UNIVERSITI PUTRA MALAYSIA

LEVEL OF FLUORIDE IN DRINKING WATER AND ITS RELATIONSHIP WITH URINARY FLUORIDE AMONG, TIDRTEEN YEAR-OLD SCHOOL CIDLDREN IN A SECONDARY SCHOOL IN KUALA LUMPUR

FAIZ AMIN BIN AMINUDDIN

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FAIZ AMIN BIN AMINUDDIN

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Report submitted to the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Medical Doctor

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Level of Fluoride in Drinking Water and Its Relationship with Urinary Fluoride among Thirteen year-old School Children in a Secondary School in Kuala Lumpur

Faiz A.A.[1], Goh S.L.[1], Shaharuddin M.S.[2] & Anisah B.[2]

[1] *Second Year Medical Student*
[2] *Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia*

ABSTRACT

A cross-sectional study was carried out to determine the level of fluoride in drinking water and its relationship with urine among thirteen year-old school children in Sekolah Menengah Kebangsaan Aminuddin Baki, Kuala Lumpur. Drinking water and urine samples were collected from respondents for three consecutive days. The level of fluoride in drinking water and urine were analyzed by SPADNS method using the spectrophotometer HACH DR/2500. Questionnaires were administered to obtain the demographic data. A total of 65 respondents were recruited into this study, where 41 were males while 24 were females. Results showed that the mean level of fluoride in drinking water was 0.34 ± SD 0.20 mg $L^{-1}$and the mean for urinary fluoride was 1.37 ± SD 0.39 mg L^{-1}. The fluoride level in drinking water, 39 (60.0%) samples were underranged, 19 (29.2%) were normal and while 7 (10.8%) were overranged. All urinary fluoride level were within the normal range. Based on the Pearson test, there was no significant relationship between level of fluoride in drinking water and urine (p = 0.694). Independent samples t-test showed that there was no significant difference in urinary fluoride content between males and females (p= 0.082), while one-way ANOVA showed that there was no significant difference in fluoride levels at different sampling locations (P = 0.514). It was concluded that there are other sources of fluoride intake such as foods and beverages and not only specified to fluoridated drinking water.

Key word: fluoride, drinking water, urine, school children, Kuala Lumpur

Kepekatan Fluorida dalam Air Minuman dan Hubungannya dengan Fluorida dalam Urin dalam kalangan Pelajar Sekolah Tiga Belas tahun di Sekolah Menengah di Kuala Lumpur

Faiz A.A.[1], Goh S.L.[1], Shaharuddin M.S.[2] & Anisah B.[2]

[1] *Pelajar Perubatan Tahun Dua*

[2] *Jabatan Kesihatan Komuniti, Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia*

ABSTRAK

Satu kajian keratan rentas telah dijalankan untuk mengenalpasti kepekatan fluorida dalam air minuman dan hubungannya dengan fluorida dalam urin dalam kalangan pelajar sekolah tiga belas tahun di Sekolah Menengah Kebangsaan Aminuddin Baki, Kuala Lumpur. Sampel air minuman dan urin dikumpulkan selama tiga hari berturut-turut. Kepekatan fluorida dalam air minuman dan urin telah diukur dengan mengunakan kaedah SPANDS pada spektrofotometer HACH DR/2500. Soalan kaji selidik telah diedarkan untuk mendapatkan maklumat demografik. Sebanyak 65 responden telah dimasukkan ke dalam kajian, di mana 41 adalah lelaki manakala 24 adalah perempuan. Keputusan menunjukkan purata kepekatan fluorida dalam air minuman adalah 0.34 ± SD 0.20 mg/L dan purata bagi kepekatan fluorida dalam urin adalah 1.37 ± SD 0.39 mg/L. Dalam kepekatan fluorida dalam air minuman, 39 (60.0%) sampel adalah kurang dari julat yang normal, 19 (29.2%) adalah normal manakala 7 (10.8%) adalah melebihi julat yang normal. Semua kepekatan fluorida dalam urin adalah dalam julat yang normal Berdasarkan ujian Pearson, didapati tiada perhubungan yang signifikan antara kepekatan fluorida dalam air minuman dan urin ($p = 0.694$). Ujian independent samples t-test menunjukkan tiada perbezaan yang signifikan dalam kepekatan fluorida dalam urin antara lelaki dan perempuan ($p = 0.082$). Ujian one-way ANOVA menunjukkan tiada perbezaan yang signifikan dalam kepekatan fluorida di antara lokasi-lokasi sampel ($P = 0.514$). Secara kesimpulannya, terdapat sumber pengambilan fluorida yang selain daripada makanan dan minuman dan tidak hanya tertentu kepada air minuman berfluorida.

Kata kunci: fluorida, air minuman, urin, pelajar sekolah, Kuala Lumpur

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LIST OF ABBREVIATIONS

EFSA	*European Food Safety Authority*
EDTA	*Ethyelene Dinitrolotetraacetic Acid*
HF	*Hydrogen Fluoride*
IPCS	*International Programme on Chemical Safety*
MOH	*Ministry of Health*
NIOSH	*National Institute of Occupational Safety and Health*
SPSS	*Statistical Package for Social Science*
UPM	*University Putra Malaysia*
USNRC	*United States Nuclear Regulatory Commission*
WHO	*World Health Organization*

CHAPTER 1

INTRODUCTION

1.1 Background

Fluoride is one of the chemical elements on Earth that is not really essential to the human body as it does not have any particular function in the human body except that fluoride is essential in prevention of a tooth-related disease (Finkelman et al., 2011). Fluorine is a highly reactive gas and the lightest and most electronegative member in Periodic Table of Elements. Therefore, fluorine in the environment can be found as fluorides due to its strong tendency to acquire negative charge. Fluorides represent 0.06–0.09 % of the earth's crust (WHO, 2006). The usage of fluoride has been very important in preventing dental caries (Roumeliotis, 2011). Another usage of fluoride is the formation of denser bones which could prevent osteoporosis (Kaminsky et al., 1990).

However, a high level of fluoride is closely associated with the human health problem. Particularly fluorosis which is a result of the destruction of metabolic calcium and phosphorus by the element fluoride, that causing the inhibition of active enzymatic process in the human body, which also interrupts the function of the endocrine system; that will leads to the formation of fluorosis especially at the calcified tissues namely bones and teeth (Xiang et al., 2004).

Nowadays, the recent human activities and the recent revolutionize of the product manufacturing industries have a significant role in the increasing of fluoride exposures to the environment and eventually to the biosphere of the Earth. Not only that fluoride can be found in dental products and any other household items, but it also can be found in foods and drinks which will endanger humans when consuming it in large quantities (Finkelman et al., 2011).

Fluoridation process in the public drinking water system is considered essential in many countries as it is the process of turning raw water from the river into a drinking water that is safe and suitable for human consumption. Normally, the levels of fluoride that reach households are ranging from 0.5 mg/L to 1.0 mg/L. Fluoridation of public drinking water system thus far is the most effective way to ensure healthy teeth of the community (Ericsson & Ribellius, 1971).

1.2 Problem Statement

Dean (1954) found out that fluoride levels of 1.0 mg/L would not increase the severity of dental fluorosis. Therefore, fluoride levels must be controlled and the standard of 1.0 mg/L of fluoride level is still maintained until today. Fluoride however has the potential to become toxic if consumed a lot. The toxic effect of fluoride can be either chronic or acute. Acute toxicity will be resulted in death when a person ingests doses of 2.5 g to 5.0 g which is proportionate to 2500 to 5000 litres of water containing 1.0 mg/L of fluoride (Horowitz et al., 1984).

Consumption of water containing 1.0 mg/L or less of fluoride during tooth calcification and development may somehow contribute to the development of dental fluorosis, but it was not categorized as serious (Jackson et al., 1995). Furthermore, the World Health Organization (WHO) concluded that at a fluoride level of 0.9 mg/L to 1.2 mg/L, a very mild dental fluorosis can be occurred at these levels of consumption (World Health Organization, 1997).

Like most of the soluble substances, fluoride compounds are readily absorbed by the stomach and intestines, and are excreted through urine. Urine tests have been used to ascertain rates of excretion in order to set upper limits in exposure to fluoride compounds and thus, urine has been a biomarker for fluoride level in the body prior to fluoride intake (Baez et al, 2000).

Ministry of Health Malaysia (MOH) report indicated that 62.2% of the Malaysian public receives processed water with artificial fluoridation and the recommended levels are at 0.5 mg/L to 0.9 mg/L (Ministry of Health Malaysia, 1996). Another study by the Ministry of Health Malaysia in the year 2002 stated that fluoride levels in a few Malaysian states were above the recommended levels and this may be a factor that contributes to the higher prevalence of dental fluorosis among people (Ministry of Health Malaysia, 2002).

So, the aim in this study is to determine the level of fluoride in drinking water and its relationship with urinary fluoride to observe if the drinking water is contributing to the level of fluoride in urine among 13-year-old school children in a secondary school in Kuala Lumpur.

1.3 Objectives

1.3.1 General Objective

To determine the level of fluoride in drinking water and its relationship with urinary fluoride among 13-year-old school children in a secondary school in Kuala Lumpur.

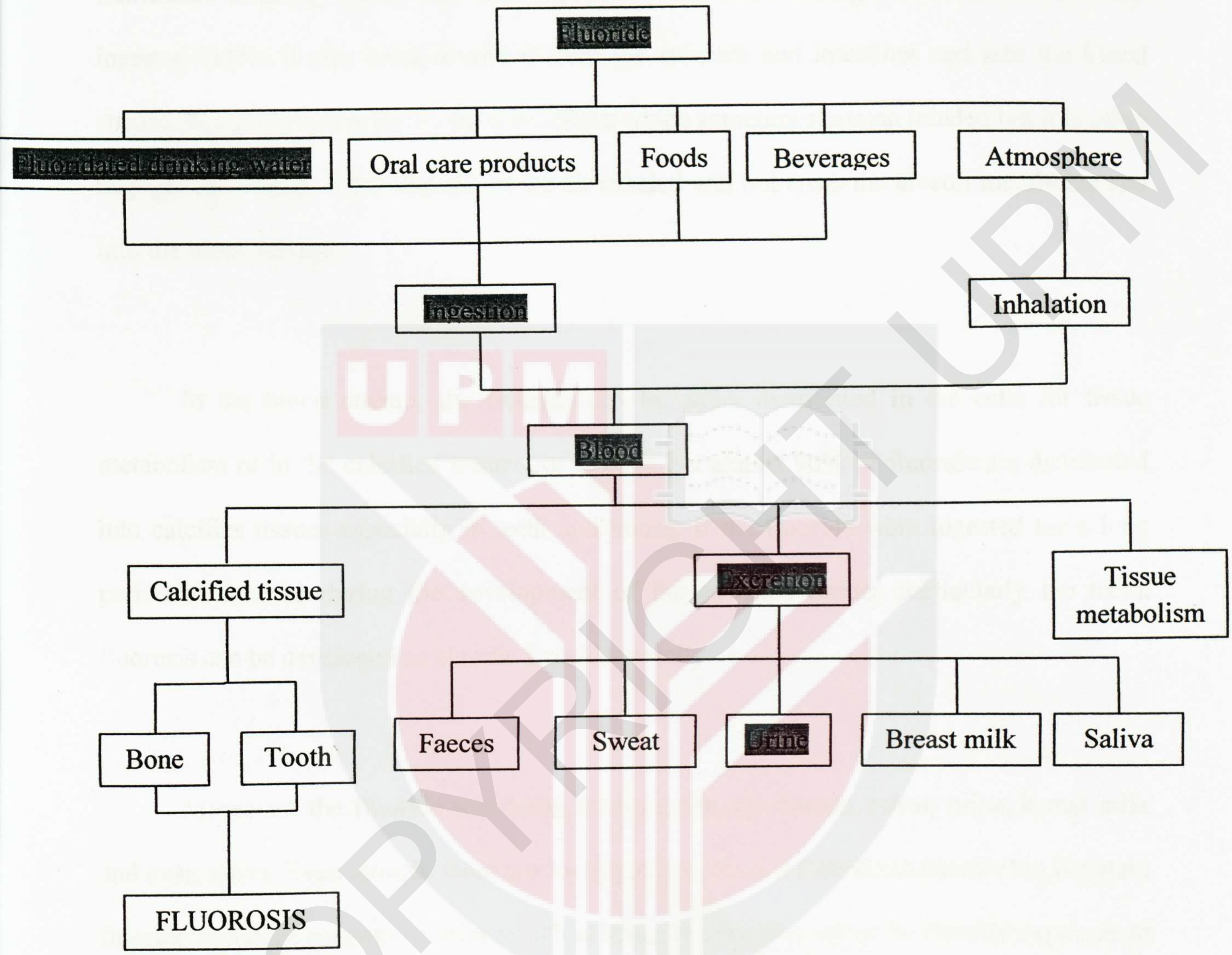
1.3.2 Specific Objectives

1. To determine level of fluoride in drinking water.
2. To determine urinary fluoride level of respondents.
3. To determine the difference in urinary fluoride level among male and female respondents.
4. To determine the difference in fluoride levels between different sampling locations.
5. To determine the relationship between fluoride in drinking water and urinary fluoride of respondents.

1.4 Research Hypothesis

1. There is a significant difference in urinary fluoride level among male and female.
2. There is a significant difference in fluoride levels between difference sampling locations.
3. There is a significant relationship between fluoride in drinking water and urinary fluoride of respondents.

1.5 Conceptual Framework



LEGEND: Highlighted area represents focus of our research.

Figure 1: Conceptual framework of fluoride metabolism

The conceptual framework shows the flow chart of our study from the fluoride element which is ubiquitous in nature and even in the human consumption products such as fluoridated drinking water, oral care products, foods and beverages that human used and ingested before it was being absorbed from the stomach and intestines and into the blood stream. Fluoride even exists in the atmosphere which sometimes human inhaled but it is often negligence as most of the fluoride in the air inhaled will not cross the alveoli membrane and into the blood stream.

In the blood stream, the fluoride can be either distributed in the cells for tissue metabolism or in the calcified tissues for storage but almost 90% of fluoride are distributed into calcifies tissues especially in teeth and bones. If the fluoride were ingested for a long period of time or during the development of the calcified tissues particularly the teeth, fluorosis can be developed as chronic fluoride toxicity.

Moreover, the fluoride was being excreted through faeces, sweat, urine, breast milk and even saliva. Even though, there is a lot of pathway for the fluoride to excrete but the main fluoride excretion pathway is urine which is being used as biomarker for fluoride exposure or fluoride intake during a particular period of time.

Therefore, the highlighted part is where our study was focused on especially the fluoridated drinking water and the urine.

CHAPTER 2

LITERATURE REVIEW

2.1 Fluoride

Fluorine is a highly reactive gas and is the lightest and most electronegative member in Periodic Table of Elements. Therefore, fluorine in the environment can be found as fluorides due to its strong tendency to acquire negative charge. Fluorides represent 0.06–0.09 per cent of the earth's crust (WHO, 2006).

Traces of fluoride are found in our daily life products. Fluoride is found in many dental products to prevent dental decay, especially the toothpaste, mouth rinse and gel for topical treatment. Besides that, almost every food and beverages contain fluoride. Vegetables and fruits have low levels of fluoride which is approximately 0.1-0.4 mg kg^{-1} while barley and rice normally have higher fluoride levels, approximately 2 mg kg^{-1} (Murray, 1986). Fluoride accumulates in bone, even though the level is relatively low, naturally occurring fluoride is found in fish and sardine-canned fish. Mechanically deboned meat such as chicken fingers and nuggets contain elevated levels of fluoride due to the contamination from bone particles during meat processing. In facts, meat and fish contain approximately about 0.2-1.0 mg kg^{-1} and 2-5 mg kg^{-1} respectively in diet (Murray, 1986). Instant tea, iced tea, brick tea are favorites of the public because of its sweet taste. However, many do not know that tea contains fluoride. The fluoride content of tea has been found to range from 0.1 to 4.2 parts per million (ppm) fluoride, with an average of about 3 ppm (Levy & Guha-Chowdhury, 1999).

2.2 Benefits of fluoride

A study from Roumeliotis (2011) shows that there is significant relationship between dental caries prevalence and fluoridated drinking water. Dental caries are local infections which can sometimes be dangerous when spread to eyes and brain. The study concluded that water fluoridation at recommended level contributes to decreases of dental caries in non-fluoridated area. Health Canada has stated that the ideal level of fluoride in water is 0.7 mg/L while Ontario's recommended levels are within 0.5 to 0.8 mg/L. Tooth decay is caused by bacteria in the mouth. Bacteria cause production of acid that remove minerals from surface of tooth. This is known as 'demineralization' which can lead to dental caries. Fluoride promotes remineralization of enamel to strengthen the teeth. Therefore, water fluoridation at recommended level is safe and effective to reduce tooth decay at all stages of life.

Another beneficial effect of fluoride is formation of denser bones. Thus, fluoride has been suggested as a therapeutic agent in the treatment of osteoporosis by stimulates the osteoblastic activity and increases the hardness of bones (Kaminsky et al., 1990).

2.3 Harmful effects of fluoride

Over consumption of fluoride from different sources can result in health problems. Adverse effects of fluoride can be divided into acute toxicity and chronic toxicity.

2.3.1 Acute toxicity

Acute toxic doses range from 1 to 5 mg/kg whereas doses exceeding 15 to 30 mg/kg may be fatal (Kaminsky et al., 1990). Acute toxicity can occur due to a single ingestion of a large amount of fluoride. Acute fluoride poisoning is characterized by gastrointestinal symptoms such as nausea, vomiting, paresthesias, abdominal pain, and diarrhea before systemic poisoning set in. Signs of acute systemic poisoning include generalized malaise, pallor, weakness, tachycardia, hypotension, central nervous system depression, respiratory depression, paralysis, seizures, tetany and ultimately can be fatal (Frank,1993).

2.3.2 Chronic toxicity

2.3.2.1 Dental effects

Chronic fluoride toxicity is due to long-term consumption of low dose fluoride. High levels of fluoride present in levels up to 10 mg/L were found to have associated with dental fluorosis. (WHO, 2006) Chronic ingestion of fluoride in drinking water can lead to dental fluorosis which is easier to recognize compared to pathological effect in bones. Dental fluorosis is a diffuse symmetric hypomineralization disorder of ameloblasts. Fluorosis that caused by exposure of fluoride can be irreversible if the enamel is still developing. Normal enamel is creamy-white translucent color, whereas fluorosed enamel is porous and opaque, teeth resemble a ghastly white chalk color. While other characteristics are cloudy striated enamel, white specks or blotches, 'snow- capping', yellowish-brown spots, or brown pits on teeth. In severe form, fluorosed enamel is structurally weak, brittle and prone to erosion and breakage (Dhar & Bhatnagar, 2009).

2.3.2.2 Skeletal effects

Excessive fluoride more than 8 ppm in drinking water daily for years is able to cause skeletal fluorosis. Cases are more severe in warm climates where drinking water contains relatively high level of fluoride. Bone density slowly increases due to chronic consumption. As a result, toxicity accumulates in the bone, joints become stiff and painful. However, skeletal effects are not clinically obvious until the advanced stage. Early cases are often misdiagnosed as rheumatoid or osteoarthritis because some of the clinical symptoms are arthritis mimicking. Though fluoride increases the stability of the crystal lattice in bone, it makes bone more brittle. Severity of skeletal fluorosis depends directly on the level and duration of exposure (Dhar & Bhatnagar, 2009). Crippling skeletal fluorosis is a harmful effect of chronic fluoride exposure, which resulted from osteosclerosis, ligamentous and tendinous calcification and extreme bone deformity. Total intake of 14 mg per day there is a clear excess risk of skeletal adverse effects and there is suggestive evidence of an increased risk of effects on the skeleton at total fluoride intakes above about 6 mg per day (IPCS, 2002). Epidemiologically, endemic skeletal fluorosis is known to occur at several parts of the world, including India, China and northern, eastern, central and southern Africa, with range of severity (WHO, 2006).

2.3.2.3 Others health effects

Other health effects of chronic fluoride toxicity have been associated with thyroid changes, growth retardation, kidney changes, and even urolithiasis. Some people are unusually susceptible to the toxic effects of fluoride and its compounds. The populations at risk are normally the elderly, people with deficiencies of calcium, magnesium, and/or vitamin C, and people with cardiovascular and kidney problems (Dhar & Bhatnagar, 2009).

2.4 Fluoride metabolism

2.4.1 Absorption

The predominant route of absorption of fluoride is via the gastrointestinal tract. Atmospheric fluoride may also be inhaled and followed the respiratory tract. While dermal absorption is almost negligible except in the cases of hydrofluoric acid burns (WHO, 1984).

When fluoride ions enter the acidic environment of the stomach lumen, fluoride ions are largely converted to weak acid hydrogen fluoride (HF) with a pKa of 3.45 (WHO, 2002). The higher acidity in the stomach speeds up the process of absorption by passive diffusion from both the stomach and small intestine, it is suggesting that the fluoride is absorbed from the stomach as an undissociated hydrogen fluoride rather than as a fluoride ion (Cerklewski, 1997; Whitford & Pashley, 1984). Most of the fluoride that is not absorbed from the stomach will be rapidly absorbed from the small intestine. There is no evidence suggesting that the active transport processes are involved (WHO, 2002).

2.4.2 Distribution

Once fluoride is absorbed into the bloodstream, it is readily to be distributed throughout the body. Approximately 99% of fluoride is retained in the calcium-rich areas such as bones and teeth where it is incorporated into the crystal lattice. In infants, about 80-90% of the absorbed fluoride is retained however, in adults; this level falls to 60%. Fluoride is able to cross the placenta and is found in mother's milk at low levels (WHO, 1996; IPCS, 2002). The level of fluoride found in bone is varied according to the part of the bone examined, age and gender of the individual. Bone fluoride is considered to be a reflection of long-term exposure to the fluoride (IPCS, 2002).

2.4.3 Excretion

Mostly fluoride is excreted in the urine but there is some fluoride is excreted in the faeces, sweat and even been deposited in the skin which later will be shed. Fluoride even occurs in milk, saliva and tears as a trace element. Fluoride is probably not exhaled in the breath even though there is no evident of it. But the most principal excretion of fluoride is through urine in the kidney (IPCS, 2002). The clearance of urinary fluoride level increases with urine pH due to the decreasing in level of hydrogen fluoride. Several factors for example, total intake of fluoride, health status of the individual, diet and even drugs; can affecting the urine pH and thus affecting fluoride clearance and fluoride retention (USNRC, 1993).

2.5 Biomarkers of fluoride exposure and their status

2.5.1 Plasma, saliva and urine as contemporary markers

The levels of fluoride in bodily fluids namely plasma, saliva and urine give some indication of recently fluoride intake. Fluoride ion itself does not produce any metabolites and so it is suitable for measured indicator. Despite that however, this indicator does not well indicate the fluoride body burden or the accumulation of fluoride in the body, as the relationship between the fluoride level of fluoride in bone and in extracellular fluids is incompletely defined. Furthermore, the level of fluoride in plasma, urine, saliva, and dental plaque is dependent on the intake of fluoride via water, diet, fluoride supplements and fluoride-containing dental products (Ekstrand et al., 1977; Ekstrand, 1977; Sjogren et al., 1993; Ekstrand, 1997; Twetman et al., 1998; Stephan Eakle et al., 2004).

2.5.2 Nails and hair as recent markers

The level of fluoride in nails and hair appears to be proportional to intake over longer periods of time, taking into account of their growth rate. Daily intake from food, water, dental products or fluoride supplements may contribute to this exposure of fluoride in the environment at the place of residency including occupational exposure. The major advantage of nails and hair over fluids and tissues as a biomarker for the fluoride exposure is that they can be easily access by non-invasive manner. Different from bodily fluids whose fluoride levels provide a snapshot at a certain point of time and they are subjecting to change due to recent fluoride intake and certain physiological variables, the level of fluoride in nails and hair is cumulative and reflects the average level of intake over a period of time, but depends on how often the nails are clipped or hair cut (Schamschula et al., 1985; Czarnowski et al., 1996; Kokot et al., 2000; Whitford et al., 2005).

2.5.3 Calcified tissues as historical markers

About more than 99% of body burden fluoride is found in the calcified tissues. Generally, it is agreed that the level of chronic exposure towards fluoride extending over a period of years is best assessed by determining fluoride levels in bone and teeth (Y. Ericsson et al., 1973). Calcified tissues namely bone and dentine which accumulate fluoride throughout life and whose levels are proportional to the absorbed dose of fluoride, fluoride in enamel however is not an appropriate biomarker as most of its fluoride was taken up during tooth development (EFSA, 2005). The post-eruptive fluoride uptake of enamel is expressed only in the outer layer and depends on the level of fluoride in the oral cavity (WHO, 1994).

2.6 Urinary fluoride

Urine is considered one of the most commonly biomarker for fluoride excretion in public health and epidemiology studies on normal children (Ketley & Lennon, 2001). Approximately 30-50% of fluoride is excreted through urine in children (Ketley & Lennon, 2001; Villa, Anabalon & Cabezas, 2000).

Some dietary factors that can increase or decrease the absorption and excretion of ingested fluoride, making the body's retention of fluoride quite important yet variable needs to be considered and it somehow affects the urinary fluoride level (Maguire et al., 2007).

Fluoride excretion is influenced by a number of factors particularly the health of the kidneys, including glomerular filtration rate, urinary flow and urinary pH. The excretion of fluoride in urine is obviously reduced in individual with an impaired kidney function. Urine fluoride excretion is 0.79 mg/day in humans with normal kidney function, while 0.53 mg/day in those with questionable health issue and 0.27 mg/day in those with impaired kidney function (Singer et al., 1982).

The main pathway for urine excretion was through the kidney and urinary fluoride level excretion is corresponding to about 50% of fluoride intake during that time (Cross et al., 2003). Many studies have shown that urinary fluoride level was correlated to the amount of fluoride intake, and thus the urinary fluoride level is considered to be a good indicator for indication of fluoride exposure (Symonds et al., 1988).

CHAPTER 3

METHODOLOGY

3.1 Study location

This study was conducted at Sekolah Menengah Kebangsaan Aminuddin Baki, Kuala Lumpur.

3.2 Study design

A cross-sectional study design was used in this study. In this study, the exposure status (level of fluoride in drinking water) and the outcome (urinary fluoride level) were measured in a target population at a point of time. The objective was to determine the relationship of level of fluoride in urine and drinking water.

3.3 Study duration

The study started from 25th March to 6th September 2013, a total of approximately six months' duration. Data collection was conducted from 1st July to 10th July 2013.

3.4 Sampling

3.4.1 Study population

The target population was 13-year-old students from Sekolah Menengah Kebangsaan Aminuddin Baki.

3.4.2 Sampling population

a. Inclusion criteria

The inclusion criteria were 13-year-old students who studying at Sekolah Menengah Kebangsaan Aminuddin Baki, using tap water as drinking water, as well as residents of the study area.

b. Exclusion criteria

The exclusion criteria were those who absent on the day of study or had diabetes mellitus and kidney diseases. Respondents who were using water filter system at home were also excluded.

3.4.3 Sampling frame

List of 13-year-old students studying at Sekolah Menengah Kebangsaan Aminuddin Baki.

3.4.4 Sampling unit

13-year-old student studying at Sekolah Menengah Kebangsaan Aminuddin Baki.

3.4.5 Sampling methods

Purposive sampling was used in this study. It was a form of non-probability sampling in which decisions concerning the respondents to be included in the sample are taken by the researcher, based upon inclusion and exclusion criteria.

3.4.6 Sample size

The sample size was single mean sampling. In order to estimate the sample size, the formula of Lu & Cornelius (2006) was used: -

$$N = \frac{Z^2_{1-\alpha/2}\delta^2}{d^2}$$

Where:

N = sample size

$Z_{1-\alpha/2}$ = standard errors associated with 95% confidence interval

= 1.96

δ = estimated standard deviation of fluoride level in drinking water. (Fatin, Lim & Chow, 2012)

= 0.13

d = desired precision

= 0.03

So, when substitute:-

$$N = \frac{(1.96)^2(0.13)^2}{0.03^2}$$

= 72

So the minimum sample size was 72. After considering 10% of the dropout rate or missing and incomplete data, the final sample size calculated was around 79.

3.5 Instruments and data collection

3.5.1 Instruments/Questionnaire

A validated and standardized questionnaire was distributed among respondents to collect information. The questionnaire consisted of four sections: Section A: Socio-demographic factor, Section B: Information on dental health status, Section C: Information on fluoride exposure, and Section D: Information on family background. Next, morning urine and drinking water samples were collected from respondents for three consecutive days. This was then analyzed within 72 hours using direct reading spectrophotometer. SPADNS method was used to measure the level of fluoride in drinking water and urine samples.

3.5.2 Data collection

Eligible respondents were gathered at the hall after permitted by principal of Sekolah Menengah Kebangsaan Aminuddin Baki. The details of the research were explained and every student was given a consent form to obtain parents' approval. After collected the consent form, questionnaire was then distributed to all 13-year-old students and they were given time to answer all the questions on the spot. The questionnaire section was under supervision of researchers. 50 ml of morning urine and 250 ml of home drinking water were collected for three consecutive days. The urine sample bottles were added with 0.2 g of EDTA to preserve the urine and placed in cooler box and finally transported to the laboratory. Level of fluoride in urine and drinking water were analyzed using SPADNS method.

3.5.3 Quality Control

The quality control was the pre-test questionnaire to 10% of the total number of respondents. Standardization and calibration of the equipments such as spectrophotometer was done for every 10 samples examined.

3.6 Data analysis

Data was analyzed by using Statistical Package for Social Sciences Program, SPSS version 20.0. Descriptive analysis was used to produce raw and basic statistical data of fluoride level in both drinking water and urine. The data was presented in the form of mean, variance, standard deviation maximum and minimum value. The significant difference of urinary fluoride level between genders was determined by using 2 independent sample t-test. The significant difference of fluoride level at different sampling locations was determined by one-way ANOVA. The relationship of fluoride level in drinking water and urine was determined by Pearson test.

3.7 Study ethics

Ethical clearance to carry out this study was first obtained from the Ethical Committee of Faculty of Medicine and Health Sciences, UPM. With the approval of the target school authorities, consent forms were given to eligible students to get permission from them as well as their parents.

3.8 Variables

3.8.1 Dependent variables

The dependent variable was urinary fluoride level among 13-year-old students in Sekolah Menengah Kebangsaan Aminuddin Baki.

3.8.2 Independent variables

The independent variables were fluoride levels in drinking water, gender, socio-economic status, and demographic factors.

3.9 Definition of terms

1. Urinary fluoride level:

   The level of fluoride in urine which act as a biomarker to measure the level of fluoride intake at that particular period of time (Ketley & Lennon, 2001).

CHAPTER 4

RESULTS

4.1 Socio demographic data

This study took place at Sekolah Menengah Aminuddin Baki, Kuala Lumpur. It involved 65 13-year-old school children. Our minimum sample size was 79 respondents. The number of questionnaires distributed was 79. However, 65 were returned. Thus, the response rate of our study was 82.28%. From 65 respondents, 41 (63.1%) were male and 24 (36.9%) were female. Table 4.1 shows the respondents' gender distribution.

Table 4.1: Respondents' gender distribution

Gender	Number of respondents (n)	Percentage (%)
Male	41	63.1
Female	24	36.9

Out of 65 respondents, 35 (53.8%) of them were Malays, followed by 19 (29.2%) Chinese, 8 (13.8%) Indians and 3 (3.1%) were other ethnic group such as Sikh and Punjabi. Please refer Table 4.2.

Table 4.2: Respondents' ethnic group distribution

Races	Number of respondents (n)	Percentage (%)
Malay	35	53.8
Chinese	19	29.2
Indian	8	13.8
Others	3	3.1

Regarding the residential locations where the water samples were collected, it can be categorized into 7 different locations namely, Kuala Lumpur, Ampang, Cheras, Wangsa Maju, Kajang, Petaling Jaya and Puchong. From 65 respondents, 36 (55.4%) respondents stayed in Kuala Lumpur, 14 (21.5%) respondents stayed Ampang, 9 (13.8%) respondents lived in Cheras and 3 (4.6%) respondents stayed in Wangsa Maju. Only Kajang, Petaling Jaya and Puchong each have 1 respondent. Please refer to Table 4.3.

Table 4.3: Distribution of water samples based on various residential locations

Location	N	%
Kuala Lumpur	36	55.4
Ampang	14	21.5
Cheras	9	13.8
Wangsa Maju	3	4.6
Kajang	1	1.5
Petaling Jaya	1	1.5
Puchong	1	1.5
Total	65	100.0

In the occupation category, 38 (58.46%) of the students' fathers worked in the government sector, 25 (38.46%) worked in the non-government sector and 2 (3.08%) of them worked as self-employment. Please refer to Table 4.4.

Table 4.4: Respondents' fathers occupation distribution

Occupation	Number of respondents (n)	Percentage (%)
Government sector	38	58.46
Non-government sector	25	38.46
Self-employment	2	3.08

Regarding respondents' mothers occupation, 37 (56.92%) of them were housewives and 28 (43.08%) worked either in the government or non-government sectors. Please refer to Table 4.5.

Table 4.5: Respondents' mothers occupation distribution

Occupation	Number of respondents (n)	Percentage (%)
Housewife	37	56.92
Employment	28	43.08

4.2 Fluoride levels in drinking water and urine

Mean for urinary fluoride level was 1.37 ± SD 0.39 mg/L. The minimum reading obtained was 0.44 while the highest value was 2.19. And the variance of urinary fluoride level was 0.15.

Mean for fluoride in drinking water was 0.34 ± SD 0.20 mg/L. The minimum reading obtained was 0.02 while the highest value that can be seen was 0.73. And the variance for fluoride in drinking water was 0.04. Please refer to Table 4.6.

Table 4.6: Fluoride levels in drinking water and urine

	Drinking water	Urine
Mean	0.34	1.37
Standard deviation	0.20	0.39
Variance	0.04	0.15
Maximum value	0.73	2.19
Minimum value	0.02	0.44

According to Table 4.7, the fluoride level in drinking water and urine can be categorized into three namely, underranged, normal and overranged. The normal range for fluoride level in drinking water is between 0.4 to 0.6 mg/L while the normal range for urinary fluoride level is between 0.2 to 3.2 mg/L. In fluoride level in drinking water, 39 (60.0%) water samples were underranged, 19 (29.2%) were normal and while 7 (10.8%) were overranged.

In urinary fluoride level, all the urine samples were normal range.

Table 4.7: Categorization of fluoride levels in drinking water & urine

	Drinking water		Urine	
	N	%	N	%
Underrange	39	60.0	0	0
Normal	19	29.2	65	100
Overrange	7	10.8	0	0
Total	65	100.0	65	100

4.3 Difference in urinary fluoride level among male and female respondents.

When comparing the level of urinary fluoride based on gender, the mean of urinary fluoride level in males was higher than in females. The mean of urinary fluoride level in males was (1.44 ± SD 0.40) mg/L while the mean of urinary fluoride level in females was (1.26 ± SD 0.37) mg/L. The Shapiro-Wilk test was used to determine the normality of the level of urinary fluoride. From the test, level of urinary fluoride was normally distributed ($p = 0.078$). Therefore, the relationship of urinary fluoride level with gender was studied using independent samples T-test. Levene's test showed equal variance assumed ($p = 0.899$). However, there was no significant difference in mean of urinary fluoride level between male and female respondent [$t (63) = 1.769$, $p = 0.082$]. Please refer to Table 4.8.

Table 4.8: Difference of urinary fluoride level between males and females

Gender	Average urinary fluoride level (mg/L)		t	Df	P
	Mean ± SD	Mean difference			
Male	1.44 ± 0.40	0.18	1.769	63	0.082
Female	1.26 ± 0.37				

4.4 Difference in fluoride level of drinking water from different sampling locations.

Mean fluoride level in drinking water was the highest at Kajang (0.44mg/L), which is within the normal range, while the lowest at Wangsa Maju (0.17 ± SD 0.20) mg/L, which is under range. The Shapiro-Wilk test was used to determine the normality of the level of fluoride in drinking water. From the test, level of fluoride in drinking water was normally distributed ($p = 0.078$). Next, Levene's test was used to determine equality of variance. From this test, the equal variance assumed ($p= 0.316$). Hence, one-way ANOVA was used to determine the difference in fluoride levels from different sampling locations. From the results, there was no significant difference in fluoride levels from different sampling locations ($P = 0.514$). Please refer to Table 4.9.

Table 4.9: Relationship of fluoride level in drinking water from all 7 locations

Locations	Fluoride level in drinking water (mg/L)				ANOVA test
	Mean	Standard deviation	Maximum	Minimum	
Kuala Lumpur	0.34	0.20	0.02	0.73	F (6, 40) = 0.825, P= 0.514
Ampang	0.38	0.22	0.02	0.73	
Cheras	0.30	0.14	0.10	0.52	
WangsaMaju	0.17	0.20	0.02	0.40	
Kajang	0.44	-	0.44	0.44	
Petaling Jaya	0.41	-	0.41	0.41	
Puchong	0.30	-	0.30	0.30	

4.5 Relationship between fluoride in drinking water and urinary fluoride of respondents

The Shapiro-Wilk test was used to determine the normality of the level of fluoride in drinking water and urine. From the test, level of fluoride in drinking water ($p = 0.078$) and urine ($p = 0.078$) were normally distributed. Hence, Pearson test was used to determine the relationship between level of fluoride in drinking water and urine. From this parametric test, there was no significant relationship between level of fluoride in drinking water and urinary fluoride level of respondents ($r = 0.05$, $p = 0.694$).

CHAPTER 5

DISCUSSION, CONCLUSION, AND RECOMMENDATION

5.1 DISCUSSION

5.1.1 Socio demographic data

This research consisted of 65 13-year-old school children from Sekolah Menengah Kebangsaan Aminuddin Baki, Kuala Lumpur. The reason this school was being chosen because there was no study that had been done in this school yet. Therefore, we aimed our study to determine the level of fluoride in drinking water and its relationship with urinary fluoride among these 65 respondents. The final sample size (65 respondents) was less than the expected sample size (79 respondents) due to inadequate samples or respondents and time constraint in collecting the data. Moreover, there were even some incomplete data and some respondents were unable to obtain their parents' consent, which needed to be considered. The method of selection of respondents was based on the description which was previous discussed in Chapter 3.

The respondents were selected according to the inclusion criteria which were 13-year-old school children from Sekolah Menengah Kebangsaan Aminuddin Baki who used the tap water for drinking and cooking without the usage of any water filtering system, this included those who do not have diabetes and kidney disease as well as the permanent residents in the study area. The diabetic patients were excluded in the study due to the fact that most diabetic patients would consume water in large quantities and thus, this could directly increase the fluoride intake (Banu et al., 1997). Furthermore, individuals with kidney disease were excluded in the study because of the impaired kidneys have decreased the ability to excrete the fluoride in urine, and thus the urinary fluoride level might be inaccurate in these individuals (Bansal & Tiwari, 2006).

According to the questionnaire, majority of the respondents were Malay. Furthermore, most of the respondents were living in Kuala Lumpur while the rest were in Ampang, Cheras, Wangsa Maju, Kajang, Petaling Jaya and Puchong. While, in the term of the parents' occupation distribution, it covered a range of jobs especially in the government sector, non-government sector, self-employment and even housewife. Most of the father's respondents worked in the government sector with 58.46% while most of the mother's respondents worked as housewives with 56.92%.

5.1.2 Fluoride levels in drinking water and urine

The level of fluoride in drinking water and urine was determined by using the HACH brand DR/2500 direct reading spectrophotometer with wavelength of 580 nm. From the analysis that was obtained, the level of fluoride in drinking water and urine was normally distributed.

Based on the Ministry of Health Malaysia (2002), the normal range of level of fluoride in drinking water is within 0.4-0.6 mg/L. However, the mean value that was obtained in this study was 0.34 ± SD 0.20 mg/L. This was below the recommended level of fluoride range recommended by the Ministry of Health Malaysia. This might be due to the dilution of the fluoride content in the fluoridated water over the distance from the water preserve plantation to the residential areas (Foulkes & Anderson, 1994). Although the mean value is below recommended level but the maximum level of fluoride in drinking water recorded was 0.73 mg/L. This showed that some houses had exceeded the level recommended from the Ministry of Health Malaysia. This might be due to run off from weathering of fluoride-containing rocks and soils that were leaching from the soil into the groundwater (Doull et al., 2006).

A local study by Shaharuddin et al (2009) in the area around Sri Serdang, Selangor which showed the mean value of the level of fluoride in drinking water was 0.71 ± SD 0.12 mg/L which was above the recommended level according to the Ministry of Health Malaysia. A study in China by Zhang et al (2003) showed that the mean value of level of fluoride in drinking water was 3.0 mg/L which was very high according to WHO recommended level.

Based on the National Institute Occupational Safety and Health (NIOSH, 1984), the normal range of urinary fluoride level is 0.2–3.2 mg/L. Fortunately in this study, the mean value that had obtained was 1.37 ± SD 0.39 mg/L which was within the normal range according to NIOSH. Similarly, the maximum value of urinary fluoride level was 2.19 mg/L while the minimum value was 0.44 mg/L which were within the normal range. The maximum and the minimum values of urinary fluoride level reflected the fluoride intake at that particular period of time. The fluoride intake had been varied from foods to beverages, and not only specific to fluoridated drinking water.

Similarly, a study in India at the Jhajjar District, Haryana by Yadav & Lata (2003) which had a normal range of fluoride in drinking water, was found that the mean urinary fluoride level was 1.50 mg/L which was within the normal range. However, a study in China by Lu et al (2000) showed that areas with high fluoride in drinking water resulted in a high mean value of urinary fluoride level (4.99 ± SD 2.57 mg/L). While areas with low fluoride content in drinking water resulted in urinary fluoride level which was within the normal range of 1.43 ± SD 0.64 mg/L.

5.1.3 Difference in urinary fluoride level among male and female respondents.

When comparing the level of urinary fluoride based on gender, the mean of urinary fluoride in males was higher than in females. The mean of urinary fluoride in males was (1.44 ± SD 0.40) mg/L while the mean of urinary fluoride in females was (1.26 ± SD 0.37) mg/L. A study done by Czarnowski and Krechniak (2002) reported that the urinary fluoride level was significantly higher in males than females. This may be caused by greater physical activity of the boys.

The relationship of urinary fluoride level with gender was studied using independent samples T-test. From the result, there was no significant difference in mean of urinary fluoride level between males and females [t (63) = 1.769, p= 0.082]. This may be due to the average consumption of fluoride from drinking water and diet is the same for both genders. A previous study by Héctor et al. (2009) showed that the urinary fluoride level did not affected by gender. Another study done by Acevedo et al. (2007) also reported similar findings, that there was no difference in urinary fluoride level between males and females.

5.1.4 Difference in fluoride level of drinking water at different sampling locations.

The mean fluoride level in drinking water was highest at Kajang (0.44mg/L), while lowest at Wangsa Maju (0.17 ± SD 0.20) mg/L. The maximum reading recorded was at Kuala Lumpur and Ampang, both were 0.73 mg/L (overranged), while the minimum reading recorded was 0.02mg/L (underranged), at Kuala Lumpur, Ampang and Wangsa Maju. In a local study, Shaharuddin et al. (2009) reported that Sri Serdang, Selangor was the highest, at 0.71 ± SD 0.12 mg/L (overranged), while the level of fluoride in the samples from Kota Kinabalu had the lowest mean level of fluoride, at 0.08 ± SD 0.06 mg/L (underranged). The mean levels of fluoride in the majority of samples were lower than the level recommended by the health authorities, for example 0.5–0.7 mg/L, which is insufficient for eradicating dental caries.

Fluoride content in drinking water varies around the world depending on geographical locations. A study in Egypt by Abu Zeid (2010) reported that source of tap water was mainly the River Nile and the range of fluoride in tap water was 0.330-0.337 mg/L, while the source of drinking water in Marsa Matrouh and Arish, however, was groundwater coming from artesian wells. This water contained higher fluoride level with an average of 0.761-0.926 mg/L.

One-way ANOVA was used to determine the significant difference in fluoride levels at different sampling locations. From the results, there was no significant difference in fluoride levels at different sampling locations (P= 0.514). This can be due to same sources of water supply from Empangan Semenyih. However, a study from Akpata et al. (2009) stated that the fluoride levels in the various water sources were significantly higher in North Central geopolitical zone than in the other zones ($p < 0.05$). High fluoride levels have been reported in natural waters at the rift valley extending to Kenya and Ethiopia, especially in the low land areas with recent volcanic eruptions. Fluoride level varied in different parts of the country could be due to different sources of water supply.

5.1.5 Relationship between fluoride in drinking water and urinary fluoride of respondents.

Pearson test was used to determine the relationship between level of fluoride in drinking water and urine. From this parametric test, there was no significant relationship between level of fluoride in drinking water and urinary fluoride of respondents ($r = 0.05$, $p = 0.694$). This may due to majority sources of the fluoride consumed are not from drinking water. A study from Wang et al. showed that there was no positive correlation relationship was found between fluorine levels in urine and those in drinking water in urban and rural areas of northwestern China. Another study done by Forte et al. (2008) also supported this finding, that there was no significant correlation between fluoride level in drinking water and urinary fluoride in a tropical area of Brazil.

5.2 Limitation

The study used cross-sectional design which only measured exposure status and outcomes at a point of time. This was due to limited period of time and budget to conduct the research. The number of students was less than the minimum sample size required. Hence, this may affect the result of findings. In addition, purposive sampling had a potential bias in the researcher's criteria and resulting sample selections might not represent the whole population.

5.2.1 Bias and confounders

Recall bias might affect the accuracy of the results when respondents had to recall the past event. The confounding factors such as other sources of drinking water, liquids consumed, and usage of dental products should be noted in this study.

5.3 CONCLUSION

From the study, the mean value of the level of fluoride in drinking water was 0.34 ± SD 0.20 mg/L with the range value between 0.02-0.73 mg/L while the mean value of the level of urinary fluoride was 1.37 ± SD 0.39 mg/L with the range value between 0.44-2.19 mg/L.

Independent t-test was used to determine the relationship of urinary fluoride with gender. However, there was no significant relationship of urinary fluoride between male and female ($t (63) = 1.769$, $p= 0.082$). Thus, the hypothesis of the relationship of urinary fluoride between male and female was rejected.

Furthermore, one-way ANOVA was used to determine the difference in fluoride levels from different sampling locations. From the results that was obtained, there was no significant difference in fluoride levels from different sampling locations ($P = 0.514$). So, the hypothesis of the difference in fluoride levels from different sampling locations was also rejected.

Pearson test was used to determine the relationship between level of fluoride in drinking water and urine. From this parametric test, there was no significant relationship between level of fluoride in drinking water and urinary fluoride of respondents ($r = 0.05$, $p = 0.694$). Thus, the hypothesis of the relationship between level of fluoride in drinking water and urine was rejected.

In conclusion, all of the objectives in this study were achieved.

5.3 RECOMMENDATION

The study was conducted by using the cross sectional study with only 65 13-year-old school children as respondents from Sekolah Menengah Kebangsaan Aminuddin Baki, Kuala Lumpur. With such a very small respondents, this study was not suitable to be represented for all the school children population in that area of study. Thus for the future study, it was suggested to have a large respondents number and also must not be confined to only one school in that particular area so that the study could represent a particular population to a particular area of study.

It was also suggested that the experience lab technicians should conduct the analysis of the fluoride in drinking water and urine while using a manually reading machine or if convenient, using the automated and expensive reading machine can avoid any technical reading error which was very common when obtaining the primary data.

Furthermore, health education should be given regardless of their ages in order to increase the community awareness towards the effect of fluoride on health. Nowadays, the community was still lacking the knowledge regarding the fluoride even though most of this element had been in human consumer products especially in foods and beverages. Health care association should really need to be more active in contributing to awareness of the pros and cons of fluoride towards human health.

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Appendix 1
Ethical Approval Letter from UPM

Attachment 2

JKEUPM Ref No. : FPSK_Mei(13)04

Members of the JKEUPM who reviewed the documents:

Prof. Dr. Zamberi Sekawi

Date of approval: 29/7/2013

Endorsed at JKEUPM Meeting on **2/8/2013**, attended by:

NAME	DESIGNATION	GENDER	TICK IF PRESENT
Prof. Dr. Norlijah Othman	Paediatrics& Dean, Faculty of Medicine and Health Sciences	Female	√
Prof. Dr Zamberi Sekawi	Medical Microbiologist & Deputy Dean of Research and Internationalization, Faculty of Medicine and Health Sciences	Male	√
Prof. Dato' Dr. Lye Munn Sann	Medical Statistician, Dept of Community Health, Faculty of Medicine and Health Sciences	Male	
Prof. Dr. Tengku AizanAbd Hamid	Gerontologist & Director, Institute of Gerontology	Female	√
Prof. Dr. Lekhraj Rampal	Medical Statistician, Dept of Community Health, Faculty of Medicine and Health Sciences	Male	
Prof. Dr. Elizabeth George	Pathologist, Dept of Pathology, Faculty of Medicine and Health Sciences	Female	√
Prof. Dr. Lim ThiamAun	Anesthesiologist, Dept of Surgery, Faculty of Medicine and Health Sciences	Male	
Prof. Dr. Wan Omar Abdullah	Medical Parasitologist, Dept of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences	Male	
Prof. Dr. Patimah Ismail	Professor of Biomedicine, Dept of Biomedical Sciences, Faculty of Medicine and Health Sciences	Female	√
Assoc. Prof. Dr. Johnson Stanslas	Pharmacologist, Dept of Medicine, Faculty of Medicine and Health Sciences	Male	√
Assoc. Prof Dr. Mansor Abu Talib	Assoc. Professor of Guidance and Counselling, Dept of Human Development and Family Studies, Faculty of Human Ecology	Male	
Assoc. Prof. Dr. Noritah Omar (Lay Person)	Assoc. Professor of English Language, Dept of English Language, Faculty of Communication and Modern Languages	Female	√
Dr. RojanahKahar (Lay Person)	Lecturer of Dept of Human Development and Family Studies, Faculty of Human Ecology	Female	√
Tan Sri Dato' Napsiah Omar (Lay Person)	Chairman, National Population and Family Development Board	Female	

Appendix 2

Permission Letter from Ministry of Education Malaysia

Appendix 3

Parents Consent Form

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

CONSENT FORM (PARENTS/GUARDIAN)

STUDY TITLE : Level of Fluoride in Drinking Water and Its Relationship with Urinary Fluoride among Thirteen year-old School Children in a Secondary School in Kuala Lumpur.

RESEARCHER : FAIZ AMIN BIN AMINUDDIN
GOH SOO LING

I .... Identity Card No. .... address...
....hereby voluntarily agree to allow my *son / daughter / ward.. to take part in the clinical research *(clinical study, questionnaire study/ drug trial) specified above.

I have been informed about the nature of the clinical research in terms of methodology, possible adverse effects and complications (as written in the Respondent Information Sheet). I understand that my *son / daughter / ward has the right to withdraw from this clinical research at any time without assigning any reason whatsoever. I also understand that this study is confidential and all information provided with regards to the identity of my* son / daughter / ward will remain private and confidential.

I* wish / do not wish to know the result of the tests performed on any samples taken from my *son / daughter / ward.

* delete where necessary

Signature .... (*Parent/Guardian*)

Signature .... (*Witness*)

Date :..

Name :..

I/C No. :..

I confirm that I have explained to the respondent's parent/guardian the nature and purpose of the above –mentioned clinical research.

Date ....

Signature .... (*Researcher*)

Appendix 4

Respondent's Information Sheet

JAWATANKUASA ETIKA UNIVERSITI UNTUK
PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG,
SELANGOR, MALAYSIA

RESPONDENT'S INFORMATION SHEET

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

STUDY TITLE

Level of Fluoride in Drinking Water and Its Relationship with Urinary Fluoride among Thirteen year-old School Children in a Secondary School in Kuala Lumpur.

INTRODUCTION

This research is to study the fluoride consumption in drinking water and its relationship with urinary fluoride. Unconsciously, fluoride can be found not only in drinking water, but also in food and the air that we are exposed to. An excess level of fluoride will bring various adverse effects, including dental fluorosis and crippling skeletal fluorosis. These effects can be detected through the level of fluoride found in urine.

WHAT WILL YOU HAVE TO DO?

You will need to sign the consent form which states your interest in participating in the research as a respondent. This will be done after you have read and understand the contents of this information sheet. The consent form has to be returned to the researcher. Each respondents will be given a set of questions that has to be answered before sample collection begins. The respondent's drinking water and urine sample will be taken to determine the fluoride level.

WHO SHOULD NOT ENTER THE STUDY?

Respondents who are absent on the day which the study is being carried out and respondents who use water filter at their home.

WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

You are able to know the level of fluoride in your body and the fluoride content in your drinking water.

b) TO THE INVESTIGATOR?

The result of this study will be used to improvise the knowledge and management of the fluoride level in the near future. This information can be used for policy formulation and implementation of programs in an effort to improve the health status of future generations.

WHAT ARE THE POSSIBLE RISKS?

There are no risks associated with this research.

WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

All information regarding the respondent will be remained private and confidential. There will be no elaboration of the individual from all aspects of discussion.

WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

If you have any queries, you can contact any members of the research team.

FAIZ AMIN BIN AMINUDDIN	012-7085474
GOH SOO LING	017-2120294

Thank you for your help and cooperation.

FAIZ AMIN BIN AMINUDDIN
GOH SOO LING

Researcher,
Medical Doctor
Universiti Putra Malaysia

Appendix 5
Questionnaire Form

UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

No Siri: | | | | |

JABATAN KESIHATAN KOMUNITI
FAKULTI PERUBATAN DAN SAINS KESIHATAN
UNIVERSITI PUTRA MALAYSIA

"KEPEKATAN FLUORIDA DALAM AIR MINUMAN DAN HUBUNGANNYA DENGAN FLUORIDA DALAM URIN DALAM KALANGAN PELAJAR SEKOLAH TIGA BELAS TAHUN DI SEKOLAH MENENGAH DI SEKITAR KUALA LUMPUR"

Adalah dimaklumkan bahawa satu kajian tentang kesihatan akibat pendedahan fluorida sedang dijalankan di tempat kerja anda. Sehubungan dengan itu, sukacita dimaklumkan bahawa anda telah terpilih untuk menjadi salah seorang responden kajian ini. Oleh demikian, anda diminta menjawab semua soalan yang dikemukakan dengan mengikut arahan yang telah diberikan. Segala maklumat berkenaan responden akan dirahsiakan dan hanya akan digunakan untuk kajian ini.

ID Responden: | | | | | | | | | | |

Tarikh kajian: ....

ARAHAN SOALAN:

1. Kertas soalan ini mengandungi empat (4) bahagian iaitu:

   Bahagian A: Latar Belakang Responden

   Bahagian B: Maklumat Kesihatan Pergigian Pelajar

   Bahagian C: Maklumat Pendedahan Fluorida

   Bahagian D: Maklumat Keluarga

2. Anda diminta menjawab semua soalan yang ada dalam buku soalan ini.

3. Untuk menjawab, anda perlulah menandakan (√) pada ruangan kotak yang disediakan.

4. Buku soalan ini hendaklah diserahkan semula kepada pengkaji setelah selesai menjawab semua

Bahagian A (Latar Belakang Responden)

1. Tarikh :..

2. Jantina:..

3. Bangsa :..

4. Darjah :..

5. Nama Sekolah:..

6. Alamat Rumah:..

7. Maklumat Sekolah Rendah Darjah 1 hingga Darjah 6

Bilangan	Nama sekolah rendah	Dari darjah	Ke darjah

Bahagian B (Maklumat Tentang Kesihatan Pergigian Pelajar)

8.Sejauh manakah anda berpuas hati dengan keadaan gigi anda sendiri?

Tandakan (/) pada jawapan yang paling tepat

a. Sangat memuaskan …terus kesoalan 11

b. Memuaskan …terus kesoalan 11

c. Tidak Memuaskan …terus kesoalan 12

9. Berapa kerapkah anda berjumpa doctor gigi?

a. 3 bulan sekali

b. 6 bulan sekali

c. 12 bulan sekali

d. Tidak pernah

10. Sejauh manakah anda berpuas hati dengan warna gigi hadapan anda?

Tandakan (/) pada jawapan yang paling tepat

a. Sangat memuaskan

b. Memuaskan

c. Tidak memuaskan

11. Jika anda menjawab c = tidak memuaskan pada soalan 11, Mengapa?

Untuk menjawab soalan ini anda boleh menjawab lebih dari satu jawapan. Sila tandakan (/) pada kotak berkaitan.

a. Kerana gigi saya berjalur/tompok putih

b. Kerana gigi saya berwarna kuning/coklat/kelabu

c. Sebab-sebab lain

(Nyatakan……………………………………………)

12. Adakah anda melakukan perkara berikut kerana keadaan warna gigi hadapan anda?

Untuk menjawab soalan ini anda boleh menjawab lebih dari satu jawapan. Sila tandakan (/) pada kotak berkaitan.

a. Menutup mulut bila senyum dan ketawa ☐

b. Mengelak dari keluar bersama rakan-rakan ☐

c. Menggunakan bahan-bahan pemutih gigi ☐

d. Berjumpa doktor gigi ☐

13. Jika anda berjumpa doctor gigi, apakah yang doctor gigi lakukan?

a. Memberikan rawatan ☐

b. Memberikan nasihat ☐

14. Adakah anda pernah mengalami perasaan berikut kerana keadaan gigi hadapan anda?

Untuk menjawab soalan ini anda boleh menjawab lebih dari satu jawapan.Sila tandakan (/) pada kotak berkaitan.

a. Runsing kerana warna gigi ☐

b. Tidak yakin dalam pergaulan ☐

c. Perasaan lain ☐

(Nyatakan:…………..)

Bahagian C (Maklumat Pendedahan Fluorida)

15. Jenis kawasan tempat tinggal?

a. Bandar

b. Luar Bandar

c. Pekan (Separuh Bandar)

16. Jenis bekalan air yang anda terima di rumah?

a. Paip JBA

b. Telaga

c. Gabungan mana-mana di atas

d. Lain-lain

(Nyatakan:………………………………)

17. Apakah jenama ubat gigi yang anda selalu gunakan?

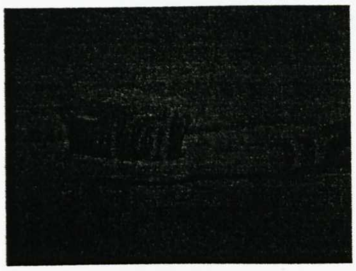
(Nyatakan:………………………………)

17. Berapa kerapkah anda menggosok gigi dalam sehari?

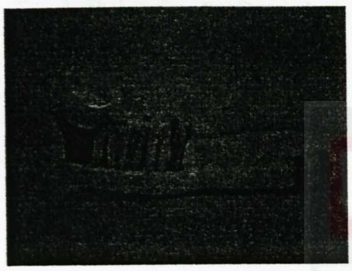
……..kali sehari

18. Berapakah kuantiti ubat gigi berfluorida yang anda gunakan setiap kali menggosok gigi?

(Rujuk gambar dan tandakan (/) pada kotak)



☐



☐

☐

19. Berapakah gelas air masak atau air tapisan yang anda minum sehari?

…………………gelas sehari

20. Apakah air minuman kegemaran anda dan berapa gelas anda minum sehari?

…………………gelas sehari

21. Pernahkah anda mengalami kesakitan buah pinggang baru-baru ini?

Ya ☐

Tidak ☐

Bahagian D (Maklumat Keluarga)

22. Pekerjaan bapa atau penjaga (tandakan (/) pada kotak)

    a. Professional ☐

    b. Peniaga ☐

    c. Pegawai kerajaan ☐

    d. Pekerja upahan ☐

    e. Pesara ☐

    f. Bekerja sendiri ☐

    g. Lain-lain (Nyatakan:……………….)

23. Pekerjaan ibu (tandakan(/) pada kotak)

    a. Professional ☐

    b. Pegawai kerajaan ☐

    c. Peniaga ☐

    d. Pesara ☐

    e. Pekerja upahan ☐

    f. Suri rumah ☐

    g. Lain-lain (Nyatakan:……………….)

24. Pendapatan ibu-bapa (penjaga) sebanyak RM………..

Appendix 6

USEPA SPANDS Method

Fluoride

★Method 8029

SPADNS Method*

Reagent Solution or AccuVac® Ampuls

(0.02 to 2.00 mg/L F⁻)

Scope and Application: For water, wastewater and seawater; USEPA accepted for reporting for drinking and wastewater analyses (distillation required; see *Distillation on page 3*)**

* Adapted from *Standard Methods for the Examination of Water and Wastewater, 4500-F B & D*

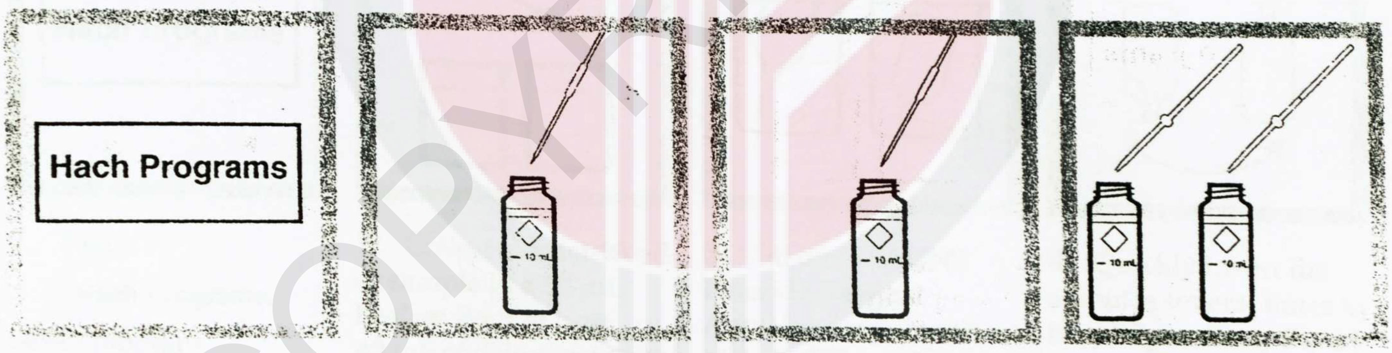
** Procedure is equivalent to USEPA method 340.1 for drinking water and wastewater.

Tips and Techniques

- The sample and deionized water should be at the same temperature (±1 °C). Temperature adjustments may be made before or after reagent addition.
- SPADNS Reagent is toxic and corrosive. Use care while handling the reagent.
- For best results, measure the volume of SPADNS Reagent as accurately as possible.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.

  SPADNS Reagent contains sodium arsenite. Final solutions will contain arsenic (D004) in sufficient concentration to be regulated as a hazardous waste for Federal RCRA. See *Section 4* for information on proper disposal of these materials.
- If the instrument displays **OVER!**, dilute a fresh sample (5 mL) with an equal volume of deionized water and repeat the test, using this solution in *step 2*. Multiply the result by 2.

Using SPADNS Reagent — Method 8029

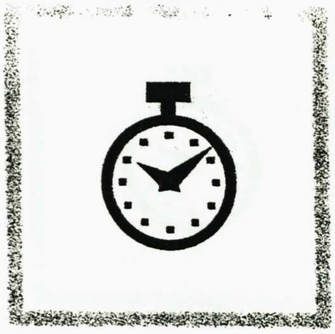


1. Touch **Hach Programs**. Select program **190 Fluoride**. Touch **Start**.

2. Pipet 10.0 mL of sample into a dry, round sample cell (this is the prepared sample).

3. Pipet 10.0 mL of deionized water into a second dry, round sample cell (this is the blank).

4. Carefully pipet 2.0 mL of SPADNS Reagent into each cell. Swirl to mix.



5. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.

7. Touch **Zero**.

The display will show:

0.00 mg/L F–

8. Place the prepared sample into the cell holder.

Results will appear in mg/L F–.

AccuVac Ampul — Method 8029

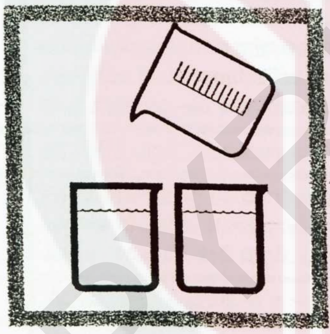
1. Touch

Hach Programs.

Select program

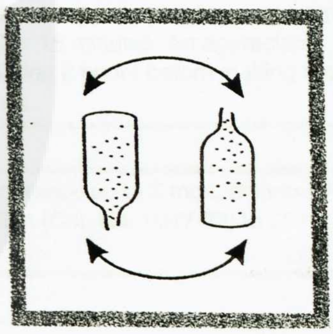
195 Fluoride AV.

Touch **Start**.

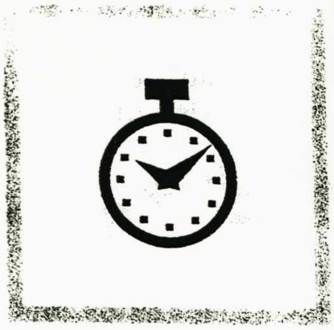


2. Collect at least 40 mL of sample in a 50-mL beaker. Pour at least 40 mL of deionized water into a second beaker.

3. Fill one SPADNS Fluoride Reagent AccuVac Ampul with sample. Fill another ampule with deionized water (the blank). Keep the tips immersed while the ampules fill completely.



4. Quickly invert the ampules several times to mix. Wipe off any liquid or fingerprints.



5. Touch the timer icon.

Touch **OK**.

A one-minute reaction period will begin.

6. When the timer beeps, place the blank into the cell holder.

7. Touch **Zero**.

The display will show:

0.00 mg/L F⁻

8. Place the AccuVac Ampul that contains the sample into the cell holder.

Results will appear in mg/L F⁻.

Interferences

This test is sensitive to small amounts of interference. Glassware must be very clean (acid rinse before each use). Repeat the test with the same glassware to ensure that results are accurate.

Interfering Substance	Interference Levels and Treatments
Alkalinity (as $CaCO_3$)	At 5000 mg/L it causes a –0.1 mg/L F^- error.
Aluminum	At 0.1 mg/L it causes a –0.1 mg/L F^- error. To check for interferences from aluminum, read the concentration one minute after reagent addition, then again after 15 minutes. An appreciable increase in concentration suggests aluminum interference. Waiting 2 hours before making the final reading will eliminate the effect of up to 3.0 mg/L aluminum.
Chloride	At 7000 mg/L it causes a +0.1 mg/L F^- error.
Chlorine	SPADNS Reagent contains enough arsenite to eliminate interference up to 5 mg/L chlorine. For higher chlorine levels, add one drop of Sodium Arsenite Solution (Cat. No. 1047-32) to 25 mL of sample for each 2 mg/L of Chlorine.
Iron, ferric	At 10 mg/L it causes a –0.1 mg/L F^- error.
Phosphate, ortho	At 16 mg/L it causes a +0.1 mg/L F^- error.
Sodium Hexameta-phosphate	At 1.0 mg/L it causes a +0.1 mg/L F^- error.
Sulfate	At 200 mg/L it causes a +0.1 mg/L F^- error.

Distillation

1. Most interferences can be eliminated by distilling the sample from an acid solution as described below:
2. Set up the distillation apparatus for general purpose distillation. Refer to the Distillation Apparatus manual for proper assembly. Use a 125-mL Erlenmeyer flask to collect the distillate.
3. Turn on the water and maintain a steady flow through the condenser.
4. Measure 100 mL of sample into the distillation flask using a 100-mL graduated cylinder. Add a magnetic stir bar and 5 glass beads.
5. For proof of accuracy, use a 1.0-mg/L Fluoride Standard Solution in place of the sample.

6. Turn the stirrer power switch on. Turn the stir control to 5.
7. Using a 250-mL graduated cylinder, carefully add 150 mL of StillVer® Distillation Solution into the flask. (StillVer Distillation Solution is a 2:1 mixture of concentrated sulfuric acid and water.)

Note: *When distilling samples with high amounts of chloride, add 5 mg of Silver Sulfate (Cat. No. 334-14) to the sample for every mg/L of chloride in the sample.*

8. With the thermometer in place, turn the heat control to 10. The yellow pilot lamp indicates the heater is on.
9. When the temperature reaches 180 °C or when 100 mL of distillate has been collected, turn the still off (requires about 1 hour).
10. Dilute the distillate to a volume of 100 mL, if necessary. The distillate may now be analyzed by the SPADNS or the fluoride ion-selective electrode method.

Sample Collection, Storage and Preservation

Samples may be stored in glass or plastic bottles for at least seven days when cooled to 4 °C (39 °F) or lower. Warm samples to room temperature before analysis.

Accuracy Check

Standard Solution Method

A variety of standard solutions covering the entire range of the test is available from Hach. Use these in place of sample to verify technique.

Minor variations between lots of reagent become measurable above 1.5 mg/L. While results in this region are usable for most purposes, better accuracy may be obtained by diluting a fresh sample 1:1 with deionized water and retesting. Multiply the result by 2.

To adjust the calibration curve using the reading obtained with a Fluoride Standard Solution:

1. Touch **Options** on the current program menu. Touch **Standard Adjust: Off**.
2. Touch **On**. Touch **Adjust** to accept the displayed concentration (the value depends on the selected units). If an alternate concentration is used, touch the number in the box to enter the actual concentration, then touch **OK**. Touch **Adjust**.

See *Section 3.2.3 Adjusting the Standard Curve* on page *38* for more information.

Method Performance

Precision

Standard: 1.00 mg/L F^-

Program	95% Confidence Limits of Distribution
190	0.95–1.05 mg/L F^-
195	0.88–1.12 mg/L F^-

See *Section 3.4.3 Precision* on page *42* for more information, or if the standard concentration did not fall within the specified range.

Sensitivity

[illegible]	[illegible]	[illegible]	[illegible]
at 0	0.010	0.03 mg/L F^-	0.02 mg/L F^-
at 1 ppm	0.010	0.03 mg/L F^-	0.03 mg/L F^-
at 2 ppm	0.010	0.04 mg/L F^-	0.04 mg/L F^-

See *Section 3.4.5 Sensitivity* on page 43 for more information.

Summary of Method

The SPADNS Method for fluoride determination involves the reaction of fluoride with a red zirconium-dye solution. The fluoride combines with part of the zirconium to form a colorless complex, thus bleaching the red color in an amount proportional to the fluoride concentration. This method is accepted by the EPA for NPDES and NPDWR reporting purposes when the samples have been distilled. Seawater and wastewater samples require distillation. Test results are measured at 580 nm.

Required Reagents

Description	Quantity Required per Test	Unit	Cat. No.
SPADNS Reagent Solution	4 mL	500 mL	444-49
or SPADNS Fluoride Reagent AccuVac® Ampuls	2 ampuls	25/pkg	25060-25
Water, deionized	10 mL	4 liters	272-56

Required Apparatus (Using Solution)

Pipet Filler safety bulb	1	each	14651-00
Pipet, volumetric, Class A, 2.00-mL	1	each	14515-36
Pipet, volumetric, Class A, 10.00-mL	1	each	14515-38
Sample Cells, 10-mL, w/cap	2	6/pkg	24276-06
Thermometer, -10 to 110 °C	1	each	1877-01

Required Apparatus (Using AccuVac® Ampuls)

Beaker, 50-mL	2	each	500-41H

Required Standards

Fluoride Standard Solution, 0.2-mg/L F^-	500 mL	405-02
Fluoride Standard Solution, 0.5-mg/L F^-	500 mL	405-05
Fluoride Standard Solution, 0.8-mg/L F^-	500 mL	405-08
Fluoride Standard Solution, 1.0-mg/L F^-	1000 mL	291-53
Fluoride Standard Solution, 1.0-mg/L F^-	500 mL	291-49
Fluoride Standard Solution, 1.2-mg/L F^-	500 mL	405-12
Fluoride Standard Solution, 1.5-mg/L F^-	500 mL	405-15
Fluoride Standard Solution, 2.0-mg/L F^-	500 mL	405-20
Fluoride Standard Solution, 100-mg/L F^-	500 mL	232-49

Distillation Reagents and Apparatus

Cylinder, graduated, 100-mL	1	each	508-42
Cylinder, graduated, 250-mL	1	each	508-46
Select one:			
Distillation Heater and Support Apparatus Set, 115 VAC, 50/60 Hz	1	each	22744-00
Distillation Heater and Support Apparatus Set, 230 VAC, 50/60 Hz	1	each	22744-02
Distillation Apparatus Set, General Purpose	1	each	22653-00
Flask, Erlenmeyer, 125-mL	1	each	20897-43
Glass Beads	5	100/pkg	2596-00
StillVer® Distillation Solution		500 mL	446-49
Stir Bar, magnetic	1	1	10764-16

HACH®

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In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

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Telephone: (970) 669-3050
FAX: (970) 669-2932