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**Determining an upper reference value for the urinary fluoride-creatinine ratio in healthy children younger than 7 years**

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**Declaration of interests**

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## Abstract

The urinary fluoride/creatinine ratio ( $U_{F/Cr}$ ) in a spot urine sample could be a useful systemic F exposure monitoring tool. No reference value for  $U_{F/Cr}$  currently exists, therefore this study aimed to establish an upper reference value for a  $U_{F/Cr}$ , corresponding to excessive systemic F exposure ( $>0.07\text{mgF/kgbw/day}$ ) in children. Subsidiary aims were to examine the relationship between; (i) total daily F intake (TDFI) and 24-hour urinary F excretion (DUFE); (ii) DUFE and  $U_{F/Cr}$ , and; (iii) TDFI and  $U_{F/Cr}$ . Simultaneously collected TDFI, DUFE and urinary Creatinine ( $U_{Cr}$ ) data in children  $<7$  years were taken from UK studies conducted from 2002-2014 to calculate  $U_{F/Cr}$  (mg/g) for each child. For the 158 children (mean age 5.8y), included in the data analysis, mean (SD) TDFI and DUFE were 0.049(0.033) and 0.016(0.008)mg/kgbw/day, respectively and mean  $U_{F/Cr}$  was 1.21(0.61)mg/g. Significant ( $p<0.001$ ) positive linear correlations were found between TDFI and DUFE, DUFE and  $U_{F/Cr}$ , and TDFI and  $U_{F/Cr}$ . The estimated upper reference value for  $U_{F/Cr}$  was 1.69mg/g; significantly ( $p=0.019$ ) higher than the  $U_{F/Cr}$  (1.29) associated with optimal F exposure (0.05-0.07mg/kgbw/day). In conclusion, the strong positive correlation between TDFI and  $U_{F/Cr}$  confirms the strong association of these 2 F exposure variables and the value of a spot urine sample for prediction of TDFI (i.e. the most important risk factor in determining fluorosis occurrence and severity) in young children. Establishing an estimation of an upper reference value of 1.69mg/g for  $U_{F/Cr}$  in spot urine samples could simplify and facilitate their use as a valuable tool in large epidemiological studies.

## Introduction

The continuing decline in dental caries has been attributed to both the pre- and post-eruptive effects of fluoride (F) [O'Mullane et al., 2016]. Current evidence confirms the importance of topical F in caries prevention and contemporary F-based preventive methods are focussed on ensuring that elevated F concentrations are sustained in the oral cavity while ingestion of F from oral health products is minimised [Wong et al., 2010]. Nevertheless, the use of topical F, primarily through F toothpastes in young children with developing teeth is often associated with inadvertent ingestion of toothpaste and consequently systemic absorption of F. Chronic excessive F ingestion during the first five years of life, with the first three years being the most critical period, may result in an increased risk of development of dental fluorosis on the aesthetically important anterior teeth [Levy et al., 2010].

An empirically-based total daily F intake of 0.05-0.07 mg/kg bw is currently accepted as an estimate of “a useful upper limit for F intake in children” [Burt, 1992]; an intake which provides effective protection against dental caries without increasing the risk of dental fluorosis. Conversely, an F intake of >0.07 mg/kg bw/day, during enamel formation, might increase the potential for development of dental fluorosis.

Therefore, monitoring F intake and examining for signs of excessive exposure to F in early childhood can help to ensure that total F intake from all sources does not exceed these thresholds and upper limits and thus minimises the risk of development of dental fluorosis. Accurate assessment of F intake from all sources (including diet as well as unintentional ingestion of topical F such as from F toothpastes) is complex and presents significant challenges, both practical- and cost- related, associated with the collection of data/samples [Omid et al., 2015, 2017]. Urine is the main route for the elimination of the absorbed (i.e. bioavailable) F from the body [Whitford, 1996]. Consequently, urinary F assessments have been suggested as alternative tools, capable of detecting changes in concurrent F intake about 6 years earlier than examination for dental fluorosis [O'Mullane et al., 2016]. It has been shown that daily (24-hour) urinary F excretion data can be used to obtain satisfactory estimations of total daily F intake, in children, at a community level [Villa et al., 2010]. However, despite the usefulness of 24-hour urine samples to objectively determine total daily F intake, it is difficult to achieve complete sample collection in an adequately representative number of children due to the associated participant burden for children and their parents and the logistical challenges. Collecting 24-hour urine samples is, specifically, difficult in infants and young children who are not toilet-trained, while this age group represents the most critical period for development of dental fluorosis. An easier substitute for a 24h urine sample is the collection of a single-voided (spot) urine sample and determination of the ratio of a contained desired analyte (such as F, Ca, I etc) to the urinary creatinine ( $U_{cr}$ ). Estimation of 24-hour excretion of the analyte can then be made by

multiplying this ratio by the daily urinary creatinine excretion (Ucr) reference values which are in routine use. This method has now overtaken 24-hour urine collection in clinical investigations and screening for some diseases [Remer et al., 2002; Sethuram et al., 2011]. A mean Ucr reference value of 15 mg/kg bodyweight (bw)/day with 5<sup>th</sup> and 95<sup>th</sup> percentiles of 8 and 22 mg/kg bw/day has been reported as the standard for young children [Osiki et al., 1994].

Limited research has studied the correlation between urinary excretion of fluoride and creatinine. A strong positive linear correlation ( $r=0.94$ ,  $p<0.001$ ) between 24-hour urinary F excretion and fluoride/creatinine (F/Cr) ratio of a spot urine sample has been reported for pre-school children in Chile [Villa, 1994]. Studies in young children under customary F intake [Kertesz et al., 1989; Zohouri et al., 2006; Szekely et al., 2008] have also shown a positive correlation between daily urinary F excretions estimated using the F/Cr ratio in a spot urine sample and a 24-hour urine sample. The use of the F/Cr ratio of a spot urine sample as an index of 24-hour urinary F excretion, when conditions mean that obtaining 24-hour urine sample is not feasible, has now been suggested for monitoring F intake in community prevention programmes for oral health [World Health Organization, 2014]. However, there are no reference values for a urinary F/Cr ratio ( $U_{F/Cr}$ ) in normal children to allow the interpretation of the results of F monitoring. Since the dental caries-preventive effect of F is topical whereas dental fluorosis is usually a consequence of chronic excessive systemic ingestion of F, the reference value for a  $U_{F/Cr}$  should correspond to an upper limit of F intake – i.e. a threshold value that would predict/assess the risk of development of dental fluorosis. Therefore, the main aim of this investigation was to establish an upper reference value for a  $U_{F/Cr}$ , corresponding to excessive F intake in children. The subsidiary aims were to explore the: i) appropriateness of daily urinary F excretion (DUFE) as a predictor of total daily F intake (TDFI) by examining the relationship between TDFI and DUFE; ii) correlation between DUFE and the  $U_{F/Cr}$ , and; iii) suitability of  $U_{F/Cr}$  as a predictor of TDFI by examining the relationship between TDFI and the F/Cr ratio of urine.

## **Material and methods:**

### *Data sources and participants*

The study was based on a number of research projects on the intake and urinary excretion of F in young children, conducted in the UK from 2002 to 2014 [Zohouri et al., 2006; Maguire et al., 2007; Zohouri et al., 2013; Omid et al., 2017]. Only studies in which individual data for measured total daily F intake (TDFI) together with simultaneously measured daily urinary F excretion (DUFE) values were included in the present study. Collectively, those studies provided data pairs for 193 children aged 18 months to 7 years. All those studies used the same methodology to measure F and creatinine

excretion and F intake from toothpaste ingestion. Although two different dietary methods (i.e. 3-day food diary and 2-day duplicate plate) were used to measure F intake from diet, validity assessment showed no significant differences in estimated total daily dietary F intake obtained by the two methods [Omid et al., 2015].

#### *Measurements of F intake and excretion variables*

The materials and methods used in those studies were the same as those reported in detail in previous papers [Zohouri et al., 2006; Maguire et al., 2007; Zohoori et al., 2013; Omid et al., 2017].

In brief, TDFI from diet (including food, water and drinks) and toothpaste ingestion was measured for each individual child. Dietary information was collected using a 3-day food diary and/or a 2-day duplicate plate method. To measure F intake from diet, food and drink samples were collected from parents/households/schools/shops, as appropriate, and analysed for F contents. F intake from toothpaste ingestion was measured by weighing each child's toothbrush before and after dispensing toothpaste onto the toothbrush and collecting all expectorated saliva, rinsing liquids and toothpaste during toothbrushing as well as rinsing and collecting information on toothbrushing habits, including frequency of toothbrushing.

A 24h urine sample was collected for each child using the method described by the World Health Organisation (WHO) [WHO, 1999; World Health Organization, 2014] and used in the studies providing the data pairs [Zohouri et al., 2006; Maguire et al., 2007; Zohoori et al., 2013; Omid et al., 2017]. The 24h urine volume (l) was recorded and aliquots were taken for subsequent F and Cr analysis.

All samples were analysed, in triplicate. F concentrations of non-milk-based drinks, waters and urine samples were determined directly using a F-ion-selective electrode (model 96-09, Orion: Analytical Technology, Inc., Boston, MA, USA) in conjunction with an ISE Meter (Model 720A, Orion) after adding TISAB III, and the other samples (solid foods, toothpastes and toothbrushing expectorate samples) by silicon-facilitated diffusion methods [Martínez-Mier et al., 2011]. The urinary creatinine ( $U_{Cr}$ ) of each urine sample was measured by the Jaffe method [Bones and Taussky, 1951].

#### *Data handling and analysis*

DUFE (mg) for each child, was calculated by multiplying the measured F concentration of 24-h urine sample (mg/l) by the urine volume (l). To calculate 24-h  $U_{Cr}$  (g), the measured Cr concentration of each 24-h urine sample (g/l) was multiplied by its urine volume (l). The  $U_{F/Cr}$  ratio (mg/g) was then calculated by dividing DUFE (mg) by  $U_{Cr}$  (g).

The TDFI, DUFE and  $U_{Cr}$  were calculated on body weight basis, for each individual child, by dividing each variable by the child's body weight (kg).

Considering the 5<sup>th</sup> and 95<sup>th</sup> percentiles of 8 and 22 mg/kg bw/day as the standard range of urinary excretion of creatinine for young children [Oski et al., 1994; Remer et al., 2002], any child with a  $U_{Cr}$  value of less than 8 mg/kg bw/day or more than 22 mg/kg bw/day was excluded from data analysis in this study.

To establish the F/Cr ratios corresponding to an F exposure meeting or exceeding the suggested optimal intake, each TDFI was categorized into one of three major groups based on IoM [Institute of Medicine, 1997] and EFSA [European Food Safety Authority (EFSA), 2008] guidance for: i) low F intake (<0.05 mgF/kg bw/day) – an F intake which might not provide effective protection against dental caries, ii) optimal F intake (0.05–0.07 mgF/kg bw/day) – an F intake which might protect against dental caries, and; iii) excessive F intake (>0.07 mgF/kg bw/day) – an F intake which might increase the risk of dental fluorosis.

The data were analysed using SPSS Statistics software (Version 21 Chicago, IL, USA). Following descriptive analysis to report the mean ( $\pm$ SD) of the variables, Pearson's correlation and regression analyses were used to investigate the relationship between: (i) DUFE and TDFI; (ii) DUFE and  $U_{F/Cr}$ , and; (iii) TDFI and  $U_{F/Cr}$ . Statistically significant differences between boys and girls were detected using a t-test. Statistically significant differences among the three TDFI groups were detected using ANOVA followed by a post-hoc test (Tukey) with statistical significance set at  $\alpha < 0.05$ .

## **Results:**

Of the 193 children, who took part in the 2002-2014 UK studies, 35 did not meet the inclusion criteria of a  $U_{Cr}$  value of 8-22 mg/kg bw/day and therefore were excluded from further data analysis.

Table 1 presents the age, weight, intake and excretion data for the 158 children (74 girls and 84 boys) by gender. There were no statistically significant differences between genders for any of the outcome variables. The overall mean (SD) age and weight of the 158 children were 5.8 (1.2) y and 21.8 (4.5) kg, respectively. Mean (SD) TDFI was 0.049 (0.033) mg/kg bw/day, of which 48% was due to inadvertent toothpaste ingestion during toothbrushing. The mean (SD) DUFE and F/Cr ratio was 0.016 (0.008) mg/kg bw/day and 1.21 (0.61) mg/g, respectively.

Figure 1 shows the linear relationship obtained when DUFE was plotted against TDFI for the 158 data pairs. The results showed a statistically significant positive correlation; “DUFE (mg/kg bw/day) =  $0.01 + [0.12 \times \text{TDFI (mg/kg bw/day)}]$ ”; Pearson’s correlation = 0.5,  $p < 0.001$ .

There was also a very strong positive correlation between  $U_{F/Cr}$  and DUFE, which was statistically significant (Figure 2):  $U_{F/Cr} \text{ (mg/g)} = 0.16 + [65.05 \times \text{DUFE (mg/kg bw/day)}]$ ; Pearson’s correlation = 0.87,  $p < 0.001$ .

Figure 3 shows the linear relationship obtained when the  $U_{F/Cr}$  was plotted against TDFI for the 158 data pairs and describes a statistically significant positive correlation between the  $U_{F/Cr}$  and TDFI; “ $U_{F/Cr} \text{ (mg/g)} = 0.74 + [9.57 \times \text{TDFI (mg/kg bw/day)}]$ ”; Pearson’s correlation = 0.512,  $p < 0.001$ .

The numbers of children with estimated low, optimal and excessive F exposures were 96, 34 and 28, respectively with an overall mean (SD) F intake of 0.029 (0.011), 0.058 (0.005) and 0.107 (0.030) mg/kg bw/day, respectively.

The estimated  $U_{F/Cr}$  associated with low, optimal and excessive F intake for children is presented in Table 2. For low F exposure ( $< 0.05$  mgF/kg bw/day) the  $U_{F/Cr}$  was 1.05 mg/g, while it was 1.29 mg/g with optimal F exposure (0.05-0.07 mgF/kg bw/day), and 1.69 mg/g with excessive F exposure ( $> 0.07$  mgF/kg bw/day). There was no significant difference in  $U_{F/Cr}$  between the low and optimal F exposure categories ( $p = 0.089$ ), while the  $U_{F/Cr}$  estimated for excessive F exposure category was statistically significantly higher at 1.69 compared with the other two TDFI categories (low vs excessive  $p < 0.001$ , and optimal vs excessive  $p = 0.019$ ) (Table 2).

## Discussion

To reduce the risk of development of dental fluorosis, total F intake in infancy and early childhood should be monitored and examined for signs of excessive F exposure [O’Mullane et al., 2016]. This study showed a strong positive linear relationship between TDFI and  $U_{F/Cr}$ , which suggests value in the use of a  $U_{F/Cr}$  for estimation of TDFI in young children. This study has also established, for the first time, an estimated upper reference value of a  $U_{F/Cr}$  of 1.69 corresponding to excessive F intake currently suggested as being  $> 0.07$  mg/kg bw/day [Burt, 1992].

This investigation was based on a number of studies which concurrently measured F intake and excretion in children, providing 158 data pairs. The results showed a positive linear correlation between TDFI and DUFE in children younger than 7 years (Figure 1) which is in agreement with a previous report [Villa et al., 2010], generated from 212 data pairs from children aged 0.15-7 years.

These findings confirm that DUFE data could be used to estimate TDFI at community levels. Although DUFE is best measured with 24-hour urine collection, it is very difficult to obtain complete and valid samples using such a collection method in infants and young children, especially those who are not toilet-trained or do not stay dry at night. In contrast, collection of a single-voided (spot) urine is a non-invasive and relatively inexpensive method with less burden on children and their families. The reported positive correlation between DUFE estimated using the  $U_{F/Cr}$  ratio in a spot urine sample and from a 24-hour urine sample in young children [Kertesz et al., 1989; Zohouri et al., 2006; Szekely et al., 2008] supports the use of the  $F/Cr$  ratio of a spot urine sample for estimation of DUFE when the collection of 24-hour samples is not feasible. To estimate DUFE using the  $U_{F/Cr}$ , the 24-hour  $U_{Cr}$  (g/day) for each child is firstly estimated by multiplying their body weight (kg) by the standard creatinine value of 15 mg/kg bw/day. This estimated 24-hour  $U_{Cr}$  (g/day) is then multiplied by the urinary  $F/Cr$  ratio (mg/g) to estimate the DUFE which can then be used to predict TDFI [Zohouri et al., 2006]. The limitation of this approach is the use of a standard  $U_{Cr}$  value of 15 mg/kg bw/day, suggested for white children [Remer et al., 2002], which might not be appropriate for all populations; for example, the mean  $U_{Cr}$ , in the present study (13.5 mg/kg bw/day) was less than the suggested standard  $U_{Cr}$  value of 15 mg/kg bw/day. The other limitation of this method is that TDFI is estimated based on another estimate (i.e. the estimated DUFE).

However, estimating TDFI directly from the  $U_{F/Cr}$  could be a straightforward, valuable alternative measurement for large epidemiological studies as well as for dental public health authorities to facilitate monitoring/evaluation of F exposure in a community prior to and following the introduction of any additional F programmes for caries prevention. The present study is the first to directly investigate the correlation between TDFI and  $U_{F/Cr}$  and it showed a very strong positive correlation between  $U_{F/Cr}$  and DUFE (Figure 2) as well as a good correlation between the  $U_{F/Cr}$  and TDFI (Figure 3), which suggests that the  $U_{F/Cr}$  can be used to obtain reasonably good estimations of TDFI in children. However reference values for this ratio are a prerequisite for a straightforward and simple assessment of signs of excessive F exposure in populations.

The present study showed a mean (SD)  $U_{F/Cr}$  of 1.29 (0.46) g/mg was associated with a F exposure of between 0.05-0.07 mgF/kg bw/day; a level of F intake which is regarded as optimal, providing effective protection against dental caries without causing dental fluorosis. A mean (SD) threshold  $U_{F/Cr}$  value of 1.69 (0.85) mg/g was associated with a F exposure of >0.07 mgF/kg bw/day; regarded as excessive in terms of F body burden if sustained over long periods. Therefore a  $U_{F/Cr}$  of 1.69 mg/g is proposed as an estimate for an upper reference value corresponding to a potentially excessive F intake in children. Although this estimated upper reference value, developed in the present study, provides a simple biomarker for F which can be used to estimate TDFI (i.e. the main risk factor for development of dental fluorosis) in children, until more research is done and  $U_{F/Cr}$  data collected,

some caution, outlined below, should be taken in its use. The proposed estimated upper reference value has been developed based on the current range of estimated optimal F intake (0.05-0.07 mgF/kg bw/day) which was suggested in the absence of the detailed pharmacokinetic and metabolic data necessary for developing scientifically based recommendations [Burt, 1992]. Therefore, there is also a need to re-evaluate the upper limit of F intake by conducting a well-designed longitudinal study with individual assessments of F intake and excretion.

The intake and excretion data collected in the four studies included in the present investigation [Zohouri et al., 2006; Maguire et al., 2007; Zohoori et al., 2013; Omid et al., 2017] were based on thorough assessments of total intake and urinary excretion of F at individual levels, which strengthened the findings of the present investigation. However, in order to generate relatively larger numbers of data, all age groups were combined to create one large group (n=158) with an average age of 5.8 y (Table 1). Although the intake and excretion data, in the present study, were normalised for body weight, to cover the differences in body size by age, larger studies with adequate numbers of data within narrower age groups are needed to establish reference values for the  $U_{F/Cr}$  by age group to allow for any possible effect of skeletal growth rate and consequently F retention in the body. Another aspect worth noting is that all children were from one geographical area (north-east of England), although investigated over a 12-year period (i.e. from 2002 to 2014). The observations seen in this study are well supported by the available although limited literature. A study in Chile [Yévenes et al., 2010] collected spot urine samples (one per child) from two groups of children aged 3-5 years and reported mean concentrations of F and Cr of 0.51 mg/l and 0.44 g/l, respectively, for those living in a fluoridated water (0.48mgF/l) community (n=100) and 0.15 mg/l and 0.45 g/l, respectively for the group living in a non-fluoridated water (0.1mgF/l) community (n=100). In the Chilean study, the  $U_{F/Cr}$  was not reported, but by using the reported mean urinary concentrations of F and Cr, the  $U_{F/Cr}$  would be calculated as 1.16 mg/g (0.51 mgF/0.44 gCr) for the fluoridated water community and 0.33 mg/g (0.15 mgF/0.45 gCr) for the non-fluoridated water community, values which are in agreement with the findings of the present study.

The other points worth highlighting are the numbers of samples and times of day used for collection of spot urine samples. While urine is a biomarker for short-term F exposure, an overnight fasting urine sample has been suggested as a useful potential indicator of chronic F exposure or bone F concentration [Waterhouse et al., 1980]. In addition, due to the urine being retained in the bladder for a longer period overnight, the morning urine sample is more representative of 24-hour urine sample. Therefore for an epidemiological study aiming to collect baseline data in a community, collection of one fasting morning spot urine sample from each participant might be adequate to predict F intake in the absence of dietary F supplementation such as fluoride-tablets or fluoridated milk. However, when monitoring inadvertent F ingestion, or assessing a F-based intervention or community-based

fluoridation programme, a minimum of two spot urine samples (pre- and post-intervention) should be collected to allow appropriate evaluation of the programme. In terms of monitoring for inadvertent F ingestion in particular, a baseline  $U_{F/Cr}$  of 0.42 mg/g has been reported in 42 Spanish children aged 5-8 years living in a non-fluoridated water (<0.3 mgF/l) community, compared with the corresponding ratio of 1.38 mg/g following the application of 0.35 ml fluoride varnish (Duraphat®) [Garcia-Hoyos et al., 2012]. The same authors [Garcia-Hoyos et al., 2014] also reported  $U_{F/Cr}$  ratios of 0.26 mg/g and 1.58 mg/g, respectively, at pre- and post-use of a 10ml 0.2% sodium-fluoride mouthwash in 58 children aged 5-8 years living in a non-fluoridated water (<0.3 mgF/l). Although the results of these two studies clearly indicated unintentional ingestion of F therapies aimed at topical use only but which had resulted in some systemic absorption in the studied children, no further information was given on the administered doses of F, which may have been due to a lack of reference values for a  $U_{F/Cr}$  corresponding to an upper limit of F intake. Since even short-term increases in systemic F ingestion during tooth formation can potentially increase the risk of development of dental fluorosis [Broffitt et al., 2004], the utilisation of a simple biomarker for F, such as the  $U_{F/Cr}$  ratio, is important in potentially bringing the benefits of F exposure monitoring to wider populations. The strong associations shown between  $U_{F/Cr}$  and TDFI in this paper, and the well documented associations seen between TDFI, F body burden and fluorosis provide the opportunity for a simple spot urine collection method and the  $U_{F/Cr}$  ratio to be utilised in determining whether an individual's TDFI reaches a level that could be regarded as a concern in children.

In conclusion, a strong positive linear relationship between TDFI and  $U_{F/Cr}$  was found in this study, which suggests that the  $U_{F/Cr}$  can be used to estimate DUFE and consequently predict/assess TDFI in young children. The estimated upper reference value of F/Cr ratio (1.69 mg/g) in a spot urine sample, proposed in this study, could facilitate F exposure monitoring using a spot urine sample as a simple and useful tool in epidemiological studies. This simplified approach could be valuable for public health authorities and policy makers when monitoring and/or implementing F-based community prevention programmes for oral health.

**Author contributions:**

FVZ and AM designed and supervised the studies; FVZ and AM drafted the paper.

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**Table headings:**

**Table 1.** Mean (SD) age, weight, F intake and urinary excretion data for 158 children aged 18 months to 7 years.

**Table 2.** Mean (SD) estimated  $U_{F/Cr}$  ratio (mg/g) associated with low, optimal and excessive F exposure (mg/kg bw/day) for children aged 18 months to 7 years.

**Figure Legends:**

**Figure 1.** Relationship between estimated daily urinary F excretion (mg/kg bw/day) and total daily F intake (mg/kg bw/day) for 158 data pairs from children aged 18 months to 7 years.

$DUFE$  (mg/kg bw/day) = 0.01 + [0.12 x  $TDFI$  (mg/kg bw/day)]; Pearson's correlation= 0.5,  $p < 0.001$ .

**Figure 2.** Relationship between estimated  $U_{F/Cr}$  (mg/g) and daily urinary F excretion (mg/kg bw/day) for 158 data pairs from children aged 18 months to 7 years.

$U_{F/Cr}$  (mg/g) = 0.16 + [65.05 x  $TDFI$  (mg/kg bw/day)]; Pearson's correlation= 0.87,  $p < 0.001$ .

**Figure 3.** Relationship between estimated  $U_{F/Cr}$  (mg/g) and total daily F intake (mg/kg bw/day) for 158 data pairs from children aged 18 months to 7 years.

$U_{F/Cr}$  (mg/g) = 0.74 + [9.57 x  $TDFI$  (mg/kg bw/day)]; Pearson's correlation= 0.512,  $p < 0.001$

**Table 1.** Mean (SD) age, weight, F intake and urinary excretion data for 158 children aged 18 months to 7 years.

Variables	Mean (SD)		
	All n=158	Girls n=74	Boys n=84
Age (years)	5.8 (1.2)	5.9 (1.1)	5.8 (1.2)
Weight (kg)	21.8 (4.5)	21.8 (4.4)	21.8 (4.6)
Total daily F intake (mg/kgbw/day)	0.049 (0.033)	0.048 (0.036)	0.051 (0.030)
24h urine volume (l)	0.527 (0.236)	0.529 (0.240)	0.526 (0.233)
Daily urinary F excretion (mg/kgbw/day)	0.016 (0.008)	0.015 (0.008)	0.017 (0.008)
Urinary Creatinine excretion (mg/kgbw/day)	13.5 (3.2)	13.1 (3.0)	13.9 (3.3)
U <sub>F/Cr</sub> (mg/g)	1.21 (0.61)	1.18 (0.66)	1.24 (0.58)

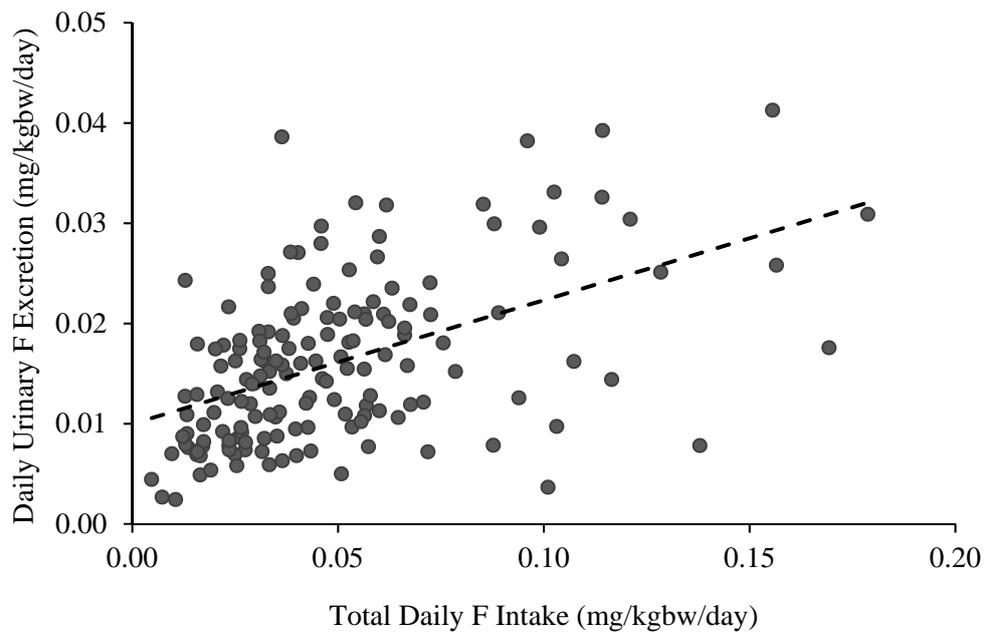
**Table 2.** Mean (SD) estimated  $U_{F/Cr}$  ratio (mg/g) associated with low, optimal and excessive F exposure (mg/kg bw/day) for children aged 18 months to 7 years.

Categories of total F intake	n	$U_{F/Cr}$ (mg/g)	P value
Low (<0.05 mg/kgbw/day)	96	1.05 (0.50)	<sup>a</sup> Low vs Optimal: p=0.089
Optimal (0.05-0.07 mg/kgbw/day)	34	1.29 (0.46) <sup>a</sup>	<sup>b</sup> Low vs Excessive: p<0.001
Excessive (>0.07 mg/kgbw/day)	28	1.69 (0.85) <sup>b,c</sup>	<sup>c</sup> Optimal vs Excessive: p=0.019

**Figure 1.** Relationship between estimated daily urinary F excretion (mg/kg bw/day) and total daily F intake (mg/kg bw/day) for 158 data pairs from children aged 18 months to 7 years.

$$\text{DUFE (mg/kg bw/day)} = 0.01 + [0.12 \times \text{TDFI (mg/kg bw/day)}]$$

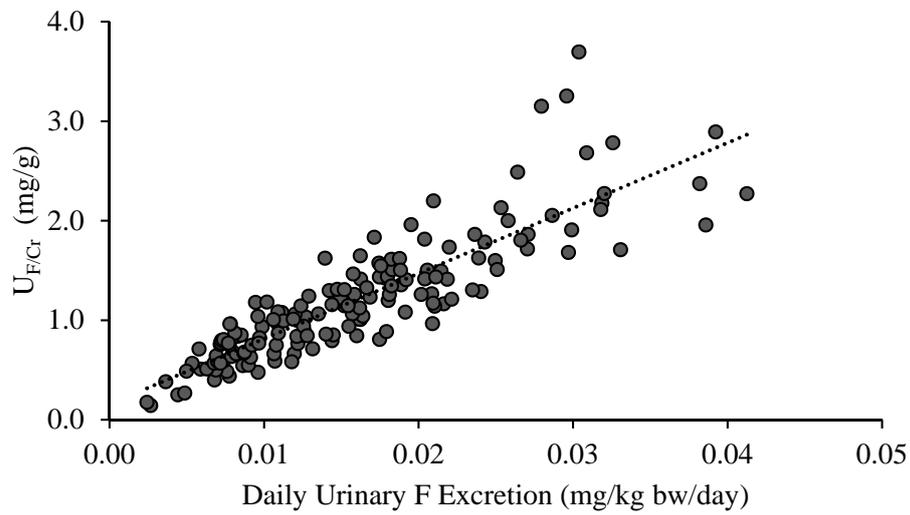
Pearson's correlation= 0.5,  $p < 0.001$ .



**Figure 2.** Relationship between estimated  $U_{F/Cr}$  (mg/g) and daily urinary F excretion (mg/kg bw/day) for 158 data pairs from children aged 18 months to 7 years.

$$U_{F/Cr} \text{ (mg/g)} = 0.16 + [65.05 \times \text{TDFI (mg/kg bw/day)}]$$

Pearson's correlation= 0.87,  $p < 0.001$ .



**Figure 3.** Relationship between estimated  $U_{F/Cr}$  (mg/g) and total daily F intake (mg/kg bw/day) for 158 data pairs from children aged 18 months to 7 years.

$$U_{F/Cr} \text{ (mg/g)} = 0.74 + [9.57 \times \text{TDFI (mg/kg bw/day)}]$$

Pearson's correlation= 0.512,  $p < 0.001$

