



## Assessment of fluoride intake from drinking water and toothpaste in 3-year-olds: preliminary results in Belgrade, Republic of Serbia

Procena unosa fluorida putem vode za piće i paste za zube kod dece uzrasta od tri godine: preliminarni rezultati u Beogradu, Republika Srbija

Danijela Djukić-Ćosić\*, Evica Antonijević\*, Zoran Mandinić†, Marijana Ćurčić\*,  
Dejana Ćupić Miladinović‡, Biljana Antonijević\*, Vesna Matović\*

University of Belgrade, Faculty of Pharmacy, \*Department of Toxicology “  
Akademik Danilo Soldatović” and Center for Toxicological Risk Assessment, School of  
Dental Medicine, †Clinic for Preventive and Paediatric Dentistry, Faculty of Veterinary  
Medicine, ‡Department of Pharmacology and Toxicology, Belgrade Serbia

### Abstract

**Background/Aim.** Fluoride has beneficial effect on dental caries prevention and enables high hardness of enamel. However, fluoride intake above optimal levels can have adverse effects on teeth and bones, especially in young children during the period of intense growth and teeth development. The aim of this study was to assess fluoride intake from water and toothpaste among 3-year-old children in Belgrade, Serbia, in the municipalities of Vračar and Novi Beograd. **Methods.** A questionnaire for the parents ( $n = 40$ ) was used to provide information on the water consumption (tap and/or bottled water) and the brand of toothpaste used by children as well as the frequency of tooth brushing and the amount of toothpaste during brushing. Fluoride concentrations in water and toothpaste samples were determined electrochemically by using fluoride-selective electrode. Fluoride intake was estimated through a mathematical model commonly used by the U.S. Environmental Protection Agency. **Results.** The obtained results indicate no significant difference in daily fluoride intake through drinking water and tooth-

paste in 3-year-old children in Vračar ( $n = 19$ ) compared to Novi Beograd ( $n = 21$ ) ( $p > 0.05$ ). However, all estimated fluoride levels (0.089–0.625 mg/day) are significantly lower than the optimal daily intake level for caries protection (0.7 mg/day for children up to 4 years, FNB-USA National Institute of Medicine) and two to six times lower than tolerable upper fluoride level for the children of same age (1.3 mg/day, FNB-USA National Institute of Medicine). Furthermore, calculated daily fluoride intake per kilogram body weight confirm very low fluoride intake by water and toothpaste in children of investigated municipalities in Belgrade, being significantly below the recommended adequate intake (0.05 mg/kg/day, EFSA). **Conclusion.** This preliminary study has shown that daily fluoride intake in 3-year-olds is lower than tolerable upper fluoride level, even not sufficient for the prevention of dental caries.

### Key words:

fluorides; child, preschool; drinking water; toothpastes; tooth diseases; surveys and questionnaires.

### Apstrakt

**Uvod/Cilj.** Fluoridi imaju pozitivan efekat na prevenciju karijesa zuba kao i povećanje čvrstine zubnog emajla. Međutim, njihov unos iznad optimalnog može imati štetne efekte na zube i kosti, posebno kod male dece tokom intenzivnog rasta i razvoja zuba. Cilj ovog rada bio je da se proceni unos fluorida putem vode za piće i paste za zube kod dece uzrasta od tri godine u Beogradu, na teritoriji dve opštine, Vračar i Novi Beograd. **Metode.** Anketnim upitnicima ( $n = 40$ ) za roditelje dobijeni su podaci o vrsti vode koju deca piju (česmenska i/ili flaširana), proizvodima koje koriste za čišćenje i negu zuba, uč-

stalosti pranja zuba kao i količini paste za zube koja se koristi po jednom pranju. Sadržaj fluorida u vodi za piće i pastama za zube određen je elektrohemijski sa fluoridnom jonselektivnom elektrodom. Unos fluorida procenjen je korišćenjem matematičkog modela datog od strane Američke agencije za zaštitu životne sredine. **Rezultati.** Dobijeni rezultati pokazuju da nema statistički značajne razlike u dnevnom unosu fluorida putem vode za piće i paste za zube između dece uzrasta od tri godine na Vračaru i Novom Beogradu ( $p > 0,05$ ). Međutim, sve procenjene vrednosti unosa fluorida (0,089–0,625 mg/dan) značajno su niže od optimalnog dnevnog unosa za prevenciju karijesa (0,7 mg/dan za de-

cu uzrasta do 4 godine, FNB-Nacionalnog instituta medicine, SAD) i dva do šest puta niže od tolerišućeg gornjeg nivoa unosa fluorida za decu istog uzrasta (1,3 mg/dan, FNB-Nacionalnog instituta medicine, SAD). Štaviše, izračunati dnevni unosi fluorida izraženi po kilogramu telesne mase potvrđuju veoma nizak unos fluorida kod dece ispitivanih opština u Beogradu, čak značajno niže od preporučenog optimalnog unosa (0,05 mg/kg/dan, EFSA). **Zaključak.**

Ovim preliminarnim istraživanjem pokazano je da je dnevni unos fluorida kod trogodišnjaka u Beogradu značajno niži od tolerišućeg gornjeg nivoa unosa fluorida, čak niži od optimalnog unosa za prevenciju karijesa.

**Ključne reči:**  
**fluoridi; deca, predškolska; voda za piće; dentifriciji; zub, bolesti; ankete i upitnici.**

## Introduction

Fluoride intake in an optimal dose is a safe and efficient way to minimize a risk of dental caries and prevent enamel demineralization<sup>1-3</sup>. However, the range between optimal levels and the concentrations that lead to adverse effects such as dental and skeletal fluorosis is narrow, and indicates the importance of fluoride intake assessment<sup>2,4,5</sup>. Furthermore, the opinion of the European Food Safety Authority (EFSA) is that data on fluoride exposure are insufficient<sup>5</sup>.

The important sources of fluoride intake for general population are water, drinks and food, particularly some kinds of tea, sea fish, fluoridated foods and fluoride dentifrices<sup>6-8</sup>. Epidemiological studies from the 70's have shown that the most important source of fluoride intake is drinking water which contains 0.5 to 1 mg/L fluoride, depending on climate conditions<sup>4</sup>. On the other hand, fluorosis can be related not only to the regions rich in fluoride but also to the fluoride overuse, particularly from fluoride toothpastes, mouthwashes, gels, etc.<sup>9,10</sup>. High fluoride intake can be of a special risk in children up to six years of age when tooth growth and development occurs, especially during the phases of enamel formation<sup>10-12</sup>. The period between the 15th and 30th month of life is considered to be of the highest risk, when the fluoride intake should be carefully controlled and balanced between the need for dental caries protection and the risk of fluorosis<sup>13</sup>. A special caution should be taken in two- and three-year-olds since they can swallow high amount of toothpaste as a result of inadequate swallowing reflex<sup>5,14,15</sup>. Thus, the Academy of Paediatric Dentistry [American (AAPD) and European (EAPD)] gave detailed recommendations for fluoride content in toothpastes and its amounts for brushing in relation to the age of child<sup>16,17</sup>. For the majority of European communities, the EAPD recommends the use of appropriate fluoride toothpaste in conjunction with good oral hygiene to be the basic fluoride regimen<sup>17</sup>. The approach of the EAPD was adopted in Serbia, having in mind the importance of adequate fluoride intake through toothpaste for reducing dental caries in children<sup>18</sup>.

However, despite a significant reduction in caries in Western and Northern European countries, available data indicate that the prevalence and occurrence of dental caries in Serbian children is high, even the highest in the countries of Eastern and Central Europe (DMFT index 3.4)<sup>19</sup>. According to the DMFT index value (number of decayed, missing due to caries and filled teeth in the primary dentition) relative risk for tooth decay in our country is almost two times higher than the regional average<sup>19</sup>. The prevalence of dental caries in early childhood in Serbia is

8.3% for three-year-olds, while in six-year-olds with permanent teeth the percentage is 0.8%<sup>18</sup>. Data from the latest research in the Autonomous Province of Vojvodina, Republic of Serbia show that the prevalence of early childhood caries is extremely high, approaching 50%<sup>20</sup>.

Drinking water with optimal fluoride concentration is the most important source of fluoride intake in childhood, but in the areas with water and food, poor in fluoride, the dominant route of fluoride intake is via toothpaste<sup>7,8,21</sup>. The results of de Almeida et al.<sup>21</sup> indicate that toothpaste alone is responsible for about 80% of the daily fluoride intake of 1 to 3-year-old children. Thus, the aim of this study was to assess fluoride intake through drinking water and toothpaste in three-year-olds and obtain preliminary results of fluoride intake in children in Belgrade, Republic of Serbia. To achieve this aim, it was necessary to: conduct a questionnaire for parents to obtain information on the type of water that children consume (tap and/or bottled), the type of toothpaste they use, the frequency of tooth brushing and the amount of toothpaste used per tooth brushing; determine fluoride concentrations in the samples of non-carbonated bottled water, tap water and toothpastes selected on the basis of the questionnaire for parents; estimate the daily fluoride intake in mg/day and mg/kg/day and compare the obtained results with the recommended an adequate intake and tolerable upper level of fluoride intake for children of this age, proposed by the Food and Nutrition Board, U.S. National Academy of Sciences Institute of Medicine and EFSA<sup>5,22</sup>.

## Methods

### Questionnaire

A previously validated questionnaire for parents was used in order to obtain the general data (sex, age, body weight and dental health of children), information on the type of water that children drink (tap, bottled, type of bottled water), the type of toothpaste their children use, the frequency of tooth brushing, and the amount of toothpaste used per tooth brushing<sup>23</sup>. The questionnaire was anonymous and voluntary. Filling out the questionnaire by the parents of 3-year-olds was carried out in two kindergartens in Belgrade – in one from the municipality of Vračar and one from the municipality of Novi Beograd.

### Drinking water and toothpaste

Different non-carbonated bottled waters and toothpastes available on the market in Belgrade, were selected for fluo-

ride determination on the basis of the completed questionnaire for parents. All non-carbonated bottled waters available on the market had no fluoride content, while the content of fluoride was indicated on toothpastes and ranged in a very wide range of 500 to 1450 mg/kg fluoride. Tap water from the territories of Vračar and Novi Beograd was also used for fluoride determination.

#### Fluoride determination

Fluoride in water was determined directly without previous preparation, while the preparation of toothpastes depended on the chemical form of fluoride present in the toothpaste (NaF, Na-monofluorophosphate, aminofluoride). For the preparation of toothpaste samples the protocol given by Omena et al.<sup>24</sup> was applied. All samples were prepared in duplicate. The fluoride content was determined electrochemically, using fluoride-selective electrode (WTW, ISE type 800 – Consort, Belgium; pH meter Iskra MA 5735). Before measurement, all samples were mixed with the TISAB buffer solution in ratio 1 : 1. All chemicals, obtained from the commercial sources, were of analytical grade purity. The analytical method for fluoride determination was linear in the range of 0.05–5 mg/L ( $r = 0.9998$ ). The obtained limits of determination (LOD) and quantification (LOQ) were 0.003 and 0.009 mg/L, respectively. The recovery values from 98% to 107% indicated an adequate accuracy of the method, while repeatability was confirmed by corresponding coefficient of variation from 2.2% to 4.4%.

#### Estimation of fluoride intake

Fluoride intake (mean and 95th percentile, P-95) was estimated through a mathematical model employed by the U.S. Environmental Protection Agency (EPA) according to the equations for water and toothpaste<sup>25</sup>:

$$EDI_w = \frac{C \times IR \times CF}{BW}$$

Where:  $EDI_w$  – Estimated daily intake by drinking water (mg/day or mg/kg/day)

C – Fluoride concentration in drinking (mg/L)

IR – Intake rate, amount of water intake per day (L) – 0.9 L for 3 years age<sup>26</sup>

CF – Conversion factor

BW – Body weight (kg)

$$EDI_t = \frac{C \times IR \times AF \times EF \times CF}{BW}$$

$EDI_t$  – Estimated daily intake by toothpaste (mg/day or mg/kg/day)

C – Fluoride concentration in toothpaste (mg/kg)

IR – Ingestion or intake rate, amount of toothpaste per one tooth brushing (mg)

EF – Exposure frequency (number per day), or the frequency of tooth brushing

AF – Absorption factor (amount of fluoride swallowed, for the age of 3 years 48%)<sup>14</sup>

CF – Conversion factor

BW – Body weight (kg)

Total daily fluoride intake in mg/day and mg/kg/day by these sources was obtained adding the estimated values for  $EDI_w$  and  $EDI_t$ .

#### Data analysis

Statistical analysis was performed using the computer program STATISTICA 7.0 and the MS Excel package 2007. The Student *t*-test was applied to determine statistically significant difference between water and toothpaste intake as well as for the total intake between the two examined groups. Statistical significance was set for  $p < 0.05$ .

## Results

#### Data obtained from the questionnaire

A questionnaire was filled in by parents from two Belgrade municipalities, Vračar and Novi Beograd. The total number of filled in questionnaires was 40, 19 from Vračar and 21 from Novi Beograd. Twenty questionnaires referred to girls, 19 to boys, and in one questionnaire the gender was not specified. The majority of parents in Vračar considered the dental health of their children to be excellent (47.83%), followed by good (43.48%), moderate (4.38%), while one parent did not provide the answer. The parents in Novi Beograd evaluated the dental health of their children mostly as good (47.62%), followed by moderate (28.57%) and bad (14.29%), while there was only one answer for excellent (4.76%) as well as for very bad (4.76%).

Based on the data obtained from the questionnaire shown in Table 1, about one half of the children drink both tap and bottled water (Vračar 52.6%, Novi Beograd 47.6%). The consumption of tap water only is more common in Novi Beograd (38.1%) than in Vračar, while on the other hand, the consumption of bottled water only, is higher in Vračar (31.6%) than in Novi Beograd (4.8%).

**Table 1**

#### Type of water consumed by the 3-year-old children in Vračar and Novi Beograd

Type of water	Vračar n (%)	Novi Beograd n (%)
Tap	3 (15.8)	8 (38.1)
Tap purified	0 (0)	2 (9.5)
Bottled	6 (31.6)	1 (4.8)
Tap and bottled	10 (52.6)	10 (47.6)
Total	19 (100)	21 (100)

**n – number of children.**

Table 2 presents data obtained from the questionnaire: the type of toothpaste, frequency of tooth brushing and the amount of toothpaste used per tooth brushing. The frequency of tooth brushing in the 3-year-olds in Novi Beograd is once per day, in Vračar it is once or two times per day, while only one child brushes teeth more than two times per day.

The largest percentage of children in both localities (Vračar 52.6%, Novi Beograd 100%) applies a pea-sized amount of toothpaste on the brush. The results of the conducted questionnaire show that the children use toothpastes with a fluoride content that varies to a great extent, from toothpastes which does not contain fluoride, to toothpastes with 500 mg/kg fluorides, to those which contain 1,450 mg/kg fluoride-content equivalent to adult toothpaste. The majority of children in Vračar use the toothpaste *Vademecum*

*junior 2 in 1* – 1,450 mg/kg (26.3%), followed by *Chicco* non-fluoride (21.1%) and *Colgate Smiles* – 1,000 mg/kg (15.8%), while children in Novi Beograd mostly use *Vademecum junior 2 in 1* (33.3%) – 1450 mg/kg, *Vademecum* (23.8%) – 500 mg/kg and *Aquafresh kids* (23.8%) – 500 mg/kg (Table 2). The obtained results also show that at that time no child took fluoride supplements in the form of tablets, gels or solutions.

Table 2

**The frequency of tooth brushing, the amount of toothpaste per brushing and the most commonly used toothpastes by the 3-year-olds in Vračar and Novi Beograd**

Questionnaire	Municipality	
	Vračar n (%)	Novi Beograd n (%)
How often do you brush your child's teeth?		
1x day	9 (47.3)	21 (100)
2x day	9 (47.3)	0 (0)
> 2x day	1 (5.4)	0 (0)
When tooth brushing, do you use a pea-sized amount of toothpaste?		
yes	10 (52.6)	21 (100)
no	9 (47.4)	0 (0)
Which toothpaste does your child most commonly use?		
Aquafresh kids (500 mg/kg)*	1 (5.2)	5 (23.8)
Lacalut (500 mg/kg)*	0 (0)	0 (0)
Vademecum (500 mg/kg)*	2 (10.5)	5 (23.8)
Vademecum junior 2 in 1 (1,450 mg/kg)*	5 (26.3)	7 (33.3)
Colgate Smiles (1,000 mg/kg)*	3 (15.8)	1 (4.8)
Chicco – non fluoride	4 (21.1)	3 (14.3)
Other	4 (21.1)	0 (0)

n = number of children; \*fluoride content in tooth paste.

#### Fluoride concentrations in drinking water and toothpaste

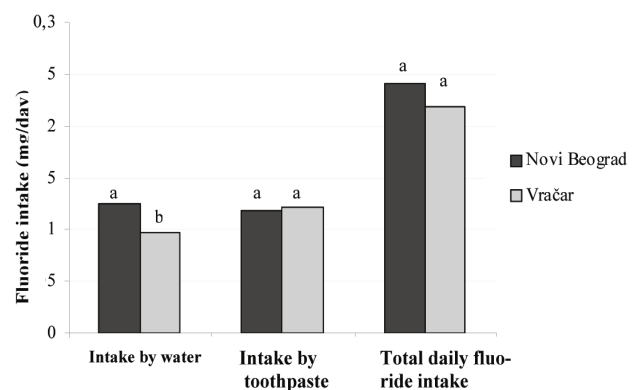
Since the results from the questionnaire showed that the children used only a few brands of non-carbonated bottled drinking water and toothpaste, fluoride content was determined in those brands. All the examined non-carbonated bottled waters (n = 8) contained very low fluoride levels in the range from 0.077 to 0.185 mg/L. Fluoride content in tap water was also low, in Novi Beograd  $0.153 \pm 0.004$  mg/L and in Vračar  $0.127 \pm 0.003$  mg/L.

The results of fluoride content in toothpastes showed that the fluoride concentration in the analyzed toothpastes was in the range of 475–1,475 mg/kg (declared content 500–1,450 mg/kg). The most commonly used toothpastes according to the questionnaire (*Aquafresh kids*, *Colgate smiles*, *Vademecum junior 2 u 1*) had fluoride content similar to the declared.

#### Estimation of the total daily fluoride intake

Based on the mathematical model commonly used by the U.S. EPA, the total daily fluoride intake by drinking wa-

ter and toothpaste was calculated and the results are presented in Figures 1 and 2 and Table 3.



**Fig. 1 – Daily fluoride intake by the 3-year-olds in Belgrade (mg/day).**

Values are presented as the mean intake of fluoride in Novi Beograd (n = 21) and Vračar (n = 19). Statistical significance of differences was tested by the *t*-test; different letters indicate statistical significance between fluoride intake in 3-year-old children from two investigated municipalities,  $p < 0.05$ .

Table 3

## The lowest and highest calculated daily fluoride intake from drinking water and toothpaste in 3-year-olds in Belgrade

Fluoride intake	Value mg/day	Type of water	Fluoride content in toothpaste (mg/kg)	Amount of toothpastes per brushing	Frequency of tooth brushing
Min – Vračar	0.089	T/B	without fluoride	pea-sized*	once daily
Min – Novi Beograd	0.135	T	without fluoride	pea-sized	once daily
Max – Vračar	0.625	T	500 mg/kg	tooth brush sized**	twice daily
Max – Novi Beograd	0.322	T/B	1450 mg/kg	pea-sized	once daily

T – tap water; T/B – tap and bottled water; \*a pea-sized amount of toothpaste is equivalent to the mass of 0.25 g; \*\* a tooth brush sized amount of toothpaste is equivalent to the mass of 0.75 g<sup>14</sup>.

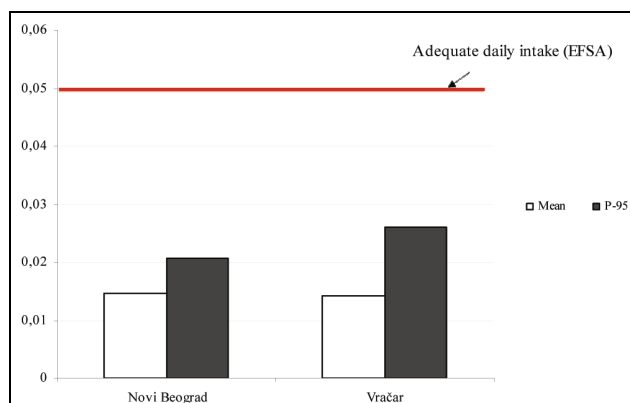


Fig. 2 – Mean and P-95 of the daily fluoride intake from drinking water and toothpaste in the 3-year-olds from two Belgrade municipalities, Novi Beograd and Vračar (mg/kg/day) in comparison with adequate daily intake according to the European Food Safety Authority – EFSA (0.05 mg/kg, based on all fluoride sources).

Figure 1 shows the mean daily fluoride intake by water, toothpaste and the total intake by these sources in Vračar and Novi Beograd, while Figure 2 gives mean and P-95 values for total fluoride daily intake per kilogram body weight in the investigated municipalities in Belgrade. In Novi Beograd, the fluoride intake was somewhat higher through water, in Vračar through toothpaste, although the difference was not statistically significant. A significant difference was obtained only between consumed water in Vračar and Novi Beograd. The total fluoride daily intake was in the range of 0.089 to 0.625 mg/day for children living in Vračar and 0.135 to 0.322 mg/day for children from Novi Beograd, corresponding to 0.0143 mg/kg/day (mean value) and 0.026 mg/kg/day (P-95), and 0.0146 mg/kg/day (mean value) and 0.020 mg/kg/day (P-95), respectively (Figure 2). No significant difference in the total daily fluoride intake among three-year-olds from the two Belgrade municipalities was found (Figure 2).

Table 3 presents the lowest and highest calculated daily fluoride intake from drinking water and toothpaste in 3-year-olds in Belgrade. The lowest calculated daily fluoride intake for both municipalities (0.089 mg/day and 0.135 mg/day in Vračar and Novi Beograd, respectively) was in children who use non-fluoride toothpaste. In Vračar, the highest daily fluoride intake (0.625 mg/day) was calculated for the children who used fluoride toothpaste (500 mg/kg), brushing teeth two times a day and applied a toothbrush-sized amount of the

toothpaste per brushing. In other investigated municipalities, the highest daily fluoride intake was two times lower than the values in Vračar (0.322 mg/day) and the result was obtained for the children who used a toothpaste with fluoride (1,450 mg/kg) and applied pea-sized toothpaste per brushing, but brushed the teeth only once day (Table 3).

### Discussion

In this study, daily fluoride intake was estimated in 3-years-old children in Belgrade on the basis of the content of fluoride in drinking water and toothpaste. Although fluoride can be ingested by various sources (water, drinks, food, toothpaste, supplements, etc.), drinking water and toothpaste represent the most important sources of fluoride intake in preschool children<sup>27</sup>. When containing the optimal fluoride levels of 0.5–1 mg/L as declared by the World Health Organization (WHO), drinking water is the main contributor to the total daily fluoride intake<sup>4-6</sup>. In many countries, drinking water is fluoridated, although not in our country. Furthermore, the use of bottled water is becoming considerably more common, both in the world and in our country, but nonetheless many of the brands do not declare fluoride content. The results of this study show that the non-carbonated bottled waters consumed by children have levels of fluoride in the range from 0.077 to 0.185 mg/L which are significantly below the recommended ones. The results of the conducted questionnaire show that the majority of children consume both bottled and tap water, tap water in both locations also containing rather low levels of fluoride. These results are in accordance with other recent studies conducted in our country, which indicate low fluoride levels in tap and bottled non-carbonated drinking water, with the exception of a minor area with endemic fluorosis<sup>28-32</sup>. Daily fluoride intake could not be significantly increased by food intake, since food is prepared with poor fluoride level water<sup>5, 17</sup>. Therefore, in areas with a deficit of fluoride in drinking water, toothpaste is an important source of this anion<sup>21</sup>. Besides, the toothpaste has an important role in caries prevention, not only due to fluoride content but also due to its local action on the tooth surface<sup>18, 33</sup>. The local availability of fluoride from the toothpaste was shown to prevent caries by primarily three mechanisms: inhibiting demineralization of tooth enamel; enhancing remineralization of tooth enamel prior to lesion progression; and inhibiting the enzyme activity of cariogenic bacteria<sup>34</sup>.

Data obtained from the questionnaire show that the majority of children in Novi Beograd (85.7%) and Vračar (70.8%) use toothpaste with fluorides ranging from 500 mg/kg to 1450 mg/kg while a small number of children brush their teeth with a toothpaste without fluoride. However, it should be emphasised that the obtained levels of fluoride in the toothpastes were under the levels recommended by the EAPD and national protocol in Serbia – 1,000(+) mg/kg for children 2 to 6 years old<sup>17, 18</sup>. Similar results were obtained from toothpastes for children in Chile<sup>35</sup>, although in another South American country Brazil fluoride levels > 1,000 mg/kg was determined<sup>21, 36</sup>.

Furthermore, fluoride intake depends on the amount of used toothpaste as well as on the frequency of tooth brushing. The results show that most of the children (Vračar 63.6% and Novi Beograd 100%) use the amount of toothpaste corresponding to pea-sized amount of toothpaste, the amount that is in accordance with the national protocol<sup>18</sup>. When considering the frequency of brushing, two-times brushing was reported for the half of investigated children in Vračar, while in Novi Beograd all children brush their teeth once per day.

Estimated total daily fluoride intake through tap and/or non-carbonated bottled water and toothpaste in 3-year-olds in investigated municipalities indicates no significant difference between fluoride intakes in children living in two different municipalities. A significant difference was observed only for fluoride intake via water, and the 3-year-olds in Novi Beograd had a higher intake by water than children in Vračar. Fluoride intake by toothpaste does not differ between the examined groups indicating no influence of the frequency of teeth brushing on the total fluoride intake. That could be explained by higher levels of fluoride in toothpastes used in Novi Beograd. However, the obtained values for total fluoride intake are lower than optimal fluoride intake (0.7 mg/day) for this age, and two to six times lower than tolerable upper intake level 1.3 mg day<sup>-1</sup> for children up to 4 years of age proposed by the Food and Nutrition Board, U.S. National Academy of Sciences Institute of Medicine<sup>5, 22</sup>.

The minimum observed fluoride intake level was 0.089 mg/day in Vračar and 0.135 mg/day in Novi Beograd, while the highest fluoride intake 0.625 mg/day was estimated for Vračar and 0.322 mg/day for Novi Beograd. Low levels of fluoride intake in both municipalities can be explained by low fluoride levels in consumed water and used toothpastes without fluorides. On the other hand, the application of fluoride toothpaste with fluoride content of 1450 mg/kg resulted in the higher total fluoride intake 0.322 mg/day for Novi Beograd. The highest level of total fluoride level (0.625 mg/day) estimated for Vračar can be explained by 2-times brushing with bigger amounts of applied toothpaste (a toothbrush-sized – 0.75 g).

Furthermore, in this study the estimated daily fluoride intake per kg b.w. confirm very low fluoride intake by water

and toothpaste, being significantly below the adequate intake level from all sources, including non-dietary sources (0.05 mg/kg/day, EFSA)<sup>5</sup>. Contrary to our results, Omena et al.<sup>24</sup> estimated a daily fluoride intake of 0.128 mg/kg/day in 18 to 36-months-old children consuming water with 0.94 mg/L and toothpastes with > 1,000 mg/kg fluoride. Other literature data also estimated daily fluoride intake through water with optimal and high fluoride concentration, fluoridated food (milk, salts and other dietary sources) and dental products above the recommended values, indicating the opposite risk of fluoride overdosage<sup>37–40</sup>.

According to our investigation, the total intake of fluoride in young children, estimated on the basis of consumption water with low fluoride content and toothpaste application is far below the levels known to be beneficial for caries prevention. Moreover, these results indicate that children aged 3 years can safely use the toothpaste with a maximum fluoride concentration of 1450 mg/kg, especially as tooth brushing with fluoride toothpaste is a fundamental measure for the primary prevention of early childhood caries<sup>41</sup>. These findings can serve as a starting point for developing an optimal caries prevention national plan, in addition to other measures as well as patient/parents education, dietary advice, use of nonfluoride caries-preventive agents (xylitol lozenges, sucrose-free chewing gum, chlorhexidine, etc.) and periodic clinical examinations<sup>17, 42</sup>.

## Conclusion

Based on these preliminary results it can be concluded that the daily fluoride intake by drinking water and toothpaste in children aged 3 years on the territory of two Belgrade municipalities is significantly lower than the adequate intake level thus contributing to the occurrence of dental caries. Further research is needed to cover all children of preschool age from 2 to 7 years of age and other municipalities in Belgrade and elsewhere in Serbia in order to obtain more comprehensive information on the assessment of fluoride intake in children.

## Conflicts of interest

The authors declare no conflict of interest.

## Acknowledgements

We thank kindergartens and parents for filling out the questionnaire. Also, we thank Ana Aleksić for participating in the experimental part of the study. The authors thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for partial support within research Project no. III46009.

## R E F E R E N C E S

- Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: The WHO approach. *Community Dent Oral Epidemiol* 2004; 32(5): 319–21.
- Everett ET. Fluoride's effects on the formation of teeth and bones, and the influence of genetics. *J Dent Res* 2011; 90(5): 552–60.
- Clark MB, Slayton RL. Fluoride use in caries prevention in the primary care setting. *Pediatrics* 2014; 134(3): 626–33.
- World Health Organization (WHO). Guidelines for Drinking-water Quality. 4th ed. Geneva: World Health Organization; 2011.
- European Food Safety Agency (EFSA). Panel on Dietetic Products, Nutrition and Allergies. Scientific opinion on Dietary Reference Values for fluoride. *EFSA J* 2013; 11(8): 3332.
- Fawell J, Bailey K, Chilton J, Dahi E, Fentrell L, Magara Y. Fluoride in Drinking-water. U: WHO Water Series. ISBN: 1900222965. London, UK: World Health Organization, IWA Publishing; 2006.
- 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. *EFSA J* 2005; 192: 1–65.
- Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine. Atlanta, Georgia: U. S. Department of Health and Human Services, Public Health Service; 2013.
- Pereira AC, da Cunha FL, Meneghim MC, Werner CW. Dental caries and fluorosis prevalence study in a nonfluoridated Brazilian community: Trend analysis and toothpaste association. *ASDCJ Dent Child* 2000; 67(2): 132–5.
- Wong MC, Clarkson J, Glenny AM, Lo EC, Marinho VC, Tsang BW, Worthington HV. Cochrane reviews on the benefits/risks of fluoride toothpastes. *J Dent Res* 2011; 90(5): 573–9.
- Ekambaram M, Itthagarun A, King NM. Ingestion of fluoride from dentifrices by young children and fluorosis of the teeth: A literature review. *J Clin Pediatr Dent* 2011; 36(2): 111–21.
- Lockner F, Twetman S, Stecksén-Blicks C. Urinary fluoride excretion after application of fluoride varnish and use of fluoride toothpaste in young children. *Int J Paediatr Dent* 2017; 27(6): 463–8.
- Evans RW, Stamm JW. An epidemiologic estimate of the critical period during which human maxillary central incisors are most susceptible to fluorosis. *J Public Health Dent* 1991; 51(4): 251–9.
- Ellwood RP, Cury JA. How much toothpaste should a child under the age of 6 years use?. *Eur Arch Paediatr Dent* 2009; 10(3): 181–7.
- Kobayashi CA, Belini MR, Italiani FM, Pauleto AR, Araújo JJ, Tesarolli V, et al. Factors influencing fluoride ingestion from dentifrice by children. *Community Dent Oral Epidemiol* 2011; 39(5): 426–32.
- American Academy of Pediatric Dentistry (AAPD). Guideline on Fluoride Therapy. *Clin Pract Guidelines* 2014; 37(6): 177–9.
- European Academy of Paediatric Dentistry (EAPD). Guidelines on the use of fluoride in children: An EAPD policy document. *Eur Arch Paediatr Dent* 2009; 10(3): 129–35.
- Ivanović M, Carević M, Marković D, Vulićević Z, Stevanović R, Petrović V, et al. The protocol for the use of fluoride in caries prevention in children and youth in Serbia. In: Ivanović M, Carević M, Marković D, Vulićević Z, Stevanović R, Petrović V, et al. Protocols in dentistry. Belgrade: University of Belgrade, School of Dentistry. 2009. p. 21–47. (Serbian)
- da Silveira Moreira, R. Epidemiology of dental caries in the world. In: Viridi M, editor. Oral Health Care – Pediatric, Research, Epidemiology and Clinical Practices. Rijeka: InTech; 2012. Available from: <http://www.intechopen.com/books/oral-health-care-pediatric-research-epidemiology-and-clinical-practices/epidemiology-of-dental-caries-in-the-world> [accessed 2016 December 28].
- Tušek I, Tušek J, Ukropina S. Risk factors associated with early childhood caries in autonomous province of Vojvodina, Republic of Serbia. *Vojnosanit Pregl* 2017; 74(6): 511–9.
- de Almeida BS, da Silva Cardoso VE, Buzalaf MA. Fluoride ingestion from toothpaste and diet in 1- to 3-year-old Brazilian children. *Community Dent Oral Epidemiol* 2007; 35(1): 53–63.
- Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington (DC): National Academies Press (US); 1997.
- Dukić-Čosić D, Antonijević E, Vićentijević N, Malešević N, Čurčić M, Dilber S, et al. Validation of a questionnaire for assessing fluoride intake in preschool children. *MD-Medical Data* 2017; 9(2): 95–100. (Serbian)
- Omena LM, Silva MF, Pinheiro CC, Cavalcante JC, Sampaio FC. Fluoride intake from drinking water and dentifrice by children living in a tropical area of Brazil. *J Appl Oral Sci* 2006; 14(5): 382–7.
- U.S. Environmental Protection Agency (EPA). Guidelines for exposure assessment. *Fed Reg* 1992; 57: 22887–938.
- Food and Nutrition Board (FNB). Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington DC: Institute of Medicine National Academy Press; 2004.
- Erdal S, Buchanan SN. A quantitative look at fluorosis, fluoride exposure, and intake in children using a health risk assessment approach. *Environ. Health Perspect* 2005; 113(1): 111–7.
- Glavaški M, Čurčić M, Dukić-Čosić D, Plamenac-Bulat Z, Matović V. Fluoride content in bottled mineral waters of Serbia. *Arh Farm* 2009; 59: 321–30. (Serbian)
- Petrović TM, Zlokolica Mandić M, Veljković N, Papić PJ, Poznanović MM, Stojković JS, et al. Macro and microelements in bottled and tap waters of Serbia. *Hem Ind* 2012; 66(1): 107–22. (Serbian)
- Mandinić Z, Čurčić M, Antonijević B, Charles P, Lekić CP, Carević M. Relationship between fluoride intake in Serbian children living in two areas with different natural levels of fluorides and occurrence of dental fluorosis. *Food Chem Toxicol* 2009; 47(6): 1080–4.
- Mandinić Z, Čurčić M, Antonijević B, Carević M, Mandić J, Djukić-Čosić D, et al. Fluoride in drinking water and dental fluorosis. *Sci Total Environ* 2010; 408(17): 3507–12.
- Antonijević E, Mandinić Z, Curčić M, Djukić-Cosic D, Milicevic N, Ivanovic M, et al. "Borderline" fluorotic region in Serbia: Correlations among fluoride in drinking water, biomarkers of exposure and dental fluorosis in schoolchildren. *Environ Geochem Health* 2016; 38(3): 885–96.
- Twetman S, Axelsson S, Dahlgren H, Holm A, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: a systematic review. *Acta Odontol Scand* 2003; 61(6): 347–55.
- Weyant RJ, Tracy SL, Anselmo T, Beltrán-Aguilar ED, Donly KJ, Frise WA, et al. Topical fluoride for caries prevention: executive summary of the updated clinical recommendations and supporting systematic review. *J Am Dent Assoc* 2013; 144(11): 1279–91.
- Giacaman RA, Carrera CA, Muñoz-Sandoval C, Fernández C, Cury JA. Fluoride content in toothpastes commercialized for children in Chile and discussion on professional recommendations of use. *Int J Paediatr Dent* 2013; 23(2): 77–83.

36. *Cury JA, Oliveira MJ, Martins CC, Tenuta LM, Paiva SM.* Available fluoride in toothpastes used by Brazilian children. *Braz Dent J* 2010; 21(5): 396–400.
37. *Oganesian E, Ivanakova R, Lencova E, Broukal Z.* Alimentary fluoride intake in preschool children. *BMC Public Health* 2011; 11: 768.
38. *Nascimento HA, Soares FJ, Granville-Garcia AF, Brito CE, Almeida CA, Sampaio FC.* Estimation of toothpaste fluoride intake in preschool children. *Braz Dent J* 2013; 24(2): 142–6.
39. *Cochran JA, Ketley CE, Duckworth RM, van Loveren C, Holbrook WP, Seppä L,* et al. 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. *Community Dent Oral Epidemiol* 2004; 32(Suppl 1): 47–53.
40. *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(10): 1190–201.
41. *Kumar S, Tadakamadla J, Johnson NW.* Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis. *J Dent Res* 2016; 95(11): 1230–6.
42. *Rethman MP, Beltrán-Aguilar ED, Billings RJ, Burne RA, Clark M, Donly KJ,* et al. Nonfluoride caries-preventive agents. Executive summary of evidence-based clinical recommendations *J Am Dent Assoc* 2011; 142(9): 1065–71.

Received on July 21, 2017.

Accepted on September 15, 2017.

Online First September, 2017.