socio-demographic characteristics of the Dunedin and Timaru participants were similar. The average age of the Dunedin children was 9.5 years and the average age of the Timaru children was 9.6 years. The body mass index (BMI) of Dunedin and Timaru children was 18.4 and 16.6 kg/m$^2$, respectively, as Timaru children had a lower average body weight (33.4kg) than the Dunedin children (37.0kg). Thirteen (65%) of the participants were male and seven (35%) were female. Of the 20 children who participated in the study, 11 completed two 24-hour periods of data collection.
Table 4 Summary of socio-demographic characteristics of participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dunedin</th>
<th>Timaru</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 10</td>
<td>n = 10</td>
<td></td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Age (years) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European/Pakeha</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>Māori</td>
<td>20</td>
<td>0</td>
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<td>Asian</td>
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<tr>
<td>Other</td>
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</tr>
<tr>
<td>Height (m)</td>
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</tr>
<tr>
<td>Weight (kg)</td>
<td>37</td>
<td>33.4</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>18.4</td>
<td>16.6</td>
</tr>
<tr>
<td>Deprivation level$^b$</td>
<td>4.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>

$^a$ Area deprivation index as determined by the 2006 Census of Population and Dwellings, supplied by Statistics New Zealand (68)

The mean daily energy intake for Dunedin children was 7418kJ/day, and 7074kJ/day for Timaru children. During the first data collection period, only one child from Dunedin underreported their energy intake and no children underreported during the second data collection period. On average, Dunedin children met the estimated average requirement (EAR) for protein intake and nearly met the EAR for calcium intake. On average, children from Timaru cities met the EAR for daily protein but not for calcium.
Table 5 Mean (SD) energy, protein and calcium intake in Dunedin and Timaru.

<table>
<thead>
<tr>
<th>City</th>
<th>n</th>
<th>Energy (kJ/day)</th>
<th>Protein (g/day)</th>
<th>Calcium (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunedin</td>
<td>10</td>
<td>7418 (1136)</td>
<td>69 (25)</td>
<td>865 (400)</td>
</tr>
<tr>
<td>Timaru</td>
<td>10</td>
<td>7074 (1646)</td>
<td>63 (16)</td>
<td>704 (289)</td>
</tr>
</tbody>
</table>

NRV

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<tr>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td>6475-8870&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>800&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boys</td>
<td>7812-10480&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>800&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Estimated using weight, height, BMR (Basal Metabolic Rate) and a PAL (Physical Activity Level) of 1.55 (37)

<sup>b</sup> EAR (37)

Daily average fluoride intake from diet and toothpaste ingestion are shown in Table 6. The mean daily dietary fluoride intake for Dunedin children was 0.7mg/day (0.02mg F/kg bw/day), while for Timaru children it was 0.2mg/day (0.01 mg F/kg bw/day). As shown in Figure 1, the primary dietary source of fluoride for Dunedin children was fluoridated water, which contributed to 75% of dietary fluoride intake. Whilst not artificially fluoridated, water, followed by breads and cereals, were the primary sources of dietary fluoride for Timaru children, contributing to 38% and 35% of dietary fluoride intake, respectively. On average, diet alone contributed to 43% of total daily fluoride intake (TDFI) in Dunedin, and 18% in Timaru.
Table 6 Mean (SD) fluoride intake from diet and toothpaste

<table>
<thead>
<tr>
<th>City</th>
<th>n</th>
<th>Mean (SD) Daily Fluoride Intake</th>
<th>AI (mg/day)a</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diet</td>
<td>Toothpaste</td>
<td>Diet + Toothpaste</td>
</tr>
<tr>
<td>Dunedin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/day</td>
<td>10</td>
<td>0.705 (0.357)</td>
<td>0.951 (0.414)</td>
<td>1.656 (0.555)</td>
</tr>
<tr>
<td>mg F/kg bw/day</td>
<td>10</td>
<td>0.018 (0.008)</td>
<td>0.038 (0.015)</td>
<td>0.056 (0.015)</td>
</tr>
<tr>
<td>Timaru</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg/day</td>
<td>10</td>
<td>0.212 (0.060)</td>
<td>0.987 (0.524)b</td>
<td>1.199 (0.600)</td>
</tr>
<tr>
<td>mg F/kg bw/day</td>
<td>10</td>
<td>0.006 (0.001)</td>
<td>0.030 (0.013)</td>
<td>0.032 (0.018)</td>
</tr>
</tbody>
</table>

a Ministry of Health NRVs for children aged 9-10-years-old (36)
b One participant excluded from toothpaste analysis. If outlier included, mean F intake from toothpaste = 1.7 (2.3) mg/day

Figure 1 Contribution of food groups to dietary fluoride intake
Toothpaste ingestion was a major source of fluoride in both Dunedin and Timaru, on average contributing 1.0 ± 0.4 mg/day (57%) and 1.0 ± 0.5 (82%) to TDFI, respectively. The mean amount of toothpaste used by Dunedin children each day was 1.5g, while Timaru children used 1.1g. Children in both Dunedin and Timaru brushed their teeth an average of two times a day (ranged from 1-3 times per day). No Dunedin children swallowed their toothpaste after brushing, but in Timaru during the first data collection period, three children swallowed their toothpaste and during the second data collection period, two children swallowed their toothpaste (i.e. there was no expectorated toothpaste and saliva). The average fluoride concentration of toothpaste used by the children was 1000ppm in Dunedin and 1200ppm in Timaru. No children reported using fluoride tablets, therefore diet and toothpaste were their only sources of fluoride. Toothpaste ingestion data were excluded for one participant because of an unusually high fluoride intake from toothpaste (8.12mg/day).

Overall, neither the Dunedin or Timaru children met the AI for fluoride intake. Dunedin children had average total daily fluoride intake of 1.7 ± 0.6mg (0.06 ± 0.02mg F/kg bw/day) which is 83% of the fluoride required to meet the AI for daily fluoride intake. On average, Timaru children had a total daily fluoride intake of 1.2 ± 0.6mg (0.03 ± 0.02 mg F/kg bw/day) which is 65% of the daily fluoride required to meet the AI. No children exceeded the UL for fluoride intake of 10mg/day.
Table 7 Mean (SD) urinary fluoride excretion in Dunedin and Timaru.

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) Daily Urinary Fluoride</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dunedin n=10</td>
<td>Timaru n=9a</td>
</tr>
<tr>
<td>Mean volume of urine (L/day)</td>
<td>0.790 (0.244)</td>
<td>0.908 (0.430)</td>
</tr>
<tr>
<td>DUFE: mg/day</td>
<td>0.378 (0.155)</td>
<td>0.249 (0.123)</td>
</tr>
<tr>
<td>mg F/kg bw/day</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>

a One participant excluded due to an incomplete urine sample
b FUFE = (DUFETDFI)*100

Data on daily urinary fluoride excretion (DUFET) are presented in Table 7. Data from one child were excluded due to an incomplete urine sample. Dunedin children had an average daily urine volume of 0.790L which is lower than that of Timaru children who, on average, had a daily urine volume of 0.908L. On average, Dunedin children excreted 0.4 ± 0.6 mg of fluoride per day whilst Timaru children excreted 0.2 ± 0.1 mg of fluoride per day in their urine. Based on average body weight, mean DUFET was 0.01 ± 0.003 mg F/kg bw/day in Dunedin children and 0.008 ± 0.004 mg F/kg/bw/day in Timaru children. Both Dunedin and Timaru children excreted approximately 22% of ingested fluoride in their urine, with up to 78% of ingested fluoride being retained.

The results from the follow-up survey showed that 12 caregivers found urine collection to be the easiest aspect of data collection and six found the duplicate diet to be the most difficult, closely followed by completing the diet record. Five caregivers
responded saying they found no part of the study difficult to complete. Most (87%) caregivers said they did not change how their child ate during the study, but 7% said they made meals that were cheaper and 7% said they made meals that were easier to make, thus easier to write down in the diet record. Children did not drink less to avoid, or reduce urine collection.
6 Discussion

This pilot study demonstrated that it is feasible to conduct a study to assess the fluoride intake of 9-10-year-old children living in New Zealand. The results of this study also show that the fluoride intake of children living in both fluoridated and non-fluoridated areas of New Zealand falls below the Adequate Intake. However, since this is a pilot study and only 20 children participated, some caution is needed when interpreting the results.

6.1 Fluoride Status of 9-10-year-old children in Dunedin and Timaru

The mean total daily fluoride intakes for Dunedin and Timaru was 0.06 ± 0.02mg F/kg bw/day and 0.03 ± 0.02 mg F/kg bw/day, respectively. These values are similar to those for 6-7-year-old children living fluoridated and non-fluoridated areas of the United Kingdom, who had intakes of 0.05 ± 0.01 and 0.03 ± 0.03mg F/kg bw/day, respectively (7). The fluoride intake of Dunedin and Timaru children were higher than the intakes reported for 4-6-year-old children living in artificially (0.6-0.8ppm) and non-fluoridated areas of Brazil (40), who had fluoride intakes of 0.04 and 0.01 mg F/kg bw/day, respectively.

The fluoride content of Dunedin drinking water is 0.75ppm, which falls within the level recommended for CWF of 0.7-1.0ppm as set by the New Zealand Ministry of Health. The fluoride concentration of Timaru drinking water is 0.12ppm. The fluoride water concentrations are similar to water from fluoridated and non-fluoridated areas in Brazil (40) and the United Kingdom (9). As shown by the difference in mean dietary fluoride intake and total daily fluoride intake between Dunedin and Timaru children, fluoridated water plays a key role in improving the daily fluoride intake of children and increased
fluoride intake by 0.5mg/day. Before fluoridated water was included in the dietary analysis, Dunedin children had a similar dietary fluoride intake to Timaru children. However, the results of the pilot study show that even with fluoridated water, diet alone does not provide sufficient fluoride to achieve the recommended fluoride intake of 2mg/day.

Dietary fluoride intake has been underestimated in the present study because New Zealand fluoride values were not available for many foods, however, the extent of the underestimation is likely to be small. Fluoride values for dairy, meat and seafood groups were not determined as part of the 2016 New Zealand Total Diet Study because of methodological difficulties. These are important and frequently consumed food groups for 9-10-year-old children. In 6-7-year-old children living in optimally fluoridated areas of the United Kingdom, fish and seafood, and meat and meat products contributed <1 and 5µg to total daily fluoride intake, respectively (9). However, in non-fluoridated areas of the United Kingdom, seafood and fish contributed 7µg of fluoride to children’s total daily fluoride intake, and meat and meat products contributed 17µg of fluoride (9), similar to the daily fluoride intake from bread (20µg/day). Bread has previously been noted as a primary dietary source of fluoride for children living in non-fluoridated areas (4, 9) but meat and meat products have not. In both areas of the United Kingdom, milk provided 2-3µg of fluoride to mean total daily fluoride intake (9).

If the fluoride content values of meat and meat products, dairy, and fish and seafood in the United Kingdom (69) were used for the quantities of these foods eaten by Dunedin and Timaru children, this might increase fluoride intakes in New Zealand children
of this age by 6.5-8.5µg/day. Similar to the United Kingdom children (9), milk may have added 2-3µg to the total daily fluoride intake of both groups of children, and a similar amount added again by cheese. In the present study, few children ate beef, chicken or seafood, and sausages were the most popular meat product which might have contributed an extra 2.5µg/day of fluoride to their total daily fluoride intake. The theoretical contribution of meat and seafood to dietary fluoride intakes are quite similar for Dunedin children and 6-7-year-old children living in fluoridated areas of the United Kingdom. However, the fluoride contribution from these food groups is much lower for Timaru children, compared to the children living in non-fluoridated areas of the United Kingdom as mentioned above (9). Fluoride values provided by a United Kingdom database (69) could not have been used in the present study because the fluoride content of New Zealand foods may differ to foods grown and purchased in the United Kingdom.

Toothpaste was the primary source of fluoride for Dunedin and Timaru children, and although the amount of fluoride ingested from toothpaste was similar between cities, toothpaste contributed to a greater proportion of total daily fluoride intake in Timaru children (82%). The higher contribution of toothpaste to the total daily fluoride intake of Timaru children is consistent with previous studies that report toothpaste as the primary source of fluoride in non-fluoridated areas (7, 32). However, no studies have reported a contribution to total daily fluoride intake as high as 82%, observed for Timaru children. This could be due to younger children (or their parents) putting less toothpaste on their toothbrushes and/or the impact of some Timaru children swallowing their toothpaste which is usually more common in much younger children (7). It is also uncommon for toothpaste
to provide the greatest amount of fluoride to total daily fluoride intake for children living in fluoridated areas, as it did in Dunedin (i.e. 57%). Previous studies have reported lower toothpaste contributions to total daily fluoride intake of 47% for both 3-4-year-olds in fluoridated areas of New Zealand (32) and 6-7-year-old children living in optimally fluoridated areas in the United Kingdom (7). These differences in toothpaste fluoride contribution may be due to Dunedin children using more toothpaste and drinking less fluoridated water than in other populations. The results of the present study suggest that toothpaste ingestion is the most important source of fluoride for 9-10-year-old children, regardless of whether they have access to fluoridated water or not. However, it is important to note that, as residual fluoride on the toothbrush was not determined in the present study, it is likely that fluoride intake from toothpaste was overestimated which may have resulted in the lower FUFE values seen compared to other values published in the literature (8, 11).

Based on the results of this pilot study, if CWF was implemented throughout New Zealand it is unlikely that 9-10-year-old children would exceed the UL of 10mg/day. Diet alone contributes very little to total daily fluoride intake, and whilst fluoridated water and toothpaste contribute more fluoride, on average Timaru and Dunedin children did not meet the AI of 2mg/day. For 9-13-year-olds, the recommended intake (AI) for water consumption is 1.9 and 2.2L a day for girls and boys, respectively (37). If Dunedin children had met the AI for daily water consumption, fluoridated water alone could have contributed 1.4-1.7mg of fluoride per day to total daily fluoride intake which in addition to the average dietary and toothpaste fluoride ingestion, may have resulted in an average total daily fluoride intake of 3.03-3.33mg/day. This is above the AI but still well below the UL.
6.2 Feasibility & Recommendations for Future Research

Completed data collection and analysis show that it is feasible to assess fluoride intake in 9- and 10-year-old children in New Zealand. Although the children were old enough to complete some aspects of data collection on their own, such as urine collection and filling in parts of the diet record, parental guidance and assistance during the study was very important. If the study was to be undertaken with children younger than 9- and 10-years-old, heavy parental involvement would be required to ensure that data collection was completed correctly. Younger children may have more difficulty collecting 24-hour urine samples, so it may be more practical to use spot morning urine samples to estimate 24-hour fluoride excretion, as detailed by Zohoori et al (55). Many parents reported that completing the duplicate diet was the most difficult part of the study. If the fluoride content of the duplicate diets and diet records was very similar, only the diet record would need to be completed in future studies which would be less burdensome for participants. The analysis of expectorated toothpaste and saliva was successful and quick to complete, as was the urine analysis so it would be feasible to undertake this study on a larger scale. For future studies, the collection of and analysis of 24-hour diet records, expectorated toothpaste and saliva samples, and 24-hour urine samples is suggested. Furthermore, one data collection period, not two, is recommended and supported by a recent study that found there was no weekly significant variation in children’s total daily fluoride intake (8).
6.3 Strengths and Limitations

6.3.1 Strengths

This is the first study conducted that measured the actual fluoride intakes of 9-10-year-old children living in fluoridated and non-fluoridated areas of New Zealand, using 24-hour diet records, 24-hour urine samples and expectorated toothpaste/saliva samples. This pilot study identified the methods that are the most important for assessing daily fluoride intakes in New Zealand children and that could be replicated reliably in future studies.

The samples and information collected during this pilot study provide a good estimate of fluoride intakes because the children’s caregivers reported that their child did not drink less to avoid, or reduce, urine collection and the majority did not change how their child ate during the data collection periods. The accuracy of the diet records and duplicate diets were checked by comparing the sum of the weights recorded in the diet record, and the total weight of the food and drink collected in the duplicate diet bucket. This pilot study used the most recently available information on the fluoride content of New Zealand foods which was sourced from the 2016 New Zealand Total Diet Survey (62).

6.3.2 Limitations

As discussed above, fluoride data was not available for all food and beverages in New Zealand and this may have resulted in lowering dietary fluoride intake values have been underreported. Some assumptions had to be made about the children’s diets if there were missing or incorrectly recorded values in the diet record. These assumptions can be found in Appendix F. The fluoride content of urine samples may have been underreported
slightly because some children forgot to collect one urine void during the data collection period. Fluoride intake from toothpaste may be overestimated because any fluoride left on the toothbrush after brushing was not accounted for.

6.4 Conclusion

The results of this pilot study confirm that it is feasible to measure the mean daily fluoride intakes of 9- and 10-year-old children living in fluoridated and non-fluoridated areas of New Zealand. Furthermore, the results show that 9- and 10-year-old children living in Dunedin and Timaru are not meeting the AI for fluoride intake. Toothpaste was the primary source of fluoride for children in both cities and fluoridated water was an important source of fluoride for Dunedin children. Further research is required on a larger scale to determine the mean total daily fluoride intakes of New Zealand children aged across a wider range of ages including 5-8-years old.
7 Application of Research to Dietetic Practice

The skills used this pilot study can be applied to both public health and clinical areas of dietetic practice.

This research project has taught me that effective communication skills can take a lot of practice to develop but that they are key when interacting with the public. If a patient is asked to do something, such as complete a diet record, and they do not completely understand what the dietitian has meant, the diet record might be filled in incorrectly. This is frustrating for the patient and the dietitian. It is the responsibility of the dietitian to ensure they explain information and instructions clearly to the patient, without using scientific jargon. Often, referring to a visual aid, such as an example of a diet record, can enhance the patient’s understanding and teaches the dietitian what language and information are helpful when educating members of the public, thus improving the dietitian’s communication skills. Another strategy that can enhance a patient’s understanding and a dietitian’ communication skills, is getting the patient to repeat back what the dietitian said or getting the patient to describe what they are going to do.

During this whole process, I have also learnt the importance of new research. Currently, in New Zealand, fluoride fortification is a controversial topic. Many members of the public are concerned that community water fluoridation is going to provide some of the population with too much fluoride. Our research showed that this is unlikely to happen, and in fact, community water fluoridation is much more likely to improve health, rather than be a detriment to it. A dietitian is required to use evidence-based practice during all
aspects of their job. Therefore, a dietitian has a responsibility to keep up to date with new research being published in order to give evidence-based advice to whoever needs it, especially since nutrition is an ever-evolving area. I have developed the ability to ascertain whether scientific articles are of good quality or not by looking at the study design, the study size and demographics and the methods used. In addition, I have learnt how to interpret the findings of the study and apply them to what I am working on, which will be a valuable skill to have when working as a dietitian.

In dietetic practice, the actions of dietitians must be always guided by ethical principles. These include maintaining a patient’s autonomy, acting in the best interest of the patient, causing no harm to the patient and acting fairly, and in a non-biased or judgmental way. A way to ensure these principles are achieved during research is to obtain ethical approval. Ethical approval indicates that researchers have abided by the accepted ethical standards when designing their study which minimises any risk for the participant. Participants and patients have a right to be protected during any research or treatment they are involved with. They also have a right to know what information will be collected from them and what will happen with it. During each step of completing this research, it was important to reflect on the ethical principles and make sure they were being met. This is an important habit to develop as it is an integral part of working as a dietitian.
Appendices

Appendix A. Ethical approval

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:

gary.witte@otago.ac.nz

jo.farrondediaz@otago.ac.nz

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 and Consent Form (child)

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: __________________________

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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 HE17001

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

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

Where do you live?

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

Is there fluoride in the water where you live?
Appendix C. Information Pack and Consent Form (Parent)

Fluoride intake of primary school children

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

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: ..............................................................
### 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:

| Dr. Sheila Skeaff                                | Contact phone numbers: |
| Department of Human Nutrition                    | 03-479-7944            |
| Associate Professor                              |                         |

| Chontelle Watts                                  | Study Cell phone#:      |
| Department of Human Nutrition                    | 021 114 2155            |
| Masters of Dietetics Student                     |                         |

| Caitlin Davenport                                | Study Cell phone#:      |
| Department of Human Nutrition                    | 021 114 2155            |
| Masters of Dietetics Student                     |                         |

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.
We welcome any comments on your experience!


Duplicate Diet & Urine Collection Instructions

FLOSS
The Fluoride in School-children Study
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 whole grain 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.
Appendix D. Survey Monkey Questionnaires (Baseline and Follow-up)

1. What is your name?

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

3. When is your birthday?
   Birthdate: 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?
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 E. 24-hour Diet Record

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>Crans</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>
</tbody>
</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</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pot, except for half of cheese</td>
</tr>
<tr>
<td>Cheese</td>
<td>Mainland Cheddar</td>
<td>100g</td>
<td>which was sprinkled on top</td>
</tr>
<tr>
<td>Mixed veg</td>
<td>McCans - peas, corn, carrot</td>
<td>500g</td>
<td></td>
</tr>
</tbody>
</table>
We appreciate the time you have taken to complete this diet record!

We welcome any comments:
Appendix F. Kaiculator 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>Olivari 75% fat (J1009)</td>
</tr>
<tr>
<td>Beauty and the Beast cereal</td>
<td>Cocoa pops (D1041)</td>
</tr>
<tr>
<td>Nequi cereal</td>
<td></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>Healthenes 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 chips 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</td>
</tr>
<tr>
<td></td>
<td>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
8 References

45. Centers for Disease Control and Prevention. Fluoridation Statistics United States: U.S Department of Health and Human Services; 2016 [ ]
68. Prebble C. Mashblock New Zealand [Internet]. Statistics New Zealand; 2017 [Available from: http://www.mashblock.co.nz/.
69. Zohoori FV, Maguire A. Database of the Fluoride (F) content of Selected Drinks and Foods in the UK. United Kingdom: Newcastle University and Teesside University; 2015.