

Development of a standardized method for comparing fluoride ingested from toothpaste by 1.5–3.5-year-old children in seven European countries. Part 2: Ingestion results

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Abstract – Objectives: To develop a standardized method for measuring the variables affecting fluoride ingestion from toothpaste in young children between the ages of 1.5 and 3.5 years, and to use the method at seven European sites. **Methods:** Random samples of children were invited to take part in the study. Parents who gave consent were visited at home. The children brushed their teeth using the toothpaste brand and toothbrush type currently in use. The difference between the fluoride dispensed onto the toothbrush and the fluoride recovered after accounting for losses was deemed to be the fluoride ingested. Details of other oral health-care habits were collected by questionnaire. For each child, the fluoride concentration of the toothpaste used was measured in the laboratory, from which an estimate of total daily fluoride ingestion was made. **Results:** There was considerable variation between countries in the types of toothpaste used and in the amounts of toothpaste applied and ingested. The amount of fluoride ingested ranged from 0.01 to 0.04 mg fluoride per kg of body weight per day. **Conclusion:** The amount of fluoride ingested that is likely to be a risk factor for the development of dental fluorosis during tooth formation is equivocal and was found to vary widely between European countries. There appears to be a need for clearer health messages regarding the use of fluoridated toothpaste by young children.

Key words: children; dental fluorosis; fluoride ingestion; standardization; toothpaste

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Reports of an increased prevalence of enamel fluorosis in permanent incisors have attributed this in part to regular ingestion of fluoride-containing toothpastes by very young children (1). More recently (2) in a study of children where the prevalence of fluorosis was 12.9% using the Thylstrup and Fejerskov (TF) index, beginning brushing before the age of 2 years increased the severity of fluorosis

significantly ($P < 0.001$). Amongst research workers and public-health dentists two schools of thought are emerging on this issue. On the one hand, there are those who strongly believe that the fluoride concentration in toothpastes should be reduced to less than 600 ppm for children aged 6 years and younger, at low risk of developing caries (3). Such children often swallow some of the toothpaste they

use and some subsequently develop unacceptable levels of enamel fluorosis. Decision-makers and legislators are becoming increasingly aware of this point of view and discussions on the elimination or reduction of the fluoride content of toothpastes have commenced in some countries. A recent national survey estimated that 34% of children in Great Britain aged between 1.5 and 4.5 years used toothpastes formulated for children containing less than 600 ppm F (4). The opposing school of thought is that reducing fluoride concentrations in toothpastes to below 600 ppm F inevitably leads to an increase in caries levels (5). In addition, this latter group regards the levels of enamel fluorosis seen as minor and not a public-health problem. They accept that a very small number of children who seriously misuse the product when aged less than 3 years may suffer from unsightly fluorosis affecting the permanent incisors. However, they argue that adopting procedures or technologies aimed at controlling the amount of toothpaste used at each brushing by young children could solve this problem.

The overall aim of 'Project FLINT' was to address this gap in current knowledge concerning a possible link between fluoride ingestion from toothpaste by young children and dental fluorosis (6). The first part of the work aimed to collect standardized epidemiological information on the important individual variables that may affect fluoride ingestion from toothpaste (7). This second part of the study aimed to identify and characterize the practices and habits of those children at risk of developing fluorosis of the permanent central incisor teeth following ingestion of fluoride from toothpaste alone. Fluoride concentration in toothpaste used by young children was measured and the amount of fluoride ingested during toothbrushing was determined, from which the total daily fluoride intake could be estimated.

This paper describes the laboratory-based aspects of fluoride analysis of the toothpastes used and of the expectorates and rinse water collected, the statistical analysis of the data, the inferred levels of fluoride ingestion and the implications of these data as risk factors for fluorosis.

Materials and methods

Details of the study sites, sample selection and methodology are given elsewhere (7).

Briefly, samples of about 100 children, aged 1.5–2.5 and 2.5–3.5 years, were chosen (where possible) at random at each of seven sites in different

European countries: Almada/Setubal (Portugal), Athens (Greece), Cork (Ireland), Haarlem (the Netherlands), Knowsley (UK), Oulu (Finland) and Reykjavik (Iceland). In practice, a third age group, >3.5 years, was also included.

Fluoride ingestion from toothpaste

During home visits each parent/child was given a new toothbrush and a preweighed tube of toothpaste, both of which were identical to those in current use. The parent/child conducted toothbrushing in their normal manner. Double de-ionized water was provided for rinsing if required. A large plastic bowl was used to collect expectorate. Toothpaste or expectorate dropped elsewhere was either fully recovered (usually by scraping the child's face with a spatula) or recorded as a case with losses.

Fluoride analysis – postbrushing samples

The used toothbrushes, expectorate and recovered toothpaste were placed in sealed plastic containers. At the laboratory each brush and each container was rinsed in 20 mL de-ionized water. The brush was placed in an ultrasonic bath for 10 min to extract any remaining toothpaste. The resulting solution was then mixed with the expectorate sample and rinse water, and the mixture was made up to a constant volume (120 mL) and vortex stirred for 5 min. Sub-samples of 10 mL and 5 mL were frozen and the 5 mL sub-samples were sent to the central (Cork) laboratory on dry ice for determination of fluoride concentration.

Samples containing sodium monofluorophosphate (SMFP) were treated as described by Duckworth et al. (8). After the addition of 10% by volume of total ionic strength adjustment buffer (TISAB), samples were stirred for 24 h before fluoride analysis using a fluoride ion-selective electrode (ISE, 720 A series, Orion Research). To minimize electrode hysteresis effects, particularly at low fluoride concentration, samples were analysed on two separate occasions. The second readings, carried out in order of concentration, were used in the calculations. Fluoride ingestion from toothpaste was determined as the mass of fluoride dispensed minus the total mass of fluoride recovered.

Fluoride analysis – toothpaste

The fluoride concentration in the toothpaste used by each individual child was determined by ISE at the central laboratory and, when necessary, by gas chromatography (GC; Hewlett Packard 5890) at Unilever Research (9).

The fluoride in toothpastes that contained either sodium fluoride or amine fluoride was analysed by ISE. SMFP pastes were analysed by ISE or GC depending on the abrasive system.

When using the fluoride ISE, 1 g of toothpaste ± 0.0001 g was used to form a slurry (1:10 with deionized water) by vortex mixing for 10 min. TISAB was then added to the slurry (1:1) and the sample was stirred continuously for 24 h. The toothpaste slurry was filtered through a 0.2- μ m filter. The filtrate was further diluted 0.1 g to 10 g of deionized water before analysis. Samples containing SMFP were further treated as described by Duckworth et al. (8). Reagents were prepared as described by Duckworth et al. (10).

Validation of the fluoride ISE measurements was conducted by analysis of a set of toothpastes, representing the different fluoride sources, by both ISE and GC.

Statistical analysis

Analyses of variance were carried out on key parameters by country and by age group, and a regression analysis was carried out to characterize children at risk of developing fluorosis of the permanent central incisor teeth following ingestion of fluoride from toothpaste alone. The outcome/dependent variable for fluoride ingested was mg F/kg/day. The analysis used the following restrictions: subjects had to be aged 1.5 years or more with no losses of toothpaste at the time of toothbrushing during the home visit, who used their usual toothpaste and brushed at least once per week.

Results

Methodological error

Resting salivary levels of fluoride are reported to be between 0.01 and 0.03 ppm F (11) and were therefore deemed insignificant in the calculations of fluoride ingested from toothpaste.

For all countries except Portugal, nine out of 1073 measurements suggested that a child expectorated more fluoride than was known to have been dispensed. Methodological error could account for four values and these cases were deemed to represent zero ingestion. The remaining five values were thought to be genuine outliers and were excluded from the statistical analysis.

The data from Portugal presented a challenge: 72 out of 198 samples suggested more fluoride was expectorated than was possible from the toothpaste

dispensed. The decision was made to exclude all the Portuguese fluoride ingestion data as it was deduced from local information that the Portuguese children might have consumed fluoride tablets close to the time that the measurements for fluoride ingestion from toothpaste were made.

Fluoride concentration in toothpaste used

Of the 188 different brands and batches of toothpaste analysed, 151 were analysed by ISE and 37 by GC. Validation of the ISE method showed no statistically significant difference between the readings obtained from ISE and from GC (*t*-test, $P > 0.4$).

For various reasons the fluoride concentration measured in a tube of toothpaste may differ from the amount stated on the manufacturer's label. Of the 188 tubes of toothpaste with different batch numbers used in the study, 25% of the laboratory values agreed with the label concentration, 59% were lower and 16% were higher. In most cases (77%), differences were ± 100 ppm F. Remarkably, four tubes contained 400–500 ppm F more than the stated amount.

For the toothpaste categories 'children's' and 'regular' there was a difference between the mean fluoride concentrations at the various study sites. In Haarlem the mean concentration of children's toothpaste was 420 ppm F and in Oulu it was 950 ppm F. The difference in mean fluoride concentration of the regular toothpastes was not so great. For example, the lowest mean concentration for regular toothpaste was in Cork (1221 ppm F) and the highest was in Almada/Setubal (1399 ppm F). The smallest difference between the mean concentration of a children's paste and a regular paste was in Oulu (289 ppm F). The greatest difference between the two mean values was in Haarlem (814 ppm F).

Fluoride ingested during toothbrushing

Table 1 lists the fluoride ingested per kg body weight by each child from the observed episode of toothbrushing. These data were then multiplied by the reported brushing frequencies to give estimates of mg fluoride ingested per kg body weight per day (Table 2).

Estimated percentage fluoride ingested from the amount dispensed

Table 3 shows the upper estimates of fluoride that may have been ingested as a proportion of the fluoride dispensed. The data indicate that children between 1.5 and 2.5 years old ingested an estimated average of 64% (Athens) to 84% (Reykjavik) of the

Table 1. Mean fluoride ingestion from toothpaste during the observed toothbrushing

Age group	Study site	Mean F intake (mg/kg body weight)
1.5–2.5 years	Athens (n = 32)	0.017
	Cork (n = 44)	0.016
	Haarlem (n = 94)	0.008
	Knowsley (n = 49)	0.017
	Oulu (n = 59)	0.013
2.5–3.5 years	Reykjavik (n = 23)	0.017
	Athens (n = 79)	0.014
	Cork (n = 96)	0.010
	Haarlem (n = 66)	0.009
	Knowsley (n = 69)	0.014
>3.5 years	Oulu (n = 103)-	0.010
	Reykjavik (n = 65)	0.023
	Athens (n = 88)	0.010
	Cork (n = 18)	0.007
	Haarlem (n = 21)	0.008
	Knowsley (n = 36)	0.015
	Oulu (n = 26)	0.006
	Reykjavik (n = 46)	0.018

toothpaste dispensed. For the children between the ages of 2.5 and 3.5 years, this percentage was between 53% (Athens) and 82% (Reykjavik). Percentage fluoride ingested was highly significantly related to age group and to country ($P < 0.0001$). Differences between the age groups were not consistent across the countries ($P > 0.28$).

Table 2. Percentage frequency distribution of estimated mg F/kg body weight/day ingested from toothpaste

Study site	0.000– <0.004	0.004– <0.008	0.008– <0.020	0.020– <0.040	0.040– <0.060	0.060– <0.080	0.080– <0.100	≥0.100	Mean ingested (SD)
1.5–2.5 years									
Athens (n = 28)	32	21	21	11	7	0	4	4	0.02 (0.03)
Cork (n = 44)	18	11	30	32	5	0	2	2	0.02 (0.02)
Haarlem (n = 88)	28	24	32	14	1	0	0	1	0.01 (0.04)
Knowsley (n = 47)	9	4	32	34	9	9	2	2	0.03 (0.03)
Oulu (n = 57)	28	25	33	7	2	2	0	4	0.01 (0.03)
Reykjavik (n = 23)	26	26	4	22	17	0	4	0	0.02 (0.02)
2.5–3.5 years									
Athens (n = 66)	42	17	23	9	5	3	0	2	0.01 (0.02)
Cork (n = 96)	21	29	33	8	5	2	1	0	0.01 (0.02)
Haarlem (n = 66)	23	23	36	12	2	2	0	3	0.02 (0.03)
Knowsley (n = 69)	12	12	41	17	9	6	3	1	0.02 (0.02)
Oulu (n = 100)-	27	26	27	16	3	1	0	0	0.01 (0.01)
Reykjavik (n = 64)	8	14	30	20	17	6	0	5	0.04 (0.08)
>3.5 years									
Athens (n = 78)	41	28	21	6	3	0	1	0	0.01 (0.01)
Cork (n = 18)	33	28	22	11	0	6	0	0	0.01 (0.02)
Haarlem (n = 21)	38	14	29	14	5	0	0	0	0.01 (0.01)
Knowsley (n = 35)	6	11	40	23	9	6	3	3	0.03 (0.03)
Oulu (n = 25)	36	28	36	0	0	0	0	0	0.01 (0.00)
Reykjavik (n = 44)	11	20	25	25	11	2	0	5	0.03 (0.03)

Shaded columns indicate children who ingested more than the ‘safe’ amount of fluoride.

Children at risk of developing fluorosis

None of the data from Portugal were used in the analysis. Of a possible remaining 978 subjects, 955 matched the inclusion criteria. The factors that were found to be significant were: age, $P < 0.0001$ (age was also highly correlated with body weight); weight of toothpaste used, $P < 0.0001$; fluoride concentration of the toothpaste used, $P < 0.0001$; and country, $P < 0.0001$. The following interactions were also significant: country and weight of toothpaste ($P < 0.0001$) and country and fluoride concentration ($P < 0.0001$).

Discussion

Fluoride content of toothpaste

The validation checks carried out on the use of the ISE confirmed that the methods used were acceptable. Possible reasons for lower fluoride than that stated on the tube are beyond the scope of this discussion. Leading manufacturers have restricted their regular brands in Europe to 1450 ppm F to avoid exceeding the EU upper limit of 1500 ppm F (12). It is of some concern that four tubes of toothpaste contained between 400 and 500 ppm more fluoride than the claimed amount. There would appear to be no other reported literature on quality control issues concerning fluoride concentration of toothpaste.

Fluoride ingestion

The approach used for determining fluoride ingestion in this study may overestimate the true ingested amount for two reasons. First, the percentage of F absorbed into blood is less after eating (13, 14), and second, some fluoride from toothpaste is retained in the oral soft tissues, plaque and teeth. Hence, in many cases estimated ingestion may overestimate fluorosis risk and values reported should therefore be regarded as an upper limit. Most of the fluoride in toothpaste ingested on an empty stomach is absorbed into the blood stream (15). The bioavailability of this absorbed fluoride is then dependent on factors including acid–base balance, renal clearance and bone fluoride concentration (16). The effects of fluoride from toothpaste must therefore be considered in terms of both plasma peak concentration following ingestion and the percentage contribution to the total body intake of fluoride.

The level of fluoride intake (from all sources) beyond which 'unacceptable' dental fluorosis will occur has been reported as 0.05–0.07 mg F/kg body weight/day (17–19). In terms of plasma fluoride levels it has been shown that ingestion of 0.036 mg F/kg body weight from fluoride toothpaste in 3–4-year-old children was insufficient to produce a peak of 0.1 ppm, which was considered the threshold required for fluorosis (20). The average ingested amounts at each site are within this 'safe' level of 0.036 mg F/kg body weight per brushing.

For the three age groups of children, the overall mean amount of fluoride potentially ingested from

toothpaste was 0.02 mg F/kg body weight/day. Similar results were obtained in 3–4-year-old children from both a fluoridated and nonfluoridated area in New Zealand where the fluoride ingestion from toothpaste was found to be 0.017 and 0.019 mg F/kg/day, respectively (21).

The mean contribution to the total daily fluoride intake from toothpaste in children of this age has been reported to be in the range of 43–71% (22, 23). If it is accepted that the daily intake should not exceed 0.05–0.07 mg F/kg body weight/day (24), then the mass of fluoride ingested from toothpaste should not exceed 0.022–0.036 mg F/kg body weight/day. Table 2 can be used as a guide to some of the children for whom potential fluoride ingestion from toothpaste may be a risk.

Estimated percentage fluoride ingested from the amount dispensed

The overall average percentage of dispensed fluoride ingested was 64%. A high percentage of children in the two youngest age groups appeared to ingest between 80% and 100% of the fluoride dispensed (Table 3). This percentage reduced markedly after the age of 3.5 years. The exception to this was Haarlem where the percentages of children ingesting 80–100% of the fluoride dispensed were 48%, 42% and 38% for the three age groups, respectively. This may reflect national differences in oral-hygiene practices in children and possibly also their parents. For all the countries combined children over 3.5 years ingested a mean of 53% (range of averages 39% to 67%) of the

Table 3. Distribution (percentage of children) of the estimated percentage fluoride ingested from the amount dispensed onto the toothbrush during brushing

Age group	Study site	0–<20%	20–<40%	40–<60%	60–<80%	80–100%	Mean percentage F ingested (SD)
1.5–2.5 years	Athens (<i>n</i> = 28)	4	11	29	21	36	64.3 (24.1)
	Cork (<i>n</i> = 47)	0	2	21	30	47	75.5 (18.0)
	Haarlem (<i>n</i> = 89)	1	3	11	36	48	76.2 (17.1)
	Knowsley (<i>n</i> = 47)	4	4	6	36	49	74.9 (20.6)
	Oulu (<i>n</i> = 57)	0	4	14	32	51	77.1 (16.3)
	Reykjavik (<i>n</i> = 23)	0	0	0	30	70	83.9 (8.1)
2.5–3.5 years	Athens (<i>n</i> = 66)	11	27	17	26	20	52.7 (25.1)
	Cork (<i>n</i> = 96)	7	24	33	20	16	53.3 (23.2)
	Haarlem (<i>n</i> = 67)	3	4	28	22	42	69.6 (23.0)
	Knowsley (<i>n</i> = 69)	3	9	17	33	38	68.5 (20.7)
	Oulu (<i>n</i> = 100)	3	12	35	23	27	61.0 (22.8)
	Reykjavik (<i>n</i> = 64)	0	5	5	22	69	81.9 (17.1)
>3.5 years	Athens (<i>n</i> = 78)	33	27	17	17	6	38.7 (24.4)
	Cork (<i>n</i> = 18)	0	61	28	6	6	40.2 (18.3)
	Haarlem (<i>n</i> = 21)	5	24	10	24	38	62.7 (28.5)
	Knowsley (<i>n</i> = 35)	3	14	40	31	11	57.7 (19.4)
	Oulu (<i>n</i> = 25)	24	12	32	8	24	49.8 (30.4)
	Reykjavik (<i>n</i> = 44)	2	11	25	34	27	67.3 (21.0)

fluoride dispensed. This compares with averages of 40% for 3–6-year-olds (18) and 62% for children aged 40–48 months (24) reported in previous studies.

Children at risk of developing fluorosis

Children were identified as being at risk of fluorosis by regression analysis. Age, fluoride concentration of the toothpaste used and country were all highly significant. Factors such as amount of toothpaste used, brushing frequency, whether the child expectorated and whether the child expectorated more than three times entered the regression analysis but were not found to be significant risk factors for fluorosis. It has been reported that the use of toothpaste flavoured for children has been related to higher ingestion than that for regular pastes (25). Use of children's toothpaste, eating toothpaste and liking the taste of toothpaste were therefore included in the regression analysis model in this study but were not found to be significant factors for fluorosis risk. Mascarenhas and Burt (2) in a study on risk from early exposure to fluoride toothpaste found significantly ($P < 0.001$) increased severity of fluorosis when brushing commenced before the age of 2 years. Other factors such as eating or swallowing fluoride toothpaste and higher frequency of use, did not show a statistically significant increased risk for prevalence or severity of fluorosis. Other factors that entered the model and were also found not to be significant included the educational status of the parent, duration of toothbrushing, size of toothbrush head and whether the parent or the child placed the toothpaste on the brush. Levy et al. (26) also found that there was a negative association with parental assistance in brushing and fluoride ingestion. The authors intend to conduct a follow-up study to assess clinically the actual fluorosis levels that develop in the children. By that means, they will also be able to assess the validity of the deductions made here concerning fluorosis risk.

When considering the most appropriate concentration of fluoride in toothpaste for children, there is obviously a need for compromise to balance the benefits and risks. More specifically, so that the user of the product can be given simple clear messages, it is important for manufacturers, dental professionals and policy-makers to agree. Collaborative work is therefore required to optimize the benefits of fluoride toothpaste and reduce the risk of fluoride ingestion.

In this paper standardized data collected from seven European countries have been presented which identify the practices and habits of those

children at risk of developing fluorosis of the permanent central incisor teeth following ingestion of fluoride from toothpaste. For the first time this has enabled direct comparisons to be made across regions and apparent differences have been highlighted. Much more effort needs to be given to promoting the appropriate use of toothpaste as a topical vehicle for fluoride delivery. It is important not to neglect our role in educating and encouraging parents to start training their children to expectorate at an early age and to use small amounts of toothpaste. This has been demonstrated by van Loveren et al. (27) in his paper investigating further the relationship between fluoride dispensed and fluoride recovered from the toothbrush, the expectorate and the after brush rinses at the Dutch and Irish study sites.

Since age and fluoride concentration of toothpaste were significant factors in the regression analysis for fluorosis risk it is time that there was a consensus from the toothpaste industry about fluoride concentration and age-related marketing strategies. Longitudinal studies may be necessary to fully understand the relationships between fluoride ingestion over time and development of fluorosis.

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