

Total daily Fluoride Intake and Fractional Urinary Fluoride Excretion in 4-6-year-old children living in a fluoridated area: weekly variation?

Narges Omid^a, Anne Maguire^b William T O'Hare^c, Fatemeh V Zohoori^{a*},

^a Health and Social Care Institute, Teesside University, Middlesbrough, UK

^b Centre for Oral Health Research, School of Dental Sciences, Newcastle University, Newcastle-upon-Tyne, UK

^c School of Science and Engineering, Teesside University, Middlesbrough, UK

Key words: Fluoride, intake, urine, excretion

*** Corresponding author**

Professor FV Zohoori

Health and Social Care Institute

Teesside University

Middlesbrough, TS1 3BA, UK

Tel: +44 (0) 1642 342973

Fax: +44 (0) 1642 342770

Email: v.zohoori@tees.ac.uk

Word Count: 3666

Abstract

Objectives Risk of development of dental fluorosis may increase with even a short-term increase in fluoride (F) intake during tooth formation. Considering the wide variations in F concentrations of different food and drinks, it is important to assess short-term differences in F intake and consequently fractional urinary F excretion (FUFEx) in children, which provide an indication of F body burden. Therefore, the aim of this study was to investigate weekly variation in total daily F intake (TDFI) and its sources and fractional urinary F excretion (FUFEx) in 4-to-6-year-olds living in a fluoridated area in the UK.

Methods Sixty-one children were surveyed twice with a one-week gap between surveys. Dietary F intake was assessed by 'food-diary' and 'duplicate-plate collection'. Toothbrushing expectorate (saliva/toothpaste) was collected to estimate F intake from toothpaste ingestion. TDFI was calculated from dietary F intake and toothpaste ingestion. Daily urinary F excretion (DUFEx) was estimated by collecting 24h urine samples and FUFEx was calculated from DUFEx and TDFI [$FUFEx = (DUFEx/TDFI) \times 100$].

Results The overall mean TDFI, DUFEx and FUFEx for all children was 0.056 (SD 0.036) mg/kgbw/day, 0.018 (SD 0.007) mg/kgbw/day and 39 (SD 20)%, respectively. The mean (95% CI) difference between the two weeks studied was 0.004 (-0.004, 0.011) mg/kgbw/day for TDFI, 0.002 (-0.001, 0.004) mg/kgbw/day for DUFEx and 1 (-6, 8)% for FUFEx.

Conclusions Mean TDFI and FUFEx did not vary statistically significantly with week and therefore one set of data collection from a group of children living in a temperate climate could be sufficient to monitor F exposure and F body burden in community prevention programmes for oral health.

Introduction

Several studies, conducted in both water fluoridated and non-fluoridated areas, in the 1980s-2000s indicated an increased dental fluorosis prevalence^{1,2}. The increase over this period, as a result of chronic excessive exposure to fluoride (F), is thought to be mainly due to an increase in different sources of F contributing to overall exposure and a rise in its “halo effect” through increasingly extensive worldwide movements of food and drink products. The greatest susceptibility of the permanent dentition to dental fluorosis is during the first 7 years of life due to the ongoing process of calcification of the crowns of erupting teeth^{3,4}. Apart from age (that is, the phase of mineralisation of developing tooth enamel reached), the prevalence and severity of dental fluorosis at an individual level depends also on body weight as well as the amount and duration of F exposure^{5,6}.

Fluoridated toothpastes as well as foods and drinks produced in fluoridated water areas are the common sources of F exposure in developed countries. The literature shows a wide variation in the contributions of diet and toothpaste ingestion to total F intake in children, depending on their age, oral hygiene and dietary habits. The longitudinal Iowa F study showed diet as the major component of total daily F intake in children up to 6 years; comprising 95% of total daily F intake in 1-year-olds, 65% in 3-year-olds and 75% in 6-year-olds⁷.

In children, a total daily F intake of 0.05-0.07 mg/kg body weight (bw) has been suggested as optimal for dental health benefit while minimising risk of dental fluorosis⁸, whereas an intake of 0.1 mgF/kgbw/day has been termed as the Tolerable Upper Intake Level (UL)^{9,10}. However, the longitudinal Iowa Fluoride study¹¹, in which F intake in children with- and without dental fluorosis was compared from infancy through to 9 years of age, reported an overlap in F intake for groups with- and without fluorosis, indicating that absolute F intake may not be the complete predictor of dental fluorosis prevalence.

Several factors, such as composition of diet, age and body size can alter the degree of F absorption and therefore assessing F retention and F body burden might be more important than estimating F intake, when considering dental fluorosis risk in children. With urine being the prime metabolic pathway for absorbed F excretion F from the body, due to practical difficulties in collecting faeces in children, most F body burden studies have focused on measuring the fraction of ingested F excreted in urine, known as the fractional urinary F

excretion (FUFE). These studies ¹²⁻¹⁶ have reported a wide variation in FUFE, ranging from 30% to 80% in children due primarily to methodological variations, participant age and between-population/between-individual differences in dietary and oral hygiene patterns/habits. However, another explanation for these differences could be daily or weekly variations in dietary and oral hygiene patterns within an individual. Since the reliability of outcome variables is greater with repeated measurement¹⁷, it is important to establish the minimum number of measurements which reflect a sufficiently accurate assessment of FUFE for a group of individuals.

No study has examined within-individual variation in urinary F excretion and FUFE due to the practical difficulties in collecting dietary data and 24h urine samples from young children on several occasions. As a part of a larger project ^{18, 19} to evaluate the validity of two different methods (Food- Diary and Duplicate-Plate) of F intake assessment in children, data on total dietary F intake as well as 24h urinary F excretion were collected on two occasions, approximately one week apart. The results showed no significant differences in estimated mean total daily dietary F intake assessed by the two methods. The data on F exposure and excretion, collected on two occasions, was subsequently used to examine weekly variation in F intake and excretion in children. The aim of this paper was therefore to investigate weekly variations in i) total daily F intake (TDFI) and its sources, ; ii) daily urinary F excretion (DUFE) and, consequently; iii) FUFE, in 4-6 year old children living in a fluoridated area in the UK.

Methods

Ethical approval for the study was obtained from Teesside University Research Ethics Committee and County Durham and Tees Valley 1 Research Ethics Committee (Ethics no. 08/H0905/116). The study was carried out in areas of north-east England (Newcastle) where the F concentration of the water supply is adjusted to 1ppm. After obtaining permission from the relevant educational authorities in the study area, parents of healthy children aged 4, 5 and 6 years who were lifelong residents of the area, were contacted through schools. The eligibility criteria for children to take part in the study included being healthy and lifelong residents of the area which were checked with parents through a face-to-face interview. An informed written parental consent was obtained for 61 children who met the inclusion criteria.

The data collection phase included three home visits. At the 1st home visit, the weights of children, without shoes and jacket, were measured to the nearest 0.1 kg using a calibrated portable digital balance (SOEHNLE, Slim Design Linea, Germany) and information about each child's toothbrushing habits collected. At the 2nd home visit dietary data, expectorated saliva/toothpaste and 24h urine samples were collected and a similar set of data and samples was collected in the 3rd home visit, approximately one week after Visit 2.

Dietary F intake of children was assessed by both "food-diary" and "duplicate-plate collection" methods ¹⁹. It was emphasised to parents that they should ensure that their child's usual dietary habits were maintained over the study period. For duplicate-plate collection parents were asked to duplicate the exact portions of food and drink items consumed by their children as accurately as possible over two days; one weekday and one weekend day. Food parts not habitually eaten (e.g. bones, fruit cores/ skin etc.) were removed from the duplicate portions by parents before the food and drink samples were collected into separate containers. For food-diary based dietary F intake estimation, parents were provided with a food-diary and accompanying instructions and asked to record all food and drink consumed by their children over three days; two weekdays and one weekend day. During the sample collection period on weekdays when the children were at schools, collection of "duplicate-plate" and food records were undertaken by the researcher on-site. The day following duplicate-plate collection and food-diary, the parents and children were interviewed at their homes (2nd and 3rd home visits) to ensure that all food and drink items were collected and/or entered into the food-diary, to clarify the nature of the food or drink and to determine the weights of food/drink entered into the food-diary, if necessary.

The method of collection of expectorated saliva/toothpaste in this study has been reported in detail elsewhere ¹⁸. In summary, during the 2nd and 3rd home visits, children were asked to brush their teeth, based on their usual habits, using their normal toothbrush and toothpaste, with or without parental help. The toothpaste brand used by the child was recorded. The child's toothbrush was weighed before and after dispensing the toothpaste onto the brush using a portable electronic compact balance (A&D Instruments Ltd, Model HL-100, UK) to calculate the amount of toothpaste dispensed onto the toothbrush. During tooth brushing, all expectorated saliva, liquids and toothpaste were collected in a wide-mouth polystyrene bowl.

Each child provided two 24h urine samples with sample collection starting on the same weekend day (that is, Saturday) as the dietary assessments. Parents were provided with urine collection kits which comprised disposable cups, jugs, a potty, funnels and screw top plastic bottles. All the urine voided over the 24-h period was collected and pooled into one container by parents and collected, by the researcher, from the family at the 2nd and 3rd home visits.

All drinks, apart from drinking tap water and milk, collected for each day for each individual child at home and at school were mixed together and the total volume recorded. The collected drinking tap water samples from home and school, for each child, were also mixed together and the volume recorded. Milk was classified as a food and mixed with solid foods, and the mixed sample was weighed and then homogenised using an industrial blender (Vorwek, Thermomix TM31, Germany). Expecterated saliva/toothpaste samples were weighed and vortexed for 30s. All the urine samples collected for each child were pooled and the total volume recorded for each 24h sample.

The concentrations of F in urine, water and drink samples were measured in triplicate at room temperature directly using a F-ion-selective electrode (Thermo Scientific Orion, Model 9609BNWP, USA) coupled to a potentiometer (Thermo Scientific Orion, Model 720A, USA) after adding total ionic adjustment buffer (TISAB) III. The F concentration of each expecterated saliva/toothpaste sample as well as each toothpaste collected was also measured directly using a F-ISE, based on the procedure of Duckworth et al ²⁰. Food samples were analysed for F concentration after overnight hexamethyldisiloxane-facilitated diffusion using a F-ISE. Validity of the F analysis method was evaluated by determining F recovery in 10% of samples and reliability was measured through repeat analysis of 10% of samples.

Completeness of 24h urine samples was checked against the validity criteria suggested by the World Health Organization which provide, lower limits of 5ml/h and 9ml/h for urinary flow rate in <6y and ≥6y olds ²¹, respectively. All children in the present study met the criteria and therefore were included in the data analysis. Daily urinary F excretion was estimated from 24h urine volume and F concentration of the urine sample.

F ingestion (µg) from toothpaste per toothbrushing was estimated by subtracting the total amount of F in expecterated saliva/toothpaste from the total amount of F dispensed onto the toothbrush and multiplying that value by the child's corresponding frequency of daily

brushing to calculate the daily F intake from toothpaste ingestion. Each child's food-diary was analysed using the Weighed Intake Software Package (WISP)²², a computerised standard food composition table with in-house added F concentrations of food and drink items²³, to estimate daily dietary F intake. Daily F intake from diet for each child was estimated by multiplying the weight of each food sample (g) by its corresponding F concentration ($\mu\text{g/g}$). F intakes from diet and toothpaste ingestion were then added to obtain TDFI, since no child used a F supplement in this study.

To estimate the number (%) of children with F exposure meeting or exceeding the suggested optimal or upper limits, TDFI was categorised into three major groups based on IoM and EFSA guidance: i) sub-optimal (<0.05 mgF/kg bw/day) – an intake which might not provide effective protection against dental caries, ii) optimal (0.05-0.07 mgF/kg bw/day) – an intake which might protect against dental caries, and iii) supra-optimal (>0.07 mgF/kg bw/day) – an intake which might increase the risk of dental fluorosis. Group (iii) was further categorised into two groups: 0.07- <0.10 mgF/kg bw/day and ≥ 0.10 mgF/kg bw/day (Upper Limit of F exposure)^{9,10}.

Fractional Urinary Fluoride Excretion (FUFE %) was calculated from the following equation: $(\text{DUFE}/\text{TDFI}) \times 100$, where TDFI and DUFE were estimated in mg/d and mg/kgbw/d.

Statistical analysis: Since this study was the first on weekly variation in TDFI, DUFE and FUFE, sample size calculation was based on the confidence interval, derived from the differences in mean TDFI of 0.504 (SD 0.138) and 0.552 (SD 0.192) mg/d reported²⁴ for 12 children living in an optimally fluoridated area (1 ppmF), when dietary data were assessed by food diary and duplicate plate methods. Therefore a minimum of 44 participants was sufficient to provide 80% power. However, a sample size of 61 was sought to allow an attrition rate of up to 30%.

Data were descriptively analysed using SPSS version 17.0. The mean difference between week one and week two was presented together with the 95% CI for the difference. Weekly variations in TDFI, DUFE and FUFE were examined by paired t-tests. Analysis of variance was used to evaluate the effect of age groups on FUFE.

Results

All 61 children who took part in the study completed all aspects and comprised 20 (33%), 22 (36%) and 19 (31%) children aged 4, 5 and 6 years, respectively. The overall mean weight for all children was 21(SD 4) kg, the mean weight being 19 (SD 5), 21 (SD 2) and 24 (SD 4) kg for 4, 5 and 6 year olds, respectively.

The overall mean recovery of added F was 99.3% with a range of 98.7-100%. The mean difference in F concentration from analysis to re-analysis was 0.011 μ gF/g, ranging from 0.003 to 0.018 μ gF/g.

The mean urinary flow rate was 20 (range 7 - 43) ml/h in 4-year-olds, 20 (range 7 - 46) ml/h in 5-year-olds and 22 (range 13 - 32) ml/h in 6-year-olds. The mean F concentration of drinking water for all children was 0.97 (SD 0.02) mg/l.

The weekly variations in mean TDFI, DUFE and FUFEE for all children are summarised in Table 1 and show no major differences between weeks. The mean difference in FUFEE between Week one (40%) and Week two (39%) was 1% (95% CI -6%, 8%) which was not statistically significant.

Table 2 shows the differences between weeks in mean TDFI, DUFE and FUFEE by age group. The mean TDFI, when expressed by body weight, was fairly similar in 4-, 5- and 6-year-olds. The mean contribution of F from toothpaste ingestion to TDFI was 46 (SD 16)%, 45(SD 18)% and 42(SD 24)% in 4, 5 and 6 year old children, respectively. The mean DUFE, when expressed by body weight, was also similar for all three age groups. There was no statistically significant difference in the mean FUFEEs (%) between different age groups which were 42%, 39% and 36% in 4-, 5- and 6-year-olds, respectively (Table 2).

In addition, no statistically significant correlation ($p=0.265$) was found between TDFI and children's weight (Figure 1). Table 3 presents the number (percentage) of children with estimated sub-optimal, optimal and supra-optimal F intake by data collection week. In Week one, 28% of children were receiving supra-optimal F intake, while in Week two this proportion decreased to 20%. Based on the average of the two data collection weeks, only 15% of the children had a TDFI within the so-called "optimal F intake range" (0.05-0.07

mg/kgbw/day), with 53% of their daily F intake being due to toothpaste ingestion (Table 3). For the 61% of children with suboptimal F intake (TDFI < 0.05mg/kgbw/day), diet was the main contributor of TDFI (65%), whereas toothpaste ingestion provided 62% of TDFI for the 24% of children with a F intake >0.07mg/kgbw/day (Table 3).

Discussion

The present study, which provides the first data on weekly difference in F intake and excretion, reported a relatively low variation in mean TDFI, DUFE and FUFE between weeks in a group of children living in a fluoridated area. This finding suggests that collection of a set of data at one time point could be adequate to estimate TDFI, UFE and consequently FUFE in a community.

The caries prevention effect of F is now believed to be mainly attributable to its local topical effect at the tooth surface, whereas dental fluorosis results from the chronic high systemic ingestion of F from different sources during crucial periods of tooth development. The optimum F concentration of water has been set as 1.0 mg/l in many countries; however the US Public Health Service Recommendation, published in 2015, suggested a reduction in optimal F concentration of drinking water to 0.7 mg/l to lessen the chance of development of dental fluorosis²⁵. Periodic assessment of total F exposure in populations has been recommended by the World Health Organization^{21,26} to enable public health administrators to make appropriate decisions on fluoridation/ supplementation policies and programmes for prevention of dental caries.

Since several factors, including the composition of diet and certain vitamins or drugs (e.g. ascorbic acid, ammonium chloride), can alter the rate of F absorption and excretion²⁷, an estimate of body F retention in children would appear to be more valuable than estimation of F intake when considering dental fluorosis prevention. Several reports in the literature on FUFE in children report a range from 39 to 78% in infants and toddlers younger than 3 years²⁸⁻³⁰ and 30 to 80% in children aged 3 to 7 years¹²⁻¹⁶; indicating body F retention of 20% to 70% in children. The wide variation in F retention could be due to the physiological and dietary/oral hygiene habit differences between-individuals and/or variations in the patterns of dietary/oral hygiene habits within-individual. Therefore, longitudinal cohort studies looking at fluoride retention, focusing on both total daily intake and excretion of F in early life and

development of dental fluorosis, are needed to establish clear predictors of dental fluorosis development.

The present study is the first to report on weekly variation in intake and excretion of F as well as FUFEx in children. The present study showed no statistically significant weekly variations in mean daily F intake from diet or toothpaste ingestion nor in mean daily F excretion. The main weakness of the study was that only two sets of sample/data, with a one-week gap, were collected from the study participants. This was mainly to minimise the burden to the families as the study required parental cooperation for close supervision of their child's diet in order to complete food diaries and the collection of food samples and 24h urine samples on two occasions. Due to the challenges of multiple data collection, particularly 24h urine samples from children, there is no report in the literature on variation in urinary F excretion.

However, a few studies have investigated seasonal variations in dietary F intake in children. A higher dietary F intake in summer compared with winter was reported for 4 year old Iranian children³¹, with the higher F intake due to higher water consumption as a result of the higher temperature (29°C in summer vs 5°C in winter). Additionally, the Iowa Fluoride Study³² reported a small seasonal variation in F intake from beverages with increasing monthly temperature, ranging from 0.025 mgF/kgbw/day at <-5°C to 0.030 mgF/kgbw/day at 25°C, in 1-6 year olds. No significant seasonal difference in dietary F intake was also reported for Brazilian children aged 20-30 months living in subtropical regions where the temperature range was smaller - from 25°C in autumn to 31°C in summer³³. In the present study undertaken in the north-east of England, sample collection took 9 months from April to December 2009 and seasonal and diurnal variations in temperature are relatively low. The mean annual temperature that year was 9°C, ranging from 2°C in December to 16°C in August with average seasonal temperatures of 9, 15, 10 and 3°C in the spring, summer, autumn and winter, respectively³⁴.

In the present study, the proportion of children with a TDFI of more than the UL (0.10 mgF/kgbw/day) was 8%. Interestingly, a recent study³⁵ also conducted in Newcastle, UK reported a dental fluorosis (of aesthetic concern) prevalence of 7.1% in 11-13 year olds. Despite living in an optimally water fluoridated area, only 15% of children (based on average data) in the present study received an optimum F intake, with similar mean proportions of F being consumed from diet (47%) and toothpaste (53%) ingestion in this group (Table 3).

Conversely, 61% of children received a sub-optimal F intake with diet (65%) as the main source of F intake, whereas F intake from toothpaste ingestion was the main source of F (62%) for those 24% of children who had supra-optimal F intake. The highest total daily F intake (4.439 mg/day = 0.22 mg/kgbw/day) was estimated for a 5-year-old child (Figure 1) of which 72% (3.217 mg/day = 0.16 mg/kgbw/day) was from toothpaste ingestion. Our previous paper on toothbrushing practices by the children in the present study¹⁸ indicated that F intake from toothpaste ingestion was significantly ($p < 0.001$) influenced by the weight of toothpaste used, the F concentration of the toothpaste and age. These findings confirm that living in an optimally fluoridated area *per se* is unlikely to be a risk factor in young children for development of dental fluorosis but the additional ingestion of a fluoridated toothpaste may put a child at increased risk of dental fluorosis. Moreover, according to a recently conducted consumer survey in England and Wales³⁶, only 2% of children up to 5 years old consumed about one litre of tap water per day, equivalent to a F intake of 0.05mgF/kgbw/day for a 20kg child residing in a water fluoridated area (1ppmF). That survey also reported that 4% of the children consumed more than one litre water per day while most (39%) consumed 201-500ml tap water per day (~0.010-0.025mgF/kgbw/day for a 20kg child, if water was fluoridated at 1ppm). Additionally, a study in England³⁷ reported the small contribution that water as a drink itself made to TDFI for 6-7 year old children; 4% in non-fluoridated areas (0.08ppmF) and 11% in fluoridated areas (0.82ppmF).

A small decrease in FUF_E with increasing age was observed in the present study. The wide variations in FUF_E reported in the literature make it difficult to explore between-age variation in FUF_E. The negative trend, although very small, between FUF_E and age found in the present study could be due to a lower growth rate at 4 years of age and therefore less F retention in calcified tissues, similar to the pattern seen with calcium retention. Daily skeletal gains of calcium with age seen from birth to puberty follow a 'V' shape with the peak gains being during the first months of life and then again during the adolescent growth spurt, whilst the lowest gain is at about 4 years of age³⁸.

To conclude, the magnitude of the weekly variation in TDFI, DUFE and FUF_E was very low for 4-6 year old British children, suggesting that estimation of FUF_E (and therefore F body burden by direct extrapolation) at group levels could be achieved fairly accurately by collecting only one set of F intake and urinary excretion data.

Acknowledgements

This study was kindly supported by grants from Teesside University Research Doctoral Scholarships and The Borrow Foundation. We also thank the families who participated in the study for their invaluable assistance during data/sample collection.

The authors report no conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

References:

- 1 Whelton HP, Ketley CE, McSweeney F, O'Mullane DM. A review of fluorosis in the European Union: prevalence, risk factors and aesthetic issues. *Community Dent Oral Epidemiol* 2004;32: Suppl 1:9-18.
- 2 Pendrys DG. Risk of enamel fluorosis in nonfluoridated and optimally fluoridated populations: considerations for the dental professional. *J Am Dent Assoc* 2000;131:746-55.
- 3 Bårdsen A, Klock KS, Bjorvatn K. Dental fluorosis among persons exposed to high- and low-fluoride drinking water in western Norway. *Community Dent Oral Epidemiol* 1999;27:259-67.
- 4 Levy SM. An update on fluorides and fluorosis. *J Can Dent Assoc* 2003;286-91.
- 5 DenBesten P, Thariani H. Biological mechanisms of fluorosis and level and timing of systemic exposure to fluoride with respect to fluorosis. *J Dent Res* 1992;71:1238-43.
- 6 Ellwood RP, Cury JA. How much toothpaste should a child under the age of 6 years use? *Eur Arch Paediatr Dent: Official Journal of the European Academy of Paediatric Dentistry* 2009;10:168-74.
- 7 Levy SM, Warren JJ, Broffitt B. Patterns of fluoride intake from 36 to 72 months of age. *J Public Health Dent* 2003;63:211-20.
- 8 Burt BA. The changing patterns of systemic fluoride intake. *J Dent Res* 1992;71:1228-37.
- 9 Institute of Medicine. *Dietary Reference Intakes for Calcium, Magnesium, Vitamin D, and Fluoride*. Washington DC: National Academy Press, 1997.
- 10 European Food Safety Authority (EFSA). *Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Fluoride*. *The EFSA Journal* 2005;192:1-65.
- 11 Levy SM, Broffitt B, Marshall TA, Eichenberger-Gilmore JM, Warren JJ. Associations between fluorosis of permanent incisors and fluoride intake from infant formula, other dietary sources and dentifrice during early childhood. *J Am Dent Assoc* 2010;141:1190-201.

- 12 Zohoori FV, Walls R, Teasdale L, Landes D, Steen IN, Moynihan P, et al. Fractional urinary fluoride excretion of 6-7-year-old children attending schools in low-fluoride and naturally fluoridated areas in the UK. *Br J Nutr* 2013;109:1903-9.
- 13 Villa A, Anabalón M, Cabezas L. The fractional urinary fluoride excretion in young children under stable fluoride intake conditions. *Community Dent Oral Epidemiol* 2000;28:344-55.
- 14 Haftenberger M, Viergutz G, Neumeister V, Hetzer G. Total fluoride intake and urinary excretion in German children aged 3-6 years. *Caries Res* 2001;35:451-7.
- 15 Franco AM, Saldarriaga A, Martignon S, Gonzalez MC, Villa AE. Fluoride intake and fractional urinary fluoride excretion of Colombian preschool children. *Community Dent Health* 2005;22:272-8.
- 16 Maguire A, Zohoori FV, Hindmarch PN, Hatts J, Moynihan PJ. Fluoride intake and urinary excretion in 6- to 7-year-old children living in optimally, sub-optimally and non-fluoridated areas. *Community Dent Oral Epidemiol* 2007;35:479-88.
- 17 Hackett AF, Rugg-Gunn AJ, Appleton DR. Use of a dietary diary and interview to estimate the food intake of children. *Human Nutrition Applied Nutrition*. 1983;37(4):293-300.
18. Zohoori FV, Duckworth RM, Omid N, O'Hare WT, Maguire A. Fluoridated toothpaste: usage and ingestion of fluoride by 4- to 6-yr-old children in England. *Eur J Oral Sci* 2012;120:415-21.
- 19 Omid N, Maguire A, O'Hare WT, Zohoori FV. Estimation of daily dietary fluoride intake: 3-d food diary v. 2-d duplicate plate. *Br J Nutr* 2015;114:2103-9.
- 20 Duckworth RM, Maguire A, Omid N, Steen IN, McCracken GI, Zohoori FV. Effect of rinsing with mouthwashes after brushing with a fluoridated toothpaste on salivary fluoride concentration. *Caries Res* 2009;43:391-6.
- 21 Marthaler TM. Monitoring of renal fluoride excretion in community preventive programmes on oral health. Geneva: World health Organisation;1999.
- 22 Weighed Intake Software Package (WISP), Version 3. Intakes, Recipes, and Menu Analysis. UK: Tinuviel Software; 2006.

- 23 Zohoori FV, Maguire A. Database of the Fluoride (F) content of Selected Drinks and Foods in the UK. UK: Newcastle University & Teesside University; 2015.
- 24 Martinez-Mier EA, Kelly SA, Eckert GJ, Jackson RD. Comparison of a dietary survey and the duplicate plate method for determining dietary fluoride ingested by young children: a pilot study. *Int J Paediatr Dent* 2009;19:99-107.
25. Public Health Service. U.S. Public Health Service Recommendation for Fluoride Concentration in Drinking Water for the Prevention of Dental Caries, USA: Department of Health and Human Services Federal Panel on Community Water Fluoridation; 2015.
- 26 World Health Organization. Fluorides and oral health. Report of a WHO Expert Committee on Oral Health Status and Fluoride Use. *Tech Rep Ser* 1994;846:1-37.
- 27 Whitford GM. *Metabolism and Toxicity of Fluoride*. Basel:Karger,1996.
- 28 Ekstrand J, Hardell LI, Spak CJ. Fluoride balance studies on infants in a 1-ppm-water-fluoride area. *Caries Res* 1984;18:87-92.
- 29 Ekstrand J, Ziegler EE, Nelson SE, Fomon SJ. Absorption and Retention of Dietary and Supplemental Fluoride by Infants. *Adv Dent Res* 1994;8:175-80.
- 30 Zohouri FV, Swinbank CM, Maguire A, Moynihan PJ. Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? *Community Dent Oral Epidemiol* 2006;34:130-8.
- 31 Zohouri FV, Rugg-Gunn AJ. Total fluoride intake and urinary excretion in 4-year-old Iranian children residing in low-fluoride areas. *Br J Nutr* 2000;83:15-25.
- 32 Broffitt B, Levy SM, Warren JJ, Heller KE. Seasonal variation in fluoride intake: the Iowa fluoride study. *J Public Health Dent* 2004;64:198-204.
- 33 Lima YBO, Cury JA. Seasonal variation of fluoride intake by children in a subtropical region. *Caries Res* 2003;37:335-8.
- 34 Met Office UK. . Met Office UK. England, <http://www.metoffice.gov.uk/climate/uk/2009/>
- 35 McGrady MG, Ellwood RP, Maguire A, Goodwin M, Boothman N, Pretty IA. The association between social deprivation and the prevalence and severity of dental caries and

- fluorosis in populations with and without water fluoridation. *BMC Public Health* 2012;12:1122.
- 36 Ipsos MORI. Tap water drinking behaviour: a study of children aged 0-15. Report for Defra's Drinking Water Inspectorate (DWI). England: Social Research Institute; 2012.
- 37 Zohoori FV, Maguire A, Moynihan PJ. Sources of Dietary Fluoride Intake in 6-7 Year Old English Children Receiving Optimally, Sub-optimally, and Non-fluoridated water. *J Public Health Dent* 2006;66:227-34.
- 38 Kanis JA, Passmore R. Calcium Supplementation of the Diet 1. *Br Med J* 1989;298:137-40.

Legends

Table 1. Weekly variation in mean (SD) daily F intake (mg/day) from diet and toothpaste ingestion, total daily F intake (TDFI) (mg/day, mg/kgbw/day), daily urinary F excretion (DUFE) (mg/day, mg/kgbw/day) and fractional urinary F excretion (FUFE) (%) in 61 children aged 4 to 6 years living in a fluoridated area.

Table 2. Weekly variation in mean (SD) total daily F intake (TDFI), daily urinary F excretion (DUFE) and fractional urinary F excretion (FUFE) by age group.

Table 3. Number (%) of children with suggested sub-optimal (<0.05 mgF/kg bw/day), optimal (0.05-0.07 mgF/kg bw/day) and supra-optimal (>0.07 mgF/kg bw/day) F intake by data collection week, and overall mean (SD) contribution of toothpaste ingestion to total daily F intake (TDFI) by F intake group.

Figure 1. Relationship between average total daily fluoride intake (mg/day) and body weight in 61 children aged 4 to 6 years living in a fluoridated area. The dotted line shows the best fit straight line for that relationship. Total daily F intake (mg/day) = $[0.02 \times \text{Weight (kg)}] + 0.65$, $R^2=0.02$

Table 1. Weekly variation in mean (SD) daily F intake (mg/day) from diet and toothpaste ingestion, total daily F intake (TDFI) (mg/day, mg/kg bw/day), daily urinary F excretion (DUFE) (mg/day, mg/kg bw/day) and fractional urinary F excretion (FUFE) (%) in 61 children aged 4 to 6 years living in a fluoridated area.

	Week of Collection		Difference between the two weeks		P value
	1	2	Mean	95% CI	
Daily F intake from					
- Diet (mg/day)	0.568 (0.291)	0.548 (0.296)	0.021	-0.039, +0.081	0.491
- Toothpaste ingestion (mg/day)	0.636 (0.748)	0.580 (0.531)	0.057	-0.102, +0.216	0.475
TDFI					
- mg/day	1.205 (0.842)	1.127 (0.663)	0.078	-0.072, +0.227	0.302
- mg/kg bw/day	0.058 (0.042)	0.054 (0.034)	0.004	-0.004, +0.011	0.337
DUFE					
- mg/day	0.387 (0.186)	0.354 (0.189)	0.033	-0.019, +0.086	0.206
- mg/kg bw/day	0.018 (0.009)	0.017 (0.009)	0.002	-0.001, +0.004	0.179
FUFE (%)	40 (20)	39 (27)	1	-6, +8	0.776

Table 2. Weekly variation in mean (SD) total daily F intake (TDFI), daily urinary F excretion (DUFE) and fractional urinary F excretion (FUFE) by age group.

	TDFI		DUFE		FUFE
	mg/day	mg/kg bw/day	mg/day	mg/kg bw/day	%
4 year olds (n=20)					
Week 1	1.061 (0.719)	0.058 (0.043)	0.355 (0.171)	0.019 (0.010)	43 (24)
Week 2	1.063 (0.803)	0.057 (0.046)	0.312 (0.173)	0.017 (0.010)	41 (39)
Average	1.062 (0.699)	0.057 (0.040)	0.334 (0.160)	0.018 (0.009)	42 (29)
5 year olds (n=22)					
Week 1	1.282 (1.078)	0.062 (0.054)	0.389 (0.166)	0.019 (0.008)	39 (17)
Week 2	1.178 (0.646)	0.057 (0.032)	0.373 (0.167)	0.018 (0.008)	39 (23)
Average	1.223 (0.827)	0.060 (0.041)	0.381 (0.124)	0.018 (0.006)	39 (15)
6 year olds (n=19)					
Week 1	1.267 (0.655)	0.053 (0.024)	0.420 (0.222)	0.018 (0.009)	37 (19)
Week 2	1.136 (0.541)	0.048 (0.023)	0.375 (0.229)	0.015 (0.008)	35 (17)
Average	1.202 (0.549)	0.050 (0.022)	0.398 (0.187)	0.017 (0.007)	36 (13)
All Children (n=61)					
Average	1.166 (0.700)	0.056 (0.036)	0.371 (0.157)	0.018 (0.007)	39 (20)

Table 3. Number (%) of children with suggested sub-optimal (<0.05 mgF/kg bw/day), optimal (0.05-0.07 mgF/kg bw/day) and supra-optimal (>0.07 mgF/kg bw/day) F intake by data collection week, and overall mean (SD) contribution of toothpaste ingestion to total daily F intake (TDFI) by F intake group.

F intake group (mgF/kg bw/day)	Number (%) of children by week		Based on overall average of the two data collection weeks	
	Week 1	Week 2	Number (%) of children	Mean (SD) percentage contribution of toothpaste ingestion to TDFI
Sub-optimal (<0.05)	34 (56)	35 (57)	37 (61)	35 (14)
Optimal (0.05-0.07)	10 (16)	14 (23)	9 (15)	53 (20)
Supra-optimal (>0.07)	17 (28)	12 (20)	15 (24)	62 (17)
- 0.071-<0.10	8 (13)	7 (12)	10 (16)	62 (17)
- ≥0.10*	9 (15)	5 (8)	5 (8)	62 (16)
All			61 (100)	45 (19)

* Tolerable Upper Intake Level (UL)

Figure 1. Relationship between average total daily fluoride intake (mg/day) and body weight in 61 children aged 4 to 6 years living in a fluoridated area. The dotted line shows the best fit straight line for that relationship. Total daily F intake (mg/day)= $[0.02 \times \text{Weight (kg)}] + 0.65$, $R^2=0.02$

