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Daily dietary intake of fluoride by Slovenian Military based on analysis of total fluorine in total diet samples using fluoride ion selective electrode

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Abstract

The purpose of this study was to develop an analytical procedure for determination of the amount of total fluoride in total diet samples, including drinking water and beverages. Samples were taken by the duplicate portion technique and decomposed by alkali carbonate fusion using $KNaCO_3$, and the amount of fluoride in solution was determined by fluoride ion selective electrode using the multiple known addition technique. The mean amount of total fluoride determined in 20 total diet samples obtained from the Slovenian Military was 1.84 ± 0.70 mg/kg on a dry matter basis. Accordingly the estimated daily intake was 1.50 ± 0.56 mg.

Keywords: Fluoride; Diet; Daily intake; Decomposition; Fluoride ion selective electrode

1. Introduction

Fluorine is one of several trace elements receiving much attention owing to its possible harmful effects on health and the environment. The effect of fluoride on humans is a dual one; it is an essential trace element at low levels and is a potentially toxic element at higher levels.

In general, drinking water, beverages, dentifrices and other dental agents are regarded as the main dietary contributors to human fluoride intake. The adequate intake for fluoride from all sources is set at 0.05 mg/day/kg body weight; this intake is recommended for all ages greater than 6 months because it confers a high level of protection against dental caries and is associated with no known unwanted health effects (DRI, 1997); accordingly adequate intakes of 3.1–3.8 mg/day of fluoride for adult men and women were set and accepted by the German and Austrian Nutrition Society and the Swiss Nutrition Association (Reference Values for Nutrition Intake, 2002). These guidelines were also accepted in Slovenia in 2004 (Referenčne vrednosti za vnos hranil, 2004).

The amount of fluoride in food is usually within the range 0.1–1 mg/kg on a fresh weight basis; exceptions to this include fluoridated water, beverages and some infant formulas that are made or reconstituted with fluoridated water, teas, and some marine fish (Lopez & Navia, 1988; Singer & Ophaug, 1986; Taves, 1983). San Filippo and Battistone (1971) estimated 0.8 to 0.9 mg, Singer, Ophaug, and Harland (1980) 0.5 to 1.1 mg and Taves (1983) 0.4 mg as the daily intakes of fluoride from food with no intake of drinking water taken into account; regrettably, energy values regarding the fresh and dry weights of the total daily diets were not reported. Haldimann and Zimmerli (1993) reported a total daily intake of 0.9 mg of fluoride per adult from food in an unfluoridated area on the basis of a mean daily intake of 460 g dried matter. No data on the daily intake of fluoride through foodstuffs has been reported to date in Slovenia.

The entire fluoride content in drinking water is present in ionic form as free fluoride (F_f^-), which can be determined by a fluoride ion selective electrode (ISE) (Frant &

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Ross, 1966). Fluoride in diet is present in both ionic and bound forms so total decomposition of the sample, which seems to be the critical step of the entire analytical procedure, is a prerequisite for the purpose of determining the amount of total fluoride (F_t^-) (Liebman & Ponikvar, 2005; Lopez & Navia, 1988). Among various decomposition procedures the most important ones are open ashing, fusion, oxygen combustion and acid digestion, that could be followed by separation or concentration steps such as distillation, diffusion, pyrolysis and solvent extraction; the amount of fluoride in thus prepared samples is most often determined by a fluoride ISE, spectrophotometrically or by the use of gas chromatography (Haldimann & Zimmerli, 1993; Venkateswarlu, 1990).

Several studies reported dry ashing with alkali as an ashing aid for total fluoride (Inkilewicz, Czarnowski, & Krechniak, 2003; Kjellevold Malde, Bjorvatn, & Julshamn, 2001; Singer & Ophaug, 1986), while the use of fusion with alkali metal carbonates, which is generally appropriate for dealing with insoluble or inorganic complex fluorides (Bock, 1979), was not reported for use in decomposition of food or diet samples. In addition, it is also interesting to note that calibration of the fluoride ISE for determination of fluoride at low levels was usually performed using the direct calibration technique, while the use of the automated standard addition technique was not reported. This technique termed also multiple known addition technique by the producer assures (Orion, 1991): (1) minimization of matrix effects because it enables calibration of the electrode in the sample solution; (2) greater precision of the analysis; (3) a spike recovery test is performed on each sample and; (4) shorter time and greater convenience of the analysis.

The aim of our study was to develop a procedure for determination of total fluoride in total diet with decomposition by alkali carbonate fusion and subsequent determination of fluoride by fluoride ISE using the semiautomatic multiple known addition technique. The proposed method should enable determination of the amount of F_t^- in total diet samples, which were obtained from the Slovenian Military and an estimation of the daily intake of men aged between 18–28 years.

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade and all solutions were prepared using double-distilled water. A 50% solution of sodium hydroxide (Merck) was prepared by dissolving the reagent pellets in water. For alkaline carbonate fusion, solid $NaKCO_3$ (Merck) was used and for the acidification of samples, conc. H_2SO_4 (Merck).

A citrate buffer solution (CBS buffer) (Peters & Ladd, 1971) was prepared by dissolving 35.3 g of CDTA (diaminocyclohexanetetraacetic acid, Merck) in water followed by the addition of 50% NaOH. Once all the salt had dissolved, 600 g of tri-sodium citrate dihydrate (Carlo Erba) and 120 g of sodium chloride (Carlo Erba) were added. The solution was then diluted to approximately 1.9 l, the pH adjusted to pH 6.0 using conc. HCl (Merck) and made up to volume (2 l) with water.

A 5.0 mg/l fluoride standard solution was prepared daily by dilution of a 100.0 ± 0.5 mg/l standard solution of fluoride (Orion). A 0.050 mol/l working solution of NaF was prepared by dissolving NaF (Merck) in water.

The accuracy and precision of the methods employed were checked by the analysis of a standard reference material, SRM 2695 Fluoride in Vegetation, consisting of a standard with a high level and a standard with a low level of fluoride (National Institute of Standards and Technology).

2.2. Sample collection and preparation

Total diet samples were collected in Slovenian Military barracks from different regions, where more than 100 meals per day were prepared. Soldiers nutrition is prepared in compliance with official recommendations (Slovene Ministry of Defence, 1994) that are harmonized with USA standards (Nutrition Standard & Education, 2001). Soldiers consume four daily rations (breakfast, snack, lunch and dinner) and beverages composed of food typical in the Slovene diet. At least twice a day meals were composed of meat or fish and their products (fish paste, liver pâte, meat pie, sausages). So, 10 different daily menus were prepared. The duplicate portion technique was used to collect total diet samples (including beverages and drinking water) over a period of two months in the year 2002. Twenty composite total diet samples were sampled five times from four barracks, giving 20 samples. To collect a composite total daily diet sample, servings of all foodstuffs (as served to each soldier at the counter) were randomly taken for each of the four meals, and this in triplicate. Total diet samples were homogenized in a titanium blender, frozen at −24 °C and then lyophilized and finally milled in an agate mill. The mean energy value of the daily diet was 3770 ± 538 kcal. The fresh mass of the total diet samples ranged from 2900 to 4600 g, average 3957 ± 401 g; the mass of lyophilized samples ranged from 790 to 1150 g, average 959 ± 134 g; the mass of dry samples ranged from 600 to 1000 g, average 820 ± 122 g, (dried at 105 °C to the constant mass) and the moisture content ranged from 77.1–82.9% with a mean value of $79.3\% \pm 1.7\%$ (Smrkolj, Pograjc, Hlastan-Ribič, & Stibilj, 2005).

2.3. Sample decomposition

Approximately 5 g of sample was weighed into a 70 ml platinum dish with a precision of ±0.05 mg and soaked with 1 ml of 50% NaOH. After the sample had been evaporated to dryness in a sand bath, it was decomposed by fusion with 6 g of $KNaCO_3$ using a Bunsen burner and melted until a transparent melt with no solid remains was obtained. This was then cooled and quantitatively transferred to a 100 ml polyethylene volumetric flask. Before determining the fluoride content, the digestates were neutralized by addition of

conc. H_2SO_4, acidified to pH 2–3 and diluted with water. A blank solution of all reagents used during the digestion of the samples was also prepared.

2.4. Determination of fluoride

An Orion 960 autochemistry system with a temperature sensor and combined fluoride ISE (Thermo Orion model 96-09) was used for the potentiometric determination of fluoride. Polyethylene flasks and beakers were used throughout. Calibration solutions were prepared by adding 2 ml of 5.0 mg/l standard solution of fluoride to a 50 ml flask. Before final dilution with water to 50 ml, a 25 ml aliquot of CBS buffer was added to assure a constant ionic strength, an appropriate pH and the complexing and masking of interfering ions. This solution was then transferred to a beaker. At least six consecutive determinations of fluoride in the calibration solution were conducted daily to determine the mean amount of fluoride. The amount of fluoride in samples was determined in a 20 ml aliquot with 25 ml CBS, spiked by the addition of 2 ml of 5.0 mg/l standard solution of fluoride.

The amount of fluoride was determined by the multiple known addition technique using a fluoride ISE at room temperature and a 0.050 mol/l working solution of NaF (Orion, 1991). Adjustments made to the factory settings of the apparatus were the stability criteria of the electrode, which was set to be 1 mV/min (vs. a proposed 3 mV/min) and the constant increment, which determines the standard additions of working solution, was set to be 18 mV (vs. a proposed 10 mV/min). The results reported were within the required ranges if: (1) results calculated after the first addition were similar to the result from the final addition; (2) the electrode slope was 59 ± 2 mV/DEC; (3) the level of precision was equal or better than the required 2%; (4) spike recovery was $100 \pm 2\%$; and (5) no warning messages appeared.

When determining the amount of fluoride in samples the mean amount of fluoride determined in the calibration solution was converted to mmol (amount of fluoride present in 50 ml flask) and entered into the program that automatically subtracted this value from the final result of the determination reported in mg/kg (Orion, 1991). The amount of fluoride in the calibration solution was checked every 2 h. In order to clean the sensing element before each determination, the electrode was soaked for 5 min in distilled water, blotted dry and then soaked for 5 min in sample solution, and the program then started.

The analysis was performed on duplicate sample portions. The amount of fluoride in each portion was measured in at least twice.

3. Results and discussion

3.1. Methods

Low amounts of fluoride were expected in samples of total diet, so 5 g of sample was weighed in the platinum dish; this is the highest amount of the sample that could be decomposed in a 70 ml dish.

Different approaches to the decomposition were studied. First experiments were conducted by fusion of the sample directly with $KNaCO_3$ using a Bunsen burner without prior ashing of the sample with an ashing aid. Since samples tended to ignite, use of an oven is not recommended. While determining amount of fluoride in a sample decomposed in this way it was shown that the entire content fluoride was not detected in solution.

In order to fix the fluoride in the sample prior to open fusion with $KNaCO_3$, the sample was made strongly alkaline by an addition of 1 ml of 50% NaOH and evaporated to dryness on a sand bath. Further, it was decomposed by fusion with 6 g of $KNaCO_3$. First attempts were made by melting the sample in the oven. Low and irreproducible results obtained in that way were ascribed to the fact that the entire dish glows in the oven so the melt is slowly lost from the dish and with it some of the fluoride. Instead of using an oven, a Bunsen burner was used for the purpose of melting the sample so that only the bottom of the dish was glowing. The decomposition was finished when a transparent melt was obtained.

The multiple known addition (MKA) technique was selected as the technique of choice for determination of low concentrations of fluoride. First, appropriate parameters of the MKA technique at low levels of fluoride had to be determined. For this purpose the concentrations of fluoride in different standard solutions of fluoride were first determined and compared to the theoretical amount. These experiments revealed that a good compromise between precision, accuracy and speed of analysis. The speed was achieved when determining low concentrations of fluoride using a 0.050 mol/l standard solution of NaF as a working standard solution and modification of the built-in program of the apparatus so that the electrode drift was 1 mV/min and the standard increment 18 mV. The memory effect of the electrode had to be minimized before each repeated measurement (see Experimental). The accuracy and precision of the fluoride determinations at low concentrations was than systematically studied. The results of these experiments are presented in Table 1.

The results revealed the presence of a positive error under the selected operating conditions of the fluoride ISE. This error could be lowered and thus the accuracy improved by reducing the electrode drift and/or the standard increment, but then the time of analysis would increase substantially because longer times would be required to establish stable electrode potentials during each measurement; consequently the precision of the measurements would worsen.

The mean amounts of fluoride determined were then plotted against the theoretical amounts of fluoride and a straight line was fitted to the measured points by the least squares method. The regression line coefficient (R) indicated high linearity ($R = 1.000$) of the curve. The intercept of the derived least-squares line at 0.011 mg/l shows the

Table 1
Accuracy and precision of the determination of fluoride at low concentrations

$C(F^-)_{theor.}$ (mg/l)	Number (n)	$C\,(F^-)_{det.}$ (mg/l)	$C(F^-)_{det.} - C(F^-)_{theor.}$ (mg/l)	SD[a] (mg/l)	RSD[b] (%)
0.050	3	0.060	0.010	0.003	4.76
0.100	3	0.110	0.010	0.004	3.51
0.200	5	0.213	0.013	0.005	2.49
0.400	3	0.412	0.012	0.012	2.95
1.000	4	1.013	0.013	0.028	2.80

[a] SD is the standard deviation.
[b] RSD is the relative standard deviation.

presence of a positive error throughout the entire investigated concentration range that is equal to the blank value; that is the case if no fluoride is present in the investigated solution.

The precision of the results is higher and the time of analysis shorter, when working in the linear operating range ($1–10^{-5}$ mol/l) of the fluoride ISE. According to the manufacturers declaration the linear range begins at an approximate fluoride concentration of 0.2 mg/l (Orion, 1991). With the purpose of obtaining accurate and precise results in a short time, we examined whether it would be advantageous to spike samples by an addition of fluoride standard solution for the purpose of the bringing electrode into the linear operating range. The amount of fluoride present in the solution added, (which should be determined in the preliminary experiments), would be then subtracted from the final result obtained when analyzing the sample solution. In that way the sensitivity of the electrode would be enhanced and the detection limit of the method improved. Such a calibration solution would also act as an internal standard.

A calibration solution was prepared by adding 2 ml of fluoride standard solution ($C = 5.00$ mg/l) per 50 ml flask resulting in a final concentration of fluoride of 0.20 mg/l. The mean determined amount of fluoride found in the calibration solutions was 0.213 ± 0.0044 mg/l ($n = 11$). Since samples of total diet undergo a decomposition procedure with amounts of reagents that could ultimately significantly contribute to the blank value, a second series of experiments was performed. In these experiments a 2 ml aliquot of fluoride standard solution was added to a 20 ml aliquot of a solution of reagents used during the preparation of the sample and diluted to 50 ml. The mean amount of fluoride determined in such calibration solutions was 0.213 ± 0.0047 mg/l ($n = 12$). Using the t-test for comparison of the two experimental means (Miller & Miller, 2000), it was confirmed that the difference between the means of calibration solutions is not significant at the $P = 0.05$ level and further shown that the mean value of the calibration solutions can be determined without addition of a solution of the reagents used during preparation of the sample. These experiments also showed that 25 ml of CBS buffer adequately buffers a 20 ml aliquot of reagent blank with pH 2–3 to pH ≈6, as required for determination of fluoride with a fluoride ISE. The amount of fluoride in the calibration solution should be determined daily by running at least six consecutive determinations. The RSD of these determinations must be equal or lower than 2.5%. The calibration solution should be checked every two hours between measurements and the result obtained must be within the range of mean blank value ±SD. As a result of ageing and deposition of dirt on the membrane, the performance of the electrode slowly changes over time and electrode response becomes slower. In order to maintain and prolong the electrode performance, we suggest that one electrode is assigned for low level measurements of fluoride.

At this point it is important to note that while numerous reference materials with certified element concentrations, mainly essential and toxic elements, are available for testing the accuracy of analytical methods, values for fluoride are normally lacking, but there are a number of reference materials for which an indicative value of the fluorine concentration has been published. NIST SRM 2695 Fluoride in Vegetation is an exception; however the major disadvantage of SRM 2695 for the purpose of validation of our method was that the levels are elevated above fluoride concentrations representative of the baseline in uncontaminated plant materials. Lower concentrations of fluoride were expected to be present in samples of total diet, but nevertheless it was decided to apply the analytical procedure developed to the analysis of CRM 2695 for the purpose of examining: (1) the accuracy; (2) the precision of the method developed and; (3) potential influences of the matrix effect. Since SRM 2695 High level contains about 100-fold higher amounts of fluoride than expected in food, only 1 g of sample was prepared for the analyses, while NIST SRM 2695 Low level contains about 10-fold as much of fluoride so 5 g were analyzed in order to consider possible matrix effects. The results of these determinations are presented in Table 2.

The results revealed high accuracy and precision of the results using the developed method with no interferences caused by the sample matrix. This was additionally proven by the spike recovery test automatically performed during

Table 2
Results of determination of fluoride in NIST SRM 2695 material

Sample	n	$F^-_{certified}$ (mg/kg)[a]	F^-_{found} (mg/kg)[b]
SRM 2695, High level	2	277 ± 27	280 ± 3
SRM 2695, Low level	2	64.0 ± 8.4	67 ± 2

[a] The uncertainties of the certified values are at 95% confidence intervals.
[b] Mean of two parallel determinations ± standard deviation of measurements.

Table 3
The reproducibility and recovery of fluoride determination in selected samples of total diet

Sample no.	n	F^-_{added} (μg)	F^-_{found}(mg/kg)[b]	RSD (%)	Recovery (%)
6	5	0	1.25	6.0	
	5	5[a]	2.31	3.0	104.8
16	5	0	1.08	4.9	
	5	5[a]	2.01	2.7	93.5
19	3	0	0.90	13.8	
	3	5[a]	1.65	9.2	72.2

[a] 5 μg of fluoride added to 5.000 g of sample represents a final amount of fluoride of 1.00 mg/kg, if no fluoride is present in the sample.
[b] On a lyophilized matter basis.

each measurement. It was also examined whether deviation from the determined value would be observed where lower aliquots of sample taken (1, 2, 5 ml) but the accuracy and precision of the results remained unaffected.

The limit of detection (LOD) of the method was then defined as the sum of the mean fluoride concentration in the blank plus 3 times the standard deviation (SD) of its measurement. For the purpose of determining the LOD, the mean concentration of fluoride in the calibration solution was first determined and this was then subtracted from the results obtained during subsequent measurements of fluoride concentration, again in calibration solutions. The LOD of fluoride determined in this way was found to be 0.013 mg/l and was improved in comparison to the LOD of the electrode reported by the manufacturer (LOD ≈0.02 mg/l) (Orion, 1991).

The limit of quantification (LOQ) could be calculated as the sum of the mean fluoride concentration in the blank plus ten times the SD of its measurement. The LOQ obtained in this way was estimated to be 0.042 mg/l and corresponded to an amount of fluoride of 2.1 mg/kg per sample of total diet (if 5 g of sample were decomposed and then the amount of fluoride determined in a 20 ml aliquot). However, the amount of fluoride to be found in samples of total diet was expected to be substantially lower than the LOQ of the method, and therefore the LOQ was re-estimated based on the analysis of real samples. Repeatability and recovery tests of randomly selected samples (sample 6, 16 and 19) of total diet were first conducted. Recovery in this paper is defined as the proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is recovered and presented for measurement (Cuadros-Rodríguez et al., 2005). Sample 19 represented a sample with a low amount of fluoride. Five parallel determinations of samples 6 and 16 and three of sample 19 were conducted without spiking and with spiking the samples with fluoride. Results of these analyses are presented in Table 3.

The results obtained for higher concentrations (Table 3) showed the good reproducibility of the procedure with relative standard deviations (RSDs) being below 6%. Recovery of the method was better when higher amounts of fluoride were present in the sample. RSDs increased to 10% and higher, when samples containing amounts of fluoride close to the LOD of the method were analyzed, though recovery still remained within the suggested range (Cuadros-Rodríguez et al., 2005), being between 70–110%. Recovery factor was not used to correct the results obtained without spiking. Hence it was concluded that the amount of fluoride that could be determined in diet samples is equal and higher than 0.90 mg/kg of lyophilized matter, corresponding to 1.04 mg/kg of dry matter, so this value is proposed as the LOQ parameter of the developed method.

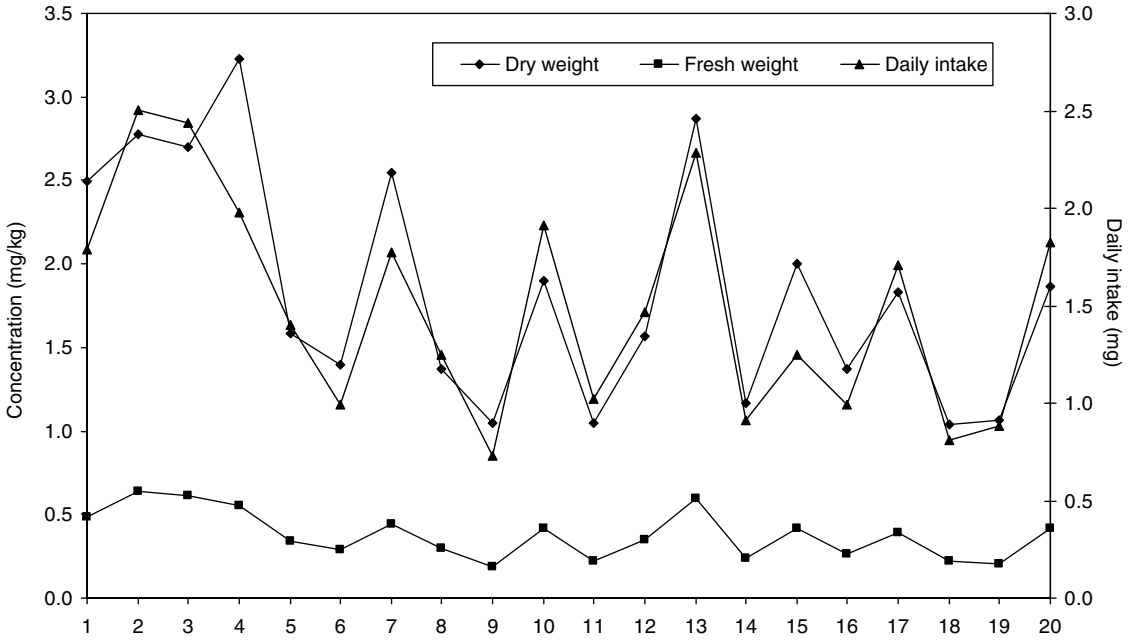

Fig. 1. The average content of fluoride in twenty total diet samples from the Slovenian Military, expressed on a fresh weight and a dry weight basis of each sample separately (mg/kg) and as a daily intake of fluoride (mg/day).

3.2. Amount of fluoride in total diet samples

The amount of fluoride was determined in twenty lyophilized total diet samples obtained from the Slovenian Military. In order to estimate the daily intake of fluoride, the mass of total diet was taken into account. The results of the analysis are expressed on a dry weight and a fresh weight basis are presented in Fig. 1.

It is evident that the concentration of F_t^- expressed on a dry weight basis (calculated based on dry, respectively fresh weight of each sample separately) ranged from 1.04 to 3.23 mg/kg with a mean value of 1.84 ± 0.70 mg/kg. The concentration of total fluoride on a fresh weight basis is considerably lower, being within the range 0.19–0.64 mg/kg with a mean value of 0.38 ± 0.14 mg/kg. The average daily intake based on these data was estimated to be within the range 0.73–2.50 mg with a mean of 1.50 ± 0.56 mg.

3.3. Conclusion

Total sample decomposition by fusion with $KNaCO_3$ after prior ashing of the sample with NaOH is appropriate for determination of total fluoride in samples of diet with a fluoride ISE and the modified MKA technique. The method was developed in accordance with a recent suggestion by Kjellevold Malde, Bjorvatn and Julshamn (2001) who stated that in order to enable comparison of results from different studies, methods for determination of fluoride that give the total amount of fluoride are preferred.

The mean total daily intake of fluoride of 1.50 mg per solder on the basis of 820 g of dry diet matter estimated in this study is in excellent agreement with the results of Haldimann and Zimmerli (1993) who reported a mean total daily intake of 0.9 mg/day per adult on the basis of mean daily intake of 460 g dry matter; both studies were conducted in an unfluoridated area with a fluoride concentration in drinking water of less than 0.1 mg/l. The estimated daily intake is well below the suggested 4 mg/day for males aged 19 and over (DRI, 1997), but other sources such as fluoride-containing dentifrices may, if not used properly, significantly increase the daily intake. Regrettably the results of this study cannot be compared to the results of other studies cited at the beginning of this paper because the data reported in these studies, such as number of samples, energy values of the diets, weight of dry and or fresh matter are insufficient to make a reliable comparison.

Finally it is important to note that CRMs based on total diet are not available. Due to the increasing awareness of food as a possible source of fluoride, the preparation of such materials is recommended.

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