AMERICAN UNIVERSITY OF BEIRUT

NATIONAL STUDIES OF URINARY FLUORIDE STATUS OF LEBANESE SCHOOL CHILDREN (AGED 6-10)

by

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AN ABSTRACT OF THE THESIS OF

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Fluoride is a trace element; its proper intake and usage is beneficial to bone and tooth integrity. Its level of exposure may decline the prevalence of dental caries, or raise fluorosis cases and influence children's neurodevelopment. Urinary fluoride excretion is the primary metabolic pathway for fluoride removal from the body and a useful way to estimate the overall fluoride intake of a population. Due to the impracticality of 24-h urinary collection, the F/Cr ratio of a morning spot urine sample was reported to be a good indicator of F excretion.

This study is a national cross sectional school-based study that intended to investigate urinary fluoride status of the Lebanese children (aged 6 to 10 years old) by analyzing urine samples of 1350 children. Schools from the 8 Lebanese districts were recruited according to the population load. Subjects were randomly selected from 26 elementary schools. Anthropometric measurements and morning spot urine samples were collected and analyzed for fluoride and creatinine. F/Cr ratio was calculated, and daily fluoride excretion and total daily fluoride intake (TDFI) were estimated.

The F/Cr ratio was significantly different between males and females and among age categories, districts and school types. Daily fluoride excretion (mg/d) also varied by age, districts and schools types. The estimated TDFI was for the majority lower than the recommended adequate intake (AI) for this age range (0.05 mg/kg/d of fluoride).TDFI (mg/kg/d) was significantly higher in males than in females. Cultural factors related to eating habits might explain such a difference. TDFI (mg/d) increased significantly with age whereas TDFI (mg/kg/d) did not possibly implying that higher weight with age is correlated to the increasing intake. Students attending public schools and from the Northern district of Lebanon had significantly higher TDFI. These might be respectively influenced by socio-economic and geographical factors.

Further research on fluoride intake in Lebanese children is needed to inform public health policies aiming to decrease dental caries while protecting them from the effects of excess fluoride on bone, teeth and neurodevelopment.

CONTENTS

viii

Appendix:

IILUSTRATIONS

TABLES

ABBREVIATIONS

%	Percent
&	and
/	Per
<	Less than
>	Greater than
±	Plus or minus
µS	Average number of students per school
a.m.	Before noon
AI	Adequate intake
AUB	American University of Beirut
BMI	Body Mass Index
cm	Centimeter
Cr	Creatinine
d	Day
et al.	and others
F	Fluoride
F/Cr	Fluoride to creatinine ratio
g	Gram
h	Hour
H_2O	Distilled Water
kg	Kilogram
l	Liter
MEHE	Ministry of education and higher Education
mg	Milligram
ml	Milliliter
n	Sample Size
p.m.	After noon
PPS	Probability Proportionate to Size
RDA	Recommended Dietary Allowance
SD	Standard Deviation
TDFI	Total daily fluoride intake
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Fluoride is a natural element found at varying concentrations in drinking water as well as in soil (Palmer *et al.*, 2005). It was reported by the World Health Organization (WHO) in 2000 that its beneficial as well as its toxic effects in humans have important public health implications. Although daily intake of 1–3 mg of fluoride prevents dental caries, long-term exposure to higher amounts may have damaging effects on tooth enamel and bone (WHO, 2000). The intelligence of children is also affected, some studies showed the possibility of an adverse effect of high fluoride exposure on children's neurodevelopment (Choi *et al.*, 2012). Acute toxicity may occur by consuming single doses of 5–10 mg/kg body weight, and death was reported following ingestion of 16 mg/kg. "The usual lethal concentration range is 70–140 mg/kg" (WHO, 2000).

Worldwide interventions have been implemented to achieve adequate fluoride intake among different populations. For 65 years, community water fluoridation has been a secure and healthy way to successfully prevent dental caries. The United States Centers for Disease Control and Prevention (CDC) have recognized water fluoridation as one of 10 great public health achievements of the 20th century (Centers for disease control and prevention, 2014). Other interventions include mouth washes, toothpaste, systemic supplementation and salt fluoridation.

But the primary issue to be discussed remains the baseline intake of the population, from which we can deduce the need for supplementation within the safe range away from fluorosis. In fact, in case of adequate fluoride intake and no deficiency, excessive fluoride intake may lead to fluorosis. Even though fluoridation

proponents argue that a decrease in the recommended level of fluoride used for water fluoridation promotes tooth decay, particularly among low-income groups who cannot afford dental care, this has been countered and it has been suggested that a high intake of fluoride can place people at risk of bone abnormalities, fractures and impaired cognitive development. The optimal level "provides the best balance of protection from dental caries or tooth decay while limiting the risk of dental fluorosis". (George, 2011) However, the European Food Safety Authority (EFSA) stated that no Average Requirement for the performance of essential physiological functions was found to be clarified since fluoride was declared to be not an essential nutrient; but still EFSA considered that the setting of an AI is appropriate because of the beneficial effects of dietary fluoride on prevention of dental caries. (Agostoni *et al*., EFSA, 2013).

An epidemiological investigation was carried out at the national level in Lebanon to provide data on the oral health condition in the country. The results of this survey showed that almost 56% of 6 years old (y.o.), 93% of 12 y.o. and 97% of 15 y.o. school children in Lebanon have tooth decay. In fact, experience clearly indicates that dental caries cannot be successfully eliminated nation-wide through curative measures only and that preventive measures must also be implemented. This study recommended efforts in the field of oral health care in order to focus primarily on health promotion and education, leading in turn to improved oral hygiene. (Doumit *et al.*, 2002)

In response to that, the Lebanese Ministry of Education, with medical and professional staff cooperation, sent circular 186 to all public schools in the year 2005. It includes having a well-organized dental care follow up for every student as well as a fluoride mouth wash applied in every public school country-wide; this circular was renewed in 2012 (Appendix I). While in private and private free schools this decision

has not been applied, Lebanese parents of students attending these schools are always asked to follow up for their children's health generally and dental health specifically.

Moreover, the Lebanese Ministry of Health recently issued the law 178 in year 2011 that stipulates adding potassium fluoride (FK or $KF_2 H_2O$) to table or cooking salt that is imported or manufactured in Lebanon (Appendix VI). All salt producing industries are required to follow certain standards decreed by this law. Potassium Fluoride percentage added to the salt must be mentioned on the package and should be at the level of 250 mg per 1 Kg.

This study aimed to investigate fluoride status of the Lebanese children (age 6 to 10 years old) in order to determine baseline fluoride status through urinary fluoride excretion before the implementation of salt fluoridation law. Urine is the main excretion route for ingested fluoride and a useful way to estimate the overall fluoride intake of a population; so the results would be used by public health professionals to prevent fluoride deficiency or toxicity. (Zohouri, 2006).

CHAPTER II

LITERATURE REVIEW

A.   Background on Fluoride

Fluoride is the ionic form of fluorine (USDA, 2010) that exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil (Dhar *et al.*, 2009). Fluorine is the first member of halogens and has unique physical and chemical characteristics (Tsunoda *et al*., 1985). Discovered by Henri Mossan in 1886, fluorine is a corrosive pale yellow gas. It is the world's 13th most abundant element, constituting 0.08% of the Earth crust and having the highest electronegativity of all elements. As for fluoride, it is widely distributed in the environment, occurring in the air, soils, rocks, and water (United States Environmental Protection Agency, 2000). It may be bound to metal, nonmetal, or organic compounds. It predominates in nature and is present in trace amounts in the body (USDA, 2010).

B.   Food and other sources

Fluoride is provided in mainly two ways, systemic and topical. Systemic fluoride represents the fluoride that is ingested and later incorporated into teeth. Examples are the fluoride in water, dietary supplemental fluoride (drops, lozenges or tablets) and food sources. Topical fluoride is the type that is applied locally to the teeth. Topical fluoride is also applied through toothpastes, mouth rinses, gels, and varnishes. (Mady, 2007). A portion of mouth wash has been reported to pass to the digestive system, highlighting the importance of systemic fluoride even through topical sources (García-Hoyos *et al.*, 2014). Table 1 summarizes these systemic and topical fluoride sources.

Table 1. Sources of fluoride

Fluoride sources	Topical fluoride	Systemic fluoride
Natural sources		Water
		Foods
		Beverages
		Soil
		Several minerals such as fluorite and fluorapatite
Synthetic sources	Fluoride containing dentifrices	Water Fluoridation &School Water fluoridation
	Fluoride containing Prophylaxis pastes	Dietary supplements of fluoride tablets
	Solutions & gels	Fluoridation of milk & Infant formulas
	Supervised self- application of fluoride (Such as mouth-rinsing with fluoride solutions, tooth-brushing with fluoride solutions and gels…)	Addition of fluoride to salt
		Drugs
		Occupational exposure through the air

(Ghosh *et al.*, 2013- Buzalaf *et al.*, 2011- García-Hoyos *et al.*, 2014)

The use of fluorine in industry leads to occupational exposure to fluoride in air. In addition, prolonged use of certain drugs has been associated with the chronic adverse effects of fluoride excess; including the use of NaF for the treatment of osteoporosis. Furthermore, fluoride preparations for dental purposes, may contain low (0.25–1 mg per tablet: 1,000–1,500 mg of fluorine per kg of toothpaste) or high concentrations (liquids containing 10,000 mg/L and gels containing 4,000–6,000 mg/kg are used for local applications). (Ghosh *et al.*, 2013).

Fluoride content of edible items varies geographically depending on the contents of the soil and water used for irrigation. In general, fish, seafood and tea contain relatively high levels of fluoride. (Dhar *et al.*, 2009)

Tea is a naturally rich source of fluoride; it absorbs fluoride from the soil in which it grows. Because it is a fluoride accumulator, tea consumption may contribute

significantly to fluoride intake. Historically, tea was grown in natural soil; however, many tea growers now use fluoride-containing fertilizers which may further increase fluoride content of the tea. (Dhar *et al.*, 2009)

Levels in dry tea range between 3 and 300 mg/kg with an average of 100 mg/kg. Accordingly, two to three cups of tea contain approximately 0.4–0.8 mg of fluoride. Hence, brewed teas may contain fluoride concentrations of 1-6 ppm or mg/l depending on the used amount of dry tea, the water fluoride concentration and the brewing time. (Dhar *et al.*, 2009- Pehrsson, 2005, USDA). The fluoride content of the common food commodities is presented in table 2. Finally, the main sources of fluoride in children are infant formulas and water, fluoride ingested from toothpaste and dietary fluoride supplements. Around 90% of children swallow about 0.5 g of toothpaste per day and about 7% of the children swallow between 1 and 2 g per day. Young children's toothpaste use should be controlled, and the use of only small quantities of it should be emphasized. Dietary fluoride supplements should be considered a targeted preventive regimen only for children at higher risk for dental caries and with low levels of ingested fluoride from other sources (Levy *et al.*, 2007- Piva *et al.*, 2003- Zohouri *et al.*, 2000).

Table 2. Fluoride content in agricultural products and other edible items.

	Food items	Reported range of fluoride (ppm)*
Cereals	Wheat	2.59–4.60
	Rice	2.00–4.03
	Maize	~5.60
	Cereal	~4.60
Pulses and legumes	Bengal gram	3.84–14.8
	Green gram dal	2.34–21.2
	Soybean	~4.0
	Red gram dal	2.34–52.8
Leafy vegetables	Spinach	0.77–4.14
	Cabbage	1.28–3.3
	Lettuce	5.7
Other vegetables	Cucumber	2.57–4.10
	Tomato	0.33–3.40
	Ladies finger	1.74–4.00
Roots and tubers	Carrot	1.90–4.90
	Potato	1.27–2.92
	Onions	1.00–3.70
	Beet root	4.20
	Sweet potato	3.20
Beverages	Tea (dry leaves)	39.8–112.0
	Tea infusion (1 g boiled for 5 min)	18.13–56.19
	Aerated drinks	0.77–1.44
	Coconut water	0.43–0.60
Nuts and oil seeds	Almond	~4.0
	Cashewnut	~4.1
	Coconut	~4.4
	Mustard seeds	~5.7
Spices and condiments	Coriander	~2.3
	Garlic	~5.0
	Ginger	~2.0
Foods from animal sources	Mutton	3.0–3.50
	Beef	4.0–5.0
	Fishes	1.0–6.50
	Pork	3.0–4.50
Fruits	Banana	0.84–2.90
	Grapes	0.84–1.74
	Mango	0.80–3.70
	Apple	1.05–5.7

*ppm= part per million (mg/kg)

(Gropper *et al.*, 2009 -Ghosh *et al.*, 2013- Food and Nutrition Board, 1997)

C.   Fluoride metabolism and regulation

1.   *Fluoride Absorption*

Systemically, fluoride is mainly absorbed at the level of the stomach and intestines especially the small intestines, mostly by passive diffusion (Fawell *et al.*, WHO, 2006- WHO, Geneva, chapter 15, 1996- Gropper *et al.*, 2009, chap. 12). Ingested fluoride is almost 75–90 per cent absorbed in the body (Fawell *et al.*, WHO, 2006- USDA-DRI, 2010- Ananian *et al.*, 2006). Some studies even showed that the drinking-water fluoride is frequently absorbed with an efficiency exceeding 90% (WHO, Geneva, 1996). The percentage of fluoride absorption at the level of the stomach is affected by the gastric pH. An acidic stomach leads to fluoride conversion into hydrogen fluoride (HF) and almost 40 per cent of the ingested fluoride is absorbed from the stomach as HF. In case of high stomach pH, due to Proton Pump Inhibitor consumption for instance, the concentration uptake of HF decreases, leading to a consequent decrease in gastric absorption. The non-absorbed fluoride in the stomach is then absorbed in the intestine where it is unaffected by pH.

At the level of absorption, fluoride may interact with other cations. Gastrointestinal fluoride absorption may be clearly decreased by high concentrations of cations, relative to the amount of fluoride ingested; cations such as calcium, magnesium and aluminum, may form insoluble complexes with fluoride (Fawell *et al.*, WHO, 2006- USDA-DRI, 2010).

As for the topical level of fluoride absorption, fluoride present in low, sustained concentrations (sub-ppm range) in the oral fluids during an acidic challenge is able to absorb to the surface of the apatite crystals, inhibiting demineralization. When the pH is re-established, traces of fluoride in solution will make it highly supersaturated

with respect to fluorohydroxyapatite; this will speed up the process of remineralization. The mineral formed under the nucleating action of the partially dissolved minerals will subsequently & preferentially include fluoride and exclude carbonate, rendering the enamel more resistant to future acidic challenges (Buzalaf *et al.*, 2011).

2. ***Distribution***

Once absorbed into the blood, fluoride is distributed throughout the body, with almost 99% of the retained fluoride getting associated in calcium rich areas or hard tissues such as bone and teeth where it is incorporated into the crystal lattice (Fawell *et al.*, 2006- Whitford, 1990)**.** Bone fluoride usually reflects the long-term exposure to fluoride since mobilization from calcified tissues is dependent on previous fluoride intake, mostly in drinking-water (Zohoori *et al.*, 2012- WHO 2000). Fluoride is rapidly distributed by the systemic circulation to the intracellular and extracellular water of tissues; but usually, the ion accumulates only in calcified tissues primarily bone and teeth. "In the blood, the ion is asymmetrically distributed between plasma and the blood cells, so that the plasma concentration is approximately twice as high as that associated with the cells." (Gropper *et al.*, 2009)

In addition, fluoride is distributed from the plasma to all tissues and organs. The rates of delivery are normally determined by the blood flow to the tissues in question. Moreover, normal ranges for ionic fluoride have been established at 0.01 to 0.2 μg F^-/m L in the plasma and 0.2 to 1.1 mg F^-/mL in urine (Gropper *et al.*, 2009).

3. ***Excretion***

A number of pharmacokinetic parameters such as plasmatic, renal and extra renal clearances of fluoride were examined to determine the routes of the removal of

fluoride from the body. (Liteplo *et al.*, 2002). Fluoride is excreted primarily in urine, but it is also found in feces, in low concentration in sweat, saliva and breast milk. Fluoride also passes through the placenta (Ghosh *et al.*, 2013- WHO, 2000).

a.   Urinary excretion

Fluoride is excreted primarily via urine. (Fawell *et al.*, 2006 - Liteplo *et al.*, WHO, 2002).The renal clearance of fluoride in the adult usually ranges from 30 to 50 ml/min, whereas clearance rates of the other halogens (chloride, iodide and bromide) are generally less than 1.0 ml/min. Fluoride is reabsorbed at the level of the renal tubules. The degree of reabsorption depends mainly on the pH of the tubular fluid, urinary flow and renal function (Liteplo *et al.*, WHO, Geneva, 2002). Urinary fluoride excretion is the most important metabolic pathway for fluoride elimination from the body (Ekstrand *et al*., 1994).

b.   Fecal excretion

Fecal fluoride excretion is approximately 10% of the total daily fluoride intake (TDFI) in children and adults (Ekstrand et al., 1994).

c.   Excretion in sweat

Early works overestimated fluoride content in sweat (0.3-0.9 mg/l, Buzalaf *et al*., 2011). Henschler *et al.* (1975) studied the predicted losses in workers whose urinary fluoride levels are about 5 mg/l, the sweat could account for approximately 5% of the fluoride excreted (Liteplo *et al*, 2002)**.** However, with the introduction of the ion-specific electrode, estimates were much lower. Whitford stated that fluoride concentrations in sweat is 0.019-0.057 mg/l (Buzalaf *et al.* 2011). Presently, issues of

collection including contamination and lack of supporting data preclude the use of sweat as a viable marker of contemporary fluoride exposure.

d.   Salivary excretion

The concentration of fluoride in saliva is approximately two-thirds of the plasma fluoride concentration and seems to be independent of flow rate. The levels of fluoride in saliva are important, because this potentially provides further topical exposure of the teeth to fluoride (Liteplo *et al*., WHO, Geneva, 2002).

e.   Excretion in breast milk

Studies of breast feeding mothers have shown that there is some transfer of fluoride from plasma to breast milk. Breast milk is the main source of fluoride during the first 6 months of life. (Liteplo *et al*., 2002)**.**

4.   *Interactions*

Fluoride may interact with some other ions in the absorption process. Some cations such as calcium, magnesium and aluminum, may form insoluble complexes with fluoride, thus leading to a reduced uptake of fluoride. While some other ions may increase fluoride uptake especially by increasing its bioavailability (USDA-DRI, 2010). Phosphate, Sulfate and Molybdenum increase the uptake of F^-.

The rate and extent of fluoride absorption from the gastrointestinal tract are reduced to some extent by ingestion with solid foods and some liquids, especially those rich in calcium, such as milk or infant formulas. Results from studies with rats that had chronically elevated plasma fluoride concentrations showed that a diet high in calcium increases fecal fluoride excretion such that fluoride loss can equal or exceed fluoride intake. It has been suggested that the co-ingestion of fluoride and caffeine or some other

methylxanthines increases the bioavailability of fluoride, however other studies failed to confirm this effect (Whitford, 1994).

Biological interactions between fluoride and calcium have been studied extensively. While a diet high in calcium increases fecal fluoride excretion, fluoride toxicity may occur and is reported in population groups whose calcium nutritional status is poor. (Narasinga Rao *et al*, 1979).

D.   Fluoride Functions:

Fluoride is a natural element found at different concentrations in drinking water, soil and food. Its main functions in optimal levels are:

- Prevention of dental caries due to its capacity to increase tooth mineralization, reduce dental enamel demineralization, promote dental enamel remineralization and reduce dentin hypersensitivity (Palmer et *al*., 2005).

- Strengthening of the bones due to its capacity to induce bone formation by stimulating osteoblasts.

- Neurological effects and cognitive capacity of children (Saxena *et al.*; 2012).

The impact of fluoride on human teeth was recognized in 1909 in Colorado, United States, when the two dental surgeons, Frederick McKay and Grant Black, launched an investigation into the causes of mottled enamels. (Peckham, 2014). Fluoride has both useful and harmful effects on human health. The prevalence of dental caries and osteoporosis is inversely related to the concentration of fluoride in drinking water; while excess of fluoride is associated in a dose-response relationship with the prevalence of dental and bone fluorosis. In addition, many studies have examined the

effects of high fluoride consumption on the children's intellectual ability and other aspects. (Trivedi *et al.*, 2007)

1.   Dental caries

Fluoride is an important protective factor against dental caries which is a multifactorial infectious disease that is the most common chronic condition of childhood and has various risk factors. (Buzalaf *et al*., 2011-Palmer *at al*., 2005). The widespread use of both systemic and topical fluorides was linked to the reductions in dental caries. However, the topical use of fluorides has gained greater popularity than its systemic use with fluoride toothpastes being the most widespread form of topical fluoride usage. (Wong *et al*., 2011- Buzalaf *et al*., 2011).

Ingested fluoride or in other words systemic fluoride becomes incorporated into forming tooth structures. Fluoride ingested on a regular basis during teeth development is deposited throughout the entire surface of the tooth and contributes to long lasting protection against dental decay. Also, evidence supports fluoride's systemic mechanism of caries inhibition in pit and fissure surfaces of permanent first molars when it is incorporated into these teeth pre-eruptively**.** (Dhar *et al*., 2009- Buzalaf *et al*., 2011).

On the topical level, fluoride can provide antimicrobial action. Fluoride concentrations as found in dental plaque have biological activity on critical virulence factors of *Streptococcus mutans in vitro*, such as acid production and glucan synthesis, but the *in vivo* implications of this are still not clear. (Buzalaf *et al*., 2011). It has been demonstrated that topical fluoride is more effective and safer than systemic intake in tooth decay prevention. (Limeback *et al.*, 1999- García-Hoyos *et al.*, 2014).

Dental caries is the net result of consecutive cycles of de- and remineralization of dental tissues at the interface between the biofilm and the tooth surface. Demineralization is caused by the production of acids by oral bacteria after sugar consumption. Five categories of fluoride were described in the enamel structure and are: FO is the outer fluoride, present outside enamel (in the biofilm or saliva); FS is the fluoride present in the solid phase, incorporated in the structure of the crystals, also known as fluorhydroxyapatite; FL is the fluoride present at the enamel fluid; FA is the fluoride adsorbed to the crystal surface; and CaF_2 is the globule deposited on enamel and biofilm after application of highly concentrated fluoride products and acts as a pH-controlled fluoride and calcium reservoir.

In case of fluoride presence in plaque fluid (FL) when bacteria produce acids, it will penetrate along with the acids at the subsurface, adsorb to the crystal surface (FA) and protect crystals from dissolution. When the whole crystal surface is 100% covered by FA, it will not dissolve upon a pH fall caused by bacterial- derived acids, since this type of coating makes the characteristics of the crystal similar to those of fluorapatite. On the other hand, when the coating of FA is partial, the uncoated parts of the crystal will undergo dissolution. While FA has effectively the protective role for the crystals from dissolution, the role of fluoride present in solution (FL) is equally important, because the higher the concentration of FL, the higher the probability that it adsorbs (FA) and protects the crystals but even low concentrations can inhibit acid dissolution of tooth minerals. Calcium fluoride (CaF_2) is important as a source of fluoride to the oral fluids (FL). It is known as pH- controlled fluoride and calcium reservoir. This compound forms when the fluoride concentrations in the solution bathing enamel are higher than 100 ppm. In the remineralization fluoride role, salivary flow containing

fluoride buffers the acids produced by the bacteria after an acidic challenge. Once the pH is higher than 5.5, remineralization will occur since saliva is supersaturated with respect to the dental mineral. Traces of fluoride in solution throughout dissolution of hydroxyapatite will make the solution highly supersaturated with respect to fluorhydroxyapatite. (Buzalaf *et al*, 2011)

Accordingly, prior studies have shown that fluoride mouth rinsing programs are effective in reducing caries among children and adolescents. One of these studies was done through national surveys of child dental health in the UK and it confirmed that there is a variation in oral health. In particular, it showed that children of low socioeconomic status in Scotland have an excessively high incidence of dental disease. This study concluded that fluoride rinsing can be successfully targeted at children from deprived areas through school-based initiatives. (Levin *et al*., 2009)

A meta-analysis of the effects of fluoride toothpastes on the prevention of dental caries in the primary dentition of preschool children pointed out the role of a topical fluoride source in caries prevention, since it showed that standard fluoride toothpastes are effective in reducing dental caries in the primary teeth of preschool children and so their use should be recommended to this age group (Santos *et al*., 2013).

These studies aimed to analyze a topical source effect on dental caries. While a study done in South Korea aimed to evaluate the systemic effect of water fluoridation on dental caries prevalence and experience. By using a cross-sectional survey at two schools where water fluoridation had ceased 7-years prior and at two schools where the water had never been fluoridated including similar population size in both schools.. It showed that 1 year exposure to fluoride is not enough for protection against caries while 4 years was significantly protective. Moreover, they suggested that better oral hygiene

in the never fluoridated area might be the cause of a less caries incidence among its children and that the systemic effect of fluoride intake through water fluoridation might be important for the prevention of dental caries. (Cho *et al*., 2014)

Sugars are known to be the major dietary factor in the development of dental caries. Sugars and caries still have a significant relationship despite the increase in a regular use of fluoride toothpaste and fluoridated water. A study was done to examine the quantitative relationship between sugar intake and the progressive development of dental caries. It highlighted the fact that sugar intake is still high in populations and that even regulations are not sufficiently low to protect against dental caries; it shed the light as well on the importance of a radical approach to limiting sugar intakes and ensuring that fluoride use is well controlled in water and/or toothpaste is needed. In addition, this study endorsed that it is important not to consider fluoridation as a replacement for main efforts in reducing population sugar intakes (Sheiham *et al*., 2014).

2. *Dental fluorosis*

In a report conducted by WHO trace elements have been divided into three groups from the point of view of their nutritional significance in humans. These three groups were essential elements, which are probably potentially toxic elements, but some may have some essential functions at low levels. Fluoride was considered a part of the third group since the toxicity of ingested fluoride is well documented. (WHO, 1996)

Since, fluoride ingestion may be from different potential sources; it is important to consider safety issues related to the level of fluoride ingested. Excessive fluoride during tooth development can cause dental fluorosis which is a developmental disturbance of enamel and cause white spots and, in severe cases, brown stains, pitting, or mottling of the enamel. "It has been shown that dental fluorosis and fluoride content

of enamel, plaque, saliva, urine, nails and hair are directly related to fluoride levels of drinking water and dietary fluoride intake."

For instance, a study was conducted in India to assess the fluoride excretion in the population exposed to environmental fluoride, and revealed that more than 50% of the individuals were found to be affected with fluorosis in this district where fluoride concentration in the drinking water was high (Yadav, Lata, 2003). A similar study showed that higher fluoride level in the drinking water was also associated with a higher prevalence and severity of dental fluorosis. (Narbutaite *et al*., 2007). Another study identified higher fluoride intake from infant formula, beverages, and toothpaste ingestion as a risk factor for dental fluorosis. (Levy *et al*., 2010).

Dental fluorosis, whether minor or more noticeable, is the consequence of excessive fluoride intake as mentioned before. Unfortunately, the manifestations of such fluorosis can only be assessed several years after the individual has been exposed to higher-than-recommended fluoride levels (WHO, 1994).

3.   *Skeletal and non-skeletal fluorosis*

Fluoride is one of only a few identified agents that can stimulate bone cell proliferation and increase new mineral deposition in cancellous bone. Fluoride incorporation into bone increases the size and, thus, decreases the solubility of the bone crystals. Clinical studies have shown that ingested fluoride rapidly enters mineralized tissues like bone and developing teeth, and studies showed that there is a risk to develop skeletal fluorosis when intake of fluoride is high (Rawlani *et al*., 2010).

Non-skeletal forms of fluorosis are indicated as disturbances in soft tissues in chronic intoxication with fluorine and these develop early, usually long before the onset of typical changes in teeth and skeletal bones; thus these changes characterize the

preskeletal phase of fluorosis. Non-skeletal fluorosis may reach the central nervous system, skeletal musculature, stomach, liver, kidney, cardiovascular system, retina, and the skin. (Dhar *et al*., 2009).

Fluoride enhances the stability of the crystal lattice in bone but it makes bone more brittle. The most important factor in determining the clinical course of skeletal fluorosis was found to be the total quantity of fluoride ingested; in addition, the severity of symptoms correlates directly with the level and duration of exposure. Bone changes observed in human skeletal fluorosis are structural and functional, with a combination of osteosclerosis, osteomalacia, osteoporosis and exostosis formation, and secondary hyperparathyroidism in a proportion of patients. At very high fluoride concentrations, stages 2 and 3 of skeletal fluorosis are likely to occur. The clinical signs of these stages are chronic joint pain, dose related calcification of ligaments, osteosclerosis, possible osteoporosis of long bones, and in severe cases, muscle wasting and neurological defects. Because some of the clinical symptoms mimic arthritis, the first 2 clinical phases of skeletal fluorosis could be easily misdiagnosed (Dhar *et al*., 2009).

Moreover, skeletal fluorosis cases are divided into three categories mild, moderate, and severe and four types: constrictive, raritas, mixed, and soft. A thickening of bone trabeculaein the pelvis, bone spots, dense patches, and os in os phenomenon in the pelvic region are observed in the constructive type skeletal fluorosis. The second type which is the raritas type skeletal fluorosis is characterized with less thickening of the bone trabeculae in the pelvis. The third type which is the mixed type skeletal fluorosis is diagnosed when constrictive and raritas types exist at the same time. While the last, soft type, is characterized by lower density of the bony structure, thickening of bone trabeculae, raritas, and veil construction.

A cross-sectional study done in severe coal-burning endemic fluorosis area of southwest China used these types definition to assess the prevalence and pathogenic stage of skeletal fluorosis. About 23% of students in the study population were identified as high-risk. Moreover, about 97% of the high-risk children were identified with dental fluorosis indicating an association between the prevalence of both diseases among children. Skeletal fluorosis among children may lead to poor health and reduced productivity when they reach adulthood (Qin *et al*., 2009). Similarly, another study considered the long term occupational exposure to fluoride after a 50-year period of exposure and with a diagnosed bone fluorosis, and reported that the accumulation of fluorides in the organism appears only after many years of exposure as a bone-joint impairment (Buchancová *et al*., 2008). Furthermore, fluorosis was found to be endemic throughout the East African Rift valley, including parts of Tanzania and a study conducted in that area identified that low body mass index, drinking predominantly well water for 3 years, not being weaned on bananas, the use of fluoride salts in cooking during childhood and drinking more cups of tea per day were independent predictors of juvenile skeletal fluorosis (Jarvis *et al*., 2013).

4. *Osteoporosis*

Osteoporosis is a multifactorial disease, and a major cause of mortality and morbidity in elderly population, especially in women. Nutrition, nutritional status and its relationship with bone mass density are important modifiable factors in the development and maintenance of bone mass (Paknahad *et al*., 2014).

The concentration of fluoride in drinking water influences the quantity of fluoride accumulation in the skeleton, although large deviations from normal levels of fluoride in the diet also affect it. Many studies showed that fluoride is bound within the

bone replacing hydroxyl or bicarbonate groups normally associated with hydroxyl-apatite structures and it increases the crystallinity or crystal structure of the apatite (Dhar *et al.*, 2009).

For instance, 2 studies aimed to analyze the effect of sodium fluoride in the treatment of osteoporosis reported that it increases bone mass density even more than calcitonin in patients with osteoporosis who received calcium plus vitamin D. However, they suggested that it may reach progressive bone changes similar to skeletal fluorosis (Mowla, 2007- El Khoury *et al.*, 1982).

5. *Children's intelligence*

Fluoride is known to inhibit the enzyme acetylcholinesterase, which is involved in transmitting signals along nerves. Clinical and physiological studies from Russia published in 1974 demonstrated that patients with fluorosis exhibited disturbed nervous activity & brain dysfunction. China as well as India has areas with high levels of endemic fluorosis. Many possible adverse effects of fluorides were the subject of several researches in those two countries. In 1982, the first suggestions that fluoride could affect the brain were published in China. Following that, many studies have been conducted into the role of fluoride in brain development and its effects on intelligence (Groves, 2011).

Excess fluoride was reported to be able to pass through the blood brain barrier and exert a negative impact on the brain tissue. Fluoride can have an effect on the content of monoamine neurotransmitters, such as noradrenaline (NA), dopamine (DA) and serotonin (5-HT), which could lead to impaired neurotransmission. Different studies were done on rats in order to explain the metabolism of fluoride excess on the neurodevelopment. It was reported that developmental fluoride exposure leads to

cognitive decline and anxiety- and depression-like behaviors and reduces the total amount of ambulation in adult mice. NaF-induced memory impairment was found to be associated with NA and 5-HT increases in discrete rat brain regions. Fluoride may also affect the activity of some enzymes in the brain such as significantly reducing acetylcholinesterase (AchE) content in rat brain. (Liu *et al*., 2014)

Mullenix *et al.* (1995) studied the neurotoxicity of sodium fluoride in rats and also found that fluoride accumulated in brain tissues and that the severity of the effect on behavior increased directly as fluoride levels rose in the blood and the brain.

Similar to the effect on dental and bone health, both excess and deficit of fluoride seems to have an influence on brain. Fluoride has been used as a treatment for Alzheimer's disease (Moss *et al*., 2013) but excess of fluoride has been incriminated in an elevation of an Alzheimer's incidence (Isaacson, 1992).

Furthermore, some studies discussed the relationship between exposure to different drinking water fluoride levels and children's intelligence. For example a cross-sectional study done in Madhya Pradesh state, India included children from low and high fluoride areas. And showed that children in endemic areas of fluorosis are at risk for impaired development of intelligence (Saxena *et al*.; 2012). A meta-analysis showed that children in high-fluoride areas had significantly lower IQ scores than those who lived in low-fluoride areas. (Choi *et al*., 2012).

E.   Recommendations and current intakes

Recommendations aim to have optimal safe levels of intake required especially that "the dose makes the difference". Body fluoride status depends on many factors, including the fluoride content of natural drinking-water, the total amount ingested daily, the duration of ingestion and the efficiencies of intestinal absorption and renal excretion

(WHO, 1996- USDA, 2005). The average daily dietary intake of fluoride (expressed on a body weight basis) by children residing in optimally fluoridated (1 ppm) communities is 0.05 mg/kg/day. One ppm is equivalent to 1 milligram of something per liter of water (mg/l) or 1 milligram of something per kilogram of soil (mg/kg). In communities without optimally fluoridated water, average intakes for children are about 50% lower. (Dhar *et al*., 2009- USDA, DRI, 2010).

The Institute of Medicine (IOM, 1997) recommended an AI of 0.05 mg/kg/day beyond 6 months of age, to prevent dental caries. Upper limits of 0.10 mg/kg/day in children less than 8 years and 10 mg/day for those older than 8 years are recommended for prevention of dental fluorosis. Similar levels have been authorized by the American Dental Association (ADA, 1994) and the American Dietetic Association (ADA, 2000- USDA, 2005). Table 3 represents the dietary reference intakes set by the IOM of the US National Academy of Sciences.

Table 3. Dietary reference intakes for fluoride

Age group	Reference weights g (lb)	Adequate intake (mg/day)	Tolerable upper limit (mg/day)
Infants 0-6 mo	7 (16)	0.01	0.7
Infants 6-12 mo	9 (20)	0.5	0.9
Children 1-3 y	13 (29)	0.7	1.3
Children 4-8 y	22 (48)	1.0	2.0
Children 9-13 y	40 (88)	2.0	10
Boys 14-18 y	64 (142)	3.0	10
Girls 14-18 y	57 (125)	3.0	10
Males 19 y and older	76 (166)	4.0	10
Females 19 y and older	61 (133)	3.0	10

(Food and Nutrition Board, 1997)

In 1984, World Health Organization (WHO) conducted an extensive review about fluoride. One of their findings was noticing that mottling of teeth (i.e. dental fluorosis) is sometimes associated with fluoride levels in drinking-water above 1.5 mg/l and crippling skeletal fluorosis can ensue when fluoride levels

exceed 10 mg/l. As a result, WHO recommended a guideline value of 1.5 mg/l as a level at which dental fluorosis should be minimal. The 1.5 mg/l fluoride guideline value that was set in 1984 was subsequently re-evaluated by WHO during the years 1996 and 2004 and it was concluded that there was no evidence to suggest that it should be revised. The 1.5 mg/l guideline value of WHO is not a "fixed" value but is intended to be adapted to take account of local conditions such as diet, water consumption or other conditions. (Fawell *et al*., WHO, 2006 - WHO, 1996)

A study investigated the fluoride intake from fluids consumed by children in Lebanon. It showed that dietary fluids provide a substantial proportion of the recommended safe and adequate intake of fluoride for younger children in Lebanon, and that this proportion is significantly higher in rural than urban children. Another study was conducted to assess the fluoride levels in herbal teas available in the Lebanese market. Fluoride concentrations ranged from 0.620 to 1.680 mg/l for tea. Fluoride levels in herbal teas varied with the type of tea, brand, and method of preparation. Contribution of fluoride in tea to the total fluoride consumed by Lebanese families appears to be considerable (Jurdi *et al*., 2001).

F.   Methods of Fluoride intake assessment

When investigators seek to assess dietary intakes at the population level, many factors should be considered before choosing the type of dietary assessment tool, including how detailed, population-specific, practical and expensive the tool will be (Subar, 2004). The levels of fluoride in plasma, serum and urine have been considered useful biomarkers for fluoride exposure and that was proven by multiple studies (Ekstrand & Ehrnebo, 1983 & 1986- Whitford, 1996- Lund *et al.,* 1997). The concentrations of fluoride in parotid saliva have also been used to assess plasma levels

of fluoride. There are, however, ethical and technical limitations of the use of these fluids for large-scale monitoring of the body burden of fluoride in humans.

Other methods have been suggested to assess fluoride levels like concentration in nails and hair. However, there is only limited information on the reliability of these methods. Information on proper preparation methods as well as analytical techniques needs to be further evaluated. (Liteplo *et al*., WHO, 2002).

1.   *Questionnaires*

One way of assessing fluoride intake is by questionnaires. Two main types of questionnaires are possible: food diaries and food frequency questionnaires (FFQ). Food diaries must be filled on daily basis which may reduce compliance but improves accuracy. FFQs may be more practical to complete. However, a study showed that a quantitative FFQ are correlated to diaries but not as accurate (Rankin *et al*., 2011).

2.   *Urine*

Fluoride excretion in the urine is a biomarker of contemporary F intake. Since percentages of gastrointestinal absorption, fecal excretion, retention in calcified tissue, and renal excretion have ranges of variation, assumptions of those percentages must be done to deduce intake from urinary F level. Fluoride excretion is more suitable for population studies than for individual analysis. Villa *et al*. showed a linear relationship between total fluoride intake and daily fluoride renal excretion (Villa *et al*., 2010).

Ranges of fluoride excretion associated with ranges of total daily fluoride intake have been defined for specific age groups in specific conditions, for example different fluoride concentrations in drinking water (Villa *et al*., 2010). However, the width of the 95 % confidence interval (10-15 %) of the linear relationship indicates that

fluoride excretion in the urine is suitable to predict fluoride intake for groups only, and not individuals (Rugg-Gunn *et al*., 2011).

Since 24 h urine collection is difficult to achieve in large studies, spot urine was studied as an alternative. Creatinine has been used to correct the daily variation in urinary dilution, because total urinary creatinine excretion is relatively constant in healthy subjects. Standards for 24-h urinary creatinine by different age groups are available. Zohouri *et al.* demonstrated that F/Cr ratio in a spot urinary sample was representative of the fluoride content of a 24-h urine sample. (Zohouri *el al*., 2006)

3.   *Plasma*

Plasma fluoride concentrations are dependent on the total fluoride intake, dose frequency and the plasma half-life. (WHO, 1994). More recently, the plasma concentration has been shown to be associated with total fluoride intake, and with fluoride dentifrice use, but not with dietary fluoride intake (Cardoso *et al*., 2006). There are still insufficient data across age groups to define normal plasma concentrations in individuals (Rugg-Gunn *et al*., 2011).

4.   *Saliva*

Fluoride concentrations in ductal and glandular saliva closely correlate to the plasma concentration, but at a lower level. Kaiser *et al.* found that salivary fluoride content rose significantly within five minutes from baseline following the consumption of different meals rich with fluoride and almost returned to baseline at 60 minutes (Kaiser *et al*., 2006). It was found that only ductal saliva is a reliable marker of plasma fluoride concentration however, it is not easily obtained. (Whitford *et al*., 1999).

5.   Sweat and milk

Fluoride concentrations in sweat are similar to those in plasma, but conclusions cannot be drawn regarding its intake because of difficulties in standardized sample collection and lack of available data. The available data on F concentration in human milk do not permit a conclusion to be drawn on dietary intake of lactating women (Rugg-Gunn *et al*., 2011- Buzalaf *et al*. 2011).

6.   Bone and dentin and enamel

Fluoride retention in bone & dentin is proportional to long-term F intake; it is as well dependent on the turnover rate of bone, age, sex and the type of bone (Caraccio *et al*., 1983). The F content of surface bone and enamel may reflect contemporary F intake, whilst F in mature bone or in enamel reflects historical F intake related to their formation (Buzalaf, 2011). Post-eruptive F uptake of enamel is dependent on the F concentration in saliva, food, dental plaque and dental products (WHO, 1994). For instance, the F content in enamel biopsies was elevated with higher F concentration in drinking water (0.09 versus 1.9 mg/l) and higher in superficial than in deeper enamel biopsies (Schamschula *et al.*, 1985, Agostoni *et al*, 2013).

7.   Hair and nails

The F content in hair and nail reflects its intake over longer periods of time. (Mandinic *et al*., 2010). The Panel of the EFSA notes that there are insufficient data for defining a dose-response relationship. (Agostoni *et al*., EFSA, 2013).

G.   Studies on urinary fluoride excretion, intake and retention

Recent reviews have considered the suitability of biomarkers for fluoride exposure. One review looked at contemporary biomarkers which is those measuring

present or very recent exposure, and looked at biomarkers for both recent and historical exposure. The contemporary biomarkers considered were blood, bone surface, saliva, milk, sweat and urine; the biomarkers of recent exposure considered were nails and hair; and the biomarkers of historical exposure considered were bone and teeth. The reviews concluded that, at present, urine is the most useful biomarker of contemporary fluoride exposure.

Ingested fluoride from all sources is excreted primarily in the urine as mentioned above. This makes urinary fluoride measurement ideal for assessing the intake of fluoride in populations. (WHO, 2014) The curve in the figure 1 below shows the transition between the risk of caries and the risk of fluorosis with water fluoride intake. We notice that 1 ppm of fluoride in drinking water is the level at which dental caries risk reduction is slowed down and fluorosis risk is accelerated (Hodge, 1950).
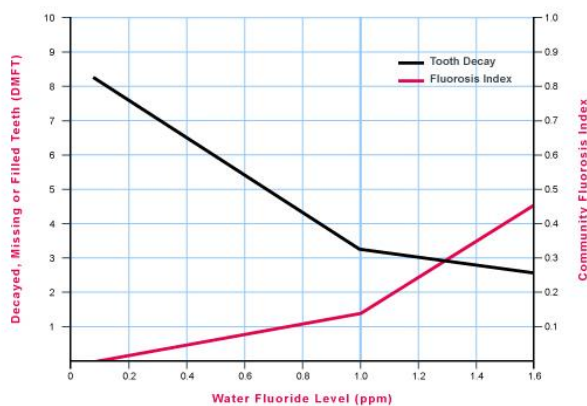
Figure 1. the transition between the risk of caries and the risk of fluorosis with water fluoride intake. (Hodge, 1950).

Urinary fluoride excretion was studied in different age groups at different fluoride exposure levels for different or similar purposes. Some studies are summarized in table 4.

Table 4. Summary of other studies on urinary fluoride excretion, intake and retention from different countries

Study	Country	Age of Children (years)	Sample size	Mean fluoride content in water (ppm or mg/l)	Method of assessment	Fluoride (mg)/ Creatinine (g) ratio	Calculated or Estimated mean daily fluoride intake (Mean ±SD)	Mean urinary fluoride excretion concentration	Percentage of urinary excretion
Hoyos *et al.*, 2014	Spain	5-8	74	<0.3ppm	Urine collection 1) Before mouth wash 2) after 2 hrs	1) 0.26 mg/g 2) 1.58mg/g	-	-	-
Shaoxian *et al.*, 2013	Southern china	6-12	120	0.11	Urine sample collection at 11am		-	0.43±0.38 mg/l	-
Akpata *et al.*, 2013	Kuwait	1-9	400	Tap water: 0.04±0.02 Bottled water: 0.28±0.4	Early morning spot urine & estimation of 24 hour urinary fluoride using Fluoride/Creatinine ratio	0.52±0.52 mg/g	0.013-0.018 mg/Kg/d	0.128-0.220mg/d	-
Zohoori *et al.*, 2012	UK	6-7	33	1) Low F area: 0.3 2) Natural F area: 1.06	Assessing dietary F intake and F ingestion from toothpaste. Collection of 24 h urine samples for 2 days.	-	1) 0.945±0.621 mg/d 0.038±0.027 mg/kg/d 2) 1.707±0.799 mg/d 0.076±0.038 mg/kg/d	1) 0.297±0.131 mg/d 0.012±0.006 mg/kg/d 2) 0.393±0.209 mg/d 0.017±0.007 mg/kg/d	1) 40% 2) 30%
Franco *et al.*, 2009	Venezuela	1-6	31	0.1±0.02	Assessment of daily F intake and 24 h urinary fluoride	-	1.308± 0.474 mg/d 0.08±0.03mg/kg/day	0.80±0.4 mg/l 0.368±0.183 mg/d	30%
Soares *et al.*, 2008	Brazil	2-7	42	1) (n=10)0.5-1.0 2) (n=17)1.1-1.5 3) (n=15)>1.5	Collection of 24 h urine twice. Two phases of toothpaste application for a week each: a. Fluoridated b. non fluoridated	-	-	1) a. 0.024±0.012 mg/kg/d 0.453±0.290 mg/d b. 0.026±0.024 mg/kg/d 0.453±0.187 mg/d 2) a. 0.026±0.013 mg/kg/d 0.451±0.224 mg/d b. 0.028±0.025 mg/kg/d 0.430±0.352 mg/d 3) a. 0.031±0.017 mg/kg/d 0.0592±0.390 mg/d b. 0.032 (±0.021) mg/kg/d 0.624 (±0.409) mg/d	-
Maguire *et al.*, 2007	UK	6-7	29	• Optimally fluoridated ⇨ 0.82±0.13 • Sub-optimally	Measuring urinary fluoride excretion and urine volume over 24 h and estimation of fractional urinary fluoride	-	1) (n =5) a. 1.043±0.220 mg/d b. 0.047±0.008 mg/kg/d	1) (n =3) a. 0.323±0.184 mg/d b. 0.014±0.006 mg/kg/d	1) (n =3) 32% 2) (n =8) 40% 3) (n = 18) 44%

Reference	Country	Age	N	Water F	Method		Intake	Excretion	Retention
				fluoridated ⇨ 0.47±0.09 • Non-fluoridated ⇨ 0.08±0.03	excretion and retention		2) (n =9) a. 0.883±1.011 mg/d b. 0.038±0.038 mg/kg/d 3) (n = 18) a. 0.736±0.533 mg/d b. 0.031±0.025 mg/kg/d	2) (n =8) a. 0.239±0.108 mg/d b. 0.011±0.005 mg/kg/d 3) (n = 18) a. 0.203±0.099 mg/d b. 0.008±0.004 mg/kg/d	
Zohouri *et al*., 2006	UK	1-3	7		Measuring intake and collection of 24h urine and spot urine	1.49±0.63 mg/g	0.71±0.41 mg/d 0.05±0.02 mg/kg/d	0.33±0.16 mg/d	48%
Ketley *et al*., 2004	6 European countries 1) Ireland 2) England 3) Finland 4) Iceland 5) Netherlands 6) Portugal	1.5-3.5	86: ➢ 9 ➢ 18 ➢ 18 ➢ 4 ➢ 6 ➢ 2	Ireland: 0.8-1 All others: <0.15	Collection of 24 hr urine sample	-	-	1) 0.37±0.11 mg/d 0.22±0.008 mg/kg/d 2) 0.2±0.14 mg/d 0.014±0.010 mg/kg/d 3) 0.16±0.08 mg/d 0.011 ±0.007 mg/kg/d 4) 0.17±0.01 mg/d 0.011±0.001 mg/kg/d 5) 0.21±0.15 mg/d 0.014±0.010 mg/kg/d 6) 0.33±0.27 mg/d 0.022±0.019 mg/kg/d Total: 0.26± 0.18 mg/d 0.017±0.013 mg/kg/d	-
Haftenberger *et al*., 2001	Germany	3-6	11	0.25	2 days intake assessment (duplicate-diet approach) and 24 h urine collection	-	0.931±0.391mg /d or 0.053 ±0.0 21 mg/kg/d	0.91±0.45 mg/l 0.476±0.192 mg/day	51.5%
Baez *et al*., 2000	US (Texas)	4-6	29	0.1-3.2	Urine collection in three time periods of the day	-	-	0.749 mg/d Morning : 1.261 mg/l Afternoon:1.419 mg/l Night: 1.334 mg/l	-
Villa *et al*., 2000	Chile	3-5	20	0.5-0.6	Two successive 24-h periods determination of: - total amount of fluoride ingested from liquid and	-	0.064±0.015 mg /Kg/d 1.02 mg/d	0.928±-0.217 mg/l 0.358±0.076 mg/d	35.5 % (estimated fractional F retention

					solid food, and toothpaste -amount of urinary fluoride excretion				54.5%)
Ketley *et al.*, 2000	UK	4-5	8	<0.1	Part 1: Total urinary fluoride excretion in normal conditions of fluoride intake for 3 days and 2 nights Part 2: 24-h urinary fluoride excretion for 2 days after 3 days of low fluoride intake and then for 2 days while giving a 0.5 mg/d fluoride tablet	-	-	Part 1: 0.33±0.15 mg/d 0.017±0.008 mg/kg/d Part 2: (n=7) ➤ Day 1 & 2: 0.18± 0.04 mg/d ➤ Day 3 & 4: 0.32±0.05 mg/d	30.1%
Heintze *et al.*, 1998	Brazil	5-50	545	Garca 0.9±0.18 Bauru 0.64±0.30 Itapolis 0.02±0.02	Urine collection at 3 different times of the day 8-10 am 2-4 pm 7-9 pm	-	-	1.31±0.61 mg/l (in Garca) 0.88±0.49 mg/l (in Bauru) 0.39±0.21 mg/l (in Itapolis)	-

CHAPTER III

MATERIALS AND METHODS

A.   Study design

This is a national cross sectional school-based study that was conducted from March till June 2013 and November till January 2014. It is part of a wider study investigating mineral status in Lebanese elementary school children (aged 6-10). The focus of the present study is on their urinary fluoride status. A multi-stage cluster sampling method was used to select random sample of Lebanese 6 to 10 year old children attending elementary schools. The study protocol was approved by the Institutional Review Board committee of the American University of Beirut, under the code NUT.0013. This research was partially funded by the URB (University Research Board of the American University of Beirut).

B.   Study Population

The study recruited 1403 healthy male & female subjects (781 males and 622 females). The age range was between 6 and 10 years. A list of schools in Lebanon, provided by the Ministry of Education and Higher Education, was the source to obtain numbers of students within grade 1 to grade 5. Since 2003, according the "Center de Resources sur le développement local" (Localiban, 2009), Lebanon has been divided into 8 districts (North, Akkar, Bekaa, Baalbeck/Hermel, South, Nabatiyeh, Mount Lebanon, and Beirut). Schools were grouped within clusters of the 8 districts according to the population load in each district, aiming to keep the sample representative. Twenty six schools accepted to contribute to the study in order to obtain the required sample

size. The response rate of schools was 72.22%. Subjects were then randomly selected from the 26 public, private, and private free schools; they were recruited by direct approaching to schools with the coordination of the Ministry of Education and Higher Education. The participants' parents signed consent forms prior to collection. School children were picked in a representative way of the Lebanese 6-10 year old children given that to the World Bank data, in 2012 present the average enrollment rate of children in the elementary school as 93 %.

1.	*Inclusion criteria for the student were:*

- The student's age range is within 6 to 10 years old
- The student is in one of the chosen schools in one of the 8 districts
- The student is healthy
- Informed consent signed by the parents or responsible of the child
- A sufficient amount of urine was provided in order to be analyzed

2.	*Exclusion criteria for the student were:*

- The student has renal impairment
- The student was not of Lebanese nationality
- The student was consuming any medication

**C.	Sampling process**

The probability proportionate to size (PPS) method was used in order to maintain a high degree of representativeness.

The first step was to determine the sample size. The following formula was used: n=$\dfrac{1.96 \times 1.96 \times p\,(1-p)(DEFF)}{d \times d}$

This was used to reach a required sample size of 1537.

Multi-stage cluster sampling was then used at the Mohafazat (District) level, school level and class level. Number of schools required per district was calculated proportional to total number of schools per district.

According to the proportional distribution of schools in each particular district, the types of schools in each district were selected. Table 5 shows the distribution of the types and the number of schools needed in each district.

In total 17 public, 11 private, and 6 private free schools were included.

Table 5. Distribution of Types and Number of Schools in each district

District	Number of schools	Public	Private	Private Free
Akkar	5	3	1	1
Baalbeck/Hermel	3	1	1	1
Beirut	3	1	2	-
Bekaa	3	2	1	-
Mount Lebanon	5	1	3	1
Nabatiyeh	4	2	1	1
North	8	4	3	1
South	5	3	1	1

Within districts, schools were selected using simple random selection from a school listing. Number of children per school was calculated proportionate to population size.

As a final step, in each school, a random sampling was adopted. Every student aged between 6 and 10 years old had the same probability of being selected in the study.

D.   Practical Procedures

Several procedures took place before the launching of data collection. The Director of Guidance and Counseling in the Lebanese Ministry of Education and Higher Education (MEHE) notified all the Lebanese public schools about the project; while

selected Lebanese private and private free schools were directly contacted by the researchers. An official letter and a consent form (Arabic and English forms-in the appendix section) were given to the directors of schools in a first visit. After receiving the acceptance of participation, consent forms were sent to all students in particular classes (Grade 1- Grade 5) suitable for the age range of the study (6-10 years old) and only students with signed consents participated in the study in the second visit.

E.   Data collection & Laboratory analysis

After providing assent, socio-demographic information, anthropometric measurements (weight and height), and urine samples were collected by 4 researchers. The research staff received training for ethical, professional, and standardized field work. Data collection detailed process is the following:

1. Anthropometric measurements

Anthropometric data were obtained using standardized techniques and calibrated equipments. Weight was measured using Tanita scale (±0.1Kg) in light indoor clothing. By means of a Shorrboard portable stadiometer (±0.1cm) received from UNICEF. BMI is calculated using the following formula weight/height[2] and expressed in kg/m^2.

2. Urine collection and storage

All the necessary information about the study and the urine collection were explained to each participant by one of the 4 researchers who obtained assent from each student afterwards (Arabic and English forms-in the appendix section). Non-fasting

random urine samples were collected in chemicals free cups between 9 a.m. and 1 p.m. then were transported to the laboratory on ice.

After collection, samples were transferred to tubes, which were firmly sealed with screw tops to avoid contamination and evaporation. An amount of 10 ml of urine stored with 0.5 ml EDTA (0.2 g/ml concentration) solution is required for fluoride analysis with ion selected electrode method (Tolos, 1994). Other urine milliliters were aliquoted for creatinine & other minerals analysis. Some urine samples had to be excluded and discarded because of the insufficient urine volume to measure both creatinine and fluoride; thus 1345 samples were included in this study. Urine specimens were stored in $-20^{o}C$ freezers to date of analysis. Zohouri *et al* concluded that the F/Cr ratio of a morning spot urine sample may be used to estimate mean 24-h urinary excretion of fluoride. (Zohouri *et al*., 2006)

3. *Fluoride testing standards*

Before fluoride analysis of urine samples at least five working standards had to be prepared in the range 0.1 to 100 μg F /mL by appropriate dilutions of the calibration stock solution with distilled water. Then this set of working standards together with the samples and blanks were analyzed (urinary fluoride analysis mentioned below) starting with the lowest concentration. Following that, a calibration graph was plotted on three-cycle semi-log paper scheming millivolts on the linear scale and fluoride concentration, μg/mL, on the log scale. In order to maintain standardization and quality assurance, the first sample analyzed in each day of laboratory analysis was used as a control after every 10 specimens analyzed.

Fluoride Testing

- NaF + Distilled water
- 100 mg NaF in 1000 ml H_2O → 0.1mg/ml => 100 µg/ml
- 100 mg (NaF) → 1000 ml Distilled water
- S0= 5ml (NaF solution) + 45ml (distilled water)

Table 6. Fluoride Testing Standards

Standards	Concentration and preparation
S1 → 50 mg/ml	20ml NaF + 20ml H_2O
S2 → 25 mg/ml	20ml S1 + 20ml H_2O
S3 → 12.5 mg/ml	20ml S2 + 20ml H_2O
S4 → 6.25 mg/ml	20ml S3 + 20ml H_2O
S5 → 3.12 mg/ml	20ml S4 + 20ml H_2O
S6 → 1.56 mg/ml	20ml S5 + 20ml H_2O
S7 → 0.15 mg/ml	2ml S6 + 18ml H_2O
S8 → 0.03 mg/ml	4ml S7+ 16ml H_2O

An internal control (IC) was prepared to show systematic and consistent results for creatinine values. Every 40 tests were interrupted by IC measurements of the creatinine having an average of 3 IC per run. Table 9 shows the mean, standard variation and the coefficient of variance of the test of the internal control's creatinine. The dispersion of the results was 3.8 % for creatinine which shows credibility & reliability of the methods used.

Table 7. Creatinine Internal Control Biochemical Results

Test	Mean±SD	CV
Cr	88.2±3.4	3.8

All means were reported as mg/dl.

4.   *Biochemical measurements*

Urine samples were defrosted prior to any mineral analysis. Urine specimens were homogenized using vortex then centrifuged using EPPENDORF Centrifuge 5810R at a speed of 3500 rpm for 10 minutes on $20^{\circ}C$.

Fluoride was analyzed using Fluoride Ion Selective Electrode (Thermo scientific, Beverly, MA 01915 USA).

Subsequently, the fluoride content of the urine samples collected was analyzed. In order to analyze for fluoride anions, dilution of equal volumes of urine with TISAB, (*total ionic strength adjustment buffer*) any fluorine-containing substances that can be metabolized to fluoride (F-) can be monitored using this procedure. Inorganic compounds of fluoride can be absorbed by the body, resulting in the excretion of fluoride ions as sodium fluoride. "Dietary and domestic water sources of fluoride must be considered, as well as dental treatments." This method is P & CAM 114 in a revised format. Other methods that have been used are those described in the NIOSH criteria documents on inorganic fluorides and hydrogen fluoride (Tolos, 1994)

Creatinine was measured using Vitros 350 analyzer (Ortho Clinical Diagnostics, Johnson and Johnson, 50-100 Holmers Farm Way, High Wycombe, Buckighamshire, HP 12 4DP, United Kingdom) in the NFSC department (AUB, Beirut, Lebanon).

Values are reported in mg/dl. The conversion from mg to mmol was done by dividing the values by the molecular weights.

Creatinine diffuses to the reagent layer, where it is hydrolyzed to creatine in the rate-determining step. The creatine is converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine, in the presence of sarcosine oxidase, is oxidized to

glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product. Following addition of the sample, the slide is incubated. During the initial reaction phase, endogenous creatine in the sample is oxidized. The resulting change in reflection density is measured at 2 time points. The difference in reflection density is proportional to the concentration of creatinine present in the sample.

5. *Fluoride to creatinine ratio*

Fluoride results are expressed in ratio of its concentration in spot urine to creatinine concentration as F/Cr in addition to the direct fluoride excretion values.

6. *Predictive values*

Predictive values of total creatinine per 24 hours (mg/d) were calculated based on the equation of Remer, Neubert, & Maser-Gluth (2002): $\log y = 0.0102x - 0.6854$ (y: 24 hour creatinine expressed in mmol/d; x: height expressed in cm) of which $R^2 = 0.87$ and $P < 0.0001$. Total F per 24 hours (mg/d) was estimated based on the predictive 24-hour creatinine values and the F/Cr ratio of concentrations in spot urine. Adequate F intake was calculated using the formula "0.05* body weight (mg/d)". Estimated F intake was calculated using the formula (estimated 24 h F excretion (mg/d)-0.03)/0.35 (Villa *et al.*, 2010).

F. Statistics

1. *Data entry*

Paper forms were used in the data collection process. Then, socio-demographic, anthropometric data were entered on the Statistical Package for the Social

Sciences (SPSS version 21.0). Biochemical results were entered primarily on Microsoft Office Excel 2007 and then copied back on SPSS. Thus, a double data entry process was applied in order to minimize the occurrence of errors. Data cleaning was then done to identify and correct all possible mistakes.

2.   *Statistical analysis*

Data were statistically analyzed using Mini-Tab 16 for Windows. Statistical significance was set at p-value < 0.05. Results were presented as means ± standard deviation. Data were stratified by gender or by 5 age groups (6-7, 7-8, 8-9, 9-10, and 10-11). Two-sample t-Test was used to identify statistically significant differences between genders. One way ANOVA with 95% of individual confidence interval evaluates differences among age groups. The subgroup analysis, differences among age groups, was performed using Fisher's test with 95% of confidence interval. Frequencies and descriptive statistics were performed for the different variables.

CHAPTER IV

RESULTS

The following chapter will detail the results of the study: subject characteristics, fluoride and creatinine concentration in spot urine, estimation of 24h urinary fluoride excretion and estimated total daily fluoride intake (TDFI) and their relationship to age, gender, body weight, BMI, school types and districts.

A.   Subject Characteristics

One thousand four hundred and three healthy children aged 6 to 10 years were recruited. This study on urinary fluoride status is part of a wider study investigating minerals status in Lebanese elementary school children (aged 6-10). There was not enough urine for 53 students and therefore 1350 subjects were tested. Five subjects were outliers since they were outside the 95 % CI (confidence interval): 3 had very low creatinine values and 2 had high fluoride values. Thus, 1345 children were included in the study.

The sample comprised 747 males with a mean age of 7.681±1.386 and 603 females with mean ±standard deviation (SD) age of 7.844±1.349. The subjects were divided in 5 age groups (6-7, 7-8, 8-9, 9-10 and 10-11) and distributed as follows: 314 subjects were 6-7 years old, 328 subjects were 7-8 years old, 291 subjects were 8-9 years old, 211 subjects were 9-10 years old, and 206 subjects were 10-11 years old. The mean ±SD weight, height, BMI and age of the entire sample were 28.34±7.92 kg, 127.14±9.77 cm, 17.24±2.75 kg/m^2, and 7.753±1.371 years respectively. Figure 2

illustrates that only 6% of the study population was underweight, the majority with a 65% normal and 29% overweight and obese.
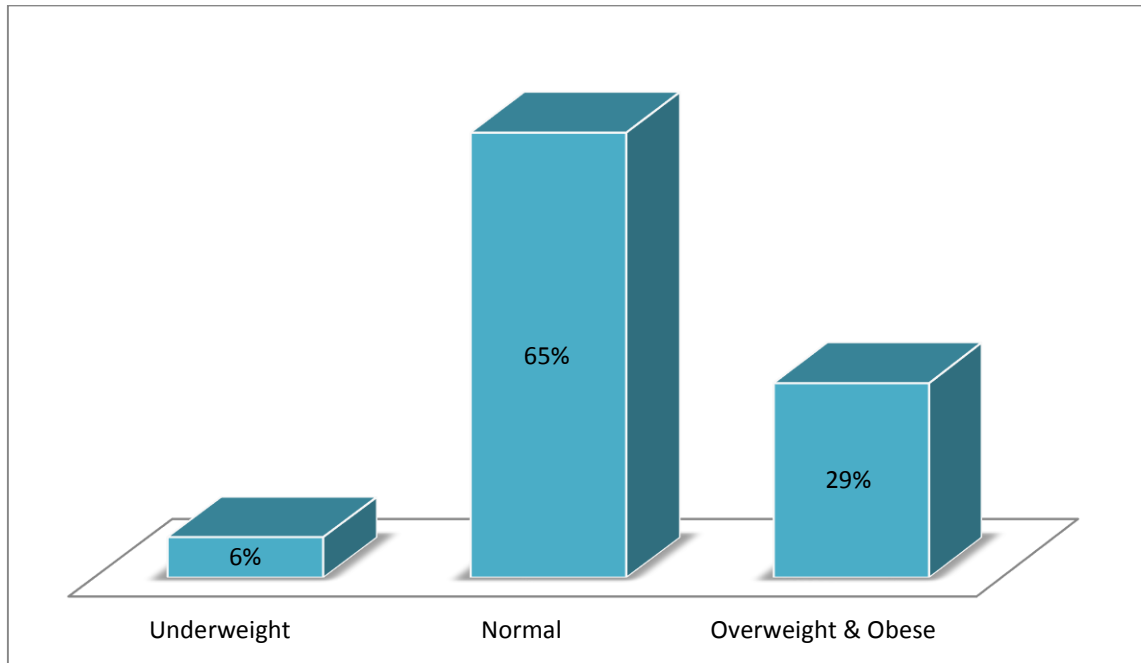
Figure 2. Nutritional Status of the Study Population.

Table 8 shows the data split by age group and gender. Table 9 shows that there was no sex related statistical significant difference for neither weight, nor height, nor BMI. But, the differences of weight, height, and BMI by age category were significant (P < 0.001) (table 14). As for BMI, 2 age categories (7-8 and 8-9) were not different. Schools and districts distributions strongly depended on the response rate.

Table 8. Anthropometric Characteristics of Study Population.

Age (years)	Sample		Weight (kg)	Height (cm)	BMI (kg/m^2)
	Gender	n			
6-7	M	195	22.52±4.08	117.04±5.80	16.36±1.97
	F	119	22.12±4.05	116.40±5.35	16.24±2.01
	T	314	22.37±4.07	116.80±5.64	16.31±1.98
7-8	M	180	26.56±4.97	124.61±6.06	17.01±2.28
	F	148	26.56±5.43	124.22±5.76	17.08±2.35
	T	328	26.56±5.17	124.44±5.92	17.04±2.31
8-9	M	150	28.64±6.14	128.20±5.66	17.29±2.59
	F	141	28.00±6.31	128.14±6.41	16.91±2.58
	T	291	28.33±6.22	128.17±6.02	17.10±2.59
9-10	M	113	31.68±7.43	132.91±6.03	17.79±3.10
	F	98	30.88±6.49	132.44±6.51	17.48±2.56
	T	211	31.31±7.00	132.69±6.25	17.64±2.86
10-11	M	109	36.61±9.70	139.16±6.59	18.75±3.97
	F	97	37.91±9.48	141.13±8.19	18.81±3.35
	T	206	37.22±9.60	140.08±7.43	18.78±3.68
Total	M	747	28.16±7.81	126.73±9.49	17.27±2.81
	F	603	28.55±8.06	127.65±10.09	17.22±2.68
	T	1350	28.34±7.92	127.14±9.77	17.24±2.75

 All the values in the table are represented as mean ±SD.

M, F, and T refer respectively to Male, Female, and Total.

n= sample size.

Table 9. Anthropometric Characteristics of the Study Population by Gender.

Variable	Males (n=747)	Females (n=603)	p-value
Weight	28.16±9.49	28.55±8.06	0.374
Height	126.73±9.49	127.6±10.1	0.089
BMI	17.27±2.81	17.22±2.68	0.727

All values are reported as mean ±SD.

n= sample size.

t-test is used aiming to compare genders and significance is set at p<0.05.

Table 10. Anthropometric Characteristics of the Study Population by Age.

Age (years)	**Anthropometric Characteristics**		
	Height (cm)	Weight (kg)	BMI (kg/m^2)
6-7	116.8±5.640[a]	22.37±4.066[a]	16.31±1.984[a]
7-8	124.4±5.920[b]	26.56±5.175[b]	17.04±2.306[b]
8-9	128.2±6.020[c]	28.33±6.219[c]	17.10±2.586[b]
9-10	132.7±6.250[d]	31.31±7.003[d]	17.64±2.863[c]
10-11	140.1±7.430[e]	37.22±9.598[e]	18.78±3.678[d]
p-value	<0.001	<0.001	<0.001

All values are reported as mean ±SD.

One way ANOVA is used to detect baseline characteristics significant differences between age groups. Significance is set at p<0.05.

The subgroup analysis is performed using Fisher Method. Means not sharing the same letter are significantly different.

B.   Fluoride analysis

1.   Fluoride excretion and intake analysis

The mean F excretion for the study population was found to be 0.027±0.024 mg/l, 0.170±0.162 mg/d or 0.006±0.006 mg/kg/d. While the mean Cr excretion and F/Cr, were found to be 474.7±113.7 mg/d and 0.372±0.357 mg/g, respectively (Table 11).

The mean estimated AI for the study population was found to be 1.416±0.396 mg/d while TDFI was found to be 0.399±0.462 mg/d and 0.015±0.018 mg/kg/d. (Table 12).

2.   Gender related differences

The mean F excretion of the study population in mg/l is significantly different being higher in males than in females. However when corrected through the ratio F/Cr to be estimated in mg/d it loses its significance (Table 11). Still mean F excretion (in mg/kg/d) and mean F/Cr (mg/g) are significantly higher in males than in females. The difference is not significant for Cr (mg/d).

The difference was not significant for means estimated AI and TDFI (mg/d) but significant for mean TDFI (mg/kg/d). Results show that mean TDFI (mg/kg/d) was significantly higher in males than in females (Table 12)

Table 11. Estimation of Fluoride and Creatinine Excretion in the Study Population by Gender

Variable	Mean ±SD			
	Total (n=1345)	Male (n=744)	Female (n= 601)	P value
F excretion (mg/l)	0.027±0.024	0.029±0.026	0.024±0.022	<0.001
F excretion (mg/d)	0.170±0.162	0.177±0.165	0.161±0.157	0.077
F excretion (mg/kg/d)	0.006±0.006	0.007±0.006	0.006±0.006	0.046
Cr excretion (mg/d)	474.7±113.7	469.2±107.5	481.5±120.7	0.051
F (mg)/Cr(g)	0.372±0.357	0.389±0.359	0.350±0.354	0.043

All values are reported as mean ±SD

The number presented between brackets is the sample size.

t-test is used comparing genders. Significance is set at p<0.05 for the difference between genders.

Table 12. Estimation of Fluoride Intake in the Study Population by Gender

Variable	Mean (±SD)			
	Total (n=1345)	Male (n= 744)	Female (n= 601)	P value
AI* (mg/d)	1.416±0.396	1.408±0.391	1.427±0.403	0.388
TDFI (mg/d)	0.399±0.462	0.419±0.471	0.375±0.449	0.077
TDFI (mg/kg/d)	0.015±0.018	0.016±0.018	0.014±0.017	0.048

Expected AI: expected intake of fluoride requirement (AI = 0.05 Body weight (mg/d))

All values are reported as mean ±SD

n= sample size.

t-test is used comparing genders. Significance is set at p<0.05 for the difference between genders.

3. *Age related differences*

Fluoride and creatinine excretion by age categories is presented in table 13. Fluoride excretion in mg/d, Cr excretion in mg/d and F/Cr increased significantly with age. There was no significant difference for fluoride excretion in mg/l and in mg/kg/d. Fisher method for subgroup analysis is also shown in table 13.

First, for the fluoride excretion in mg/d, there was no significant difference among the group of ages 6-7, 7-8, and 8-9. The same applies for the ages 8-9 and 9-10; as well as the ages 9-10 and 10-11. But the significant difference was between the age ranges of groups mentioned above except for the same age category shared among two groups.

Second, for the Cr excretion (mg/d) there was a significant difference between every age category. Third, there was a significant difference amid the age category 6-7 and all the other age groups except for the ages 7-8 and 8-9 concerning the F/Cr ratio (mg/d)

Table 14 shows the estimated AI in mg/d and TDFI in mg/d and mg/kg/d by age categories. The estimated AI and TDFI in mg/d increased significantly with age. This was not the case of TDFI in mg/kg/d. Fisher method for subgroup analysis is as well shown in table 14. It illustrates a significant difference between all the age categories for the adequate intake (mg/d). Regarding the TDFI in mg/d, the ages 6-7, 7-8 and 8-9 has no significant difference. The age categories 8-9 and 9-10; as well as 9-10 and 10-11, have no significant difference.

Table 13. Age Related Difference of F Excretions of the Study Population

Age	n	F excretion			Cr excretion	F/Cr
		mg/l	mg/d	mg/kg/d	mg/d	mg/g
6-7	314	0.026±0.021	0.152±0.123[a]	0.007±0.006	365.9±50.23[a]	0.420±0.333[a]
7-8	327	0.025±0.027	0.155±0.162[a]	0.006±0.007	438.1±61.07[b]	0.367±0.399[a b]
8-9	290	0.026±0.024	0.168±0.184[a b]	0.006±0.007	478.5±69.21[c]	0.359±0.389[a b]
9-10	209	0.029±0.025	0.193±0.165[b c]	0.006±0.006	532.3±79.87[d]	0.371±0.326[b]
10-11	205	0.029±0.023	0.198±0.170[c]	0.006±0.005	635.5±111.3[e]	0.324±0.294[b]
P-Value		0.257	0.02	0.216	<0.001	0.041
Total	1345	0.027±0.024	0.170±0. 162	0.006±0.006	474.7±113.7	0.372±0.357

All values are reported as mean ±SD

n= sample size.

One way ANOVA is used to detect F and Cr excretions differences between age groups. Significance is set at p<0.05.

The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

Table 14. Age Related Difference of F Intake of the Study Population

Age	n	AI	TDFI	
		mg/d	mg/d	mg/kg/d
6-7	314	1.118±0.203[a]	0.349±0.351[a]	0.016±0.016
7-8	327	1.328±0.259[b]	0.358±0.463[a]	0.014±0.019
8-9	290	1.417±0.311[c]	0.395±0.525[a b]	0.015±0.020
9-10	209	1.563±0.349[d]	0.467±0.473[b c]	0.016±0.016
10-11	205	1.862±0.481[e]	0.480±0.485[c]	0.014±0.015
P-Value		<0.001	0.002	0.598
Total	1345	1.416 ±0.396	0.399 (±0.462)	0.015±0.018

All values are reported as mean ±SD

n= sample size.

One way ANOVA is used to detect AI and TDFI differences between age groups. Significance is set at p<0.05.

The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

4. Schools

Fluoride excretion, F/Cr and TDFI by school types are shown in table 15 and 16. All values are significantly different among school types showing higher excretion and thus higher TDFI in public schools. Fisher method for subgroup analysis is as well shown in table 15 and 16. It shows that there is neither significant difference between the private and private free schools for fluoride excretion in all the units mentioned in the table 15 (mg/l, mg/d and mg/kg/d), nor for the F/Cr (mg/g), nor for the TDFI in mg/d or in mg/kg/d).

There was a significant difference in the public schools compared to the other school types. The values reported for the public schools, in each of the variables in tables 15 and 16, are even double or more than double the values of the private and private free schools. The p value is <0.001 for each of the variables analyzed by school types.

Table 15. F Excretion in Different School Types of the Study Population.

Schools	n	F excretion			F/Cr
		mg/l	mg/d	mg/kg/d	mg/g
Public	676	0.037 ± 0.029^a	0.232 ± 0.193^a	0.009 ± 0.007^a	0.520 ± 0.433^a
Private	445	0.017 ± 0.012^b	0.115 ± 0.097^b	0.004 ± 0.003^b	0.229 ± 0.168^b
Private free	224	0.017 ± 0.009^b	0.093 ± 0.046^b	0.003 ± 0.002^b	0.211 ± 0.111^b
P value		<0.001	<0.001	<0.001	<0.001

All values are reported as mean ±SD

n= sample size.

One way ANOVA is used to detect F excretion and F/Cr differences between school types. Significance is set at p<0.05.

The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

Table 16. F Intake in Different School Types of the Study Population.

Schools	n	TDFI	
		mg/d	mg/kg/d
Public	676	0.576 ± 0.552^a	0.022 ± 0.215^a
Private	445	0.242 ± 0.277^b	0.008 ± 0.009^b
Private free	224	0.181 ± 0.130^b	0.007 ± 0.005^b
P value		<0.001	<0.001

All values are reported as mean±SD

n= sample size.

One way ANOVA is used to detect TDFI differences between school types. Significance is set at p<0.05.

The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

5. *Districts*

Fluoride excretion, F/Cr and TDFI by districts are shown in table 24 and 25. All values are significantly different among districts. Fisher method for subgroup analysis is as well shown in table 24 and 25. F excretion (mg/d) and TDFI (mg/d) are significantly higher in Akar, Hermel and North districts than in Bekaa which is significantly higher than in Beirut, Nabatiye and South, which are significantly higher than in Mount Lebanon.

Akar and North share similar values and are not significantly different regarding the F excretion in mg/l, and mg/d, also regarding the TDFI in mg/d. Conversely, this is not the case when the unit is mg/kg/d for the F excretion and TDFI nor when the value analyzed is the ratio F/Cr. This is typically similar to the South and Nabatiye districts.

Table 17. F Excretion in Different Lebanese Districts of the Study Population.

Districts	N	F excretion			F/Cr
		mg/l	mg/d	mg/kg/d	mg/g
Akar	150	0.042±0.028[a]	0.281±0.200[a]	0.012±0.008[a]	0.689±0.476[a]
Beirut	209	0.021±0.017[c]	0.125±0.158[c]	0.005±0.006[d]	0.286±0.358[d]
Bekaa	166	0.023±0.014[b c]	0.192±0.122[b]	0.006±0.004[c]	0.360±0.213[c]
Hermel	49	0.029±0.018[b]	0.273±0.188[a]	0.010±0.006[a b]	0.597±0.361[a b]
Mount Lebanon	158	0.012±0.008[d]	0.072±0.033[d]	0.002±0.001[f]	0.151±0.071[f]
North	243	0.044±0.030[a]	0.264±0.200[a]	0.010±0.007[b]	0.574±0.415[b]
Nabatiye	191	0.020±0.027[c]	0.107±0.084[c]	0.004±0.003[d e]	0.253±0.205[d e]
South	180	0.021±0.015[c]	0.107±0.059[c]	0.003±0.002[e f]	0.204±0.118[e f]
P value		<0.001	<0.001	<0.001	<0.001

All values are reported as mean ±SD

n= sample size.

One way ANOVA is used to detect F excretion and F/Cr differences between districts in Lebanon. Significance is set at $p < 0.05$.

The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

Table 18. F Intake in Different Lebanese Districts of the Study Population.

Districts	N	TDFI	
		mg/d	mg/kg/d
Akar	150	0.717±0.572[a]	0.030±0.023[a]
Beirut	209	0.271±0.453[c]	0.010±0.017[d]
Bekaa	166	0.462±0.347[b]	0.015±0.011[c]
Hermel	49	0.695±0.537[a]	0.026±0.019[a b]
Mount Lebanon	158	0.119±0.094[d]	0.004±0.003[f]
North	243	0.668±0.571[a]	0.025±0.021[b]
Nabatiye	191	0.221±0.240[c]	0.009±0.009[d e]
South	180	0.220±0.170[c]	0.007±0.005[e f]
P value		<0.001	<0.001

All values are reported as mean±SD

n= sample size.

One way ANOVA is used to detect TDFI differences between districts in Lebanon. Significance is set at $p < 0.05$.

The subgroup analysis is performed using Fisher Method. Categories not sharing the same letter are significantly different.

6. *Comparison between TDFI and AI of the study population.*

Figure 3 shows the distribution of TDFI (mg/d) of the study population. The higher percentage of distribution is reported below 1.2 mg/d of TDFI. Figure 4 shows the distribution of TDFI (mg/kg/d) compared to the AI set by the Institute of Medicine (IOM, 1997) which is 0.05 mg/kg/day for infants beyond 6 months of age. (USDA, chapter 8, 2010).

The frequency of the study population consuming more than 0.05 mg/kg/d is 4.7% thus 95.3% consume less than this recommendation as illustrated in figure 4.The majority of the Lebanese elementary school children aged 6-10 receive a total daily fluoride intake lower than the recommended. The line drawn on figure 11 separates the two categories of TDFI lower and higher than the AI. It illustrates visually this finding.
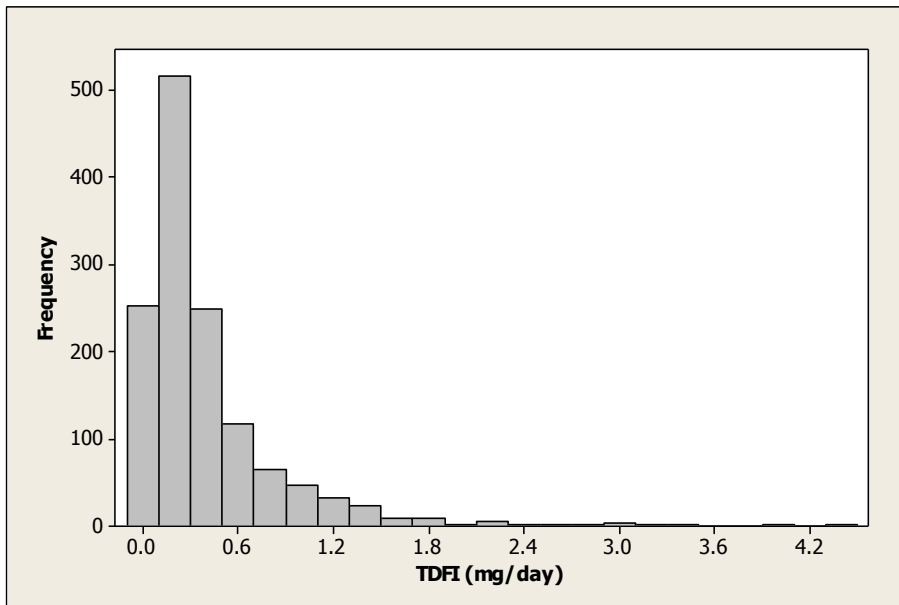
Figure 3: Histogram representing the distribution of total daily fluoride intake (TDFI) in mg/d of the study population
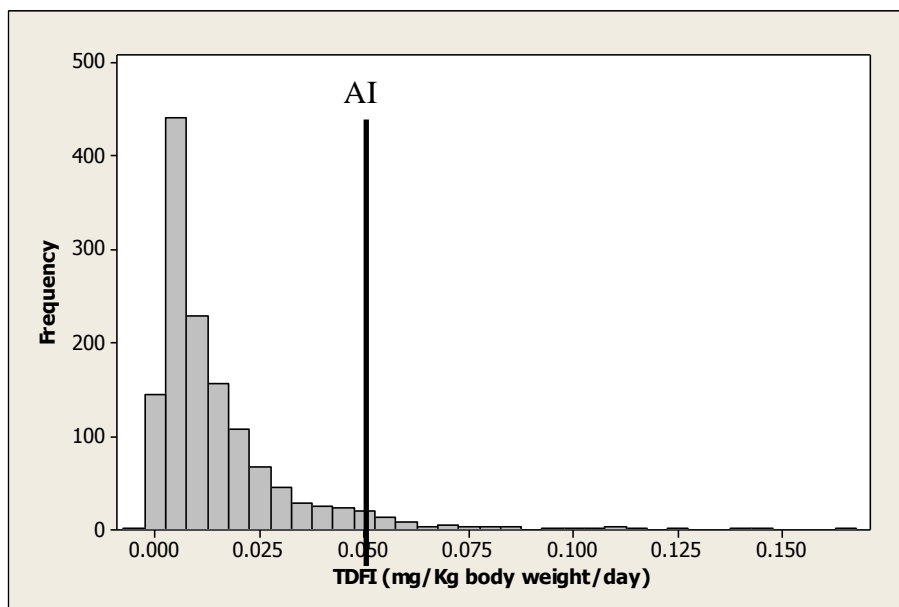

Figure 4: Histogram representing the distribution of total daily fluoride intake (TDFI) in mg/kg/d of the study population compared with the adequate intake (AI) for this age range which is 0.05mg/kg/d.

CHAPTER V

DISCUSSION

The present national study was intended to be representative of the Lebanese population aged 6-10 years. The mean weight (28.16 kg for males and 28.55 Kg for females), height (126.73 for males and 127.6 cm for females) and BMI (17.27 kg/cm^2 for males and 17.22 kg/cm^2 for females) of the children in the present study were similar to the weight, height and BMI values reported for 3636 Lebanese children in the same age group: 29.61 Kg for males and 28.85 Kg for females; 128.44 cm for males and 127.50 cm for female; 17.58 cm/kg^2 for males and 17.52 cm/kg^2 for females during the year 2009. (Nasreddine *et al*., 2012). Weight, height, and BMI increased with age as normally expected but it did not differ significantly between genders.

Only 6% of the study population was found to be underweight, the majority (65%) were normal and 29% overweight and obese, showing a normally distributed population (figure 2).

Concerning the assessment method chosen, assessing dietary intake at the population level needs more considerations than assessing it on an individual level. Several factors should be included to choose the type of dietary assessment tool, including how detailed, population-specific and expensive the tool will be for the purpose of the study (Subar, 2004).

In the present study urinary biomarker was used to measure fluoride excretion in Lebanese elementary school children aged 6 to 10 years in order to then estimate their fluoride intake.

Urinary fluoride assessment method and others such as plasma and serum levels of fluoride were proved to be useful biomarkers for fluoride exposure (R. Liteplo

et al, 2002). Even more methods have been suggested to assess fluoride intake like using food questionnaires; saliva; sweat and milk; bone and dentine; enamel; hair and nails levels of fluoride.

However, there is a high probability of recall biases when food questionnaires are used to assess food intake. In addition, the food sources section in the literature review highlighted that fluoride content of edible items differ on a wide scale from place to another depending on the contents of the soil and water used for irrigation (Dhar *et al*., 2009). Thus to assess the intake, measuring a biological marker is better than assessing the intake from questionnaires or based merely on food sources content.

Furthermore, serum and plasma assessment methods are invasive. This is particularly important to consider when dealing with children. This study meant to reach a national level and recruited a big sample size. Hence, using an invasive method such as plasma fluoride content assessment would not be feasible nor accepted by parents or responsible of the selected children.

Moreover, technical and ethical limitations, as well as limited information on reliability of the other methods of assessment, as discussed in the literature review, made them less adequate to be chosen for our study. (Liteplo *et al*., 2002)

Finally, urine collection is a cost effective, practical, and reliable way to reach the goal needed with the large sample size of this study. Twenty four hours urine collection is the classical method for urinary assessment, since urinary concentrations of various substances like fluoride are prone to variation throughout the day, and their concentration in a spot urine sample measured may not be representative of 24-h urinary fluoride excretion. Many studies adopted the 24-h urine collection in their methodology as a way to assess the fluoride such as some studies done in UK by Zohoori *et al.* and by

Maguire *et al*., in Venezuela by Franco *et al*., in Brazil by Soares *et al*, in Germany by Haftenberger *et al*, in Chile by Villa *et al* and also in 6 European countries by Ketley *et al*. (Zohouri *et al*, 2006- Zohoori *et al*, 2012- Maguire *et al.*, 2007- Franco *et al*, 2009- Soares *et al*, 2008- Haftenberger *et al.*, 2001- Villa *et al*., 2000- Ketley *el al*, 2004). However, spot urine samples are easier to be collected and more cost effective in large scale studies. Therefore urinary creatinine concentration was found as a solution to estimate the 24-h excretion rates of certain substances including fluoride from spot urine. The ratio of the substance studied to creatinine in spot urine is determined. Estimation of 24 h creatinine excretion from weight is used to deduce 24 h excretion of the concerned substance. Twenty four hours urinary creatinine output was proved to be relatively constant during the day in healthy subjects and proportional to weight. Consequently it is a useful method to help correct urinary dilution of substances. Zohouri *et al*., evaluated the representativeness of the F/Cr ratio in a spot urine sample to the fluoride content of a 24-h urinary sample in children. They suggested that this ratio can be used for estimating 24-h urinary fluoride excretion which is needed in large scale studies of children. (Zohouri *et al*., 2006). Relying on those results, spot urine was chosen as a cost effective assessment tool in this study. Other recent studies used the spot urine method of assessment as well. (Hoyos *et al*, 2014-Shaoxian *et al*, 2013- Akpata *et al,* 2013).

Villa *et al*. designed a study in order to examine the relationship between total daily fluoride intake (TDFI), daily urinary fluoride excretion (DUFE) and fractional fluoride retention. They analyzed many recent published studies that simultaneously measured the TDFI and DUFE for the relationship between these two variables. Their results showed that there is a strong linear relationship between TDFI and DUFE but

with different slopes for young children and adults. Their findings derived a formula for the estimation of average TDFI in children and adults from average DUFE values on a community basis and not on an individual basis. Therefore, this study adopted their formula to estimate the TDFI of the Lebanese elementary school children. (Villa *et al*, 2010)

Results showed that the mean F excretion is 0.170±0.162 mg/d or 0.006±0.006 mg/kg/d and that the mean TDFI is 0.399±0.462 mg/d or and 0.015±0.018 mg/kg/d. These were similar to the same variables found by a study done recently in Kuwait which found 0.128-0.220 mg/d for the mean F excretion and 0.013±0.018 mg/kg/d for the mean TDFI (Akpata *et al*., 2013).

Conversely, the Lebanese values are lower than the values reported for as Venezuela, Germany, Chile, UK and Texas. For instance, TDFI mean in Venezuela is 1.308± 0.474 mg/d or 0.08±0.03mg/kg/day (Franco *et al.*, 2009); in Germany is 0.931±0.391mg/d or 0.053 ±0.021 mg/kg/d (Haftenberger *et al*., 2001) and in Chile is 1.02 mg/d or 0.064±0.015 mg/Kg/d (Villa *et al*., 2000). As for the UK, one study was conducted by Zohoori *et al* in 2006 resulted in 0.71±0.41 mg/d or 0.05±0.02 mg/kg/d as a mean TDFI, while in 2012 another study by Zohoori *et al.* included a low F area and a natural F area having 0.945±0.621 mg/d and 0.038±0.027 mg/kg/d for mean TDFI in the low F area. It should be noted that the TDFI mean in the present study is even lower than its mean in the low F area in the latter study. (Zohouri *et al*., 2006- Zohoori *et al*, 2012). This is also the case comparing to Maguire et al study on TDFI in UK involving an optimally fluoridated area, a sub-optimally fluoridated area and a non- fluoridated area; TDFI means were respectively in the three areas: 1.043±0.220 mg/d or 0.047±0.008 mg/kg/d; 0.883±1.011 mg/d or 0.038±0.038 mg/kg/d; and 0.736±0.533

mg/d or 0.031±0.025 mg/kg/d. (Maguire *et al*., 2007). In addition, F excretion in the present study is lower than the value found in a study done in Texas. (Baez *et al*, 2000) Although F in natural water in Venezuela and Germany is not higher than in the Lebanese natural water sources, the studies done in these 2 countries found toothpaste and fluoride tablets respectively as the main sources of the F intake (Franco *et al.*, 2009-Haftenberger *et al*., 2001). The fluoride in Texas natural water ranges between 0.1 and 3.2 mg/l which may explain the higher F excretion in this study. (Baez *et al*, 2000)

Furthermore, Lebanese natural water sources were studied in different regions in Lebanon to determine the fluoride concentration in mg/l. The result of this investigation showed that the water concentration range in Beirut district was 0.10-0.18 mg/l, in Bekaa & Hermel districts was 0.00-0.54 mg/l, in Mount Lebanon was 0.05-0.83 mg/l except for Sibline water at Barouk which was 2.50 mg/l, in the North & Akkar districts was 0.00-0.60 mg/l, and in the South & Nabatiyeh districts was 0.10-0.94 mg/l. (Doumit *et al,* 2004). The optimal level of fluoride was established by the WHO at 1 mg/liter and this level was related to low prevalence of dental caries (WHO, 2000). So, almost all the natural water in Lebanon was considered low in fluoride. Concerning bottled water, fluoride is often under this limit too; some examples are Tannourine containing <0.2 mg/l of fluoride, Sohat water 0.01 mg/l of fluoride, and Rim 0.1 mg/l.

On the other hand, a Lebanese study has found a high percentage of tea consumption among families and children under 2 years of age (100.0% of urban families and 89.4% of rural families, 83.1% vs 57.1% of children). Another study evaluating fluoride content of Lebanese tea found values of 0.620 to 1.680 mg/l (Jurdi *et al*., 2001). This suggests a considerable proportion of fluoride intake from tea. But we cannot deny the famous cultural higher tea consumption in European countries, and

particularly in UK. This might be partly explained by the cold weather for longer periods of the year and could account for the higher TDFI values cited above.

Figure 4 illustrates the distribution of total daily fluoride intake (TDFI) in mg/kg/d of the study population compared with the adequate intake for this age range which is 0.05 mg/kg/d. It showed that the frequency of the study population consuming more than the AI is 4.7% and 95.3% consume less than this recommendation. Although these findings show a low fluoride level of intake, this might reflect only the intake from the drinking water that was already shown as mostly low in Lebanon. In fact, a correlation between fluoride concentration in drinking water and fluoride concentration in morning urine is suggested by some studies (Saleh *et al* 2000, Grimaldo *et al* 1995). Regression analysis in the study by Grimaldo *et al* showed an increment of 0.54 ppm (p<0.0001) of fluoride in urine for each ppm of fluoride in water. Grimaldo et al also noted that fluoride urinary levels were higher in samples collected during the afternoon (18:00) when compared with sample collected during the morning (11:00). Those values on spot urine were not corrected for creatinine level, so the difference can be simply due to difference in urine concentration. It might however note a possible variation of fluoride excretion during the day independent of urine concentration effect linked to dietary intake.

Concerning gender related difference, TDFI (mg/kg/d) was significantly higher is males than in females although AI (that takes weight into consideration) was not higher and there it loses its significance. An Indian study in 2010 found also higher incidence of dental fluorosis in males than in females. (Choubisa *et al*, 2010) This finding is different from a study conducted at Kuwait in 2013 which found no statistical difference between genders. (Akpata *et al*, 2013).

As for age related differences, TDFI (mg/d) increased significantly with age whereas TDFI (mg/kg/d) didn't increase significantly with age. This would imply that increasing weight with age is correlated to the increasing intake. This is similar to the results of Akpata *et al.* since they found that mean total fluoride ingestion per kilogram was the same among the children aged 4–6 and 7–9 years, but was lower at age 1–3 years. (Akpata *et al*, 2013)

Analyzing the differences among school types, public schools students had significantly higher TDFI than private and private free schools. This reflects the findings of Akpata *et al*. in Kuwait as well, which pointed at the lower socioeconomic status association with a higher fluid consumption in Children in this country. The authors suggested that higher socioeconomic status was associated with longer hours in air-conditioned rooms and thus lower fluid consumption. Diet habits might also differ between socioeconomic categories, with more tea consumption possibly in low income classes. Fluoride mouth wash policy applied in public schools must not account for this difference since the urinary sampling was done on another day than the mouth wash and the latter is supposed to affect urinary fluoride after a couple of hours (García-Hoyos *et al*, 2014), but will be mostly cleared by renal excretion within 24h.

Finally, the districts distribution showed that F excretion (mg/d) and TDFI (mg/d) are significantly higher in the Northern districts than in Bekaa which is significantly higher than in Beirut, and South districts, which are also significantly higher than in Mount Lebanon. This is different than the study done in Lebanon in 2004 (Doumit *et al* 2004) where the F excretion is highest is Beirut, followed by South, than North and Mount Lebanon and lowest in Bekaa, noting that statistical significance was not specified in their case. Regular follow up of fluoride intake in districts might be

necessary to avoid fluorosis since it seems to change dynamically with time, in case any supplementation policy is to be started.

Numerous studies done in industrialized and developing countries have shown an increase in the prevalence of dental fluorosis in populations from communities with and without water fluoridation (Pendrys *et al*, 1990). This suggested that the threshold of fluoride exposure in order to maximize caries prevention while minimizing the potential risk of dental fluorosis has been exceeded. Reaching the best balance between caries reduction and the avoidance of dental enamel fluorosis is of critical importance to public health planners especially that risk factors of high level of fluoride intake are seriously dangerous. It was reported by the European Food Safety Authority (EFSA) that there are no identified signs of fluoride deficiency in humans. EFSA considered the relationship between fluoride intake and the growth of infants and observed that there is no enough evidence to prove a causal relationship among them. It was as well mentioned that fluoride may decrease the susceptibility of enamel to acid attacks after eruptions, however caries is not a fluoride deficiency disease and fluoride is not an essential nutrient. (Agostoni *et al*., EFSA, 2013)

Several health systems and different factors may have an impact on dental caries prevalence. Fluoride is one of these factors that has been used and has a role in the prevention of dental caries in both systemic and topical ways. Toth *el al* demonstrated an increase in fluoride concentration of saliva after ingestion of fluoridated milk, salt or tablets. (Toth *et al*., 2005). A study found that the use of topical fluoride and fluoridated salt are negatively associated with dental caries occurrence (Schulte *et al*, 2001). However, salt fluoridation was not effective in the setting of another study which included the same age category and done in a similar area. Total dental caries proximal

progression rate was the same whether salt fluoridation intervention was applied or not. (Wennhall *et al*, 2014).

In addition, dental caries experience was association with preventive behaviors of children like tooth brushing and oral hygiene. Supervised tooth brushing and parental knowledge about fluoride in toothpastes was significantly correlated with lower dental caries incidence while systemic fluoride use was poorly related to dental caries. (Tubert-Jeannin *et al*, 2008). For instance, better oral hygiene in a never fluoridated area was suggested to be the cause of a less caries incidence among its children (Cho *et al*., 2014).

Dental caries is the net result of successive cycles of de- and remineralization of dental tissues at the interface between the biofilm and the tooth surface. Fluoride was found to be not an essential nutrient. It is more effective to be used topically than systemically in order to protect against dental caries; when it is ingested, it was emphasized that despite being classified as a "systemic" method of fluoride delivery, the mechanism of action of fluoridated water to control caries is mainly through its topical contact with the teeth while in the oral cavity or when redistributed to the oral environment by means of saliva. The high frequency of contact of fluoride present in the water with the tooth structure or intraoral fluoride reservoirs, especially that fluoridated water is consumed many times a day, helps to explain why water fluoridation is so effective in controlling caries, despite having fluoride concentrations much lower than fluoride toothpastes, for instance (Buzalaf *et al*., 2011).

Furthermore, caries incidence is influenced by sugar consumption. The quantitative relationship between sugar intake, the progressive development of dental caries and fluoride use were studied. It was done analyzing other studies on a global

level, with emphasis on marked differences in both national sugar intake and fluoride use and where one factor such as sugar intake changed progressively without changes in other factors over a decade or more. It was highlighted that the much greater adult burden of dental caries indicates the need for very low sugar intakes throughout life like 2–3% of energy intake, whether or not fluoride intake is optimum. (Sheiham *et al*., 2014). Two other studies done on Italy and UAE considered the point that good oral hygiene and restriction in sugar consumption have been associated with dental caries reduction. These two studies concluded that that frequency of sugar intake, snacking frequency in between meals and socio-economic status may play an important role in caries prevalence among children (Rehman *et al*., 2008- Petti *et al*., 1997).

On the Lebanese public health planning level, the Lebanese ministry of health issued the law 178 in year 2011 that consists of adding the potassium fluoride (FK or $KF_2 H_2O$) to table or cooking salt that is imported or manufactured in Lebanon. This law has not been applied yet in the country. On another hand, the Lebanese ministry of education with a medical staff and professionals sent a circular to all public Lebanese schools to have a well-organized dental care follow up for every student as well as a fluoride mouth wash applied in every public school all over Lebanon. In general, dental health of private and private free schools students is expected to be followed up by their parents through medical supervision; they were not included in this decision done by the Lebanese ministry of education. This study was designed to assess urinary fluoride levels in Lebanese elementary school children aged 6 to 10 years old, aiming to estimate their fluoride intake since it has not been evaluated before on a large scale.

The limitations of this study include first the estimation of intake from spot urine. F/Cr ratio was used to correct the dilution of urine that might change during the

day but there are other possible modifying factors that might affect urinary fluoride clearance. In fact, this clearance increases with urine pH due to a decrease in the concentration of hydrogen fluoride. Numerous factors (e.g. diet and drugs) can affect urine pH and thus affect fluoride clearance and retention (Fawell *et al*., 2006). Moreover dietary modification from day to day can account for daily variation in urinary fluoride excretion. In order to eliminate such modifying factor, more than 24 h urine collection would have been necessary which is very hard to achieve in young children and in epidemiological large scale studies.

Whereas the strength of this study is its big sample size on a national level, distributed on all districts. A strength also is that school children in Lebanon are representative of the whole pediatric population in this age category since the average enrollment rate of children in the elementary school was 93 % in 2012.

Many studies evaluated the risk-benefit balance of fluoride exposure providing evidence to assist in the formulation of appropriate guidelines for fluoride use. In addition, the topical use of fluorides such as the fluoride toothpastes, mouth rinses, gels, and varnishes has gained greater popularity than the systemic use of fluorides like the use of fluoride tablets and the addition of fluoride to drinking water. Fluoride toothpaste was the most widespread form of topical fluoride usage. (Wong *et al*, 2011) Topical fluoride seemed to be more effective and safer than systemic intake in tooth decay prevention. (Limeback *et al*, 1999- García-Hoyos *et al*, 2014). It is important then after assessing the status of elementary school Lebanese children to consider all the sources that might be available through natural ways or supplementations. Potential sources of fluoride for Lebanese children might be mainly toothpastes, mouth wash and tea

sources; these may increase the fluoride intake estimation by their contribution in

children intake at this age in the Lebanese population.

CHAPTER VI

SUMMARY AND CONCLUSION

The results of the present study provided the fluoride status of the elementary school children aged 6-10 years on the national level. It seemed that the fluoride intake in the majority was not reaching the recommended level. This was similar to the Kuwait study findings and lower than European and American studies values. However, Lebanese water sources were found to be as well lower in fluoride than other countries and water is one of the main sources of fluoride. The topical use of fluoride was more spread recently than the systemic use of fluoride and both methods must be well planned for prior to use on a national level. This study adopted a feasible and effective method for fluoride assessment being a large scale study. The findings of this study can provide the basis for public health planning; more specifically these can lead to recommending and providing mandatory or optional ways of fluoride supplementation for the Lebanese population especially in the age range studied.

Moreover and since the kidneys play the major role in the removal of fluoride from the body, some consequences will be expected in case of renal malfunction or impaired kidneys. So, if supplementation policy is obligatory it may put people with impaired renal function at risk of fluorosis.

Lastly, fluoride status in the Lebanese population must be followed up with the dietary changes occurring and after any intervention. This would help provide a better protection against dental caries while avoiding an increase in fluorosis cases.

APPENDIX I

LEBANESE MINISTRY OF EDUCATION: ORGANIZED DENTAL CARE FOLLOW UP & MOUTH WASH DECISION IN LEBANESE PUBLIC SCHOOLS

الجمهورية اللبنانية
وزارة التربية والتعليم العالي
المديرية العامة للتربية

تعميم رقم........١٨٦

حول استكمال تنفيذ الحملة الوطنية للوقاية من امراض الفم والاسنان .

عطفا على التعميم رقم 55/م/2005 تاريخ 2005/11/19، الذي يتعلق بتنفيذ الحملة الوطنية للوقاية من امراض الفم والأسنان لتلامذة المدارس الرسمية.

واستكمالاً للحملة الوطنية للوقاية من أمراض الفم والأسنان تقوم اللجنة الوطنية لرعاية صحة الفم والأسنان عبر فريق من أطباء الأسنان المعتمدين، بالكشف الميداني على أسنان تلامذة المدارس الرسمية،
لذلك ،

وحرصا" على صحة التلامذة وحمايتهم من أمراض الفم والأسنان، نؤكد على جميع المسؤولين عن المدارس الرسمية للتزام ما يلي:

- الزامية الكشف على الاسنان لجميع تلامذة التعليم الاساسي بما فيها رياض الاطفال وتسهيل عمل الأطباء المعتمدين ومساعدتهم على القيام بمهماتهم.

- ضرورة البدء بتنفيذ الكشف الطبي على الاسنان فور استلام اللوائح الاسمية بالأطباء المعتمدين للسنة الدراسية 2012-2013، وذلك عبر الاتصال بطبيب الاسنان المعتمد او المؤسسة الداعمة وفق اللوائح المرفقة بهدف اجراء الكشف والحصول على توقيع الطبيب على التعهد المرفق بالتعميم 55/م/2005، على ان يصار الى المبادرة الى اعلام الارشاد والتوجيه وحدة التربية الصحية مباشرة في حال تعذر الاتصال بالطبيب لتأمين البديل وفق الاصول.

- تنفيذ عملية المضمضة بمحلول الفليور لتلامذة الحلقة الاولى من التعليم الأساسي حفاظا على سلامة التلامذة ووقاية أسنانهم من التسوس والأمراض.

- الطلب الى المرشد الصحي في المدرسة تنفيذ المهمات التالية :
أ. ملء الاستمارات المطلوبة قبل حضور الطبيب.
ب- تحديد موعد بدء الحملة بالتنسيق مع الإدارة والطبيب.
ج - تحديد موعد جملة التوعية للأهل وتوزيع استمارات نتائج الكشف الطبي عليهم (النسخة البيضاء) بالتنسيق مع الإدارة والطبيب.
د- القيام بحملة إعلامية حول الموضوع في المدرسة (الهيئة التعليمية والتلامذة بالتنسيق مع طبيب الأسنان)، وإعلامهم بمواعيد الكشف مسبقا والاحتفاظ بنسخة عن نتيجة الكشف لإعداد التقارير النهائية وضمها الى الملف الصحي الشخصي لكل تلميذ.
ذ- ارسال نتائج الكشف على الاسنان إلى مكاتب التربية الصحية الفرعية في المناطق في مهلة أقصاها نهاية آذار.

إن المديرية العامة للتربية، اذ تعلق اهمية كبرى على دور مديري مدارس رياض الأطفال ومرحلة التعليم الأساسي في الاهتمام بصحة التلامذة من خلال الخدمات الصحية المختلفة، وتحليهم بأعلى درجات المسؤولية، تؤكد ضرورة التقيد بما ورد أعلاه على ان يقدم الارشاد والتوجيه التقارير الدورية اللازمة بالمشاكل التي تعيق تنفيذ الكشف الطبي بهدف المتابعة والمساءلة والمحاسبة.

المدير العام للتربية
فادي يرق

بيروت في: ٢١ كانون٢ ٢٠١٢

- الموقع الالكتروني

APPENDIX II

CONSENT FORM (ENGLISH)

Permission for Child to Participate in Research
AUB

Study Title: The assessment of urinary iodine status of children in Lebanon
Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Co-Investigator: Dr. Hala Ghattas/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut
Researchers: Dareen Shatila/
Address : American University Beirut, Cairo Street, Hamra, Beirut – Lebanon/01 – 350000
Where the study will be conducted: Schools all over Lebanon (private and governmental schools)

This is a permission form for your child/child for whom you are legal guardian to participate in a research study. It contains important information about this study and what to expect if you decide to permit your child/child for whom you are legal guardian to participate.
Your child's participation is voluntary.
Please consider the information carefully before you decide to allow your child to
participate. If you decide to permit participation, you will be asked to sign this form and will receive a copy of the form.

A. Purpose of the Research Study: Iodine is an essential trace mineral that plays an important physiological role in the body. In Lebanon, endemic goiter was previously reported to be a serious public health problem with the greatest incidence occurring in the high mountain valleys. Currently, Lebanon is still identified as a country with mild to moderate iodine deficiency. Salt iodination was partially initiated in 1992 and was implemented in the year 1995 in a uniform manner by the Ministry of Health. No comprehensive study was conducted to determine urinary iodine status of schoolchildren since the introduction of salt iodination (1992-1995). Therefore it is essential to conduct a study in order to determine the iodine status in children. This would be of importance to determine the success of the salt iodization program and to determine whether the level of iodination is sufficient. Recent data regarding iodine status exists for Lebanon is lacking. Thus, the objectives of the following study are to: assess the prevalence of IDD and ascertain the extent and severity of the problem and identify high-risk areas.

B. Project/Procedures Description: Subjects' recruitment will be done by direct approaching to the school director. The selected children from each school will be given consent forms and questionnaires to share it with their parents for approval and filling the questionnaire once approved. Children of signed consent forms will be recruited afterwards to collect the urine samples needed for this research.
Urine samples will be collected for the measurement of urinary <u>iodine, sodium, potassium fluoride, phosphorus, magnesium, calcium and creatinine</u> concentrations. The assessment of iodine status will include concurrent assessment of household use of iodized salt through surveys that will be filled by parents. These surveys will also include information regarding the socioeconomic status of the participants.

In this study subjects will be asked to maintain their regular dietary and physical activity habits during the entire study course, as well as any unusual strenuous exercise 24 hours prior to the study. Exclusion criteria include: any significant medical diseases and subjects out of the age range (5-10years).

Researchers will go to schools where: anthropometric measurements (height, weight) will be taken, a socio-demographic questionnaire will be collected after being filled from the children's parents or legal guardian and a sample of urine will be collected in special tubes.

This study is a randomized study and a total of 1500 schoolchildren (age between 5 to 10 years) would be required; from 35 schools recruited randomly from all over Lebanon; for its completion.

C. Duration: The estimated time to complete this study is approximately one year. The researchers will have to visit the allocated schools spread all over Lebanon. Only two visits will be needed one for asking for the parents' or legal guardian's permission for participation in this study and one to undergo the study. The duration of each visit will be approximately 30 minutes.

Your child may leave the study at any time. If you decide to stop your child's participation in the study, there will be no penalty to you, or your child and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship, or that of your child, with AUB.

D. **Risks, Discomforts and Benefits:** Your child participation in this study does not involve any physical risk or emotional risk to you beyond the risks of daily life.

You or your child will receive no direct benefits from participating in this research; however, when the prevalence of IDD is detected, growth retardation can be prevented. Moreover, the results obtained are interesting in increasing our knowledge and in the modification of the fortification method used since 1995. This significant new finding will be conveyed to subjects.

E. **Confidentiality:** Efforts will be made to keep your child's study-related information confidential. All data from this study will be maintained in a secure locked drawer in a locked office or on a password protected computer. Data will only be reported in the aggregate. No names of individual children will be disclosed in any reports or presentations of this research. However, there may be circumstances where this information must be released. For example, personal information regarding your child's participation in this study may be disclosed if required by law. Also, your child's research data may be reviewed by the following groups (as applicable to the research):

• U.S. Office for Human Research Protections or other federal, state, or international regulatory agencies, required;

• The AUB Institutional Review Board or Office of Human Research Protections;

• The sponsor, if any, or agency supporting the study.

After the conclusion of the study, the Principal Investigator will retain all original study data in a secure location for at least three years to meet institutional archiving requirements. After this period, data will be responsibly destroyed.

F. Compensation/Incentive: No costs have to be paid by you. There will neither be anticipated expenses for participating and costs for transportation, parking etc will not be reimbursed.

G. Payment for Research-related Injury: In case of any adverse event as a result of the study, there will be no compensation to cover such expenses, in case it is not covered by a third party or governmental insurance. If you are injured as result of participating in this study or for questions about a study-related injury, you may contact Dr. Omar Obeid at 01/355555-ext 4440 or send him an email at oo01@aub.edu.lb.

H. **Contact Information and Questions:**

1) If you have any questions or concerns about the research you may contact:
Dr. Omar Obeid, 01/355555-ext 4440; oo01@aub.edu.lb.
2) If you have any questions, concerns or complaints about your rights as a participant in this research, you can contact the following office at AUB:
Social & Behavioral Sciences Institutional Review Board: irb@aub.edu.lb, 00961 1 350000-ext 5440 or 5445

I. Participant Rights: You may refuse to allow your child to participate in this study without penalty or loss of benefits to which you are otherwise entitled. If you are a student or employee at AUB, your decision about whether or not you allow your child to participate in this research will not affect your grades or employment status. If you choose to allow your child to participate in the study, you may discontinue his/her participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you or your child may have as a participant in this study. The Institutional Review Board responsible for human subjects' research at AUB has reviewed this research project and found it to be acceptable, according to applicable Lebanese and U.S. federal regulations and AUB policies designed to protect the rights and welfare of participants in research.
Do you have any questions about the above information? Do you wish your child to participate in this study?

J. Future Contact

Would you like to be contacted for future research?   Yes _____   No _____
Please notify that the investigator has the right to end subject's participation in this study.

Participant Consent:
Signing the consent form
I have read (or someone has read to me) this form and I am aware that I am being asked to give permission for my minor child (or child under my guardianship) to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to give permission for my child/child under my guardianship to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

_____
Printed name of subject
_____
Printed name of person authorized to give permission for minor subject/participant
_____
Signature of person authorized to give permission for minor subject/participant (when applicable)
_____   _____ AM/PM
Relationship to the subject   Date and time

Investigator/Research Staff

I have explained the research to the parent or legal guardian of the child subject/participant before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the parent/legal guardian of the child participant/subject.
_____   _____

Printed name of person obtaining permission   Signature of person obtaining permission

_____ AM/PM

APPENDIX III

CONSENT FORM (ARABIC)

عنوان البحث: تقييم حالة اليود في البول عند الأطفال في لبنان

إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

الباحثين المساعدين: د. هلا غطاس/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

منسقي البحث: دارين شاتيلا

العنوان: الجامعة الأميركية في بيروت، شارع الحمرا، بيروت – لبنان   01-350000

مكان إجراء البحث: المدارس الخاصة و الرسمية من كل لبنان.

هذا بيان موافقة للسماح لإبنك أو إبنتك بما أنك الوصي الشرعي المشاركة ببحث علمي سيجرى من قبل الجامعة الأميريكية في بيروت.   الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) السماح لإبنك أو إبنتك المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الإستمارة أو عن هذه الدراسة ككل من طبيبك. مشاركة إبنك/إبنتك غير إلزامية. أذا تمت الموافقة على المشاركة، سوف يطلب من حضرتك كوصي شرعي على توقيع هذا البيان و سوف تحصل على نسخة لك من هذا البيان.

أ) هدف هذا البحث: اليود هو معدن أساسي و له دور مهم اذ يساعد في النمو الذهني عند الأطفال. ان نقص اليود هو واقع صحي يهدد الأطفال في العالم و لا توجد في لبنان معلومات عن نسبة نقص هذا المعدن و غيرها من المعادن الاساسية. مع أن لبنان اتبع برنامجا لتدعيم الملح باليود سنة 1995 من قبل وزارة الصحة. ان هدف هذه الدراسة هو قياس نسبة اليود عند الاطفال الذين تتراوح أعمارهم بين 5-10 سنوات و ذلك لضمان نجاح برنامج التدعيم و بالتالي التأكد من أن كمية اليود المستعملة في التدعيم هي كافية .إن هدف البحث هو تحديد المناطق التي تعاني من نقص في اليود في لبنان.إن هدف البحث أطروحة وستنشر في صحيفة طبية ومن الممكن تقديمها في المؤتمرات الأكاديمية.

ب) **وصف الإجراءات والمشروع**: ستتم عملية اختيار المشاركين في الدراسة عن طريق الاتصال المباشر بمدير المدرسة التي تم اختيارها. سوف يعطى الأطفال الذين تم اختيارهم من كل مدرسة بيان طلب موافقة للمشاركة في هذا البحث و استمارة لاعطاؤها للأهل أ, الوصي الشرعي. بعد الموافقة من قبل الأهل او الوصي الشرعي سوف يطلب منهم التوقيع على البيان و ملئ الاستمارة و ارجاعها مع الطفل الى المدرسة. و من ثم سوف يتم اختيار هؤلاء الأطفال لجمع عينات من البول المطلوب لهذا البحث. سوف يتم جمع عينات البول لقياس نسبة اليود،البوتاسيوم،الصوديوم،والفلورايد و الفسفور و الكالسيوم و المغنيزيوم و الكرياتنين في البول. و من خلال الاستمارات التي تم ملؤها من قبل الأهل أ, الوصي الشرعي سوف يتم قياس نسبة استعمال الملح المعالج باليود. هذه الاستمارات تتضمن ايضا معلومات عن الوضع الاجتماعي و الاقتصادي لعائلة الطفل.

سوف يطلب من الاشخاص الذين تم اختيارهم للمشاركة في هذا البحث متابعة تناولهم للطعام وممارسة نشاط بدني بشكل طبيعي خلال مدة الدراسة وتفادي النشاطات الكثيفة قبل 24 ساعة من بدء الدراسة. سوف يطلب من الأشخاص الذين يعانون من أمراض مزمنة معينة عدم المشاركة في هذا البحث. كما سيتم استبعاد الأطفال الذين لا تتراوح أعمارهم بين ال5 و ال10 سنوات.

سيتوجه الباحثون الى المدارس حيث سوف يتم قياس الوزن و الطول لكل طفل، و الحصول على الاستمارة التي تم ملؤها من قبل الاهل أو الوصي الشرعي، و الحصول على عينات من البول في عبوات مخصصة. هذه دراسة عشوائية و عدد الاطفال المطلوب مشاركتهم في هذا البحث هو 1500 تلميذ تتراوح أعمارهم ما بين ال5 و ال10 سنوات من أجل اكمال هذه الدراسة.

ج) **المدة**:إن الوقت المقدر لانهاء البحث هو تقريبا سنة. مطلوب زيارة موقع الدراسة مرتين (مرة للحصول على موافقة المشاركة و مرة لاجراء البحث .مدة كلّ زيارة 30 دقيقة تقريباً.
يمكن لطفلك الانسحاب من البحث في أي وقت. إن أردت من طفلك التوقف عن المشاركة، ما من عقوبة تفرض عليك أو على طفلك ولن تخسر أي من الفوائد التي تملكها وقرارك لن يؤثر على أي علاقة مستقبلية لك أو لطفلك مع الجامعة الأمريكية في بيروت.

د) **المخاطر والمضايقات والفوائد:** مشاركة طفلك في هذه الدراسةلا تتضمن أية مخاطر جسدية أو نفسية أكثر من مخاطر الحياة اليومية. لن تتقاضى أنت أو طفلك أي أجر لهذه الدراسة، و لكن اهمية هذه الدراسة تكمن عندما يتحدد نسبة نقص اليود عند الاطفال، سوف يحد من مشكلة طمور النموعند الاطفال. كما أن النتائج التي سوف يتم الحصول عليها ستمكننا من معرفة و ايجاد طرق لتغيير برامج تدعيم الملح باليود التي تستعمل منذ عام 1995. سيتم الاخبار عن نتائج هذا البحث في نهاية الدراسة.

ه) **السرية:** سوف تبذل الجهود لتأمين سرية المعلومات التي تتعلق بإبنك، إسمك والمعرفات الأخرى لن تكون معلقة مع أجوبتك لضمان السرية. جميع المعلومات والمدونات ستحفظ في غرفة مغلقة أو حاسوب لديه رمز سري. الوصول إلى المعلومات مسموحٌ فقط للباحث الأساسي والباحثين الذين يعملون مباشرةً على الدراسة. جميع المعلومات ستدمر بشكل مسؤول من بعد الوقت المطلوب .سيحافظ على سريتك في جميع المعلومات المكتوبة والمنشورة عن نتائج هذا البحث. لن يستعمل إسمك أو أي معلومة متعلقة بهويتك في تقاريرنا أو مقالاتنا المنشورة. من الممكن أن توجد ظروف حيث يجب نشر معلوماتك السرية. مثلاً يمكن للمعلومات الشخصية المتعلقة باشتراكك أن تعطى لمجلس المراجعة المؤسسية في الجامعة الأمريكية في بيروت إن طلبت و للجان الأخلاق المهنية المستقلة، ومفتشين من الإدارات الحكومية المنظمة، مكتب حماية البحث الإنساني للولايات المتحدة أو أي وكالة تنظيمية فدرالية أو دولية أخرى، أو راعي البحث، إن وجد أو أي وكالة تسند البحث. بعد انتهاء البحث سوف يتم حفظ المعلومات من قبل الباحث المسؤول في مكان امن و سري لمدة 3 سنوات بعدها تتلف بمسؤولية.

و) **التعويض / الحافزة:** ليس هناك أية تكاليف مطلوبة منك أن تدفعها و لن تتقاضى أي أجر لهذه الدراسة، ولن تتقاضى أجر التنقل أو كلفة موقف السيارة الخ.

ز) **الدفع للإصابات ذات صلة بالبحث:** ما من تغطية لحصول الحوادث الغير متوقعة، في حال لم تكن هناك تغطية من قبل تأمين صحي أ, ضمان اجتماعي. إن تعرضت إلى إصابة جراء البحث، أو لأي سؤال عن الإصابات المتعلقة بالبحث، يرجى الاتصال بالدكتور عمر عبيد   350000 (01) مقسم 4440، email: oo01@aub.edu.lb

ح) **أسئلة ومعلومات الاتصال**
١) لأي أسئلة أو أي مخاوف حول البحث، يمكنك الاتصال بالدكتور عمر عبيد، قسم التغذية وعلم الطعام الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان   350000 (01) مقسم 4440، email: oo01@aub.edu.lb.
٢) لأي أسئلة أو أي مخاوف حول حقك كمشارك في هذا البحث يمكنك الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت:مجلس المراجعة المؤسسية

أو 5445 مقسم 350000 (01) الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان

5440 ،email: irb@aub.edu.lb

ح) حقوق المشاركين:

مشاركة إبنك أ, إبنتك في هذا البحث طوعية. يمكن لابنك أو ابنتك مغادرة البحث في أي وقت من دون أي عقوبة. إن قرارك بعدم المشاركة لن يؤثر بأي شكل ممكن على علاقتك بالجامعة الأمريكية في بيروت.

إذا اخترت السماح لطفلك الاشتراك في هذه الدراسة، يمكنك وقف اشتراكه فى اى وقت بدون عقوبة او فقدان الاستحقاقات. بتوقيع هذا البيان, لن تتخلى عن اى حقوق قانونية أو شخصية اذا قمت أنت أو طفلك بالمشاركة فى هذه الدراسة.

مجلس المراجعة المؤسسية في الجامعة الأمريكية في بيروت المسؤولة عن الابحاث التي تتعلق بالبشر قد قامت باستعراض هذا المشروع البحثى,و وجدت أنه مقبول حسب القضاء اللبناني و اللوائح الفيدرالية في الولايات المتحدة الأمريكية . إن الجامعة الامريكية لها سياسات تهدف الى حماية حقوق ورفاهية المشاركين فى البحث.

هل لديك أي أسئلة حول المعلومات الواردة أعلاه؟ هل ترغب في المشاركة في هذه الدراسة؟

أ. **الاتصال في المستقبل:**
هل ترغب في الاتصال بك للمشاركة في أبحاث أخرى في المستقبل؟ نعم _____ لا _____
ملاحظة: للباحث الحق الكامل بايقاف أي مشارك عن متابعة مشاركته في هذا البحث.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. وبناء عليه فأنني، حرا مختارا، أجيز الموافقة لابني أو ابنتي تحت وصيتي إجراء هذا البحث و أوافق أن يشارك فيه .

لن أتخلى عن أية حقوق قانونية عند امضائي لهذا البيان كما أنني سأستلم نسخة من هذا البيان.

إسم المشترك _____ التاريخ_____توقيع المشترك_____

الإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:_____

العلاقة بالشخص:_____

إمضاء الشخص المأذون للموافقة:_____ التاريخ:_____

توثيق الموافقة:

الإسم المطبوع للشخص الذي يطلب الموافقة:_____

إمضاء الشخص الذي يطلب الموافقة:_____

التاريخ و الوقت:_____

الباحثون:

لقد شرحت كل التفاصيل التي تتعلق بهذا البحث لأهل الطفل المشارك أو للوصي الشرعي قبل الحصول على امضاء الأخير. لا يوجد فراغات في هذه الوثيقةز و قد تم اعطاء نسخة لأهل الطفل المشارك أو للوصي الشرعي.

لإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:_____

إمضاء الشخص المأذون للموافقة:_____ التاريخ و الوقت:_____

APPENDIX IV

CHILD ASSENT FORM (ENGLISH)

Child Assent Form

Study Title: The assessment of urinary iodine status of children in Lebanon

Principal Investigator: Dr. Omar Obeid/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut

Co-Investigator: Dr. Hala Ghattas/ Faculty of Agricultural and Food Sciences/ Department of Nutrition and Food Science/ American University of Beirut

Researchers: Dareen Shatila

- **You are being asked to be in a research study. Studies are done to find better ways to treat people or to better understand how kids think about things or how kids and adults may behave at different times.**

- **This form will tell you about the study to help you decide whether or not you want to participate.**

- **You should ask any questions you have before making up your mind. You can think about it and discuss it with your family or friends before you decide.**

- **It is okay to say "No" if you don't want to be in the study. If you say "Yes" you can change your mind and quit being in the study at any time without getting in trouble.**

- **If you decide you want to be in the study, an adult (usually a parent) will also need to give permission for you to be in the study.**

1. **What is this study about?**

This study is about iodine, a mineral needed for all children to grow. Iodine is mainly derived from marine sources and is added to salt. We want to measure its level in the urine to check if children are getting enough of this mineral and are growing properly.

2. **What will I need to do if I am in this study?**

If you want to be in this study, you need to take your parent's permission first, and then fill this tube with urine, and then we will take your height and weight.

3. How long will I be in the study?

We will visit you two times, the first time to distribute the consent forms to ask for your parents' permission. Once we get your parents' permission we will come for the second visit and be with you for 30 minutes only.

4. Can I stop being in the study?

You may stop being in the study at any time. If there are limitations, such as you may discontinue completing the test/survey at any time, but you must remain at your desk in this room until the survey period ends.

5. What bad things might happen to me if I am in the study?

No health risks will happen to you.

6. What good things might happen to me if I am in the study?

The good things is that you will help us a lot in providing good information needed to help your friends who are not growing well.

7. Will I be given anything for being in this study?

No you will not be given any reward.

8. Who can I talk to about the study?

For questions about the study you may contact **us the researchers, your parents, and your teachers.**

To discuss other study-related questions with someone who is not part of the research team, you may contact the AUB Institution Review Board at 961-1-350000 or oo01@aub.edu.lb

Signing the assent form

Investigator/Research Staff

I have explained the research to the participant before requesting the signature above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

_____   _____
ed name of person obtaining assent   ture of person obtaining assent

                                            'M

                                            _____
                                            Date and time

APPENDIX V

CHILD ASSENT FORM (ARABIC)

عنوان البحث: تقييم حالة اليود في البول عند الأطفال في لبنان

إسم الباحث: د. عمر عبيد/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

الباحثين المساعدين: د. هلا غطاس/ قسم التغذية وعلم الطعام/ الجامعة الأمريكية في بيروت.

منسقي البحث: دارين شاتيلا

- المطلوب منك هو مشاركتك في هذه الدراسة البحثية. هذه الأبحاث تجري عادة لايجاد طرق جديدة و فعالة لمعالجة الناس أو لفهم بطريقة أفضل كيف يفكر الأطفال في بعض الاشياء أو كيف يتصرف الاطفال و الراشدين في بعض الاوقات.
- هذا البيان سوف يخبرك أكثر عن هذه الدراسة لمساعدتك في تحديد ما اذا كنت تود المشاركة .
- يرجى طرح أي أسئلة قبل أن تقرر. بإمكانك أن تفكر و تناقش الموضوع مع أهلك أو أصدقائك قبل أن تقرر.
- من الممكن أن تقول "لا" في حال كنت لا تود المشاركة في هذه الدراسة.و اذا قلت "نعم" يمكنك أن تغير رأيك و تنسحب من هذه الدراسة في أي وقت و دون مشاكل.
- اذا قررت أن تكون في هذه الدراسة، يجب أن تحصل على موافقة من شخص راشد (عادة من الاهل) كي تشارك.

1- عن ماذا تدور حول هذه الدراسة؟

هذه الدراسة هي عن معدن اليود وهو معدن مهم من اجل نمو الاطفال. يتواجد اليود في المأكولات البحرية و يضاف الى الملح. سوف نقوم بقياسه في البول لنتأكد أن جميع الاطفال يحصلون عليه بشكل كاف و بالتالي ينمون بشكل طبيعي.

2ـ ماذا سأفعل اذا كنت مشاركا في هذه الدراسة؟

اذا كنت ستشارك يجب الحصول على موافقة الاهل اولا و من ثم اعطاء عينة من البول في هذا الانبوب المخصص و سنأخذ طولك ووزنك.

3ـ كم من الوقت سوف أكون في هذه الدراسة؟

سوف نقوم بزيارتكم مرتين. المرة الاولى لتوزيع بيان الموافقة . فور الحصول على موافقة مشاركتكم سوف نقوم بالزيارة الثانية التي ستستغرق 30 دقيقة فقط.

4ـ هل بإمكاني التوقف عن مشاركتي في هذه الدراسة؟

بإمكانك التوقف عن المشاركة في أي وقت. اذا كان هناك تجاوزات يمكنك التوقف عن اكمال هذه الدراسة في أي وقت و لكن عليك لزوم مقعدك حتى نهاية وقت البحث.

5- ما هي المخاطر التي قد اتعرض لها اذا كنت مشاركا في هذا البحث؟

لا توجد أية مخاطر قد تتعرض لها.

6ـما هي الامور الجيدة التي ستحصل من جراء مشاركتي في هذا البحث؟

هناك العديد من الامور الجيدة منها انك ستساعدنا كثيرا باعطائنا معلومات مهمة لمساعدة أصدقائك الذين لا ينمون بشكل جيد.

7ـهل ساحصل على أي شئ لانني شاركت في هذه الدراسة؟

كلا لن تحصل علي اي شئ.

8- مع من يمكنني التحدث عن هذه الدراسة؟
لأي أسئلة حول البحث، يمكنك الاتصال بالباحثين، أهلك، و اساتذتك.
للحصول على أية معلومات مع أشخاص لا علاقة لهم في هذا البحث يرجى الاتصال بالمكتب التالي في الجامعة الأمريكية في بيروت:مجلس المراجعة المؤسسية
أو 5445 مقسم 350000 (01)الجامعة الأمريكية في بيروت، شارع القاهرة، بيروت، لبنان

5440 ،email: irb@aub.edu.lb

الباحثون:

لقد شرحت كل التفاصيل التي تتعلق بهذا البحث للطفل المشارك قبل الحصول على امضاء الأخير . لا يوجد فراغات في هذه الوثيقة. و قد تم اعطاء نسخة لأهل الطفل المشارك أو للوصي الشرعي.

الإسم المطبوع للشخص المأذون للموافقة من أجل الشخص:_____

إمضاء الشخص المأذون للموافقة:_____ التاريخ و الوقت:_____

APPENDIX VI

LAW 178: POTASSIUM FUORIDE (FK or KF2 H2O) ADDITION TO TABLE OR COOKING SALT THAT IS IMPORTED OR MANUFACTURED IN LEBANON

بموجبها أحكام مبرمة.

المادة الثالثة: يعمل بهذا القانون فور نشره في الجريدة الرسمية.

بيت الدين في ٢٩ آب ٢٠١١

الامضاء: ميشال سليمان

صدر عن رئيس الجمهورية

رئيس مجلس الوزراء

الامضاء: محمد نجيب ميقاتي

رئيس مجلس الوزراء

الامضاء: محمد نجيب ميقاتي

قانون رقم ١٧٨

اضافة مادة إيودور البوتاسيوم او إيودات البوتاسيوم ومادة الفليورور او فليورايد البوتاسيوم الى الملح المعد للمائدة او للمطبخ

أقر مجلس النواب،

وينشر رئيس الجمهورية القانون التالي نصه:

المادة الاولى: يجب أن يحتوي الملح المعد للمائدة أو للمطبخ في جميع الاراضي اللبنانية على مادة إيودور البوتاسيوم (KI) أو إيودات البوتاسيوم (KIO3) ومادة فليورور البوتاسيوم (FK) أو فليوراد البوتاسيوم (KF2 H2O).

المادة الثانية: يجب على أصحاب المعامل والمحامص ومستوردي الملح في لبنان أن لا يسلموا الى الأسواق ملحا للمائدة أو للمطبخ للاستهلاك المحلي الا اذا كان ممزوجا بمادة إيودور البوتاسيوم (KI) أو إيودات البوتاسيوم (KIO3) ومادة فليورور البوتاسيوم (FK) أو فليورايد البوتاسيوم (KF2 H2O).

المادة الثالثة: يلزم باستعمال الملح المستورد والمحتوي على مادة الايودور أو الايودات والفيورور والفليورايد البوتاسيوم اذا كان موضوعا في أوعية فنية كتيمة (محكمة الغلق) واذا كان نوع الملح مكررا وجافا. وتخضع نسب اليود والفليور في الملح المستورد والمصنع محليا للمواصفات المحددة من قبل مؤسسة المقاييس والمواصفات اللبنانية شرط أن تكون النسبة المئوية لليود والفليور مذكورة بوضوح تام على الأوعية.

بحيث تكون النسبة = ٢٥٠ ملغ (250 mg) من

فليورايد البوتاسيوم لكل ١ كلغ (1 Kg) من الملح المعد للمائدة أو للمطبخ.

المادة الرابعة: تحدد نسبة اليود والفليور المضاف الى ملح المائدة والمطبخ المصنوع محليا حسب مواصفات مؤسسة المقاييس والمواصفات اللبنانية على أن تكون النسبة مدونة على الأوعية الفنية الكتيمة وشرط التقيد بنفس الشروط الواردة في الملح المستورد المشار اليه في المادة السابقة.

المادة الخامسة: يمنع منعا باتا تداول واستعمال ملح المائدة أو المطبخ المخالف للشروط الواردة في هذا القانون.

المادة السادسة: يطلق على الملح المكرّر الجاف الممزوج بايودور البوتاسيوم أو ايودات البوتاسيوم وفليورور وفليورايد البوتاسيوم اسم «ملح للمائدة أو للمطبخ مزوّد باليود والفليور» أو «ملح ميوّد ومفلور».

المادة السابعة: يفرض على أصحاب معامل تكرير الملح المنتجة للملح الميوّد والمفلور في الاراضي اللبنانية:

١ ــ تجهيز معاملهم بالمعدات اللازمة لعملية مزج الملح باليود والفليور.

٢ ــ مراجعة رئيس فرع الكيمياء في المختبر المركزي للصحة العامة لتزويدهم بطرق ضبط الكمية وتعيير النسبة المئوية لليود والفليور.

٣ ــ تعبئة الملح الميّود والمفلور بأوعية فنية كتيمة (محكمة الغلق) لا يزيد محتوى واحدها عن كيلوغرام واحد.

٤ ــ تدوين ما يلي على الأوعية:

أ ــ نوع الملح «ملح ميوّد ومفلور» أو «ملح للمائدة أو للمطبخ مزوّد باليود والفليور».

ب ــ نوع الاضافات الكيماوية الى الملح ومقاديرها.

ج ــ تاريخ صنع الملح الميّود والمفلور وانتهاء الصلاحية.

د ــ اسم المعمل وعنوانه.

٥ ــ الالتزام بتركيبة الملح الصالح للاستهلاك حسب المواصفات المحددة من قبل مؤسسة المقاييس والمواصفات اللبنانية.

ملح المطبخ		ملح المائدة	
حد أدنى	٩٧٫٥٠ %	٩٨٫٥٠ %	كلور الصوديوم
حد أعلى	١٫٠٠ %	٠٫٥٠ %	رطوبة
حد أعلى	٠٫٢٥ %	٠٫١٠ %	مجموع المغنيزيوم
حد أعلى	٠٫٣٣ %	٠٫٣٣ %	مجموع الكالسيوم
حد أعلى	٠٫٧٦ %	٠٫٧٦ %	مجموع السلفات
حد أعلى	٠٫٢٠ %	٠٫٢٠ %	مواد غير ذوابة بالماء

مجموع اليود: ٦٠ ـ ٨٠ مليغرام بالكيلو الواحد ٦٠ ـ ٨٠ مليغرام بالكيلو الواحد (KIO3)

مجموع الفليور: ٢٥٠ ميلغرام بالكيلو الغرام الواحد حد أعلى

على أن تكون مواصفات فليورور أو الفليورايد البوتاسيوم (KF, KF2H20) متطابقة لما يلي:

١ ـ يجب أن يحتوي الملح المعد للمائدة أو للمطبخ على ٢٥٠ مليغرام بالكيلوغرام الواحد من مادة الفليورايد البوتاسيوم (KF, KF2H20).

٢ ـ إن فليورور أو فليورايد البوتاسيوم المستعمل يجب أن يخضع للمواصفات المدرجة في الجدول المرفق بهذا القانون.

المادة الثامنة:

١ ـ كل شخص طبيعي أو معنوي يخالف أحكام هذا القانون يعاقب بالحبس من شهرين الى سنة وبغرامة تتراوح بين أربعمئة ألف ليرة لبنانية الى ثلاثة ملايين ليرة لبنانية أو باحدى هاتين العقوبتين، إضافة الى مصادرة البضائع موضوع المخالفة.

٢ ـ للمحكمة الحق بإيقاف المؤسسة أو الشركة أو الوكالة المصنّعة أو الموزّعة أو المسوّقة مؤقتا عن العمل.

٣ ـ في حال تكرار المخالفة تضاعف العقويات المذكورة اعلاه. وتقفل المؤسسة أو الشركة أو الوكالة المصنّفة أو الموزّعة أو المسوّقة للمُنتج المصنّف.

المادة التاسعة: يعطى اصحاب معامل تكرير الملح في الاراضي اللبنانية مهلة سنة لتطبيق ما خصهم في هذا القانون بعد صدوره ونشره في الجريدة الرسمية. ويطبق هذا القانون على مستوردي الملح بعد ثلاثة أشهر من نشره في الجريدة الرسمية.

المادة العاشرة: تحدد دقائق تطبيق هذا القانون بمراسيم تتخذ في مجلس الوزراء بناء على اقتراح وزير الصحة العامة. يمكن تعديل النسب والمواصفات الواردة في هذا القانون بموجب مرسوم يتخذ في مجلس الوزراء بناء على اقتراح وزير الصحة العامة، وذلك وفقا للمواصفات التي تضعها منظمة الصحة العالمية.

المادة الحادية عشرة: يلغى القانون الموضوع موضع التنفيذ بالمرسوم رقم ١٧٨١ تاريخ ١٩٧١/٩/١ المتعلق باضافة مادة ليودور البوتاسيوم أو ليودات البوتاسيوم الى الملح المعدّ للمائدة أو للمطبخ، وجميع القوانين والنصوص التي تتعارض وأحكام هذا القانون.

المادة الثانية عشرة: يعمل بهذا القانون فور نشره في الجريدة الرسمية.

بيت الدين في ٢٩ آب ٢٠١١
الامضاء: ميشال سليمان
صدر عن رئيس الجمهورية
رئيس مجلس الوزراء
الامضاء: محمد نجيب ميقاتي
رئيس مجلس الوزراء
الامضاء: محمد نجيب ميقاتي

APPENDIX VII

FLUORIDE ANALYSIS PROTOCOLS

Reagents needed for fluoride analysis procedure:
- Double distilled or deionized water. (DDI)
- Sodium citrate ($Na_3C_6H_3O_7 \cdot 2H_2O$).
- Ethylene dinitrilotetracetic acid (EDTA), disodium salt.
- Acetic acid, glacial.
- Sodium chloride.
- Sodium hydroxide, 5 M. Dissolve 20 g NaOH in distilled water; dilute to 100 mL.
- Sodium fluoride.
- Calibration stock solution, 100 μg F /mL. Dissolve 0.2211 g dry sodium fluoride in distilled water. Make 1000 mL solution.
- Total ionic strength activity buffer (TISAB), pH 5.

TISAB preparation method:
   Add 57 mL glacial acetic acid, 58 g sodium chloride, and 0.30 g sodium citrate to a 1-L beaker containing 500 mL distilled water. Stir to dissolve. Place beaker in water-bath for cooling. Slowly add 5 M sodium hydroxide until the pH is between 5.0 and 5.5. Cool to room temperature; dilute to 1 L with distilled water.

Test technique: ion selective electrode (ISE)
Procedure applied for fluoride analysis in urine:
- Defrost samples
- Wash each tube with HCl and rinse with double deionized water.
- Mix 10ml of urine + EDTA, with 10ml TISAB and shake before reading.
- Immerse electrodes. Allow sample to mix for 2 to 3 min and then record millivolt reading.
- Wash electrode with de-ionized double distilled water (DDI) between each sample reading.
- Run test in the control sample after the reading of 10 samples.

   In term of interference, hydroxide is the only positive interference and it is eliminated by use of the buffer. Negative interferences from complexation of fluoride by cations, such as calcium, are minimized by EDTA preservative and the high ionic strength buffer. (Tolos, 1994)

APPENDIX VIII

DEFINITION OF VARIABLES

Definition of statistical variables.

Name of variable	Description
Student ID	Continuous variable
School Type	1: Public* 2: Private* 3: Private Free*
District	1: Akkar 2: Beirut 3: Bekaa 4: Hermel 5: Mount Lebanon 6: North 7: Nabatiyeh 8: South
Gender	1: Male 2: Female
Altitude	Continuous variable
Age	Continuous variable
Class	1: Grade 1 2: Grade 2 3: Grade 3 4: Grade 4 5: Grade5
Height	Continuous variable
Weight	Continuous variable
BMI	Continuous variable calculated from weight and height
Nutritional Status	1: Sever Thinness 2: Thinness 3: Normal 4: Overweight 5: Obese
Creatinine	Continuous variables measured using Vitros 350
Estimated Creatinine	Continuous variable calculated as suggested by Remer, Neubert, & Maser-Gluth (2002)
F	Continuous variables measured using ISE
F/Cr	Continuous variable calculated from F/Cr
Estimated 24 h F excretion	Continuous variable calculated from the cross multiplication using the predicted creatinine values: $\frac{F \times estimated\ 24\ hour\ Cr}{Cr}$
TDFI (total daily fluoride intake)	(estimated 24 h F excretion (mg/d)-0.03)/0.35 (Villa et al, 2010)
Adequate intake (0.05 mg/kg/day)	0.05* body weight (mg/d) (USDA-DRI, chapter 8, 2010)
Date of collection	Date (dd-Mon-yy)

BIBLIOGRAPHY

C. Palmer, SH. Wolfe (2005) "Position of the American Dietetic Association: The Impact of Fluoride on Health". Journal of the American Dietetic Association.Vol. 105, number 10, pp. 1620-1628

S. Saxena, A. Sahay, and P. Goel. (2012). "Effect of fluoride exposure on the intelligence of school children in Madhya Pradesh, India". Journal of neuroscience in rural practice,Vol 3, pp. 144-149

Zohouri F.V. Swinbank C.M. , Maguire A. , Moynihan P.J. (2006)."Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? Community Dent Oral Epidemiol, vol. 34, pp. 130–8

W. Tolos (1994). "FLUORIDE in urine: METHOD 8308". NIOSH Manual of Analytical Methods (NMAM), Fourth Edition, Issue 2, NIOSH/DBBS, pp. 1-4

A. Villa, M. Anabalon, V. Zohouri, A. Maguire, A.M. Franco, A. Rugg-Gunn (2010). "Relationships between Fluoride Intake, Urinary Fluoride Excretion and Fluoride Retention in Children and Adults: An Analysis of Available Data. Caries Res.;Vol 44, pp. 60–68

KA. Levin,CM. Jones, C Wight, C. Valentin, GVA. Topping, R. Naysmith. (2009) "Fluoride rinsing and dentalhealth inequalities in 11-year-oldchildren: an evaluation of asupervised school-based fluoriderinsingprogramme in Edinburgh". Community Dent Oral Epidemiol Vol. 37,pp. 19–26

JP Yadav, S Lata (2003). "Urinary fluoride levels and prevalence of dental fluorosis in children of Jhajjar District, Haryana."Vol. 57, pp. 394-9

A. Ananian, B. H. Solomowitz, I. A. Dowrich (2006). "Fluoride, a controversy revisited". New York State Dental journal, Vol 72, pp.14-18

World Health Organization, (2000), Air quality guidelines, chapter 6.5, Fluorides

Doumit M, Doughan B (2002), "Oral health in school children in Lebanon" Santé, Vol. 2:223-8

H. Tsunoda , M.H. Yu (1986). "Fluoride research 1985" book, ELSEVIER 1986, Amsterdam - New York - Oxford – Tokyo.

National Research Council. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride (2010 updated). Washington, DC: The National Academies, United States Department of Agriculture (USDA), Press 1997, chapter 8: Fluoride

J. Fawell, K. Bailey, J. Chilton, E. Dahi, L. Fewtrell and Y. Magara, (2006). "Fluoride in drinking water", World health organization (WHO), London, UK, chapter 3.

United States Environmental Protection Agency, (2000) "Fluoride," Report to Congress section 112 (n) (16), Clean Air Act, Washington, DC, USA.

S. Peckham, and N. Awofeso (2014). "Water Fluoridation: A Critical Review of the Physiological Effects of Ingested Fluoride as a Public Health Intervention" the Scientific world journal, vol. 2014, Article ID 293019, 10 pages

BS. NarasingaRao, KA Krishnamachari, C. VijayaSarathy (1979). "^{47}Ca turnover in endemic fluorosis and endemic genu valgum". British Journal of Nutrition. Vol. 41, pp. 7-14.

V. Dhar, M. Bhatnagar (2009). "Physiology and toxicity of fluoride". Indian J Dent Res 2009;20:350-5

GM. Whitford (1990). "The physiological and toxicological characteristics of fluoride". J Dent Res;69:539-49

M. Wong, J. Clarkson, A.Glenny, E. Lo, V. Marinho, B. Tsang, T. Walsh, and H. Worthington (2011). "Cochrane reviews on the benefits/risks of fluoride toothpastes". J Dent Res 90, pp. 573-579.

A. Villa, M. Anabalón M, L. Cabezas (2000). "The fractional urinary fluoride excretion in young children under stable fluoride intake conditions." Community Dent Oral Epidemiol 2000; 28: 344–355.

ES.Akpata, J. Behbehani, J. Akbar, L. Thalib, O. Mojiminiyi (2013)."Fluoride intake from fluids and urinary fluoride excretion by young children in Kuwait: a nonfluoridated community." Community Dent Oral Epidemiolvol

A. Maguire, FV. Zohouri, PN. Hindmarc, JHatts, PJ Moynihan (2007). "Fluoride intake and urinary excretion in 6- to 7-year-old children living in optimally, sub-optimally and non-fluoridated areas". Community Dent Oral Epidemiol Vol. 35, pp. 479–488.

M. Haftenberger, G. Viergutz, V. Neumeister, G. Hetzer (2001)."Total Fluoride Intake and Urinary Excretion in German Children Aged 3–6 Years". Caries Res. Vol. 35, pp. 451-457.

R. J. Baez, M. X. Baez, and T. M. Marthaler (2000). "Urinary fluoride excretion by children4–6 years old in a south Texas community". Pan Am J Public HealthVol. 7, pp. 242-247.

B. A. Groves (2001). "Fluoride drinking ourselves to death?" Newleaf, pp. 63-143

P. Mullenix, P. Denbesten, ASchunior, W. Kernan (1995). "Neurotoxicity of sodium fluoride in rats." J. NeurotoxicolTeratol Vol. 17, pp. 169-177.

D. Moss, R. Fariello, J. Sahlmann, I. Sumaya, F. Pericle, E. Braglia (2013) "A randomized phase I study of methanesulfonyl fluoride, an irreversible cholinesterase inhibitor, for the treatment of Alzheimer's disease".Br J ClinPharmacol Vol. 75, pp. 1231–1239.

R. Isaacson (1992) "Rat studies link brain cell damage with aluminium and fluoride in water". State University of New York. Wall street Journal article by Marilyn chase, Vol. B-6.

CA. Palmer, JA. Gilbert (2012). "Position of the Academy of Nutrition and Dietetics: the impact of fluoride on health." J AcadNutr Diet. Vol. 112, pp.1443-1453.

F. V. Zohoori, R. Walls, L. Teasdale, D. Landes, I. N. Steen, P. Moynihan, N. Omidand A. Maguire (2012). "Fractional urinary fluoride excretion of 6–7-year-old children attendingschools in low-fluoride and naturally fluoridated areas in the UK" British Journal of Nutrition, pp. 1-7

M. Buzalaf, S. Levy (2011). "Fluoride intake of children: considerations for dental caries and dental fluorosis". Fluoride and the oral environment, Monogr Oral Scl. Basel, Karger, vol 22, pp. 1-19.

S Paiva, Y. Lima, and J. Cury (2003). " Fluoride intake by Brazilian children from two communities with fluoridated water". Community Dent Oral Epidemiol, vol. 31, pp. 184-191.

F.V. Zohouri and A.J. Rugg-Gunn (2000). "Sources of dietary fluoride intake in 4-year-old children residing in low, medium and high fluoride areas in Iran." International Journal of Food Sciences and Nutrition Vol. 51, pp. 317–326

S. Gropper, J. Smith (2009). "Advanced Nutrition and Human Metabolism". Chapter 12, Micro-minerals, pp. 530-532

J. Ekstrand, EE. Ziegler, SE. Nelson, SJ Fomon (1994). "Absorption and retention of dietary and supplemental fluoride by infants". Adv Dent Res Vol. 8, pp. 175–180

GM. Whitford (1994). "Intake and metabolism of fluoride". Adv Dent Res Vol. 8, pp. 5-14

D. Mady (2007). "Fluoride an excellent, safe nutrient for teeth". Windsor, Ont, Body & Health, Vol B, p. 5

M.H. Trivedi, R.J. Verma, N.J. Chinoy, (2007). "Effect of high fluoride water onintelligence of school children in india". Fluoride Vol. 40 pp. 178–183.

A. L. Choi, G. Sun, Y. Zhang, and P. Grandjean (2012). "Developmental Fluoride Neurotoxicity: A Systematic Review and Meta-Analysis". Environmental health perspective, vol. 120 (10), pp.1362-1368

AF Subar (2004). "Developing dietary assessment tools". J Am Diet Assoc. Vol. 104, pp. 769–770.

USDA (2005) "USDA National Fluoride Database of Selected Beverages and Foods, Release 2" Nutrient Data Laboratory Beltsville Human Nutrition Research Center Agricultural Research Service U.S. Department of Agriculture, pp.1-9

H. Limeback, A. Ismail, D. Banting, P. DenBesten, J. Featherstone, PJ. Riordan (1999). "Canadian Consensus Conference on the appropriate use of fluoride supplements for the prevention of dental caries in children". J Can Dent Assoc. Vol. 64(9), pp. 636-9.

A. Ghosh, K. Mukherjee, S. Ghosh, B. Saha (2013). "Sources and toxicity of fluoride in the environment" Res ChemIntermed, Vol. 39, pp. 2881–2915

P. Pehrsson, R. Cutrufelli, K. Patterson, K. Phillips, A. Rasor, J. Heilman, C. Perry, and J. Holden "The Fluoride Content of Brewed and Microwave Brewed Black Teas" (2005). USDA, Agricultural Research Service. http://www.ars.usda.gov/ba/bhnrc/ndl

F. García-Hoyos, C. Cardoso Silva, E. Barbería (2014). "Renal excretion of fluoride after fluoride mouth rinses in children". European Journal of Pediatric Dentistry vol. 15/1/2014, pp. 35-38.

World Health Organization, Geneva, (1996). "Trace elements in human nutrition and health". Macmillan/Ceuterick. Chapter 15.

S. Gropper, J. Smith (2009)."Advanced Nutrition and Human Metabolism". Chapter 12, Microminerals, pp. 530-531

Standing Committee on the Scientific,Institute of Medicine, National Academy of Sciences, Food and Nutrition Board (1997). "Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin." Washington, DC: National Academies Press

APP. Santos, P. Nadanovsky, BH. Oliveira (2013). "A systematic review and meta-analysis of the effects of fluoride toothpastes on the prevention of dental caries in the primary dentition of preschool children". Community Dent Oral Epidemiol Vol. 41, pp. 1–12

H. G. Jarvis, P. Heslop, J. Kisima, W. K. Gray, G. Ndossi, A. Maguire and R. W. Walker (2013) "Prevalence and aetiology of juvenile skeletal fluorosis in the south-west of the Hai district, Tanzania – a community-based prevalence and case–control study". Tropical Medicine and International Health, vol. 18 no 2 pp. 222–229

R. J. Weyant, S. L. Tracy, T. Anselmo, E. D. Beltrán-Aguilar, K.J. Donly, W. A. Frese, P. P. Hujoel, T. Iafolla, W. Kohn, J. Kumar, S. M. Levy, N. Tinanoff, J. T. Wright, Domenick Zero, K. Aravamudhan, J. Frantsve-Hawley and D. M. Meyer (2013). "Topical fluoride for caries prevention: Executive summary of the updated clinical recommendations and supporting systematic review". The Journal of the American Dental Association Vol. 144(11), pp. 1279-1291

MA. Buzalaf, JP.Pessan, HM.Honório,JM. ten Cate (2011). "Mechanisms of action of fluoride for caries control." Monogr Oral Sci. Vol. 22, pp. 97-114.

R. Liteplo, R. Gomes, P. Howe, H. Malcolm (2002). "Environmental Health Criteria 227: Fluorides". World Health Organization, Geneva.

M.A.R. Buzalaf (2011) "Fluoride and the Oral Environment." Monographs in oral science Vol. 2, pp 37-51, (chapter: Contemporary Biological Markers of Exposure to Fluoride. Rugg-Gunn, A.J. (Newcastle upon Tyne); Villa, A.E. (Santiago); Buzalaf, M.A.R. (Bauru))

HJ. Cho, BH. Jin, DY. Park, SH. Jung, HS. Lee, DI. Paik, KH. Bae (2014). "Systemic effect of water fluoridation on dental caries prevalence." Community Dent OralEpidemiol Vol. 42, pp. 341–348.

C. Agostoni, R. B. Canani, S. Fairweather-Tait, M. Heinonen, H. Korhonen, S. La Vieille, R. Marchelli, A. Martin, A. Naska, M. Neuhäuser-Berthold, G. Nowicka, Y. Sanz, A. Siani, A. Sjödin, M. Stern, S. (J.J.) Strain, I. Tetens, D. Tomé, D. Turck and H. Verhagen. (2013). "EFSA Panel on Dietetic Products, Nutrition, and Allergies". EFSA Journal Vol. 11(8), p. 1-46

S. J. Rankin, S. M. Levy, J. J. Warren2, J. E. Gilmore and B. Broffitt. (2011) "Relative validity of an FFQ for assessing dietary fluoride intakes of infants and young children living in Iowa" Public Health Nutrition, Vol. 14(7), pp. 1229–1236

AJ. Rugg-Gunn, AE .Villa and MR.Buzalaf(2011). "Contemporary biological markers of exposure to fluoride". Monographs in Oral Science Vol. 22 pp. 37-51.

WHO (World Health Organization) (1994)."Report of an Expert Committee on oral health status and fluoride use. Fluorides and oral health." WHO Technical Report Series No. 846, pp. 37

VE. Cardoso, GM. Whitford and MA. Buzalaf MA(2006). "Relationship between daily fluoride intake from diet and the use of dentifrice and human plasma fluoride concentrations" Archives of Oral Biology Vol. 51. pp. 552-557.

Ekstrand J, 1977. Fluoride concentrations in saliva after single oral doses and their relation to plasma fluoride. Scandinavian Journal of Dental Research, 85, 16-17

Whitford GM, Thomas JE and Adair SM, 1999a. Fluoride in whole saliva, parotid ductal saliva and plasma in children. Archives of Oral Biology, 44, 785-788.

Toth Z, Gintner Z and Banoczy J, 2005. The effect of ingested fluoride administered in salt, milk, and tablets on salivary and urinary fluoride concentrations. Fluoride, 38, 199-204.

Kaiser D, Neumeister V, Stößer L and Hetzer G, 2006. "Fluorid kon zentrationimSpeichel und in der Plaque nach Verzehr fluorid salzhaltiger Speisen." Oralprophylaxe & Kinderzahnheilkunde, 28, 110-114.

Caraccio T, Greensher J and Mofenson HC, 1983. The toxicology of fluoride. In: Clinical management of poisoning and drug overdose. Eds Haddad L, Winchester J. WB Saunders, Philadelphia, Pennsylvania, USA, 1012 pp.

Pessan JP and Buzalaf MR, 2011. Historical and recent biological markers of exposure to fluoride. Monographs in Oral Science, 22, 52-65.

Schamschula RG, Sugar E, Un PS, Toth K, Barmes DE and Adkins BL, 1985. Physiological indicators of fluoride exposure and utilization: an epidemiological study. Community Dentistry and Oral Epidemiology, 13, 104-107.

Mandinic Z, Curcic M, Antonijevic B, Carevic M, Mandic J, Djukic-Cosic D and Lekic CP, 2010. Fluoride in drinking water and dental fluorosis. Science of the Total Environment, 408, 3507-3512

Czarnowski W and Krechniak J, 1990. Fluoride in the urine, hair, and nails of phosphate fertiliser workers. British Journal of Industrial Medicine, 47, 349-351.

Whitford GM, Sampaio FC, Arneberg P and von der Fehr FR, 1999b. Fingernail fluoride: a method for monitoring fluoride exposure. Caries Research, 33, 462-467.

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Fluoride. The EFSA Journal 2005, 192, 1-65. doi:10.2903/j.efsa.2005.2192

NRC (National Research Council), (1993). Health effects of ingested fluoride. Subcommittee on Health Effects of Ingested Fluoride. National Academy Press, Washington DC, USA, 206 pp.

A. Sheiham, and W P. T James (2014). "A new understanding of the relationship between sugars, dental caries and fluoride use: implications for limits on sugars consumption". Public Health and nutrition, Vol. 17 (10), pp. 2176-2184

SM. Levy, B. Broffitt, TA. Marshall, JM. Eichenberger-Gilmore, JJ. Warren. (2010). "Associations between fluorosis of permanent incisors and fluoride intake from infant formula, other dietary sources and dentifrice during early childhood". J Am Dent Assoc Vol. 141(10), pp. 1190-1201.

J. Narbutaite, MM. Vehkalahti, S. Milciuviene (2007). "Dental Fluorosis and Dental Caries among 12-Year-Old Children from High- and Low-Fluoride Areas in Lithuania". S. Eur J Oral Sci , Vol. 115, pp. 137-42

Z. Paknahad, N. Mohammadifard, Z. Bonakdar, A. Hasanzadeh (2014). "Nutritional status and its relationship with bone mass density in postmenopausal women admitted in osteodensitometry center, Isfahan-Iran". Journal of Education and Health Promotion, Vol. 3 pp. 78-82

Sud. Rawlani, Sho. Rawlani, Shi. Rawlani (2010). "Assessment of Skeletal and Non-skeletal Fluorosis in Endemic Fluoridated Areas of Vidharbha Region, India: A Survey". Indian Journal of Community Medicine, Vol 35, Issue 2, pp. 298-301

X. Qin, S. Wang, M. Yu, L. Zhang, X. Li, Z. Zuo, X. Zhang, and L. Wang (2009). "Child Skeletal Fluorosis from Indoor Burning of Coal in Southwestern China" Journal of Environmental and Public Health, Vol. 2009, 7 pages

J. Buchancová , H. Poláček, H. Hudečková, L. Murajda, O. Osina, J. Valachová (2008). "Skeletal fluorosis from the point of view of an occupational exposure to fluorides in former Czechoslovakia". InterdiscToxicol, Vol. 1(2), pp. 193–197.

S. Petti, G. Tarsitani, P. Panfili, A. Simonetti D'Arca (1997). « Oral hygiene, sucrose consumption and dental caries prevalence in adolescent systemic fluoride nonusers." Community Dent Oral Epidemiol, Vol. 25: pp. 334-6.

M. MustahsenurRehman, N. Mahmood, B. urRehman (2008). "The relationship of caries with oral hygiene status and extra-rola risk factors". J Ayub Med Coll Abbottabad, Vol. 20(1), pp. 103-108

K. Mowla (2007). "Treatment of osteoporosis in patients with rheumatoid arthritis: sodium fluoride or calcitonin". Jundishapur journal of natural pharmaceutical products, vol. 1, pp. 8-12

G.Y. El Khoury, T. E. Moore, J. P. Albright, H. K. huang, R. K. Martin. (1982). "Sodium Fluoride treatment of osteoporosis: radiologic findings". AJR., vol. 139, pp. 39-43.

S.D. Heintze, JRM. Bastos, R. Bastos (1998). "Urinary fluoride levels and prevalence of dental fluorosis in three Brazilian cities with different fluoride concentrations in the drinking water". Community Dent Oral Epidemiol, Vol. 26, pp. 316-23.

S. Chen, B. Li, S. Lin, Y. Huang, X. Zhao, M. Zhang, Y. Xia, X. Fang, J. Wang, S. A. Hwang and S. Yu (2013). "Change of urinary fluoride and bone metabolism indicators in the endemic fluorosis areas of southern china after supplying low fluoride public water". Chen et al. BMC Public Health, Vol. 13, p. 156

C. E. Ketley, J. A. Cochran, W. P. Holbrook, L. Sanches, C. Van Loveren, A.M. Oila, DM. O'Mullane (2004). "Urinary fluoride excretion by preschool children in six European countries". Comm Dent Oral Epidemiol, Vol 32, pp. 62-68

C.E. Ketley, M. A. Lennon (2000). "Urinary fluoride excretion in children drinking fluoridated school milk". International Journal of Pediatric Dentistry, vol. 10, pp. 260-270.

M. Jurdi, D. AbiSaid, and M. Al KouatlyKambris (2001). "Decision to fluoridate. 1. Fluoride levels in herbal teas used in Lebanese communities". The United Nations University, Food and nutrition bulletin, vol. 22, pp. 62-66

M. Jurdi, D. Abi Said, and M. Al KouatlyKambris (2001). "Decision to fluoridate. 2. Intake of fluoride from non milk fluids by children under two years of age in Lebanon." The United Nations University, Food and nutrition bulletin, vol. 22, pp. 67-70

World health organization (2014). "Basic methods for assessment of renal fluoride excretion in community prevention programmes for oral health". WHO Library Cataloguing-in-Publication Data, Geneva.

H.C. Hodge (1950). "The concentration of fluorides in drinking water to give the point of minimum caries with maximum safety". Journal of the American Dental Association, vol. 40, pp. 436-39

Franco H., Acevedo A. M., Petrone M., Volpe A. and Rojas-Sánchez Fátima (2009). "Fluoride intake and urinary fluoride excretion in children attending a daycare center in Maracay, Aragua state, Venezuela". Journal of Dentistry and Oral Hygiene Vol. 1(3), pp. 027-035

Vitros 350 instructions for use Crea retrieved from
http://www.cmmc.org/cmmclab/IFU/CREAT_020.PDF, on 29/12/14.

F. D. Soares, S. A. Saliba, F. Correia (2008). "Urinary Fluoride Excretion in Children
Exposed to Fluoride Toothpaste and to Different Water Fluoride Levels in a
Tropical Area of Brazil". Braz Dent J, vol. 19(3), pp. 214-218

DG Pendrys & JW Stamm (1990). "Relationship of total fluoride intake to beneficial
effects and enamel fluorosis". J Dent, Res 69, pp. 529–538.

AF Subar (2004). "Developing dietary assessment tools". J Am Diet Assoc. Vol. 104,
pp. 769–770.

L. Nasreddine, F. Naja, M. C. Chamieh, N. Adra, A.M. Sibai and N. Hwalla (2012).
"Trends in overweight and obesity in Lebanon: evidence from two national cross-
sectional surveys (1997 and 2009)". BMC Public Health, vol. 12, pp. 798.

M. Doumit, B. Doughan, R. Baez. (2004). "Programme pour la Sante Bucco-dentaire au
Liban." L'assistance technique pour le développement d'études préliminaires á la
fluoration du sel. Rapport final sur la collecte des données.

I. Al-Saleh, I. Al-Doush (2000) "Urinary fluoride levels in Saudi individuals drinking
tap and bottled water." International Journal of Environmental Health Research,
vol. 10, pp. 21– 26

M. Grimaldo, VH. Borja-Aburto, AL. Ramírez, M. Ponce, M. Rosas, F. Díaz-Barriga
(1995). "Endemic fluorosis in San Luis Potosi, Mexico. Identification of risk
factors associated with human exposure to fluoride." Environ Res. Jan;68(1):25-
30.

SL. Choubisa, L. Choubisa, D. Choubisa (2010). "Osteo-dental fluorosis in relation to
age and sex in tribal districts of Rajasthan, India". J Environ Sci Eng., vol 52(3),
pp. 199-204.

S. M. Levy DDS, M. C. Kiritsy, and J. J. Warren DDS (2007). "Sources of Fluoride
Intake in Children". Journal of Public Health Dentistry. Vol. 55 (1), pp. 39–52.

A. Schulte, R. Rossbach, P. Tramini (2001). "Association of caries experience in 12-
year-old children in Heidelberg, Germany, and Montpellier, France, with different
caries preventive measures." Community Dent Oral Epidemiol, vol. 29, pp. 354–
61.

I. Wennhall, S. Hajem, S. Ilros, K. Ridell, K. R. Ekstrand, S. Twetman (2014). "Fluoridated salt for caries prevention and control – a 2-year field study in a disadvantaged community". International Journal of Pediatric Dentistry Vol. 24, pp.161–167

S. Tubert-Jeannin, P.J. Riordan, R. Manevy, M.M. Lecuyer and E. Pegon-Machat (2008). "Caries prevalence and fluoride use in low SES children in Clermont-Ferrand (France)". Community Dental Health Vol. XX, pp. 1–06

F. Liu, J. Ma, H. Zhang, P. Liu, YP. Liu, B. Xing, YH. Dang (2014). "Fluoride exposure during development affects both cognition and emotion in mice." Physiol Behav. Vol. 124, pp. 1-7

MA. Buzalaf, JP. Pessan, HM. Honório, JM. ten Cate (2011). "Mechanisms of action of fluoride for caries control. Monogr Oral Sci., vol. 22, pp. 97-114